SunGrant INITIATIVE

Science for Biomass Feedstock Production and Utilization

Proceedings of the 2012 Sun Grant National Conference

Volume 1

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1 ENERGY CROP DEVELOPMENT

1.1
THE SUNGRANT POPLAR WOODY CROPS RESEARCH PROGRAM: ACCOMPLISHMENTS AND IMPLICATIONS

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Abstract
Members of the genus *Populus* are considered some of the most promising taxa for use as woody energy crops due to their rapid growth, ease of propagation, repeated multiple harvests through coppicing, desirable raw material properties and potential for genetic improvement through inter-specific hybridization followed by clone selection. The aim of the Sungrant Poplar Feedstocks Team is to increase yield and genetic diversity of poplar for use as an energy crop. To that end, a total of 93 research sites are actively measured in a large national field study network. This includes sites that pre-date the DOE/Sun Grant program and those established since the program began. Data are collected from clonal trials, yield tests, spacing trials, and large-scale genetics tests with some locations containing over 900 genotypes. Information is reported on progress in breeding, field testing of new genotypes and yield analyses in the various regions of the United States.

Keywords: Biomass Production, Poplar, Genetics, Populus deltoides, Populus nigra, Clone Screening

Introduction
Hybrid poplars are grown commercially throughout the United States to produce fiber, biofuels feedstock and for phytoremediation (Zsuffa et al. 1996). Genotypes of Eastern cottonwood (*P. deltoides* Bartr. ex Marsh) and hybrids between eastern cottonwood and Asian black poplar, European black poplar, and western black cottonwood (*P. maximowiczii* A. Henry, *P. nigra* L., and *P. trichocarpa* Torr. & Gray, respectively) have been identified that are capable of producing yields ranging from 9 metric tons hectare^{-1} year^{-1} in the northern United States to 20 tons hectare^{-1} year^{-1} in the Pacific Northwest as a result of varying climate, fertility, and cultural practices.

Several traits make poplar species a potentially ideal energy crop. Plantations of clonal populations can be established using dormant hardwood cuttings producing adventitious roots reducing propagation and planting costs (Heilman et al. 1995). Similar to willow, these species exhibit the ability to resprout after dormant-season harvest allowing multiple harvests without replanting. Other important traits are relatively high inherent biomass density, low ash content, a wide natural range and the ability to readily hybridize among several species. Also, natural variation in important traits such as growth rate, form and disease resistance facilitates high gain and significant increases in yield are expected through genetic improvement (Fig. 1).

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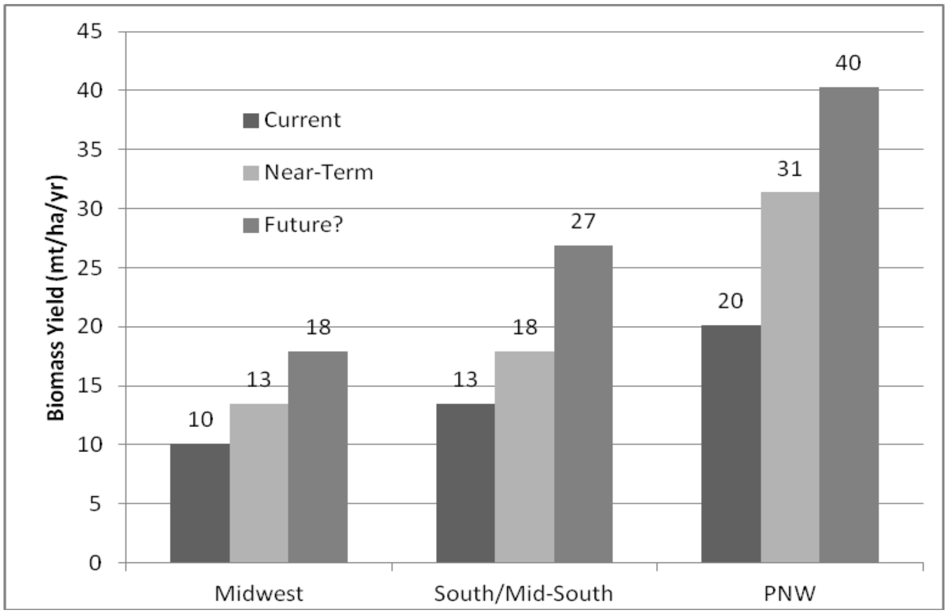

Figure 1. Current, Near-Term and Expected Future Yields of Poplar in the Midwest, South/Mid-South and Pacific Northwest.

The Sun Grant Poplar Woody Crops Program is a collaboration of industry and academic institutions including the University of Minnesota, Mississippi State University, Michigan State University, GreenWood Resources LLC and ArborGen. This group is conducting research to improve yield and disease resistance of poplar by sampling natural populations, conducting breeding and field-testing new clones at various locations across the U.S. Also, once superior genotypes are selected, work is being done to document yield improvements in larger blocks. The Poplar Feedstock Team study network includes a total of 93 field sites (Table 1, Fig.2).

Table 1. Sungrant Poplar Woody Crops study sites by institution, state and study type

Institution	State	Clone Test	Yield Study	Large Scale Genetics	Nursery	Notes
University of Minnesota	MN	20	20	13	2	70 clones in clone tests, 12 clones/site in yield blocks, breeding nursery
Michigan State	MI	6	1			70 clones/site in clone tests, Escanaba, MI yield tests
Mississippi State	MO	1				Consolidated Clone Test - 80 clones, 2 tests
Mississippi State	MS	2			2	Consolidated Clone Test - 80 clones 2 tests totally 140 clones; Populus deltoids Progeny Test; Populus nigra study; and two new stoolbeds
ArborGen	SC	6			2	Consolidated Clone Test - 80 clones, other clone trials, P. nigra nursery test
GreenWood Resources	OR	9			1	Boardman, Westport - includes CCTs, varietal trials, breeding nursery
GreenWood Resources		8				Varietal Site Trials - OR, ID, NM, WA

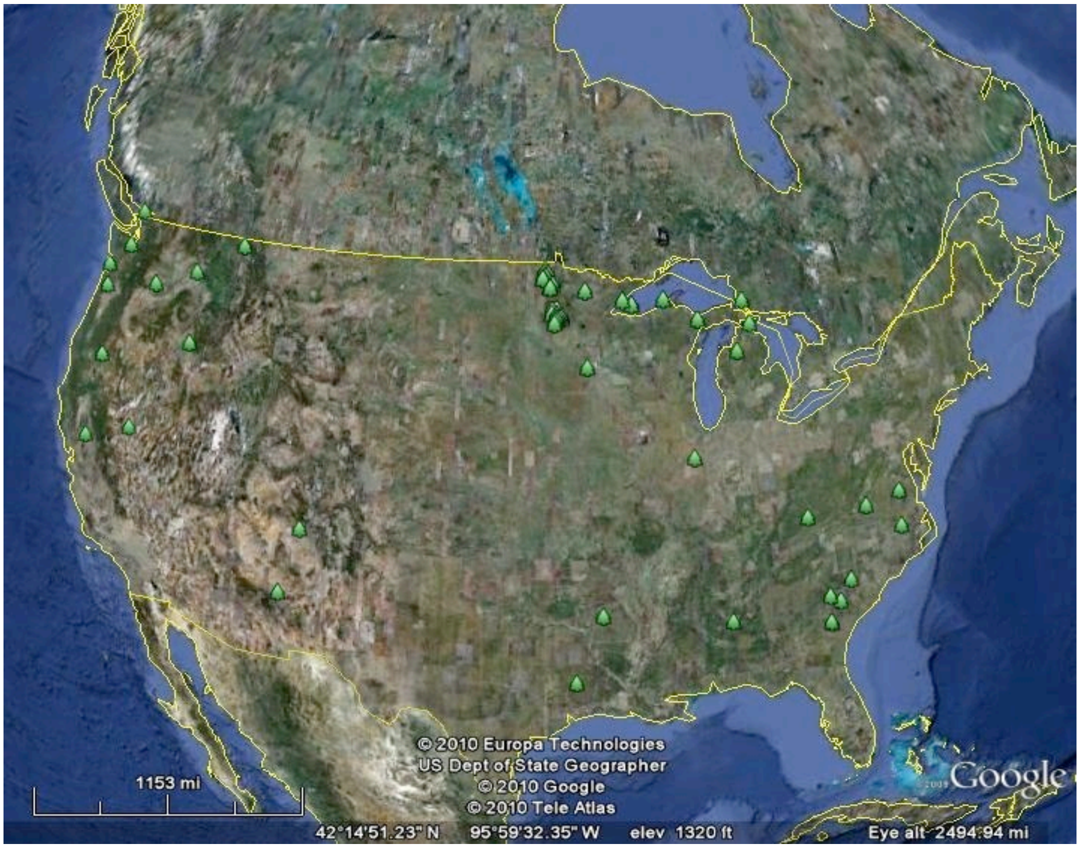

Figure 2. Poplar research site locations in the Sungrant study network.

Genetic Improvement Background

Genetic improvement research has varied considerably across the U.S. depending on the region. In the Upper Midwest prior to the mid-1990s, most genetic improvement work in woody crops consisted of a series of field tests using clones that were developed in other programs; notably the Oxford Paper program which began in the 1920s by Ernest Schreiner and those produced in Europe. In the mid-1980's, clone tests of this material were planted at multiple locations to assess growth rate and susceptibility to disease. Of the more than 70 clones that were tested, only three clones, DN34, DN5 and NM6, were found to be disease resistant and fast growing under a range of conditions (Hansen et al. 1994). The resulting set of clones was not genetically diverse which posed a risk to commercial production.

In the southern U.S., *P. deltoides* clones were developed for sites along the Lower Mississippi Alluvial Valley (LMAV). While these clones can be used on sites outside of the LMAV area, they perform best on those sites that are high in nutrient availability and have consistent rainfall. Unlike pure *P. deltiodes*, limited testing of hybrid poplars in the South has produced mixed results due to *Septoria* canker, a serious disease of some poplars. In the Pacific Northwest, work began in the early-1980s to identify superior clones of *P. trichocarpa* and produce hybrids of *P. deltoides* and *P. trichocarpa* for commercial production. While genetic improvement programs changed ownership due to mergers among the industry, the region has a long history of genetic improvement efforts with GreenWood Resources being the only currently active program in the Pacific Northwest. In light of the relatively short history of poplar development as an energy crop and opportunities for yield improvement, the need clearly exists to produce new populations of poplar and test this material nationally.

The goal of breeding of *Populus* as an energy crop is to bring together commercially important traits in one genotype. *P. deltoides*, while potentially fast growing and disease resistant, roots from hardwood cuttings at a lower rate than other species resulting in higher establishment costs. *P. nigra* as a group isn't necessarily fast growing. However, rooting ability of most clones of *P. nigra* is high and fast growing genotypes can be identified. *P. trichocarpa* and *P. maximowiczii* are generally fast-growing but lack widespread resistance to *Septoria* canker. Due to the ability to hybridize among these species, it is possible to produce hybrids that have characteristics that any one species alone does not possess.

Based on experience, poplar clones must be developed for adaptability and high productivity for specific regions. While some energy crops such as switchgrass appear to exhibit a relatively wide regional adaptability (Casler et al. 2007), poplar hybrids appear to have a more limited adaptability. Even though *P. deltoides* occurs across a wide geographic range, segregation has resulted in subpopulations that are suited to a limited geographic range. This is particularly true in the case of northern climates where harsh winters eliminate clones of more southerly origin. Also, differences in disease susceptibility among regions are evident. *Septoria* canker is a serious disease limiting clone selection in the Upper Midwest and potentially, in the Mississippi River Valley. However, *Septoria* canker is not a significant problem in the Pacific Northwest and evidence suggests it may not be as prevalent in the eastern U.S. and South. As a result, breeding underway at the two Sun Grant breeding centers is not targeting the same species and commercially successful clones will not be the same among regions.

Refinement of Parent Populations

Prior to conducting controlled crossing, refinement of parent populations is important. Random crossing using parents with no prior local performance data is not a good strategy to increase the genetic quality through time. A guiding principle in the breeding program is refinement of parental populations using collections of natural populations planted on field sites in target regions to identify pure-species parents suitable for regional breeding. In 2005, University of Minnesota staff contacted members of the EUFORGEN network to obtain a pan-European collection of *Populus nigra* for field testing. The EUFORGEN network is a collaboration of government and academic institutions aimed at preservation of *P. nigra* in Europe. A total of 34 sources were obtained comprising over 2,000 clones. After field testing, a subset of clones was selected and an archive established in Minnesota. A sampling of this population and from a collection obtained by GreenWood Resources was distributed to Sun Grant cooperators in the South. Over time, these plantings will provide a source of regionally-adapted *P. nigra* for future breeding. Similar work is underway in refinement of *P. deltoides* and *P. trichocarpa.* To our knowledge, this approach has not been used before in the U.S and we view selection of regionally-adapted parental material as the next major step forward in genetic improvement of poplar in the U.S.

Breeding

Poplar breeding is being done at two locations as part of the Sungrant program; in the Pacific Northwest by GreenWood Resources (GWR) and at the University of Minnesota's, Natural Resources Research Institute (UM-NRRI). Production of interspecific hybrids is ongoing using parental populations of *P. deltoides* crossing with *P. nigra*, *P. trichocarpa* and *P. maximowiczii*. Breeding underway by GWR includes 90 crosses of DxD, DxN, DxM, NxN and P. deltoides x P. fremontii, a species native to the western U.S. Breeding at the UM-NRRI in 2012 includes 313 attempts with emphasis on DxN crosses. This includes crosses using *P. deltoides* females of northern and southern origin to provide new DxN clones for testing in the Midwest, South and Mid-South. Of the northern populations, a total of 99 full-sib families were produced representing over 15,000 new genotypes; a very large number given the logistics associated with field-testing this amount of material.

Regional Clone Testing

Due to past work done by the Sun Grant poplar team members, we are in a unique position to test an extensive array of taxa on a national scale. Introduction of new clones for commercial use is a multi-step process. New taxa generated through breeding are subject to a field-testing regimen whereby populations are reduced with only a small subset ultimately selected for further testing. Tests are then done on more sites with increased clone-replication to evaluate growth and disease resistance over a wider geographic range. Finally, a limited number of elite clones are planted in larger-scale yield blocks for evaluation under conditions resembling a commercial plantation.

As part of the overall effort under the SunGrant Feedstock Partnership, members of the Poplar Woody Crops Team have been establishing a series of Consolidated Clone Tests (CCTs) at various locations. These tests are comprised of clones from each of four partners (University of Minnesota, Mississippi State University, GreenWood Resources and ArborGen) for a total of 80 to 90 clones in each test. The purpose of these trials is to determine the adaptability of those genotypes identified as superior under climatic conditions prevalent at each member's location and evaluate the range of climatic adaptability. Identification of the potential adaptability of genotypes is important to the design of a national genetic improvement program and affects the potential range over which collaborative clone exchanges among research institutions could occur.

The oldest of the Consolidated Clone Tests is now three years of age with the most recent data collected in the fall of age two. As expected, results of the CCTs planted in Minnesota show significant mortality among southerly *P. deltoides* clones. The overall mean height at age two in this trial is 3.0 meters with superior clones reaching 4.4 meters in height. Trials planted in Michigan show similar growth with the five fastest growing clones averaging 3.6 meters at age two at Escanaba, Michigan. Height growth in studies in Missouri is highly variable even among southerly-derived material. The average height growth of the five fastest growing clones at the New Madrid, Missouri site is 7.7 meters after two years with clones of southerly origin having higher growth rates. These trials are useful to identify those clones for further yield testing. Also, superior clones will be used as a source of parental stock for breeding.

A series of clone tests planted in Minnesota in 2008 demonstrates the potential to improve poplar yield through genetics. Six sites were planted using an identical set of clones and experimental design. Data were collected in the fall of 2011, four years post-planting. Of the 70 clones tested, NM6, the current commercial standard in Minnesota ranked 66th in biomass index. The average diameter-squared of the top twenty clones was 1.98 times that of NM6 after four years. Thus, significant forward strides in biomass yield are expected through genetic improvement.

One example of the utility of testing clones adapted to one region for use in another region is in the southeastern U.S. Hybrid poplar clones developed by GWR have been planted in a series of trials established between 2008 and 2010. Analyses of the 2011 measurements showed that the top performing hybrid poplar varieties (DM and TD hybrids) were producing 20 to 48 percent more volume than the commercially available eastern cottonwood clones and were the top performers in trials comparing the performance of over 400 eastern cottonwood and hybrid poplar clones.

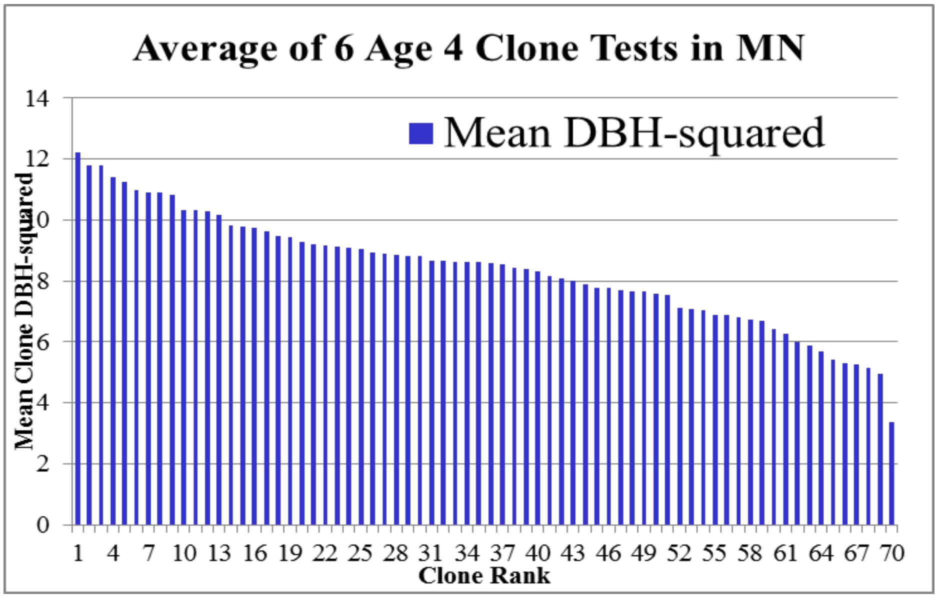

Figure 3. Mean clone rank in a six-site clone test network at age 4 in central Minnesota.

Biomass Yield Studies

Tests such as those in the CCT network provide data on performance relative to other clones. However, biomass yield of single-tree plots is not useful to estimate closed-canopy, long-term biomass yield. After selection for high growth rate and disease resistance in clone tests, the next step is to verify production in blocks that are sufficiently large to eliminate edge-effect and bias toward overestimation of yield. The Sun Grant Poplar Team is measuring a series of yield trials to estimate biomass yield under conditions similar to commercial management.

Studies underway indicate that biomass yields of superior clones in the Upper Midwest are likely to range from 7.5 to 11 mt ha^{-1} yr^{-1} of ovendry biomass. Tests ongoing in the Pacific Northwest have shown yields exceeding 19 mt ha^{-1} yr^{-1}. A new series of yield tests is underway using clones selected from regional tests to evaluate yield gain over commercial clones. At this time, new yield tests indicate gains ranging from 16 to 30% over commercial clones. These tests will provide needed information on biomass yield of poplar using new, previously untested, genetic material.

Conclusions

The wide natural range of poplar and the ease of inter-specific hybridization point to the fact that poplar is one of the most promising species groups for national development of fast growing woody crops. Past research has documented acceptable yields using genetic material that is essentially one generation away from wild populations. Genetic improvement research underway as part of the Sun Grant Poplar Woody Crops Program demonstrates that significant gains in biomass yield are possible and underscores the need for continued, concerted national breeding and field testing.

While results thus far indicate that poplar has the potential to produce high yields on moderately productive sites, this is an important trait requiring a better understanding particularly on less productive agricultural sites.

A series of yield tests of multiple clones having documented regional adaptability should be planted on lands specifically selected for lower agricultural crop productivity. This information is needed to optimally target energy crops on appropriate sites and better inform the “food-versus-fuel” debate.

Based on the work of the Sun Grant Poplar Program to date, the following next steps are recommended to further the work nationally. These include:

1) Continued enhanced breeding using performance data from regional field trials to select parents for future intraspecific crossing;
2) Selection of superior genotypes from the existing field test network, propagation of these clones and testing in clone trials and short-rotation coppiced production systems in multiple regions and;
3) Expansion of new test sites targeting soils and landscapes less attractive for traditional agriculture due to flooding risk or lower crop yield.

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1.2
INITIAL WILLOW BIOMASS YIELD TRIAL RESULTS FOR MICHIGAN

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Abstract

Yield trials comprised of 20-26 willow clones per site were established in Michigan from 2008 to 2011 on sites ranging from south-central Michigan north through the Upper Peninsula in an effort to evaluate the potential for the development of willow as a biofuel feedstock species in Michigan. Initial results show productivity varying greatly among clones and performance rankings shifting between harvests in year 1 and year 4. Survival was also highly variable among clones within site, ranging from 10-99%. Results to date show that are some, but not many clones which are good "general performers" across this geographic region, while the performance of other clones is highly site specific, thus offering the possibility of two different strategies for the production of commercial planting stock for the region. However, it is clear that many of the better clones are those that perform well at specific sites, indicating the importance of local adaptation and broad clonal testing in the development of planting stock recommendations for willow planting stock for biofuel plantations.

Keywords: willow, coppice production, clonal variation, yield, short-rotation energy plantation, biomass.

Introduction

Short rotation, intensively managed plantations of willow species have shown great potential for the sustainable production of biomass and for uses such as phytoremediation in the northeastern and mid-western regions of the United States (Volk *et al*., 2004; Kopp *et al*., 2001;). In establishing such production systems, maximization of yield is essential in order to make energy plantations economically-viable alternatives to other land uses. Accomplishing this depends to a large degree on the identification and development of elite genotypes that are well adapted for rapid growth, as well as disease and pest resistance, on the sites on which the plantations will be established (Smart et al., 2005).

Assessment of genotype performance across regions in forest trees has typically been accomplished through the use of common garden experiments, provenance tests, planted on sites that are typical of those on which commercial production will occur (Wright *et al*., 1972, Bloese and Keathley, 1988) due to genotype X environment interactions that impact growth. Extant breeding programs have produced dozens of willow and poplar clones that show potential for rapid growth as single clone plantations or for use in mixed clone plantations in short rotation coppice systems on other sites and in other regions (Volk, *et al*., 2005; McCracken *et al*.2011), but which have not been tested in Michigan.

Currently there is a need to gain a better understanding of clonal performance in Michigan's climate and soils, and the identification of elite genotypes for that region is needed in order to sustainably provide the woody biomass feed stocks necessary for the development of a biofuel industry. However, to date, the evaluation of elite hybrid genotypes for use in establishing coppice system energy plantations of willow in Michigan has been lacking. The results reported here are from the first such tests in Michigan. This network of plantations was established to evaluate the growth rate and disease and pathogen resistance of willow genotypes planted in short rotation, coppice system plantations across the range of site conditions

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found in Michigan. The network of plantations was established in order to evaluate available genotypes to see if there are clones which exhibit generally high performance across the region or whether it will be necessary to use locally adapted clones for each specific micro-climate or soil type. The results reported here are the first analysis of these plantations and give insights as to whether it will be possible to identify genotypes that show generally high performance across the region and which could be deployed throughout the state, as well pointing to specific genotypes that are highly adapted for growth and survival in each region of the state and which can be used for the establishment of commercial energy plantations in Michigan.

Methods

Yield trials of 33 hybrid willow clones planted in tests using 20-26 clones per site were established in Michigan on sites ranging from south-central Michigan north through the Upper Peninsula. Sites included the Forest Biomass Innovation Center at Escanaba (FBIC), Skandia, Brimley, Onaway, Lake City, and Albion. Un-rooted hardwood cuttings of each hybrid were planted in 78-tree clonal plots at a density of 14,346 tree/ha. The tests are designed to be harvested on three year rotations following an initial coppice cutting at the end of the first growing season. The plantations were established between 2008 and 2011. The specific clones planted at each site are shown in the accompanying tables and all sites are being scored for survival, yield, and pest and pathogen susceptibility/resistance. All trials are designed to be coppiced at the end of the first growing season and harvested thereafter on a three year cycle. Data presented here include survival and oven dry weights expressed as proportions of the site means. Data are from the first year harvest at all sites, as well as the year 4 harvest of the plantation at the FBIC site.

Figure 1. Locations of Hybrid Willow Test Plantations in Michigan.

Results and Discussion

These initial results show productivity varying greatly among clones and across sites. Due to the small initial small samples sizes and large differences in growth rates between the northern and more southern plantations, growth data are presented as a proportion of the plantation mean to facilitate comparison between and within sites. Empty cells in the tables reflect that only subsets of the total group of clones that was tested were planted at the various sites, and so some clones are missing on some sites. As can be seen in Table 1., growth rates differed widely among genotypes at the various test locations, with values ranging from 29% to 184 % of the plantation mean. This large range in the yields among the individual clones at the various sites points to the necessity of testing genotypes thoroughly in order to identify the elite hybrids to be used in commercial plantings.

Table 1. Oven Dry Yield of Hybrid Willow Clones at Six Test Sites in Michigan Expressed as a Proportion of the Respective Site Means.

	Plantation Location					
Clone	FBIC	Skandia	Brimley	Onaway	Lake City	Albion
Allegany	**1.84**	**0.98**	**0.78**	**1.26**	**1.10**	**0.88**
Otisco	1.62	1.13	0.95	.	.	1.57
Owasco	1.61	1.41	1.06	.	.	1.10
Fabius	**1.60**	**1.32**	**0.65**	**1.05**	**1.38**	**1.27**
Tully Champion	1.58	1.43	1.03	1.25	1.79	.
Truxton	**1.52**	**1.13**	**1.07**	**1.26**	**0.80**	**0.74**
SX67	1.40	.	.	0.96	0.96	.
99208-038	1.40	.	.	.	.	.
SV1	**1.32**	**0.73**	**1.37**	**0.90**	**0.49**	**0.72**
Verona	1.17	1.04	1.06	.	.	.
SX64	**1.12**	**1.05**	**0.94**	**0.72**	**1.08**	**0.83**
9882-41	0.90	.	.	1.02	1.01	0.53
Taberg	0.88	.	.	.	.	.
Onondaga	0.87	0.80	1.04	.	.	.
Fish Creek	**0.84**	**1.00**	**0.95**	**1.08**	**0.80**	**0.69**
Marcy	0.81	0.87	1.47	.	.	1.25
Oneida	**0.81**	**1.08**	**1.06**	**0.95**	**0.62**	**0.54**
94001	0.78	0.65	0.97	.	.	.
00X-032-094	0.71	.	.	.	.	.
Sherburne	**0.66**	**0.88**	**0.99**	**0.70**	**0.84**	**0.79**
Saratoga	0.58	.	.	0.99	0.98	.
Millbrook	**0.53**	**0.75**	**1.12**	**1.23**	**0.75**	**0.92**
Cicero	0.47	.	.	.	.	0.76
SX61	**0.45**	**0.83**	**0.66**	**1.13**	**0.66**	**1.07**
Canastota	0.37	0.89	0.90	.	.	1.01
S25	0.29	1.07	0.94	0.78	0.32	.
01X-268-015	.	.	.	.	.	1.71
01X-268-016	.	.	.	1.04	1.56	1.30
Oneonta	.	0.82	0.83	.	.	1.27
01X-265-020	.	.	.	1.31	1.47	0.99
01X-266-016	.	.	.	0.96	2.30	.
S365	.	.	.	0.96	0.70	.
9837-77	.	.	.	0.81	0.39	.

*Clones highlighted in bold were grown on all six test sites. Shaded entries indicate they were above average for all sites in either the Upper or Lower Peninsula.

Differences were not restricted to just variation among genotypes within test locations. Average growth differed significantly across the six sites (ANOVA not shown) based on the set of ten clones that that were common to all six sites (Boldfaced font in the Table1). Clonal growth performance varied dramatically among sites and between the peninsulas, with only three clones growing above the mean at all three Upper Peninsula sites, and two clones above average at all Lower Peninsula sites (shaded). Analysis within sites showed significant growth differences attributable to clone for two sites in the Upper Peninsula, FBIC and Skandia (ANOVA not shown). However in the Lower Peninsula clone effects only approached significance at a single site (Lake City, P = 0.07).

The impact of the variations in local climate and soils on growth and survival was evidenced by separating the plantations into Upper Peninsula and Lower Peninsula groupings for statistical analysis (Tables 2 &3). When grouped geographically in this fashion, overall growth rates varied and clonal performance differences within site were highly significant. Survival also varied significantly between sites and for clones within sites. In the Lower Peninsula differences among sites accounted for 69% and 24% of the total variation in year one harvest mass and survival respectively. This once again emphasizes the lack of individual clones with plasticity for high performance across the region and need to identify specific groups of high performing clones for the varied combinations of soils and climate found within Michigan if economically viable biomass production systems are to be developed and implemented on fallow or abandoned farm acreage that is currently available for planting within this region. One of the more interesting comparisons came from contrasting the growth performance of the plantation at FBIC between Years 1 and 4. Although comparison of the actual harvest yields would be meaningless given the difference in numbers of stems as well as time period for the harvest, an examination of the relative performance in Year 1 as compared with Year 4 showed shifts in ranking among the clones sufficiently large as to warn against use of preliminary data for selection of elite genotypes (Table 4). For example, Allegany was the top ranked clone in Year 1, but ranked 13th in Year 4. Similarly Otisco ranked 2nd in Year 1, but dropped to 9th in Year 4. However, there also were clones that ranked among the best in both samples: Owasco, Tully Champion, SV1, and Truxton. Thus while early data may be useful in culling some of the poorer performers from a testing population, it would not be advisable for selecting clones to be recommended for commercial planting.

Table 2. ANOVA of Clones Common to the Three Upper Peninsula Sites.

		Oven Dry Yield in Year 1		Year 1 Survival	
	DF	F sig	%var	F sig	%var
Site	2	0.0001	0.10	0.0001	0.61
Blk(site)	9	NS	0.02	NS	0.00
Clone	18	NS	0.04	NS	0.01
Clone*site	36	0.04	0.10	0.0001	0.43
Error	162		0.74		0.42

Table 3. ANOVA of Clones Common to the Three Lower Peninsula Sites.

		Oven Dry Yield in Year 1		Year 1 Survival	
	DF	F sig	%var	F sig	%var
Site	2	0.0001	0.69	0.0001	0.24
Blk(site)	9	0.01	0.03	0.001	0.10
Clone	12	NS	0.02	0.023	0.08
Clone*site	24	NS	0.00	NS	0.01
Error	154		0.26		0.57

Table 4. Clone Performance Expressed as a Proportion of the Plantation Mean for Oven DryYields for Years 1 and 4 Coppice Production and Year 4 Survival at FBIC.

Clone	Oven Dry Yield Year 4	Survival in Year 4	Oven Dry Yield Year 1
Truxton	1.33	0.94	1.52
Tully Champion	1.29	0.99	1.58
SV1	1.25	0.92	1.32
Owasco	1.23	0.58	1.61
94001	1.22	0.93	0.78
Fish Creek	1.19	0.96	0.84
Fabius	1.16	0.69	1.60
SX64	1.15	0.63	1.12
Otisco	1.14	0.78	1.62
Oneida	1.06	0.75	0.81
Millbrook	1.05	0.64	0.53
Marcy	0.95	0.68	0.81
Allegany	0.90	0.96	1.84
Verona	0.88	0.89	1.17
Canastota	0.83	0.42	0.37
Sherburne	0.80	0.46	0.66
SX61	0.77	0.50	0.45
Onondaga	0.76	0.60	0.87
S25	0.56	0.29	0.29

Overall the results to date show that the genotypes that were tested vary widely for survival and growth rate both within individual test sites as well as across this network of test sites in Michigan. Very few, only two of the thirty-three clones that were evaluated, are good “general performers” across the full range of soil and climatic conditions found in this geographic region. Tully Champion and Owasco were the only clones that exceeded the site mean for yield for every site on which they were tested. All other clones that were evaluated had test sites at which they performed below the site average for yield. That does not mean the others were generally poor performers. Many of the clones had above average performance for yield at one or more of the test locations. In fact only eight clones were below average on all of the sites on which they were evaluated. Clearly the performance of most of the clones in the work reported here was highly site specific. So while the results indicate that the possibility of identifying a mix of high “general performers” for this region does exist, the more probable strategy is to find groups of clones that give high yields and have high survival rates at the individual locations. Both breeding strategies may be successful in this region, but since it is clear that many of the better clones are those that perform well at specific sites, continued testing should emphasize the importance of local adaptation and broad clonal testing in the development of planting stock recommendations for willow plantations for commercial biofuel production in Michigan.

Funding for this work was provided by Michigan State University’s AgBioResearch and US Department of Energy awards numbered DE-EE-0000280 and DE-FC36-05GO85041. Technical assistance from Brad Bender, Kile Zuidema, Paul Irving, and Jeremy Mason is gratefully acknowledged.

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1.3
DEVELOPMENT OF A BLACK WILLOW IMPROVEMENT PROGRAM FOR BIOMASS PRODUCTION IN THE LOWER MISSISSIPPI RIVER ALLUVIAL VALLEY

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Abstract

Black willow (*Salix nigra* Marsh.) has the potential to be a significant feedstock source for bioenergy and biofuels production in the Lower Mississippi Alluvial Valley (LMAV). This potential is based on a number of primary factors including rapid growth, ease of vegetative propagation, excellent rooting, and the ability to regenerate from coppice following harvest. To date, there has been no directed black willow improvement effort for the LMAV and production rates of this species in dedicated energy plantations is unknown. The focus of this program is to identify genetically superior black willow clones and define planting stock for use in regeneration of marginal agricultural sites. Mississippi State University Forestry Department and the USDA Forest Service Center for Bottomland Hardwoods Research formed a joint venture in 2008 to pursue this effort. The initial selection strategy incorporated five geographic areas, four stands within each area, and five clones within each stand. The five geographic areas included two along the Mississippi River and one each along the Atchafalaya, Trinity, and the Brazos Rivers. From each stand five to eight one to two year-old stems were collected during the winter of 2009. Over a two-year period a total of four screening trials were established. Data from ages one and two have provided insight into geographic origin performance and heritability. These early results allowed us to design a more highly replicated clone test of the better performing clones as well as increasing selections for the base population.

Keywords: Black Willow, Selection Strategy, Geographic Origin, Clone Testing

Introduction

Various willow species and their hybrids (*Salix spp*.) have been used in Europe and proposed for use in the northeastern United States as a fast growth forest species for the bioenergy and biofuels production (Abrahamson 1998; Dickmann 2006; Kopp et al. 2001). Although black willow (*Salix nigra* L.) grows throughout the southern United States, it has received very little attention as a biomass species. In this area and especially in the Mississippi Alluvial Valley (MAV), the majority of the research has focused on eastern cottonwood (*Populus deltoides* Bartram ex Marsh.), which was used by the pulp and paper industry. While various *Populus* species and their hybrids are still being evaluated for biomass production, the sites now being examined for large-scale production are considered “marginal”. The reasoning behind the use of marginal sites for biomass production is to avoid further controversy in the "food for fuel” debate; thus productive agricultural sites are simply avoided. Marginal sites include those that are characterized by heavy clay soils and prolonged flooding. Although, eastern cottonwood may survive on these types of sites it will not reach its biological potential. While black willow is adapted to these types of sites biomass production of plantation grown black willow is currently unknown. But with the development of silvicultural tools and the inclusion of genetically improved planting stock black willow may prove to be a viable biomass species on these sites.

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Black willow is easily vegetatively reproduced from stem cuttings allowing the selection of superior genotypes and exploitation of genotype by environmental interactions. The exceptional rooting characteristics of black willow dormant unrooted cuttings typically results in extremely high survival rates. Benefits from high survival rates could include reduced competition control, uniform production rates, increased yields during the first rotation, and greater potential from coppice regeneration (Ceulemans et al. 1996; Kuzovkina and Quigley 2005).

The main objective of the program was to define genetically superior genotypes for use in biomass plantations along the lower Mississippi River Alluvial Valley.

Methods

The initial sampling scheme for the program included five geographic areas (i.e. sources) all of which were found along four southern rivers (i.e. Mississippi, Atchafalaya, Trinity, and Brazos Rivers). Two areas were sampled along the Mississippi River, with one located near Rosedale, MS and the other near Tunica, Mississippi. The area sampled on the Atchafalaya River was located near Patterson, LA and was the most southern location of the five areas. The two locations along the east Texas Rivers of the Trinity and the Brazos were near Antioch, TX and College Station, TX, respectively. From each of the five geographic areas four stands were randomly selected and from each stand at least five randomly selected clones were collected. An additional 13 clones were also randomly collected, resulting in 113 total clones. During the sampling, a number of sandbar willow clones were mistakenly collected and later isolated in the stoolbed and the tests. All of the clonal material was collected during the winter of 2008-2009 from either one or two-year-old juvenile willows, and placed into a stoolbed during the spring of 2009. During January 2010 whips from each clone were harvested and used either in establishment of two clonal screening trials in 2010 or in the formation of a new stoolbed. At this time the sandbar willow collection was isolated to ensure a separation of the two species. With two stoolbeds a sufficient number of cuttings can be produced for a variety of tests but additional stools for some clones will be necessary when testing transfers from low to high replication numbers.

Screening Trials

In the context of this paper, a screening trial is defined as the initial clonal evaluation of genotypes where clonal replicates are held to minimal numbers. The split-plot design of the 2010–2011 Screening Trials is comprised of four blocks, five geographic areas, 20-25 clones per area, and planted in two-tree row plots, at a spacing of three by nine feet. In addition to the main plot being the five geographic sources a pseudo sixth source was also created and included all of the sandbar clones regardless of the geographic origin. In 2010, two screening trials were established, with one being located at Stoneville, MS and the second located at Prairie, Mississippi. The Stoneville, MS site included all five geographic areas and 111 clones, of which 99 were black willow clones and 12 were sandbar willow clones. The Prairie, MS site included all five geographic areas but only 82 clones, of which 72 were black willow clones and 10 were sandbar willow clones. In 2011, two additional screening trials were established, with one test located near Hollandale, MS and the second trial located near Prairie, Mississippi. The Hollandale, MS site included all five geographic areas and 111 clones of which 99 were black willow clones and 12 were sandbar clones. The 2011 Prairie site also included all five geographic areas but only 84 clones of which 72 were black willow clones and 10 were sandbar willow clones. Measurements for all four screening trials included total eight and number of stems at age one, and total height, number of stems, and DBH at age two.

Results and Discussion

Since the sampling scheme was initially limited, the decision was made to sample geographic areas that produced genetically superior eastern cottonwood clones for alluvial sites along the lower Mississippi River Valley. In addition, the inclusion of screening trials provides a quick evaluation of all the sampled clones without the need for a large number of cuttings per clone. Elimination of inferior clones during the screening trial stage allows for a more efficient use of land and time. It also allows for additional time to increase ramet numbers per clone which will be needed during the next step where only the most promising clones will be included in highly replicated clone tests, thus maintaining an efficient test size and the ability to test across a number of sites.

Survival of the 2010 Stoneville test site was excellent averaging 98% at age one and 97% at age two. In comparison, survival of the 2010 Prairie test site averaged 88% at ages one and two. The survival of the 2011 test sites located on the Hollandale site and the Prairie was nearly identical to the 2010 test sites, with the Hollandale site averaging 99% and the Prairie site averaging 88% survival. In both cases, the difference in survival between the two locations was the mortality rate among the sandbar willow at the Prairie site. The one aspect that can be tied to the mortality of the sandbar willow at the Prairie site was the acidic soil pH, which is 4.6. Mortality in the Stoneville and the Hollandale sites was random, with no source or clonal pattern being detected. Test site differences were significant for all traits, with height differences accentuated in year two. Test means for the age-one total height, age-two total height, age-2 DBH, and growth between ages one and two for the 2010 Stoneville site was 6.3ft, 13.5ft, 1.1in., and 7.2ft, respectively while the Prairie Site means were was 7.0ft, 11.1ft, 0.7in., and 4.2ft, respectively. The reduction in height growth on the Prairie site was originally attributed to increased weed competition but may also be tied to the acidic soil condition.

Both the separate site analysis and the combined analysis of the 2010 trials indicated significant differences among the five geographic areas and clones within geographic areas. The Atchafalaya River source was the tallest source at age one (7.7ft) and remained the tallest at age two (12.1ft), in the combined analysis. In addition, this source also had the largest age-two diameter (0.81inch.). The performance of the geographic sources showed a general clinal trend. When each test site was analyzed separately, age-one height among the five geographic sources was very similar with the exception of the Trinity River source. The major difference was the Trinity River source, which was the shortest at the end year one and two at the Stoneville site but among the tallest at Prairie site for both ages. The faster growing Stoneville site showed a greater range among the geographic sources for height and diameter at age two of approximately 3.0ft. and 0.37in., respectively. The age–two range for height and diameter among sources at the Prairie site is nearly half of that of the Stoneville site at 1.5ft. and 0.18in., respectively.

Significant clonal differences were shown for diameter and height in the combined analysis. The difference between the tallest clone and the test mean was approximately 1.5ft. at age one and increased to nearly 3.0ft. at age two. Four of the top seven clones that were the tallest at age-one remained among the largest at age two. It is also interesting to note that four of the top seven clones that originated from the Atchafalaya River source had the greatest age-two heights and diameters. When the test sites are examined separately, there are greater similarities at age two between the clones that performed well at both 2010 test sites than at age 1. With the exception of age-two height as an indicator over 50% of the clones in the top 10% of the test population originated from the Atchafalaya River. The two clones that remained stable across both test sites at age two were ATCR 2-3 and ATCR 2-5. Other clones that performed well at both sites were ATCR 1-1, BRZ 2-2 and MROS 1-2.

The results of the 2011 Screening Trials revealed that, with the exception of the Trinity River source the other four sources showed very little difference for age-one height. The Trinity River source, which was significantly shorter than the other four sources in the 2010 tests showed the same pattern in the 2011 tests. Although the Atchafalaya River source was the tallest at age one, it was not significantly different from the Rosedale, Brazos, and Tunica sources. Clonal performance in the 2011 trials was also similar to the clonal performance of the 2010 trials. Clones such as BRZ 2-2, MROS 1-2, ATCR 2-3, and ATCR 2-5 showed similar rankings in 2011 as well as 2010.

The genetic variability indicated in the 2010-2011 trials showed that considerable gain can be made, especially at the clonal level. The most southern sources produced many of the largest clones through age two. However, a number of fast-growing clones originated from a variety of sources. Although these results are from two-years of growth, the expected rotation for biomass production is three to five years, thus dictating early-age selection. In 2012, all of the available data were used to determine the top 25 clones to be included into a series of highly replicated clone tests. The intent is to establish a series of highly replicated clone tests in 2013 and 2014 that will yield a small number of clones to be recommended for willow biomass plantations in the LMAV. Future work will involve additional collections of native germplasm, creation of new stoolbeds, and the establishment of screening trials.

Summary

It is encouraging that with such a limited initial sampling scheme, the results suggest that significant gains can be made in growth. The extremely high survival rates of black willow are due to excellent rooting characteristics and should greatly aid in biomass yields. Clonal screening trials will provide the best genotypes for further highly replicated clone tests from which a small deployment population will be selected. Additional infusion of new germplasm will broaden the genetic variability and should provide greater gains.

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1.4 REGIONAL TESTING OF ENERGYCANE (SACCHARUM SPP) GENOTYPES AS A POTENTIAL BIOENERGY CROP

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Abstract

Sugarcane (*Saccharum* spp.) has been a cash crop in the Deep South since 1795, but the area of production has been limited by its lack of cold hardiness. Energycanes are complex hybrids derived from crosses of domestic sugarcane varieties and *S. spontaneum* (a cold-hardy relative). They are typically low in sugar, but high in fiber and biomass yield. The objective was to evaluate energycane hybrids for biomass yield. Replicated field trials of five genotypes (Ho 02-144 & 147; Ho 06-9001 & 9002; and Ho 72-114) were conducted across five states in the Southeast to evaluate the potential production and sustainability of energycane as a bioenergy feedstock resource. Test locations were: Tifton & Athens, GA; Starkville & Raymond, MS; St. Gabriel, LA: and Beaumont & College Station, TX. Data collected by location years included: date of emergence, monthly height and °Brix, and end of season biomass yield. After two full year's growth, data indicated greatest plant height was observed during mid to late September at all locations. Termination of growth corresponded to a decrease in soil temperature below 30 °C. Brix varied with location and genotype, but maximum °Brix was observed in mid-October. A genotype by location interaction was also observed for yield. Generally, clones that did the best at southern most locations had lower biomass yields at the northern most locations. Yields in the first full year of production ranged from 8.72 Mg/ha (Ho02-144 @ Raymond, MS) to 57.04 Mg/ha (Ho 06-9001 @ Beaumont, TX). Record cold weather did impact yield, but no clones were lost.

Keywords: spontaneum, biomass, yield, Brix, height, cold hardy, ratoon

Introduction

Sugar production from cane (*Saccharum* spp.) has been occurring in the southern parts of Louisiana since 1795 (Gravois 2001). Early production of cane was 16-20 tons/acre; modern yields of sugarcane range from 30-50 tons/acre/year; with extractions of 180-240 lbs sugar/ton of cane (Gravois 2001). Sugarcane is bred for large stalk diameter, low fiber content and high sugar content under Louisiana conditions. However, the northern limits of these sugarcane varieties have always been determined by the tropical origins of their parents. During the 1960s mosaic virus threatened the sugarcane industry in Louisiana. USDA-ARS at Houma imported wild cane (*Saccharum spontaneum*) and screened it for resistance to mosaic virus (Hale, personal communication). Greater emphasis was placed on the development of

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energycane with the "oil shocks" of 1973 and 1979. In 2007, Louisiana State University released L79-1002 a cane specifically as a biomass feedstock (Bischoff et al. 2008). However, as will most alternative crops, when the crisis goes away, so does interest in the crop. Because energycane was bred for biomass and high fiber content there was a corresponding reduction in sugar concentration, making it unattractive to the sugar industry.

USDA-ARS at Houma continued a small program on energycane development throughout the 1990s, but added cold hardiness to the list of desirable traits. Passage of the Energy Independence and Security Act of 2007 set the stage for a resurgence of all biomass crops, among them energycane. In fall of 2007, Drs. Tew and Richard sent germplasm to Mississippi State University (Starkville, MS) for general assessment and winter screening.

It should be noted that energycane, like sugarcane is a perennial that is vegetatively propagated; meaning it comes back for several years after initial planting and additional fields are established from mature canes of existing plants. By using canes from existing plants, vigor observed in F_1 hybrids of the sugarcane x *S. spontaneum* cross is maintained from field to field, because all subsequent plantings are from the original vegetative material.

With the renewed interest in biomass and bioenergy, energycane piqued the interest of researchers. In addition to the high biomass tonnage, infra-structure and equipment that supports Louisiana's $2 billion sugarcane industry could be directly applied to production of energycane.

Methods

Screening of potential energycane germplasm began with a small test of seven energycane and two sugarcane lines at Mississippi State University in fall of 2006. Billets were cut into individual nodes and sprouted. These plants were maintained in a greenhouse throughout the winter of 2006/2007. Plants were crown divided and planted to the field in May 2007. Fertilizer and lime applications were as recommended by Louisiana State University. Canes were harvested during fall 2007. Crowns were subjected to ambient winter temperatures and precipitation during the winter of 2007/2008. Survival and emergence ratings were taken in late March, April and May 2008 on regrowth of the surviving crowns. March 2008 ratings at Starkville indicated sugarcane types were as good as the high fiber low sugar energycane. However, damage to the shoots from late spring frosts severely set back growth of early emerging germplasm. Therefore, five of the seven energycane lines tested at Starkville were selected for broader testing across the Southeast as part of the Regional Biomass Feedstock Partnership. These genotypes were: Ho 02-147; Ho 02-144, Ho 72-114. Ho 06-9001, and Ho 06-9002. In August and September of 2008, mature cane (seed cane) of these genotypes was distributed to seven test sites (Tifton, GA; Auburn, AL; Raymond and Starkville, MS; St. Gabriel, LA; Beaumont and College Station, TX) (1). Crop failure at the Auburn site caused an alternate site to be selected at Athens, GA. Waimanalo, HI was added in 2009. Planting was accomplished at all locations within three days of seed cane delivery. Some sites included other sugar or energycane genotypes, but all locations had the same five genotypes in common. Individual genotypes were planted in plots 32 ft long x 3 rows (18 ft) wide. Two rows would be harvested for yield estimates, the third would be used for growing season data. Plots of all five genotypes occupied a space 32 ft by 96 ft; this was replicated four times within a field at each location. During the following spring (2009) emergence data (shoots/plot), date of 50% emergence, and soil temperature @ 6" was monitored. Over the course of the growing season, mean height and °Brix (a measure of sugars in the sap) were recorded. Site scientists were asked to record any major factors (drought, hurricanes, and extremes of temperature, insects or disease) observed at their location. Harvest date varied by location, depending on frost date and weather conditions. At the end of the growing season, stalk count, final mean height, a general frost damage rating, final °Brix, fresh harvest weight were recorded. From the sacrifice row, sap yield, stalk moist weight (after pressing sap out) and dry weight were recorded. Dry stalks were ground and submitted for structural carbohydrate analysis (cellulose, lignin, sugar).

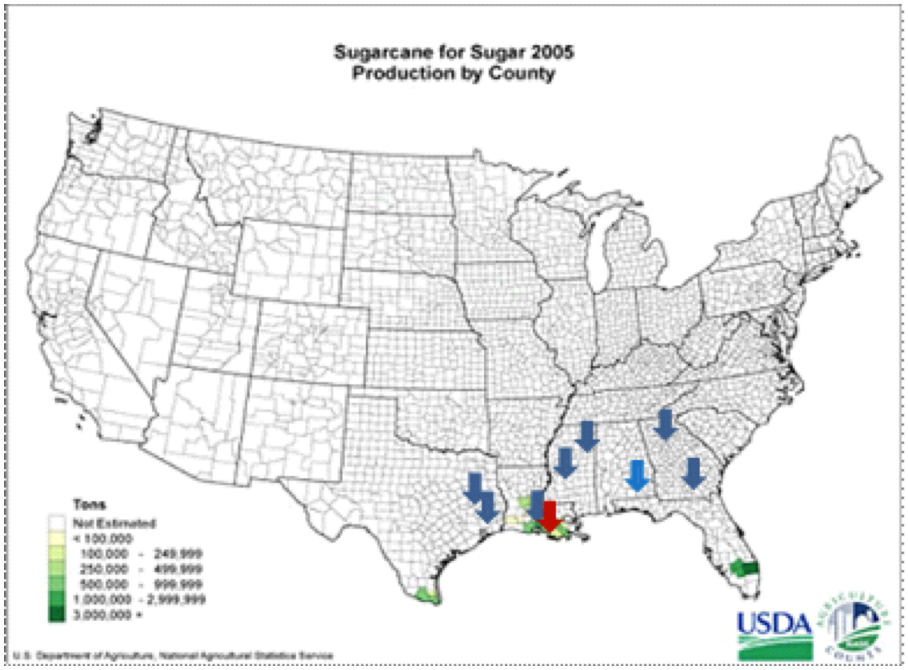

Figure 1. Location of test sites for energycane (*Saccharum* spp. (blue arrows)as part of the Regional Feedstocks Partnership (2009-2012). Green shaded areas indicate location of commercial sugarcane production. Light blue = Auburn, AL; later replaced with Athens, GA. Red arrow = Houma, LA, source of all germplasm used in this testing. (Source of image: USDA-National Agricultural Statistics Service)

Results and Discussion

Height of all germplasm increased throughout the summer into the fall. Comparison of heights at northern and southern locations showed substantially greater heights (2 ft) at more southerly locations, with the exception of Bryan, TX. Lack of rainfall limited height of germplasm at Bryan. Mean height was monitored over the course of the growing season. Height measurements indicated onset of "grand growth" (the point at which growth accelerates rapidly) occurred in June or July. While the date of onset differed for each of the sites, regardless of location or year, it corresponded to a mean ambient temperature of 85°F. Grand growth ceased (heights remained level) after mid-September, which corresponds to daytime temperatures cooling below 85°F.°Brix, a measure of sugars and simple starches dissolved in the sap of the plant, varied by location, variety, and time of growing season. Brix readings ranged from 5 at the beginning of the season to 20 at frost. Brix values dropped sustainably two weeks after frost. Germplasm differences for °Brix were not uniform across locations; indicating some varieties accumulated more sugar at one location, while others accumulated more sugar at a different location.

Efforts to extract sap varied by variety also; Ho 02-144, Ho 06-9001 and Ho 06-9002 had less extractable sap than Ho 02-147 and Ho 72-114. The difference is due to the woody nature of the former, and the pulpy nature of the latter varieties.

With regard to biomass production, dry matter yield is the most important attribute. Of the seven locations in 2009 (the first year); highest yields were observed at Tifton, GA for every variety (Table 1). This was followed by Raymond, MS and St. Gabriel, LA. Lowest yields were observed at the two most northern sites; Athens, GA and Starkville, MS (Table 1). Beaumont didn't report yields because a hurricane destroyed the plots.

Table 1. First year, seed crop, energycane dry matter yield by location (2009)

	Athens, GA	Starkville, MS	Raymond, MS	Tifton, GA	Bryan, TX	Beaumont, TX	St. Gabriel, LA
EnergycaneGenotype	- - - - - - - - - - - - - - - - - US tons/acre - - - - - - - - - - - - - - - -						
Ho 02-144	2.50 c	3.47 ab	7.97 a	10.88 d	4.56 a	X	6.27 c
Ho 02-147	2.81 c	2.16 b	9.85 a	13.50 bc	5.07 a	X	7.87 ab
Ho 06-9001	4.35 ab	4.66 a	6.36 a	11.10 d	6.69 a	X	7.58 abc
Ho 06-9002	3.69 bc	3.31 ab	7.56 a	12.60 cd	6.29 a	X	6.44 bc
Ho 72-114	5.14 a	3.10 ab	6.29 a	17.07 a	7.68 a	X	8.83 a
LSD 0.05	1.30	2.01	3.70	2.09	3.84	X	1.54

Second year yields (the first ratoon crop) were generally higher than the first year at all locations. Beaumont, TX had higher yields than any other site, followed by Tifton, GA (Table 2). Yields at these two sites ranged from 18 to 25 dry US tons/acre/year and 10 to 15 dry US tons/acre/year, respectively. Raymond, MS had the lowest yields in the second year of growth (Table 2). Two varieties decrease in yield from the first to second year; Ho 02-144 and Ho 02-147 decreased in yield at Raymond MS and Tifton, GA. Ho 72-114 also decreased in yield at Tifton, GA from the first to second year. (Table 1 vs 2).

Table 2. Second year, first ratoon, energycane dry matter yield by location (2010)

	Athens, GA	Starkville MS	Raymond MS	Tifton GA	Bryan TX	Beaumont TX	St. Gabriel LA
Energycane Genotype	- - - - - - - - - - - - - - - US tons/acre - - - - - - - - - - - - - - - -						
Ho 02-144	11.12 ab	7.06 ab	3.89 ab	10.09 c	11.99 a	18.28 c	6.76 d
Ho 02-147	9.85 bc	5.93 bc	6.81 a	11.15 c	9.72 a	21.22 abc	9.52 a
Ho 06-9001	12.44 a	10.08 a	5.68 a	14.98 a	9.18 a	25.44 a	8.02 bc
Ho 06-9002	11.58 a	8.83 a	6.12 a	15.18 a	10.17 a	23.44 a	7.52 cd
Ho 72-114	9.24 c	5.68 c	4.75 ab	14.26 ab	10.04 a	23.30 ab	8.92 ab
LSD0.05	1.61	2.41	3.13	2.79	5.06	4.36	1.23

Third year yields (data is not shown) included data from the site at Waimanalo, HI. Existing laws, prohibiting the importation of sugarcane germplasm, and heat treatments to destroy pathogens delayed establishment of their test. All eight locations reported yields in the third year of this trial (Hawaii's first year). Yields continued to increase for all varieties at: Starkville and Raymond, MS; Tifton, GA; and Bryan, TX; but compared to the year prior, there were strong reductions in yield at Beaumont, TX and slight reductions in yield at St. Gabriel LA; due to drought conditions at both locations. There was a severe reduction (mean decrease of 8 US tons/A) at Athens, GA; one of the two northern locations. This reduction can be attributed to five days of extremely cold weather that occurred at Athens, GA from the 8th to the 12th of January 2011. Mean winter temperatures for Athens are 52°F day / 34°F night. During this time period, daytime highs were 30 F while night temperatures dropped to 15°F. The result was a freezing of the soil profile well below the surface with corresponding damage to rhizomes that would have arisen in the spring.

All germplasm was negatively impacted (Table 1 vs Table 2), but no variety was killed out due to the extreme weather. Hawaii's yields for the five common energycane varieties ranged from 13 to 20 US tons/acre; which was similar to Tifton, GA and Beaumont, TX, but less than Bryan, TX. It is important to note, that this was Wiamanalo's first year of growth, and that is being compared to sites on the mainland in their third year of growth.

Of course no crop is without problems. At the more northerly locations, extremely cold winters will limit production. However, these locations allow the breeders at USDA-ARS @Houma to differentiate between lines that are colder hardy than others. The presence of potentially troublesome insects; sugarcane borer (*Diatraea saccharalis*) and Mexican rice borer (*Eoreuma loftini*) was reported at Beaumont, TX and Raymond, MS. Sugarcane smut (*Sporisorium scitaminea*) was reported at Tifton, GA in 2011, but only in a single variety (L79-1002).

In summary, the new germplasm/varieties tested in this work have shown that energycane can produce 10-11 dry US tons/acre/year yields at the most northern locations (33°N latitude) and in excess of 20 dry US tons/acre/year at the southern locations. It is important to note that the varieties that yield the best in the north, are slower to emergence in the spring, and therefore have lower yields compared to the other varieties in this test when grown at the southern/tropical locations. Sugar and starch in the sap of the plant is a function of the variety and rainfall at each location. When averaged across all locations, the Ho-9001 and 9002 lines gave the highest mean yield/acre, but were lowest in sugar. As a perennial crop, energycane has the potential to move cane production farther north, while producing significant biomass; and protecting and increasing soil organic matter.

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1.5
QTL FOR BIOMASS YIELD AND COMPOSITION IN ENERGY SORGHUM (SORGHUM BICOLOR L. MOENCH)

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Abstract
In the past several years, QTL studies of sorghum have identified genomic regions that influence both biomass yield and composition. Most of these studies also conclude that these traits are strongly influenced by plant maturity and height. While loci controlling height and maturity could be considered as biomass QTL, the presence of variation for these two traits biases the detection and effect of other QTL for biomass yield and composition. The objective of this study was to conduct QTL analysis in a population of bioenergy sorghum that does not segregate for maturity and height. One hundred $F_{4:5}$ recombinant inbred lines derived from the cross of two photoperiod sensitive, tall sorghum genotypes (R07018 / R07020) were evaluated in two long day environments for biomass yield and composition. Phenotypic and genotypic variation was identified in the RIL. QTL analyses of each environment identified many QTL for both biomass and composition. As expected, preliminary QTL analyses indicates that some QTL are detected across environments while others are unique to a subset of environment(s). Our study confirms that yield and composition QTL can be identified in populations in which major maturity genes are not a contributing factor and that evaluation in these populations is important to elucidate the genetic basis of biomass yield and composition in energy sorghum.

Keywords: Sorghum, QTL, photoperiod sensitive, biomass

Introduction
Sorghum (*Sorghum bicolor* L. Moench) is an important grain, forage, and more recently, energy crop that is grown throughout the world (Saballos 2008, 211). Given its relative importance, there has been significant effort to characterize and genetically improve the crop. Part of this effort has been to create high-density genetic maps useful for study and analysis of quantitative trait loci (QTL) as well as introgression of specific traits into elite germplasm (Saballos 2008, 227). Several studies have identified QTL influencing biomass yield and composition (Murray et al., 2008; Ritter et al., 2008, Felderhoff et al., 2012). As expected, different QTL were detected across these populations due to QTL segregating in some populations but not others. More importantly, these studies conclude that biomass yield and composition are strongly influenced by maturity and height genes.

There is a need to identify QTL that influence biomass yield and quality independent of the effects of maturity and plant height. Thus, the goal of this study was to identify QTL for traits affecting yield using a photoperiod sensitive recombinant inbred line (RIL) population created using two tall photoperiod sensitive parents. Under long days, the relative effects of maturity and height will be minimized if not eliminated. Identification of QTL in this population, in these environments should reduce inconsistencies in QTL identification by (i) decreasing segregation for height and maturity within the population and (ii) minimizing genotype specific QTL expression (i.e. tall vs short).

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Materials and Methods

An RIL population consisting of 100 F_5 individuals was derived from a biparental cross of R07018 x R07020. Both parents are tall, photoperiod sensitive inbred lines. R07018 is a Guinea sorghum with white grain and tan plant color while R07020 is a Caudatum sorghum with red grain and a purple plant color. The population and parental lines were planted in College Station and Weslaco, TX in 2011 and Guayanilla, PR in 2012. Agronomic notes taken prior to harvest included; stem diameter, internode length, height, exertion, and days to flower. Flowering time was recorded as the day in which the average of each plot reached mid- anthesis after planting. One meter samples were harvested and harvest yield, stalk yield, panicle yield and juice volume and brix were recorded.

DNA was isolated utilizing the FastDNA SPIN Kit, and RSA DNA sequencing identified single nucleotide polymorphism (SNP) loci for genotyping with the Illumina GAIIx DNA sequencing platform. Illumina sequencing files were aligned to the sorghum genome (Paterson et al., 2009) and SNPs were identified using the CLC SNP Detection tool. Custom pearl and python scripts were developed to compare SNP data between parental lines and RIL progeny (P.E. Klein, personal communication). A genetic map consisting of 97 individuals and 567 chromosome specific markers was constructed using JoinMapV4.0, resulting in twelve linkage groups spanning 1458cM with an average distance of 2.63cM between markers. Marker information indicated 16.5 percent heterozygosity. Population means and variances were calculated using JMP 9.0 to determine population performance relevant to each parent (Table 1). Entry means for each location were used in QTL analysis. Composite interval mapping (CIM) identified significant QTL within each location with Windows QTL Cartographer. Significance thresholds were determined through 1,000 permutations of the data at an alpha confidence level of 0.05 and a window size of 10cM and walk speed of 1cM.

Table 1. Phenotypic measurements of parental lines R07020 (020), R07018 (018) and F5 population for all locations. Measurements are reported for traits expressing significant QTL.

Trait	2011 College Station			2011 Weslaco Fall			2012 Puerto Rico		
	020	018	F5 range	020	018	F5 range	020	018	F5 range
Height,cm	427	414	339-530	342	355	298-373	237	246	215-280
Flowering time, d	na*	na*	na*	95	90	80-97	61	64	56-65
Stalk yield, Mg ha^{-1}	57.4	46.9	6.6-89.4	50.5	52.3	40.4-79.1	na*	na*	na*
Leaf yield, Mg ha^{-1}	15.4	18.8	1.9-18.1	6.9	11.6	3.66-16.5	na*	na*	na*
Panicle yield, Mg ha^{-1}	na*	na*	na*	2.8	3.1	0.12-5.5	7.6	2.1	1.7-16
Juice yield, L ha^{-1}	4303	5667	734-25282	5052	4921	1509-8464	na*	na*	na*
Brix,%	7.6	8.8	5.5-10.2	7.7	8.6	3.9-12.7	7.6	9.6	5.5-11.6
Internode length, cm	21	16	12-27	26	23	17-28	26	26	22-30
Stem diameter, mm	16	16	11-23	18	13	10-23	10	11	7.45-14
* Data not available									

Results and Discussion

While the environments did influence performance, trends were detectable across environments. For example, R07020 was generally taller and higher yielding than R07018 while R07018 had higher moisture content and greater leaf content than R07020 (Table 1). For most traits in most locations, the RIL population mean was between the parental means and in all cases, transgressive segregation was observed, indicating that genetic variation between the two parents is available for further improvement of the measured traits (Table 1).

This population was developed specifically to minimize the effect of maturity on biomass accumulation and quality. The three locations used in this evaluation differed greatly because of the effect of daylength on maturity. As expected, plants did not flower in College Station as this was a long day environment. Differences in maturity were present in the other locations as they allowed the population to flower (Table 1).

Across all locations, QTL were identified for nine traits including juice volume, internode length, stem diameter, brix, height, stalk weight, leaf weight, panicle weight, and days to flower (Table 2). Of these, only QTL for days to flower and internode length were detected at multiple locations. A QTL for days to flower was identified in Weslaco and Puerto Rico while an internode length QTL was identified in College Station and Puerto Rico (Table 2). QTL for volume and stem diameter were identified in College Station. Brix, height, stalk weight, leaf weight, panicle weight, and days to flower QTL were identified in Weslaco (Table 2).

Table 2. Genetic map position and relative effect of phenotypic QTL from collected data at three locations: College Station 2011 (CS2011), Weslaco Fall 2011 (WF2011), and Puerto Rico 2012 (PR2012)

Trait	Location	Chrom	QTL Peak (cM)	LOD 2 Interval (cM)	LOD	Additive effect	R^2
Internode length	CS2011	9	58.9	53.7-59.7	3.4975	-0.9367	0.1386
Stem diameter	CS2011	3	92.4	89.4-99.5	4.1649	-0.7585	0.1457
Juice volume	CS2011	2	64.2	54.2-70	3.3046	240.8361	0.1138
Brix %	WF2011	6	35.5	33.2-41.5	3.3421	-0.5535	0.1088
Flowering time	WF2011	2	164.5	164.3-168.2	6.5773	-1.7942	0.1907
Height	WF2011	10	107.3	97.4-109.9	4.6666	-2.6462	0.1574
Leaf yield	WF2011	10	107.3	97.3-112.5	4.1081	0.0722	0.1398
Panicle yield	WF2011	2	71.6	70.1-74.6	3.7859	0.0339	0.1263
Panicle yield	WF2011	2	78.4	74.6-79	4.0265	0.0361	0.1333
Stalk yield	WF2011	3	17.4	12.9-21.1	2.9994	-0.2652	0.0951
Flowering time	PR2012	3	90.4	88.3-92.8	3.2697	0.6804	0.1036
Internode length	PR2012	7	110.8	110.7-112.9	3.9554	0.7528	0.1426

Each QTL is reported by chromosome (Chrom) and location on each chromosome (QTL Peak). The 2- likelihood of odds interval indicates the centimorgan distance for which significant QTL were detected. Additive effect indicates phenotypic influence of parent R07018 on each trait. The variance explained by each QTL is reported as R^2.

Analysis of the RIL population in three environments varying in day lengths allowed the partitioning of the photoperiod response and analysis of genetic effects with and without the influence of maturity. The presence of QTL for height and maturity within this population indicated that the two parental genotypes vary in alleles for genes that influence these traits (Tables 1 and 2). The internode length QTL identified on linkage group (LG) 09 was coincident with Sb.Ht9.1, a previously identified QTL for plant height on chromosome 9 (Mace and Jordan 2010, 1342). The presence of internode length QTL in Puerto Rico coupled with mapping of Dw3 to the same chromosome (Mace and Jordan 2010, 1342) indicates this QTL most likely corresponds to Dw3. A flowering time QTL identified in Puerto Rico on LG-03does not coincide with previously identified maturity loci (Mace and Jordan 2010, 1341). The flowering time QTL identified in Weslaco on LG-02 may correspond to maturity loci identified in previous studies (Mace and Jordan 2010, 1341). A significant QTL identified for height in Weslaco does not map to any known height genes or previous QTL on chromosome 10 (Table 2).

Conclusion

Evaluation in short and long day environments influenced the expression of both photoperiod sensitivity and maturity loci. Because of this, it is useful to analyze the environments separately to dissect those genetic effects. As in previous experiments, different QTL are identified when populations are analyzed in different environments. Our results indicate that QTL can be identified for biomass yield and composition when maturity is not a factor although their expression is reduced in number and effect. It is recommended that in future studies of this nature to increase the number of locations and individuals within the population to increase QTL detection, as well as determine whether QTL expression and phenotypic variances are due to environmental, genetic, or a combination of both factors.

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1.6
QUANTITATIVE GENETIC ANALYSIS OF BIOMASS YIELD, PEST RESISTANCE, AND OTHER AGRONOMIC TRAITS IN PRAIRIE CORDGRASS AND CUP PLANT

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Abstract
Prairie cordgrass (*Spartina pectinata*), and cup plant (*Silphium perfoliatum*) are currently being evaluated for biomass feedstock production on marginal lands in the northern Great Plains and Midwest. Half-sib families of these species were evaluated in replicated single-row plots at Brookings, SD and Madison, WI in 2010 and 2011 for biomass production and several other agronomic traits. Significant differences were found among families of prairie cordgrass in SD and among families of cup plant in WI. Estimates of narrow sense heritability for biomass were generally moderately high, indicating ample additive genetic variation to achieve progress from selection. No genetic variation was found for resistance to the giant eucosma (*Eucosma giganteana*) in a population of cup plant evaluated in SD. It is expected that this research will identify superior families and perhaps superior individuals within families that will result in the development of new high-biomass-yielding cultivars of each species. Finding pest resistance will require screening of more germplasm.

Keywords: Spartina pectinata, Silphium perfoliatum, Eucosma giganteana, heritability, variance components

Introduction
Prairie cordgrass (*Spartina pectinata*) and cup plant (*Silphium perfoliatum*) have shown promise as herbaceous perennial biomass feedstocks on marginal land in the northern Great Plains and Great Lakes Regions (e.g., Boe et al., 2009). Both are adapted to moist prairies and cropland depressions (Stanford, 1990; Boe et al., 2009). Cordgrass is well-adapted to moderately saline wet soils (Kim et al., 2012) and is widely used for revegetation and soil stabilization in drainage areas (Jensen, 2006). Cup plant has been used for silage in areas that are subject to short-term flooding and thus are not suitable for row-crop production (Lehmkuhler et al., 2007) and is also highly desired in revegetation plantings because of its attractiveness to pollinators and other arthropods. These two species will complement switchgrass and other native species in multi-species approaches to optimizing biomass production and ecological goods and services from biomass feedstock plantings across diverse landscapes (Gonzalez et al., 2009). Cup plant was recently introduced to Chile, where it has shown to be of value for forage production in low-input farming systems (Pichard, 2012).

Large differences were found among seven natural populations of prairie cordgrass on prime land in eastern South Dakota (Boe and Lee, 2007) and on dry marginal land in east-central South Dakota (Boe et al., 2009). One of us, K. Albrecht, found differences among natural populations of cup plant from Wisconsin for biomass production. Both species have approached biomass production of 20 Mg ha^{-1} in experimental plantings (Boe et al., 2009; Pichard, 2012). However, no studies to estimate genetic variances for biomass and other agronomic traits have been conducted for either species. Therefore, the objective of this research was to estimate additive genetic variance and narrow-sense heritabilities for

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biomass and other agronomic traits in expermimental populations of prairie cordgrass and cup plant that have shown potential for biomass production over periods of several years in the northern Great Plains.

Materials and Methods

Fifty eight half-sib families of prairie cordgrass and 34 half-sib families of cup plant were established from transplanted seedlings in separate randomized complete block designs with three replications at Brookings, SD in 2010. The same experiment containing cup plant was also established at Arlington, WI in 2010. A smaller experiment containing a subset of 25 out of the 58 families of prairie cordgrass was established at Aurora, SD in 2010. For prairie cordgrass, individual family plots were composed of a single row of seven plants with 0.3-m intra-row and 1.2-m inter-row spacing. For cup plant at Brookings, individual family plots were single rows of six plants with 0.4-m intra-row and 0.9-m inter-row spacing. At Arlington, individual plots were space planted on 0.9-m centers. Transplanting was conducted during May 2010 for both species in SD and WI.

Prairie cordgrass experiments in South Dakota were harvested for biomass during late October 2011. Cup plant experiments were harvested during late September 2011 in WI and early October in SD.

Additional agronomic data collected for prairie cordgrass were rust infection by (*Puccinia sparganioides*) in 2010 and 2011 using a scale proposed by Gustafson et al. (2003) and number of panicles per plant in 2010. For cup plant at Brookings, frequency of apical mersistems infested by the giant eucosma, *Eucosma giganteana*, (Lepidoptera:Tortricidae) was determined in 2011 and 2012.

Estimates of additive genetic variance and of narrow-sense heritability were calculated from estimates of variance components from analyses of variance as described by Nguyen and Sleper (1983) and Nyquist (1991).

Results and Discussion

Significant differences were found among families of prairie cordgrass for biomass production in South Dakota. Location means and ranges in family means are presented in Table 1. The means of the 25 families that were common to both locations were 1.9 Mg ha^{-1} at Aurora and 6.2 Mg ha^{-1} at Brookings. The simple linear correlation between family means at the two locations was significant (r=0.70**). This indicated that, although the biomass production varied by nearly 3-fold between locations, the ranking of families was consistent. Narrow-sense heritabilities were moderately high at both locations, with the estimate of heritability at Brookings, which had 33 more families than the nursery at Aurora, being higher than at the Aurora location (Table 1).

Table 1. Location means, ranges in family means, and narrow-sense heritability estimates for biomass production in populations of half-sib families of prairie cordgrass during 2011 in eastern South Dakota.

Location	Location mean	Range in family means	Narrow-sense heritability (h^2)†
	Mg ha^{-1}		
Brookings, SD	5.8	3.2-9.0	0.68
Aurora, SD	1.9	1.2-3.3	0.51

†$h^2=\sigma_F^2 / (\sigma_F^2 + \sigma_E^2/r)$ where h^2= narrow-sense heritability on a family mean basis, σ_F^2=among-family variance component, σ_E^2= the error mean square, and r=number of replications.

Heritability estimates for the other agronomic traits measured during this study, i.e., rust score and number of panicles per plant, were 0.57 and 0.61, respectively. Data for these two traits were collected for individual plants, so the individual is the selection unit rather than the family, which was the intended selection unit for biomass. The magnitudes of the heritability estimates for biomass production, disease resistance, and panicles per plant (an important component of seed yield) indicated progress from selection could be expected for all of those traits.

For cup plant, significant differences were found among half-sib families for biomass production in WI, but not in SD. Location means, ranges in family means, and estimates of narrow-sense heritability for biomass production are presented in Table 2.

Table 2. Location means, ranges in family means, and narrow-sense heritability estimates for biomass production in populations of half-sib families of cup plant during 2011 in South Dakota and Wisconsin.

Location	Location mean	Range in family means	Narrow-sense heritability (h^2)†
	Mg ha^{-1}		
Brookings, SD	5.5	4.3-6.8	0.0±
Arlington, WI	8.7	5.7-11.5	0.46

†$h^2=\sigma_F^2 / (\sigma_F^2 + \sigma_E^2/r)$ where h^2= narrow-sense heritability on a family mean basis, σ_F^2=among-family variance component, σ_E^2= the error mean square, and r=number of replications
±The among family component of variance was negative.

Biomass production in WI was 60% greater than in SD during 2011. The planting designs were different at the two locations, with plant densities of 1.2 plants m^{-2} in WI compared with 2.6 plants m^{-2} in SD. In SD the highest yielding family produced 60% more biomass than the lowest yielding family; whereas, in WI the yield of the highest yielding family was two times that of the lowest yielding family. The environment in WI appeared to be superior to that in SD for differential expression of yield potential among the families. More data need to be collected to determine optimum plant density for sustainable biomass production and for identifying the best environment for detecting genetic differences among families for biomass production and thus making progress from selection for biomass production.

A large difference was found between years but not among families of cup plant for frequency of apical mersitems destroyed by the larvae of the giant eucosma, *Eucosma giganteana*, in SD. In 2011, < 1% of the apical meristems were destroyed; whereas, in 2012 >97% of the apical meristems were destroyed. The attack of the moth larvae on the meristems occurred at the 4- to 5-leaf stage, thus vertical growth was essentially stopped at about halfway of the normal height. More germplasm collection will be necessary to determine if genetic resistance to this insect occurs in natural populations from its natural natural geographic range.

The estimates of narrow-sense heritability presented here, with the exception of rust score for prairie cordgrass, are based on a single year's data at a single location. Therefore, we would expect the additive genetic variances and heritabilities to be overestimated due to genotype x environment interaction biases (i.e., family x year, family x location, and family x year x location variances) (Nguyen and Sleper, 1983). Consequently, data will be collected over the next two to three years to provide more accurate estimates.

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1.7
FIELD-BASED EXPERIMENTS ON LOW-LIGNIN SWITCHGRASS AS A FEEDSTOCK FOR LIGNOCELLULOSIC BIOFUEL PRODUCTION

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Abstract
Switchgrass (*Panicum virgatum* L.) is a warm-season C4 perennial grass that is native to the prairies of North America. High biomass production and wide adaptation has made switchgrass a leading candidate for a dedicated lignocellulosic feedstock. One of its major limitations is the recalcitrance of complex carbohydrates to hydrolysis for conversion of lignocellulosic biomass into ethanol. Lignin is a primary contributor to recalcitrance as it creates a physical and chemical barrier to enzymatic access of cell wall polysaccharides. Therefore, genetic manipulation of the lignin biosynthetic pathway in an effort to reduce lignin content is a promising approach for overcoming this inherent cell wall recalcitrance. Low-lignin transgenic switchgrass plants were produced via down-regulation of caffeic acid *O*-methyltransferase (COMT), or by overexpression of the MYB4 transcription factor, an R2R3 type MYB repressor of the lignin biosynthetic pathway. Resulting greenhouse-grown COMT transgenic plants exhibited increased ethanol yields by up to 38% compared to the control and required less severe pretreatment and 300-400% less cellulase enzyme loading (Fu *et al.,* 2011). Greenhouse-grown MYB4 overexpression transgenic plants demonstrated 3-fold increase in sugar release efficiency (Shen *et al.,* 2011). Field trials of COMT and MYB transgenic switchgrass are underway in Knoxville, Tennessee where plants are being assessed during a period of two-to-three growing seasons for 1) agronomic biomass performance (tiller height, plant width, tiller number) and biomass yield, 2) cell wall characterization, 3) biorefinery performance, including pretreatment response, sugar release efficiency, and ethanol yield, and 4) pest response, especially switchgrass rust incidence on transgenic plants and controls.

Keywords**:** bioethanol, cell wall recalcitrance, lignin, caffeic acid O-methyltransferase (COMT), MYB transcription factor, transgenic switchgrass, field evaluation.

Introduction
Switchgrass (*Panicum virgatum* L.) is a perennial warm season C4 grass and has been identified as a potential dedicated bioenergy crop for cellulosic fuels because of its rapid growth rate, nutrient use efficiency and widespread distribution throughout North America (McLaughlin and Kszos, 2005). Switchgrass reduces soil erosion, increases water quality, enhances sequestration of carbon and reduces

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greenhouse gas emissions with an estimated reduction of 94% compared to greenhouse gas emissions from petroleum fuel while producing 540% more net energy (Schmer *et al.,* 2008). Although switchgrass is a promising bioenergy crop, improved feedstock characteristics are crucial for making cellulosic biofuels economically feasible. The main obstacle for cellulosic biofuels is related to the biomass *recalcitrance*, defined as the resistance of biomass to enzymatic breakdown into sugars. This recalcitrance is inherent in the plant cell wall structure, where the desirable cellulose microfibrils are embedded in a matrix of hemicelluloses, pectin and lignin. These additional cell wall components can physically and chemically inhibit access to the cellulose, leading to the need for costly thermochemical pretreatment and enzymatic saccharification, which drastically increase the production cost of cellulosic fuels.

Genetic engineering is a promising approach for developing improved bioenergy crops (Gressel, 2008). It has been predicted that genetic modifications of plant cell walls can reduce or eliminate the pretreatment step and decrease the cost of cellulose to sugar conversion by more than 20% (Lynd et al., 2008). Current modifications to reduce cell wall recalcitrance have been focused on down-regulation of genes in the lignin biosynthetic pathway (Fu *et al.,* 2011a, 2011b; Saathoff *et al.,* 2011). Lignin, one of the major plant cell wall components, contributes significantly to biomass recalcitrance, and therefore reduction in lignin leads to a loosening of the cell wall and more facile release of cell wall polysaccharides. Studies suggest that the genetic modification of alfalfa plants with down-regulated caffeic acid *O*-methyltransferase (COMT), an enzyme involved preferentially in the formation of S lignin (Guo *et al.,* 2001), can double sugar conversion from plant biomass compared to controls (Chen and Dixon, 2007). The lignin biosynthesis pathway is also co-regulated with the secondary cell wall biosynthesis program through a master switch system, which includes R2R3-MYB transcription factors (Zhong *et al.,* 2010). Members from subfamily 4 of the R2R3-MYB family have been shown to act as transcriptional repressors of monolignol biosynthetic genes. Arabidopsis AtMYB32 (Preston *et al.,* 2004), a close homolog of AtMYB4 (Jin *et al.,* 2000), and maize ZmMYB31 and ZmMYB42 have also been identified as lignin repressors, and ZmMYB31 was proposed to be a good candidate for biotechnological applications (Fornale *et al.,* 2006, 2010; Sonbol *et al.,* 2009).

With these results in mind, transgenic switchgrass plants with reduced lignin content were produced by down-regulation of COMT (Fu *et al.,* 2011) or by up-regulation of the switchgrass PvMYB4 transcription factor (Shen *et al.,* 2011). Resulting COMT transgenic plants showed normal growth and development, and no significant changes to biomass productivity (Fig. 1A), but reduction in lignin content and S/G ratios, higher sugar release, and higher ethanol yield (Fu *et al.,* 2011). Resulting MYB4 transgenic plants showed reduction in plant height but more tillers (Fig. 1B), reduction in lignin content and unchanged S/G ratios, and higher sugar release (Shen *et al.,* 2011). Therefore, the modification of a single gene affecting lignin biosynthesis enables a significant reduction in recalcitrance of lignocellulosic feedstocks.

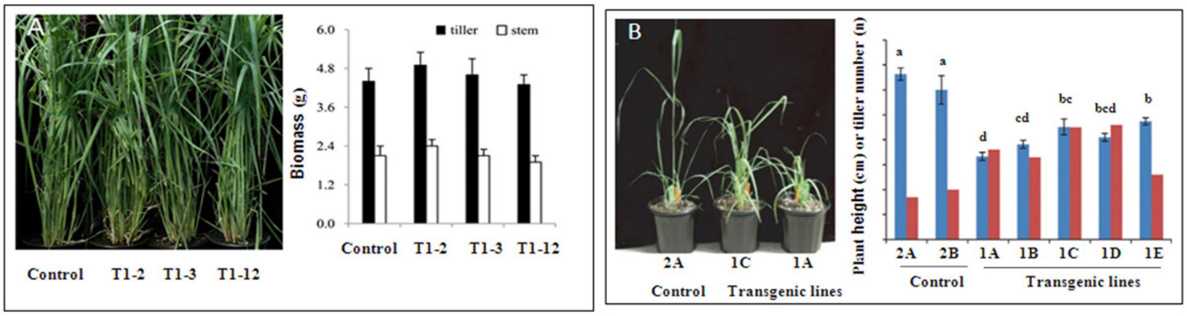


Figure 1. (A) Phenotype of T1-generation transgenic switchgrass plants with COMT down-regulation. Greenhouse-grown switchgras plants of control, transgenic T1-2, T1-3, and T1-12 (left panel). Dry weight of tillers and stems of control and transgenic switchgrass. Values are means ± SE (n = 5) (right panel). Fu *et al.,* 2011, Proceedings of the National Academy of Sciences, USA 108, 3803-3808. Permission was granted by the PNAS to use this figure. (B) Phenotype of T0-generation transgenic switchgrass plants with MYB4 over-expression. Greenhouse-grown transgenic plants have reduced stature but increased tillering (left panel). Plant height (blue bars) and tiller number (red bars) for control and transgenic plants. Values are means ± SE (n = 4). The letters indicates significant differences at the *P* ≤ 0.05 level (right panel). Shen *et al.,* 2011, New Phytologist 193, 121-136. Permission was granted by the New Phytologist to use this figure.

These analyses were performed on greenhouse-grown green plants and produced results that could be of great interest to the biofuel industry. However, productivity and performance of field-grown transgenics could potentially differ from greenhouse-grown transgenics due to exposure of plants to environmental stresses not present in a greenhouse setting. In addition, these studies used green-tissues that will vary from brown (senesced) tissue harvested after frost in the autumn. Brown tissue results from the field are more relevant to commercial biofuel production from biorefineries. Therefore, it is essential to determine whether the improved traits observed in greenhouse experiments will be maintained in the field in order to further assess the relevance of these transgenics to the biofuel industry.

Furthermore, field-grown transgenics need to be evaluated for plant susceptibility to insects and pathogens, presumed to be promoted by reduced cell wall recalcitrance. These evaluations need to be carried out on both high and low recalcitrance lines to determine the relationship between increased sugar yield and disease and insect susceptibility. Of particular interest, *Puccinia emaculata* was reported to cause rust disease on switchgrass. It has been observed in Tennessee (Zale *et al.,* 2008) and Arkansas (Hirsch *et al.,* 2010) and is likely to be ubiquitous in the eastern part of the U.S. Switchgrass rust epidemics typically start at varying times of the season, but there has been a trend for disease outbreaks to occur earlier each year (Black *et al.,* 2011). As a result, infected plants are stunted and overall yield is reduced. Li *et al.* (2009) observed that resistant switchgrass plants used hypersensitivity to impede the progress of *P. emaculata* in infected leaves. Lignin and its precursors are vital components of hypersensitive responses in many plants (Hammond-Kusack and Jones, 1996). Some, but not all, previous studies show that inhibiting expression of lignin biosynthetic genes results in enhanced disease susceptibility in plants (Maher *et al.,* 1994; Tronchet *et al.,* 2010). More specifically, RNAi-mediated transient gene silencing of COMT in wheat led to increased susceptibility to powdery mildew species (Bhuiyan *et al.,* 2009). Additionally, Moerschbacker *et al.* (1990) found that rust-resistant wheat plants treated with lignification inhibitors had increased growth of *P. graminis* as a result of the reduced hypersensitive response to infection. Therefore, down-regulating COMT in switchgrass may increase sugar conversion efficiency and ethanol yields, but simultaneously increase susceptibility to rust.

The aim of the present study is to examine the performance of COMT and MYB4 transgenic switchgrass plants under field conditions. This study evaluates the effect of lignin modifications on agronomic performance, determines whether the improved traits observed in greenhouse-grown plants are maintained under field conditions, and assesses the impact of altered cell wall structure on a plant's ability to respond to environmental stresses and how this could potentially affect performance and yield.

Materials and Methods

Generation of COMT down-regulated switchgrass and field plot design

Transgenic COMT events were generated by propagating seed-derived callus from the switchgrass cv. Alamo and transforming it using *Agrobacterium*-mediated transformation with an RNAi-hairpin loop construct containing a DNA sequence specifically designed to target suppression of the *COMT* gene. The most promising plants were regenerated from the tissue culture process and selected based on down-regulation of COMT expression levels, reduced lignin content and S/G ratios. These T0 plants were outcrossed to wild-type Alamo plants to obtain T1 generation plants containing the transgenic insert and null segregants which are used as non-transgenic controls (Fu *et al.,* 2011). Plants were replicated by vegetative propagation from tillers in a greenhouse and maintained in environmentally controlled growth chambers until transplantation to the field. The transgenic switchgrass field consists of a 22.9 m x 25.9 m plot (607.0 m^2) containing two independent transgenic switchgrass events of the COMT down-regulated gene (referred to as T1-COMT2 and T1-COMT3) with the appropriate control plants. For the T1-COMT2 and T1-COMT3 events, ten replicates for each transgenic event and five replicates for each control were selected. The field design was a completely randomized design with nine individual plants for each of the replicates mentioned above. These blocks of nine replicate plants are randomized throughout the plot, and plants are spaced 76.2 cm apart with 152.4 cm between each individual block. The investigated plants are surrounded by border plants to reduce any shading effects.

Generation of MYB4 up-regulated switchgrass and field plot design

Transgenic MYB4 events were generated from embryonic callus formed from immature inflorescences of switchgrass cv. Alamo clonal genotypes ST1 and ST2 using *Agrobacterium*-mediated transformation with an overexpression construct containing the full-length *PvMYB4* open reading frame (ORF) sequence. The most promising plants were regenerated from the tissue culture process and selected based on overexpression of *MYB4* (Shen *et al.,* 2011). Plants were replicated by vegetative propagation from tillers in a greenhouse and maintained in environmentally controlled growth chambers until transplantation to the field. The transgenic switchgrass field consisted of a 19.1 m by 16.8 m plot (323.7 m^2) containing four independent transgenic switchgrass ST1 events and five ST2 events of the MYB4 up-regulated gene (referred to as T0-ST1-MYB4 and T0-ST2-MYB4) with their corresponding controls. For the T0-ST1-MYB4 and T0-ST2-MYB4 events, three replicates for each transgenic event and for each control were selected. The field design is a randomized block design blocked on genotypes (ST1 and ST2), with four individual plants for each of the replicates mentioned above. These blocks of four replicate plants are randomized throughout the plot, and plants are spaced 76.2 cm apart with 152.4 cm between each individual block. The investigated plants are surrounded by border plants to reduce any shading effects.

Results and Discussion

Field trials of low-lignin transgenic COMT and MYB4 switchgrass plants were conducted at the UT Plant Sciences farm in Knoxville, TN, USA under USDA-APHIS-BRS release-into-the environment permits, which require, as a condition for growing plants, the removal of panicles at the R0-R1 stage. Panicle removal was conducted for both controls and transgenics equally.

COMT transgenic and control plants were transplanted in the field on May 11, 2011. Plants were cut back on August 30, 2011 to synchronize growth. Samples of whole tillers including stem, leaf, and sheath (green tissue) at the E5-E6 growth stage (Moore *et al.,* 1991) were collected in midseason 2011 from each individual plant prior to senescence. Whole aboveground plants were collected after senescence (brown tissue) at the end-of-season in December 2011 (Fig. 2). All samples were dried and ground in a Wiley mill through a 20-mesh screen. The samples are being analyzed for a range of recalcitrance analyses including cell wall characterization for lignin content, S/G ratio, cellulose, hemicellulose, ash, extractives, and index of cellulose crystallinity; and biorefinery performance for sugar release (saccharification) efficiency and ethanol yield using simultaneous saccharification and fermentation. The COMT experiment is presently in the second year of growth in the field (2012) and the plants are fully established and nearing steady-state growth patterns (Fig. 3). The plants will be further evaluated for agronomic performance by means of biomass metrics (tiller height, plant width, tiller number) and dry weight yield. Samples of green and senesced tissues will be collected in 2012 and the same recalcitrance analyses will be performed. Currently, the field is being assessed for the progression of switchgrass rust (*Puccinia emaculata*) to generate disease progress curves to determine if there are differences in rust susceptibility between transgenic and control plants. All plants are also being monitored for other pathogens and insect pests.

Figure 2. COMT transgenic switchgrass field in 2011 showing phases from transplantation to harvest during the first year growing season. Plants were cut back in August to synchronize growth.

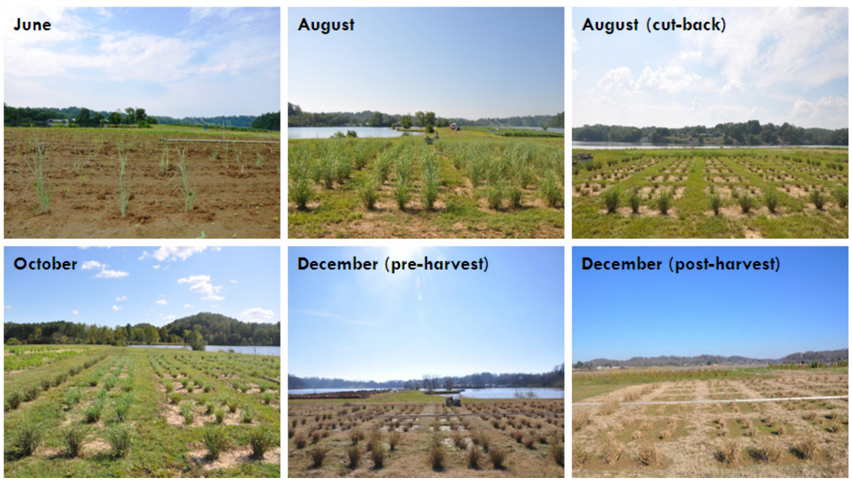
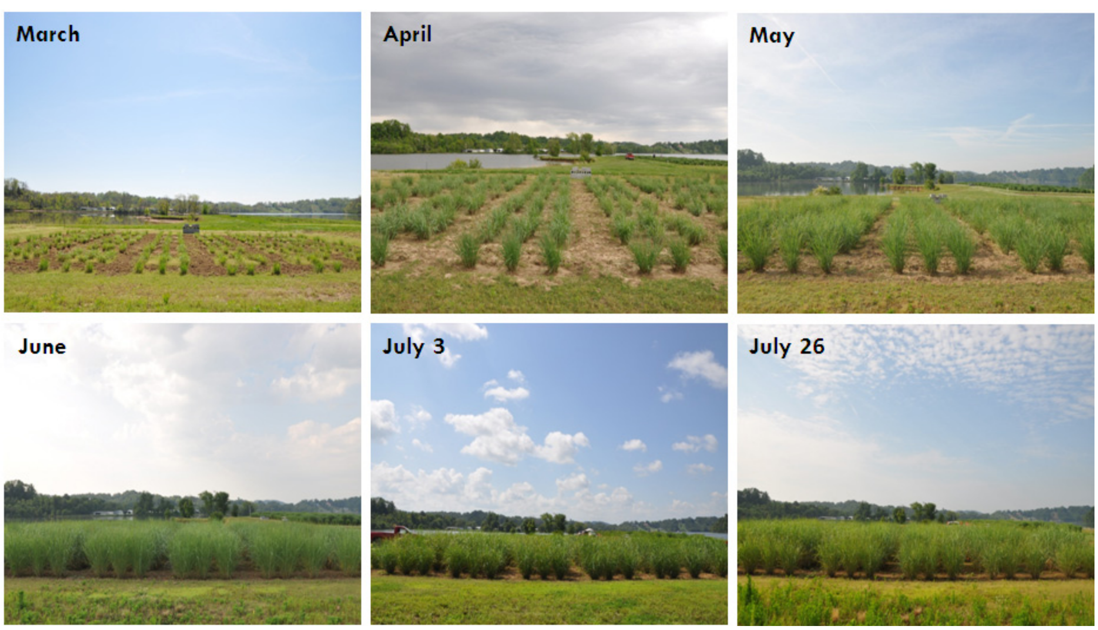


Figure 3. COMT transgenic switchgrass field in 2012 showing regrowth and establishment during the second year of the field experiment.



MYB4 transgenic and control plants were transplanted in the field on July 3, 2012. Plants are being maintained and monitored daily (Fig. 4). Plants will be monitored during first year growing season 2012 for adaptability and establishment. The identical recalcitrance analyses will be performed on green and senesced tissue collected during the first year growing season 2012. Additionally, pathogen and herbivore damage is being monitored.

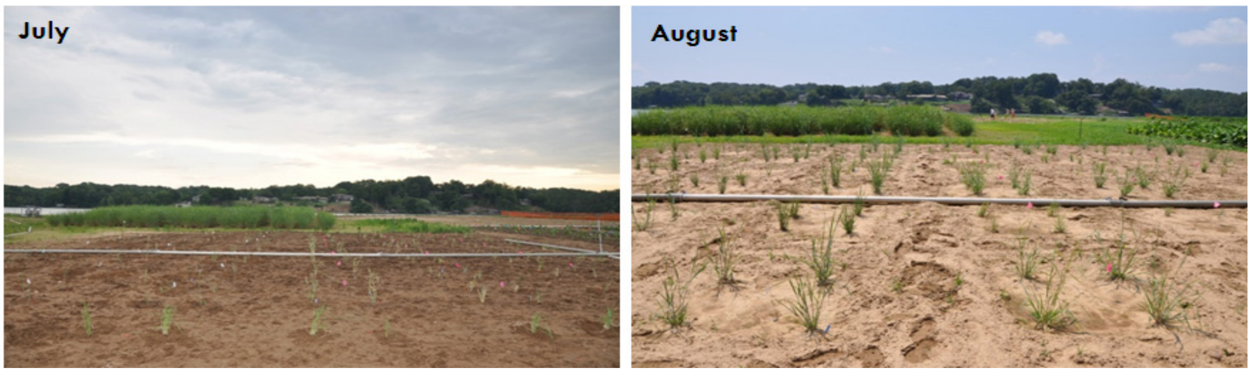


Figure 4. MYB4 transgenic switchgrass field just after transplant in July, 2012, and establishment one month later.

Collectively, candidate genes impacting recalcitrance in switchgrass have been identified and low/modified-lignin transgenic switchgrass plants were produced. Recalcitrance analyses performed on these transgenic plants grown under greenhouse conditions produced results that could be of great interest to the biofuel industry. Field evaluations of these transgenic plants are essential in assessing the commercial value of lignin-modified crops to determine whether the improved traits observed in greenhouse-grown plants are maintained under field conditions due to exposure of plants to environmental stresses not present in a greenhouse setting. These field evaluation studies provide evidence that plants generated through transgenic approaches can be incorporated into a commercialization pipeline. The knowledge gained from field study can be also useful for agronomic evaluations and incorporation into breeding programs.

Acknowledgements

We gratefully acknowledge funding by Southeastern Sun Grant and BioEnergy Science Center (BESC). BESC is a US Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.

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1.8 BIOTECHNOLOGICAL INPROVEMENT OF SWITCHGRASS FOR HIGHER BIOMASS YIELD UNDER COOL GROWING CONDITIONS

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Abstract

Biomass production of switchgrass (*Panicum virgatum*) is known to be limited by growth temperature. Under cool conditions photosynthesis is inhibited and growth reduced compared to warm conditions. In contrast, Miscanthus (*Miscanthus x giganteus*) photosynthesis and growth is not reduced by cool temperatures as much as that of switchgrass. Initial evidence suggests that this difference in cool temperature tolerance between these two important biomass fuel plants may be due to the action of pyruvate phosphate dikinase (PPDK). However, the mechanism of cool temperature inhibition of photosynthesis in switchgass is not well known and its relationship to PPDK is yet to be conclusively determined. Our goal was to fully understand the differences in photosynthesis that are associated with cool vs warm climatic conditions for switchgrass and Miscanthus. Following our characterization of photosynthetic and growth traits, we produced two transgenic switchgrass lines overexpressing PPDK from Miscanthus. Switchgrass plants grown under cool conditions (14C day/12C night) had reduced growth, reduced carboxylation efficiency, CO_2 saturated photosynthesis, and triosephosphate use, but no change in electron transport processes compared to warm conditions (28C day/25C night). Miscanthus grown under cool conditions had no significant change in carboxylation efficiency or CO_2 saturated photosynthesis. Productivity and photosynthetic parameters of the two transgenic lines were significantly less in cool conditions compared to warm. Work continues on more transgenic lines in an effort to increase cold tolerance of photosynthesis in switchgrass.

Keywords: Growth temperature, Photosynthesis, Carboxylation efficiency, light acclimation

Introduction

Switchgrass (*Panicum virgatum* L.) is one of the leading candidate feedstock species for renewable fuel production in the United States because of its favorable traits such as high yield potential, tolerance of low water and nutrient conditions, and low establishment and maintenance costs. High yield of switchgrass lines results from a C_4 photosynthetic physiology similar to that of maize (*Zea mays* L.). C_4 plants have greater water use efficiency, higher productivity and greater warm temperature tolerance than many other biomass forage species with C_3 photosynthetic physiology such as hybrid poplar. The overriding limitations to photosynthetic efficiency in C_4 plants include the enzymes responsible for regenerating phosphoenol pyruvate (PEP) from pyruvate, enzymes associated with malic acid decarboxylation, and leakage of CO_2 from bundle sheath cells back to mesophyll cells. Pyruvate phosphate dikinase (PPDK) serves to regenerate PEP in mesophyll cell chloroplasts with the use of ATP energy produced by the electron transport chain. Therefore, PPDK activity is focused in mesophyll cells, where chloroplasts specialize in C_4 carboxylation. A high rate of PPDK activity relieves the limitation by PEP availability on PEP-carboxylase activity, which increases carboxylation efficiency and carboxylation capacity. Therefore, increasing PPDK activity in switchgrass should increase photosynthetic efficiency and possibly productivity.

While C_4 plants such as switchgrass and maize have a photosynthetic advantage at warm temperatures, they are limited by a decreased performance at low temperatures (Naidu et al. 2003). Limitation of

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photosynthesis under cool conditions reduces the amount of acreage available for switchgrass forage production in the USA. This cool season limitation to carboxylation efficiency has been overcome by the C_4 grass Miscanthus (*Miscanthus* × *giganteus* J. M. Greef & Deuter ex Hodk. & Renvoize), which is an important biomass forage production species in Europe and the USA (Lewandowski et al. 2003). Photosynthesis in leaves of Miscanthus grown at 14°C were found to be equally as efficient as in the leaves of plants grown at 25°C, whereas maize grown at 14°C shows a 90% reduction in photosynthetic efficiency when compared to plants grown at 25°C (Naidu and Long 2004, Farage et al. 2006). Uninhibited photosynthetic efficiency in Miscanthus at low temperature has been attributed to increased expression of a single C4-pathway enzyme, PPDK (Wang et al. 2008b).

In order to increase the possible acreage for high yield switchgrass in the United States, more must be known about the limitations to photosynthesis under cool season conditions. We have two main goals for our research: 1) to understand the effects of cool season and warm season conditions on photosynthetic parameters of switchgrass (Alamo cultivar) and Miscanthus; 2) to investigate the potential for increasing cool season photosynthetic efficiency of switchgrass by overexpression of Miscanthus PPDK in transgenic switchgrass.

Methods

Ten seedlings of switchgrass cv 'Alamo' and ten clones of Miscanthus were grown in the Biol/VBI Plant Growth Facility at Virginia Tech. Plants were watered daily and provided with 3g slow release fertilizer (Osmocote 19-6-12) every 4 months. After 6 months of growth, each plant was propagated by dividing into 4 replicate individuals and replanting in one gallon pots with Pro-Mix BX (Premier Tech Horticulture, Quebec, Canada) to achieve the desired experimental sample size (minimum of 32). Transplants were grown in the greenhouse for two weeks before runts were culled and 8 plants were transferred into each of 4 growth chambers (Model E8, CONVIRON). Two growth chambers were set to warm (28/24°C; day/night) and two were set to cool (14/12°C) season conditions. Photoperiod was set at 14 hour light/10 hour dark and light intensity at leaf canopy height was approximately 200 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Plants were acclimated in each of the growth chambers for two weeks. After the equilibration period in the growth chambers, each individual plant was cut back to an initial state of five tillers per plant.

Switchgrass Transformation

ST1 genotype switchgrass callus produced from immature influorescence was bulked using LP9 media (Burris et al. 2009). *Agrobacterium tumafaciens* (strain EHA105) was transformed with the pANIC-6A/MgPPDK vector and grown in YEP broth to an OD_{600} of 0.50, at which time they were VIR-induced with 187 μM acetosyringone for 1 hour. Cells were re-suspended in liquid LP9 + 187 μM acetosyringone and allowed to infect ST1 callus for 30 minutes with and without vacuum on a shaker. After shaking, callus was blotted with filter paper, covered generously with LP9 + 187 μM acetosyringone, and cocultivated with *Agrobacterium* at 25 °C for 3 days. Callus was then placed on LP9 + 400 mg/L Timentin for 2 weeks and progressively moved onto higher amounts of hygromycin every following 2 weeks for 6 more weeks (20, 40, 60 mg/L hygromycin) to select for transgenic cells. Clearly fluorescent and living callus was then used to regenerate full plants. Growth and photosynthesis results from mature plants of two transformation events are included in this report.

Productivity Measurements

Newly formed tillers were counted once a week throughout the experiment as an indication of temporal changes in aboveground biomass during the experiment. After gas exchange and chlorophyll fluorescence measurements were taken, all the shoots produced in the growth chambers were harvested and dried to a constant weight. Total above ground plant production was recorded for each plant.

Gas Exchange & Chlorophyll Fluorescence

All measurements were made on the third or fourth leaf of newly formed shoots using an open path gas-exchange system (LI-6400; Li-Cor Inc, Nebraska, USA) with relative humidity between 55-75% and leaf temperature maintained at the respective growth-chamber temperature. Leaves were allowed to acclimate to cuvette conditions for five minutes prior to initiating photosynthetic response programs. The photosynthetic response to light (A/Q curve) at 400 µmol/mol CO_2 was measured on at least 4 individuals from each growth chamber to determine the light saturation point (Q_{sat}), light saturated rate of photosynthesis (A_{max}). The photosynthetic response to intercellular carbon dioxide concentration (A/C_i curve) was then measured on all eight plants per chamber at saturating light. Carboxylation capacity (A_{sat}), carboxylation efficiency (CE), triose phosphate use (TPU), and the maximum carboxylation rates of Rubisco (V_{cmax}) were calculated using Photosyn-Assistant (Dundee Scientific Ltd, Scotland, UK).

Chlorophyll fluorescence was used to assay photochemical processes by measuring F_v/F_m (indicator of photoinhibition), electron transport rate (ETR), and nonphotochemical quenching (qN), on three dark-adapted leaves from all individuals in each chamber. Leaves were dark-adapted for 10 minutes prior to measurement using a pulse-modulated fluorometer (OS-500, Opti-Sciences, New Hampshire, US)

Statistical Analysis

Results from productivity, A/Q, and A/C_i response curves were analyzed between temperature treatments (n=6-8) using a student's t-test (α=0.05) (SigmaStat 3.0, Jandel Corporation, Illinois, USA). If normality or equal variance tests failed we utilized a nonparametric rank summed test (α=0.05). In cases were a chamber effect was found, we compared results between all chamber pairs.

Results and Discussion

Comparison of Switchgrass (Alamo cultivar) and Miscanthus

Switchgrass developed new shoots more quickly than Miscanthus in both temperature treatments. Switchgrass took 102 days to reach maximum number of shoots in cool growth conditions, while Miscanthus took 193 days. The cool growing condition significantly reduced the final productivity of switchgrass ($p < 0.01$) compared with the warm growth conditions, while Miscanthus had no significant ($p = 0.09$) reduction in final productivity under cool growth conditions. Therefore, under our relatively low-light, growth-chamber conditions switchgrass had higher productivity, but was inhibited more by cool temperature conditions than Miscanthus.

There were some slight significant differences in photoinhibition (F_v/F_m) and ETR among temperature treatments for both species, but these differences were not large enough to be biologically significant. Therefore, we found no biologically significant differences in photochemical processes between temperature treatments for either species. These results differ from those for field planted switchgrass and Miscanthus due to light limitation in growth chambers. In our experiment, we found no biologically significant limitation of electron flow for photosynthesis under cool growth conditions.

Miscanthus leaf photosynthesis acclimated to the low light intensity conditions of the growth chambers evidenced by a low light saturation point (Q_{sat}) and a low light saturated rate (A_{max}) at both temperature conditions. However, switchgrass plants did not acclimate as much as Miscanthus to the low light conditions. The difference between low light acclimation of these two species may have caused the lower photosynthetic rates (A_{max}) of Miscanthus compared with switchgrass under warm growing conditions.

Switchgrass A_{sat}, CE, TPU, and V_{cmax} decreased significantly from warm to cool growth conditions. However, there were no significant temperature effects on these photosynthetic variables for Miscanthus plants, with the exception of V_{cmax} ($p < 0.01$). These results, particularly CE, demonstrate that the C_4 carboxylation pathway efficiency of Miscanthus is not significantly affected by cool conditions while that of switchgrass significantly decreases under cool growing conditions. Although photosynthetic efficiency of Miscanthus is tolerant of cool conditions, growth tended to decrease at cooler temperatures as in previous research. These results support earlier studies that also show switchgrass photosynthetic efficiency decreases under cool conditions more than that of Miscanthus . Miscanthus photosynthesis and productivity at high light intensity was found to be higher than that of switchgrass (Cave-in-Rock cultivar) in previous research, but Miscanthus required more water and nitrogen (Dohleman et al. 2009). Our results, at lower light intensity, indicate that switchgrass has higher productivity and photosynthesis under warm conditions than Miscanthus. This discrepancy is due to the different light regimes used in these two studies. Previous studies have reported that at lower growth temperatures, Miscanthus increases the expression of PPDK, and PPDK also demonstrates lower activation energy in Miscanthus at cold temperatures (Wang et al. 2008a). These reported PPDK parameters may explain the cool temperature tolerance of carboxylation efficiency we found for Miscanthus.

Comparison of Two Transgenic Lines to Control

We screened productivity and photosynthesis of two transgenic lines of switchgrass containing the gene for PPDK from Miscanthus against their control line to evaluate the possibility that incorporating the PPDK gene from Miscanthus into the genome of switchgrass would make carboxylation efficiency more tolerant of cool growth conditions. The two transgenic lines had significantly ($P<0.01$) lower productivity than the control under both growth conditions. Indicating that transformation of these two lines has impaired productivity at growth chamber light conditions.

Photosynthetic parameters of the control line were generally higher than either of the transgenic lines although not always significantly higher. For all photosynthetic parameters, including CE, the transgenic lines were similar to control under cool conditions, but significantly lower than control under warm growth conditions, which was a characteristic of Miscanthus photosynthesis in our research.

Conclusion

We found that Miscanthus was able to maintain CE and A_{sat} better than switchgrass under cool growth conditions. However, the two transgenic lines we tested had inhibited productivity and their photosynthetic parameters responded to cool conditions similarly to the control. The vulnerability of switchgrass carboxylation efficiency to cold conditions was not improved by expressing the PPDK gene from miscanthus in two transformed lines. However, the tendency of miscanthus to have lower photosynthetic capacity than switchgrass under warm conditions was found in the transgenic switchgrass lines. Further screening of more transgenic lines is necessary to fully understand the potential to minimize the decrease of carboxylation efficiency in switchgrass under cool growing conditions.

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1.9
GENOTYPIC VARIATION FOR BIOMASS AND CELL WALL POLYMERS IN *AMARANTH*

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Abstract
Thirty-five accessions belonging to genera *Amaranthus* and *Celosia* in the subfamily *Amaranthoideae*, provided by the USDA-ARS, National Plant Germplasm System (NPGS), were evaluated for biomass yield and cell wall polymer profile in greenhouse grown plants. A wide spectrum of phenotypic variation was found between species as well as among genotypes within the same species. The average dry biomass (single plant), lignin, hemicellulose and cellulose values across all accessions ranged from 10.1–166.5 g, 7.6-25.6%, 15.9-31.0% and 7.8-20.0% of dry matter, respectively. Considering biomass and cell wall composition among different species, *A. australis* had the highest biomass yield (both wet and dry matter), *A. cruentus* had the highest hemicellulose and lowest lignin content, and *A. tricolor* had the lowest cellulose content. The magnitude of genotypic variation observed for biomass and cell wall composition indicates amaranth has potential for use as a feedstock. However, to promote its use as a new bioenergy crop, a breeding strategy should be formulated. One promising approach would cross *A. australis* (biomass donor) and *A. cruentus* (cellulose donor) or *A. caudatus* (hemicellulose donor) in order to generate hybrids with lodging resistance as well as high biomass yield and high cellulose or hemicellulose content.
Keywords: Amaranthus, biomass, cellulose, hemicellulose, lignin

Introduction
The usefulness of any higher plant in biofuel production will be limited by how well and where the plant grows, success and timeliness of regrowth from season to season for perennials or annuals, and seed germination and dormancy amongst other issues.

Amaranth is an annual herbaceous crop with C4 type photosynthesis with an upright habit and grows in both temperate and tropical climates. It is known for its significantly high yield as well as quality and becoming an increasingly important resource for health food. Currently, the unprocessed biomass of *Amaranthus* species is used primarily as fodder in many countries (Viglasky *et al*. 2009). Amaranth holds many promises for use as an alternative bioenergy crop where lignocellulosic biomass could function as a feedstock. First, amaranth produces a large amount of biomass in a short period of time. When good fertilization practices are used, whole plant dry matter (DM) yield for amaranth is 7,130kg/ha on average and can reach up to 9,100 kg/ha (Svirskis 2003) which in comparison to maize averages 12,620 kg/ha DM (Olorunnisomo and Ayodele 2009). However, with fewer inputs such as without any fertilizer, amaranth DM (5,790kg/ha) can compete with maize (5,480kg/ha) (Olorunnisomo and Ayodele 2009). Pospisil *et al*. (2009) compared grain amaranth (*A. hypochindriacus*) DM with that of forage sorghum at different growth stages. Although amaranth produced lower DM (average 5,500kg/ha) than forage sorghum (9,200kg/ha), amaranth produced higher quality of crude and digestible proteins and neutral detergent fiber and acid detergent fiber concentrations (Pospisil *et al*. 2009). Amaranth grain yield ranged from

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2200-3,000 kg/ha without significant genotype difference (Gimplinger *et al*. 2007). Second, this plant can also grow on marginal fields and areas of known water deficiency. Water resources, especially surface and ground water will continue to be available in more limited quantities for agricultural uses as domestic and industrial needs increase. Water is already a limiting factor for crop production in semi-desert zones with low annual precipitation, and so future large scale biofuel crops should be better adapted to low water use. Amaranth species as a crop are naturally resistant to water deprivation. Third, amaranth has a C4 photosynthetic pathway, and is able to maintain relatively high rates of carbon dioxide fixation. Amaranth by virtue of the C4 photosynthetic process is very efficient at converting CO_2, from the air, minerals from the soil, along with the energy of the sun, and water into plant tissue. Amaranth has ability to exploit the C4 pathway due to its tolerance to stressful environmental conditions by virtue of its natural ability to adapt to marginal areas. Fourth, amaranth cell wall biochemical composition analyses indicate that it compares favorably with maize, switchgrass, and miscanthus as a biomass feedstock (Pospisil *et al*. 2009). Therefore, amaranth has unusual potential as a low-lignin fermentation feed-stock. Nevertheless, research into the biochemical composition of amaranth's cell wall is limited worldwide. Given our nation's dependence on overseas fossil fuel and increased demand for crops that can be used as a biofuel replacement, the investigation of amaranth as an alternate source of biomass becomes more strongly justified.

Utilization of amaranth biomass as a renewable energy source could be considered as a multipurpose crop with great potential in the 21st century because of its ability to absorb heavy metals from the surrounding soil as well as the other promising attributes aforementioned (Huska 1992). However, no comprehensive investigation into the biochemical composition of cell wall and biomass production of *Amaranthus* accessions has been reported. Using the diverse *Amaranthus* accessions, we systematically assessed the quality of the biomass produced by the various species under greenhouse conditions. The aim of our study was evaluate the chemical composition of cell wall components and associated biomass traits in the above-ground portion for thirty five different accessions representing seven different amaranth species.

Materials and Methods

Plant Materials

The set of 35 genotypes from six *Amaranthus* (31) and one *Celosia* (4) species (Table 1) were obtained from the National Plant Germplasm System (NPGS) to use as test subjects in the investigation of phenotypic and biomass traits. Individual plants were grown in 10-gallon containers under greenhouse conditions *e.g*., controlled temperature and relative humidity (approximately 80°C and 50%) at Texas A&M AgriLife Research Center at Dallas.

Table 1. Genotypes numbers, plant growth parameters and mean values of Fresh matter (FM), Dry matter (DM), lignin (LIG), hemicellulose (HCEL), cellulose (CEL) and ash (ASH) contents of six *Amaranthus* species and one *Celosia* species.

Species	No. of Genotypes	Plant Height (cm)	FM (g)	DM (g)	LIG (%)	HCEL (%)	CEL (%)	ASH (%)
A. australis	1	330.0	1106.0	166.5	14.3	16.8	9.1	0.7
A. blitum subsp. oleraceus	1	76.5	148.0	20.2	7.6	26.8	14.2	0.8
A. caudatus	3	76-100 (86.7)	50-153 (95.5)	7.7-16.2 (12.0)	7.8-23.2 (13.8)	27.6-35.8 (31.0)	11.7-20.4 (17.1)	0.3-2.8 (1.2)
A. cruentus	15	79-125 (104.7)	119-242 (155.7)	15.6-25.7 (21.2)	4.9-22.4 (13.8)	15.6-33.7 (19.6)	11.2-33.1 (20.0)	0.2-7.0 (1.1)
A. hypochondriacus	6	79-148 (122.2)	116-238 (156.5)	16.9-24.8 (20.6)	12.8-25.8 (19.2)	16.7-21.2 (19.2)	12.4-19.3 (15.3)	0.2-2.6 (1.1)
A. tricolor	5	40-120 (77.2)	54-153 (115.2)	6.2-16.9 (13.2)	20.3-28.8 (25.6)	13.4-18.0 (15.9)	4.9-13.7 (7.8)	1.5-4.7 (3.2)
Celosia argentea	4	54-87 (72.5)	91-149 (115.5)	8.2-12.2 (10.1)	11.9-30.2 (20.7)	15.4-20.1 (18.6)	7.3-20.9 (12.1)	0.1-1.2 (0.4)
	SD	29.3	47.4	7.0	7.1	5.7	6.8	1.5
	CV%	29.2	33.0	38.0	41.7	28.7	43.3	113.7

Note: Number in bracket is the average value.

Whole Plant Biomass and Cell Wall Polymers

Plant height, leaf no., and leaf area (length x width, cm) were measured three times when fully matured (after flowering). Then all aboveground biomass was harvested and weighed (fresh matter, FM). The samples were then oven dried at 80°C until they achieved a constant weight (dry matter, DM) (Table 1).

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined following the protocol of ANKOM Technology using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). Hemicellulose (HCEL) and cellulose (CEL) contents were determined by subtracting the values of ADF from NDF, and ADL from ADF, respectively (Hindrichsen *et al*. 2006). Ash content was determined by combustion of samples in a muffle furnace at 930 ^{o}F for 12 hours. Variance analysis of different traits and Pearson's correlation coefficients between traits is presented based on accessions mean values.

Statistical Analysis

Collected data was subjected to analysis of variance (ANOVA, $P < 0.05$) using XLSTAT for Microsoft Excel statistics package (XLSTAT software, Addinsoft, Paris).

Results and Discussion

A large amount of natural variation was observed for biomass traits for the selected *Amaranthus* accessions. The morphological differences observed could be a means of predicting the underlying variation for biomass production. Total average dry matter (DM) ranged between 10.1 g to 166.5 g per plant, revealing a wide genotypic variation (Table 1). PI 553076 (*A. australis*) was the tallest plant (330 cm) and thus produced the highest DM (166.5 g); *A. caudatus* produced the lowest amount of DM among the *Amaranthus* species (Table 1). The fresh weight ranged from 50.4 g (Ames 5363; *A. caudatus*) to 1106 g (PI 553076, *A. australis*). The coefficient of variation for plant height, no. of leaves and dry wt. was 29.23%, 38.52% and 38.04%, respectively. Variation in percentage of DM corresponds to accessions with low percentage water content and the maximum total dry biomass. The similar trend was found in rice (Jahn *et al*. 2011) and it suggests that targeting reduced water content may be a selection regime to increase total biomass in amaranth. Although one should be cautious when comparing biomass yields from single plant samples or single harvest time points, these data do however suggest that differences between accessions do exist for biomass productions and selective breeding to increase yields looks promising. However, a three-year study on biomass comparison of *A. hypochondriacus* and forage sorghum revealed that forage sorghum gave the highest fresh matter (FM) and DM yield at the tasseling stage in first year, but in the second year the amaranth cultivar gave a higher green mass yield at flowering (Ana *et al*. 2009). In our studies we found average highest FM and second highest DM for the species, *A. hypochondriacus* among the seven species tested.

Cell wall polymer composition among higher plants can differ substantially in quality and quantity (Pauly and Keegstra 2008) and is an important consideration for bioenergy production. The amounts of cell wall structural polymers and ash content were determined from whole plant tissues gravimetrically after treatment with either neutral or acidic detergent. Cellulose, hemicellulose, lignin, ash, and soluble fiber content varied widely among the accessions (data not shown). Ash content of Ames 13887 (*A. cruentus*) was about 100-fold higher than that of Ames 14960 (*C. aregentea*) (data not shown). The cellulose content ranged from 4.9% (Ames 2091, *A. tricolor*) to 33.1% (Ames 5330, *A. cruentus*). Other cell wall traits that might be involved in biofuel accumulation also exhibited large variation across the 35 accessions. Hemicellulose content ranged between 13.4% (PI 490764; *A. tricolor*) to 35.8% (Ames 5363, *A. caudatus*), but the average was 19.99%. Lignin contents ranged from 4.9% (PI 451711, *A. cruentus*) to 30.2% (PI 649287, *C. argentea*) with an average of 17.0%. Among all 35 accessions for the cell wall traits, ash content showed the highest level of co-efficient of variation (113.7%), followed by cellulose (43.3%), lignin (41.7%), and hemicellulose (28.7%).

Based on cell wall composition for the 7 different species, *A. cruentus* had the best qualities in respect of cell wall polymers. For example, it had the highest cellulose and lowest lignin content (Table 1). Previous studies by Viglasky *et al*. (2009) suggested that *A. cruentus* is one of the main crops being considered as a source for raw materials to be utilized for solid biomass based production processes since it has the potential to yield high quality biofuel. In this study, *A. australis* had the highest FM and DM. Based on these data one could develop a breeding strategy to cross *A. australis* (biomass donor) x *A. cruentus* (cellulose donor) or *A. caudatus* (hemicellulose donor) in order to produce amaranth with the potential of being the biofuel crop of the future. Additionally efforts are under way to exploit genomic tools with *Amaranthus* in order to identify loci that impact the traits of interest and use these tools to manipulate the genetic variation for traditional breeding purposes to enhance yield in this new energy crop.

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2 ENERGY CROP PRODUCTION

2.1 BIO-ENERGY FOREST PLANTATIONS FOR THE SOUTHERN UNITED STATES

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Abstract

Bio-energy forest plantations will supplement woody biomass from other sources such as logging residues. In the southern US, projections are for an increase of up to 25 million "new" tons of woody biomass demand for bio-energy. To supply this woody biomass demand will require purpose grown plantations of various species including pine, eucalypts, sweetgum, hybrid poplar and cottonwood, amongst others. Forest plantation yields can be 8-15 green tons/acre/year on rotations of 5-12 years. Utilization of this renewable and sustainable biomass resource will be as feedstock "designed" for a large number of bio-energy applications.

Introduction

Demand for renewable energy sources is increasing in the Southeastern United States. There are few opportunities in this region to achieve this with sources such as solar, wind and hydroelectric. Biomass from agriculture and forestry are available for bio-energy feedstock. In the particular case of forestry, purpose grown plantations for biomass feedstock give an opportunity for cost savings, a sustainable resource for bio-energy and an economic opportunity for forest landowners.

Bio-energy plantations include, amongst others, pine, cottonwood, hybrid poplar, sweetgum and eucalypts. Much of the emphasis has been on hardwood plantations due to their ability to coppice, continued genetic improvement programs as well as the opportunity to combine fast growth and wood properties in selected clones. In the specific case of *Eucalyptus* and *Populus*, there are a large number of commercial planting programs in countries outside the US.

A number of feedstock characteristics are important in bio-energy hardwood plantations. Firstly, the plantation hardwood species has to be adapted to the soil and climate conditions. The hardwood feedstock has to be acceptable in harvesting, field processing and ultimately for conversion to bio-energy. Lastly, the growing (stumpage), harvest, haul and preparation costs have to be favorable compared to other biomass options. In the Southeastern US there are a limited number of hardwood species that can be competitive for forest plantation biomass for bio-energy production. The most important species would be from genera such as *Eucalyptus*, *Populus* and *Liquidambar*.

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Hardwood Comparisons

Published data on commercial growth rates and final harvest yields for hardwood bio-energy plantations in the southeastern US are relatively scarce. In Table 1, a sample of published growth rates are provided.

Table 1. Growth rates in green tons/acre/year of hardwood bio-energy plantations in the southeastern US.

Species	Growth rate	Rotation (years)	Citation
Populus	4.2 to 8.6	5	Stuhlinger *et. al*., 2008
Populus	1.4 to 6.8	10	Stuhlinger *et. al*., 2007
Populus	4.2 to 9.3	5-8	NCSU, 1994
Populus	5.5 to 10.6	9-12	NCSU, 1994
Liquidambar	11 to 14	8-12	Wright and Cunningham, 2008
Eucalyptus	9.2 to 16.1	7	Dougherty and Wright, 2010, 2012

Growth rate differences are large and will depend on multiple factors including species, soils and harvest methods. In several states including Florida, eucalypt plantations are being considered for pellets (Pirraglia et. al., 2011), bio-fuel (Gonzalez et. al., 2011a,b) and combined heat and power (Dougherty and Wright, 2010, 2012).

Conclusions

Publication of commercial growth rates and final harvest yields for hardwood bio-energy plantations would be useful for those forestry and energy organizations seeking to invest in this renewable asset. It is surprising, to some, that after more than seventy years of research there are less than ten refereed publications with harvest yield values though certainly the grey "unscanned" literature contains more. In addition, including stand management details as well as stating in the publication whether it is green versus dry tons, whole tree chips versus wood chips would also assist the investors.

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2.2
SHORT ROTATION ENERGY PLANTATION DENSITY EFFECTS ON YIELD AND RETURN ON INVESTMENT IN A FIVE-YEAR-OLD HYBRID POPLAR TRIAL IN MICHIGAN

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Abstract

Short rotation energy (SRE) plantations on fallow open land can be highly productive and present property owners with an opportunity to participate in developing energy markets. Diversifying the agricultural base in the Northeastern and North Central United States through the introduction of SRE plantations also has the potential to improve rural economies which have declined as forestry and agriculture production has moved away. As biomass markets develop, adoption of SRE plantation systems by growers will depend to a large extent on the returns they can obtain from their investment. This, in turn, will be highly dependent on their choice of crops and cropping system. Hybrid poplars are one promising SRE crop but the interaction between varieties (taxa) and production systems (planting density and rotation length) strongly influences costs and yields and consequently, return on investment. Fifth-year growth in a replicated study of six hybrid poplar taxa planted at three densities in Escanaba, MI was examined here. Biomass yield varied significantly among taxa and densities (ranging from 37.6 Mg/ha for NM6 at 2,200 stools/ha to 14.2 Mg/ha for DN34 at 2,700 stools/ha) and a significant interaction between treatments was observed. When yields were projected to the end of an eight-year rotation, estimated rates of return ranged from +9.6% (for NM6 planted at 1,900 stools/ha) to -4.6% (for DN34 at 2,700 stools/ha). Understanding the interaction between taxa and planting densities will be critical to the profitability of SRE plantation systems.

Keywords: poplar, spacing, density, yield, short-rotation energy plantation, biomass, return on investment.

Introduction

Producing wood in Short Rotation Energy (SRE) plantations has become a viable method for augmenting the feedstock demand of the emerging renewable energy industry around the world. Ligno-cellulosic material (biomass) produced in SRE plantations can be combusted for the production of heat and power or upgraded to liquids or gases for transportation fuels and chemical production. Biomass is the only source of renewable carbon on the planet and so will be a vital feedstock in replacing the fossil carbon on which we now so heavily depend.

SRE plantations require a different type of forest management (silviculture) than that traditionally employed by foresters. Traditional silviculture is optimized for the production of large trees on long cutting cycles (rotations). Although the underlying fundamentals remain constant, this new SRE plantation silviculture must account for unfamiliar varieties (taxa), short rotations, and new landowner expectations and be optimized for the rapid production of this low-value forest product. Since the goal is to produce biomass quickly, regardless of individual tree size, SRE Plantation silviculture research focuses on biology and economics during the first few years of stand development.

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Many taxa and management systems have been examined during the past four decades including an unsuccessful "wood grass" system that annually harvested plantations with more than 600,000 stools[2] per hectare (s/h) and relied on sprouts to re-establish the stand each year (DeBell 1993). Experience has led us to concentrate on two main systems; 3- to 4-year "micro" rotations of *Salix* (or willow) hybrids at "high" densities of 14,000 s/h, and 6- to 10-year "short" rotations of *Populus* (or poplar) hybrids at "low" densities below 4,000 s/h. Research continues to optimize these systems across a range of taxa and planting sites. Work reported here examines the biological development and economic implications of six poplar taxa planted at three densities in the "short" rotation system on a site in northern Michigan after their first five years of development.

Early plantation biomass production per unit area, or Annual Biomass Increment (ABI), increases exponentially during the period before individual trees compete with each other for site resources, or achieve "crown closure." After crown closure trees compete with each other, ABI levels-off then gradually decreases, and mortality begins (Yoda 1963). Maximum Annual Biomass Increment (MABI) is reached earlier in dense plantings because the trees fully occupy the site more quickly than in less dense plantings. However, less dense plantings quickly catch up and maintain MABI longer than their dense counterparts because inter-tree competition is less severe (Hansen 1979; DeBell 1996). Consequently, total biomass production over "short" rotations is similar over initial planting densities ranging from 3,000 to 40,000 s/h (Johnstone 2008; Strong 1993; Ferm 1989; DeBell 1996; DeBell 1993).

Plantations with low densities (1,100 s/h and below) are less expensive to install and eventually produce larger individual trees than high density plantations (Fang 1999; van Oosten 2006). This is useful for growing traditional forest products like pulpwood, sawlogs, and veneer logs but not for biomass. Since density controls the rate at which plantings reach MABI and therefore both biological and economical rotation length, it is critical to the successful design of SRE Plantation systems.

Rates of change in ABI and absolute values for MABI are controlled by numerous factors including; planting stock genetics, initial planting density, non-crop competition, climate, soil fertility, moisture availability, and pest depredation. We limited our test to the effects of three densities on six poplar taxa at a single site in northern Michigan.

Methods

Six superior poplar taxa were selected based on previous trials in the Great Lakes region of the United States (Netzer 2002; Isebrands 2007) and on the availability of sufficient numbers of cuttings for establishing our test plantation. One taxon of *Populus deltoids* (D105), three taxa of *P.* x*canadensis* (DN5, DN34, and NE222), and two taxa of *P. nigra* X *P. maximowiczii* (NM2 and NM6) were included. Unrooted hardwood cuttings of each taxa were obtained from five regional nurseries and planted into a well-tilled field at Michigan State University's Forest Biomass Innovation Center (45º 45' 50" N latitude, 87º 11'30" W longitude) in the spring of 2008. Stems that sprouted from each of these cuttings formed a "stool." Soil in the field was a fine sandy loam from the Onaway soil series. The site receives an average annual rainfall of 45 cm during a growing season that encompasses 140 frost-free growing days and includes an average of 1,170 growing degree days (base temperature 10º C). Weeds in the plantation were controlled using a combination of herbicides and mechanical cultivation for the first two years after planting. No fertilization or irrigation was applied.

Previous poplar studies suggested that there was no yield advantage for densities more than 3,000 s/h when rotations were five or more years long and that densities less than 1,100 s/h produced inferior biomass yields. Consequently for our target rotation length of eight years we chose to test three densities between these limits. Cuttings of each taxa were planted in rows separated by 2.44m. Because Johnstone

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[2] Stems originating from a single planted seedling or cutting form a "stool."

(2008) had shown that density, rather than the degree of rectangularity of tree spacing, was the factor controlling yield, we varied the distance between cuttings within each plot's rows (2.13m, 1.83m, or 1.52m) to obtain the desired planting density of 1,900, 2,200, and 2,700 s/h respectively. The test was arranged in a randomized block design with four blocks of 0.04-hectare plots. Each complete block was composed of 18 plots; one for each of the six poplar taxa planted at each of the three densities. A shortage of NM2 cuttings resulted in two blocks being incomplete, containing only 15 plots.

Measurements were taken at the end of the third and fourth, and near the end of the fifth growing seasons from stools in the center of the larger whole plots, leaving a two-stool border to isolate the measurement plot from the edge effects created by adjoining plots. Consequently 24, 28, and 32 stools were measured in the 1,900, 2,200, and 2,700 s/h plots respectively. Stem diameters at 1.37m above the ground (DBH), number of stems per stool, and stool survival was recorded. Total stem oven-dry biomass was computed using an equation developed by Zhonglei Wang at Michigan State University's Department of Forestry from destructively sampled poplars in a similar plantation adjacent to this test (personal communication):

$$\textit{Single-tree oven-dry biomass}_{(kg)} = 0.09784 \bullet DBH_{(cm)}^{2.3}$$

Unit area biomass production was calculated by summing the biomass of all stems within a measurement plot and multiplying by the appropriate expansion factor for that particular density plot. Measurements were not made during the first two growing seasons because too few stems had reached a height sufficient for reasonable DBH measurement and biomass estimation.

Results

Biomass production varied with taxa falling into three statistically distinct groups: NM6 and NM2 were the best biomass producers, DN5 was intermediate, and NE222, D105, and DN34 produced the least (Figure 1). Significant clone by density interaction was found, indicating that the optimal density for each clone was different. The best performing group yielded nearly twice the biomass of the poorest group at their respective optimal densities. Stool survival was good, exceeding 88% for all taxa except NE222 which had a slightly lower survival rate of 83%. Stand density after five years generally increased slightly due to the tendency for certain taxa to develop more than one stem per stool (most significantly D105, which had an average of 1.4 stems/stool). In two cases (NM2 and NE222) density decreased due to stool mortality (Table1). Stool mortality occurred early in the life of the planting and so was the result of establishment failure and not inter-tree competition.

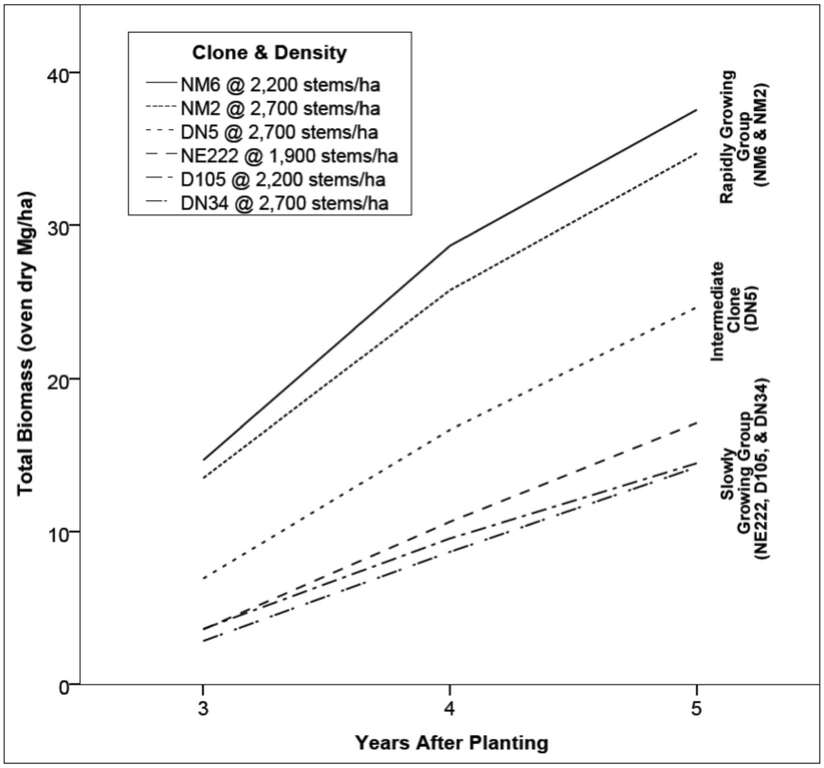

Figure 1. Biomass production of 6 poplar taxa over 5 years growing at optimal densities in a spacing trial in Escanaba, Michigan. Taxa cluster together into 3 statistically significant groups.

Table 1. Performance of 6 taxa at optimal densities after 5 growing seasons in a density trial in Escanaba, Michigan.

Clone	Planting Density (stems/ha)	*At end of the 5th growing season*					
		Density (stems/ha)	Total Biomass (OD Mg/ha)*		ABI (OD Mg/ha-yr)	Stool Survival*	
NM6	2200	2823	37.6	a	7.5	100%	a
NM2	2700	2397	34.7	a	6.9	88%	b
DN5	2700	2712	24.6	b	4.9	93%	a
NE222	1900	1702	17.1	c	3.4	83%	b
D105	2200	2923	14.5	c	2.9	94%	a
DN34	2700	3006	14.2	c	2.8	95%	a
LSD α=0.05			4.8			10%	
* Biomass and survival means followed by the same letter are not significantly different.							

Areal biomass productivity was calculated by summing the biomass of each stem and so was a function of the number and size of individuals in each plot. Taxa that quickly and completely occupied the growing space tended to produce biomass at similar rates across densities. Individual stools had begun to compete and diverge in size so the biomass per unit area produced by many smaller stools was equaled by that produced by fewer bigger ones (*e.g.* NM6 averaged 13.2 kg/stool in high density plots and 18.9 kg/stool in low density plots). Taxa that were less aggressive in occupying the growing space tended to produce stools of consistent size irrespective of density (*e.g.* DN5 stools had a consistent weight of 10.2 kg/stool). When individual stool size was consistent, areal biomass was higher when more stools were present (Figure 2).

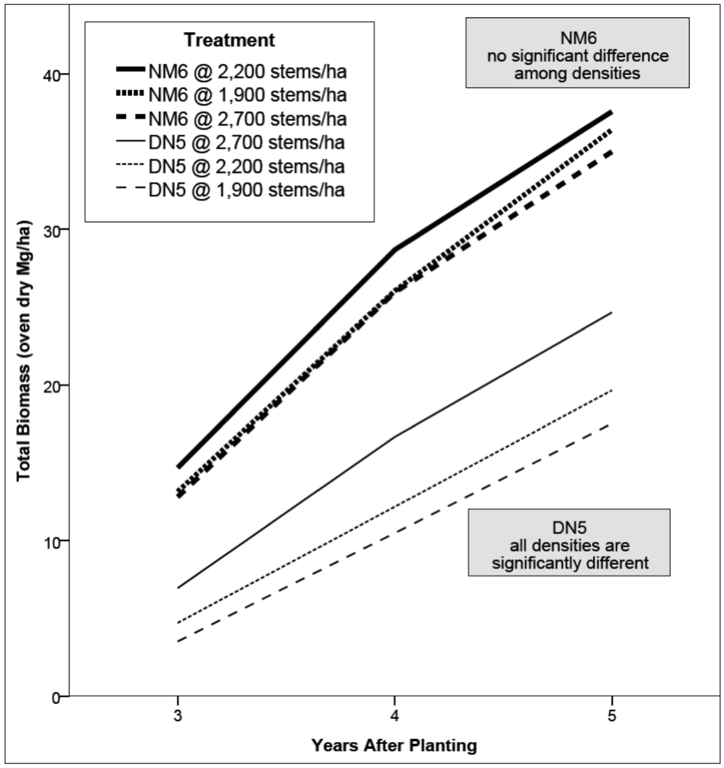

Figure 2. NM6 and DN5 biomass production over 5 years at 3 planting densities in a large-plot density trial in Escanaba, Michigan.

ABI has increased at different rates for all taxa at all densities in this test and it appears that MABI will not be attained for several more years. This will be determined through continued monitoring of this trial for at least three more years.

Discussion

Not surprisingly, biomass productivity varied among taxa, but this study also demonstrated that optimal density varied among taxa. Increasing planting density for slower growing taxa did improve biomass productivity but did not overcome the advantage that faster growing taxa had in total biomass production at the densities tested here. It is apparent that proper taxa selection combined with the choice of appropriate planting density for each taxon is critical to optimizing both the biology and economics of poplar SRE Plantation systems. For rotations of approximately eight years, it seems unnecessary to plant more than 1,900 s/h of taxa like NM6 and NM2 that aggressively occupy the available growing space. Planting densities far in excess of 2,900 s/h (perhaps as high as 4,000 s/h) would be required for less aggressive taxa like DN5 to even approach the level of productivity of the more aggressive taxa. This would nearly double planting costs and severely reduce profitability.

Mortality due to inter-tree competition had not begun at any of the densities tested here. This suggests that densities of 2,900 s/h are not excessive when planting these taxa for rotations of five years. Because MABI had not been reached in any of these clone/density combinations, the biologically-optimal rotation age had not yet been achieved after five years. It is anticipated that this point will be reached for many taxa after eight growing seasons and that mortality due to inter-tree competition will begin in high density plots as the plantation ages.

A simple linear regression was used to predict biomass yields at age eight for each clone/density treatment in this trial. In this way, after eight years, a ten-hectare plantation of the best performing treatment (NM6 at 1,900 s/h) is expected to produce 717 OD Mg while the worst performing treatment (DN34 at 2,900 s/h) is expected to produce only 313 OD Mg of poplar chips.

A cursory economic analysis of these two scenarios using a conservative set of assumptions[3] suggests that a producer could experience internal rates of return (IRR) ranging from +9.6% for NM6 at 1,900 s/h to -4.6% for DN34 at 2,700 s/h. Most of this wide range was due to differences among taxa. Clonal selection obviously had a huge impact on returns. Planting density also had a significant, albeit smaller, impact on system profitability. For taxa like NM6 whose yield was independent of spacing, IRR decreased with increasing density (+9.6% at 1,900 s/h, +9.3 at 2,200 s/h, and +7.6% at 2,700 s/h). Planting additional cuttings of this taxon did not increase yield but did increase establishment costs, making high density plantations of taxa like NM6 a poor financial choice. For taxa like DN5 whose yield decreased with decreasing density, IRR did just the opposite; increasing with increasing density (-0.3% at 1,900 s/h, +0.6% at 2,200 s/h, and +3.0% at 2,700 s/h). None of the DN5 treatments tested here were as profitable as the NM6 systems but the cost of planting additional cuttings of this taxon was offset by increased yields. Therefor increasing the density of SRE Plantations of taxa like DN5 does improve the financial outcome for the grower.

Planting density had a significant effect on profitability in either case and was determined by the underlying biology of the taxa involved. Density trials like this are necessary to understand these taxa-specific fundamentals, choose the appropriate density for each taxa, and thereby increase the profitability of SRE plantation systems.

Acknowledgements

Funding for this work was provided by Michigan State University's AgBioResearch and US Department of Energy awards numbered DE-EE-0000280 and DE-FC36-05GO85041. Technical assistance from Kile Zuidema, Paul Irving, and Jeremy Mason is gratefully acknowledged.

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[3] Site preparation @ $440/ha; Planting cost @ $0.20/cutting; 1st year weed control @ $350/ha; 2nd year weed control @ $350/ha; Annual management @ $35/ha; Harvesting and transportation @ $18/OD Mg; Revenue @ $66/OD Mg (mill gate); No fencing, irrigating, or fertilizing costs were incurred.

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2.3 STUDYING THE EFFECTS ON WOODY BIOMASS PRODUCTIVITY OF GENOTYPE-BY-ENVIRONMENT INTERACTIONS

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Abstract

There is great potential for the production and use of short rotation woody biomass for the sustainable production of biofuels, bioenergy and bioproducts in the US. The growth of perennial woody crops, such as shrub willow (*Salix* spp.), as a bioenergy feedstock offers significant advantages with respect to net energy ratio, soil conservation, nutrient management, biodiversity, and sustainable utilization of marginal agricultural land unsuitable for food crops. The deployment of perennial willow crops on marginal lands faces significant barriers, however, one of which is a lack of understanding of cultivar by site interactions that are essential for yield modeling. The NE Sun Grant Project NE 11-48 "Genetic Improvement for Yield and Establishment of Short Rotation Woody Biomass Crops on Marginal Lands" is taking a multi-institutional, multi-disciplinary approach to increase yields of shrub willow through genomics, breeding, and improving agronomic techniques. Survival and production data are being collected from a network of new and existing trials of willow clones across 10 states, including stem diameters, stem number, leaf area index, foliar nutrient concentrations, stem height, light use efficiency, stem biomass weight and composition, and site environmental characteristics. Climatic data is being compiled and soil samples analyzed, including elemental analysis and metagenomics (total soil DNA sequencing). Correlations of production, climatic and soil composition data with variety yields and stress responses will allow the development of a predictive model that will allow growers and extension educators to identify varieties that have the greatest yield potential for a particular site.

Keywords: yield; biomass; marginal land; stress response; varieties; genetic improvement; Salix

Background

The US Department of Energy (DOE) and the Biomass Research and Development Board have highlighted the unsustainable rise in demand for foreign petroleum in the US and have provided leadership in proposing a National Biofuels Action Plan (*http://www1.eere.energy.gov/biomass/pdfs/nbap.pdf*, accessed 1/31/2011) to meet a national goal of replacing 15% of gasoline usage with biofuels and to increase biofuels production in the U.S. to 36 billion gallons per year by 2022. In order to meet these goals in an environmentally sound and sustainable manner, there will need to be dramatic increases in the total production of biofuel feedstocks (Richard 2010).

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There is great potential for the production, aggregation and use of agriculturally-produced biomass other than corn grain as a feedstock for the production of biofuels in the US. The growth of perennial woody crops, including shrub willow (*Salix* spp.), as a feedstock crop for biofuels and bioproducts offers significant advantages with respect to net energy ratio, soil conservation, nutrient management, biodiversity, and utilization of marginal agricultural land, in addition to diversifying the feedstock commodities available to the biofuels industry (Verwijst 2001; Berg 2002; Dhondt *et al.* 2004; Volk *et al.* 2004; Dimitriou & Aronsson 2005; Volk *et al.* 2006; Dhondt *et al.* 2007). Shrub willow is a high-yielding perennial crop that can be grown on underutilized or marginal agricultural land, especially poorly drained sites unsuitable for food crops, and could contribute significantly to the mix of regionally optimized biomass commodity crops. Research conducted or directed over many years by the U.S. Department of Energy at Oak Ridge National Laboratory has concluded that willow has superior properties as a perennial energy crop for the Northeast and Midwest US (Tolbert & Schiller 1996; Tolbert & Wright 1998).

Shrub willow crops have a short harvest cycle, low incidence of pests or diseases in improved varieties, adaptability to a wide range of site conditions, high yield of biomass with low input of fertilizer, efficient recycling of nutrients in leaf litter, and great potential for genetic improvement. For over 30 years, academic research and development in New York (NY) and Canada has demonstrated the potential for shrub willow crops in North America, drawing from a wealth of study and experience in Sweden, the United Kingdom, and Denmark, where willow bioenergy crops are planted on over 20,000 ha (Kuzovkina *et al.* 2008). Sustained efforts in optimizing agronomic and management techniques, adapting planting and harvesting technology, and demonstrating conversion methods have led to commercial deployment of shrub willow crops on the rural agricultural landscape in NY since 2005, with over 300 ha in cultivation and dramatic expansion of commercial plantings being proposed to take advantage of over 675,000 ha of underutilized agricultural land in NY alone (Volk *et al.* 2006; Wojnar & Rutzke 2010).

Despite the numerous environmental and rural development benefits associated with shrub willow crops, these systems have not yet been widely adopted due to the current high cost to produce and deliver perennial energy crop biomass to end users (Buchholz & Volk 2011). The multi-disciplinary USDA Sun Grant research project "Genetic Improvement for Yield and Establishment of Short Rotation Woody Biomass Crops on Marginal Lands" is addressing biomass feedstock production, sustainability, and economic viability through development of high yielding, stress-tolerant cultivars of shrub willow bioenergy feedstock crops using advanced genomics-based breeding techniques. This goal is being approached through varietal performances across a range of marginal sites typical of the Northeast region. The research is fully integrated with education and extension to demonstrate improved agronomic practices and sustainability in support of commercial deployment.

Our goal is to develop short rotation shrub willow bioenergy crops with increased yields and reduced production costs, while simultaneously improving the environmental benefits associated with these systems. We are focusing on optimizing low input production on a range of sites across the NE Sun Grant Region, including marginal, degraded and idle lands, through an integration of genomics, breeding, field trials, and agronomy. Our specific objectives are to 1) Develop high-throughput, low cost molecular genetic/genomic tools for the selection of novel shrub willow cultivars with resistance to environmental stresses and increased biomass quality and yields; 2) Use the genomics tools to select new, improved varieties of shrub willow crops that display increased yields; 3) Establish a network of test sites and outreach activities that will lead to an expanded range of production, improved agronomic practices, and demonstrated sustainability of willow bioenergy crops across the Northeast region.

Breeding for Yield Improvements

Increases in yield achieved through genetic improvement will reduce the overall cost of producing willow biomass and will encourage wider adoption and long-term sustainability of the crop (Karp & Shield 2008). Economic modeling indicates that a 17% increase in yield (from 12 to 14 odt ha^{-1} yr^{-1}) increases the internal rate of return by 51% (Buchholz & Volk 2011). Willow breeding began in North America in the early 1980's at the University of Toronto in Ontario, Canada under the direction of L. Zsuffa (Zsuffa 1988) using *S. eriocephala*, *S. exigua*, *S. lucida*, *S. amygdaloides*, *S. bebbiana*, *S. pellita*, *S. petiolaris*, and *S. discolor*. They established species differences in plant biomass, moisture content and specific gravity (Mosseler *et al.* 1988) and laid the groundwork for use of interspecies hybridization (Mosseler & Papadopol 1989; Mosseler & Zsuffa 1989; Mosseler 1990).

Willow germplasm from Canada and Sweden grown in NY in the 1990s was significantly impacted by potato leaf hopper and rust, which led to the establishment of willow breeding program by L. Abrahamson, R. Kopp, and L. Smart in New York to develop varieties specifically selected for conditions in eastern North America using controlled pollinations (Kopp 2000; Kopp *et al.* 2001; Kopp *et al.* 2002a). A large and diverse breeding collection of willows was assembled with major collection efforts in 1995, 2000, and 2001 across portions of the Northeast and Midwest US (Kopp 2000; Kopp *et al.* 2001; Smart *et al.* 2005; Smart & Cameron 2008). Molecular marker demonstrated high levels of genetic diversity and heterozygosity among the natural populations of *S. purpurea* and *S. eriocephala* in NY, which was largely captured in the breeding populations (Kopp *et al.* 2002b; Lin *et al.* 2009). Heterosis in F_1 hybrids was also demonstrated (Phillips 2002; Cameron *et al.* 2008). By 2007, the willow collection contained over 700 accessions representing more than 20 species and species hybrids. More than 600 crosses were completed in Smart's breeding program and variety trials are being conducted across North America (Smart & Cameron 2008; Smart & Cameron 2011).

Genotype by Environmental Interactions

A key gap in our understanding of willow biomass production surrounds the genotype by environment interactions that can result in significant, systematic rank changes among elite varieties grown on different sites (Fig. 1) (Kiernan *et al.* 2003). This phenomenon has also been observed with poplar clonal trials (Riemenschneider *et al.* 2001; Zalesny *et al.* 2009; Bergante *et al.* 2010) and could be the result of a wide range of biotic and/or abiotic environmental factors that limit growth potential, such as soil structure and nutrient properties, precipitation, drainage, temperature range, local pest or disease pressures, weed competition, or presence/absence of symbiotic microorganisms. In order to evaluate genotype by environment interactions of commercial shrub willow cultivars, researchers at various institutions have established variety yield trials across a wide range of climate and soil conditions (Fig. 2).

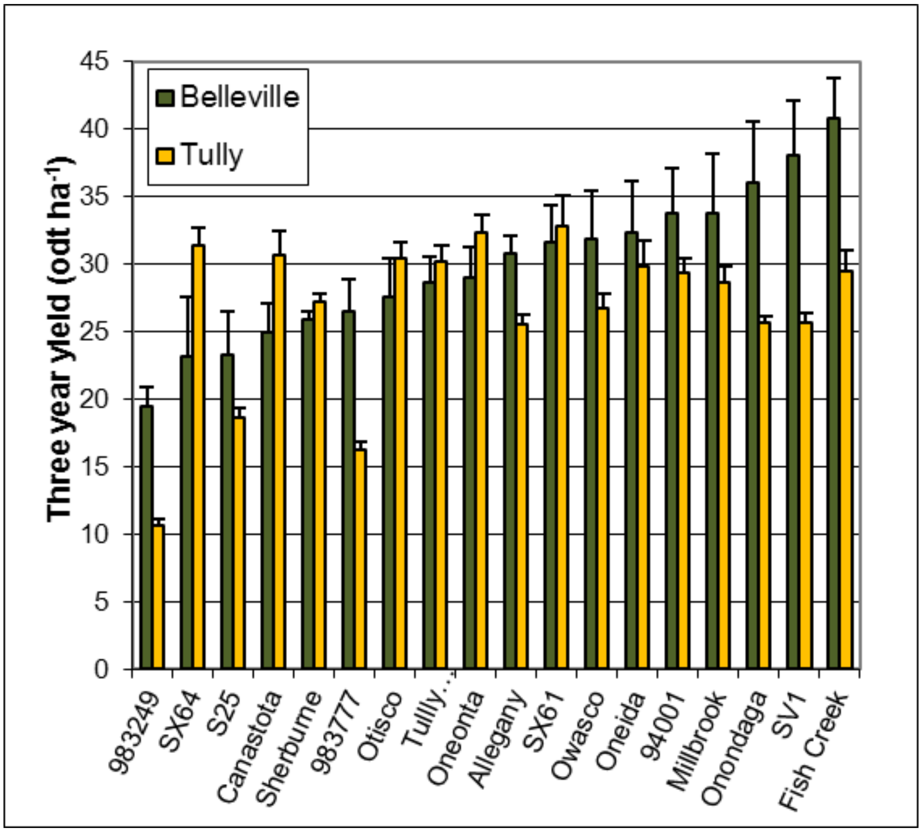

Fig. 1. Three-year first-rotation yields from two willow trials in NY.

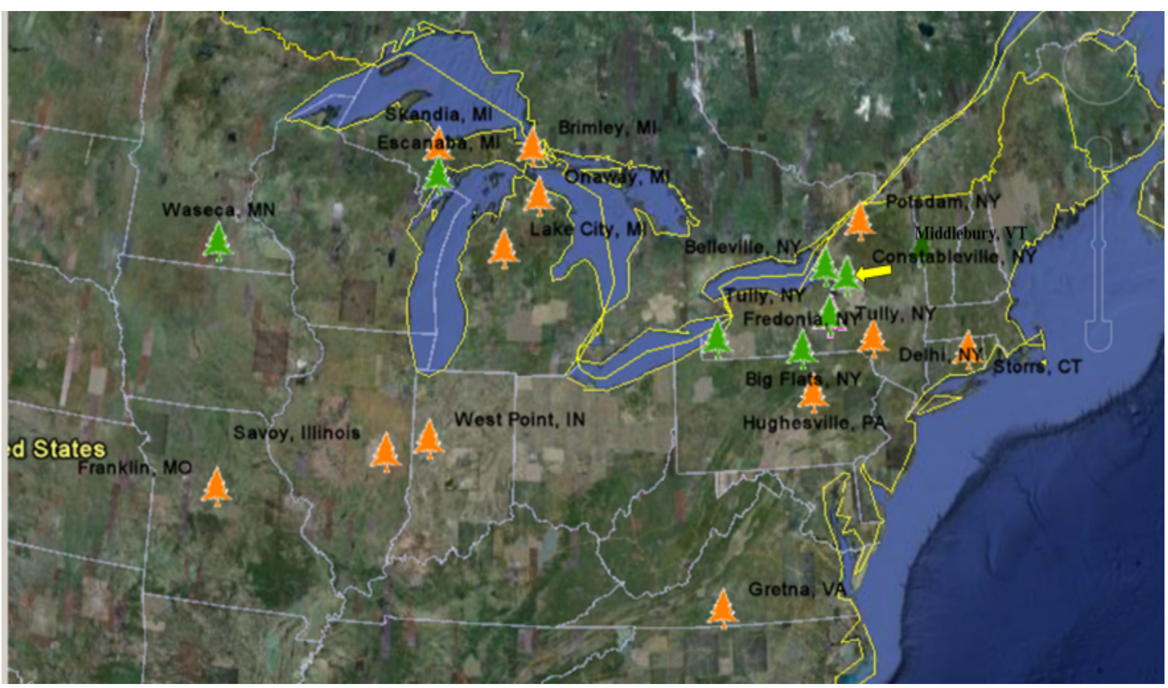

Fig. 2. Locations of field trials with current commercial willow cultivars.

One of the key tasks of this Sun Grant project is to fully analyze the site conditions at each trial and correlate those through multivariate statistical analysis with yield performance. This will allow us to develop a predictive tool based on specific site characteristics that can be used by practitioners (growers and biomass aggregators) to match willow genotypes to sites with confidence of their yield potential. By identifying the key site characteristics that limit productivity of specific varieties, we can identify parents in the breeding program that are likely to generate transgressive segregation for the traits that overcome those growth limitations, applying selection approaches that allow us to identify genotypes with improved overall yield due to plasticity to site conditions.

A new set of shrub willow genotypes bred between 2001 and 2005 is currently being tested and selected in paired 76-clone trials in Geneva and Tully, NY. A primary task of this Sun Grant project is to harvest these selection trials (76 clones, 24-plant plots, 3 reps per site, 2 sites), identify superior varieties based on weighted index selection criteria, determine genotype-by-environment interactions, and subsequently propagate cuttings for commercial scale-up and for demonstration trials.

Rapid yield enhancements through breeding of shrub willow can be accomplished through the use of genome-wide molecular markers as direct predictors of phenotypic responses, an approach referred to as "genomic selection" (Meuwissen *et al.* 2001). This approach has recently become feasible due to the advent of efficient genotyping and, recently, whole-genome sequencing technology (Heffner *et al.* 2009; Myles *et al.* 2009; Heffner *et al.* 2010; Meuwissen & Goddard 2010; Shepherd *et al.* 2010). This approach is currently being applied in *Eucalyptus* (Grattapaglia *et al.* 2009), oil palm (Wong & Bernardo 2008), and loblolly pine breeding programs on an experimental level (M. Kirst, personal communication). Using next generation sequencing technology, the genome of a diploid female purple osier willow (*Salix purpurea* L. 94006) is being completed at the US DOE Joint Genome Institute in a project lead by G. Tuskan (Oak Ridge National Lab), L. Smart (Cornell), and C. Town (JCVI). Once this assembly is completed in the next few months, it will be used in a major task of the proposed project to develop very low cost, high-throughput, and very dense markers for genetic mapping – both linkage mapping and association mapping – and genomic selection using genotyping by sequencing methods (Myles *et al.* 2010). This technology will provide a foundation for rapid selection, cutting the time needed to realize genetic gains in half relative to phenotypic selection (Heffner *et al.* 2009). Genome-wide selection capability will also vastly improve the determination of specific genotype-by-environment interactions during testing of shrub willow bioenergy cultivars.

Research Approach for Genotype-by-Environment Studies

Data from an existing network of yield trials of improved willow clones grown in 10 states (IN, IL, MI, MN, MO, NY, PA, SC, VA, VT) are being collected and compiled. In addition, this Sun Grant project is also establishing six new yield trials to demonstrate the yield potential across the Northeast region of new shrub willow varieties. The new trials are being established on marginal sites typical of those used for production of perennial bioenergy crops across the Northeast region. The marginal sites are characterized by factors such as poorly-drained soils, low pH soils, sandy and gravelly soils, mine spoils, and low fertility and/or abandoned agricultural land.

Survival and production data currently being collected include stem number and diameter for first-year post-coppice growth, which is a very good predictor of harvest-age yield measured at the end of the third growing season after coppice. Additional information on leaf area index, foliar nutrient concentrations, number of stems per stool, stem height, light use efficiency, and stem biomass composition are being collected according to established protocols (Tharakan *et al.* 2001; Tharakan *et al.* 2005; Serapiglia *et al.* 2008; Tharakan *et al.* 2008; Serapiglia *et al.* 2009) to identify characteristics predictive of yield according to associated environmental characteristics. Pest and disease surveys are being conducted at three seasonal time points each coinciding with the presence of key arthropod pests (including mites, potato leaf hopper, willow leaf beetle, Japanese beetle, leaf sawfly, stem sawfly, and poplar and willow borer) and the incidence of diseases (including *Melampsora* rusts, black canker, scab, *Leucostoma* canker, *Botryosphaeria* canker, and *Cytospora* canker). Climatic data are being compiled from local recording stations, including seasonal and annual rainfall, growing degree days, and minimum/maximum daily temperatures.

Poor soil fertility and lack of mycorrhizal symbionts can limit shrub willow establishment and productivity on marginal and abandoned sites. Thus soil samples are being collected from the sites and nutrient analysis of the soils is being conducted using a standardized chemical analysis protocols. Metagenomics (total soil DNA sequencing) is being used to characterize both mycorrhizal fungal symbionts and bacterial species composition at the yield trial sites (Martin & Nehls 2009). Correlations of soil microbiome composition and fertility with variety yields and stress responses will be evaluated and entered into the regional yield models.

Biomass yield, plant growth, soil, and climate data will be used to match willow genotypes with optimal growing conditions, identify site factors most limiting growth potential, and develop predictive yield models based on climate and soil conditions (Aylott *et al.* 2008). Identification of the biotic and abiotic factors most limiting growth potential provides the opportunity for feedback to the willow breeding program in guiding priorities for trait selection protocols and cross choices that generate transgressive segregation for those features that overcome growth barriers. The development of a predictive model will allow growers and extension educators to identify varieties that have the greatest yield potential for a particular biomass production site.

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2.4
SOIL CO2 EFFLUXES IN SHRUB WILLOW BIOMASS CROPS ALONG A 21-YEAR CHRONOSEQUENCE AS AFFECTED BY CONTINUOUS PRODUCTION AND CROP REMOVAL (TEAR-OUT)

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Abstract
Soil CO_2 efflux (F_c) is the primary pathway in the carbon (C) cycle through which the C stored in the belowground biomass is released back into the atmosphere. This study compares (a) soil CO_2 emission rates among 7, 14, 16, and 21-yr old willows and (b) between willows that were harvested and allowed to regrow (continuous production [CP]) and willows that were harvested, killed, and then ground into the soil (tear-out [TO]). Soil CO_2 emission rates, which were measured continuously for two years, showed no significant differences among the four ages ($p = 0.32$), and there was no interaction with the applied treatments ($p=0.98$). However, the mean annual soil CO_2 emission rates of CP (2.67μmol m^{-2} s^{-1}) were significantly higher than TO (2.22 μmol m^{-2} s^{-1}) ($p=0.04$). Nonzero soil CO_2 emission rates occurred year-round with highest emission during summer (July-Sept.) and lowest during winter (Jan.-Mar.). The annual cumulative soil CO_2 production ranged from 29 to 36 Mg ha^{-1} yr^{-1} for CP and 25 to 29 Mg ha^{-1} yr^{-1} for TO. F_c rates were strongly associated with soil temperature, but not with soil moisture.

Keywords: carbon cycle, cumulative soil CO_2 production, soil temperature, soil moisture

Introduction
Interest in the use of short rotation woody crops (SRWC) for the production of bioenergy, biofuels, and other bioproducts has been growing rapidly due to changes in fossil fuel prices, concerns on energy security and global warming (Nikièma et al. 2012; USDOE 2011). In northeastern and midwestern US and in Europe, shrub willow biomass crops (SWBC) are being promoted as a source of biomass feedstock (Keoleian & Volk 2005). In central and northern New York, where willow biomass research has been conducted for over 25 years, the results are being translated into a commercial scale production (over 1,400 ha) as part of the USDA Biomass Crop Assistance Program (BCAP) (www.fsa.usda.gov).

One of the environmental concerns related to SWBC production system is its potential greenhouse gas (GHG) contribution to global warming. Previous life cycle analysis (LCA) demonstrated that SWBC is a low carbon fuel source (Heller et al. 2003). But, soil CO_2 efflux (F_c) was not indicated in this analysis due to lack of data at the time of assessments. Since F_c accounts for a significant release of CO_2 emissions into the atmosphere (Schlesinger & Andrews 2000), it is important to understand this part of the C cycle in willow for an accurate assessment of the contribution of this system to global warming.

The current study assessed F_c rates among SWBC fields in production for 7, 14, 16, and 21 years for willow plots under two conditions: (1) harvested and allowed to regrow (continuous production [CP]), and (2) harvesting followed by spraying with herbicide the following spring and then grinding the stools into the top 8 – 15 cm of soil (tear-out [TO]). We hypothesized that F_c rates differed significantly among crop ages (due to the increasing sizes of below-and aboveground stools and roots) and between the two treatments.

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Materials and Methods

This study utilized four SWBC fields planted with clone SV1 (*Salix x dasyclados*). Three of these fields (ages 7, 16, and 21) were located in Tully (42° 47' 30" N and 76° 07' 30"W), NY, and the fourth (age 14) was located in Lafayette (42° 52' 42" N and 76° 06' 45 W), NY. The four SWBC fields were planted using 25 cm unrooted cuttings, spaced 1.5 m between two double rows and 0.6 m within double rows for an initial plant density of about 15,400 plants ha^{-1}. The 20 year old plot was planted with 0.9 m between two single rows, and 0.6 m within rows for an initial plant density of 36,960 plants ha^{-1} (Abegbidi et al. 2001). When the harvest treatments were applied, all the plots were two years old aboveground.

This study was designed as a split-split plot factorial with age as a whole plot factor, treatment (CP, TO) as split-plot factor, and time as a split-split plot factor. The four different sites, representing four ages, were divided and treatment was randomly assigned. For each site, four sample double row plots (5m X 4m) were established for each treatment. The stems in all four SWBC fields were harvested simultaneously on May 2010, leaving a stool (stump) ca. 10-15 cm above the soil surface. Following harvest, two treatments were applied: (1) CP – continuous production with the coppice (buds) emerging from aboveground stool allowed to grow for one rotation cycle (3 years), and (2) TO – tear out with herbicide (glyphosate at 2.2 kg ai ha^{-1}) applied to coppice regrowth and subsequent grinding of the stool/root system and incorporation into the soil using a Fecon track mulcher (FTX 350).

Total F_c (*i.e.* heterothropic and autothropic respiration) was measured continuously for two years (June 2010-May 2012), using an automated soil CO_2 flux system (LI-8100A) (Li-Cor, NE), consisting of LI-8100, multiplexer (LI-8150), long-term chamber (LI-8100-104), survey chamber (LI-8100A-102), and auxiliary sensors (LI-8100-663). Two methods of F_c measurements were employed: (a) long term chamber (LTC) measurements – designed to capture temporal variation of F_c, and (b) survey chamber (SC) measurements (point-in-time) –designed to capture spatial variation of F_c. SC measurements were carried out for two years; weekly measurements from June-November 2010 and April-November 2011, and every four weeks from December 2010-March 2011 and December 2011-March 2012 (winter). CO_2 efflux measurements using SC method were carried out between 10:00 and 18:00 h. Similarly, LTC measurements were done from August 2010 to December 2010 and March 2011-December 2011. No LTC measurements were conducted during winter (Jan-Mar) because LTC machines are not designed to operate under heavy snow conditions. Four LTCs were used: two chambers for each treatment, which were randomly placed within double and within two double rows. Observations were made at 30 minute intervals for 4 consecutive days at each site. In both methods, F_c measurements protocol were: 30 sec equilibration/deadband, 15 second air purge time, and 120 seconds observation length, which are based on Li-Cor recommendation for low to moderate soil F_c (LI-COR 2007).

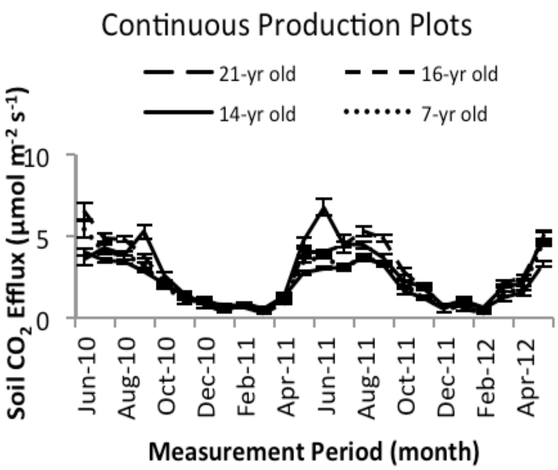
LTC and SC chambers were mounted on soil chamber collars, which were constructed from a cylindrical polyvinyl chloride pipe (PVC) (20 cm dia x 10 cm long). Soil chamber collars were inserted approximately 4 cm depth to minimize root shearing, leaving about 6 cm long soil chamber collars above the soil surface. Soil temperature and soil moisture were measured simultaneously with F_c at 5 cm depth using soil temperature and soil moisture probes (Decagon Devices), which are connected to auxiliary sensors attached to LI-8100A. In this paper, we showed only the measured soil temperature and soil moisture using the LTC method.

For SC method, we calculated the cumulative F_c per month by multiplying the mean F_c per month (*i.e* calculated from weekly SC measurements) by 30 days. Similarly, for the LTC method, we calculated the mean F_c per month by multiplying the average of eight days continuous measurements (*i.e* two 4 consecutive days/month) in each SWBC field by 30 days. In this paper, the term cumulative F_c per month refers to the mean of calculated cumulative F_c's per month from both SC and LTC methods (*i.e* [F_c of SC + F_c of LTC]/2). Cumulative F_c per year was calculated as the sum of 12 months cumulative F_c, from January to December. In this paper, the cumulative F_c per year was based on two years measurements (*i.e* [1st yr cumulative F_c + 2nd cumulative F_c]/2). Analysis of variance (ANOVA) was used to assess the effects of age, treatment, and their interaction on F_c. In all analyses, a probability level of $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using SAS statistical package (SAS 9.2).

Results and Discussion

Seasonal soil CO_2 efflux

F_c was measured year-round with highest emission during summer (July-Sept.) and lowest during winter (Jan.-Mar.). In fall (Oct.-Dec.), F_c rates showed a descending pattern, but reversed in spring (Apr.-June). In general, seasonal contributions to annual cumulative F_c averaged across ages and treatments were: 5.7 to 6.9% for winter, 33.5 to 34.4% for spring, 42.6 to 47.7% for summer, and fall 12.4 to 16.1% for fall. The mean F_c rates for winter, spring, summer, and fall seasons were: CP -0.7, 3.1, 4.1, and 1.5 µmol m^{-2} s^{-1}; TO -0.4, 2.6, 3.9, and 0.9 µmol m^{-2} s^{-1}, respectively (Fig. 1). The growing season (May, June, July, Aug) contribution to total cumulative F_c (*i.e.* 12 months [Jan. - Dec.]), averaged across ages, was 65% in CP and 60% in TO. No significant differences in F_c rates were detected among the four ages (P=0.3296), and treatment did not interact with age (P=0.9487). However, the mean annual F_c rates of CP (2.67µmol m^{-2} s^{-1}) was 20% higher than the TO (2.22 µmol m^{-2} s^{-1}) (P =0.0499).



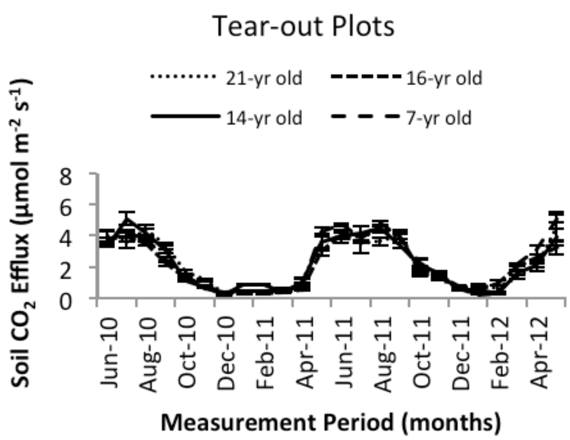


Figure 1: Mean monthly soil CO_2 efflux in continuous production and tear-out plots across the four SWBC fields. Vertical bars represent standard error.

High F_c rates during summer are related to high soil temperature and root respiration during this period (Janssens et al. 2001; Borken et al. 2006). High fine root biomass production during summer also enhances F_c rates (Arevalo et al. 2010). On the other hand, the snow cover and low soil temperature during winter did not completely stop soil CO_2 emissions. However, F_c rates during this period were very low (i.e. <0.5 µmol s^{-1} m^{-2}). Continuous F_c at very low rates, despite temperatures below the -7°C threshold for respiration, was reported for boreal forest (Winston et al. 1995) and also in northern Russian ecosystems (Zimov et al. 1996). Some authors attributed the continuous efflux during winter season to microbial activities and root respiration. A soil respiration study in alpine and sub-alpine forest in Wyoming revealed that CO_2 efflux did not cease within approx 1°C soil temperature due to microbial activity (Sommerfield et al. 1993). In the current study, mean soil temperature under deep snow, measured from January to March, ranged from 0.5°C to 1°C, for 5 and 10 cm depths, respectively. Although plants are dormant during winter, root metabolism continues; some authors estimated a contribution of about <5% (Coxson and Parkinson 1987).

Cumulative soil CO_2 efflux across ages and between treatments

The annual cumulative F_c ranged from 29.1 to 35.4 Mg CO_2 ha^{-1} yr^{-1} for CP and from 25.8 to 29.3 Mg CO_2 ha^{-1} yr^{-1} for TO (Table 1). These values agree well with Nikièma et al. (2012) estimate of 30.1 Mg $ha^{-1}yr^{-1}$ cumulative soil respiration rates of a one-year old willow field. For hybrid poplar, Arevalo et al. (2010) reported an average cumulative F_c over two growing seasons (June to November), ranging from 502 to 551 g C m^{-2} (ca. 18 to 20 Mg CO_2 eq. ha^{-1} yr^{-1}). These values are within the range of cumulative F_c (17.0 to 21.9 Mg CO_2 ha^{-1}) that we measured over the same period (Jun. –Nov.). The importance of measuring cumulative F_c is apparent because the total values in this study were 29 to 97% greater than in Arevalo et al. (2010) where non growing season measurements were not taken. Although the foregoing cumulative F_c values are large, an associated study found that annual cumulative C sequestration in fine roots and foliage offset soil CO_2 emissions in SWBC (Pacaldo et al. 2011). In fact, in another associated study, we found that the entire SWBC production system has a net GHG potential of -42.9 Mg CO_2 eqv ha^{-1} at the end of 22 years (Pacaldo et al. 2012).

Table 1: Cumulative soil CO_2 emissions between continuous production and tear-out treatments across the four age classes measured over two years. Standard errors are in parenthesis.

SWBC Age	Cumulative CO_2 emission (Mg CO_2 ha^{-1} yr^{-1})					
	Continuous Production Treatment			Tear-out Treatment		
	Year 1	Year 2	Mean	Year 1	Year 2	Mean
7	30.7(±1.9)	27.5(±1.4)	29.1(±1.7)	26.4(±1.6)	31.7(±1.7)	29.1(±1.7)
14	33.7(±2.9)	37.3(±2.9)	35.5(±2.9)	28.0(±2.4)	30.6(±2.9)	29.3(±2.7)
16	35.4(±2.2)	35.1(±1.8)	35.3(±2.0)	23.0(±1.8)	28.6(±1.6)	25.8(±1.7)
21	27.6(±1.2)	26.7(±1.8)	27.2(±1.5)	27.6(±2.7)	27.9(±2.8)	27.8(±2.8)

Climatic factors that influence soil CO_2 efflux rates

The seasonal pattern of F_c rates was similar to that of soil temperature, but was weakly related to soil moisture (Fig. 2). The strong relationship between F_c rates and soil temperature corroborates some previous reports (Borken et al. 2006; Arevalo et al. 2010). F_c rates increase at high temperature, which stimulates microbial activity (Winkler et al. 1996) and enhances root respiration (Arevalo et al. 2010). In contrast, F_c and soil moisture showed weak relationship, which could be attributed to a relatively small range of soil moisture content (Lee et al. 2007), with only small period of limited moisture stress. Effects of soil moisture on F_c are distinct only when the soil is too dry or too wet (Davidson et al. 1998). However, it is recognized that soil moisture and F_c might have good correlation, but this was not captured in our measurements due to lag effects. The soil moisture changes slowly while F_c changes rapidly (Fig 2).

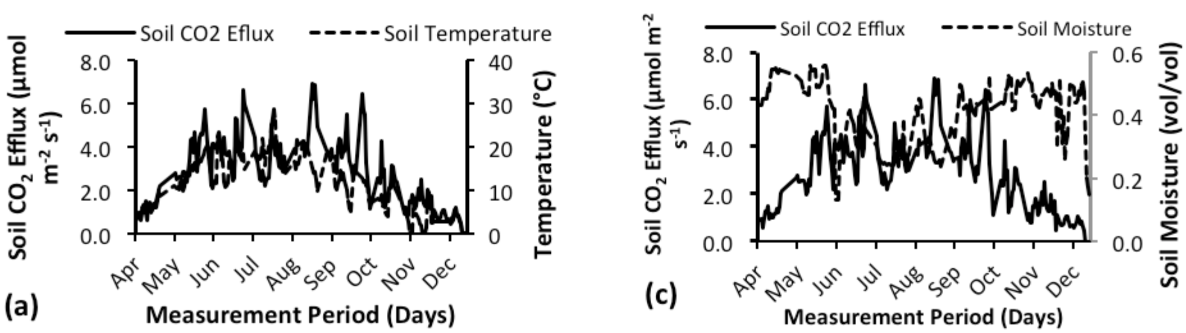


Figure 2. Relationship between soil CO_2 efflux and (a) soil temperature (b) soil moisture humidity, measured continuously at 30 minutes interval over nine months.

Conclusion

The result of this study did not support our hypothesis that as the crop ages, F_c rates also increase. Despite the multiple harvests and the aging root systems, F_c rates remained consistent across different ages. However, mean F_c rates measured over two years in tear-out plots where the willow was killed and the stool ground into the soil were significantly lower than for continuous production plots. Soil CO_2 flux occurred year round with high rates of emissions during summer and low emissions rates during winter. In this study, soil temperature strongly influenced CO_2 flux, whereas soil moisture did not have a major influence, likely due to the absence of moisture stress during the measurement period and lag effects.

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2.5
BELOWGROUND COMPETITIVE INTERACTIONS WITHIN PINE-SWITCHGRASS CO-CULTURE

Kurt Krapfl[1,] Scott Roberts[1], Jeff Hatten[1]*

Abstract

This study examines the capacity for combining the long-term productivity of a commercially important timber species, loblolly pine (*Pinus taeda* L.), with the annual yield of a dedicated bioenergy crop such as switchgrass (*Panicum virgatum* L.). Growing loblolly pine and switchgrass in co-culture could be a viable means of increasing the production potential of lands throughout the southern United States. However, co-culture production systems can inherently introduce the potential for strong competitive interactions. These interactions, occurring both above and belowground, may be especially important when incorporating fast growing herbaceous species between rows of newly established pine seedlings. This study was established in 2011 to examine how competition affects soil moisture availability and foliar nitrogen content within two newly established loblolly pine-switchgrass co-culture production systems in northeastern Mississippi. Volumetric water content within soils significantly varied by treatment, distance from tree row, and sampling depth. Foliar nitrogen content of loblolly pine significantly varied by treatment at one of our sites. Similarly, switchgrass foliar nitrogen content significantly varied by treatment at one site, and approached significance at the other. Switchgrass foliar nitrogen content was also significantly greater near non-vegetated buffer zones than in alleyways. Results of this study suggest establishing non-vegetated buffer zones around tree rows of newly established loblolly pine-switchgrass co-cultures may effectively increase soil moisture and foliar nutrient availabilities.

Keywords: belowground competition, co-culture, loblolly pine, switchgrass, volumetric water content, foliar nitrogen content

Introduction

This study investigates the capacity for combining the long-term productivity of a commercially important timber species, loblolly pine (*Pinus taeda* L.), with the annual yield of a dedicated bioenergy crop such as switchgrass (*Panicum virgatum* L.). Optimal growth of loblolly pine occurs on deep, well drained soils with high available moisture, and slightly acidic pH (Baker and Langdon 1990). Switchgrass is native to tallgrass prairies throughout most of the United States and grows well across a wide spectrum of sites (Parrish and Fike 2005). However, switchgrass makes its best growth on deep, well drained soils with moderate to high nutrient availabilities and pH ranges between 5.5 and 7. Incorporating an annually harvested biomass crop with a long-lived tree species (defined here as "co-culture") could hold negative implications for the productivity and sustainability of loblolly pine-switchgrass co-culture on marginal soils. On the contrary, the co-culture concept may provide growth advantages by means of improved soil resource utilization (Jose 2009). Furthermore, the introduction of a deep rooted, perennial grass species to lands previously cleared for agriculture may provide environmental advantages such as improved wildlife habitat and carbon sequestration while simultaneously providing an annual source of revenue to the landowner along with an intermittent revenue stream from the trees. The overall objective of this research is to examine how interspecific competition in a co-culture setting affects soil moisture availability and foliar nitrogen content.

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Materials and Methods

Study areas

Two locations in northeastern Mississippi were utilized to establish loblolly pine-switchgrass in co-culture. The first location, Pontotoc Ridge-Flatwoods Branch Experiment Station (34°07'N, 88°59'W), is approximately 1.6 ha in size and at 124 m elevation. The site has a history of agricultural use and likely residual fertility. Site preparation at Pontotoc began in May 2009 with conventional disking and harrow. Switchgrass was first sown in May 2009 by drill seeding at a rate of 6.7 kg ha^{-1} and additional switchgrass seed was sown and cultipacked in September 2009, May 2010, and July 2010 at rates of 6.7, 5.0, and 6.7 kg ha^{-1}, respectively. Loblolly pine rows were ripped to a depth of 40 cm in November 2010 and pines were planted in March 2011.

The second site is on the Noxubee Unit of the MSU John W. Starr Memorial Forest, located approximately 24 km south of the Mississippi State University campus (33°16'N, 88°53'W). This site is approximately 2 ha in size and is located at 88 m in elevation on a terrace of the Noxubee River in northern Winston County. This site has a history of forest use but has been maintained for several decades as a mowed pasture. Site preparation at Starr Forest consisted of a prescribed burn in March 2010 followed by liming in April 2010. Switchgrass was drill seeded in April 2010. Pine rows were subsoiled to a depth of 40 cm and loblolly pines were planted in March 2011.

Experimental design

This study employs a randomized complete block design utilizing a low density planting configuration (1.5 m x 9.0 m spacing) of loblolly pine planted in rows and switchgrass sown in the alleys. Treatments include: 1) pine only, 2) switchgrass only, 3) pine planted directly into switchgrass, 4) pine planted into a 1.2 m non-vegetated zone (0.6 m on either side of the pines), and 5) pine planted into a 2.4 m non-vegetated zone (1.2 m on either side of the pines). Non-vegetated zones were established and maintained mechanically and with herbicides. Four replicates at each site were utilized for monitoring volumetric soil moisture and foliar nitrogen content. Volumetric soil moisture was measured at 7.5 and 20 cm depths at four distances in proximity to the tree row: 0 m (within the tree row), 0.6 m, 1.2 m, and 2.4 m. Volumetric water content values at 20 cm were transformed to account only for VWC between 7.5 and 20 cm. Loblolly pine foliar nitrogen was measured by sampling and compositing several needles from each tree within each plot. Switchgrass foliar N was assessed by collecting and compositing several new growth grass blades from areas bordering the non-vegetated buffers (competitive edges) and in the alleys (2.4 m from tree rows).

Results and Discussion

Volumetric water content

Volumetric water content in soils at both sites significantly varied by treatment, depth, and distance from the tree row. However, significant interaction effects between treatment and sampling distance from tree row ($p < 0.0001$, Figure 1) and treatment and depth of sampling ($p < 0.05$, Figure 2), indicated differences in VWC significantly varied among treatments. Mechanisms behind the treatment by distance from tree row interaction were primarily due to the treatments themselves, as areas kept non-vegetated for treatment differences generally had greater VWC than areas with switchgrass or pine coverage. The significant treatment by depth interaction at Pontotoc demonstrates differences in VWC were only noticeable at depth and were directly related to management intensity. The treatment by depth interaction at Starr follows the same general trends as Pontotoc, but at Starr differences in VWC were found at both sampling depths. A likely cause of the discrepancy in VWC by depth between the two sites is vegetative productivity. Switchgrass yields in the 2011 harvest at Starr were less than one-quarter of the switchgrass yields at Pontotoc and switchgrass establishment at Starr was patchy and mixed with a variety of grassland species while Pontotoc had excellent establishment and formed a nearly pure monoculture.

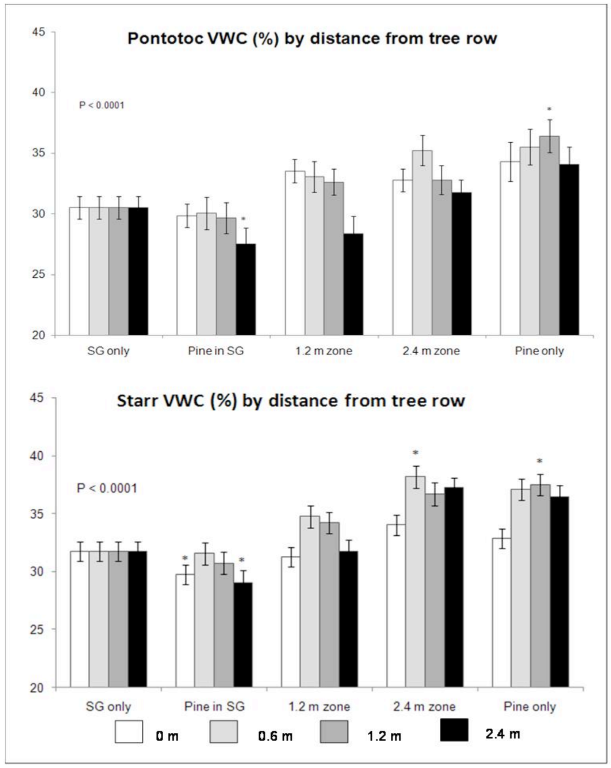

Figure 1. Volumetric water content (VWC) at the Pontotoc and Starr sites by distance from tree row. Asterisks indicate significant differences in VWC according to Tukeys HSD.

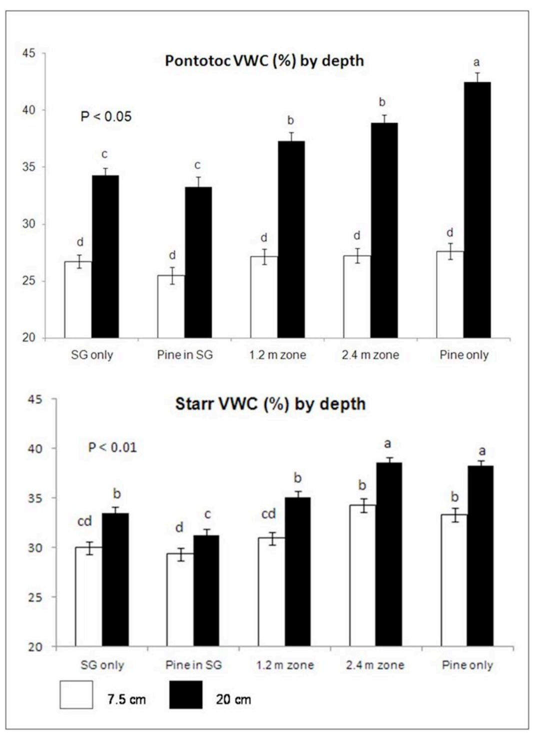

Figure 2. Volumetric water content (VWC) at the Pontotoc and Starr sites by sampling depth. Letters indicate significant differences in VWC according to Tukeys HSD.

Foliar nutrients

Loblolly pine foliar N significantly varied by treatment at Pontotoc ($p < 0.0001$). However, differences in loblolly pine foliar N at Starr were not statistically significant ($p = 0.66$). The lack of significant differences in foliar N at Starr in comparison to Pontotoc were most likely due to the lack of competitive pressures exerted by switchgrass at Starr and relatively poor switchgrass establishment. These differences may also have been due to a relative lack of inherent site fertility at Starr and less intensive site preparation techniques prior to study establishment.

Switchgrass foliar N significantly varied by treatment at Starr and approached significance at Pontotoc. Similarly, switchgrass foliar N significantly varied based upon competitive intensity at Starr but did not significantly vary at Pontotoc. There was a significant treatment by competition interaction effect at Starr ($p < 0.0001$) resulting from significantly greater switchgrass foliar N near non-vegetated treatment zones when buffers were present compared to foliar N measurements taken in alleys. The relatively low site fertility of Starr may have created larger demands for soil N and a greater foliar nutrient treatment response compared to Pontotoc. These results suggest that non-vegetated zones may effectively contribute to increased soil N availability, especially on marginal sites.

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2.6
BUILDING A BETTER BIOMASS ECOSYSTEM: COTTONWOOD-SWITCHGRASS AGROFORESTS ON MARGINAL LAND

Matthew Pelkki[1], Hal Liechty[1], Mike Blazier[2], Don White, Jr.[1], Chuck West[3]

Bioenergy markets may provide revenue to landowners on marginal lands in the Lower Mississippi Alluvial Valley (LMAV) where crop returns are low due to low fertility or risk. Cottonwood and switchgrass are native to this region, grow on a wide range of soils, and produce substantial amounts of biomass. Combining these two species in agroforests will provide both annual and periodic harvests of biomass along with valuable ecosystem services such as nutrient retention and wildlife habitat. Four levels of cottonwood and switchgrass cover were established in agroforests on marginal soils at three sites in in the LMAV in Arkansas and Louisiana in the spring of 2009. In 2011 switchgrass and cottonwood growth at the most productive sites were respectively 16.2 and 4.6 oven-dry Mg/ha. Cottonwood production has doubled each growing season and could accumulate as much as 18 Mg/ha in the fourth growing season and 34 Mg/ha on a 5-year rotation. Small mammal abundance and diversity were significantly greater in switchgrass and cottonwood agroforests than in control treatments planted to a soybean-grain sorghum rotation. Nitrate concentrations in soil water in the agroforest treatments were generally lower than those in control plots. Initial results suggest that substantial biomass production from cottonwood and switchgrass can occur on marginal soils but production differs significantly among soils. Cottonwood is very sensitive to common herbicide treatments applied to rice fields. Where establishment and growth is adequate, ecosystem services are enhanced by cottonwood and switchgrass agroforests.

Keywords: carbon sequestration, ecosystem services, short rotation woody crops

Introduction

The growing demand for renewable energy is driving the development of dedicated agricultural and forestry systems producing biomass for energy. In the US, renewable energy represented 8% of all production, and biomass was 53% of all renewalbe energy production in 2010. (US Energy Informaiton Administation 2011). The development of second-generation conversion technologies transforming cellulosic feedstocks to transportation fuels and other blendable energy commodities (de Wit et al. 2010, Londo et al. 2010) will further the demand for biomass in the US which will increase the value of these crops for farmers and producers.

The Lower Mississippi Alluvial Valley (LMAV) region of the Southern US has a high potential for cellulosic biomass production due to its long growing season and well-developed agricultural industry (Trip et al. 2009). The transportation and energy distribution systems within this region provide a suitable logistical infrastructure for the delivery of the raw and processed feedstocks, further enhancing the fitness of the region for dedicated biomass for energy production.

To avoid a “food vs. fuels” conflict, we propose to establish bioenergy crops on marginal soils where nutrient and/or moisture constraints make them poorly suited for food crops. We also propose to develop a biomass production system that will provide conservation benefits and valuable ecosystems services. To this end, we are establishing two native species, cottonwood (*Populus deltoides*) and switchgrass (*Panicum virgatum*) on marginal soils in the LMAV. We will assess how these crops might be grown in

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monocultures or in alley cropped agroforests to provide biomass for energy, wildlife habitat, and soil nutrient retention. Agroforests may not only enhance the ecosystems services of these two crops but also economically benefit landowners by diversifying the rotation length and markets for their bioenergy crops.

Methods

This study was established in 2009 at three locations; the (1) UA Pine Tree Branch Station (PTBS) near Colt, AR, (2) the UA Southeast Research and Extension Center (SREC) in Rohwer, AR, and (3) the Stephenson Farm (SF) near Archibald, LA (Figure 1). Plots were subsoiled to a 60 cm depth two months prior to planting at the two Arkansas sites, while the SF site was planted with a machine planter that created a trench approximately 44 cm in depth at the time of planting. Pre-planting herbicides included pendimethalin (2 qt./ac) and imazaquin (4 oz./ac) at PTBS and SREC, and sulfometuron methyl (1 oz./ac) at SF.

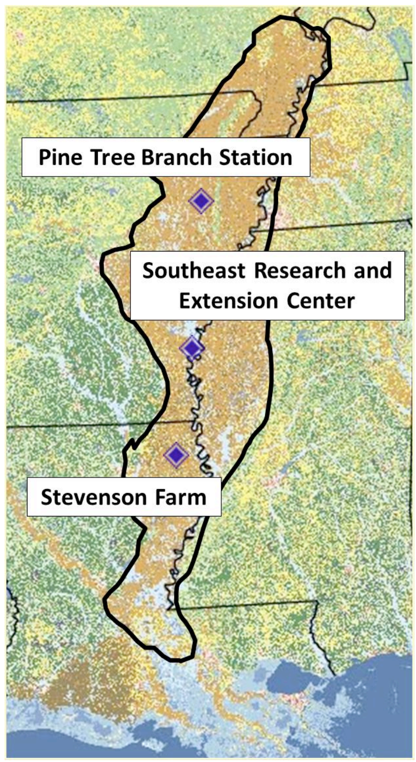


Figure 1. Location of three bioenergy agroforest study sites, UA Pine Tree Branch Station (PTBS), UA Southeast Research and Extension Center (SREC), and the Stephenson Farm (SF) within the Lower Mississippi Alluvial Valley of the Southern US.

Five different cropping treatments were established in replicate plots on marginal soils at each location in March of 2009. The five cropping treatments were 1) 100% cottonwood (CW), 2) 100% switchgrass (SG), 3) an alley cropped agroforest with 30m switchgrass alleys and 15m cottonwood alleys, (SGCW), 4) an alley cropped agroforest with 30m cottonwood alleys and 15m switchgrass alleys (CWSG), and 5) a conventional soybean-grain sorghum rotation for marginal soils in the LMAV (SGSR), which acted as a control treatment for the study. The switchgrass variety used was Alamo, seeded at a rate of 11.2 kg ha^{-1}. Two known cottonwood genomes (ST-66 and S7C20) and nursery-run mixture (Mixed) from the Louisiana Department of Agriculture and Forestry nursery in Monroe, LA were planted on 1.2 x 1.8m spacing, 4485 trees/ha. Post planting competition control was controlled using a variety of herbicides as necessary at each site. Each site also required one aerial application of imidacloprid (Provado 8 oz. /ac) to control cottonwood leaf beetle (*Chrysomela scripta* Fabricius). Trees were fertilized by banding 35.8 kg/ha of ammonium nitrate during the spring of the year following establishment. Establishment and maintenance costs for trees averaged $2200/ha and $250/ha for switchgrass. Basal diameter, diameter at breast height, and tree heights were measured annually. Biomass yields were calculated using individual tree dimensions and the equations by Jenkins et al. (2004).

Following the first growing season after establishment, the switchgrass was mowed but not removed from the plot. Yields were determined in the fall of the second and third growing seasons. Switchgrass was harvested and baled and removed from each plot following yield determinations.

The soybean-grain sorghum rotation consisted of two consecutive years of soybean followed by one year of grain sorghum. Since two of the sites (SREC and PTBS) had been planted to soybeans prior to study initiation, soybeans were planted the first and third year of the study, and grain sorghum the second year. The soybean and grain-sorghum were planted with varieties and methods commonly recommended for the soil and climate of each specific location. Herbicide, pesticides, and fertilizer were utilized as dictated by each location and climate. Grain yields as well as harvesting residue measurements were made at plant maturity using plot harvesters and manual collection methods. After sampling, grains were then harvested according to common practice in the region.

Small mammals were trapped using 36 traps baited with oatmeal within a 75 x 75-m trapping grid located in the interior of one 90 x 90-m plot from each treatment at each study site through four collection seasons (February, April, June-July, October-November) in 2011. During the winter traps were supplied with a small piece of cotton to aid the captured animal in heat retention. Trapping occurred during five consecutive nights during each season for a total of 720 trapping nights for each treatment.

Each individual collected was identified to species, with the exception of *Peromyscus* spp. and *Reithrodontomys* spp. which were identified to genus, using defining body characteristics (i.e., pelage). After identification the animals were then weighed to the nearest gram using a Pesola spring scale. Sex was determined based on urogenital distance and the presence/absence of gonads. Finally, age class (adult or juvenile) was determined based on weight and other physical characteristics. Monel, self-piercing, metal ear tags were used to uniquely identify (mark) each captured individual. All individuals were released at the site of capture.

Population composition and species diversity were determined independently for each site, treatment, and season. Shannon's diversity index (Shannon 1948), total number of individual's captured per 100 trap nights, sex/age distributions, and proportion of captures by habitat type were calculated for each treatment during each season at each site. Because sprung traps and incidental captures were minimal throughout the course of trapping, captures per 100 trap nights were simply calculated as total number of captures divided by the appropriate number of 100 trap nights.

Soil water chemistry was monitored using four tension lysimeters installed to a depth of 30 cm in each plot assigned to the SG, CW, or SGSR cropping treatments. A tension of 45 kPa was established in each lysimeter at the initiation of each soil water collection period. Soil water was collected once every two weeks starting in January 3, 2012 and ending June 20, 2012. Water was composited from the four lysimeters at a plot and analyzed for dissolved NO3-N, NH4-N, organic N, total N, and total P.

Results and Discussion

Biomass production

Cottonwood biomass accumulation was substantially higher at the SREC site than other sites (Table 1), but for different reasons. Survival of cottonwood clones at the SF site in Louisiana averaged 28-31% for the three varieties because of severe grass competition and drought in the first two growing seasons. Tree survival was much better at the PTBS site, averaging 63-64% by variety, but there, seasonal damage from rice herbicides in nearby fields killed terminal growth each year in May-June and in response to the herbicide damage the trees developed a very branchy, shrub-like form. At the SREC, tree survival averaged 78 to 86%, and tree height and diameter growth were also greatest, resulting in significantly better biomass accumulation.

Table 1. Third year cottonwood, switchgrass, and soybean biomass production at three agroforestry sites within the Lower Mississippi Alluvial Valley.

Cottonwood Clonal Variety (oven-dry kg/ha)			
	S7C20	ST-66	Mixed
PTBC[1]	1114[a]	776[a]	798[a]
SREC[2]	6974[b]	6779[b]	8087[b]
SF[3]	634[a]	387[a]	679[a]
Switchgrass (oven-dry kg/ha)			
PTBC	16,200[c]		
SREC	0.0		
SF	6,941[b]		
Soybean (green kg/ha)			
	Grain	Trash	
PTBC	2186[b]	2407[b]	
SREC	1886[b]	3233[b]	
SF	1010[a]	1349[a]	

Values with different alphabetical superscripts are different at the 5% level of significance.

1 – Pine Tree Branch Station, near Colt, Arkansas
2 – Southeast Research and Extension Center, near Rohwer, Arkansas
3 – Stevenson Farm, near Archibald, Louisiana

Switchgrass production (Table 1) was best at the PTBS and annual production in year three of the switchgrass stand was more than double the best 3-year accumulation of cottonwood treatments. Switchgrass establishment failed repeatedly at the SREC site due to the high clay content of the soils. During the mild winter of 2011-2012 an adequate stand of switchgrass was established, indicating that unique planting strategies are necessary on heavy-clay sites.

Biomass production from the control plots in 2011 (Table 1) clearly indicated the marginal nature of the three sites. Grain yields ranged from 6.8 bu./ac to 14.7 bu./ac; typical soybean yields for Arkansas are greater than 30 bu./ac (Coats and Ashlock, 2011).

While cottonwood biomass accumulation in year three is only half that of switchgrass annual production, the growth and development of cottonwood biomass accumulation at Rohwer over three growing seasons (Table 2) indicates that the optimum rotation for the cottonwood may not yet be reached. Projected cottonwood biomass accumulation at SREC for years 4 and 5 are 18 and 34 Mg/ha, respectively. And growth and development of cottonwood in future rotations from the established root systems (coppice regeneration) may also exceed what has been observed to date.

Table 2. Progression of cottonwood biomass accumulation at Southeast Research and Extension Center, Rohwer, Arkansas, 2009 to 2011.

Growing Season	Annual Growth (OD kg/ha)	Cumulative biomass (OD kg/ha)
2009	873	873
2010	1781	2654
2011	4626	7280

Small Mammals

A total of 560 captures of 289 individuals occurred at the three sites during the four seasons with 63% of the individuals captured at the SREC. Seven species or genus groups were captured: the hispid cotton rat (*Sigmodon hispidus*), *Peromyscus* spp., house mouse (*Mus musculus*), *Reithrodontomys* spp., marsh rice rat (*Oryzomys palustris*), woodland vole (*Microtus pinetorum*), and least shrew (*Cryptotis parva*). The house mouse, hispid cotton rat, marsh rice rat, and *Peromyscus* spp. represented 50.5, 18.6, 14.5, and 12.1%, respectively, of the individuals captured. The three remaining species or genus groups (*Reithrodontomys* spp., woodland vole, and least shrew) individually accounted for less than 2.0% of the captured individuals.

Where switchgrass was successfully established (PTBS and SF), the greatest number captures occurred in the SG treatment (1.94-2.92 individuals/100 trap nights). At these sites the number of captures in the SGCW and CWSG treatments was also higher than those in the CW or SGSR treatments (Table 3). At PTBS and SF, 60-84% of the captures in the CW, SGCW, CWSG, and SG cropping treatments occurred in switchgrass, indicating a preference for grass cover to tree cover. The number of captures (0.69-1.11 individuals/100 trapping nights) in the soybean-grain sorghum rotation was consistently lower than those in the SG, SGCW, or CWSG cropping treatments. At the SREC site where switchgrass had not been successfully established by 2011, the highest number of captures occurred in the CW cropping treatment (7.92 individuals/100 trapping nights) and the lowest in the SGSR cropping treatment (3.75 individuals/ 100 trapping nights).

Table 3. Small mammal community characteristics by treatment for all seasons combined at the Pine Tree Branch Station, Colt, Arkansas, 2011.

	Treatment					
Species	(CW)	(SG)	(CWSG)	(SGCW)	(SGSR)	Total
Sigmodon hispidus	0	11	1	3	0	15
Peromyscus spp.	6	9	6	8	4	33
Mus musculus	0	0	1	0	1	2
Reithrodontomys spp.	0	0	0	1	0	1
Oryzomys palustris	0	0	2	2	0	4
Microtus pinetorum	0	0	0	0	0	0
Cryptotis parva	0	0	0	0	0	0
Total	**6**	**20**	**10**	**14**	**5**	**55**
Number of ind./100 trapping nights	0.83	2.92	1.39	1.81	0.69	1.53

Where both switchgrass and cottonwood cover was available (PTBC and SF), small mammal diversity was consistently greatest in cropping treatments containing both cottonwood and switchgrass (Table 4). Shannon's diversity index was consistently greater with the SG cropping treatment than the SGSR cropping treatment.

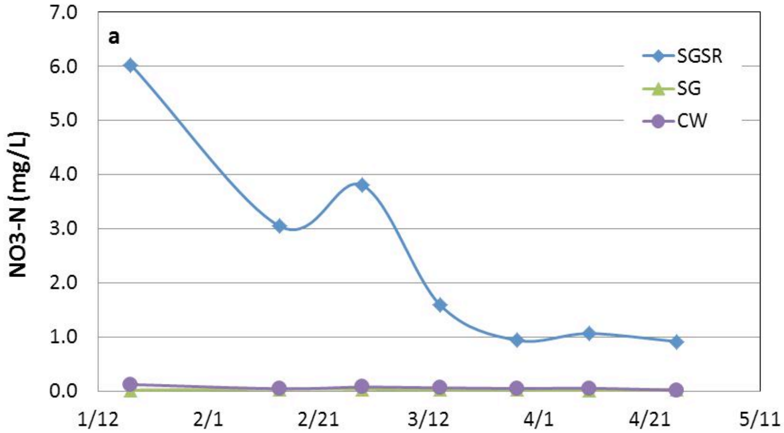
Table 4. Shannon diversity index of the small mammal community in each treatment at three biomass production sites in the Lower Mississippi Alluvial Valley in 2011.

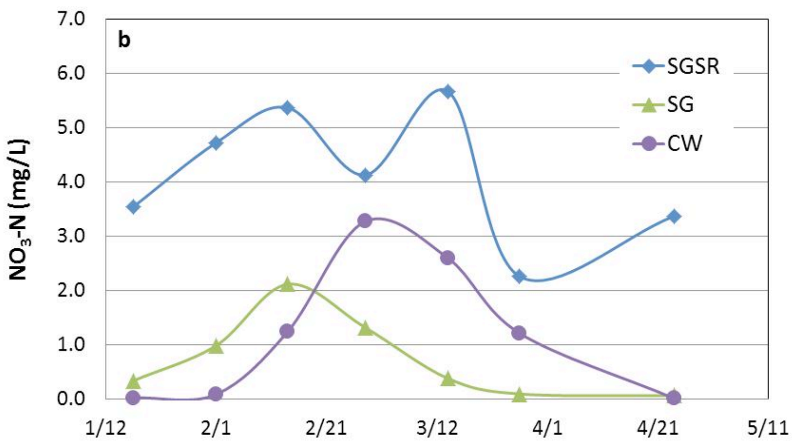
	Treatment				
Site	(CW)	(SG)	(CWSG)	(SGCW)	(SGSR)
PTBS	0.00	0.69	1.09	1.12	0.50
SREC	1.34				0.16
SF	0.67	0.52	1.27	1.04	0.00

These initial findings indicate that replacing conventional row crops with bioenergy crops such as switchgrass on marginal soils in the LMAV can increase small mammal populations. Planting alley cropped cottonwood and switchgrass would likely increase the diversity of the small mammal populations in comparison establishing any of the single-cropping systems tested.

Soil Water Chemistry and Nutrient Retention

Concentrations of N in soil water were generally greater in the soybean-grain sorghum (SGRS) then either the switchgrass (SG) or cottonwood (CW) treatments. Although NO_3-N soil water concentrations varied among sites and sampling periods, NO_3-N concentrations were consistently greater in the SGRS treatment than the other two treatments during each sampling period (Figure 2). Average NO_3-N concentrations in the SGRS cropping treatment were approximatley 5 to 6 times greater than in the SG and CW cropping treatmeants (Table 5). Nitrate-N was the dominant N ion in these soil solutions and total N concentrations like NO_3-N were significantly greater in the SGRS treatment than in the SG or CW crops. Concentrations of NH_4-N, organic N, and total P were numerically but not significantly ($p \leq 0.10$) greater in the SGRS than the other two cropping treatments (Table 5).





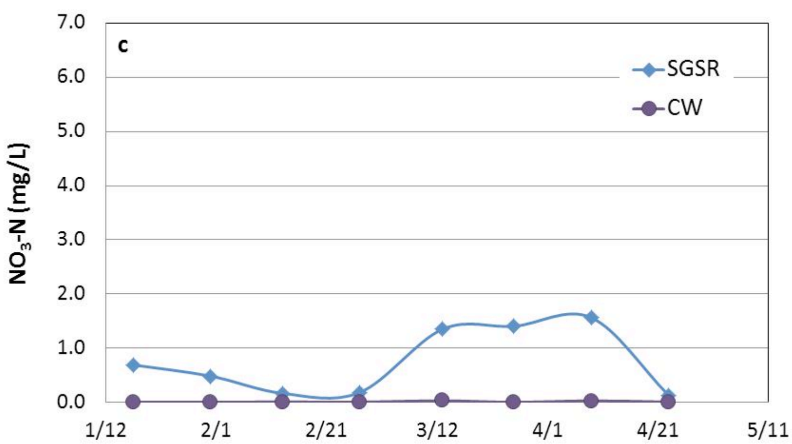


Figure 2. Soil water NO_3-N concentrations in the soybean grain sorghum rotation (SGSR), 100% switchgrass, (SG) and 100% cottonwood (CW) treatments during 2012 at the a) UA Pine Tree Branch Station, b) UA Southeast Research and Extension Station, and c) Stephenson Farm.

Table 5. Mean N and P soil water concentrations in the soybean grain sorghum rotation (SGSR), switchgrass (SG) and cottonwood (CW) cropping treatments on three biomass production sites in the Lower Mississippi Alluvial Value in 2011.

Site & Treatment	Soil Water NO_3-N (mg/L)	NH_4-N (mg/L)	Organic N (mg/L)	Total N (mg/L)	Total P (mg/L)
PTBS & SF					
SGSR	3.32*	0.88	1.26	5.14*	0.08
SG	0.39	0.09	0.24	0.72	0.03
PTBS, SREC, & SF					
SGSR	2.38*	0.69	1.01	3.84*	0.07
CW	0.41	0.10	0.29	0.78	0.04

SGSR concentrations noted with * are significantly ($p \leq 0.10$) higher than those for the SG.

The higher levels of soil water N in the SGRS treatment likely reflected the nitrogen fixing ability of the soybeans and the ferilizer applied to this cropping treatment. In addition soybeans and grain sorghum are annual crops which have rapid decomposition of harvest residues and below-ground tissues. Comparisons in root biomass among these three cropping treatments at the three study sites (unpublished data) indicated significantly higher levels of living roots in the SG and CW treatments than the SGRS. The maintenance of living roots of cottonwood and switchgrass during the entire year would likely help to absorb available N, reduce N inputs from decomposing roots, and thus increase N retention. The similar concentrations of N in the soil water of the switchgrass and cottonwood suggest that these two crops have similar N retention abilities.

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2.7
DEVELOPING A LOW INPUT SWITCHGRASS FEEDSTOCK PRODUCTION SYSTEM BY HARNESSING BENEFICIAL BACTERIAL ENDOPHYTES

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Abstract
Switchgrass represents a promising feedstock crop for US energy sustainability. However, its broad utilization for bioenergy requires improvement of biomass yields and stress tolerance. Our team has been working on harnessing bacterial endophytes to enhance switchgrass performance under poor growth conditions, and develop a low input and sustainable feedstock production system for marginal lands that does not compete with the production of food crops. We have demonstrated that the bacterial endophyte *Burkholderia phytofirmans* strain PsJN is able to colonize roots and significantly promote the growth of switchgrass cv. Alamo under *in vitro*, growth chamber, and greenhouse conditions. When grown under sub-optimal environmental conditions in field soil with no fertilizer application, PsJN-inoculated plants produced much higher biomass than controls, implying the potential benefit of PsJN inoculation for biomass production on marginal lands. Physiological parameter measurements showed that PsJN-inoculated Alamo had consistently lower transpiration, lower stomatal conductance, and higher water use efficiency compared with control plants. We also found that PsJN has a genotypic specific response, with no growth promotive effect on cv. Cave-in-Rock. Using switchgrass EST microarray analysis, we have conducted comparative global gene expression profiles following PsJN inoculation of both cultivars and are assembling regulatory network and specific pathways for growth promotion by PsJN. Some key candidate genes have been chosen for further function studies to elucidate the mechanisms by which endophytes promote plant growth.

Keywords: Bacterial endophyte; Burkholderia phytofirmans strain PsJN; Colonization; Growth promotion; Biomass increase; Global gene expression profiling; Switchgrass cv. Alamo

Introduction
Bioenergy has drawn increasing attention because it has many advantages over fossil fuels: energy security and independence, reduction of greenhouse gas emissions, environmentally friendly products, and rural economic development. It has been estimated that by 2025, the world energy demand will likely be increased by more than 50% (Erahin *et al.* 2011). To reduce the reliance on fossil fuels, the USA released the Energy Independence and Security Act of 2007 that projects an increase in the production of renewable fuels from 9.0 billion gallons in 2008 to 36 billion gallons by 2022. Switchgrass (*Panicum virgatum* L.) is one of the most promising feedstocks capable of contributing to the realization of US renewable energy goals (Sanderson *et al*. 2006). It is a native prairie grass with a diverse germplasm and

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can grow on marginal lands with low requirements of water and agrochemicals (Hill *et al*. 2006), so that it does not compete with food crops for fertile lands. The development of improved switchgrass varieties for low-cost production on marginal lands is one prerequisite for the success of the bioenergy program (Sanderson *et al.* 2006). A practical and feasible approach is to use beneficial endophytic microorganisms including bacteria and fungi (Berg 2009). Endophytes, in general, have been reported to enhance plant performance, such as growth promotion, nutrient acquisition enhancement, stress tolerance, and plant pathogen inhibition (Mei and Flinn 2010). A key component of our bioenergy crop research program involves the utilization of beneficial bacterial endophytes to improve biomass yield and enhance stress tolerance under low-input production systems. *Burkholderia phytofirmans* strain PsJN has been found to be a highly effective plant growth promoting bacterial endophyte, with a broad host range including potatoes, tomatoes, and grape vines (Sessitsch *et al*. 2005). In addition, its genome has recently been sequenced (Weilharter *et al*. 2011), providing the genomic resources needed to develop an understanding of the mechanisms associated with this endophyte's ability to promote plant growth. PsJN produces a high level of ACC deaminase (Sessitsch *et al.* 2005), enhances host plant cold stress tolerance, improves water management, and plant resistance to pathogens (Barka *et al.* 2006). In this presentation, we report the growth promotion of switchgrass cv. Alamo by *Burkholderia phytofirmans* strain PsJN under *in vitro*, growth chamber, and greenhouse conditions and mechanisms by which beneficial bacterial endophytes promote switchgrass growth.

Results and Discussion

Switchgrass Alamo colonization by bacterial endophyte PsJN

Switchgrass seeds were surface-sterilized (Kim *et al*. 2012) and grown in MS medium without hormones in an incubator at 25°C with a 16/8 hr light/dark cycle. Ten-day old seedlings were inoculated with *Burkholderia phytofirmans* strain PsJN, which was grown and prepared following Kim *et al.* (2012), and inoculated with PBS buffer alone as control. The endophytic colonization of switchgrass by *Burkholderia phytofirmans* strain PsJN-GFP could be visualized using confocal microscopy. Under the appropriate illumination, the PsJN-GFP could be clearly observed inside the roots of PsJN-inoculated plants 3 days after inoculation, while no fluorescence was observed in roots of buffer-inoculated control plants (Kim *et al.* 2012).

PsJN promotes growth of switchgrass Alamo under various conditions

PsJN significantly promoted growth of switchgrass cv. Alamo. The average total fresh weight was 56.6% higher in PsJN-inoculated plants compared to control plants from 6 experiments with significantly longer shoots and number of roots after one-month growth *in vitro*.

In order to determine growth performance of PsJN-inoculated plants in soil conditions after inoculation, the seedlings were grown *in vitro* for one month, then transferred to either a flat with 72 cavities filled with soil and grown in a growth chamber under 28/22°C day/night temperatures with 16 h light period for one month, or 4-gallon pots with 5 plants/pot and grown in the greenhouse for two months. The growth chamber experiments were repeated 5 times, and the average data showed significant growth promotion by PsJN, with a 46% and a 54% increase in fresh weight and dry weight, respectively. In a greenhouse experiment, the PsJN-inoculated plants had more early tillers with 76% more tillers than control plants in the first month. After two months growth in the greenhouse, the PsJN-inoculated plants were significantly higher in biomass yield, with a 37% increase in fresh weight and a 49% increase in dry weight. The total dry weight increase by PsJN was more than the total fresh weight increase in growth chamber and greenhouse, indicating that the PsJN-inoculated plants accumulated more biomass.

Plant growth promotion under sub-optimal conditions

In order to develop a low input and sustainable switchgrass feedstock production system utilizing beneficial bacterial endophytes, we tested growth performance of PsJN-inoculated plants in unfertilized field soil, in a glasshouse under ambient conditions during the Fall of 2010 and 2011. In an experiment conducted from September 15 to December 2 in 2010, the results showed that PsJN-inoculated plants produced 100% more total biomass than controls. In a second experiment conducted from September 7 to November 28 in 2011, the PsJN-inoculated plants had 56% more biomass than that of control plants. These results imply that beneficial bacterial endophytes could play potential roles in stimulating plant growth on marginal lands under unfavorable growth conditions.

Changes of plant physiology parameters in PsJN-inoculated plants

Plant physiology parameters of PsJN-inoculated plants were measured with a Li-COR photosynthesis system under defined conditions (1500 µMol/m^2/sec light, 25°C, and 380 ppm CO_2). The results indicated that switchgrass cv. Alamo inoculated with PsJN had consistently lower transpiration, lower leaf conductance, and higher water use efficiency compared to non-inoculated control plants (Fig. 1A-C). Although plants inoculated with PsJN had a lower photosynthesis rate per unit leaf area (Fig. 1D), their overall photosynthesis rate was significantly higher as these plants were bigger and had much larger leaf area compared to controls. Recently we developed a non-destructive photographic technique to estimate changes in leaf area over time. Photographs of the plants were taken with a known reference area placed in each photograph and then analyzed using Photoshop CS5 (Adobe Systems Inc.). The result showed that PsJN inoculation doubled the leaf area of switchgrass when the seedlings were younger than 10 weeks old (Fig. 1E). We found that PsJN inoculation benefited plant growth at the early stages, essential for biomass accumulation. The acceleration of host plant development at the early stages also indicates the potential for improvement of switchgrass stand establishment by bacterization.

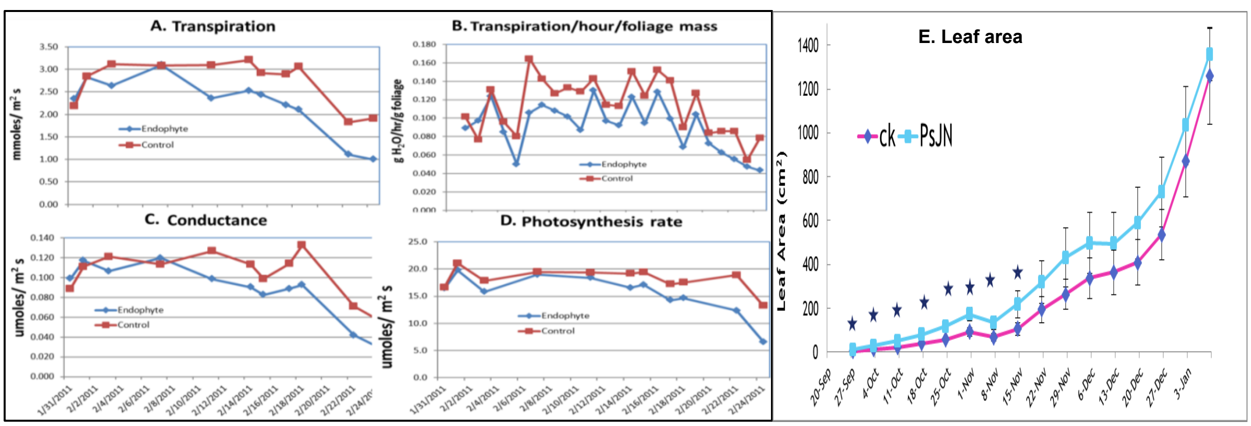

Fig. 1. Changes in plant physiology parameters (A-D) after PsJN inoculation, conducted in the greenhouse during the winter of 2011, and leaf area changes (E) during the study period (n=5, error bars are ± 1 SE and asterisks indicate significant differences (P < 0.10 between inoculation treatment and control).

Mechanisms of plant growth promotion by PsJN

Numerous reports demonstrated that host plant genotypes played a pivotal role in determining plant growth responses (Mei and Flinn 2010). We also found that PsJN has genotypic-specific responses, with no growth promotive effect on cv. Cave-in-Rock (Kim *et al.* 2012). Using switchgrass EST microarray analysis, we have conducted comparative global gene expression profiles following PsJN inoculation of both cultivars and are assembling regulatory network and specific pathways for growth promotion by PsJN using MapMan (Usadel *et al*. 2005) and PageMan (Usadel *et al.* 2006) softwares. Approximately 35,200 switchgrass ID probes were identified as showing significant differences between switchgrass cultivars Alamo and Cave-In-Rock within the first 8 days following inoculation with *B. phytofirmans* strain PsJN. Almost 17,500 identifiers were used to display the early responses of these two cultivars to the inoculation of *B. phytofirmans* strain PsJN using the rice genome as a model. Different biological pathways exhibited significant differences among the treatments and between cultivars under study using PageMan (Fig. 2) (Usadel *et al*. 2006). We have also identified more than 10 key genes, which have been verified through qPCR, and their functional studies are under way.

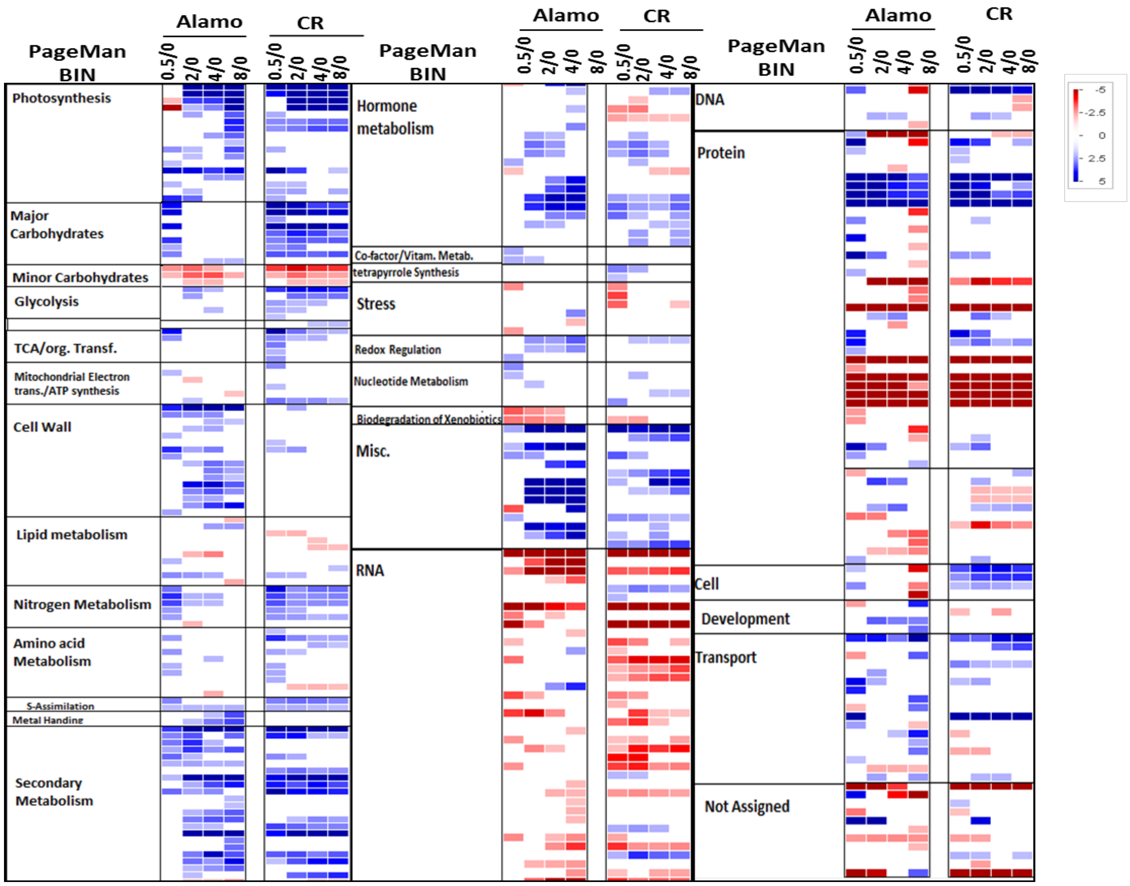

Fig. 2. PageMan display of levels of regulation of gene categories after 0.5, 2, 4 and 8 days of inoculation with *Burkholderia phytofirmans* strain PsJN.

Acknowledgements

This work was supported by the Office of Science (BER), U.S. Department of Energy for Plant Feedstock Genomics for Bioenergy Program (DE-SC0004951) and operating funds from the Commonwealth of Virginia to the Institute for Advanced Learning and Research.

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2.8
DEVELOPING LOW-INPUT, HIGH-BIOMASS, PERENNIAL CROPPING SYSTEMS FOR ADVANCED BIOFUELS IN THE INTERMOUNTAIN WEST

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Abstract
Lignocellulosic biomass studies are being conducted to evaluate perennial herbaceous feedstocks and to determine their field performance and adaptation potential for biomass production in the Intermountain West. Field performance of four biomass entries and four inputs are being evaluated over a long-term testing period at three western Colorado locations. Also, a native grass field trial was planted in 2011 to evaluate new crosses of basin wildrye (*Leymus cinereus*) x creeping wildrye (*Leymus triticoides*) as potential biomass resources. The Introduced Biomass Treatment, consisting of mostly alfalfa, has consistently had the highest biomass yield at the Fruita site. In the first cutting that occurred in 2012 in the native grass species study, tall wheatgrass (*Thinopyrum ponticum*) and intermediate wheatgrass (*Thinopyrum intermedium*) had high biomass yields. An easy-to-use crop budget enterprise tool has been developed to model the economic viability of the various plant species being evaluated. There is agronomic and economic potential to develop 200-300,000 acres of marginal land within a 50-mile radius of Rifle for the production of dedicated, herbaceous biomass. A pilot plant nearing completion at the Colorado Mountain College will convert the various perennial biomass grass species into butanol. In order to advance production, further refinement of the definition of marginal lands for bioenergy crops is needed.

Keywords: biomass, grasses, native plants, herbaceous perennial crops, dedicated energy crops, lignocellulosic biomass, biomass budget generator

Introduction
Biofuels from lignocellulosic biomass have potential to provide public benefits including increased energy independence and security, foreign exchange savings, rural development, and job creation (Rajagopal et al., 2007). Herbaceous perennial crops used for sustainable biomass crop production have requisites: 1) They should be carbon neutral- preferably, carbon negative; 2) They should have low input production requirements; 3) They should not compete with established food/feed systems or cropland; 4) They must have low irrigation water requirements; 5) They must be profitable for agriculture, industry, and others; 6) They must have desirable socioeconomic aspects.

Recent policies such as the Energy Independence Security Act of 2007 (EISA), including the second U.S. Renewable Fuel Standards (RFS), have targeted biofuel production and domestic energy independence. However, in contrast to most of the country, the mountain valleys of Colorado and similar locations in the Intermountain West (IMW) have attracted few biorefineries (http://www.ethanolrfa.org/bio-refinery-locations/). According to the EISA, arid western states are not expected to provide abundant biofuel

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development. The higher elevation environments in many areas of the IMW region are limited by the number and type of crops they can produce, yet a considerable land resource exists. Biofuel crops that facilitates energy security in the IMW contributes to regional economic development, and makes the RFS goals more attainable. Crops and cropping systems needed to produce low input herbaceous perennial crops to support a bioenergy economy in the IMW are essentially unknown. The objectives of this research are to evaluate perennial feedstocks and determine their field performance and adaptation potential for biomass production in the IMW and also to model their economic potential for commercial production.

Materials and Methods

Biomass Plant Species and Fertility Input Study

Field performance of four herbaceous biomass entries (Factor A) and four fertility input levels (Factor B) are being evaluated to assess their effect on biomass production at three western Colorado locations.

Factor A. Biomass entries

1. Native grass mixture – 'Magnar' Great Basin wildrye, 'San Luis' slender wheatgrass, 'Secar' Snake River wheatgrass, 'Rosana' western wheatgrass.
2. Switchgrass mix – 'Dacotah' and 'Blackwell'.
3. Tall fescue – 'Fawn'.
4. Introduced pasture mix – 'Cache' meadow brome; 'Fawn' tall fescue; 'Latar', 'Potomac', and 'Paiute' orchardgrass; alfalfa.

Factor B. Four input levels

1. No inputs (a control treatment without external inputs), (2) "Sustainable" inputs (composted cow manure at 5 ton/acre), (3) "Low" commercial inputs (half the level of the high input treatment at 60 lbs N/acre in split applications), and (4) "High" commercial inputs at 120 lbs N/acre in split applications (based on soil test results).

The study is being conducted at: 1) Fruita, Colorado (4600 ft); 2) Rifle, Colorado (reclaimed soil, 5400 ft); 3) Meeker, Colorado (6500 ft). Biomass plots were planted at Fruita in 2010, Rifle in 2011 and at Meeker during spring 2012. From a water perspective, preferred biomass crops would be drought-tolerant, high-yielding crops grown with limited irrigation water. Rifle and Meeker locations are sprinkler irrigated and Fruita location is furrow-irrigated.

Native Plant Species Study

A field trial was planted in 11 Oct 2011 at Fruita, CO in cooperation with the USDA-ARS Forage Lab at Logan, UT to evaluate novel crosses of basin wildrye (*Leymus cinereus*) x creeping wildrye (*Leymus triticoides*) for agronomic performance as potential biomass resources.

Results and Discussion

Biomass Plant Species and Fertility Input Study

Data were collected in the biomass plant species study at Fruita for one harvest in 2010, three cuttings in 2011, and two cuttings in 2012. A third cutting will occur at Fruita during fall 2012. At Rifle, an initial harvest occurred in 2011 and one cutting has occurred in 2012. A second cutting will occur at Rifle during fall 2012. At Fruita, the Introduced Biomass Treatment has consistently had the highest biomass yield (Table1). While the Introduced Biomass Treatment included grass species, those grass species have disappeared from the plant stand and nearly all the biomass yield in this treatment is from alfalfa. Deficit irrigation is being used in this study and plant species such as alfalfa with a deep root system can explore a large volume of soil. This gives alfalfa an advantage over shallow-rooted grass species. The native grass entry in this study has exhibited low biomass yields compared to other biomass entries. Native grass species adapted to arid environments may persist well under harsh environments, but may not be high yielding. In previous research, switchgrass has been found to be high yielding under high input conditions in some western Colorado environments, but not others (Pearson et al., 2004a, 2004b, 2004c). Establishment of some grass species, such as switchgrass, may require a few years before they begin to produce high yields. During this establishment phase of switchgrass, weeds have been a problem, particularly the winter annual weeds (data not shown). Much of the biomass produced at Rifle in 2012 in switchgrass was weeds, mainly cheatgrass (*Bromus secalinus* L.) (Table 1).

Table 1. Biomass yields of biomass entries and fertility inputs in a biomass study conducted by Colorado State University at Fruita and Rifle, CO.

	Fruita			Rifle	
	2010	2011	2012	2011	2012
Biomass entry	tons/acre^{-1}				
No of cuttings	1	3	2	1	1
Switchgrass	0.81 c*	3.27 b	2.62 b	0.18 b	0.71
Tall fescue	1.14 b	3.51 b	1.40 c	1.11 a	0.45
Introduced	1.79 a	7.68 a	7.24 a	1.21 a	0.60
Native	0.47 d	2.23 c	0.69 d	0.91 a	0.52

*Numbers in the same column followed by different letters are significantly different at the 5% level of probability.

No significant yield differences among the input treatments have occurred to date at Fruita or Rifle (data not shown). There is a trend for higher yields in the high input treatment but again a few to several years may be needed before differences among input treatments become statistically significant.

The high cost of producing biofuel feedstocks has been a major hurdle for growers, biorefineries, and distributors. Identification of parameters could lower biomass production costs to promote the economic viability of locally produced biomass. Data from our research were used to develop a crop enterprise budget tool. The enterprise budgeting tool is user friendly for a variety of audiences, including producers, crop consultants, extension agents, and others. Parameters can be adjusted to reflect variations in location, crop management, best/worst case scenarios, or optimizing a specific input. For the purposes of this paper, the parameters of the crop enterprise budget have been adjusted to reflect specific agronomic scenarios.

Native Plant Species Study

Large regions of the western United States are dominated by cool-season grasses with special adaptations to cold temperatures, sporadic and low precipitation, summer drought, salinity, high elevations with high ultraviolet radiation, and other unique and challenging growing conditions. Basin wildrye (*Leymus cinereus*) is a large native perennial grasses in western North America; however, its elevated growing point is easily damaged by grazing or mechanic harvesting. Creeping wildrye (*Leymus triticoides*) is relatively short statured (less than 5-feet) but is a strongly rhizomatous grass that recovers well following grazing, cutting, or other disturbances. Creeping x basin wildrye hybrids display a combination of plant height and rhizomatous traits that are useful in a low-input herbaceous biomass crop (Larson et al. 2011).

The biomass yields of creeping x basin wildrye species were compared to other grasses over four years, with no irrigation or fertilizer, at two research farms in Utah and Idaho. Tall wheatgrass (*Thinopyrum* *ponticum*) and intermediate wheatgrasses (*Thinopyrum intermedium*) were top entries in the first two years. The single best entry in the third and fourth harvest years was a creeping x basin wildrye hybrid.

In the first cutting that occurred in 2012 in the Native Grass Species Study being conducted at Fruita tall wheatgrass had the highest yield. Intermediate wheatgrasses also had high yields and also had low biomass plant moistures (Table 3). The creeping wildrye x basin wildrye crosses exhibited intermediate yields while Altai wildrye and switchgrass had the lowest yields with most of this biomass in the switchgrass entry coming from weeds. Switchgrass also had poor stand establishment when compared to other grasses. With additional years, yield rakings of these native grass entries are likely to change from those of this initial cutting.

Table 3. Moisture at harvest, yield, and stand establishment rating of the first cutting of native plant grass entries at the CSU Western Colorado Research Center at Fruita, CO.

Perennial grass entry	Biomass moisture	2012 First cutting yield	Stand establishment*
	%	Tons/acre	1-5
ACC 641 creeping x A636 basin wildrye hybrid	61.9	2.4	2.8
ACC 641 creeping x 'Trailhead' basin wildrye hybrid	66.0	2.3	2.8
'Continental' – basin wildrye	61.3	2.1	3.4
'Magnar' – basin wildrye	63.5	2.0	2.8
'Trailhead' – basin wildrye	60.5	2.5	2.6
'Mustang' – Altai wildrye	67.5	1.8	3.1
'Alkar' – Tall wheatgrass	59.6	3.8	4.1
'Rush' – Intermediate wheatgrass	53.3	3.0	2.9
'Oahe' – Intermediate wheatgrass	51.6	3.0	4.2
Native grass mix	60.1	2.0	3.0
Introduced mix	68.9	2.3	3.3
'Blackwell' and 'Dacotah' – switchgrass mix	74.7	1.8	1.0
LSD (0.05)	3.0	0.5	0.4

*Rating, 1=poor, 5=excellent.

A Rifle Bio-Feedstock Feasibility Study was conducted in 2008 for the City of Rifle, CO and in the statement of findings, the consultants noted that dedicated energy crops have potential in the area (TSS Consultants, 2008). A potential of 200-300,000 acres of marginal within a 50-mile radius of Rifle appears possible for production of dedicated, lignocellulosic biomass (NRCS, Aaron Waller, personal communication), although further refinement of the definition of marginal lands that could be used for bioenergy crops needs to be addressed. The construction of pilot plant is nearing completion at the Colorado Mountain College, Rifle campus and at this facility the various perennial biomass grass species will be converted into butanol.

As demonstrated in the crop enterprise budget scenarios presented in Table 2, of the four species, the introduced grass species definitively demonstrates the lowest per acre break-even price ($51.59) when grown using efficient agronomic management. In contrast, the native grass mix demonstrates a relatively lower yield and a substantially higher break-even price, at $315.35 per acre, even with efficient agronomic practices. Increases in two key costs, diesel fuel and irrigation water, not unexpectedly, directly affect production costs. Regardless of the scenario, producers with capital equipment constraints (eg. no disking) incur approximately 20% higher break-even prices due to reduced yields. The crop enterprise budget tool quantitatively shows how changing different input parameters affects potential profitability.

Table 2. Enterprise budget scenarios for biomass species trials at Fruita CO.

	Switchgrass	Tall fescue	Introduced mix	Native mix
Biomass production costs*	Assumes 2 cuttings with the average yield per species, per cutting, expressed in tons/acre			
Efficient management				
Cost per acre	$182.70	$152.10	$154.78	$157.67
Breakeven price per acre	$121.80	$152.10	$51.59	$315.35
Inefficient management				
Cost per acre	$229.97	$191.72	$195.08	$198.69
Breakeven price per acre	$153.31	$191.72	$65.03	$397.38

*Inefficient management is defined as a scenario in which the agricultural production is capital equipment constrained so that production is conducted at 80% of the efficiency of an optimal producer. The following input parameters were assumed for both management scenarios: $30/acre to deficit irrigate in a typical non-drought year, $10/hr. labor costs, 3% operating loan, $4.00/gallon diesel fuel prices, and two cuttings. Yields of 1.5, 1.0, 3.0, and 0.5 tons/acre, respectively, were used for switchgrass, tall fescue, introduced, and native species. These reflect the approximate two-year average yields for non-establishment years at the Fruita site (2011 and 2012).

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2.9
EVALUATING SWITCHGRASS VARIETIES FOR BIOMASS YIELD AND QUALITY IN MASSACHUSETTS

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Abstract
Currently there is little or no published data on switchgrass (*Panicum virgatum* L.*)* yield potential for Massachusetts. Our objective was to determine how cultivars perform in this northeastern United States climate and how time of harvest affected yield and quality of switchgrass. Five upland varieties (Blackwell, Carthage, Cave-in-Rock-, Shawnee, and Shelter) were harvested at senescence (fall), kill frost (winter), and spring between 2009-2011. Measurements were taken for yield, ash, total nitrogen, and mineral content in the feedstock and non-structural carbohydrates in roots at each time of harvest. In the first year Carthage was the highest yielding variety, and harvesting at senescence in the fall consistently produced higher yields for all varieties than harvesting in winter or spring. Harvesting Blackwell, Cave-in-Rock, Shawnee, and Shelter as the plant went into senescence in the first year caused a reduction in yield the following year, such that winter harvests were equivalent to or better than fall and spring harvests. Nutrients such as nitrogen, phosphorus, potassium, magnesium and ash all decreased in the feedstock when the harvest was delayed from fall to winter or spring. Soluble nonstructural carbohydrate concentrations in the roots were three times higher in the winter than in the fall. These levels decreased again in the spring. Biomass yields ranged from 6.8 Mg ha^{-1} to 12.6 Mg ha^{-1} across upland varieties in all years. Results of this study recommend a winter harvest after a killing frost rather than a fall post-anthesis harvest.

Keywords: Ash, Nonstructural carbohydrates, Nutrient concentrations, Time of harvest.

Introduction
An important aim of contemporary switchgrass research is to determine which cultivars grow best under local growing conditions. Switchgrass biomass production has been reported to have high variation among cultivars depending on the location (Fike et al., 2006). Hopkins et al., (1995) reported significant variation among switchgrass cultivars in date of heading and yield at heading. They also noted that early heading was associated with lower yields. Successive researchers (Casler et al., 2004; Fike et al., 2006) have shown the dramatic effects of the latitude of origin of a cultivar on its production in different geographic locations.

Switchgrass' survival during winter months and re-growth in spring to early summer depends on the extent of its root structure (Ma et al., 2001). To maintain a healthy root structure for continual crop production while applying only minimal amounts of fertilizer, it is important to determine the appropriate harvest time to allow movement of carbohydrates and nutrients from the stalk to the root system (Thomason et al., 2004). It is thought that the ideal time for harvest is after the primary nutrients have translocated from the stalk to the plant's root structure (Casler and Boe, 2003; Adler et al., 2006). Some have suggested early fall harvests may be preferable to late fall or winter harvests because weather conditions are generally more favorable requiring less time and labor to cure the crop (Samson and Mehdi, 1998; Adler et al., 2006).

Harvest time not only influences switchgrass biomass production, it also affects the biofuel quality (Adler et al., 2006). As switchgrass matures during the growing season, its ash content decreases (Sanderson and

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Wolf, 1995; Adler et al., 2006), which leads to an increase in biofuel quality. In addition, less nitrogen is required by the plant because of the translocation of nutrients into the roots (Vogel et al., 2002). Delaying harvest until spring has been shown to reduce the biomass production of some biofuel crops such as reed canarygrass (*Phalaris arundinacea* L.), Miscanthus sp. and switchgrass. However because mineral concentrations continue to decrease as well, it is as-yet unclear whether the increase in fuel quality offsets the decrease in total production (Burvall, 1997; Lewandowski et al., 2003; Adler et al., 2006).

The objectives of this study were (i) to determine high-yielding cultivars with the ability to survive winter in Massachusetts and (ii) to study how different harvest times influence switchgrass biomass yield, re-growth and the quality for energy production.

Material and Methods

Variety trials were established in 2006 at the University of Massachusetts Agricultural Experiment Station Farm in Deerfield in the Connecticut River valley (42°N, 73°W). The soil type was a Hadley fine sandy loam (nonacid, mesic Typic Udifluvent). Twelve varieties of switchgrass (Alamo, Blackwell, Carthage, Cave-in-Rock, Dacotah, Ecotype-WI, Forestburg, NE28, Pathfinder, Shawnee, Shelter, Sunburst) were obtained for an evaluation of their productive potential and adaptability to Western Massachusetts. Each variety was grown in pure cultures similar to forage grasses for permanent pastures.,After establishment trials were completed, in 2009, five highest yielding varieties (Blackwell, Carthage, Cave-In-Rock, Shawnee, and Shelter) were selected for further study. The plot size for each variety in a replication was 3 m x 6 m, allowing for a harvested sample and adequate borders. No irrigation was applied in this experiment, as that is not a common practice in Massachusetts due to adequate rainfall during the growing season. In early June of 2009, each plot was fertilized with calcium ammonium nitrate (27% N) at a rate of 136 kg N ha^{-1}.

A randomized complete block design with a split plot arrangement was conducted using the selected varieties as main plots and three harvest times (post-anthesis, killing frost, and early spring) as sub plots from 2009 to 2011. Spring harvest for each year took place the following April, such that in the 2009 trial, the spring harvest took place in April 2010. In order to keep descriptions simple the spring harvest will be referred to as in the year of 2009, since the harvested vegetation actually grew during 2009. Each plot was divided into three sections for each harvest time.

A 2.8 m^2 area of the plot was mowed using a BCS sickle mower at 10-cm stubble height and either side of the sectioned plot was discarded. Harvested switchgrass were hand gathered, and weighed in the field with a tarp and digital balance. A representative subsample was collected from each plot. The subsamples were weighed and placed in a forced air oven at 50°C for 48 hours to determine moisture content at harvest. Harvested fresh weights were then adjusted by moisture content. After drying, tissue samples were ground to pass a 1-mm screen of a Wiley mill for determination of ash and mineral content. A cup cutter was used to remove a cylinder of roots 15 cm in diameter and 15 cm deep at time of harvest to determine non-structural carbohydrates. Nitrogen content of plant tissue was determined using the Total Kjeldahl procedures. Plant tissue samples were ashed in a Furnatoral Type 53600 Controller at 500°C for 5 hours. The ash was analyzed for mineral content using an Inductively Coupled Plasma Spectro Cirsos CCD. Harvested roots along with the below-ground portion of the crown were washed and dried and then ground twice, once using a large grinder and then a second time using a 40-mesh Wiley mill. Carbohydrate analysis for the nonstructural carbohydrates of the roots was performed using High Pressure Liquid Chromatography for sucrose, glucose, and fructose. The method was developed and described in Hagidimitriou and Roper (1994).

Biomass yield, mineral content, and non-structural carbohydrate data were analyzed using the ANOVA and GLM proc (SAS institute, 2005). Means were compared using least significant differences (LSD). Results were not averaged over years when interactions of year by main effects were found significant.

Results

Switchgrass dry matter yield was influenced by year. In 2009 biomass yields averaged 11.2 Mg ha^{-1} but were reduced by 18 percent in the 2010 and then another 6.6 percent in the 2011 (Table 1). Among varieties, Carthage produced the highest biomass (12.6 Mg ha^{-1} in 2009 and 9.5 Mg ha^{-1} in 2011), whereas Blackwell was the superior variety in 2010 (10.5 Mg ha^{-1}). Shelter consistently produced lower yield compared with other varieties (Table 1). Harvest time significantly affected the dry matter yield with highest yields in the harvest that occurred during the fall of the first year (14 Mg ha^{-1}) (Table 2). Yields steadily declined as much as 43 percent in the second (9.6 Mg ha^{-1}) and third (8.0 Mg ha^{-1}) years (Table 2). Although harvest time had a significant impact on yield in 2009 and 2010, it had no effect on yield in 2011 and yields were on average at 8.5 Mg ha^{-1} for all three harvest times.

Table 1. Switchgrass dry matter yield (Mg ha^{-1}) for varieties in 2009-2011.

Variety (V)		Year (Y)		
	2009	2010	2011	Mean
Blackwell	9.9bc	10.5a	8.2ab	9.5
Cave-in-Rock	12.3ab	8.0a	9.0ab	9.7
Carthage	12.6a	9.5a	9.5a	10.6
Shawnee	11.8abc	8.4a	8.7ab	9.6
Shelter	9.6c	9.0a	7.1b	8.6
LSD $_{(0.05)}$ V×Y	2.6	2.6	2.1	

Values with the same letters are not significantly different.

Table 2. Effect of time of harvest on dry matter yield (Mg ha^{-1}) in 2009-2011.

Harvest (H)*	Year (Y)			
	2009	2010	2011	Mean
Late summer	14.0a	9.6a	8.0a	7.4
Late fall	10.1b	10.9a	8.2a	6.3
Spring	9.5b	6.7b	9.2a	6.0
Mean	11.2	9.1	8.5	
LSD $_{(0.05)}$ H×Y	2.0	2.0	1.5	

Values with the same letters are not significantly different.
*Harvest time: Late summer (Senescence), Late fall (Kill frost), Spring (Snow melt).

The effect of year and variety on ash content was not significant. Total ash in the switchgrass depended on the time of harvest. Early harvest had almost twice the ash content compared with later harvests (Table 3). There were fluctuations in the ash content by year but this is likely due to the effect of variable weather.

Table 3. Ash content (%) in feedstock as affected by harvest time 2009-2011.

Harvest (H)*	Year (Y)			
	2009	2010	2011	Mean
Late summer	4.7a	5.5a	4.7a	5.0
Late fall	1.9b	2.9b	2.6b	2.5
Spring	2.6b	2.1c	2.0b	2.2
Mean	3.1	3.5	3.3	

Values with the same letters are not significantly different.
*Harvest time: Late summer (Senescence), Late fall (Kill frost), Spring (Snow melt).

The mineral content of biomass was significantly changed for all years. The only mineral that was not affected by year was Fe. Nitrogen showed a similar trend to ash, with the highest residues occurring in the fall harvest, whereas no significant differences were observed between the concentrations in the winter and following spring harvests (Table 4). Phosphorous, K, and Mg all showed a steady decrease from the fall harvest to the spring harvest, with K showing the most pronounced difference between harvest times (Table 4). Calcium concentration remained nearly constant across all harvest times, with the largest differences in Ca concentration occurring in the winter. Iron and Al concentrations were at their lowest in the winter harvest, and there was some rise in the spring harvest (Table 4).

Table 4. Harvest time influence on chemical constituents in dry matter in 2009-2011.

Harvest (H)*	Nutrients (ppm)						
	N	P	K	Mg	Ca	Fe	Al
Late summer	0.58a	1414a	10305a	1408a	2028b	42b	66a
Late fall	0.30b	743b	5338b	1085b	2362a	40b	37b
Spring	0.33b	312c	538c	652c	2215ab	58a	59a

Values with the same letters are not significantly different.
*Harvest time: Late summer (Senescence), Late fall (Kill frost), Spring (Snow melt).

Soluble non-structural carbohydrate levels in the roots and below ground tissue of the crown were affected significantly by year, variety and harvest time. Fluctuation of sugars in various years is expected to reflect changes in weather. The levels of glucose and fructose in all five varieties were similar while sucrose which was the most abundant non-structural carbohydrate differed among varieties (data not shown). Cave-in-Rock and Shelter had the lowest levels of sucrose, while Blackwell, Carthage, and Shawnee had similar levels of sucrose. The effect of time of harvest on the sugar levels was highly significant. Sucrose level was highest when switchgrass was harvested in November and was lower in August and April harvests (Figure 1). Glucose and fructose levels were lower and less affected.

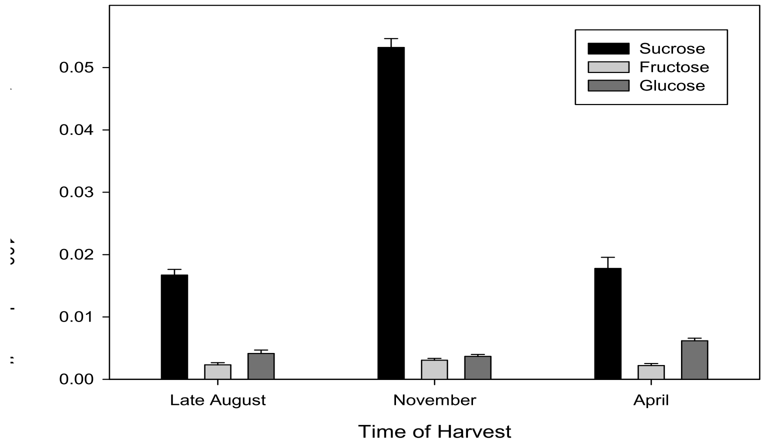

Figure 1. Soluble nonstructural carbohydrates at time of harvest for roots and crown (averaged over variety and year).

Discussion

Our experiments indicated that all varieties preformed similarly and changes depended on weather conditions. For Massachusetts conditions it appeared that Carthage and Cave-in-Rock on average were better adapted to the harsh winters and short summers found in this area. Blackwell preformed the best in 2010 but yields were the second lowest in 2011. Upland varieties; throughout the United States produce yields on average between 5-11 Mg ha^{-1} (Sanderson and Adler, 2008; Schmer et al., 2008). The trials at the University of Massachusetts across upland ecotypes ranged from 6.7-14 Mg ha^{-1}, which are similar yields to other areas in the United States. Dry matter yields were more susceptible to harvest time in the first and second year of the experiment but did not have an effect in the third year. Carthage and Cave-in-Rock produced yields at 17.0 $Mg.ha^{-1}$ and 16.2 $Mg.ha^{-1}$ in the fall of the first year and were then reduced by 28 and 51 percent, respectively, in the second year but remained more constant from the second to third year.

Many researchers claim that optimal harvest time is at senescence and that delaying the harvest until a killing frost will result in a significant decrease in yield and that harvesting prior to maturation in midsummer also negatively affects yield (Sanderson et al., 1996; Vogel at al., 2002; Sanderson and Adler, 2008). Moore et al., (1991) stated that for Cave-in-Rock optimal harvest is in the third week of August for the Midwest when switchgrass plants have just completed the senescence stage of development. In our experiment this appeared to be true for Carthage, but not for Cave-in-Rock. Cave-in-Rock yields were similar among fall and winter harvest times; so that it appears that delaying the harvest had no effect on yield in 2010 and 2011. With Blackwell, Shawnee, and Shelter delaying the harvest resulted in higher yields. In 2011 the spring harvest produced on average the highest yields at 9.2 Mg ha^{-1}, but this was still significantly less when comparing overall yield for all three years.

Switchgrass stand density declines over time, producing fewer tillers as the crop ages. This is more apparent in upland varieties than it is in low land varieties. The crop compensates for the thinning of the stand by increasing the size of the plant (Cassida et al., 2005). In the current experiment, there was a consistent decrease in dry matter yield from year to year that was more apparent when fields were harvested in fall than in the winter or spring. This might be attributed to the decrease in the number of tillers as the plants aged. More years of data are needed to determine the overall expected yield for the crop over its life span and if the decrease in fall yield is significant enough that over a ten-year period it would recommend harvesting in the winter or spring when yields are more stable.

Ash concentrations decreased with a later time of harvest as the plants matured resulting from changes in mineral content. This result confirmed prior findings reported by Sanderson and Wolf (1995). Ash content is an important factor when considering grass for combustion. Across all years and all varieties, nitrogen and ash content showed similar trends, with the highest residues occurring in the fall harvest and no significant difference between concentrations in the winter and following spring harvest. Harvesting after kill frost decreased nitrogen in plant tissue compared to the higher level at the beginning of senescence. With respect to nutrients such as P, K, and Mg which had initial concentrations greater than 1000 ppm, a delay in the harvest until at least the winter period, lowered the nutrient levels and improved the feedstock quality for combustion. Calcium concentrations were not reduced as the plant matured over the season. One of the appeals of using switchgrass as a biofuel is that it efficiently recycles its nutrients. It was a consistent finding that harvesting in fall removed vital nutrients in the harvested biomass, such that N, P, and K removal over successive years would likely cause depletion in nutrients and require more fertilizer to be used. Harvesting later in the winter period would lessen this removal. Casler and Boe (2003) stated that switchgrass had the ability to mobilize nutrients to the root system before a killing frost. Changes in levels of Fe and Al would have less effect on ash levels because of their low concentrations.

Parrish and Wolf (1993) claimed that the reduction in yield from September to November was due to the remobilizing of carbohydrate reserves and nitrogen from the stem to the roots and that remaining loss in yield was due to leaf loss. Anderson et al. (1989) showed that peak concentration in total nonstructural carbohydrates (TNC) were present in the above ground tissue in September. Figure 1 is consistent with this finding. There was three times more sucrose in the winter harvest than in the fall, which might be expected as the plant prepares for dormancy due to cold acclimation. By spring the carbohydrate levels were again low, due to the plants presumably having consumed some of their reserves to survive the winter. An analysis of the nonstructural carbohydrates in the roots sampled at each harvest date showed sucrose to be the primary sugar, with much lower quantities of fructose and glucose which is consistent with finding by White (1973), that warm season grasses store reserves in the form of sucrose and starch.

Conclusion

Given that i. yields in the fall fell dramatically enough that a winter harvest was equivalent to a fall harvest and sometimes better, ii. ash content and nutrients decreased when the harvest was delayed, and iii. soluble nonstructural carbohydrate concentrations in the roots were three time higher in the winter than in the fall we recommend a winter harvest after a killing frost rather than a fall post-anthesis harvest.

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2.10 AGRONOMIC RESULTS FROM THE SORGHUM REGIONAL BIOMASS FEEDSTOCK TRIAL

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Abstract
Sorghum (*Sorghum bicolor* L. Moench) is one of four herbaceous dedicated bioenergy crops identified by the US Department of Energy due to its high yield potential and stress tolerance. Of this group, it is the only annual crop and it is tractable to breeding and improvement. Recent breeding efforts are now producing dedicated energy sorghums. The purpose of the current study was to assess the biomass yield potential and composition of existing sorghum genotypes across different production sites in the U.S. Five sorghum hybrids and one variety were evaluated across eight locations in seven states over four years. For most agronomic and compositional traits, significant variation was detected for genotypes while the significance of environments and genotype by environment interactions depended on the particular trait. The results indicate that sorghum has excellent potential as a biomass crop and that certain environments are especially conducive to energy sorghum production.

Keywords: sorghum; bioenergy; ethanol; Regional Biomass Feedstock Trial

Introduction
Sorghum (*Sorghum bicolor* L. Moench) has been identified by the United States Department of Energy (DOE) as one of four herbaceous dedicated bioenergy crops selected to help meet the increasing demand for alternative fuels created from renewable resources. Sorghum is an ideal choice because it has high biomass yield potential, drought tolerance, established production systems, and the genetic resources and potential for further improvement. Sorghum is the fifth most widely produced cereal crop in the world with grain production in 2010 of 55.6 million metric tons grown on 40.5 million hectares (FAOSTAT, 2012). Although statistics are not maintained, it is likely that forage production is even greater.

In the U.S., the vast majority of ethanol is produced from agricultural products high in starch, primarily corn or sorghum grain. While this is a critical component of biofuel production, further production from corn is limited by other uses of the grain crop. For example, if the entire corn crop in the U.S. was

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converted to ethanol, roughly 30 billion gallons would be produced, displacing only 25% of the fossil fuels used for transportation (Rooney *et al.,* 2007). An alternative method of ethanol production is to use biomass high in cellulose and hemicellulose, or to use crops such as sugarcane or sweet sorghums with a high soluble sugar content in the stalk.

Recent breeding efforts have resulted in high biomass sorghum hybrids that are photoperiod sensitive (PS), meaning they do not typically flower in temperate regions. The drought tolerance of sorghum is accentuated in these photoperiod sensitive hybrids since sorghum is more resistant to drought when it is in the vegetative stage of growth (Rooney *et al.,* 2007).

The resources for further improvement of sorghum for bioenergy are well established in the U.S. Sorghum has undergone significant improvements since formal breeding on the crop began in the early 1900's with the genetic basis of many qualitative and quantitative traits being well understood. With a completed genome sequence and many genetic and molecular tools available, there are many established resources available to further improve the crop for different end uses.

While yield is the most important trait when selecting superior hybrids, the composition of the biomass can be just as important to the end user. Genotypes high in lignin may be desirable for a processor who will burn biomass to generate electricity, but undesirable to the processor who uses biomass to produce cellulosic ethanol because the cellulose and hemicellulose must be extracted from the lignin for processing (Lynd 1996). High ash and protein content are consistently undesirable in a biomass crop. Ash must be removed after processing and protein is correlated with the amount of nitrogen that is being removed from the soil with the harvested crop. High values of glucan and xylan are important when the biomass will be used for cellulosic ethanol since glucan is the major portion of cellulose and xylan is the major portion of hemicellulose (Lynd 1996).

Sorghum was identified as one potential dedicated bioenergy crop that could be used to aid in the production of at least one billion tons of dry biomass in the U.S. (Perlack *et al.,* 2005). This amount of biomass would be enough to displace 30% or more of the fossil fuels consumed in the U.S. with ethanol. The Department of Energy followed that study with another designed to validate their claims with real yield data on various bioenergy crops planted in multi-location, multi-year yield trials across the U.S. (U.S. Department of Energy, 2011). The purpose of the current study was to determine the potential yield and composition of six different sorghum genotypes grown at eight locations in seven different states over a span of four years. The resulting data will be used to establish the yield potential and the composition of biomass sorghums in different regions of the U.S. and the stability of production over years in these locations.

Materials and Methods

Six sorghum genotypes were evaluated across seven different states at eight locations over a span of four years. The six hybrids evaluated were: Graze All, a photoperiod insensitive (PI) sorghum-sudan forage; Graze-N-Bale, a PS sorghum-sudan forage; TX08001, a PS bioenergy hybrid; M81-E, a PI sweet sorghum variety; Sugar T, a PI sweet sorghum silage; and 22053, a PS brown midrib (bmr) silage. TX08001 was developed by Texas Agrilife Research, M81-E was developed by the Mississippi USDA-ARS, (Broadhead and Zummo, 1983) and the remaining four hybrids were produced by Advanta, Inc. The eight locations were: Manhattan, Kansas (KS); College Station, Texas (CS); Corpus Christi, Texas (CC); Ames, Iowa (IA); Lexington, Kentucky (KY); Raymond, Mississippi (MS); Yutan, Nebraska (NE); and Roper, North Carolina (NC). The yield trials were strictly rain fed. Trials were planted in 2008, 2009, 2010, and 2011 with variable plot sizes and number of replications. The IA location was added in 2009, the NE location was added in 2010, and the CC location was not planted in 2009 due to severe drought conditions. Standard production practices were observed for each location for fertilizer, tillage, etc. Target plant densities were 50,000 plants per acre for the sweets (Sugar T and M81-E), 60,000 plants per acre for the bioenergy types (22053 and TX08001), and 80,000 plants per acre for the forages (Graze All and Graze-N-Bale). The agronomic traits evaluated at each location were fresh weight, moisture content, dry weight, brix, grain yield, plant height, days to flowering, lodging, and disease and insect damage. Samples were collected at harvest, dried for a minimum of 72 hours, ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA.), and analyzed for ash, protein, lignin, glucan, and xylan composition on a FOSS near-infrared XDS Rapid Content Analyzer (FOSS NIRSystems, Inc., Laurel, MD.)

All data were analyzed using the general linear model (GLM) procedure of SAS version 9.3 (SAS Institute, 2011). Genotypes were considered fixed effects and all other sources of variation were considered random.

Results and Discussion

The eight locations used for this experiment varied widely in terms of rainfall, temperature, length of growing season, and possibly adaptation for the six genotypes tested. This variability is reflected in the mean and range for several traits important for bioenergy sorghum (Table 1). NE had the highest average fresh weight, however NE did not have the highest mean dry weight due to the high mean moisture content of the harvested biomass. The high moisture content can be undesirable when the biomass is intended to be used for cellulosic ethanol production since it adds extra weight and increases transportation costs. The low mean fresh weights and dry weights for the two Texas locations were due to the dry environments that occur there most years (Table 1). In the severe drought of 2011, the PI genotypes at CC and CS failed to enter the reproductive phase due to the lack of water, indicating that the plants had gone nearly dormant in an effort to conserve water and stay alive. While this drought tolerance mechanism exhibited by sorghum does indeed keep the plants alive, it also results in low fresh and dry weights when harvested.

Table 1. Agronomic data for all genotypes at each location across years.

Site	Fresh Weight (lbs./acre)		Moisture (%)		Dry Weight (lbs./acre)		Brix (%)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
CC, TX.	33219e†	7291-75342	75.2b	45.8-90.7	7203e	2501-23022	10.1cd	7.9-13.7
CS, TX.	33315e	5122-79408	72.7c	33.0-83.8	8731d	1575-25942	11.5b	6.2-18.2
IA	50479c	26126-90786	72.3cd	66.4-75.8	13840a	7959-25417	13.7a	7.5-19.3
KS	37000d	12393-71227	64.8f	25.8-79.9	10727c	3941-21914	13.2a	8.4-16.3
KY	27373f	4465-60981	71.4d	50.1-89.6	8726d	1988-19308	11.1bc	0.4-17.2
MS	53302c	15569-97460	73.1c	53.3-85.0	14112a	3645-30394	9.1d	4.6-15.4
NE	65655a	37204-94750	81.0a	73.0-86.5	12390b	5014-17650	.	.
NC	57420b	13758-114047	70.1e	54.4-80.8	14266a	1662-36685	10.9bc	4.0-20.0

†Means within a trait followed by the same letter were not significantly different at the 0.05 probability level based on Duncan's multiple range test (Duncan, 1955).

The ANOVA revealed that there was a highly significant (α=0.01 level) effect due to location x year and genotype x location x year for fresh weight, moisture, and dry weight. This indicates that the genotypes performed differently at each location from year to year, which is expected due to differences in rainfall, temperature, and length of growing season.

There was significant variation among locations for the five compositional traits that were measured (Table 2). There was a highly significant (α=0.01 level) effect due to location x year and genotype x location x year for all five traits with the location x year effect accounting for the majority of the variation observed in the mean trait values. This indicates that the environment plays a key role in determining the overall composition of the sorghum genotypes at each location. The effect of genotype was significant at the 0.01 or 0.05 α level for each compositional trait, indicating that breeding for each trait would be effective for influencing the composition as well.

Table 2. Compositional data for all genotypes at each location across years.

Site	Ash (%)		Protein (%)		Lignin (%)		Glucan (%)		Xylan (%)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
CC, TX.	8.5b†	6.9-10.2	4.0b	1.7-5.4	14.1a	10.4-16.7	30.9b	23.5-35.8	17.1b	13.9-19.1
CS, TX.	8.4b	6.2-10.1	4.3a	2.4-6.1	13.4bc	9.7-16.9	28.9c	24.7-33.3	16.4c	13.1-19.3
IA	6.4f	4.5-10.6	3.3d	2.4-5.9	11.3f	8.5-15.5	27.7e	23.6-32.4	14.6f	11.8-17.1
KS	7.1d	3.8-9.4	3.1de	0.1-5.0	11.6f	8.4-13.7	28.2d	24.8-31.7	15.2e	11.8-17.2
KY	6.8e	4.9-8.8	3.2de	1.5-4.7	11.9e	10.1-13.7	27.4e	24.8-29.0	15.0e	13.2-17.6
MS	7.4c	5.4-14.2	4.0b	2.1-9.3	12.7d	9.5-15.6	27.5e	23.9-31.6	15.2e	11.3-17.7
NE	9.5a	7.8-11.1	3.5c	2.1-4.9	13.5b	11.8-15.1	31.5a	26.7-35.6	17.6a	15.4-19.1
NC	7.3c	5.7-8.8	3.1e	1.5-4.6	13.2c	9.4-15.5	28.4d	24.4-31.1	16.0d	12.8-17.7

†Means within a trait followed by the same letter were not significantly different at the 0.05 probability level based on Duncan's multiple range test (Duncan, 1955).

The highly significant genotype x environment interaction observed for every trait measured indicates that the six genotypes tested performed differently across locations and years. Given that five of the six genotypes were developed in Texas and M81-E was developed in Mississippi, it is not surprising that interactions were detected. This genotype x environment interaction could potentially be minimized if breeding for high biomass sorghum hybrids was performed in the region where they were to be produced. Analysis of the data by year and by location revealed that there was a consistent year and genotype x year effect for most of the traits across most locations. This indicates that the environment will have a significant effect on the yield and composition of sorghum grown in these regions.

Conclusions

The results from this project indicate that sorghum has excellent potential as a biomass crop for various end uses. The environment plays a large role in determining the yield and composition realized from production fields. The extensive resources available to sorghum breeders and geneticists should allow future improvements to the yield and quality traits in bioenergy sorghum to meet the needs of multiple end users.

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2.11
REGIONAL PARTNERSHIP CORN STOVER MANAGEMENT EFFECTS ON SOIL AGGREGATION AND PYSICAL PROPERTIES

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Abstract
Removal of corn stover as a biofuel feedstock is being considered. It is important to understand the implications of this practice when establishing removal guidelines ensuring long-term sustainability to the biofuel industry as well as ensuring we maintain soil health. Above- and below-ground plant residue is one of the soil's main sources of organic materials that bind soil particles together into aggregates and increase soil carbon. Serving to stabilize soil particles, soil organic matter assists in supplying plant available nutrients, increases water holding capacity, and helps reduce soil erosion. Data obtained from three Corn Stover Regional Partnership sites (Brookings, SD; Morris, MN; and Lincoln, NE) will be utilized to evaluate the impact of removing corn stover on soil physical properties and aggregate stability. Field sites represent a range in soil type; climate; yield capacity and study duration. Each site consisted of three residue removal rates (low - removal of grain only; intermediate - ~50% residue removal; high- maximum amount of residue removal). Preliminary results from the Brookings, SD site showed that overall dry aggregate size distribution was less desirable in the high reside removal treatment than those treatments with greater amounts of residue remaining on the soil surface. Surface residue provides protection not only from the erosive forces of wind and water in the destruction of these smaller aggregates, but also in their creation from the breakdown of larger aggregates Incorporation of higher yielding sites as in Lincoln, NE will be included to extend this information evaluate the impact of residue removal on soil aggregation.

Keywords: corn stover management, aggregate stability, particulate soil organic matter

Introduction
In recent years, a new biomass biofuels industry has materialized out of a need to reduce U.S. dependency on imported fuel. Cellulosic fuel sources have been sought out and investigated, sometimes without extensive investigation of long-term environmental impacts. In 2010, approximately 35.7 million ha (USDA-NASS, 2010) of cropland were dedicated to corn production, producing a sizeable amount of stover with high levels of cellulose for possible conversion into ethanol. Removal of corn stover for alternative fuel production potentially affects long-term productivity of soils, which in turn may influence physical, chemical and biological soil characteristics (Wilhelm et al., 2007).

Above- and below-ground plant residue is one of the soil's main sources of organic materials that bind soil particles together into aggregates and increase soil C. Serving to stabilize soil particles, SOM assists in supplying plant available nutrients, increasing water holding capacity, and helping reduce soil erosion.

Soil aggregates have been found to form around POM (Gale et al., 2000; Golchin et al., 1994), the microbially active and labile fraction of SOM consisting of fine particles of partially decomposed plant

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tissues (Cambardella and Elliott, 1992; Soil Science Society of America, 2008) and various studies have highlighted the role of POM in aggregate formation. Golchin et al. (1994, 1995) found POM has a greater effect on stability of soil aggregates than total SOM (tSOM), because these plant and root materials have a cohesive effect on soil particles. Six et al. (1998) found that coarse intraaggregate POM (cPOM) is tied to the amount of aggregation occurring whereas fine intraaggregate POM (fPOM) increases with decreasing rate of aggregate turnover. The objective of this study was to examine the effects of corn residue removal treatments on specific soil quality indicators including dry aggregate size distribution (DASD), POM, and SOM.

Materials and Methods

Site Descriptions

Brookings SD: In the spring of 2000, a corn/soybean rotation experiment was established at the North Central Agricultural Research Laboratory near Brookings, SD. Soil types included a Kranzburg-Brookings silty clay loam complex; Kranzburg (fine-silty, mixed Udic Haploborolls), Brookings (fine-silty, mixed Pachic Udic Haploborolls). From 1995 to 1999, prior to establishing this experiment, the location was continuously cropped to alfalfa [Medicago L. (Fabaceae)] which was chemically removed without tillage in the fall of 1999.

The experimental design between 2000 and 2005 was a randomized complete block with three replications. Treatments initially included removal of corn residue at three levels: low, medium and high residue removal (LRR, MRR and HRR respectively). Low residue removal (LRR) consisted of harvesting grain with all stalks, leaves and cobs remaining on the soil surface. With medium residue removal (MRR), grain was combined and stalks chopped, windrowed using a Loftness Windrow Crop Shredder, and immediately baled. High residue removal (HRR) consisted of cutting stalks 0.15 m from the ground and removing. Individual plot dimensions were 30 x 30 m under a no-tillage soil management.

Each year, both corn and soybean phases of the rotation were present. In the fall of 2005 the residue removal treatments were split and a cover crop treatment (with or without cover crop) was integrated into the overall design, thus adjusting the experimental design from a randomized complete block design to a split-plot design, with residue removal remaining as the whole-plot treatment and cover crops representing the split-plot treatment. Cover crop consisted of winter lentils [Lentilla lens var. Morton] broadcast into soybeans at the end of R6 and slender wheatgrass [Agropyron caninum (L.) Beauv.] broadcast into corn at tasseling. Additional sampling and experiment details are reported in Hammerbeck et al, (2012).

Morris MN: The experimental sites were located at the Swan Lake Research Farm (45o 41' N lat; 95o 48'W long; elevation 370 m). Thirty-year (1971-2000) average annual precipitation recorded at the University of Minnesota West Central Research and Outreach Center in Morris, MN, is 645 mm and 30-year (1971-2000) mean monthly temperatures ranges from -13.1°C in January to 21.7°C in July (NOAA-NCDC, 2002). The area averages 1204 growing degree days (base 10°C, 1992-2005). This study took advantage of three adjacent experimental (~0.5 ha) fields at the Swan Lake Research Farm managed with contrasting primary tillage (described below).

Replication and treatment randomization occurred within tillage fields (Chisel, NT1995, or NT2005), but not among fields. Within each tillage-field, three stover removal treatments (described below) and crop phase were arranged in a randomized complete block design with four replications. Each field had 32 (6.1 m by 22.9 m) plots. These fields have Barnes-Aastad soils (USDA-SCS, 1971). Barnes is classified as a fine-loamy, mixed, superactive, frigid Calcic Hapludoll and Aastad is classified as a fine-loamy, mixed, superactive, frigid Pachic Hapludoll with <2% slope.

Based on the USDA-SCS (1971) map, three of the four replications of the Chisel field were on Barnes soil, with the fourth on Aastad, thus soil variability should be captured within replication. The NT2005 and NT1995 fields were established on an area mapped as primarily as Barnes. Stover harvest treatments were established on each field based on three rates of corn stover removal (Grain-only, HiCut/4R, and LoCut/8RLoCut/8R stover removal), four replications, and both crop phases present. The method used for harvesting stover was based on equipment availability. The Grain-only treatment removed only grain with all stover and cob material retained in the field. From 2005 through 2008, the HiCut/4R and LoCut/8R treatments were achieved by harvesting four (4R) or eight (8R) of eight rows with the forage harvester, respectively. Beginning in 2009, a one-pass harvester was used at two cutting-heights; HiCut/4R, which was just below the ear and LoCut/8R, which was as close to the soil surface as possible. Additional sampling and experiment details are reported in Johnson et al, (2012).

Results and Discussion

Soil Aggregate Size Distribution

Brookings SD: The impacts of corn residue removal following four complete rotational cycles (eight years) on selected soil quality parameters were evaluated. Due to a lack of significant difference in response to the cover crop sub-plot treatments, all interpretation of results will be based upon the whole plot residue removal treatments within the specified dry aggregate size distribution groups.

Dry aggregate size distribution data showed a higher proportion of total sample in the larger (5 and 6) aggregate size groups than in the smaller (1, 2 and 3) groups (Table 1). Evaluation of the different residue removal treatment data showed that the proportion of soil samples in aggregate size group 6 was greater for the LRR compared to the MRR and HRR treatments. In contrast the MRR and HRR treatments had a greater fraction of soil in the smaller aggregate size groups (1, 2 and 3) compared to the LRR treatments (Table 1).

Table 1. Fraction of dry soil aggregate size distribution (DASD) from the top 50 mm of soil under three different rates of residue removal, Brookings, SD. July 2008.

Residue Removal Level	Dry Aggregate Group						
	<0.42	0.42-0.84	0.84-2.0	2.0-6.4	6.4-19.2	>19.2	
	Fraction of total soil (g/kg)						
LRR	59 C[§]	34 C	75 B	150	266	416 A	
MRR	91 B	54 B	105 A	155	248	347 B	
HRR	116 A	68 A	118 A	156	242	300 B	
P > F	0.0004	<0.0001	0.0006	NS[¶]	NS	0.0378	

[§] Values marked with the same letter within the aggregate group are not significantly different (Tukey Test, α = 0.05); [¶]NS – Non Significant at $P < 0.05$ level

The fraction of soil within size groups four and five were similar for all residue removal treatments and could possibly indicate that these groups are a transition stage between the build up and break down of aggregates. This could support the concept that the building of macroaggregates from microaggregates is a function of time and availability of organic matter as the major binding agent.

The overall dry aggregate size distribution was less desirable in the HRR treatment than those treatments with greater amounts of residue remaining on the soil surface. The HRR treatment showed a lower overall mass of large aggregates and a higher mass distribution of smaller aggregates (Table 1). This illustrates that availability of residue can have an impact on the ability of the soil to form macroaggregates. The distribution of aggregates obtained within this experiment suggest that to maintain soil aggregates (formed during 5 years in alfalfa), residue should be returned to the soil surface.

Morris MN: In the chisel field, stover harvest treatments differences in aggregate distribution were observed in class sizes (5-9, 1-2 mm) (Fig 1A). At the 5-9 mm class-size, the Grain-only treatment had 12.5% and HiCut/4R treatment had 6.3% more aggregates this class size compared to the LoCut/8R stover harvest treatment. In contrast, at the 1-2mm class size the HiCut/4R and LoCut/8R stover treatments at had 10% more aggregates compared to the Grain-only treatment.

In the NT2005 field, we observed significant stover harvest treatment differences in three aggregate size fractions for the NT2005 field (Fig 1b). The Grain-only treatment had 8% and 14% more aggregates in the 5-9 mm aggregate size compared to the LoCut/8R and HiCut/4R treatments, respectively. The LoCut/8R had 15% and 60% more aggregates compared to the Grain-only treatment in the0 -0.5 and 0.5 to 1 mm classes, respectively.

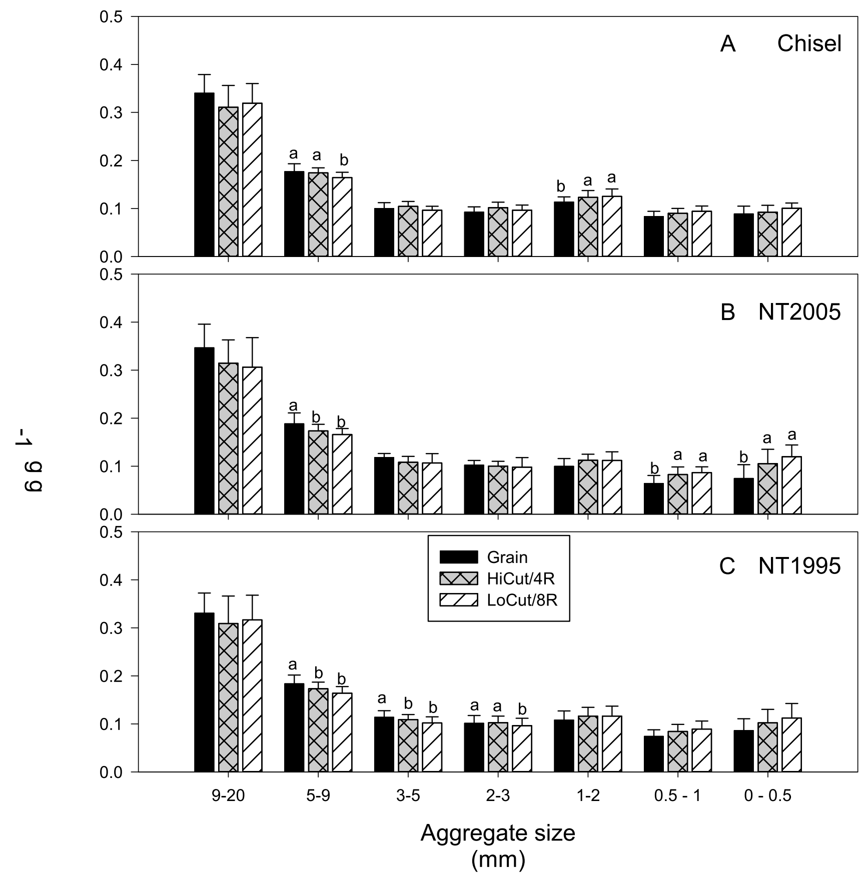

Figure 1. Dry aggregate size distribution from A) Chisel field; B) NT2005 field and C) NT1995 field. Bars label with different labels, differ at P≤0.05 by LSMEANS – pdiff (main effects). Bars represent mean with standard error (n=12). Morris, MN. July 2011.

In the NT1995 field, the Grain-only treatment had 15% and 20% more aggregate mass in the 5-9 mm size fractions compared to the LoCut/8R and HiCut/4R treatments, respectively. A similar pattern was observed that 3-5 mm class size, In the 2-3 mm class size, the grain-only and HiCut/4R treatments were similar and with more about 16% aggregates in this size class compared to the LoCut/8R treatment.

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2.12 ENERGYCANE CROP ESTABLISHMENT AND FLOOD TOLERANCE IN A TEMPERATE CLIMATE

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Abstract

Most energycanes have more vigor than sugarcane because energycane usually contains a higher percentage of genes from *Saccharum spontaneum* compared to commercial sugarcanes. Two studies were conducted to determine the relative difference between energy and sugarcanes when grown under less than optimal growing conditions. In study one, yields of cane, sucrose, and fiber of three sugarcane varieties and one energycane variety were compared when planted on August 1 (optimal time), September 1, and October 1. Fiber yields were 2.4 Mg ha^{-1} higher in cane planted in August than the averages in September and October for all varieties. There were no differences between the fiber yields for cane planted in September and October for all varieties, but sucrose yields declined with each later planting date. Thus, when planting cane at non-optimal times, energycane may be a better option than sugarcane in a sugarcane/energycane companion cropping system. In study two, two high biomass energycane varieties and two sugarcane varieties were planted and periodically flooded to determine which performed better under these stress-induced field conditions. The evaluation was conducted for two complete cane-production cycles. Energycane tolerated the flooded conditions better than sugarcane when fiber and sucrose yields were compared between treatments. Tolerance to flooding was demonstrated in the plant cane and ratoon crops of L 79-1002, and in the ratoon crops of Ho 01-12. Therefore, an alternative to sugarcane in flood prone areas would be energycanes.

Keywords: high fiber sugarcanes; biomass feedstock; water logging.

Introduction

Most energycanes have more vigor than sugarcane because energycanes usually contain a higher percentage of genes from *Saccharum spontaneum* compared to commercial sugarcanes (Legendre and Burner 1995). Sugarcane breeders use clones of *S. spontaneum* as source of genes for disease resistance, ratooning ability, and increased hardiness (Bischoff et al. 2008; Giamalva et al. 1984). Progeny from crosses with wild-type germplasm such as *S. spontaneum* as a parent have exhibited high levels of vigor, which potentially impart cold tolerance; greater ratooning ability; enhanced levels of tolerance to moisture extremes, insects, and diseases; and more efficient nutrient utilization (Bransby et al. 2010).

Unlike most other row crops, sugarcane is a perennial with the ability to produce several ratoon crops from a single planting. Therefore, management choices may not only affect the crop during the current season, but practices may also impact future crops for several additional growing seasons. With the development of an energycane industry, energycane may increase management flexibilty and expand production into marginal areas with poorer soil fertility, high salts, or prone to flooding. With increased vigor, energycane may have consistent yields with both optimal and delayed planting periods in a sugarcane/energycane farming system, and this biomass crop may be well suited for marginal sites which do not favor food crop production. Thus, this research was initiated to determine if planting date affects yields of recently released sugar and energycanes and to investigate the use of energycanes in flood-prone areas.

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Material and Methods

In the first experiment, the effects of three planting dates in 2005 and 2006 (August 1, September 1, and October 1) with three different sugarcane varieties (HoCP 96-540, L 99-226, and L 99-233) and one energycane variety (L 79-1002) were investigated on a Cancienne silt loam at the USDA-ARS Sugarcane Research Unit's Ardoyne Farm located near Schriever, LA (29°38'11" N, 90°50'25" W). Treatments were arranged in a split-plot design with planting date as whole plot and variety as split-plot. Split-plots consisted of three 1.8 m wide rows that were 4.9 m long.

In the second experiment, field experiments were planted on August 2005 and 2006 at the USDA, Natural Resources Conservation Service (NRCS), Plant Materials Center located in Galliano, LA (29°25'N, 90°16'W) on a Rita muck soil. Two energycane varieties, L 79-1002 and Ho 01-12, and two sugarcane varieties, HoCP 96-540 and L 99-226, were evaluated for tolerance to flooding. To simulate flooding, sections of the field were flooded with fresh surface water for 7 d at the beginning of each month from February to August of each year of the three-year crop cycle for plant-cane, first-ratoon, and second-ratoon. The flood treatment was imposed during this time period to mimic the seasonal period of greatest rainfall during which flooding is most likely to occur in south Louisiana. Iron plates were installed in the drainage ditches to maintain flood height at approximately 4 cm above the top of the raised beds. Other sections were levied off to provide a control representing adequate drainage. Flood treatments served as whole-plots; the four varieties served as split-plots.

For both experiments, all treatments were replicated four times and were repeated twice over time. Cane height, stalk population, cane yield, sucrose yield, and fiber yield data was collected each year. Data was analyzed using PROC MIXED, and means of significant effects were separated using the PDIFF option with the SAXTON macro at the P=0.05 level.

Results and Disccusion

Date of planting had significant effects on sugarcane yield (wet weight of stalks), sucrose yield, fiber yield (dry weight of stalks), stalk height, and stalk population for plant cane. For the plant-cane crop, planting in August increased sucrose yields by 1600 and 2300 kg ha^{-1} relative to the September and October dates, respectively, primarily as a result of an increase in cane yield. Planting in August resulted in increased fiber yields of 2.4 Mg ha^{-1} relative to the average of cane planted in September and October. For the plant-cane crop, planting in September resulted in 6.5 Mg ha^{-1} more sugarcane and 700 kg ha^{-1} more sucrose than what was produced from the October planted cane. These differences in sucrose and cane yield were not reflected in differences in stalk height or number. Fiber yields were consistent among September and October plantings. Planting date effects had no carryover into first and second ratoon yields.

Garrison et al. (2000) reported an advantage to a mid-August planting over a mid-October planting, but this research did not indicate differences with mid-August and September plantings with sugarcane. Previous research also reported yield losses with September and October planting dates relative to an August planting for sugarcane variety HoCP 96-540 (White et al. 2010). Research on elephantgrass and energycane in Florida demonstrated better establishment if they were planted during the winter season and allowed to grow the entire warm season prior to the next winter instead of spring planting (Woodard and Prine 1993). It was noted that the establishment of these grasses by planting in early fall increases levels of establishment compared to winter plantings, and authors concluded that the crop should be allowed to emerge and develop a below ground stool with above-ground tillers before a killing frost.

For study two, the effect of flooding on fiber and sucrose yields differed among the four varieties in the plant-cane crop. Fiber yields were not affected by flooding for L 79-1002, but were reduced by 16, 22, and 23% for Ho 01-12, HoCP 96-540, and L 99-226, respectively. Sucrose yields were not affected by flooding for the two energycanes but were reduced by 16 and 38% for HoCP 96-540 and L 99-226. The differences in fiber and sucrose yields can be somewhat explained by the yield parameters measured. Cane yields were not significantly different between the two treatments for L79-1002, but cane yields for Ho 01-12, HoCP 96-540, and L 99-226 were reduced by flooded conditions by 16, 21, and 24%.

Similar to plant-cane, flooded conditions affected ratoon fiber and sucrose yields among varieties. Fiber yields were not affected by flooding for the two energycanes but were reduced by 27 and 20% for HoCP 96-540 and L 99-226. Within the flood treatment, L 79-1002 produced 5.4, 7.7, and 8.4 Mg ha^{-1} more fiber than Ho 01-12, HoCP 96-540, and L 99-226, respectively. Ho 01-12 produced 2.3 and 3.0 Mg ha^{-1} more fiber than HoCP 96-540 and L 99-226. For the control treatment, both energycanes produced 5.1 Mg ha^{-1} more dry biomass than the two sugarcanes. Cane yields were not significantly different between the two treatments for L 79-1002 and Ho 01-12, but cane yields for HoCP 96-540 and L 99-226 were reduced by 25.8 and 15.9 Mg ha^{-1} when flooded.

Overall, the energycane varieties tolerated the flooded conditions better than the sugarcane varieties, with L 79-1002 showing tolerance to flooding both in plant and ratoon crops, and Ho 01-12 demonstrating tolerance in the ratoons in terms of fiber yields. Yamada et al. (2010) reported that certain energycane/biomass clones produced more biomass under poorly-drained conditions compared to the commercial sugarcane varieties. Moreover, Deren et al. (1991) reported that crosses made with commercial sugarcane with *E. arundinaceus and S. spontaneum* had flood tolerance due to rapid establishment and high biomass production of the wild relatives. An alternative to planting sugarcane in flood-prone land would be to plant energycanes. Future expansion of energycane production could be established on periodically flooded, low-lying marginal sites, which currently cannot economically sustain the production of sugarcane or other types of food and fiber crops.

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2.13
CELLULOSIC BIOENERGY CROP PRODUCTION WITH MARGINAL QUALITY WATER

Girisha Ganjegunte[1,], John A. Clark[1], Yanqi Wu[2]*

Abstract
In the recent years, interest in biobased fuels is increasing and the congressionally mandated goal is to use at least 36 billion gallons of bio-based transportation fuels by 2022. Meeting this stated goal requires a comprehensive regional strategy that includes bringing addition area from different regions within the country under bioenergy crops. In the southwest U.S., bringing vast abandoned crop lands and areas having permeable soils under bioenergy crops can be a part of such a regional strategy. While the region has adequate supply of land, finding reliable source of water to produce bioenergy crops is the main challenge. This challenge can be met by developing marginal quality (brackish/ saline) water sources for bioenergy crops production. However, information on bioenergy crops performance under elevated salinity is not well documented. This study evaluated switchgrass (*Panicum virgatum* L.) performance under treated urban wastewater irrigation using soil columns prepared from salt affected land over two years under greenhouse conditions. Results indicated that among the cultivars evaluated, "Alamo" was the most salt tolerant; both biomass yield and quality under treated urban wastewater were comparable to that produced under freshwater irrigation. Soil salinity increased with time under both freshwater and wastewater irrigation, however, sodicity of the soils remained below threshold level. This indicated that most of the salinity increase was due to solubilization of calcium salts, which is not expected to affect soil productivity.

Keywords: Bioenergy crop, biomass quality, Marginal quality water, soil salinity, sodicity, switchgrass

Introduction
Biomass production on a scale that will permit a significant substitution for fossil fuels will require availability of adequate irrigation water and land. The congressionally mandated Renewable Fuels Standards (RFS2) goal is to use at least 36 billion gallons of bio-based transportation fuels by 2022 (USDA, 2010). There is a huge gap between the current capacity and the mandated goal. USDA estimates that about 27 million acres of land needs to be brought under bioenergy crops to produce 36 billion gallons of bio-based fuels. Meeting this challenge of bridging huge gap requires a comprehensive regional strategy that includes bringing addition area from different regions within the country under bioenergy crops. In the southwest U.S. such as west Texas, bringing vast abandoned croplands and areas having permeable soils under bioenergy crops can be a part of such a strategy. While the region has adequate supply of land, finding reliable source of water to produce bioenergy crops is the main challenge. This challenge can be met by developing marginal quality water sources for bioenergy crops production. Use of marginal quality waters such as brackish groundwater or treated urban wastewater with elevated salinity to irrigate bioenergy crops may prove beneficial, if the bioenergy crops can grow under elevated salinity and the effects on soil and shallow groundwater can be minimized by appropriate management.

The region has enormous potential for marginal quality water irrigation to produce bioenergy crops for a greater farm return. For example, only 13% of the 64,000 acre feet treated urban wastewater in El Paso County of west Texas is reused every year. Far west Texas has about 200,000 acres of idled or abandoned

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farmland that can be irrigated with brackish groundwater available in the region for bioenergy crops production. However, there is limited information on the performance of bioenergy crops under elevated salinity. Developing beneficial and economically feasible strategies to utilize these marginal quality waters will benefit the region while providing bioenergy for our growing population. Switchgrass (*Panicum virgatum* L.) has been identified as a strong candidate for bioenergy production and is known to grow on different soil types under a variety of environmental conditions (McLaughlin *et al*., 2006). Its many beneficial attributes and wide distribution can make it a suitable crop for the arid southwest region. However, there is limited information on its salinity tolerance. It is critical to understand effects of marginal quality water irrigation on the crop performance and soil properties before advocating its use over a large area. Objectives of this study were to evaluate (i) salinity tolerance of selected cultivars of switchgrass crops, (iii) performance of salt tolerant switchgrass cultivar under marginal quality water irrigation and (iii) effects of marginal quality water irrigation on soil salinity to develop suitable salinity management practices.

Methods and Materials

Salinity Tolerance of Selected Switchgrass Cultivars

Seed germination and seedling survival rates of nine switchgrass cultivars (included both upland and lowland types) were determined under treated wastewater and control water salinity levels. Twenty seeds of each cultivar were sown 1 cm deep in plastic containers (27.5 cm diameter and 20 cm deep) filled with acid-washed sand. Four replications for each cultivar were maintained at 24°C in the light with a 14 h photoperiod and 18°C in the dark for 10 h photoperiod in a growth chamber (Cleland International Inc, Model 300TLR). Same amounts of waters were applied to maintain moisture levels. Percent germination after ten days and seedling mortality rates after six weeks were recorded (modified Almodares et al., 2007).

Bioenergy Crop Performance and Soil Salinity Changes

Soil columns were constructed using 46 cm diameter and 60 cm long PVC tubes with a provision to collect drainage at the bottom. Composited soil samples from a salt affected cotton field were packed into the in columns simulating field bulk densities (Table 1). Separate cores were collected from four depths (0-15, 15-30, 30-45 and 45-60 cm) at three random locations in an abandoned/salt affected cotton field to determine bulk density as described by Grossman and Reinsch (2002). Dominant soil map unit in the field was Harkey silty clay loam (Coarse-silty, mixed (calcareous), thermic Typic Torrifluvents) (USDA-NRCS, 2011). Constructed soil columns were placed in the greenhouse and maintained under conditions that mimicked long-term El Paso climate. A split plot design with two water qualities (fresh and treated wastewater) as main-plots, soil treatments (gypsum, polymer, control-no amendments) as sub-plots and soil depths as sub-sub plots was used to determine effects on plant performance and soil salinity. All columns received 25 g of elemental S, and 69 cm of respective waters applied by flood-irrigation. Control columns received 10 g of 20:20:20 fertilizer and as wastewater had appreciable N (Table 2), wastewater columns received only P and K as (2 g) monopotassium dihydrogen phosphate (KH_2PO_4). Gypsum treatment columns received 154 g of gypsum and polymer treatment columns received 10 mg/L PAM with the first irrigation. All treatments were replicated three times.

Table 1. Initial Soil Physical Properties

Soil Depth	Sand (%)	Silt (%)	Clay (%)	Bulk Density ($Mg\ m^{-3}$)
0-15 cm	40	33	27	1.34
15-30 cm	31	37	32	1.48
30-45 cm	30	39	31	1.42
45-60 cm	36	36	28	1.36
60-75 cm	39	35	26	1.35

Table 2. Irrigation Water Properties

Parameters	Control	Treated Wastewater
pH	7.3	7.3
EC ($dS\ m^{-1}$)	0.65	2.50
Ca ($mg\ L^{-1}$)	42	105
Mg ($mg\ L^{-1}$)	5.5	20.9
Na ($mg\ L^{-1}$)	168	364
Cl ($mg\ L^{-1}$)	117	375
SO_4 ($mg\ L^{-1}$)	189	980
NO_3-N ($mg\ L^{-1}$)	<0.1	18.4
SAR ($mmol^{1/2}\ L^{-1/2}$)	6.5	8.4

In each column, five seeds of the cultivar that had the greatest salt tolerance (Alamo) were sown 1 cm deep on August 10, 2009. Since we wanted to ensure good growth and development, plants were not harvested in 2009. Thereafter plants were harvested two times a year (July and October), leaving 15 cm stubble. Biomass yields at each harvest were recorded. After each harvest sub-samples were sent to Dairy One Laboratory (Madison, WI, USA) for determining forage quality parameters. These included crude protein (used to derive N), acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, lignin, and ash using NIRS.

At the end of each year (Dec 2010, and 2011), from each column soil samples were collected from four depths (0-15, 15-30, 30-45 and 45-60 cm), air dried, ground to pass through 2 mm sieve and stored for analyses. Sub-samples were used to determine soil texture by using the hydrometer method (Gee and Or, 2002); saturated paste EC_e, pH, Na, Ca, and Mg concentrations using standard methods (Sparks *et al.*, 1996). Sodium adsorption ratio (SAR) of the soil samples were determined by Ca, Mg, and Na by employing the empirical equation.

Results and Discussion

Among the nine cultivars, "Alamo" performed well under elevated salinity of wastewater and it was selected for further evaluation in the greenhouse (Fig 1). Biomass yield of Alamo during 2010 ranged from 435 g in freshwater-gypsum to 546 g/column in wastewater-polymer treatment. Biomass yields were lower in 2011 in both freshwater and wastewater irrigated columns. Dry matter yield in 2nd year (2011) ranged from 315 g in freshwater-gypsum to 394 g/column in wastewater control treatment. On area basis, biomass yields obtained in this study were towards the higher end of the range reported for switchgrass (McLaughlin *et al*., 2006). However, biomass yields produced under controlled greenhouse conditions need to be interpreted carefully. Switchgrass biomass quality did not differ significantly between fresh and wastewater treatments (Fig. 2). Carbohydrates (simple sugars, hemicellulose and cellulose) did not differ significantly between two water qualities and among soil treatments. As expected, due to elevated salinity, ash content in the biomass was greater than that reported for biomass produced under lower salinity conditions (Pasangulapati *et al*., 2012). Soil pH decreased with time probably due to greater root respiration resulting in greater CO_2 concentrations in soil air (Fig. 3). Soil salinity generally increased with time even in freshwater columns probably due to solubilization of salt as a result of application of water to abandoned salt affected land soils (Fig.4). Gypsum applied columns showed maximum salinity values. However, data showed that most of the treatments had SAR less than the threshold value of 13 (non-sodic) indicating Ca salts were responsible for the increase in salinity (Fig. 5).

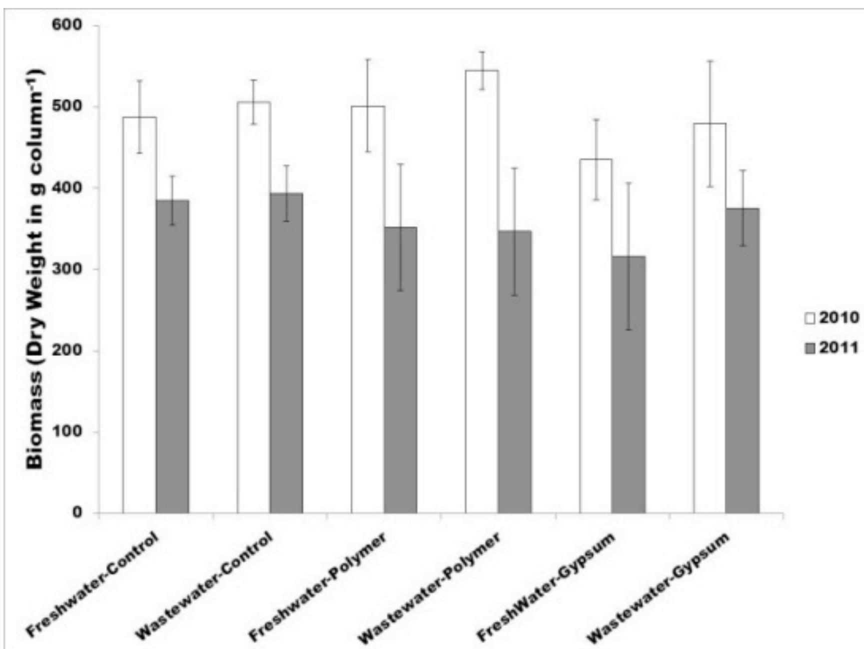

Figure 1: Switchgrass biomass yield under different treatments during 2010 and 2011.

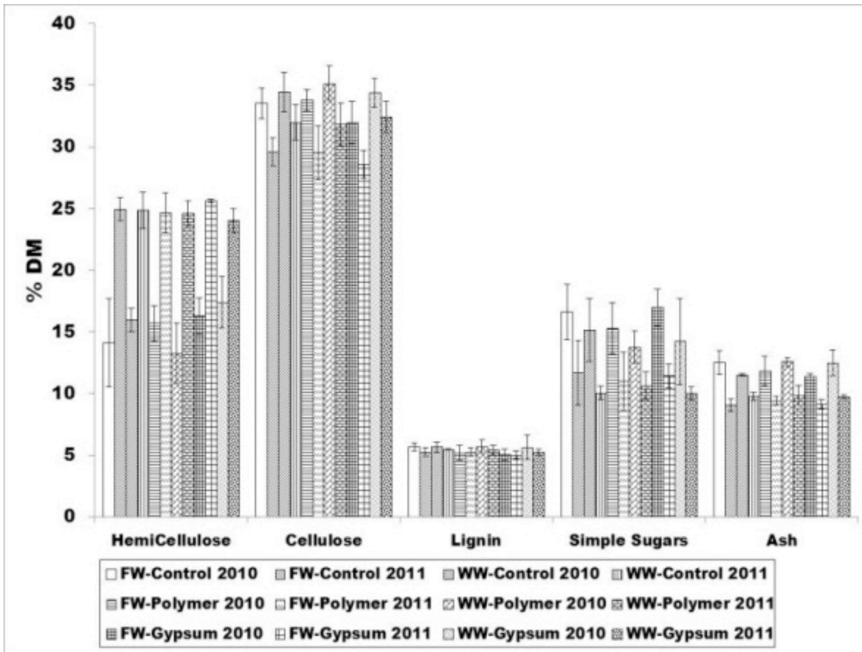

Figure 2: Biomass quality under different treatments during 2010 and 2011.

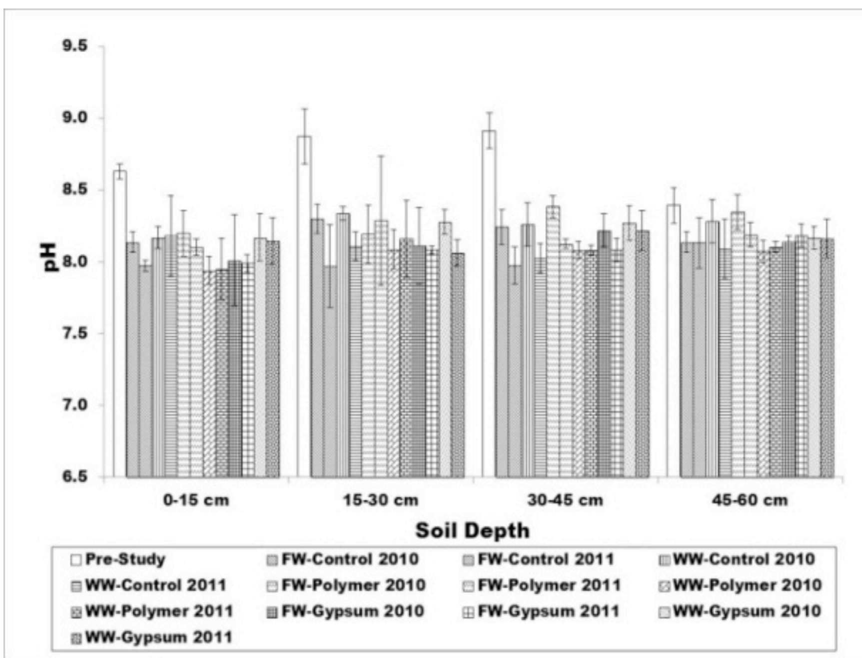

Figure 3: Changes in soil pH under different treatments during 2010 and 2011.

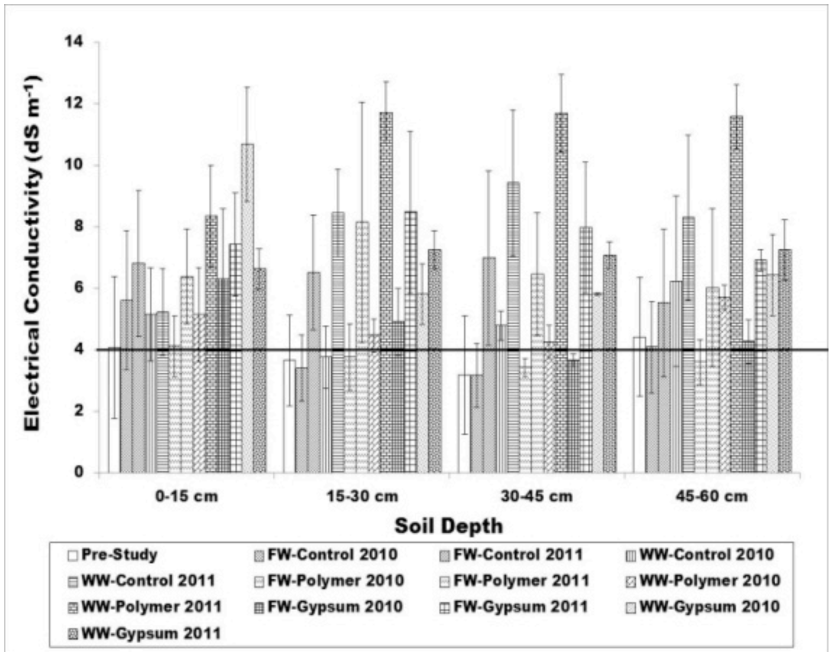

Figure 4: Changes in soil EC under different treatments during 2010 and 2011.

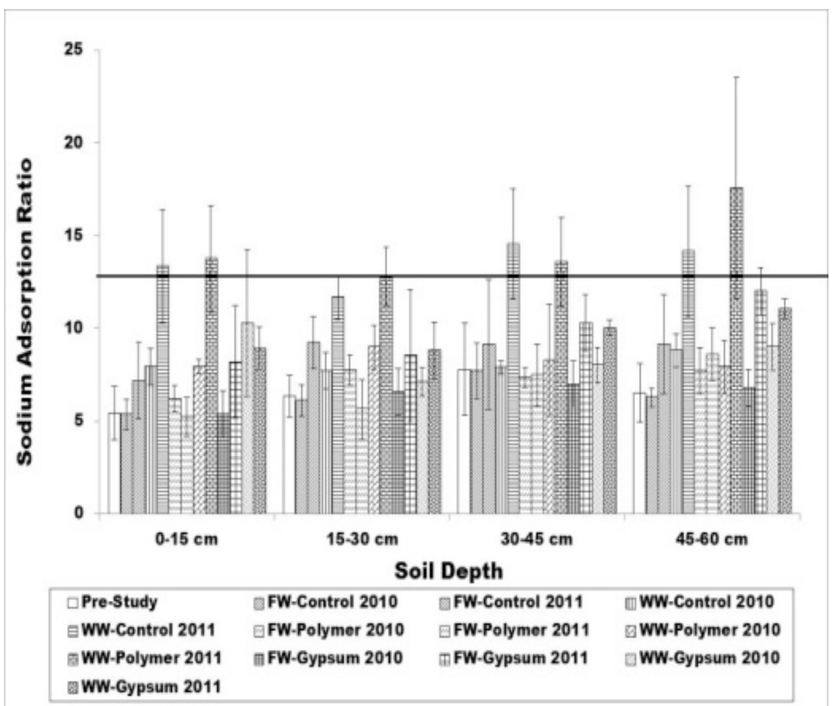

Figure 5: Changes in soil SAR under different treatments during 2010 and 2011.

Conclusions

The study results indicated that among the different cultivars evaluated, "Alamo" had the maximum germination and least seedling mortality under elevated salinity. Biomass yield and quality under elevated salinity were similar to that produced under freshwater irrigation. After two years of irrigation, soil salinity increased compared to pre-study levels, however, most of the increase in salinity came from solubilization of Ca salts, which is not expected to affect soil productivity and soil sodicity remained under the threshold level. Results of this study indicate the potential for marginal water utilization to produced bioenergy crops on salt affected lands.

Acknowledgement

Authors gratefully acknowledge the funding support from EPWU to conduct this study. Technical assistance from Carlos Castro, Sahara Jordan, and Luis Rumirez is appreciated.

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2.14
HIGH-YIELD TROPICAL BIOMASS FOR ADVANCED BIOFUELS

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Abstract
This paper describes a project to develop advanced biofuels involving a public-private collaboration of Hawaiian Commercial & Sugar Company, Hawaii BioEnergy LLC, ZeaChem Inc., the University of Hawaii, Oregon State University, Washington State University, and the Agricultural Research Service, with funding from the U.S. Departments of Agriculture, Energy and Navy. The project goals are to develop tropical feedstocks (feedstocks of strategic importance but not prevalent on the contiguous United States) for commercial biorefining; study the productivity and input efficiencies for reducing feedstock cost; analyze harvesting logistics, pretreatment and conversion technologies for these feedstocks; assess life-cycle and community impacts; and support decision-making through the development of integrated models for economic, environmental, and production sustainability.

Keywords: Energycane, Napiergrass, energy sorghum, tropical biomass, sustainability, advanced biofuels

Introduction
Hawaii is the western most state of the USA, and its location in the middle of the Pacific Ocean makes it the most isolated populated landmass in the world. As such, it plays an increasingly significant national security role as the financial and geopolitical focus shifts to the Asia-Pacific region. Hawaii is vulnerable to fluctuating oil prices since it meets over 90% of its energy needs through import of fossil fuels, and has the nation's highest energy costs. Between 2005 and 2009, Hawaii spent between $3.4 to 5.4 billion annually for fossil fuel reflecting the volatility of oil prices (Hawaii Energy Statistics 2010). Hawaii's strategic and remote location along with its high-energy costs makes it an ideal location to establish one of the first advanced biofuel refineries in the nation.

The State of Hawaii used 182 million gallons of aviation fuel, 132 million gallons of non-highway diesel, and 52 million gallons of highway diesel in 2010 (State of Hawaii Data Book 2010). Thus, it is important that feedstocks, logistics and conversion processes for advanced biofuels appropriate for Hawaii be developed. On a landmass of 6,250 square miles, Hawaii has 10 of the 14 microenvironments and 10 of the 12 soil orders of the world. Hawaii's tropical climate provides excellent growing conditions for high-yielding energy crops and allows for year-round harvesting and minimal feedstock storage requirements.

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The collaborators on this project represent the State's four largest private landowners that have significant contiguous land holdings on the various inhabited islands. Hawaiian Commercial and Sugar Company owns 38,000 acres on Maui and grows sugarcane with 800 employees. Major partners of Hawaii BioEnergy include Grove Farm (40,000 acres), Kamehameha Schools (365,000 acres), and Maui Land and Pineapple (25,000 acres). ZeaChem leads the effort on developing biochemical conversion processes to convert feedstock into advanced biofuel blend components. Agricultural Research Service scientists lead efforts on water use and efficiency, carbon balance, and crop modeling. Oregon State University leads efforts on techno-economic and life cycle assessments. Washington State University leads the efforts to develop improved energy crop harvesters. This collaborative effort is supported by grants from the U.S. Departments of Agriculture and Energy, Office of Naval Research, and the Western Sun Grant Center.

Objectives

1. Develop advanced feedstock systems for tropical grasses, with special emphasis on: developing high, sustainable biomass yields; measuring input requirements like water and nutrients; determine carbon balances; modifying existing sugarcane mechanical harvesting equipment to handle energy crops when grown on sloped and rocky marginal lands common to Hawaii; and optimizing "just in time" harvesting and processing of feedstock to take advantage of the year-round growing environment of the tropics and value-added, co-product development.

2. Develop advanced biofuel production processes, with special emphasis on: converting tropical grasses into advanced biofuels; evaluating biomass characteristics as they impact biochemical and thermochemical conversion of the feedstock; developing rural-based decentralized pre-processing systems to stabilize high-moisture grasses immediately after harvest; and providing improvements in pretreatment, hydrolysis, and fermentation technology to reach high advanced biofuel factory yields.

3. Use a suite of assessment and decision-support tools to guide sustainable development of advanced biofuel production, with special emphasis on: supporting informed decision making to improve sustainability metrics for biorefinery enterprises; measuring and estimating economic, environmental, and social impacts; validating the agronomic predictions of the test plot trials at independent locations; and adapting the framework of the Global Bioenergy Partnership 24 Sustainability Indicators (GBEP 2011) to provide a systematic evaluation of the impact of biofuel production in the Hawaiian Islands.

Results to Date

The project will focus on the C4 grasses, *Pennisetum purpureum* (Napiergrass), *P. purpureum* hybrids such as Banagrass and crosses developed by the USDA Natural Resources and Conservation Service, *Saccharum officinarium X S. spontaneum* (energycane), *Sorghum bicolor* (L.) (sweet sorghum), and *S. officinarium* (sugarcane). Field plots have been established to evaluate the effects of elevation (100, 1000 and 3000 feet above sea level) and irrigation (50%, 75% and 100% of commercial sugarcane application rates) at 100-foot elevation. The trials were started in June 2011; however, sweet sorghum and Napiergrass had to be replanted in August/September because of pest damage. First-year results showed no significant effects of irrigation level on sweet sorghum or Napiergrass dry matter yields. Energy cane dry matter yields were significantly lower at the 50% irrigation level than the 75 and 100% levels. The annualized dry matter yields (dry tons/acre-year, DT/A-Y) ranged from 8.5 to 9.4 for sweet sorghum, 8.4 to 10.6 for Napier grass, and 15.3 to 21.8 for energy cane at 100-foot elevation. Sugarcane is grown on a two-year cycle in Hawaii, so yields for that crop are not available at this time. Annualized Napiergrass yield at 3000-foot elevation was 57 DT/A-Y (34 DT/A-Y at 8 months). Preliminary results indicate genotype-environment interactions occurring with the energy crops being studied.

Compositional analyses of the energy crops are ongoing to evaluate how feedstock characteristics may affect biochemical or thermochemical processes to produce biofuels and/or co-products. Compositional analyses of Napiergrass and its ratoons at ages 2, 4, 6, and 8 months were conducted to assess changes with age and potential for generating co-products and biofuel (Takara *et al.* 2012). Juice and water-soluble elements were removed from fresh Napiergrass using a screw press. Treatments were found effective in reducing ash content including alkali and alkaline earth metals, as well as chlorine. Fuel analysis of gasified feedstock indicated that the treated Napiergrass had lower ash content, improved heating value, higher ash deformation temperatures, and higher volatile matter to fixed carbon ratios, than the untreated material. The elemental composition of the residual liquids from the treatment process was determined, appear to be suitable for irrigation or use as fertilizer.

Extensive monitoring of the test sites is occurring to quantify inputs and outputs. Hydrologic studies are being conducted to measure crop water use and losses through seepage and evapotranspiration. This information and climate data are being incorporated into an island-wide Soil and Water Assessment Tool (SWAT) simulation (Arnold *et al.* 1998). The change in soil carbon stock and pools are being measured for targeted species and varieties of feedstock grasses. The baseline carbon stock of agricultural land to be used for the production of bioenergy feedstocks has been calculated for benchmark locations. Greenhouse gas flux (CO_2, CH_4, and N_2O) from the soil surface at key stages in the growing cycle of feedstock grasses are being monitored at benchmark locations. Root morphology, biomass, and turnover in candidate bioenergy feedstock grown at a range of irrigation rates are being measured to determine the impact of deficit irrigation on yield, water use efficiency, and soil carbon storage.

Life cycle analyses of biofuel feedstock will be used to determine direct and indirect (embodied) energy in crop production. Net energy balance assesses the use of energy inputs to produce energy output in all operations of the growing cycle. Direct energy is associated with fuels for transportation and utilities. Indirect energy includes energy embodied not only in manufactured fertilizers, but also in farm machinery. This research proposes use of a net energy balance that accounts not only for direct energy but also for indirect energy use. Preliminary estimates of a net energy value (NEV) for agricultural biomass production in Hawaii (Sakuda 2011) indicate that the largest shares of energy used in Napiergrass production are embodied in chemical usage (48%), fuel consumption (20%), equipment (16%) and labor (14%). Adaptations of NEV calculations for corn in the US were utilized to validate the embodied energy accounting procedure.

Future Work

Four additional years of energy crop trials, as described in the previous section, are planned. Data from these trials will be used to parameterize existing ALMANAC (Kiniry *et al.* 1992) and DSSAT (Hoogenboom *et al.* 2003) crop simulation models, which will be then validated on similar energy crops on the island of Kauai. This will allow us to understand the appropriate conditions to use these simulation models.

Effective and efficient harvest of biomass is critical for the overall success of biofuel production systems. The energy crops in this study are all morphologically similar to sugarcane, and often stand over 12 feet tall and can have a very strong, tough stem (Henderson and Preston 1977). An effective and efficient harvesting system should be able to achieve high cutting and collection efficiency with minimal harvest cost and biomass loss. We will evaluate the need for different harvesting machinery for different terrain, stage of growth, acreage harvested, plus the relative energy requirements and economics of the various feedstock logistics scenarios, and then we will develop practical, energy-sustainable and cost-effective mechanisms for energy crop harvesting. Finally, the harvesting system will be evaluated at field scale.

We will analyze costs and returns for energy crop production, co-products (front-end derived juice as nutrient supplement, lignin for hydrogen production), and advanced biofuels production using ZeaChem's laboratory and pilot facilities. Annual equivalent costs and revenues will be calculated to account for the time value of money (Tran *et al.* 2011). Break-even prices of the energy crops, co-products and advanced biofuel will be used as indicators of economic viability of the production/processing operations.

Environmental effects will be measured by the value of carbon sequestration. The economic value of sequestering carbon will be measured using (i) historical stock market prices of carbon and (ii) estimating the energy value ($/Btu) of carbon stock. The environmental value of carbon balance will be integrated into the economic analysis and the results will be analyzed along with the results of energy accounting to determine the cost-effectiveness of advanced biofuel production from energy crops.

Comprehensive techno-economic analyses will be performed for conversion of energy crops to advanced biofuels using some of the most common pretreatment technologies (dilute acid and hot water). Process models incorporating feedstock handling, conversion to advanced biofuel, coproduct and wastewater handling will be developed.

We will conduct comprehensive attributional and consequential life cycle assessments (LCA) to evaluate the potential impacts on the environment. Important questions such as overall net energy value (NEV) of advanced biofuels, use of fertilizers and pesticides, natural resources such as fresh water, impact on green house gasses, NOx emissions, eutrophication, acidification and long term sustainability will be addressed in the attributional LCA. These metrics are similar to the bioenergy sustainability indicators suggested by the Global Energy partnership (GBEP 2011).

The Hawaii State IMPLAN (IMpact analysis for PLANning) model will be used to determine short- and long-term impacts on island communities. Evaluation overtime is important to analyze structural changes that may occur in local communities. To examine more aggregate economic impacts from biofuels production, a Computable General Equilibrium (CGE) model will be used to estimate statewide impacts (Coffman *et al.* 2007).

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2.15 SCREENING MICROALGAE SPECIES FOR BIODIESEL FEEDSTOCK PRODUCTION

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Abstract

Thirty-four species of microalgae were screened indoors for growth rate and lipid content at water temperatures of 35 and 25° Celsius, representing predicted average local water temperatures for high and moderate temperature production periods, respectively. Specific growth rate (μ) and Non-Polar Lipid Content (LPLC) were identified as key species selection parameters for maximizing biodiesel production. A production factor (PF) combining the lipid content and highest recorded eight hour growth rate of each species was used to rank species for net lipid productivity. The top eight performers at each temperature level where tested outdoors during corresponding seasonal temperature conditions. *Nannochloris sp., Dunaliella tertiolecta, Nannochloropsis oculata, and Neochloris oleoabundans* were the top performers for the warm temperature runs, with production factors of 0.945, 0.990, 1.105, and 1.520, respectively.

Keywords: Microalgae, Biodiesel, Species Selection, Growth Rate, Lipid Content

Introduction

Algal biodiesel, a second generation biofuel, is a drop-in-ready alternative fuel that directly addresses the essential logistical and energy limitations of first generation biofuels, and offers one of the few biomass based renewable energy options theoretically scalable to national or global consumption levels (Chisti 2008; Schenk, Thomas-Hall et al. 2008). According to one estimate, microalgae would only require a total of 5% of existing US crop acreage to meet 100% of present transportation fuel needs (Chisti, 2007). However, as of today, there are no verifiable data from field plots to validate these projections. Biological growth models can be used to predict the biomass and oil productivity from a given strain at a particular location. These mathematical models can offer immeasurable benefits during scale up and process calculations. However, the validity and reliability of the model simulation depends on the accuracy of input parameters. Currently, most existing kinetic screening experiments are based on indoor, flask-based experiments under artificial lighting. Therefore, there is a critical need for assessing the kinetic parameters and suitability of numerous microalgal strains for growth under outdoor conditions. Use of improper kinetic parameters in simulation models can detrimentally affect the accuracy of the output from biological growth models. For this project, a total of 34 species of microalgae were successfully screened for growth rates and non-polar lipid contents (NPLC) at two different water temperatures of 35 and 25 degrees Celsius, representing the average local (Southern Louisiana) water temperatures for high and moderate temperature production periods, respectively. The top eight performing species at each temperature level where tested outdoors during corresponding seasonal conditions in open, well mixed bioreactors, representing cultures in outdoor raceways. This research is by far the most comprehensive research aimed at screening microalagal species for suitability to produce biodiesel lipids.

Materials and Methods

Culture Origin

The species in this study were chosen from the Solar Energy Research Institute list of promising algal strains, which was compiled during the Aquatic Species Program (Sheehan et al., 1998). All species

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characterized in this study were purchased from the UTEX Culture Collection of Algae at The University of Texas, Austin or the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA). Two additional unknown species were isolated from lake water found on Louisiana State University's primary campus in Baton Rouge, Louisiana

Indoor Screening

Indoor screening experiments took place in a temperature controlled environmental chamber. Triplicate 1L batch cultures were performed for each species at 35 and 25 degrees Celsius. Cultures received a continuous illumination of 400 μmol-PAR/m^2/s from 6,100 K fluorescent bulbs. Total Suspended Solids (TSS in mg/L) was measured every eight hours until cultures reached stationary phase. Maximum eight-hour growth rates ($\mu_{max(8hr)}$) were determined from composite curves using Equation 1.

$$\mu_{max(8hr)} = \ln(N_2\text{-}N_1)/(t_2\text{-}t_1)\ldots.(1)$$

where, N_1 and N_2 = Biomass concentration at times t1 and t2 respectively

Upon reaching terminal culture density, each species underwent a 2:1 chloroform: methanol extraction, non-polar lipid assay as described in Folch et al., 1957. Gravimetric analysis provided non-polar lipid content as a percentage of dry weight.

Calculating the Production Factor

Equation 2 defines the Production Factor (PF) as a function of the growth rate and non-polar lipid content. The PF was used to rank species based on indoor performance.

$PF = \mu * NPLC$……….(2), Where, PF = Production Factor; μ = Growth rate (hr^{-1});
NPLC = Non-polar lipid content as % dry weight

Outdoor Cultures

Outdoor cultures were grown in triplicate 55 liter buckets outfitted with custom airlifts. Airlifts were powered by a regenerative blower fit with a HEPA intake filter that maintained an average airflow of 20 SCFH through a 1" PVC riser. Diurnal variation of temperature, light intensity, rainfall, and evaporation were all monitored. Water temperature was recorded using data loggers. Light intensity, in μmol-PAR/m^2/s was measured with a LI-COR Quantum Sensors (LI-COR, Lincoln, NE). Rainfall was monitored with a standard rain gauge. Evaporation was not measured quantitatively, but each morning cultures were compensated for volume lost to evaporation with sun-aged tap water. TSS was recorded every eight hours until cultures reached terminal density, at which point a second lipid extraction was performed as described earlier.

Results

Indoor Screening Results

Table 1 displays the indoor screening results for all species at both temperatures. Maximum growth rates are reported for biomass concentrations above 100 mg/L. Cultures of species failing to achieve at least 100 mg/L biomass concentration were not reported. The top eight Production Factors at each temperature are designated by a double asterisk.

Table 1. Percent lipid content, moderate temperature max growth rate, warm temperature max growth rate, and corresponding production factors for all 34 species.

Species	Folch (% lipid dry weight)	Moderate Temp. μ_{max} (hr^{-1})	Warm Temp. μ_{max} (hr^{-1})	Moderate Production Factor	Warm Production Factor
Amphora coeffiaformis	10.1	.031	.034	0.313	0.343
Ankitrodesmus falcatus	8.9	.051	.068	0.454	0.605
Botryococcus braunii #1	9.6	.026	.083	0.250	0.797
Botryococcus braunii #2	15.5	.021	.047	0.275	0.616
Botryococcus braunii #3	13.7	.041	.034	0.303	0.252
Chaetoceros muleri	8.6	.039	.01	0.335	0.086
Chlorella ellipsoidea	6.2	-	.034	-	0.211
Chlorella sp.	12.1	.035	.039	0.424	0.472
Chlorella vulgaris	7.3	.091	.035	0.664	0.256
Dunaliella sp.#1	17.0	.065	.047	0.891**	0.644
Dunaliella sp.#2	20.5	.08	.043	1.640	0.882
Dunaliella salina	4.7	.030	.043	0.141	0.202
Dunaliella tertiolecta	21.6	.051	.043	1.102**	0.929**
Ellipsoidion sp.	8.0	.033	.019	0.264	0.152
Isochrysis sp. #1	7.73	.026	.03	0.200	0.231
Isochrysis sp. #2	15.2	.014	.027	0.213	0.410
Isochrysis galbana	9.3	.034	.036	0.316	0.335
Nannochloris sp.	21.6	.064	.046	1.382**	0.994**
Nannochloropsis oculata	26.0	.07	.085	1.820**	2.210**
Neochloris oleoabundans	36.3	.05	.062	1.815**	2.251**
Pavlova lutheri	12.4	.032	.01	0.397	0.124
Platymonas sp.	10.0	.022	.032	0.220	0.320
Prymnesium parvum	4.8	.03	.02	0.144	0.096
Rhodomonas salina	4.3	-	.025	-	0.108
Selenastrum capriconutum	29.3	.043	.071	1.260**	2.080**
Scenedesmus dimorphus	7.7	.107	.068	0.824**	0.524**
Scenedesmus obliquus	18.5	.029	.071	0.537	1.314
Scenedesmus sp.	21.2	.05	.106	1.060	2.247**
Skeletonema marinoi	4.6	.027	.04	0.124	0.184
Stichococcus sp.	19.6	.024	.039	0.470	0.764
Tetraselmis sp.	4.3	.026	.039	0.112	0.168
Thalassiosira sp.	25.2	.04	.054	1.008**	1.361**
Unknown #1	7.6	.037	.044	0.281	0.334
Unknown #2	19.2	.038	.02	0.266	0.140

** indicates selection for outdoor experiments at corresponding temperature levels

Outdoor Results

The moderate temperature outdoor experiments were conducted during the first 2-3 weeks of May, 2012. The warm temperature outdoor experiments were conducted in the months of August and September 2012. Table 2 displays the maximum recorded growth rate and final lipid contents of the top eight warm species. Figures 1 depicts the water temperatures from the warm water run.

Table 2. Warm temperature outdoor maximum recorded growth rate and lipid content.

Species	Warm Temp. $\mu_{max}(hr^{-1})$	Folch (% lipid dry weight)	Production Factor
Dunaliella tertiolecta	.060	16.5	.990
Nannochloris sp.	.068	13.9	.945
Nannochloropsis oculata	.085	13.0	1.105
Neochloris oleoabundans	.076	20.0	1.520
Selenastrum capriconutum	.059	9.7	.5723
Scenedesmus dimorphus	.056	12.0	.672
Scenedesmus sp.	.028	19.5	.546
Thalassiosira sp.	.035	23.2	.812

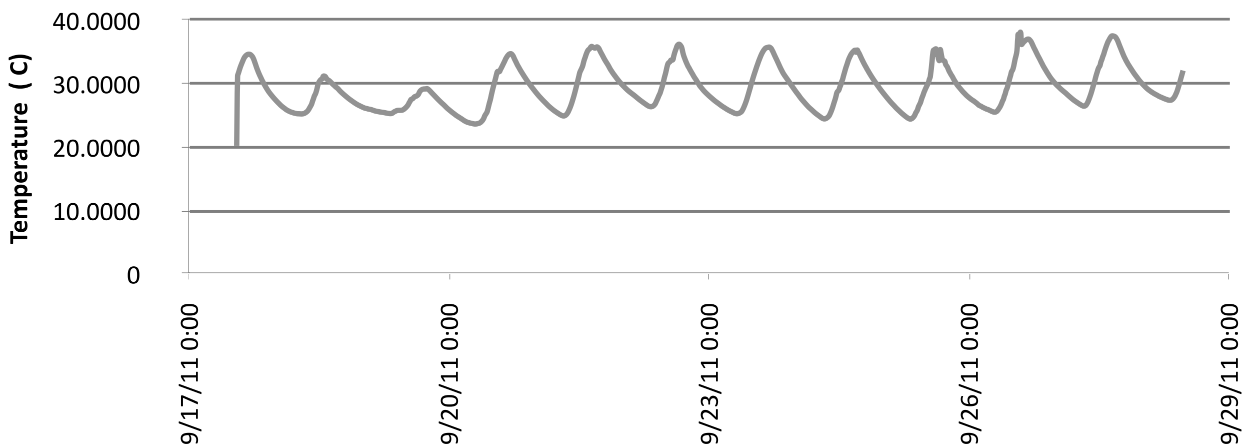

Figure 1. Water temperature recorded from the outdoor warm temperature run.

Discussion

Initial indoor screening identified several species of microalgae with promise for biodiesel production. Non-polar lipid content was highest among Chlorophytes, with *Neochloris oleobundans* reaching over 36% lipid dry weight. A notable exception was *Thalassiosira sp.* an ochrophyte diatom which reached 25% lipid dry weight. The top eight performing species at both temperatures consisted almost exclusively of Chlorophytes, with the two Ochrophytes *Nannochloropsis occulata* and *Thalassiosira sp*. being the only exceptions. The warm temperature outdoor experiment was dominated by *Neochloris oleobundans* (PF = 1.520), which exhibited moderately high growth rates coupled with a lipid content of 20%. *Thalassiosira sp.* recorded the highest lipid content (23%), but maintained significantly lower growth rates, leading to a reduced production factor. With the exception of *Neochloris oleobundans*, strains with higher lipid content generally exhibited lower growth rates, corresponding with results from literature (Sheehan et al., 1998).

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2.16
POTENTIAL OF PICOCHLORUM OKLAHOMENSIS AS FEEDSTOCK FOR BIOFUEL PRODUCTION

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Abstract
Algal biomass shows significant promise as feedstock for biofuel and bio-based product manufacturing. An understanding of microalgal biodiversity and performance of different species is of great importance when selecting a microalgae strain for an application. Properties such as strain cultivation requirements, biomass production rate, chemical composition and harvesting characteristics are critical for technical and economic feasibility of the algal production systems. *Picochlorum oklahomensis* (PO) is a microalgae strain native to Oklahoma, isolated from Salt Plains National Wildlife Refuge in Oklahoma, USA.

In this study, biomass production efficiency, downstream processing and metabolite accumulation characteristics of PO were evaluated. Chemical composition of biomass grown in Modified Artificial Seawater Medium and animal wastewater were analyzed. Biomass flocculation methods, pH adjustment, biopolymer addition and electro-flocculation, were examined for algal biomass recovery from the culture medium.

Oil content of the biomass produced by PO was about 20% (weight/weight, dry biomass basis). We have also demonstrated that swine lagoon wastewater supports algal biomass production without additional nutrients. Adjustment of the culture medium pH to 11 was effective in flocculation of algal biomass for efficient harvest.

This study demonstrated that PO can be a viable strain for biomass production. Electro-flocculation, pH adjustment and chitosan addition were effective methods to flocculate PO cells. Further research and development work is needed to determine the economic feasibility and scalability of the flocculation techniques examined in this study.

Keywords: Microalgae, biomass, chemical composition, strain selection

Introduction
Third generation biofuels particularly the ones derived from microalgae are considered to be technically viable alternative energy sources (Boucher 2012; Nigam and Singh 2011). Microalgae derived biofuels do not have the major drawbacks that are associated with the first and second generation biofuels. Main advantage of algal biofuels is the significantly lower land requirement for biomass production than that of the terrestrial crops (Chisti 2007). But most importantly, algae production does not require agricultural land. Utilization of algal biomass for renewable fuel production negates the ongoing “food vs. biofuel” debate.

Strain selection and evaluation is one of the critical steps in designing high efficiency algal systems. Overall technical viability of an algal production system hinges on the intrinsic properties of the selected

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algal strains. There are thousands of strains of algae that are being considered as biofuel feedstocks in the United States. Furthermore, there has been considerable interest in genetically modifying algae to improve biomass productivity and properties (Beer *et al.* 2009). The lack of information on the invasive potential of algae species that are in existence and being genetically modified is disconcerting (Glaser and Glick 2012). Risks associated with the cultivation of nonnative or modified algae strains on natural ecosystems and the potential impacts on biodiversity are not well understood. There are concerns that introduction of nonnative strains may change community dynamics and monopolize natural ecosystems. Indeed, several states have already started working on legislations that will regulate cultivation of biofuel feedstocks including microalgae. A recent example to such an initiative is the HB 7117, "Florida's Energy Bill" (Florida House of Representatives 2012). According to the new law "nonnative algae and cyanobacteria including genetically engineered algae, cannot be cultivated at a scale of more than two contiguous acres without a special permit". It is expected that commercial cultivation of native strains that are naturally present in local environment would be more adaptable to an algal production system than the nonnative strains and minimize the impact of algal biomass production on natural ecosystems.

According to a report released by U.S. Department of Energy, the southern U.S. states have substantial potential to establish high productivity algal production systems (U.S. Department of Energy 2010). Oklahoma is identified as one of these states based on the availability of high solar radiation, water resources and suitable land and climate for microalgae cultivation. This study evaluates the biomass production potential of microalgae strains native to Oklahoma.

Materials and Methods

Strain Selection and Cultivation

Microalgae strains investigated in this study were obtained from the Culture Collection of Algae at the University of Texas at Austin (UTEX). *Picochlorum oklahomensis* (UTEX B 2795), *Nannochloropsis oculata* (UTEX LB 2164) and SP 19, *Dunaliella sp.* and SP 20 *Dunaliella sp.* were grown in modified artificial seawater medium, Erdschreiber's medium and 5% F2 medium, respectively. The medium chemical compositions can be found at UTEX web site (UTEX 2012). The algae cultures in 2 L glass bottles (Kimble Chase Life Science and Research Products LLC, Vineland, NJ) were kept in a closed growth chamber with inner dimensions of 118.8 cm × 58.4 cm × 76.9 cm (Length × Height × Width). The growth chamber was maintained at 23 ± 4 °C. Four cool white fluorescent bulbs (General Electric Company, Fairfield, Connecticut) installed in the chamber were the light source, and the photosynthetic photon flux (PPF) was 56 ± 4 μmol x m^{-2} x s^{-1}, measured by a quantum meter (model QMSW-SS, Apogee Instruments Inc., Logan, UT). Cultures were subjected to a 12 h: 12 h cycle in which 12 h was light with aeration while the other 12 h was dark without aeration. The aeration rate was 50 mL/min and the concentration of CO_2 (Industrial Carbon Dioxide, Airgas, Stillwater, OK) in the air (Grade D Breathing Air, Airgas, Stillwater, OK) was 5%. The initial cell concentration of the culture medium was 7.6×10^4 cells/mL of medium.

Strain Characterization

Microalgae growth curves were determined as a function of absorbance (ABS) vs. time. The ABS of the sample was measured at 680 nm by a spectrophotometer (model DU 520, Beckman Coulter, Brea, CA). The pH of culture suspensions was measured daily by a pH meter (model AR20, Fisher Scientific, Waltham, MA). Cell density and dry biomass concentration were determined regularly. Oil content in the biomass was determined according to Lee *et al.* (1998). Algal biomass used for characterization tests was separated from the culture medium by centrifugation (Zhu 2012).

Flocculation Tests

Flocculation tests were carried out when algal biomass concentration reached its maximum in the culture medium (stationary phase). Three methods, pH adjustment, biopolymer addition and electroflocculation, were examined (Zhu 2012). The pH of the algae suspensions were adjusted by using either 1 M sodium hydroxide (NaOH) or 1 M hydrochloric acid (HCl). The biopolymer screening test was carried out using three non-toxic biopolymers: chitosan, sodium alginate, and cationic starch. For electroflocculation tests two aluminum plates each having a 40 cm^2 effective surface area were used as electrodes and the spacing between them was 4 cm. The effects of processing parameters, treatment time (5-20 min), current (0.2-0.8 A) and settling time (0.5-12 h) on flocculation efficiency were examined.

Results and Discussion

Microalgae strains PO, SP 19 and SP 20 are native to Oklahoma and isolated from Great Salt Plains, OK. NO is examined as a reference strain because not only some literature is available on its growth pattern and biomass characteristics but also there is some interest in utilization of this strain for commercial applications.

Each culture was grown in a medium that was optimized for the corresponding strain (UTEX 2012). Initial pH of the medium was 8 for SP19, SP 20 and PO and 7.5 for NO. A sharp drop in medium pH was observed during the first week of algal growth for all strains (Figure 1). Then medium pH stabilized around 7 except that for NO which continued to decline after a brief increase. Cell proliferation was much faster for PO than that for SP 19 and 20 (Figure 2). Although initial culture inoculation rates were similar for all the strains (7.6×10^4 cells/mL of medium), the number of PO cells in the medium was over two orders of magnitude higher than the other strains during the exponential growth phase. For PO cells the lag phase was slightly less than one week and exponential phase lasted about two weeks (Figure 3). At the stationary phase biomass concentration in the medium was highest for PO, 2.1 g/L, followed by NO, 1.2 g/L among the strains examined in this study.

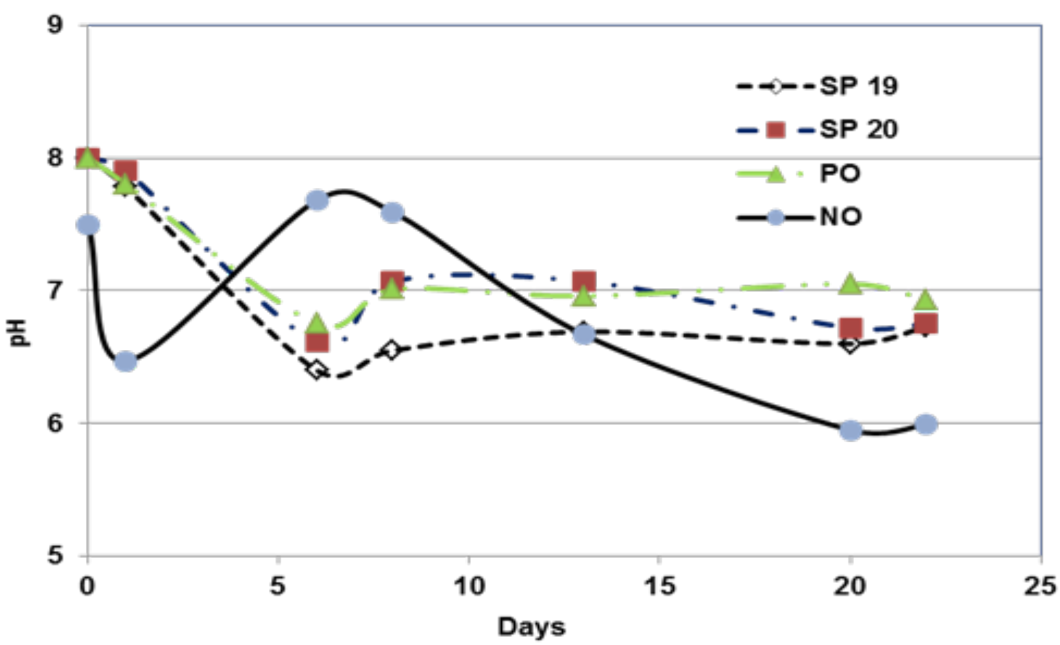

Figure 1: Culture media pH during algal growth

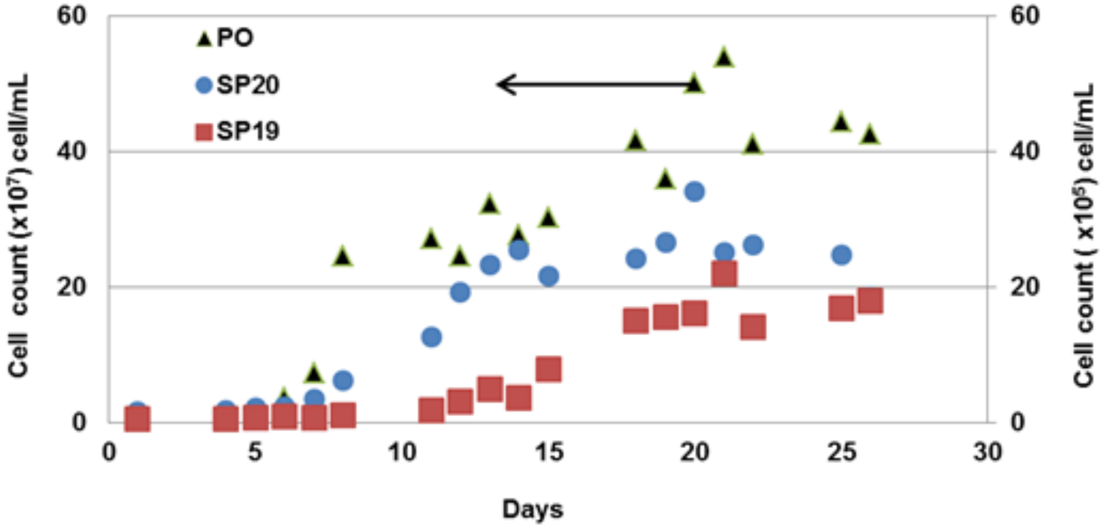

Figure 2: Cell growth pattern for three algae strains.

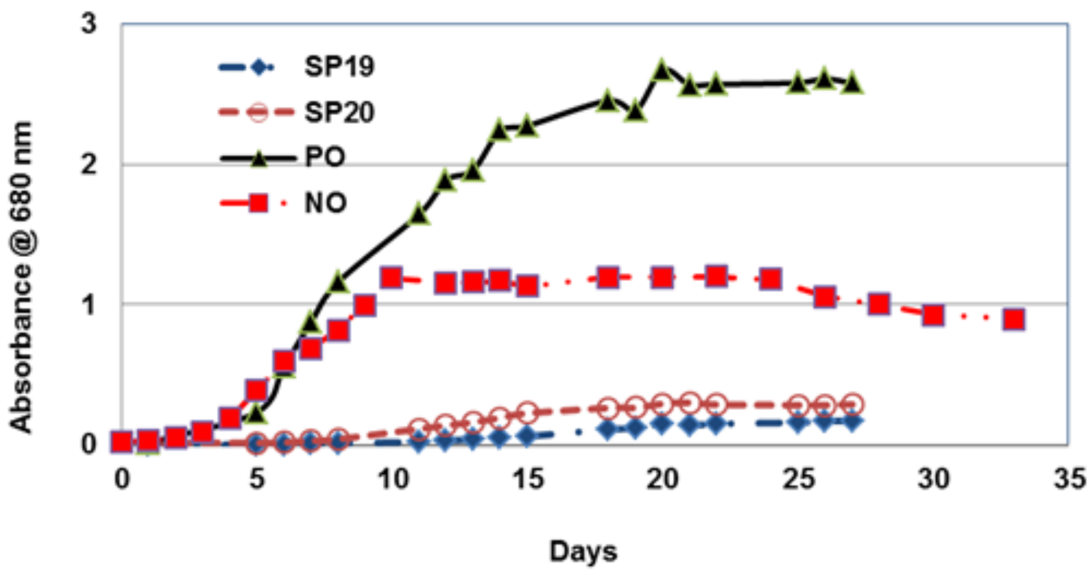

Figure 3: Algal growth pattern of algae strains as a function of light absorbance.

The oil content of the algal biomass varied between 17.9% to 36.4% depending on the strain and the growth phase and conditions. The oil content in NO biomass harvested during stationary phase was about 36.4 % (w/w, dry basis). This value was within the range of NO oil content (8-50 %) reported in literature (Brown 1991; Chiu *et al.* 2009; Converti *et al.* 2009). Oil content in NO biomass was two times higher than that for PO (20.5 %). But at the same time, the maximum biomass concentration of PO (2.1 g/L) was nearly twice as much as that of NO (1.2 g/L). Therefore, considering both biomass concentration and oil content in biomass, PO and NO contained the same amount of oil per volume of culture solution (0.431-0.436 g oil/L of culture medium). The oil contents of PO and NO biomass are comparable to the oil content in common oilseeds, 18-70 % (Salunkhe *et al.* 1992), indicating that PO and NO can be viable feedstocks for oil production.

All three flocculation techniques, pH adjustment, biopolymer addition and electroflocculation, were effective in harvesting PO and NO cells. A sharp increase in flocculation efficiency was observed over pH 10. Between pH 11 and 13, the flocculation efficiency of over 90% can be achieved (Figure 4). Although chitosan was a more effective biopolymer to flocculate PO and NO cells than sodium alginate and cationic starch, the large amount of polymer required to achieve flocculation efficiencies over 90% adversely affects the economic feasibility of this technique. The highest electroflocculation efficiency for PO, 99.7 %, was obtained at current of 0.8 ampere, treatment time of 15 min and settling time of 12 h. NO required shorter treatment time, 4 min, to achieve 99.4% flocculation efficiency.

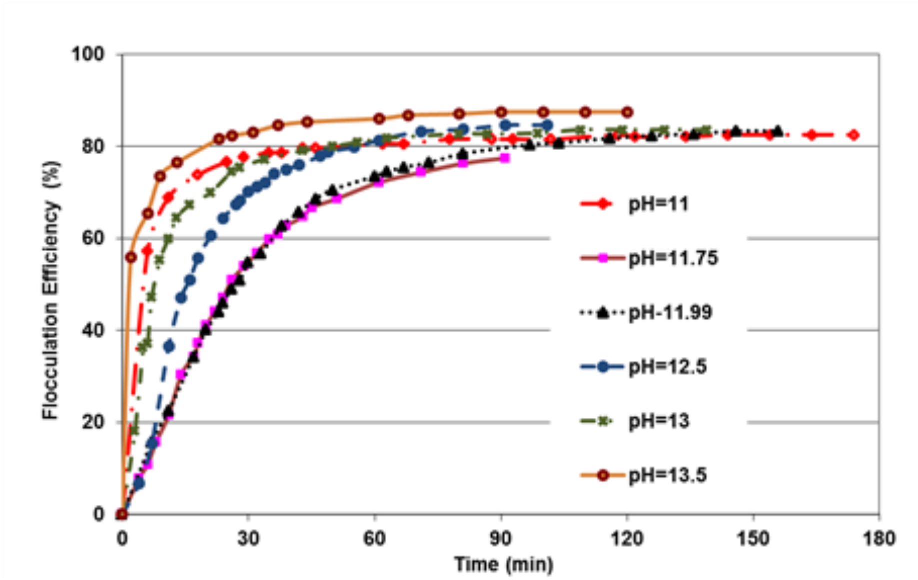

Figure 4: Flocculation efficiency of PO by pH adjustment

This study demonstrated that PO, a strain native to Oklahoma, is a robust strain with high biomass productivity and oil content similar to soybean, an oilseed commonly used in food and industrial bioproducts. PO cells can be effectively harvested by adjusting medium pH and settling. Our preliminary tests indicate that PO also grows well on swine lagoon waste water without additional nutrients. Further research is needed to examine the growth pattern of PO in open ponds.

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2.17
SWITCHGRASS YIELD RESPONSE TO NITROGEN ON FOUR SOIL TYPES IN WEST TENNESSEE

Christopher N. Boyer[1,], Roland K. Roberts[1], James A. Larson[1], Vivian Zhou[1], Burton C. English[1], Donald D. Tyler[2]*

Abstract
Research indicates that different soil types and landscapes can impact switchgrass yield response to nitrogen (N). Little is known, however, how these landscapes influence profit-maximizing N rates. Our objective is to determine profit-maximizing N rates for switchgrass produced on four landscapes in Tennessee. Switchgrass yields from 2005-2011 at one location in West Tennessee are analyzed. At the location, switchgrass was planted on four landscapes that range from well-drained to poorly-drained soils. Yield response to N is modeled using a linear response stochastic plateau function and the parameter estimates are used to find the profit-maximizing N rate for each soil type/landscape. For switchgrass grown on the well- to moderately-drained upland soil, the linear stochastic plateau response predicts the profit-maximizing N rate was 63-82 kg N ha^{-1}. For switchgrasss grown on the moderately- to well-drained flood plain soil, the profit-maximizing N rate was 61-69 kg N ha^{-1}. On the moderate- to somewhat poorly-drained eroded sloping upland soil, the profit-maximizing N rate was 97-109 kg N ha^{-1}. For poorly-drained flood plain soil the profit-maximizing N rate was 153-165 kg N ha^{-1}. The profit-maximizing N rates range with the recommend N rate for switchgrass producers in Tennessee.

Keywords: Landscapes; Linear Response Stochastic Plateau; Nitrogen; Profitability; Switchgrass; Tennessee

Introduction
Annual applications of N fertilizer are needed to produce yields large enough to make growing switchgrass (*Panicum virgatum* L.) for lignocellulosic biomass economically viable, but little attention has been given to determining the N rate that maximizes producers' profits. Several agronomic papers have estimated yield-maximizing N rates for switchgrass using analysis of variance on expected yield response to various N rates. The results have varied depending on spatial and temporal factors. A complete literature review of these studies can be found in Haque et al. (2009). A few papers have gone beyond using the analysis of variance approach by imposing a response function to find the yield-maximizing N rate for switchgrass (Aravindhakshan et al. 2011; Haque et al. 2009; Muir et al 2001; Vogel et al. 2002). However, these previous studies have discounted the influence of soil quality and landscape on switchgrass production (Fike et al., 2006).

The objective of this research was to determine the profit-maximizing N rates for switchgrass grown on four landscapes in Tennessee. For each landscape, the yield response to N was modeled using Tembo et al.'s (2008) linear response stochastic plateau function. The results should improve profit-maximizing N rate recommendations for switchgrass production for different landscapes.

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Data

Switchgrass yield data were obtained from experiments at the University of Tennessee Milan Research and Education Center at Milan, TN. Four landscapes were selected to represent the predominant landscapes found in West Tennessee: (1) a well-drained level upland (WDLU); (2) a well- to moderately well-drained flood plain (WDFP); (3) a moderate- to somewhat poorly-drained eroded sloping upland (MDSU); and (4) a poorly-drained flood plain (PDFP). The WDLU and WDFP landscapes are well suited for row crop production in Tennessee while the MDSU and PDFP landscapes represent marginal land for crop production in Tennessee. More details about the soil types can be found in Mooney et al. (2009).

A randomized complete block experiment with a strip-plot arrangement of treatments and four replications was designed for each landscape. The 'Alamo' cultivar was established in 2004. In 2005, blocks were split into strips for N fertilization at four rates: 0, 67, 134 and 200 kg ha^{-1}. Plots were harvested annually following senescence from 2005-2011.

To find the profit-maximizing N rate, we have to assume prices for switchgrass and N. Since there is no established market for switchgrass, the price of switchgrass was based on breakeven prices from the literature (Griffith et al. 2011; Mooney et al. 2009). From the results of these studies, we selected three prices of switchgrass: \$40, \$60, and \$80 Mg^{-1}. The average price of N from ammonium nitrate from 2005-2011 was selected to be the price of N, \$1.30 kg^{-1} (USDA NASS 2011).

Methods

Following the profit-maximization framework, the switchgrass producer is risk-neutral with the objective of maximizing expected net returns. The producer's objective can be expressed mathematically as

$$\max_{x} E(\pi) = pE(y) - rx \qquad [1]$$

where $E(\pi)$ is the producer's expected net returns in \$ ha^{-1}; p is the price of switchgrass in \$ Mg^{-1}; $E(y)$ is the expected switchgrass yield in Mg ha^{-1} and is a function of N; r is the price of N in \$ kg^{-1}; and x is the quantity of N applied in kg ha^{-1}.

Tembo et al. (2008) extended the conventional linear response plateau by incorporating a normally distributed year random effect in the plateau. They emphasized the effect of stochastic weather on crop yield response by including plateau-year and intercept-year random effects. The linear response stochastic plateau function assumes yield responds linearly to N until yield reaches its plateau. This response function was

$$y_{ti} = \min(\beta_0 + \beta_1 x_{ti}, \mu_m + u_t) + v_t + e_{ti} \qquad [2]$$

where y_{ti} is the switchgrass yield in Mg ha^{-1} in the tth year on plot i; β_0 and β_1 are coefficients; x_{ti} is the quantity of N applied in kg ha^{-1}; μ_m is the expected plateau yield in Mg ha^{-1}; $v_t \sim N(0, \sigma_v^2)$ is the year random effect; $e_{ti} \sim N(0, \sigma_e^2)$ is the random error term; and $u_t \sim N(0, \sigma_u^2)$ is the year plateau random effect. Independence was assumed across the three stochastic components. Tembo et al. (2008) determined the profit-maximizing N rate to be $x^* = \frac{1}{\beta_1}(\mu_m + Z_\alpha \sigma_u - \beta_0)$, where Z_α is the standard normal probability of $r/(p\beta_1)$ at the α level. The profit-maximizing yield was also derived by Tembo et al. (2008) as $E(y_{it}) = (1 - \Phi)a + \Phi(\mu_m - \frac{\sigma_u \varphi}{\Phi})$ where $a = \beta_0 + \beta_1 x^*$; $\Phi = \Phi[a - \mu_m/\sigma_u]$ is the cumulative normal distribution function; and $\varphi = \varphi[a - \mu_m/\sigma_v]$ is the standard normal density function (Tembo et al., 2008).

Results

Parameter estimates for the linear response stochastic plateau function on each landscape are presented in Table 1. For the WDFP landscape, the intercept was 10.14 Mg ha^{-1}, which was the expected yield with no applied N, and the expected plateau was16.29 Mg ha^{-1}, which was the yield when N was no longer a limiting input. The slope parameter estimate indicates that for every one kg ha^{-1} of N applied, yield increases 0.12 Mg ha^{-1}. The plateau random effect was not significant, suggesting the annual variation does not affect the plateau on this landscape. For the WDLU landscape, when no N was applied, the expected yield was 12.88 Mg ha^{-1}. If N was not a limiting input, the expected plateau was 17.50 Mg ha^{-1}, which is 7% higher than the plateau for the WDFP landscape. The parameter estimates for the MDSU landscape show that the expected yield was 7.31 Mg ha^{-1} when no N is applied, and when N was no longer limiting, the expected plateau was 17.93 Mg ha^{-1}. The expected plateau was higher on this landscape than on the WDFP and WDLU landscapes, but the intercept was lower. The slope parameter estimate suggests that switchgrass is more responsive to N on the MDSU landscape than on the WDFP and WDLU landscapes. For the PDFP landscape, the expected yield when no N was applied was 7.41 Mg ha^{-1}. When N was no longer a limiting input, the expected plateau was 15.33 Mg ha^{-1}. The establishment period was longer for the PDFP landscape causing the expected yields to be smaller than the other landscapes. The yield plateau random effect was not significant for this landscape, which indicates annual variability does not affect the plateau on this landscape.

Table 1. Parameter estimates for switchgrass yield (Mg ha^{-1}) response to N fertilization rates on four landscapes.

	Response Function			
Parameter	WDFP	WDLU	MDSU	PDFP
Intercept (β_0)	10.14***	12.88***	7.31***	7.41***
	(0.716)	(0.479)	(0.708)	(0.016)
N kg ha^{-1} (β_1)	0.12***	0.09***	0.13***	0.05***
	(0.017)	(0.009)	(0.007)	(0.002)
Yield plateau (μ_m)	16.29***	17.50***	17.93***	15.33***
	(0.949)	(0.315)	(0.455)	(0.352)
Intercept year random effect (u)	3.16	12.329***	9.06***	7.19***
	(2.131)	(2.526)	(1.716)	(0.720)
Plateau year random effect (v)	3.88	8.970**	9.28***	0.48
	(2.480)	(1.469)	(1.52)	(0.315)
Random error (e)	13.60***	9.48***	11.568***	7.42***
	(0.824)	(0.577)	(0.771)	(0.485)
−2 Log-likelihood	3084.1	2901.6	2507.4	2353.1

Note: Standard errors are in parentheses.
*Significant at the 0.1 level.
** Significant at the 0.05 level.
*** Significant at the 0.01 level.

Profit-maximizing N rates, profit-maximizing yields, and expected net returns were calculated using the linear response stochastic plateau estimates (Table 2). The profit-maximizing N rate for switchgrass on the WDFP landscape ranged from 61-69 kg N ha^{-1}, which is similar to what Haque et al. (2009) and Aravindhakshan et al. (2011) found. The expected yields on the WDFP landscape ranged from 15.96-16.16 Mg ha^{-1}, and the expected net returns ranged from \$559-1,202 ha^{-1}. On the WDLU landscape, the profit-maximizing N rate ranged from 63-82 kg N ha^{-1}, and the expected yields ranged from 16.76-17.21 Mg ha^{-1}. The profit-maximing N rates on the WDLU were also similar to what Haque et al. (2009) and Aravindhakshan et al. (2011) found. The expected net returns on the WDLU landscape ranged from \$588-\$1,270 ha^{-1}, which is higher than the WDPF landscape. The profit-maximizing N rate for switchgrass on the MDSU landscape ranged from 97-109 kg N ha^{-1}, the expected yields ranged from 17.48-17.74 Mg ha^{-1}, and the expected net returns ranged from \$572-1,278 ha^{-1}. The MDSU landscape produces the highest expected yields and net-return potential for switchgrass, even though it is considered a marginal landscape. For the PDFP landscape, the profit-maximizing N rate that ranged from 153-165 kg N ha^{-1}, the expected yields ranged from 14.90-15.18 Mg ha^{-1}, and the expected net returns for this landscape ranged from \$397-\$1,001 ha^{-1}. The profit-maximizing N rates were the highest for the PDFP landscape due to N dentrification and leaching from the poorly-drained quality of the landscape.

Table 2. Profit-maximizing nitrogen rates, expected switchgrass yields, and expected net returns by landscape for different switchgrass prices.

Switchgrass	Landscape			
Price (\$ Mg^{-1})	WDFP	WDLU	MDSU	PDFP
		Nitrogen rate (kg ha^{-1})		
\$40 Mg^{-1}	61.27	63.16	97.50	153.06
\$60 Mg^{-1}	66.24	74.76	104.36	160.73
\$80 Mg^{-1}	69.32	81.72	108.65	164.69
		Expected switchgrass yield (Mg ha^{-1})		
\$40 Mg^{-1}	15.96	16.76	17.48	14.90
\$60 Mg^{-1}	16.10	17.07	17.66	15.11
\$80 Mg^{-1}	16.16	17.21	17.74	15.18
		Expected Net Returns (\$ ha^{-1})		
\$40 Mg^{-1}	\$558.88	\$588.40	\$572.28	\$397.02
\$60 Mg^{-1}	\$879.71	\$927.31	\$923.93	\$697.53
\$80 Mg^{-1}	\$1,202.29	\$1,270.27	\$1,278.03	\$1,000.53

Note: The nitrogen price used was \$1.30 kg^{-1}.

Conclusions

The objective of this research was to determine the profit-maximizing N rates of switchgrass grown on four landscapes in Tennessee. We estimate switchgrass yield response to N using Tembo et al.'s (2008) linear response stochastic plateau function, which is unique to the switchgrass literature. The results indicate that the landscape can impact the profit-maximizing N rates, yield potential, and expected net returns, which are important to consider when recommending N rates to producers. The results also indicate the marginal landscapes can produce switchgrass yields that are comparable to the higher quality landscapes.

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2.18 BELOWGROUND COMPETITIVE INTERACTIONS WITHIN LOBLOLLY PINE-SWITCHGRASS CO-CULTURE

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Abstract

This study examines the capacity for combining the long-term productivity of a commercially important timber species, loblolly pine (*Pinus taeda* L.), with the annual yield of a dedicated bioenergy crop such as switchgrass (*Panicum virgatum* L.). Growing loblolly pine and switchgrass in co-culture could be a viable means of increasing the production potential of lands throughout the southern United States. However, co-culture production systems can inherently introduce the potential for strong competitive interactions. These interactions, occurring both above and belowground, may be especially important when incorporating fast growing herbaceous species between rows of newly established pine seedlings. This study was established in 2011 to examine how competition affects soil moisture availability and foliar nitrogen content within two newly established loblolly pine-switchgrass co-culture production systems in northeastern Mississippi. Volumetric water content within soils significantly varied by treatment, distance from tree row, and sampling depth. Foliar nitrogen content of loblolly pine significantly varied by treatment at one of our sites. Similarly, switchgrass foliar nitrogen content significantly varied by treatment at one site, and approached significance at the other. Switchgrass foliar nitrogen content was also significantly greater near non-vegetated buffer zones than in alleyways. Results of this study suggest establishing non-vegetated buffer zones around tree rows of newly established loblolly pine-switchgrass co-cultures may effectively increase soil moisture and foliar nutrient availabilities.

Keywords: belowground competition, co-culture, loblolly pine, switchgrass, volumetric water content, foliar nitrogen content

Introduction

This study investigates the capacity for combining the long-term productivity of a commercially important timber species, loblolly pine (*Pinus taeda* L.), with the annual yield of a dedicated bioenergy crop such as switchgrass (*Panicum virgatum* L.). Optimal growth of loblolly pine occurs on deep, well drained soils with high available moisture, and slightly acidic pH (Baker and Langdon 1990). Switchgrass is native to tallgrass prairies throughout most of the United States and grows well across a wide spectrum of sites (Parrish and Fike 2005). However, switchgrass makes its best growth on deep, well drained soils with moderate to high nutrient availabilities and pH ranges between 5.5 and 7. Incorporating an annually harvested biomass crop with a long-lived tree species (defined here as “co-culture”) could hold negative implications for the productivity and sustainability of loblolly pine-switchgrass co-culture on marginal soils. On the contrary, the co-culture concept may provide growth advantages by means of improved soil resource utilization (Jose 2009). Furthermore, the introduction of a deep rooted, perennial grass species to lands previously cleared for agriculture may provide environmental advantages such as improved wildlife habitat and carbon sequestration while simultaneously providing an annual source of revenue to the landowner along with an intermittent revenue stream from the trees. The overall objective of this research is to examine how interspecific competition in a co-culture setting affects soil moisture availability and foliar nitrogen content.

Materials and Methods

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Study Areas
Two locations in northeastern Mississippi were utilized to establish loblolly pine-switchgrass in co-culture. The first location, Pontotoc Ridge-Flatwoods Branch Experiment Station (34°07'N, 88°59'W), is approximately 1.6 ha in size and at 124 m elevation. The site has a history of agricultural use and likely residual fertility. Site preparation at Pontotoc began in May 2009 with conventional disking and harrow. Switchgrass was first sown in May 2009 by drill seeding at a rate of 6.7 kg ha^{-1} and additional switchgrass seed was sown and cultipacked in September 2009, May 2010, and July 2010 at rates of 6.7, 5.0, and 6.7 kg ha^{-1}, respectively. Loblolly pine rows were ripped to a depth of 40 cm in November 2010 and pines were planted in March 2011.

The second site is on the Noxubee Unit of the MSU John W. Starr Memorial Forest, located approximately 24 km south of the Mississippi State University campus (33°16'N, 88°53'W). This site is approximately 2 ha in size and is located at 88 m in elevation on a terrace of the Noxubee River in northern Winston County. This site has a history of forest use but has been maintained for several decades as a mowed pasture. Site preparation at Starr Forest consisted of a prescribed burn in March 2010 followed by liming in April 2010. Switchgrass was drill seeded in April 2010. Pine rows were subsoiled to a depth of 40 cm and loblolly pines were planted in March 2011.

Experimental Design
This study employs a randomized complete block design utilizing a low density planting configuration (1.5 m x 9.0 m spacing) of loblolly pine planted in rows and switchgrass sown in the alleys. Treatments include: 1) pine only, 2) switchgrass only, 3) pine planted directly into switchgrass, 4) pine planted into a 1.2 m non-vegetated zone (0.6 m on either side of the pines), and 5) pine planted into a 2.4 m non-vegetated zone (1.2 m on either side of the pines). Non-vegetated zones were established and maintained mechanically and with herbicides. Four replicates at each site were utilized for monitoring volumetric soil moisture and foliar nitrogen content. Volumetric soil moisture was measured at 7.5 and 20 cm depths at four distances in proximity to the tree row: 0 m (within the tree row), 0.6 m, 1.2 m, and 2.4 m. Volumetric water content values at 20 cm were transformed to account only for VWC between 7.5 and 20 cm. Loblolly pine foliar nitrogen was measured by sampling and compositing several needles from each tree within each plot. Switchgrass foliar N was assessed by collecting and compositing several new growth grass blades from areas bordering the non-vegetated buffers (competitive edges) and in the alleys (2.4 m from tree rows).

Results and Discussion

Volumetric Water Content
Volumetric water content in soils at both sites significantly varied by treatment, depth, and distance from the tree row. However, significant interaction effects between treatment and sampling distance from tree row ($p < 0.0001$, Figure 1) and treatment and depth of sampling ($p < 0.05$, Figure 2), indicated differences in VWC significantly varied among treatments. Mechanisms behind the treatment by distance from tree row interaction were primarily due to the treatments themselves, as areas kept non-vegetated for treatment differences generally had greater VWC than areas with switchgrass or pine coverage. The significant treatment by depth interaction at Pontotoc demonstrates differences in VWC were only noticeable at depth and were directly related to management intensity. The treatment by depth interaction at Starr follows the same general trends as Pontotoc, but at Starr differences in VWC were found at both sampling depths. A likely cause of the discrepancy in VWC by depth between the two sites is vegetative productivity. Switchgrass yields in the 2011 harvest at Starr were less than one-quarter of the switchgrass yields at Pontotoc and switchgrass establishment at Starr was patchy and mixed with a variety of grassland species while Pontotoc had excellent establishment and formed a nearly pure monoculture.

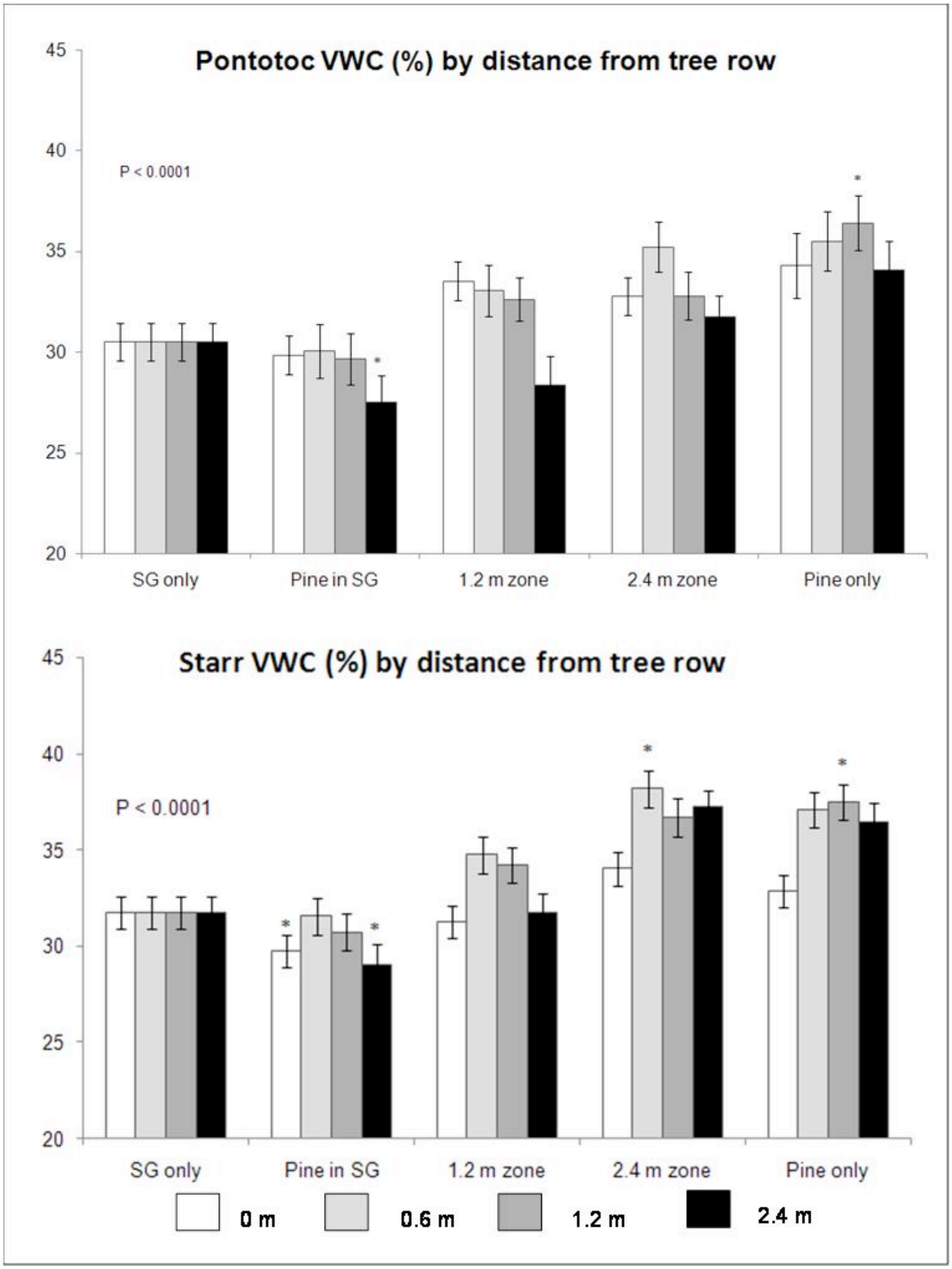

Fig. 1. Volumetric water content (VWC) at the Pontotoc and Starr sites by distance from tree row. Asterisks indicate significant differences in VWC according to Tukeys HSD.

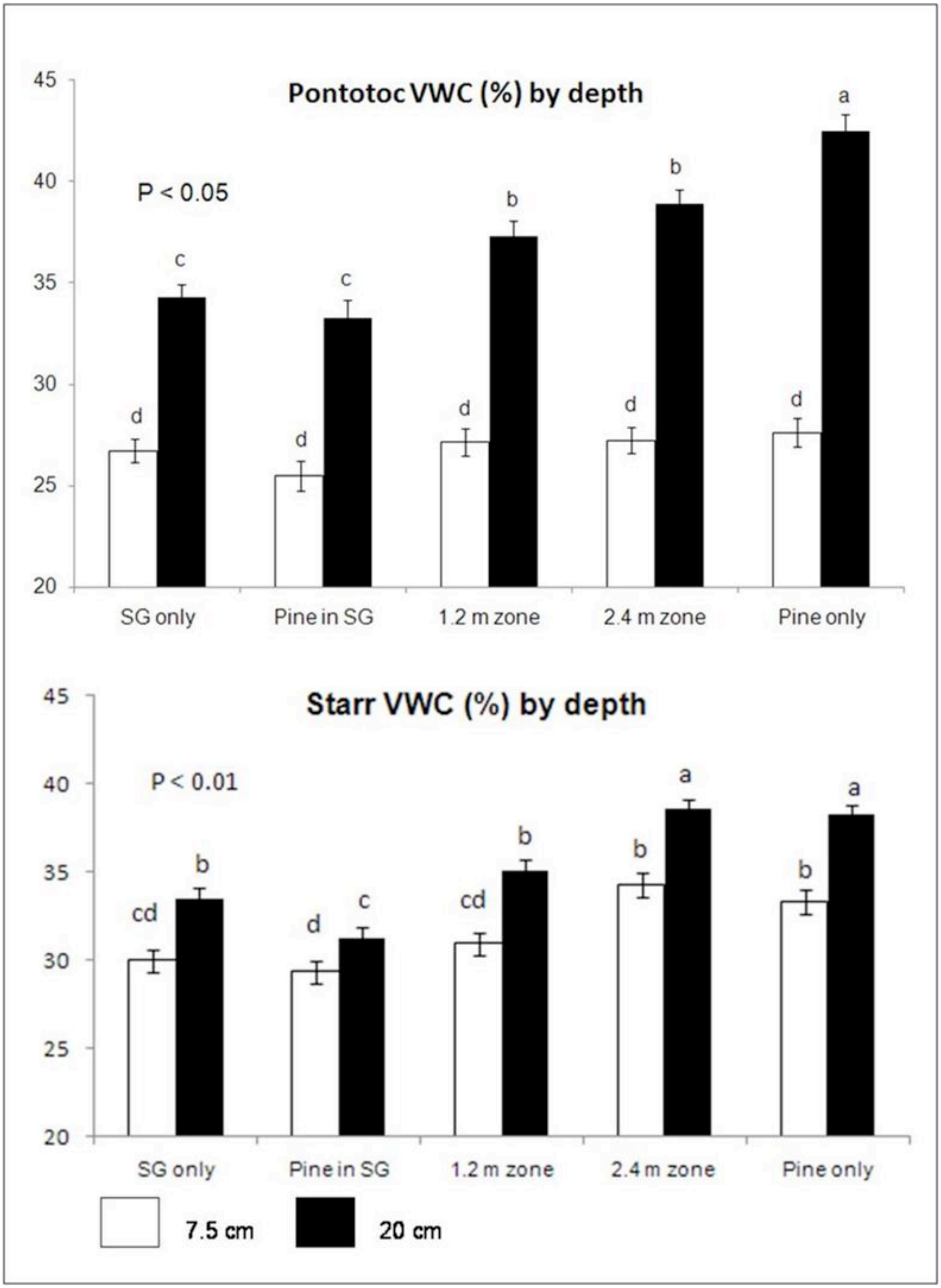

Fig. 2. Volumetric water content (VWC) at the Pontotoc and Starr sites by sampling depth. Letters indicate significant differences in VWC according to Tukeys HSD.

Foliar Nutrients

Loblolly pine foliar N significantly varied by treatment at Pontotoc ($p < 0.0001$). However, differences in loblolly pine foliar N at Starr were not statistically significant ($p = 0.66$). The lack of significant differences in foliar N at Starr in comparison to Pontotoc were most likely due to the lack of competitive pressures exerted by switchgrass at Starr and relatively poor switchgrass establishment. These differences may also have been due to a relative lack of inherent site fertility at Starr and less intensive site preparation techniques prior to study establishment.

Switchgrass foliar N significantly varied by treatment at Starr and approached significance at Pontotoc. Similarly, switchgrass foliar N significantly varied based upon competitive intensity at Starr but did not significantly vary at Pontotoc. There was a significant treatment by competition interaction effect at Starr ($p < 0.0001$) resulting from significantly greater switchgrass foliar N near non-vegetated treatment zones when buffers were present compared to foliar N measurements taken in alleys. The relatively low site fertility of Starr may have created larger demands for soil N and a greater foliar nutrient treatment response compared to Pontotoc. These results suggest that non-vegetated zones may effectively contribute to increased soil N availability, especially on marginal sites.

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3 FEEDSTOCK AGRONOMICS

3.1
RECENT DISCOVERIES AND DEVELOPMENT IN THE ENTOMOLOGY OF BIOENERGY CROP PRODUCTION

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Abstract
Since 2004 the pest insects of switchgrass (*Panicum virgatum*), prairie cordgrass (*Spartina pectinata*), and cup plant (*Silphium perfoliatum*) were studied in South Dakota and Wisconsin. The switchgrass moth (*Blastobasis repartella*) and the switchgrass midge (*Chilophaga virgati*) significantly reduce tiller biomass and destroy seed production on switchgrass. The cordgrass moth (*Aethes spartinana*) and the cordgrass bug (*Ischnodemus falicus*) together can produce devastating reductions in tiller biomass and plant health of prairie cordgrass. The giant eucosma (*Eucosma giganteana*) reduces cup plant biomass to subeconomic harvest levels. All of these pests produce economic injury levels within three years of planting in monocultural agronomic plantings, but are negligible in natural occurrences of eachplant species. Mixed species plantings involving switchgrass, prairie cordgrass, and cup plant have highly reduced infestation rates indicating the value of predators and parasites. Mixed plantings involving cup plant also provide a viable basis for maintaining or enhancing native bee pollinator populations. By producing floral and extra-floral resources the inclusion of cup plant in mixed species plantings is attractive to bees, as well as resources for predators and parasites of pest species. Our cumulative results and interpretations support ecological community stability models where more complex combinations of plants support a greater diversity of insects and a proportionately reduced loss of plant biomass. We predict that multispecies plantings for bioenergy production could be relatively free of pesticides and intensive management.

Keywords: cup plant, switchgrass, prairie cordgrass, Blastobasis repartella, Chilophaga virgati, Eucosma giganteana, native insects, biomass impact

Introduction
In the northern Great Plains and western Great Lakes regions switchgrass (*Panicum virgatum* L.), Prairie cordgrass (*Spartina pectinata* Bosc *ex* Link), and cup plant (*Silphium perfoliatum* L.) are the primary native plant species being studied as biomass feedstock crops. Published agronomic reviews on native species of plants, including these three species, typically summarize plant adapted insect associates as either seemingly nonexistent or limited in diversity and largely inconsequential (e.g., Vogel 2004, Parrish & Fike 2005, DOE 2006). This lack of recognition of host specific phytopages provides opportunities to make numerous new biological discoveries, specifically: species new to science, new arthropod/plant associations, new host/parasite relationships, new recognition of pest potential, calculations on potential biomass loss, new pollinator records and associations, new understanding on the nature of native prairie communities involving switchgrass, and new hypotheses on management of native species of arthropod on agronomic stands of native plant species. Most of these new discoveries are so recent that publication of findings remains pending.

Reports of insect associates of all three plant species are in the insect taxonomy literature with host records since the late 1800's in some cases. Ainslie's (1917) study of the cordgrass moth may be the first

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substantive ecological study involving our three target plants, with Johnson and Knapp (1996) providing the first study of the agronomic impact of the cordgrass bug (*Ischnodemus falicus* Van Duzee). The long lapse of focused study is closely correlated with the height of prairie plow-up, rural development and urbanization in the Great Plains and Midwest, causing the realized catastrophic ecological destruction and fragmentation of native communities with delayed research on their insect species.

All native plants have suites of insect and other arthropod species that feed upon them and move with their native plant hosts into agronomic environments where they express new pest dynamics. Bringing these arthropods into a cultivated monocultural environment provides ecological releases that are relatively rare in natural communities. In agronomic plantings of switchgrass, prairie cordgdrass, and cup plant insect predation is the single most important limiting factor to the maximization of biomass feedstock production. Studies on geographic differences in insect diversity, specific potential pests, insect-plant interactions, and predator/parasitoid guilds are only recently begun.

Switchgrass, *Panicum virgatum*

Nyoka et al. (2004) reported tiller damage by what became the first known host specific pest of switchgrass and was shortly thereafter recognized as *Blastobasis repartella* (Dietz) (Lepidoptera: Oecophoridae), the switchgrass moth (Adamski et al. 2010, Prasifka et al. 2010). The switchgrass midge *Chilophaga virgati* Gagné (Diptera: Cecidomyiidae) was discovered as a species new to science (Boe and Gagné 2011), and is probably the most destructive insect on switchgrass by causing 100% seed loss on infested tillers and c. 65-70% biomass reduction per tiller.

Adamski et al. (2010) reported the only known parasitoid of the switchgrass moth, *Bassus difficilis* Muesebeck (Hymenoptera: Braconidae) which was found recently in high numbers in the switchgrass canopy. The first known parasitoids of the switchgrass midge, a *Platygaster* sp. (Hymenoptera: Platygastridae), is new to science, as is at least one species of *Aprostocetus* (Hymenoptera: Eulophidae). Future work will elucidate the impact of these parasitoids on their hosts and the relative amount of seed and biomass production protected by their natural biocontrol relationship. A *Steneotarsonemus* (Acari: Tarsonemidae) mite is now know to feed on the adaxial surfaces of leaf sheaths. This is apparently a species new to science.

Prairie Cordgrass, *Spartina pectinata*

In South Dakota, *Ischnodemus falicus* (Say) (Hemiptera: Lygaeidae), the cordgrass bug, occurs in high numbers in natural and agronomic plantings, apparently producing two generations annually and frequently causing near total loss of spring growth. The adults overwinter and cause extensive damage to maturing tillers, impacting both seed viability and biomass production.

The cordgrass moth, *Aethes spartinana* (Barnes and McDonnough) (Lepidoptera: Tortricidae) has a larva that feeds in the spikelets (Ainslie 1917, Prasifka et al. 2012) and on the embryonic caryopses. Prasifka et al. (2012) reported infestation rates upwards of 70% per spike. In South Dakota, seed loss has probable normal rates of 15-20% per spike by the first two larval instars that apparently compete for food with a newly discovered gall midge larva (Cecidomyiidae). It now appears that the combination of the cordgrass moth larva and the midge, which is a species new to science, may be the cause of widely reported seed production and viability problems of prairie cordgrass. The first known parasitoid of the cordgrass moth is a species of *Euderus* (Hymenoptera: Eulophidae) and is new to science. Also new to science is a species of *Aprostocetus* wasp was found parasitizing the larvae of the new to science gall midge. Another undetermined parasitoid wasp may be parasitizing the larva of the cordgrass moth. In both South Dakota and Wisconsin, we found another *Steneotarsonemus* mite on the adaxial surfaces of leaf sheaths. This is apparently a species new to science and related to the other new species discovered on switchgrass.

Cup Plant, *Silphium perfoliatum*

The larva of the giant eucosma moth, *E. giganteana* Riley is the primary cause of massive damage to cup plant (Johnson and Boe 2012). This insect is found in all South Dakota agronomic and wild situations, but the incidence of damage in wild cup plants is negligible. In both South Dakota and Wisconsin this moth produces comparable levels of damage in plants on dry sites that is persistent, but plants in mesic sites express visually significant recovery from earlier meristem feeding damage by lateral growth. All plantings of two years of age or older are infested. Early summer damage is done by the first three larval instars feeding in the apical meristematic tissues, including floral buds. There are commonly 35-60 individuals per meristem that typically kill the apex and stop most stem growth, resulting in a 100% floral loss in most stands by late June to late July. Crude biomass loss can exceed 50% on infested sites. Some stems will produce lateral inflorescences in August that will mature, but usually at a population rate of 5-10%, if at all, on dry sites. Plants on mesic sites can produce profuse lateral growths that compensate for the damaged original leader. In late July and early August the larva leaves the stem apical feeding site and moves to the stem base ≤1.5 cm above ground level and burrows into the rhizome where it remains and feeds until winter. In early spring it spins a cocoon within the rhizome void created by feeding and the adult usually emerges in mid to late June. There are no known parasites, but the larval mortality rate ≥98% per plant.

Larvae of gall-making wasps of the genus *Antistrophus* (Hymenoptera: Cynipidae) feed in cup plan stem pith in South Dakota and Wisconsin. These wasps are parasitized by other wasps, mostly *Tetrastichus* sp. (Hymenoptera: Eulophidae), but parasitism rates and species identity require elucidation.

The larva of the tumbling flower beetle, *Mordellistena* cf. *aethiops* Smith (Coleoptera: Mordellidae) tunnels the stem pith and can cause extensive decay. This beetle appears not uncommon in southern Wisconsin.

Cup plant possesses characteristics of a keystone forb supporting native pollinators. A minimum of 12 species of native bee, fly, and beetle are already known from agronomic cup plant plots, with numerous others from wildland sites.

Summary

Observations and experimentation to date indicate biodiverse groups of arthropods on each species of native plant. In combination on switchgrass, *B. repartella* and *C. virgati* may induce upwards of 25-30% loss of potential biomass per plant in monocultural plantings. Higher levels of annual biomass loss can be expected on Prairie cordgrass infested with both *I. falicus* and *A. spartinana*. Cup plant losses in monocultural agronomic conditions will devolve to nearly 100% lost of seed crop and 50-60% loss of crude biomass due to abbreviated growth, failure of inflorescences, and overall lack of vigor. Extensive larval damage to rhizomes diminish the carbon-sink capacity of cup plant.

Experiments remain necessary to evaluate the plant community complexity to bring loss rates close to natural negligible levels without the use of costly production additives. The impact of the *Steneotarsonemus* mites on each grass is yet to be measured. Similarly, the parasitism rates by the various wasps on their hosts and the relationship to seed and biomass productivity of the plants is unmeasured.

Preliminary data suggest that cup plant intermixed with switchgrass and prairie cordgrass is a viable augmentative species for supporting and enhancing late season native pollinator guilds. Further, sampling in mixed plantings of grasses and forbs indicate that biodiverse plantings can simultaneously enhance native pollinator and parasitoid/predator guilds, and reduce pest species impacts while producing superior biomass feedstock performance.

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3.2
DISEASE CONCERNS IN ENERGYCANE

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Abstract
Diseases may be a limiting factor in the production of energycane, a perennial crop, by reducing annual yields and reducing the longevity of the crop cycle. Disease concerns also include the potential that a compatible pathogen could spread between energycane and sugarcane, sorghum, or corn. Widespread planting of energycane in the southeast may provide a corridor for transmission of diseases such as orange rust caused by *Puccinia kuehnii* throughout the entire sugarcane and energycane industries. Energycane cultivars developed at the USDA-ARS Sugarcane Research Unit are the result of crosses between clones of *Saccharum* spp. (primarily *S. spontaneum*) or near relatives and commercial sugarcane cultivars, thus energycane will likely be affected by current sugarcane diseases. Parents and progeny are screened for resistance to the major sugarcane diseases affecting U.S. sugarcane including smut caused by *Sporisorium scitamineum*, mosaic caused by *Sugarcane mosaic virus* and *Sorghum mosaic virus*, and leaf scald caused by *Xanthomonas albilineans*. Parental clones have also been screened for susceptibility to ratoon stunt caused by *Leifsonia xyli* subsp. *xyli*, a disease controlled by planting pathogen-free cuttings and preventing mechanical spread of the pathogen during harvest. Genetic variability of pathogens is another concern. For example, finding durable resistance to brown rust caused by *P. melanocephala* has been difficult to achieve because of the rapid development of new races of the rust pathogen. Research is also being conducted to determine the influence of different cultural practices, such as soil fertility and crop residue management, on the development and severity of diseases in energycane.

Keywords: biomass feedstock, diseases, pathogens

At the USDA, ARS Sugarcane Research Unit in Houma, LA, genetically wide crosses between sugarcane varieties and wild accession of *Saccharum* spp. are made to broaden the genetic base of sugarcane. Modern sugarcane varieties are hybrids of *S. officinarum* (the high sucrose, low fiber cane that was distributed and cultivated throughout the world for centuries) and wild species of *Saccharum*, primarily *S. spontaneum*. The initial crosses often produce progeny that have superior vigor and greater biomass than the parents; however, low sucrose and high fiber content makes them unacceptable for sugar production. Additional crosses are made between selected progeny and sugarcane varieties to increase sucrose content and reduce fiber content while maintaining the desirable traits of the wild accessions, such as increased tillering, better ratooning ability, disease resistance, and tolerance to environmental stresses. In the past, the early generation progeny of this program were discarded; however, those with high biomass are now retained as potential feedstock candidates. These candidate feedstock clones are referred to as energycanes. Intergeneric hybrids between *Saccharum* and *Erianthus* and *Miscanthus* have also been made and, along with interspecific *Saccharum* hybrids, are being explored as biofuel feedstocks that have expanded geographic adaptation, increased biomass, and improved ratooning ability. To date, four energycane varieties have been released as potential biofuel feedstock, L 79-1002 (Bischoff, 2008), HoCP 91-552 (Tew *et al*., 2011), Ho 00-961 (White *et al*., 2011), and Ho 02-113 (Hale *et al*., 2012). A number of other clones are at different stages of testing for future release.

Diseases may be a limiting factor in the production of energycane, by reducing annual yields and limiting the longevity of the perennial crop cycle. For example, susceptibility to sugarcane smut caused by *Sporisorium scitamineum* has limited productivity of L 79-1002, particularly as the disease incidence

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increases into the ratoon crops. Sugarcane producers have also expressed concern that an infection in an energycane crop will put added pressure on sugarcane varieties that are moderately susceptible to the disease.

Because sugarcane is a common parent of the energycanes, parents and selected progeny are screened for resistance to the major sugarcane diseases affecting U.S. sugarcane. Artificially inoculated trials include screening for susceptibility to smut, mosaic caused by *Sugarcane mosaic virus* (SCMV) and *Sorghum mosaic virus* (SrMV), and leaf scald caused by *Xanthomonas albilineans*. All experimental plots are monitored for natural infection of these pathogens, as well as, natural infection of brown rust caused by *Puccinia melanocephala* and yellow leaf caused S*ugarcane yellow leaf virus*. Orange rust caused by *P. kuehnii* was observed for the first time in the Western Hemisphere in Florida in 2007 (Comstock *et al*., 2008). The first observation of orange rust on sugarcane in Louisiana was made in 2012 on the recently release Ho 05-961 (*unpublished*). Plants of this variety have been in Florida for several years where it showed susceptibility to the orange rust pathogen. The effect that orange rust will have on sugarcane and energycane in Louisiana is unknown.

The wild parental clones have been screened for susceptibility to ratoon stunt caused by *Leifsonia xyli* subsp. *xyli*; however, susceptible clones are not necessarily eliminated because the disease in sugarcane has been effectively controlled with cultural practices (Grisham, 2004). The pathogen is spread mechanically with cutting tools and equipment. Control is achieved by planting pathogen-free cuttings and preventing mechanical spread of the pathogen during harvest by decontaminating equipment. Consequently, selecting for disease resistance to ratoon stunt has not been a major selection criterion in our sugarcane program. Three annual harvests are typical during a sugarcane crop cycle before the crop is destroyed and replanted. To increase the economic feasibility of an energycane crop, a greater number of ratoon crops will be needed. The increased number of harvests will provide a greater opportunity to introduce the ratoon stunt pathogen into a field. Resistance to ratoon stunt may be of greater importance in energycane varieties than in sugarcane varieties.

Genetic variability of pathogens is another concern. For example, finding durable resistance to brown rust caused by *P. melanocephala* has been difficult to achieve in sugarcane because of the rapid development of new races of the rust pathogen. Since 2003, 10 sugarcane varieties have been released to the Louisiana sugarcane industry. At the time of release, all were considered resistant to brown rust; however, as the varieties were increased to production levels, seven have been reclassified as susceptible or moderately susceptible to brown rust because of the development of new races of the pathogen. Genetic variability has also been observed in the viruses causing mosaic on sugarcane, SCMV and SrMV, for many years (Grisham, 2004). Studies of the populations of these two viruses revealed that at a given time the dominant strain of a virus is associated with the population of varieties being grown (Grisham and Pan, 2007).

Although disease information is abundant for *Saccharum* spp., information related to *Erianthus* and *Miscanthus* is limited. Several viruses are, however, important pathogens of *Erianthus* and *Miscanthus*, as well as, sugarcane hybrids and wild *Saccharum* spp. (Lapierre and Signoret, 2004). *Maize dwarf mosaic virus*, a major pathogen of maize and sorghum, infects *Erianthus*, *Miscanthus*, and wild *Saccharum* species. *Barley yellow dwarf virus*, a pathogen of all cultivated small grain species, occurs naturally on *Miscanthus*. SCMV and SrMV are not only important pathogens of sugarcane, but also maize, sorghum, and many other graminaceous hosts including *Erianthus*. A strain of SrMV was recently discovered infecting a clone of *Miscanthus sinenesis* in our parental collection (Grisham *et al*., 2012). Although *Sugarcane yellow leaf virus* infects most *Saccharum* spp., susceptibility of *Miscanthus* and *Erianthus* is unknown. Planting virus susceptible feedstock crops will affect not only their productivity, but, if grown in proximity to other graminaceous crops, provide a source of virus pathogens that can infect crops such as maize, sorghum, sugarcane, rice, forages, and small grains.

As the geographical range of energycane is expanded into north Louisiana, Texas, Mississippi, Alabama, Georgia, Florida, and south Carolina (Figure 1), the crop will be exposed to other pathogens that may not be present in the sugarcane production areas. As energycane farieties are grown across the Gulf Coast states, they may create a bridge between sugarcane industries in Florida, Louisiana, and Texas for the movement of diseases and insect vectors, as well as, insect pests. Trials planted in new areas where energycane may be grown need to be monitored carefully to detect diseases for which the varieties have not been tested.

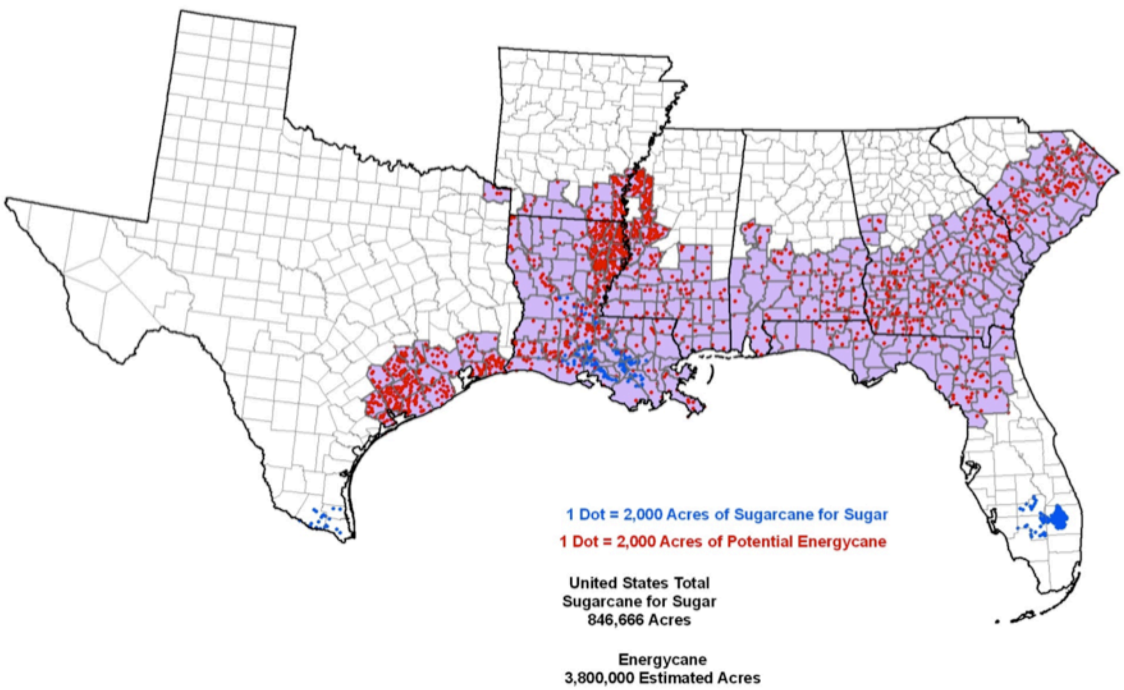

Figure 1. Potential geographical areas for energycane production.

Research is also being conducted to determine the influence of different cultural practices, such as soil fertility and crop residue management, on the development and severity of diseases in energycane. Examples of these studies include the effect of micronutrient fertilizers such as copper and nickel on the incidence and severity of brown rust and the interaction organic residue left on sugarcane fields following harvest and systemic diseases. The results will be reported at a future conference.

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3.3
STAND ESTABLISHMENT AND BIOMASS YIELD OF SWITCHGRASS IMPACTED BY SEVERAL SOIL- AND SEED-BORNE FUNGAL PLANT PATHOGENS

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Abstract
Naturally occurring soil-borne fungal pathogens of switchgrass have been isolated and identified from growers' fields and research plots in Tennessee. These fungi are typically difficult to control because they survive in soil for long periods under unfavorable conditions, even in the absence of host plants. Several of the fungi that were isolated are known pathogens of important grain and grass crops in the southeast, such as corn, wheat, sorghum, and turfgrass. Many of these pathogens are seed-borne and play a role in stand establishment problems, causing seed rots, seedling damping-off, reduced seedling vigor, and root rots. Fungal pathogens from commercial seed and field plants were characterized and identified. Rates of fungal infection among 30 switchgrass seed lots varied from <1 to 87%. Pathogens implicated in stand establishment problems and diseases of field plants in Tennessee include the following: *Alternaria alternata*, *Bipolaris oryzae*, *B. sorokiniana*, *B. spicifera*, *B. victoriae*, *Curvularia lunata*, *Fusarium acuminatum*, *F. equiseti*, *F. graminearum/ pseudograminearum*, *Pithomyces chartarum*, and *Sclerotinia homoeocarpa*. Pathogenicity and virulence of several *Bipolaris* species were determined in experimental studies with seeds and whole plants. The impact of these isolates on stand establishment, plant health, and biomass yield was measured. Although there were significant differences in the aggressiveness of isolates, even with low disease pressure, losses in biomass ranged from <5 to 70%. These studies serve to distinguish between minor pathogens with limited impact on stand establishment and biomass yield, from those that should be targets for disease resistance in switchgrass breeding programs.

Keywords: Alternaria alternata, Bipolaris spp., Fusarium spp., Panicum virgatum, Plant disease, Seed-borne pathogens, Sclerotinia homoeocarpa, Soil-borne pathogens, Stand establishment, Switchgrass

Pathogens of Switchgrass
Switchgrass is resistant to many pests and is capable of producing high yields with limited fertilizer (Parrish and Fike 2005). However, switchgrass is not resistant to all plant pathogens and information on potential disease problems is critical in order to develop sustainable, practical, and economical disease management practices that will enable growers to obtain optimal yields of high quality feedstock.

In 2008 we began isolating and identifying fungi from diseased foliage, stems, and roots of seedlings and mature 'Alamo' switchgrass plants grown in Tennessee (Fajolu *et al.* 2012, Vu 2011, Vu *et al.* 2011a, Vu *et al.* 2011b, Vu *et al.* 2011c, Vu *et al.* 2012, Zale *et al.* 2008). Most of the pathogens we isolated are known to be soil-borne, and some are considered seed-borne.

Soil-borne Fungal Plant Pathogens
Soil-borne pathogens typically have wide host ranges. These fungi are difficult to control because they are able to survive in soil for long periods, often under unfavorable environmental conditions, in the absence

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of a host plant. Some soil-borne fungi produce special, long-lived, overwintering structures, making it even more challenging to control the diseases they cause.

Our studies, over the last three years, indicate there are several fungal pathogens that may influence stand establishment, seedling vigor, and biomass production. Prevalent fungal pathogens isolated from switchgrass included *Alternaria alternata*, several species of *Bipolaris*, *Curvularia lunata*, several species of *Fusarium*, *Pithomyces chartarum*, and *Sclerotinia homoeocarpa* (Vu 2011). Pathogenicity of these species on switchgrass was confirmed. Isolates were identified based on morphological and genetic characters. While some of these fungi are secondary pathogens and may be considered nuisance and commonplace pathogenic species, others, such as species of *Bipolaris* and *Fusarium* have the potential to cause, or are possibly the major cause of poor stand establishment. In addition, many of these pathogens are known to cause disease in several agronomically important grain crops, including barley, corn, oats, rice, rye, sorghum and wheat (Table 1) (Farr *et al.* 2012).

Table 1. Soil-borne Pathogens of Switchgrass* in Tennessee that are Pathogenic on Various Grain Crops**

Fungal pathogen	Barley (*Hordeum vulgare*)	Corn (*Zea mays*)	Oats (*Avena sativa*)	Rice (*Oryza sativa*)	Rye (*Secale cereale*)	Sorghum (*Sorghum bicolor*)	Wheat (*Triticum aestivum*)
Alternaria alternata		X	X	X	X	X	X
*Bipolaris oryzae***				X			X
Bipolaris sorokiniana	X	X	X	X	X		X
Bipolaris spicifera	X	X	X	X	X	X	X
Bipolaris victoriae	X	X	X	X		X	
Curvularia lunata	X	X		X		X	X
Fusarium acuminatum	X	X	X		X	X	X
Fusarium equiseti	X	X	X	X	X	X	X
Fusarium graminearum	X	X	X	X	X	X	X
Fusarium oxysporum	X	X	X	X	X	X	X
Pithomyces chartarum		X		X			X

*Farr *et al.* 2012
**Vu 2011

Currently five species of *Bipolaris* have been reported from switchgrass in the U.S. (Farr *et al.* 2012). We isolated three of these, *B. oryzae* (Vu 2011), *B. sorokiniana* (Vu *et al*. 2011b), *and B. spicifera* (Vu et al. 2011a) from switchgrass field plants in Tennessee as well as a fourth species, *B. victoriae*, which is known mainly from oats and has not been reported on switchgrass previously (Vu 2011). Our finding of *Bipolaris spicifera* on switchgrass was a new report for the U.S. (Vu *et al.* 2011). This pathogen has also been found on turfgrass in Tennessee (Dr. Alan Windham, University of Tennessee, personal communication).

We have shown that representative isolates of the four *Bipolaris* species are virulent on 'Alamo'; however, there is variability in virulence among different species and among isolates of the same species. Inoculation of seed with *Bipolaris* species resulted in a significant reduction in percent plant stand. Depending on the species, even with low disease pressure (10 spores/100 seeds), plant stand was reduced from 38 to 52%. With higher disease pressure (100,000 spores/100 seeds), the reduction in plant stand ranged from 42 to 74%. *Bipolaris oryzae* had the greatest impact on plant stand, while *B. spicifera* had the least.

In addition to the negative impact on stand establishment, *Bipolaris* species reduced biomass. With low disease pressure (foliar spray of 10 spores/ml), *B. spicifera* reduced plant biomass by less than 5%, whereas the other three species were more virulent, reducing biomass by 64 to 70%. Higher disease pressure (foliar spray of 100,000 spores/ml), increased biomass reduction to 88% with *B. oryzae* and 67 to 68% for *B. sorokiniana* and *B. victoriae;* however, biomass was reduced by less than 5% with *B. spicifera*.

Seed-borne Fungal Pathogens

Since there is no certification program for switchgrass seed, the possibility exists that seed-borne pathogens of switchgrass are being widely distributed in the U.S. It is well-known that establishment of switchgrass plots can be problematic (Parrish and Fike 2005). Soil-borne and seed-borne fungal plant pathogens likely play a significant role in the difficulty of establishing switchgrass stands.

To identify seed-borne pathogens on switchgrass, we sampled seed from 12 commercial sources in different regions of the U.S. Our survey included seven varieties of agronomic switchgrass for a total of 30 seed lots. After surface-sterilization to remove external seed contaminants, seed were plated onto microbiological culture medium to promote growth of fungi infecting the seed internally. Plates were incubated at room temperature and the resultant fungal colonies were identified based on morphological and genetic characters. The seed infection rates of the different samples varied widely, ranging from less than 1%, to more than 87%. Many of the same soil-borne pathogens that we had previously isolated from seedlings and mature plants from fields in Tennessee were isolated from infected seed obtained from commercial sources. The most frequently isolated pathogens from seed were *B. oryzae*, *A. alternata*, and *F. graminearum*. Other species of *Bipolaris* and *Fusarium* were present, but with lower frequency.

Conclusion and Future Directions

Several soil-borne pathogens have been identified from switchgrass plants and seeds that have the potential to negatively influence stand establishment and biomass yield. Eliminating these pathogens on seed should reduce pathogen spread and improve the quantity and quality of biomass.

We are currently conducting studies to determine the impact of *Fusarium* spp. on plant health, and subsequent biomass yield and quality. We are also investigating the effect of diseased plant tissue on the bioethanol conversion process and evaluating switchgrass varieties for disease resistance.

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3.4
EARLY TREATMENT-RELATED COMPETITIVE EFFECTS IN A LOBLOLLY PINE – SWITCHGRASS CO-CULTURE SYSTEM

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Abstract
A study examining biomass yields of switchgrass grown in co-culture with loblolly pine was established on two Upper Coastal Plain sites in Mississippi. The Pontotoc site has a history of agricultural use with some likely residual fertility, while the Starr site has a history of forest use and has been maintained for several decades as a mowed field. Treatments included switchgrass only and pine only, as well as pine planted into switchgrass with 0, 0.6, and 1.2-m competition free zones on either side of the row of pines. Second year switchgrass production differed substantially between sites, but within sites switchgrass yields per square meter of were not different. However, total switchgrass yield differed by treatments, with differences related to the width of the competition free zone around the pine seedlings. There were few treatment related differences in year-1 tree heights at the Starr site where switchgrass yields were less than one-quarter of those at the Pontotoc site. At the Pontotoc site, mean year-1 tree height in the pine only and 1.2-m treatment was greater than in the other treatments, averaging 18 cm taller than the 0.6-m treatment and nearly 40 cm taller than the 0-m treatment.

Keywords: biomass production, biofuel, competition, Panicum virgatum, Pinus taeda

Introduction
Considerable interest exists in utilizing plant biomass as feedstock for production of biofuels. In the southeastern U.S., switchgrass (*Panicum virgatum* L.) has been identified by DOE as a primary species for biofuels production due to its high productivty (McLaughlin et al. 1999, Sanderson et al. 2006). Switchgrass has several positive feedstock characteristics, including high productivity, relatively low demand for moisture and nutrients, high carbon sequestration potential, and several environmental benefits (Parrish and Fike 2005, Sanderson et al. 2006, Keshwani & Cheng 2009).

Switchgrass yields have been demonstrated in excess of 20 Mg ha^{-1} yr^{-1} under research conditions (Muir et al. 2001, Thomason et al. 2004, McLaughlin and Kszos 2005, Lemus et al. 2009). While operational yields would likely be less, Parrish and Fike (2005) suggest that sustained yields of 15 Mg ha^{-1} yr^{-1} may be possible in regions receiving sufficient precipitation. As native to tall-grass prairie ecosystems, switchgrass is capable of tolerating low resource availability. However, switchgrass is sensitive to competition, particularly at time of establishment (Bryan and McMurphy 1968, Evers and Parsons 2003, McLaughlin and Kszos 2005, Parrish and Fike 2005), and its best growth is attained where growth resources are not limited by competition.

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Current relatively low values for bioenergy feedstocks, as well as the lack of markets for such materials are primary reasons for landowner concerns, especially where higher valued crops are an option. The ability to combine annual revenues from a bioenergy crop with long-term revenue from a high valued crop may provide a greater incentive to landowners. In the Southeast, loblolly pine (*Pinus taeda* L.) is a species with high value in sawtimber production over relatively short rotations (i.e. 25-30yrs). This species has demonstrated the ability to grow well on sites with relatively low moisture and nutrient. Loblolly pine also has a history of being grown in co-culture with warm-season grass species in silvopastoral systems combining sawtimber production with either grazing or haying operations. Studies have found, however, that the pine overstory will increasingly reduce herbage production as pine stocking increases (Gaines et al. 1954, Halls 1957, Halls and Schuster 1965, Wolters 1973).

This study was established to examine the competitive interactions between switchgrass and loblolly pine grown in a co-culture system designed to combine annual production of a bioenergy feedstock with long-term production of a sawtimber crop. Our objective was to quantify the reductions in switchgrass yields and pine growth resulting from these competitive interactions.

Methods

Two study sites were established in northern Mississippi. The Pontotoc site was on a Mississippi State University Agricultural Experiment Station site approximately 75 km north of Starkville, MS. The soil on the site is primarily an Atwood silt loam. The site has a history of agricultural production and likely possessed some residual fertility. The Starr site is on the MSU John Starr Memorial Forest located approximately 20 km south of Starkville, MS. The soil on the site is primarily an Ora fine sandy loam. The site has historically contained forest cover, but has been as a mowed pasture for the past several decades.

Switchgrass was seeded at each site in spring of 2010. At Pontotoc site preparation and seeding consisted of disking, drilling seed, and cultipacking. The site was seeded three times in 2010 to ensure full establishment. At Starr site preparation and seeding consisted of prescribed burning, liming, and drilling seed. Containerized loblolly pine varietal seedlings were planted at both sites in March 2011. Pine seedlings were planted into subsoiled rows spaced 9.0m apart with 1.5m within row tree spacing. Measurement plots were 15m (i.e., 10 trees) by 9m (i.e., 4.5m on either side of the tree row).

The study consisted of five treatments: switchgrass only (SG), pine only (Pine), pine planted with switchgrass with a 1.2m competition free zone on either side of the row of trees (PS-4), pine in switchgrass with a 0.6m competition free zone (PS-2), and pine planted into switchgrass with no competition free zone (PS-0). Treatments were replicated eight times at each site.

Switchgrass was harvested between Jan. 31 and Feb. 9, 2012 at the Pontotoc site. At Starr the switchgrass harvest was initiated on Jan. 6, 2012 but was not completed until Mar. 14, 2012 due to harvesting equipment issues. Switchgrass was harvested and weighed fresh in four 1m strips on either side of the tree rows. At Pontotoc, subsamples were weighed fresh and dried to allow yields to be converted to a dry weight basis. At Starr the entire samples were dried to get dry weight yields. Per hectare dry weight yields of switchgrass were evaluated relative to proximity to the tree row (0-1, 1-2, 2-3, and 3-4m), and for total plot yield. Seedling heights were measured in early February at both sites.

Results

Second year per hectare yields of switchgrass differed substantially between the two sites. Establishment and growth at Pontotoc were much greater with per hectare dry weight yields for all treatments other than pine only averaging nearly 7.1 Mg ha^{-1} and ranging as high as 12.7 Mg ha^{-1}. Yields at Starr for treatments other than pine only averaged only 1.6 Mg ha^{-1} and much of the yield consisted of herbaceous plant material other than switchgrass.

At both sites, yields per square meter of established switchgrass did not differ by treatment. However, there were treatment related differences in total switchgrass yields, with the differences being related to the width of the competition free zone maintained around the tree rows. At Pontotoc, total plot yields in the SG and the PS-0 treatments did not differ, averaging 8.0 Mg ha^{-1}. Yields from these treatments were significantly greater than yields in the PS-2 and PS-4 treatments, which averaged 6.2 Mg ha^{-1}. At the Starr site, yields from SG, PS-0, and PS-2 treatments did not differ, averaging slightly over 1.7 Mg ha^{-1}. Treatment PS-4 had a significantly lower yield of 1.3 Mg ha^{-1}.

Year-1 trees heights at the Pontotoc site were greatest in the Pine and PS-4 treatments, averaging 84 cm. Heights in the PS-0 and PS-2 treatments were significantly less, averaging 46 cm and 66 cm, respectively. These values did not differ significantly. There were fewer and smaller treatment-related differences in year-1 tree heights at the Starr site. The overall mean height across treatments was 63 cm. The only treatments that differed statistically were PS-2 (68 cm) and PS-0 (59 cm).

Discussion

The switchgrass yields observed at Pontotoc are within the expected range for a 2-year-old well-established stand on a reasonably good site. Year-3 yields are expected to be higher, but we will have to wait to see if they approach the 15 Mg ha^{-1} suggested by Parrish and Fike (2005). Switchgrass yields at the Starr site have been surprisingly low. With less intensive site preparation, the switchgrass did not establish as quickly as at Pontotoc; but even where switchgrass did become established, its development has been slow. The cause of this slow development is not clear at this point. There may be a pH limitation, although the site has been limed once. Competition from other herbaceous species is likely a major factor, illustrating the importance of getting switchgrass quickly established on a site before competing species become established.

As expected, the 1-year-old trees did not affect the production of switchgrass. However, this is expected to change in the coming years as the trees begin to overtop the adjacent switchgrass and develop extensive root systems that more effectively compete with the switchgrass for belowground growth resources. Currently, the only treatment related effects on switchgrass production is stemming from the competition free zone around the tree rows. These zones take some portion of the site out of switchgrass production, thus reducing total per hectare production.

Not surprisingly, given the slow development of switchgrass, tree growth at Starr is showing no treatment effects due to switchgrass competition. At Pontotoc, however, competitive pressures from switchgrass is affecting tree growth. Trees provided with a 1.2 m competition free buffer did not suffer any growth reduction; but trees with only a 0.6 m buffer, and particularly trees planted directly into the switchgrass, did show significant reductions in height growth. Surprisingly none of the trees at either site died in year 1, even where seedlings were planted directly into the switchgrass.

These results provide early indications that competitive interactions between the two species have begun. Future results will allow us to better quantify these interactions.

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3.5
WATER RELATIONS AND PRODUCTIVITY IN AN INTERCROPPED PINE-SWITCHGRASS STUDY EXAMINING BIOFUEL PRODUCTION IN NORTH CAROLINA, USA

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Keywords: Panicum virgatum, Pinus taeda, bioenergy, biofuels, hydrology, gross primary productivity, SPA model, loblolly pine, intensive forestry

There is increasing interest in intercropping switchgrass with forest trees, which allows for the simultaneous production of feedstock for bioenergy, and management of high quality wood products. As there are no data on the physiology or water use of loblolly pine and switchgrass when grown together, we studied gas exchange and water status in these species in an intercropped field experiment established by Catchlight Energy LLC, a joint venture between Chevron and Weyerhaeuser Company. The objectives of this study were to 1) determine the effect of intercropping on leaf-level gas exchange and water status of loblolly pine and switchgrass during the 2011 growing season; 2) parameterize a soil-plant-atmosphere (SPA) model for loblolly pine at this site, and 3) compare pine and switchgrass SPA model outputs of stand-level water use and carbon exchange.

The field site was located on the Lower Coastal Plain of eastern North Carolina, USA (35° N, 77° W), on a deep, poorly drained soil. We selected three treatments from two blocks to measure gas exchange and water relations: 1) Traditional pine etablishment; 2) Pure switchgrass plots, and 3) Pines intercropped with switchgrass. Pine trees were planted in winter 2008 at 1100 stems ha^{-1} and switchgrass (cultivar Alamo) seed was planted in June 2009 at 9 kg pure live seed ha^{-1}. Leaf-level gas exchange variables (photosynthesis (µmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1})) were measured on three replicates per plot at regular intervals four times per day, on 23 June and 7 October 2011, with a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Leaf water potential was measured five times per day on the dates described above. These measurements commenced at predawn and were made with a pressure chamber (PMS Instrument Company, Albany, OR, USA). Data were analyzed with a mixed model analysis of variance (Proc. Mixed, SAS Institute, Cary, NC, USA) and an $\alpha = 0.05$ significance level was used. We parameterized the SPA model for this site using local measurements of weather data, soil properties and plant gas exchange and water status. We used the model to predict loblolly pine and switchgrass stand-level water use and carbon exchange during 2011.

There were no intercropping effects on water potential, stomatal conductance or photosynthesis. Water potential did not differ between species for June or October 2011 measurements. Predawn water potential ranged from -0.10 to -0.54 MPa with midday values between -1.25 and -1.63 MPa. Maximum diurnal stomatal conductance rates were measured in October; these values were 125 ± 9 mmol m^{-2} s^{-1} for switchgrass, and 126 ± 3 mmol m^{-2} s^{-1} for pine. Switchgrass photosynthesized at a higher rate than loblolly pine in June and October. Maximum June photosynthetic rates were 3.7 ± 0.1 µmol m^{-2} s^{-1} for pine, and 18.4 ± 3.6 µmol m^{-2} s^{-1} for switchgrass; corresponding values for October were 6.3 ± 0.3 µmol m^{-2} s^{-1} and 15.6 ± 1.0 µmol m^{-2} s^{-1}. Modeled annual transpiration rates were 338 mm and 360 mm for

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switchgrass and pine, respectively, with associated gross primary productivity values of 1521 and 1361 g C m^{-2}. Our results indicated there was a significant species effect for photosynthesis, but no intercropping effect on leaf water status (water potential), stomatal conductance or photosynthesis for a given species. However, we anticipate that as this system develops, availability of resources such as water, light or nitrogen may change, with the potential to impact physiology and therefore switchgrass biomass production. Modeled stand-level evapotranspiration estimates (i.e. transpiration + soil evaporation + canopy evaporation) were 531 mm for switchgrass, and 567 mm per year for loblolly pine. In comparison, rainfall in 2011 was 1157 mm, indicating that at this time, there is sufficient water available to support this intercropped system.

3.6 EFFECTS OF SWITCHGRASS INTERCROPPING AND BIOMASS HARVESTING ON PLANT COMMUNITIES IN INTENSIVELY MANAGED PINE STANDS

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Abstract

Pine (*Pinus* spp.) plantations of the southeastern United States can provide biomass feedstock for biofuels via intercropping switchgrass (*Panicum virgatum*) and harvesting naturally-occurring understory vegetation (bio-baling). Added disturbance from these practices may diversify plant communities in early rotation stands. Therefore, we evaluated plant diversity responses to these practices in pine stands established and maintained by Catchlight Energy LLC (a Chevron|Weyerhaeuser joint venture) on land owned and managed by Weyerhaeuser Company in Mississippi and Alabama during summer, 2010 and 2011. We measured vegetation coverage, lateral visual obstruction, and species composition in paired intercropped and non-intercropped pine stands (n = 25 pairs) and paired bio-baled and unharvested areas within pine stands (n = 8 pairs). We observed 269 plant species across 33 pine stands and 2 years. Switchgrass intercropping increased coverage and species richness of forbs and non-switchgrass grass species and decreased woody plant coverage. During the growing season following switchgrass establishment, lateral visual obstruction was less in intercropped stands, but by the following year, lateral visual obstruction did not differ between treatments. Bio-baling did not significantly affect vegetation communities. Intercropping may promote an initially more diverse herbaceous plant community, but longer-term studies are needed to track successional trajectories relative to switchgrass intercropping.

Keywords: biomass, intercropping, managed pine, Panicum virgatum, plant community, switchgrass, Mississippi, Pinus taeda, forest management, vegetation diversity

Introduction

Growing interest in alternative fuels has supported development of biomass-based fuels that might reduce fossil fuel dependence and atmospheric CO_2 emissions (Fargione *et al.* 2009, Charles 2009). Warm-season grasses have been considered as co-firing agents in local energy plants and as potential cellulosic feedstocks (Schmer *et al.* 2008). Of these, switchgrass (*Panicum virgatum* L.) is an ideal candidate because of its ease of establishment, multiple cultivars, rapid growth, and high biomass yield (*e.g.*, Schmer *et al.* 2008, and many others). Agroforestry practices can be used in intensively managed forests to intercrop warm-season grasses between tree beds (Albaugh et al. 2012). Harvesting naturally-occurring understory vegetation (*i.e.*, bio-baling) has also been considered as a potential feedstock.

Intensively managed pine (*Pinus* spp.) forests cover an estimated 18 million ha in the southeastern United States (USDA 2007) and silvicultural methods within these forests create multiple vegetative structures, increase plant species, and diversify successional stages across a landscape, significantly impacting regional biodiversity (Hunter 1990, Franklin 1993, Carey *et al.* 1999). Forest managers are increasingly expected to incorporate conservation of biodiversity into forest management plans (*e.g.*, Forest Stewardship Council 2002, Sustainable Forestry Initiative 2010). Therefore, the potential effects of intercropping and bio-baling practices relative to landscape-level biocomplexity need to be known.

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However, studies investigating biodiversity responses to switchgrass intercropping and alternative biomass production methods (*i.e.*, bio-baling) are lacking (Riffell *et al.* 2012), and it is unknown if these practices are compatible with conservation of biological diversity within intensively managed forest landscapes. Therefore, we evaluated vegetation community responses to intercropping and bio-baling in early rotation, intensively managed pine stands of Mississippi and Alabama.

Methods

We collected plant community data in intercropped and non-intercropped stands and stands that had portions bio-baled during July-August, 2010-2011. Switchgrass stands were established by Catchlight Energy LLC on land owned and managed by Weyerhaeuser Company; other stands were owned and managed by Weyerhaeuser Company in east Mississippi and west Alabama. In 2009, live switchgrass seed was sown in between beds of planted pine (4.48-6.72 kg/ha via seed drill) following weed control application and V-shearing. Switchgrass was fertilized, mowed, and baled annually. We used GIS software to pair each switchgrass stand with a non-switchgrass stand of equal age within the closest vicinity as possible (0.6-36.1 km; $\bar{x}$ = 6.4 km). We used line transects to measure plant richness and diversity and species coverage (3, 30 m line intercepts in each stand). We placed all points > 50 m from stand edges to avoid edge effects and began transects at plot centers and oriented them perpendicular to tree beds. We recorded all plants intercepting the line, exposed debris (*e.g.*, pine needles, dead plants, etc.), and bare ground $\leq$ 1.37 m (breast height) every 3 cm. We also measured lateral visual obstruction at each sampling point using a Nudds board divided into 6, 0.3 m sections (1.8 m; Nudds 1977). We recorded percentage lateral visual obstruction per section at 5 % increments 11.3 m from the board in each cardinal direction from a 1 m viewing height at plot center.

For the bio-baler trials, naturally growing vegetation was harvested (*i.e.*, bio-baled) in stand rows from portions (5.3-8.9 ha) of stands (n=8) in east Mississippi during 2010. We established 2 random sampling points within bio-baled and un-harvested areas of the same stand for comparison (n=4 points/stand). We used a 10 m buffer between bio-baled and un-harvested areas because we did not expect bio-baling to cause as large of an edge effect as would adjacent stands of different ages (*i.e.*, young intercropped stand next to mid-rotation stand). We used 4, 22.5 m line intercepts per stand and measured lateral visual obstruction with a Nudds board at each point following the same protocol used for switchgrass versus non-switchgrass stands.

We calculated plant coverage as total additive coverage per line intercept divided by total length of line. Because line transects were subsamples (by stand or harvest treatment), we combined all transects/stand or treatment for analyses. We calculated plant species percent coverage in addition to percent coverage by growth form (*e.g.*, forbs, legumes, herbaceous vines, non-switchgrass grasses, sedges and rushes, semi-woody vines, woody plants, woody vines). We did not include switchgrass to avoid its dominance confounding analysis results.

We tested the hypothesis of no difference among vegetation coverage and species richness variables between treatments (*i.e.*, switchgrass and non-switchgrass, bio-baled and un-harvested) using Wilcoxon signed-rank tests. We calculated z-statistics as untreated variables minus treated variables within stand beds or rows. We predicted that beds of both treatments would be similar due to marginal disturbance of crop trees from intercropping or bio-baling. We tested the hypothesis of no difference in plant species composition between treatments using Multi-Response Permutation Procedures with blocking (MRBP) in PC-Ord version 6.0 with Euclidean distances as the distance measure and median alignment within blocks for analyzing within-block distances (McCune and Mefford 2011). If MRBP detected differences between groups, we used blocked indicator species analysis to identify species delineating treatments [*i.e.*, species with indicator values (IV) $\geq$ 30]. Last, we tested the hypothesis of no difference among foliage density variables between treatments using Wilcoxon signed-rank tests as above. We assumed minimal spatial correlation between years of sampling (2010 and 2011) for the 6 stands sampled twice (3 pairs). We calculated q-values with the MULTTEST procedure in SAS 9.2 by adjusting raw p-values to avoid inflating Type I error associated with multiple hypothesis testing (Storey 1982, Storey *et al.* 2004). We used bootstrap parameter $\lambda = 0.05$ for all tests (*i.e.*, false discovery rate equivalent to $\alpha = 0.05$).

Results and Discussion

Intercropping switchgrass may initially diversify plant communities among early rotation, intensively managed pine stands. Changes in vegetation structure were limited to the first growing season following intercropping (*i.e.*, Nudds board sections 2-6 with 7-29% less visual obstruction in switchgrass stands), but coverage changes in beds and rows suggested plant community responses for two growing seasons after intercropping. Our prediction of greater similarity between stand beds than rows only occurred in 2010. Switchgrass stand beds had greater forb coverage both years, greater forb species richness in 2011, and greater grass coverage in 2011. Non-switchgrass stand beds had greater exposed debris and woody plant coverage in 2011. In 2010, switchgrass stand rows had greater forb coverage (S=-58, Q=0.015), and species richness (S=-55, Q=0.023) and bare ground coverage (S=-56, Q=0.022). Non-switchgrass rows had greater woody plant species richness (S=43.5, Q-value = 0.06) and coverage (S=59, Q=0.010), woody vine coverage (S=55, Q=0.023), and semi-woody vine coverage (S=59, Q=0.010). Shannon's and Simpson's diversity indices did not differ, but understory plant species composition varied between treatments during both years in beds and rows.

Intercropping promoted herbaceous ground coverage and decreased woody plant coverage. Although direct intercropping effects were expected within rows (*i.e.*, site preparation, switchgrass planting), beds that contained crop trees also experienced changes in plant species composition (Table 1). Panic grasses [*Dichanthelium aciculare* (Desv. *Ex* Poir.) Gould and C. A. Clark, IV= 89 vs. 7; and *D. scoparium* (Lam.) Gould, IV = 84 vs. 8] indicated switchgrass stand rows, and sawtooth blackberry (*Rubus argutus* Link, IV=75 vs. 25), red maple (*Acer rubrum*, IV= 60 vs. 4), and American beautyberry (*Callicarpa americana* L., IV = 79 vs. 10) indicated non-switchgrass stand rows in 2010. Bed indicators were limited to sawtooth blackberry for non-switchgrass (IV=73 vs. 27) and openflower rosette grass [*Dicanthelium laxiflorum* (Lam.) Gould] for switchgrass (IV=82 vs. 16) in 2011. Openflower rosette grass also indicated switchgrass stand rows in 2011 (IV = 92 vs. 3).

Table 1. Descriptive statistics from Multi-response Permutation Procedures with blocking regarding plant communities of switchgrass intercropped and non-intercropped stands in east-central Mississippi and west-central Alabama sampled by line intercepts in summer 2010 and 2011.

Year	Bed or Row	Test Statistic (T)	A	Q-value
2010	Bed	-3.01	0.053	0.011
	Row	-7.87	0.205	≤ 0.001
2011	Bed	-6.92	0.208	≤ 0.001
	Row	-6.05	0.183	≤ 0.001

Additional harvesting and increased switchgrass establishment may reduce differences between beds and rows due to greater competition for growing space, nutrients, and sunlight in addition to thicker litter layers reducing seed catchment opportunities (Sparks *et al.* 1998, McCoy *et al.* 2001). Most stands used in this study were ≥ 5 years old, and diverse ground layers of younger pine plantations may be outcompeted by perennial semi-woody vines and shrubs such as sawtooth blackberry and American beautyberry. However, switchgrass intercropping in younger stands could limit plant diversity by favoring a switchgrass monoculture in rows as switchgrass becomes better established.

Bio-baling effects were absent other than a noticeable, but insignificant, decreased upper-level visual obstruction [66.2% in bio-baled areas versus 92.3% in un-harvested areas (*Q*-value = 0.308)]. Minimal effects on species diversity by bio-baling were not unusual considering similarities between bio-baling and other less intensive management practices such as mowing (McCoy *et al.* 2001). Over time and after repeated baling events, vegetation communities may favor perennial plants such as many woody species (Maron and Jefferies 2001, McCoy *et al.* 2001). However, with our short-term data set, effects of repeated harvesting are unknown and immediate bio-baling effects suggest minimal short-term impacts to vegetation composition from this alternative biomass collection tool.

Many vertebrate species, especially birds, respond to changes in vegetation structure and diversity (Roth 1976, Tews *et al.* 2004). Therefore, intercropping could increase vertebrate diversity by providing a herbaceous habitat component in rows and woody and shrub habitat structure in beds. Different switchgrass harvest regimes (summer vs. fall, once vs. multiple times per year) also may augment vegetation structure (Flaspohler *et al.* 2009, Riffell *et al.* 2012), but without switchgrass height measurements and all 2011 intercropped stands harvested in fall 2010, we were unable to evaluate harvest effects on vegetation structure. Summer harvests also could increase breeding bird nest mortality, white-tailed deer (*Odocoileaus virginianus*) fawn mortality and reduce reproductive cover (Harper 2007). Fall harvests could reduce winter protective cover (Harper 2007). Vegetation community changes in intercropped sites for 2 growing seasons after switchgrass establishment suggests improvements to plant diversity, but long-term trends of intercropping and bio-baling are unknown requiring future research to better understand successional trajectories and biodiversity impacts.

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3.7
MISCANTHUS X GIGANTEUS BIOMASS FEEDSTOCK PRODUCTION AND SUSTAINABILITY STUDIES IN THE EASTERN U.S.

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Abstract
Miscanthus x *giganteus* has been identified as a potentially productive biomass feedstock in both Europe and the U.S. The objective of this research was to determine the growth and development, productivity, and environmental sustainability of *M.* x *giganteus* in several field studies conducted in the Eastern U.S. In one study conducted in four environments (IL, KY, NE, and NJ), nitrogen was applied annually at 0, 60, and 120 kg ha^{-1} to *M.* x *giganteus*. Following the third year of growth, there were significant biomass yield differences among sites and years, but there were no significant biomass production differences among N fertilizer levels within individual sites and years. At the IL site, greenhouse gas emission fluxes, nitrate leaching, and N levels in harvested biomass were determined during the second and third growing seasons in a second study. During the third growing season, there was significantly more N_2O emitted, NO_3^- leached, and N in the harvested biomass from the 120 kg ha^{-1} plots than from the other N applications. Given that there were no biomass yield differences due to N fertilization levels, this research indicates that high levels of N fertilization may cause both financially and environmentally negative results. In a final study, *M.* x *giganteus* and *Panicum virgatum* (switchgrass) were produced in side-by-side plots in eight IL locations and 9 additional Eastern U.S. locations. Biomass yields for both *M.* x *giganteus* and *Panicum virgatum* varied greatly due to growing environment and switchgrass variety.

Keywords: *Miscanthus x giganteus*, biomass feedstocks, biomass yields

Miscanthus x giganteus
Miscanthus x *giganteus* Greef and Deuter ex Hodkinson and Renvoize (giant miscanthus) is a perennial, rhizomatous, C4 grass that has been studied in Europe and the U.S. for use as an energy crop (Hodkinson and Renvoize 2001; Jorgensen *et al.*, 2003). *M.* x *giganteus* is a triploid interspecific hybrid having the diploid M. *sinensis* and the tetraploid *M. sacchariflorus* as its parents (Hodkinson *et al.,* 2002; Scally *et al.*, 2001). It is essentially sterile and must be propagated vegetatively (Linde-Laursen 1993). Originally, giant miscanthus was imported into Europe from Japan in 1935 and used as a landscape ornamental (Scally *et al.*, 2001). Subsequently, is spread throughout Europe and the U.S. Heaton *et al.* (2004) considered *M.* x *giganteus* to be an ideal biomass energy crop because of its efficient use of available resources, it's perennial, carbon storage in the soil, high water-use efficiency, it's not invasive, and it has a low fertilizer requirements. Giant miscanthus undergoes C4 photosynthesis, giving the plant high photosynthetic efficiency and productivity (Dohleman and Long, 2009).

In Central Illinois, giant miscanthus vegetative growth begins in April and the plant often grows to 2 m by late May, flowers in late September or early October, and is typically taller than 3 m at harvest (Pyter *et al.*, 2009). Following full senescence, harvest occurs from mid-December through late March in Central Illinois (Pyter *et al.*, 2009). Inflorescences often persist throughout the winter and can typically be found in harvested biomass.

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Lewandowski *et al.* (1995) estimated that a commercial giant miscanthus planting will last 15–20 years, and in fact, several European stands have been in production for over 20 years. Giant miscanthus plots at the Rothamsted Experiment Station in the UK have experienced no yield decrease in 15 years without nitrogen fertilizer (Christian *et al.*, 2008).

Following the third growing season, early spring harvests of *M.* x *giganteus* stems in Europe were commonly 6.7–8.9 ton ac^{-1} (Clifton-Brown and Lewandowski 2002; Lewandowski *et al.*, 1995), and on German sites with deep soils and consistent precipitation, yields as high as 30 Mg/ha were achieved (Lewandowski and Kicherer 1997). Giant miscanthus yields of 13.4–17.8 ton ac^{-1} were reported on irrigated sites in Southern Europe (Clifton-Brown et al. 2001; Lewandowski and Kicherer 1997; Lewandowski *et al.*, 2000).

The Studies

University of Illinois researchers have been studying *M.* x *giganteus* for more than 10 years. The Illinois Council for Food and Agriculture Research study was initiated at three Illinois sites, Dekalb, Urbana, and Dixon Springs (Table 1). Four replications of side-by-side 10 m x 10 m plots of giant miscanthus and 'Cave-in-Rock' switchgrass (*Panicum virgatum*) were planted in 2002 (Heaton *et al*., 2008). One hundred pots of giant miscanthus were planted on 1-m spacing in four plots and four 'Cave-in-Rock' switchgrass plots were seeded at each site. The plots were not fertilized, nor irrigated following establishment. Four additional sites, Brownstown, Havana, Fairfield, and Perry, were added in 2004 (Table 1). Mean dry biomass yields from the third growing season through the 2011-growing season are presented in Table 2 (Rebecca Arundale, personal communication). Overall, higher yields come from slightly warmer sites in Central and Southern Illinois in fertile soils.

Table 1. Collaborators, planting years, species and site conditions of University of Illinois-affiliated *Miscanthus* x *giganteus* plantings.

Collaborator	Planting Year	Species[1]	Latitude (N)	Longitude (W)	Soil	Mean Annual Temp (°C)	Mean Annual Precip. (cm)
Energy Biosciences Institute Collaborations[2]							
IL	2007	1	40.06	-88.19	silt loam	10.8	104.3
KY	2009	1	38.12	-84.49	silt loams	12.9	116.6
LA	2009	1	30.41	-91.10	silt	19.4	160.2
MI	2009	1	42.39	-85.37	loam	8.9	89.3
MS	2009	1	33.42	-88.79	fine sandy loam	16.8	140.8
NJ	2009	1	40.46	-74.42	loam & sandy loam complex	11.4	123.9
OK	2009	1	35.99	-97.04	fine sandy loam	15.2	93.2
Ontario	2010	1	43.64,	-80.41	clay loam	6.3	93.9
SD	2009	1	44.36	-96.79	silty clay loam and silt loam complex	6.2	57.9
WI	2009	1	43.30	-89.35	silt loam	7.4	83.3
Sun Grant/DOE Feedstock Partnership Collaborations[3]							
U. of IL	2008	2	40.06	-88.19	silt loam & silty clay loam	10.8	104.3
U. of KY	2008	2	38.12	-84.50	silt loams	12.9	116.6
U. of NE	2008	2	41.17	-96.46	silt loam	9.8	70.4
Rutgers U.	2008	2	40.22	-74.24	sandy loam	11.4	118.8
Illinois Council for Food and Agriculture Research Sites[4]							
Havana	2004	1	40.29	-89.94	sandy loam	10.8	96.0
Perry	2004	1	39.80	-90.81	silt loam	10.8	97.7
Brownstown	2004	1	38.95	-88.96	silt loam	11.6	106.8
Fairfield	2004	1	38.38	-88.38	silt loam	12.2	114.3
Dekalb	2002	1	41.84	-88.85	silty clay loam	8.8	94.9
Urbana	2002	1	40.04	-88.23	silt loam	10.8	104.3
Dixon Springs	2002	1	37.45	-88.72	silt loam	14.5	122.5

[1] 1 = *M.* x *giganteus* + *P. viragatum* (switchgrass), 2 = *M.* x *giganteus*
[2] 4 reps; 2 treatments; 10m x 10m plots; no N
[3] 4 reps; 3 treatments (0, 60, 120 kg N ha-1); 10m x 10m plots
[4] 4 reps; 2 treatments; 10m x 10m plots; no N

Table 2. Mean dry M. x giganteus and switchgrass (Sg) biomass yields at seven IL in growing seasons 3 and beyond following planting (Heaton *et al*., 2008; Rebecca Arundale, personal communication).

Location	Years	*M.* x *g.* (Dry t ac^{-1})	Sg (Dry t ac^{-1})
Dekalb	2004-2011	7.3	3.3
Havana	2006-2011	7.4	4.4
Urbana	2004-2011	13.9	6.1
Orr	2006-2011	11.3	4.6
Brownstown	2006-2011	6.6	3.8
Fairfield	2006-2011	13.5	4.9
Dixon Springs	2004-2011	13.4	4.2

The Energy Biosciences Institute Collaborations, a similar study of side-by-side, 10 m x 10 m plots with four replications of giant miscanthus and switchgrass were established in 2007 (IL) and in seven other U.S. states and in Ontario, Canada in 2009 (Table 1). The main differences between this comparison study and the previously described Illinois study of giant miscanthus and switchgrass is that the cooperators at each site in the newer study selected the switchgrass cultivar planted at their site and the switchgrasses in the newer study were propagated in small pots and planted at a rate of 400 per 10 m x 10 m research plot. Mean 2011 dry biomass yields are presented in Table 3 (Rebecca Arundale, personal communication). While this data represents only a single growing season, it begins to sort out where *M.* x *giganteus* might successfully be produced in the U.S. Generally, it appears that giant miscanthus is best suited to the southern Midwest and the northern southeastern U.S. and from eastern KS/OK heading slightly north as the range approaches the Atlantic Ocean.

Table 3. Mean *M.* x *giganteus* and switchgrass (Sg) biomass yields at eight U.S. locations and one Canadian location in 2011(Rebecca Arundale, personal communication).

Location	*M.* x *g.* (Dry t ac^{-1})	Sg (Dry t ac^{-1})
Ontario	3.0	4.7
South Dakota	2.3	3.7
Michigan	11.0	2.9
New Jersey	3.3	5.5
Illinois	10.2	4.2
Kentucky	7.1	7.8
Oklahoma	2.8	3.7
Mississippi	8.7	5.7
Louisiana	3.8	5.0

As part of the North Central Sun Grant/DOE Feedstock Partnership Collaborations, a study that evaluates the growth and development, productivity, and environmental sustainability of *M.* x *giganteus* grown at three nitrogen rates was established in 2008 at research sites in Illinois, Kentucky, Nebraska, and New Jersey (in IL, 75% were replanted in 2009 due to winter kill). A fifth site in Virginia was established in 2010. Each site has four replications of three 10 m x 10 m plots. One plot in each replication receives an annual application of 0, 54, or 107 lbs. ac^{-1} nitrogen using urea as the N source. The 2009 and 2010 dry biomass yields presented in Table 4 are from Maughan *et al* (2012) and the 2011 yields are previously unpublished data. There have been no yield differences at any site in any year from 2009 through 2011 based on nitrogen fertility.

Table 4. 2009 – 2011 biomass yields(Maughan *et al*., 2011; unpublished data)

Location	2009 Yield (Dry t ac^{-1})	2010 Yield (Dry t ac^{-1})	2011 Yield (Dry t ac^{-1})
Nebraska	7.0	12.2	13.9
Illinois	1.3	7.0	9.2
Kentucky	7.6	8.5	8.5
New Jersey	7.5	4.3	8.3
Virginia	NA	NA	4.2

Also as part of a North Central Sun Grant/DOE Feedstock Partnership study, greenhouse gas (N_2O and CO_2) emission fluxes, inorganic nitrogen leaching, and the amounts of N in the harvested biomass for the Illinois site were determined. Results of these analyses appear in Table 5 (Behnke *et al*., In Press). During the third growing season, there was significantly more N_2O emitted and NO_3^- leached from the 120 kg ha^{-1} plots than from the other N applications (Table 3.). Given that there were no biomass yield differences due to N fertilization levels, this research indicates that applying high levels of N fertilizers may be negative from both a financial and environmental point of view.

Table 5. 2009 – 2010 N_2O and CO_2 fluxes and leached NO_3^- in Illinois in 2009 and 2010 (Behnke *et al*., 2012).

N Treatment	Mean Cumulative N_2O Flux (lb N ac^{-1})†	Mean Cumulative CO_2 Flux (lb N ac^{-1})†	Leached NO_3 (lb N ac^{-1})
2009			
0	0.67 ND	7.70 ND	5.72 ND
60	1.16	8.14	6.34
120	1.21	7.70	11.88
2010			
0	0.31*	7.95 ND	7.95**
60	0.69	8.18	13.66
120	2.60	8.00	25.81

† - Cumulative fluxes were calculated from measured fluxes corrected for temperature variations using a Q10 = 2
*, ** - Treatments are different within a given year (α = 0.05, .01); ND - no difference among treatments within a given year

Acknowledgements

The authors thank the following research supporters and collaborators: The Energy Biosciences Institute; The U.S. Department of Energy; Illinois Council for Food and Agriculture Research; Sun Grant Regional Biomass Feedstock Partnership; G. Bollero, D.K. Lee, R. Darmody, M. David, A. Parrish, A. Wycislo (U. of IL); B. Baldwin (MS State U.); R. Gaussion and M. Sousek (U. of NE); D. Williams and L. Williams (U. of Kentucky); S. Bonos, J. Murphy, and L. Cortese (Rutgers U.); J. Fike (VA Tech); K. Moss (Piedmont BioProducts, LLC); F. Miguez (IA State U.); V. Owens (SD State University); Y. Wu (OK State U.); D. Lee (U. of GA); M. Casler (U. of WI); W. Dean (U. of Guelph); S. Hamilton, D. Pennington, and K. Theilen (MI State U.); Field personnel at all sites

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3.8
GROWING MISCANTHUS FOR BIOFUELS ON MARGINAL LAND AMENDED WITH SEWAGE SLUDGE AND FLUE GAS DESULFURIZED (FGD) GYPSUM

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Abstract
Sustainable biofeedstock production can be an eco-friendly means to supplement our growing energy needs. Amending marginal land using biosolids and FGD gypsum for growing *Miscanthus x giganteus* was studied. A randomized complete block design with 2 rates of biosolids (0 and 50 KL/ha) and 2 rates of gypsum (0 and 4 Mg/ha) in split-plot arrangement was established on 20 m x 30 m replicated plots in 2010. Plant growth and bioprocessing characteristics were influenced by gypsum and biosolids. The effect of biosolids on Miscanthus was more pronounced than that of gypsum. FGD gypsum at 4 Mg/ha significantly increased the tiller numbers (11%), feedstock production (21%) and glucose content (4%) over control. Biosolids at 50 KL/ha significantly increased plant heights (29%), tiller numbers (35%), feedstock production (79%), glucose (10%), and total sugar content (3%) over control. Biosolids and gypsum in combination significantly increased the plant heights by up to 31%, tiller numbers up to 42%, feedstock production up to 80% (from 1 Mg/ha to 5 Mg/ha), glucose content up to 13%, and total sugar content up to 6%. Total carbon, nitrogen, and heavy metals concentration (Al, Pb, Cu, Cd, Cr, Zn, and Ni) in Miscanthus feedstock did not vary significantly. Total and active carbon content and soil quality increased when both biosolids and gypsum were applied, but CO_2 emission was slightly higher with only biosolids. Our results suggest that biosolids and FGD gypsum had synergistic effects on biofeedstock production and bioprocessing characteristics of Miscanthus when grown in marginal land.

Keywords: Perennial, dry-matter, tillers, waste recycling, feedstock quality, heavy metals, nutrient uptake, greenhouse gas emissions, soil quality

Introduction
Dedicated bioenergy crops, such as Miscanthus, serve as feedstock for second generation biofuel production. *Miscanthus x giganteus* is a perennial C_4 grass that originated in Southeast Asia. It has gained considerable interest as a bioenergy crop due to its high biomass yields and low nutrient requirements (Dohleman et al. 2009). Using plant biomass as a source of energy raises some concern that bioenergy crops will displace food crops in farmers' fields (Vadas et al. 2008). This issue can be resolved by reserving the highest-quality lands for food production, and growing bioenergy crops on lower-quality, marginal lands.

Marginal lands are not suitable for food production because they are often associated with one or more of the following qualities: low fertility, high acidity, and heavy metal toxicities (Selva 2008). These characteristics lead to poor agricultural yields and make growing crops on this land unprofitable. Without addressing these soil quality concerns, it is unlikely that bioenergy feedstock yields will be significant enough to convince marginal land owners to grow these crops instead of more conventional crops. The marginal soil quality issues can be ameliorated economically by recycling of industrial and municipal wastes as soil amendments (Galbally et al. 2012).

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The FGD gypsum ($CaSO_4.2H_2O$) is a by-product of coal-fired power plants (AACA 2007 and 2011). FGD gypsum enhances soil chemical properties by reducing soil acidity and heavy metal toxicity. It improves soil physical properties by promoting aggregation, aeration, and water infiltration (Dick et al., 2006). With the amount of FGD gypsum increasing rapidly, the electrical power companies are looking for ways to dispose of this waste product in an environmentally safe way. Similarly, biosolids are nutrient-rich organic materials resulting from the wastewater treatment (EPA 2012). As urban populations in the United States increase, municipalities face an increasing amount of wastewater to treat, meaning more biosolids disposal. Besides providing nutrients, biosolids can improve long-term soil quality through increases in soil organic matter content (Tsadilas et al. 2005).

Production of second-generation biofuels involves the extraction and conversion of lignocellulosic materials from Miscanthus feedstock (Melligan et al. 2012). Acid hydrolysis involves the hydrolysis of cellulose and hemicellulose into individual sugar monomers. However, it does not translate well to industrial-scale processes due to high capital costs, corrosion problems, acid consumption, and recovery costs (Gupta and Demirbas 2010). The method of enzyme saccharification is more adaptable to an industrial scale to hydrolyze and break down cellulose and hemicellulose into fermentable sugars (Le Ngoc Huyen et al. 2010). Suitable pretreatment of lignocellulosic material increases the surface area and pore volume of the biomass so that it is more accessible to enzymatic breakdown (Gupta and Demirbas 2010). The use of microwave digestion as a pre-treatment has shorter heating times, and less energy input than conventional methods.

The objectives of the study were to evaluate the simple effects of biosolids and FGD gypsum and their interaction on (i) growth and yield characteristics and nutrient and heavy metals uptake; (ii) feedstock composition, sugar recovery and theoretical ethanol yield of Miscanthus x giganteus; and (iv) soil carbon contents and greenhouse gas emissions (CO_2, CH_4 and N_2O) under Miscanthus plantations.

Materials and Methods

Site Description

The study was conducted on marginal lands at the Ohio State University South Centers at Piketon (39°02′30”N, 83°02′00”W), Southern Ohio, USA. The soil at the site is a moderately drained Omulga silt loam (fine-silty, mixed, active, mesic oxyaquic fragiudalfs) which formed in loess, colluvium, or old alluvium by underlying lacustrine sediments. Soil has a pH 5.7 to 6, bulk density of 1.2 to 1.48 g/cm^3, total N 0.8 to 1.5 g/kg, extractable P 1.5 to 4 mg/kg, K 28 to 53 mg/kg, Ca 496 to 639 mg/kg, Mg 222 to 224 mg/kg, and S 26 to 33 mg/kg, respectively.

Experimental Design and Cultural Practices

A randomized complete block design with two rates of biosolids (0 and 50 KL/ha) as main plots and 2 rates of FGD gypsum (0 and 4 Mg/ha) as subplots in split-plot arrangement was established. Treatments were replicated four times on 10 m wide by 7 m long plots, with 2 m spacing between plots. Both biosolids and FGD gypsum were surface applied. Biosolids application was followed by FGD treatment. Hybrid *Miscanthus giganteus* plugs (15 to 20 cm tall) were planted 70 cm apart within the rows in July, 2010. Irrigation was applied weekly as required while the Miscanthus was established.

Miscanthus Growth and Yield, Nutrient and Heavy Metals Content

The number of tillers per stump and heights of the Miscanthus were measured over time. The biomass was harvested at 15 cm height in February 2012; 18 months after the plants had been established. The biomass samples were oven-dried at 65°C until a constant weight was obtained, and the dry-matter production was expressed as oven-dried equivalent weight basis. The oven-dried Miscanthus biomass samples were ground using a Wiley-Mill, passed through a 500 µm mesh, and analyzed for total C and N content using dry combustion CNS analyzer. The concentration of the macronutrients (P, K, Ca, Mg, and S); micronutrients (Fe, Mn, Cu, and Zn, including Si and Na) and selected heavy metals (Al, Cr, and Ni) were determined through concentrated $HClO_4$ digestion followed by ICP Emission spectrometry (AOAC Official Method 975.03, Allen 1971, Issac and Johnson 1985). Ash content was determined using the AOAC Official Method 942.05.

Compositional Analysis and Sugar Recovery of Miscanthus Feedstock

For the acid hydrolysis, a 150 mg sample of Miscanthus feedstock was reacted with 1.5 ml of H_2SO_4 and then heated in an autoclave at 100°C for one an hour. After cooling, 43.5 mL distilled deionized water was added and the supernatant was separated from the residues by filtration. A small volume of the clear aliquot was passed through the high performance liquid chromatography to isolate different fractions of sugars and associated compounds. For the microwave digestion of the Miscanthus feedstock, a 2 g oven-dried equivalent sample of ground Miscanthus feedstock was digested (with 199.2 µl of 95% concentrated H_2SO_4 in 40 ml of distilled water) for 10 min at 190^0C. After digestion, the samples were centrifuged and the aliquot was filtered prior to analyze by high performance liquid chromatography.

Greenhouse Gas Emissions and Soil Total and Active Carbon

Greenhouse gas (CO_2, CH_4 and N_2O) emissions were measured using a Shimadzu® chromatograph over a period of 10 months (except January and February) using PVC-field gas collection chambers. The gas flux was calculated following Mosier and Klemedtsson (1994).

Field-moist composite soil samples collected from 0 to 15 and 15 to 30 cm depths were air-dried for 15-d at room temperature (25°C) prior to analyze for total and active carbon contents. Total carbon was determined on finely ground (200-µm) soils by automated dry combustion Elementar® CN analyzer. Active carbon as a measure of soil quality was measured following Weil et al. (2003).

Statistical Analysis

Significant variations in Miscanthus growth and biomass production and feedstock bioprocessing quality including heavy metals content and soil properties by biosolids and FGD gypsum application were assessed in a 2-way ANOVA procedure of the SAS (SAS Institute 2008). For all statistical analyses, significant main and interactive effects of biosolids, FGD and soil depth on dependent variables were separated by the least significant difference (LSD) test at $P \leq 0.05$ unless otherwise mentioned.

Results and Discussion

Miscanthus Growth and Yield

Biosolids treatments significantly affected the growth and yield of Miscanthus (Table 1). Biosolids significantly increased dry-matter yield, tiller numbers, and height of the plants. FGD gypsum did not significantly increase the Miscanthus growth. However, the interaction of FGD gypsum and biosolids had the highest dry-matter yields, 4.9 t/ha.

Table 1. Effect of flue gas desulfurization (FGD) gypsum and municipal biosolids on growth and yield characteristics of *Miscanthus x giganteus*

FGD (Mg/ha)	Biosolids (KL/ha)	Min. ht.	Max. ht. (cm)	Aver. ht. stump	Tillers no/ yield (Mg/ha)	Dry-matter
FGD effects						
0		98a§	203a	151a	29a	2.3a
4		104a	209a	157a	33a	3.0a
Biosolids effects						
	0	83xφ	173y	128y	25y	0.9y
	50	119x	239x	179x	38x	4.4x
FGD gypsum x biosolids						
0	0	80	171	126	23	0.8
	50	115	236	176	35	3.9
4	0	85	175	130	26	1.1
	50	123	242	183	40	4.9
$LSD_{p\geq0.05}$						
FGD x biosolids		ns	ns	ns	ns	ns

§ Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at $p\leq0.05$ between FGD treatments.
φMeans followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at $p\leq0.05$ between biosolids treatments.

The yields achieved were comparable to those found in high density Miscanthus trials (Bullard et al. 1995, Heaton et al. 2008). Significantly higher dry-matter produced from biosolids treatments is most probably due to the greater mineralization and release of organically-bound nutrients to growing Miscanthus. Miscanthus does not reach peak biomass production until three years after planting (Heaton 2009), and this study only covered half of the time necessary for Miscanthus to reach its peak yields.

Plant Nutrient and Heavy Metal Content of Miscanthus Feedstock

Biosolids and FGD gypsum application on soil did not significantly affect the concentration of N, P, K, and Ca in the Miscanthus feedstock (Table 2). However, FGD gypsum had significantly lower Mg concentration (28%) in the feedstock. Similarly, higher rates of biosolids application had significantly lower concentration of the S in the feedstock (Table 2). The interaction of gypsum and biosolids were only significant for Mn concentration (Table 3). FGD gypsum application did not have a significant effect on the concentrations of Fe, Mn, Cu, Zn, and Na in the Miscanthus feedstock. However, plants grown in biosolids amended plots had significantly decreased the concentrations of Na and Zn. Heavy metal concentrations were not affected by the application of FGD gypsum (Tables 3).

Table 2. Effect of flue gas desulfurization (FGD) gypsum and municipal biosolids on total nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur concentration in *Miscanthus x giganteus* feedstock

FGD (Mg/ha)	Biosolids (KL/ha)	N	P	K	Ca	Mg	S
		(g/kg)				(mg/kg)	
FGD effects							
0		2.8a[§]	1.5a	1.2a	1.3a	761a	385a
4		2.1a	1.7a	1.2a	1.2a	550b	343a
Biosolids effects							
	0	2.4x[φ]	1.6x	1.2x	1.5x	693x	445x
	50	2.5x	1.6x	1.2x	1.0x	619x	283y
FGD gypsum x biosolids							
0	0	2.8	1.5	1.1	1.6	811	450
	50	2.9	1.4	1.2	1.1	711	321
4	0	20	1.8	1.3	1.4	574	440
	50	21	1.7	1.1	1.0	527	246
$LSD_{p\geq0.05}$							
FGD x biosolids		ns	ns	ns	ns	ns	ns

C=Carbon, N=Nitrogen, P=Phosphorous, K=Potassium, Ca=Calcium, Mg=Magnesium, S=Sulfur.
[§]Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between FGD treatments.
[φ]Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between biosolids treatments.

Table 3. Effect of flue gas desulfurization (FGD) gypsum and municipal biosolids on total iron, manganese, copper, zinc, sodium, silica, aluminum, chromium, nickel concentration in *Miscanthus x giganteus* feedstock

FGD (Mg/ha)	Biosolids (KL/ha)	Fe	Mn	Cu	Zn	Na	Si	Al	Cr	Ni
										(mg/kg)
FGD effects										
0		119a[§]	139a	9a	34a	24a	49a	81a	12a	6a
4		89a	125a	6a	34a	22a	33a	51a	10a	5a
Biosolids effects										
	0	137x[φ]	140x	10x	45x	28x	51x	92x	13x	7x
	50	71x	125x	5x	24y	18y	32x	40x	9x	4x
FGD gypsum x biosolids										
0	0	165	116	12	42	27	61	119	15	8
	50	73	134	6	25	21	36	43	9	4
4	0	108	163	8	47	29	41	66	11	5
	50	70	115	4	22	15	24	36	9	4
$LSD_{p\geq0.05}$										
FGD x biosolids		ns	8	ns	ns	ns	ns	ns	ns	ns

Fe=Iron, Mn=Manganese, Cu=Copper, Zn=Zinc, Na=Sodium, Si=Silica, Al=Aluminum, Cr=Chromium, Ni=Nickel.
§Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between FGD treatments.
φMeans followed by same uppercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between FGD treatments.

The macronutrient uptake by the Miscanthus feedstock was not significantly influenced by FGD application, but it was significantly influenced by biosolids application (Table 4). The N, P, K, Ca, Mg, and S uptake by Miscanthus was respectively 4.6, 4.5, 4.1, 3.7, 4.3, and 3 times higher in 50 KL biosolids applied treatment than in the control. However, the macronutrient uptake by Miscanthus was not affected by the interaction of FGD gypsum x biosolids (Tables 4). The macronutrient removal (uptake) by Miscanthus was very low compared with agronomic crops. The N, P, K, Ca, Mg, and S removal by Miscanthus feedstock was only 2.3 to 12.3, 1.2 to 8, 1 to 5.3, 1.2 to 4.7, 0.6 to 2.8, and 0.4 to 1.2 kg/ha, respectively (Table 4).

Table 4. Effect of flue gas desulfurization (FGD) gypsum and municipal biosolids on total nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur uptake in *Miscanthus x giganteus* feedstock

FGD (Mg/ha)	Biosolids (KL/ha)	N	P	K	Ca	Mg	S (kg/ha)
FGD effects							
0		7.4a	3.4b	3.2a	2.6a	1.7a	0.8a
4		6.1a	4.9a	3.5a	3.0a	1.5a	0.8a
Biosolids effects							
	0	2.4y	1.5y	1.3y	1.2y	0.6y	0.4y
	50	11.1x	6.8x	5.4x	4.4x	2.6x	1.2x
FGD gypsum x biosolids							
0	0	2.4	1.2	1.0	1.3	0.6	0.4
	50	12.3	5.6	5.4	4.0	2.8	1.2
4	0	2.3	1.7	1.7	1.2	0.6	0.5
	50	9.9	8.0	5.3	4.7	2.5	1.2
LSD $p \geq 0.05$							
FGD x biosolids		ns	ns	ns	ns	ns	ns

C=Carbon, N=Nitrogen, P=Phosphorous, K=Potassium, Ca=Calcium, Mg=Magnesium, S=Sulfur.
§Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between FGD treatments.
φMeans followed by same uppercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between biosolids treatments.

Similarly, the micronutrient, beneficial and heavy metals uptake by Miscanthus was significantly higher for biosolids-applied soils (Table 5). Total Fe, Mn, Cu, Zn, Na, Si, Al, Cr, and Ni uptake by Miscanthus was significantly higher by 2.7, 4.3, 4.2, 2.9, 3.3, 3.4, 2.7, 3.5, and 4.5 times, respectively in 50 KL biosolids applied plots than in the control plots. However, the micronutrient, beneficial and heavy metals uptake was not significantly influenced except CU, Zn, and Al by FGD gypsum and biosolids interaction

(Table 5). The Cu, Zn, and Al uptake by Miscanthus was significantly reduced by FGD gypsum and biosolids interaction.

Table 5. Impact of flue gas desulfurization (FGD) gypsum and municipal biosolids on total iron, manganese, copper, zinc, sodium, silica, aluminum, chromium and nickel uptake *Miscanthus x giganteus* feedstock

FGD	Biosolids	Fe	Mn	Cu	Zn	Na	Si	Al	Cr	Ni
(Mg/ha)	(KL/ha)	(kg/ha)				(g/ha)				
FGD effects										
0		0.20a[§]	0.33a	20a	65a	51a	94a	126a	30a	10a
4		0.22a	0.37a	11a	74a	51a	75a	111a	20a	11a
Biosolids effects										
	0	0.11y[φ]	0.13y	6y	35y	24y	38y	65y	11y	4y
	50	0.30x	0.56x	25x	103x	79x	131x	173x	39x	18x
FGD gypsum x biosolids										
0	0	0.12	0.10	10	30	20	45	85	10	5
	50	0.27	0.56	30	100	83	143	168	30	15
4	0	0.10	0.17	25	40	28	30	45	13	2.5
	50	0.34	0.58	20	11	75	120	178	48	20
$LSD_{p\geq0.05}$										
FGD x biosolids		ns	ns	6	8	ns	ns	9	ns	ns

Fe=Iron, Mn=Manganese, Cu=Copper, Zn=Zinc, Na=Sodium, Si=Silica, Al=Aluminum, Cr=Chromium, Ni=Nickel.
[§]Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at $p\leq0.05$ between FGD treatments.
[φ]Means followed by same uppercase letter in each column are not significantly different by least significant different (LSD) test at $p\leq0.05$ between biosolids treatments.

Macronutrient uptake was likely increased in the biosolids applied plots because of the high yields of Miscanthus over other treatments. Similarly, the significant effect of biosolids on micronutrient and heavy metals uptake was due to higher yields of biomass. Differences in nutrient uptake could be a result of differences in crop yields and consistencies in the concentration of nutrients in the FGD gypsum and biosolids. In addition, Miscanthus' low nutrient requirements are a result of how it stores and recycles minerals. Miscanthus mobilizes mineral nutrients from the stem and leaves to the roots at the end of the growing season. Winter harvest of Miscanthus ensures that minerals will be stored in the plant's roots for the next growing season, instead of being removed with the crop (Beale and Long 1995). Several studies have reported that the heavy metal uptake in Miscanthus, and determined that the plant was metal-resistant due to its ability to restrict metal uptake and to prevent those minerals from moving from the roots to the stem and leaves (Beale and Long 1995, Galbally et al. 2012).

Compositional Analysis of Miscanthus Feedstock

Biosolids application increased (11%) the concentration of cellulose in the Miscanthus feedstock, but FGD application did not have any significant effects (Table 6). However, the composition of other compounds did not vary at all. The control treatment had a cellulose yield of 37.5% and a hemicellulose yield of 27.0%, while the combined treatment of FGD gypsum and biosolids had cellulose and hemicellulose yields of 43.0% and 26.3%, respectively. The composition of Miscanthus found in our control treatment is consistent with the findings of Melligan et al. (2012), who found cellulose and hemicellulose yields of 37.1% and 18.0%, respectively during acid hydrolysis of Miscanthus feedstock.

Table 6. Effect of flue gas desulfurization (FGD) gypsum and municipal biosolids on concentration of sugars and associated compounds released through acid hydrolysis of *Miscanthus x giganteus* feedstock

FGD (Mg/ha)	Biosolids (KL/ha)	Cellu	H-cellu	Xyl	Gly	A-acid (%)	Furfur	Ash	Total Recov.
FGD effects									
0		38.7a§	26.4a	4.2a	2.3a	12.6a	0.5a	5.6a	90.4a
4		40.2a	26.8a	4.2a	2.4a	12.7a	0.0a	4.2a	90.5a
Biosolids effects									
	0	37.4xφ	27.2x	4.2x	2.3x	12.6x	0.5x	4.7x	88.9x
	50	41.5y	26.1x	4.3x	2.4x	12.7x	0.0x	5.0x	91.9x
FGD gypsum x biosolids									
0	0	37.5	27.0	4.2	2.2	12.7	0.9	5.8	90.3
	50	40.0	25.9	4.3	2.4	12.6	0	5.4	90.5
4	0	37.3	27.4	4.2	2.4	12.6	0	3.7	87.5
	50	43.0	26.3	4.3	2.3	12.9	0	4.7	93.4
$LSD_{p\leq0.05}$									
FGD x biosolids		ns	ns	ns	ns	ns	ns	ns	ns

Cellu=Cellulose, H-cellu=Hemicellulose, Xyl=Xylitol, Gly=Glycerol, A-acid=Acetic acid, Furfur =Furfural.
§Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between FGD treatments.
φMeans followed by same uppercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between biosolids treatments.

Biosolids and FGD gypsum application on the soil had no significant effect on the concentration of cellulose and hemicellulose recovered through enzyme saccharification (Table 7). The high acid, 190°C pretreatment used to determine differences in Miscanthus feedstock composition recovered about 50% of the total composition of the material. It recovered approximately 70% of the theoretical cellulose, and 47% of the theoretical hemicellulose. This pretreatment method may have been too harsh on the material because this method did not recover much hemicellulose in the form of xylose. However, these recovery rates are lower than the recovery rates for acid hydrolysis, but this was expected because this method is meant to have lower operating and material costs than acid hydrolysis would on a large scale.

Table 7. Effect of flue gas desulfurization (FGD) gypsum and municipal biosolids on concentration of total sugars released after microwave pretreatment and enzyme saccharification of Miscanthus *x giganteus* feedstock

FGD	Biosolids	Cellu	H-cellu	Xyl	Gly	A-acid	Ethanol	HMF	Furfur	Ash	T-recov.
(Mg/ha)	(KL/ha)						(%)				
FGD effects											
0		28.4a§	10.2a	0.3a	0.7a	5.8a	0.4a	0.0a	0.1a	5.6a	51.5a
4		25.8a	9.9a	0.3a	0.7a	5.5a	0.3a	0.0a	0.0a	4.2a	46.7a
Biosolids effects											
	0	26.4xφ	10.2x	0.3x	0.7x	5.6x	0.4x	0.0x	0.1x	4.7x	48.4x
	50	27.8x	9.8x	0.3x	0.8x	5.7x	0.3x	0.1x	0.1x	5.0x	49.8x
FGD gypsum x biosolids											
0	0	28.3	10.4	0.3	0.7	5.9	0.4	0	0.1	5.8	51.9
	50	28.6	9.9	0.3	0.7	5.7	0.4	0.1	0	5.4	51.1
4	0	24.5	10.1	0.2	0.7	5.3	0.3	0	0	3.7	44.9
	50	27.1	9.7	0.3	0.8	5.6	0.3	0.1	0.1	4.7	48.5
$LSD_{p\leq0.05}$											
FGD x biosolids		ns	ns	ns	ns	ns	ns	ns	ns	n	s
	ns										

Cellu=Cellulose, H-cellu=Hemicellulose, Xyl=Xylitol, Gly=Glycerol, A-acid=Acetic acid, Furfur =Furfural and HMF=Hydroxymethyl furfural.

§Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between FGD treatments.

φMeans followed by same uppercase letter in each column are not significantly different by least significant different (LSD) test at p≤0.05 between biosolids treatments.

Overall, the recovery rates found using enzymatic hydrolysis of microwave pre-treated material were comparable to those found in similar studies. Orozco et al. (2011) found lower cellulose and total recovery rates of 16% and 18%, respectively, for their hydrolysis method of dilute acid pretreatment and hydrolysis in a microwave digester. Boonmanumsin et al. (2012) used a two-stage microwave pretreatment system to obtain 71.6% total sugar recovery for *Miscanthus sinensis*. Le Ngoc Huyen et al. (2010) found a cellulose recovery of 45.8% for late harvest (February), and 44.1% for early harvest (December) using an ammonia pretreatment on Miscanthus plants before enzyme saccharification.

Potential Ethanol Yields

The theoretical yield based on acid hydrolysis is 128.7 gal/ton of dry feedstock, which is slightly more than can be obtained with corn grain, 124.4 gal/ton of dry feedstock (DOE 2012). In our study, the Miscanthus treated with biosolids produced an average of 4.4 tons of dry biomass per hectare. This would result in ethanol yields of 1572 L/ha using microwave pre-treatment and enzyme saccharification. These results are encouraging for the development of Miscanthus as a bioenergy feedstock. Our results show that when Miscanthus is grown on marginal lands and is treated with biosolids, it can be expected to produce competitive ethanol yields. Although the expected ethanol output for this study is lower than that of switchgrass, corn, and sugarcane, it is important to note that the Miscanthus had been growing for 18 months when harvested, but Miscanthus does not reach peak biomass until 3 years after planting. It is likely that the ethanol output per hectare will increase in subsequent growing seasons and may overtake the ethanol output of switchgrass or corn despite being grown on low-quality land.

Greenhouse Gas Emissions and Total and Active Carbon Contents

The application of FGD gypsum and biosolids for growing Miscanthus influenced the emissions of CO_2 and N_2O from the soil (Table 8). The CH_4 emission did not vary significantly. Averaged across biosolids, the CO_2 and N_2O emissions increased by 1.9 and 1.6 times in the 4 Mg/ha of FGD gypsum applied plots as compared with the control. However, 50 KL/ha application of biosolids significantly increased the CO_2 emission by 2.5 times over the control. The interaction of FGD gypsum and biosolids did not significantly influence any of the greenhouse gases emission.

Table 8. Effect of flue gas desulfurization (FGD) gypsum and municipal biosolids on flux of carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) from soil under *Miscanthus x giganteus*

FGD (Mg/ha)	Biosolids (KL/ha)	CO_2 (Kg/ha/d)	N_2O (g/ha/d)	CH_4 (g/ha/d)
<u>FGD effects</u>				
0		3.0b§	10.2b	1.9a
4		5.7a	16.7a	2.4a
<u>Biosolids effects</u>				
	0	2.5xφ	11.6y	1.4x
	50	6.2y	15.2x	2.8x
FGD gypsum x biosolids				
0	0	1.6	9.6	1.3
	50	4.3	10.7	2.5
4	0	3.5	13.6	1.6
	50	8.0	19.7	3.1
$LSD_{p \geq 0.05}$	FGD x biosolids	ns	ns	ns

§Means followed by same lowercase letter in each column are not significantly different by least significant different (LSD) test at $p \leq 0.05$ between FGD treatments.
φMeans followed by same uppercase letter in each column are not significantly different by least significant different (LSD) test at $p \leq 0.05$ between biosolids treatments.

Significantly higher CO_2 and N_2O emissions under biosolids are not surprising because the application of biosolids generally results in higher emissions of CO_2 and N_2O from the soil (Granli and Bøckman 1994). This is likely due to the accelerated microbial activity by the presence of higher amount of labile or active carbon and soluble nitrogen. The active carbon and soluble nitrogen may have transformed to CO_2 and N_2O through respiration, nitrification and denitrification (Saison et al. 2006). CH_4 emissions are generally a result of anaerobic respiration of microorganisms when soils are flooded (Sass 2002), so the impact of soil amendments on CH_4 emissions can be small in comparison to the impact of soil anaerobic conditions. Other studies have shown that the application of gypsum can decrease methane emissions by 55 to 70% (Schütz et al. 1989, Denier van der Gon 1994), but a longer study period than 18 months may be necessary for these changes to become evident.

Biosolids application significantly increased (17%) the concentration of total carbon (Fig. 1) over time. However, both total and active carbon concentrations were significantly influenced by FGD gypsum and biosolids application without an interaction (Fig. 2). Biosolids increased the active carbon concentration by 36% and FGD gypsum increased the active carbon by 22%. Irrespective of soil amendments, both total and active carbon content decreased with increased soil depth. Significantly higher concentration of both total and active carbon is due to greater amount of organic matter added to the soil as biosolids (Tsadilas et al. 2005; Stietiya and Wang 2011). Since active carbon is a small fraction of total carbon, soil amended with biosolids may have invariably increased the concentration of active carbon in soil (Weil et al. 2003).

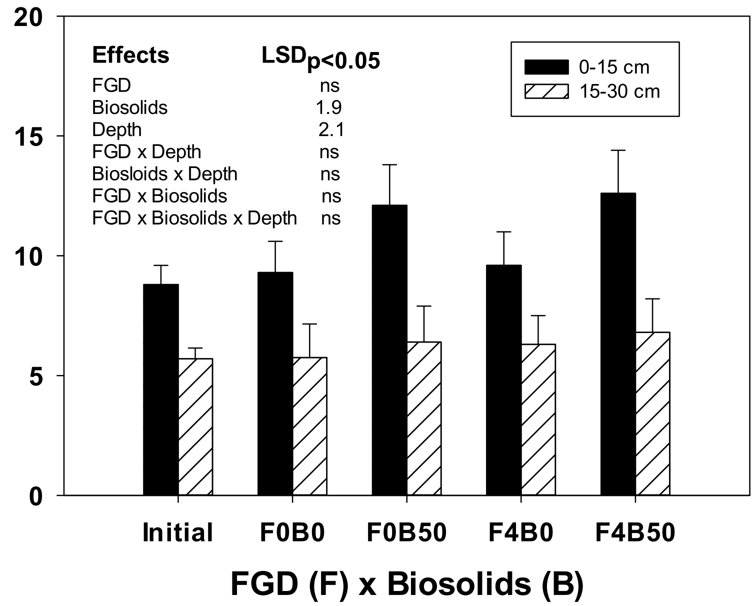

Fig. 1: Effect of flue gas desulfurized (FGD) gypsum and municipal biosolids treatment combinations on total carbon in soil at 0 to 15 and 15 to 30 cm depths (2010 vs. 2012).

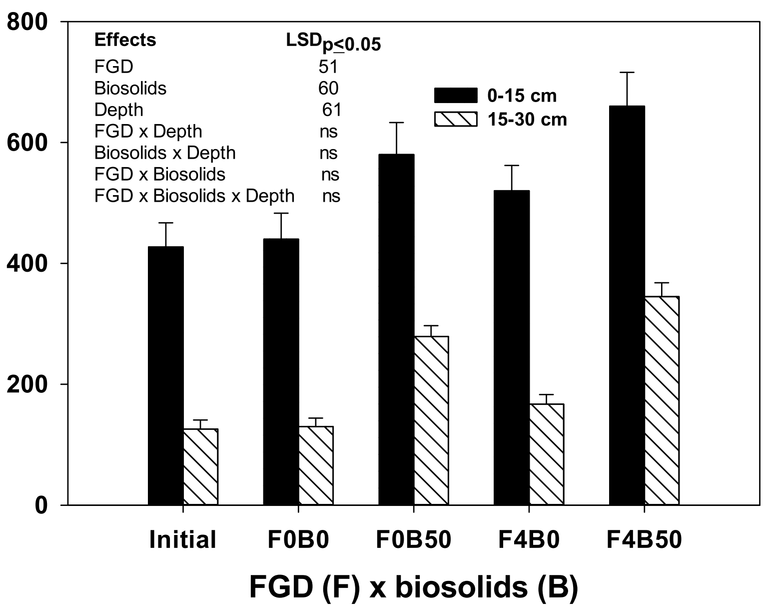

Fig. 2: Effect of flue gas desulfurized (FGD) gypsum and municipal biosolids treatment combinations on active organic carbon as a measure of soil quality at 0 to 15 and 15 to 30 cm depths (2010 vs. 2012).

Conclusions

Biosolids application significantly increased Miscanthus dry-matter yields, height, and tillers on marginal lands. However, FGD gypsum did not significantly influence the Miscanthus growth and yield. The nutrient and heavy metals concentration in the Miscanthus feedstock was not consistently affected by biosolids and FGD gypsum. There were no consistent differences in lignocellulosic composition of the Miscanthus feedstock due to biosolids or FGD gypsum.

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3.9
NITROGEN LOSSES FROM SWITCHGRASS AS AFFECTED BY NITROGEN FERTILIZER RATE

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Abstract
Little is known about nitrogen (N) losses through nitrous oxide (N_2O) emission and nitrate (NO_3) leaching as affected by N fertilization in switchgrass grown for bioenergy. Therefore, this study was conducted to evaluate how N fertilization affects N losses through N_2O emission and NO_3 leaching and the resultant N-use efficiency of switchgrass. Switchgrass was planted at Bristol, South Dakota in 2008. Three N rates (0, 56, and 112 kg N ha^{-1} applied as urea) were applied during spring from 2009 through 2011. Switchgrass was harvested during autumn each year. Biomass yield significantly increased with N application up to 56 kg N ha^{-1} but there was no benefit to 112 kg N ha^{-1}. Apparent fertilizer N recoveries were 3.8 and 5.1 % at 56 and 112 kg N ha^{-1} year^{-1}. However, nitrogen use efficiency was higher with N fertilization at 56 kg N ha^{-1} than that at 112 kg N ha^{-1}. Cumulative N_2O emissions during the growing season increased with N fertilization. Cumulative NO_3 leaching with N fertilization at 56 kg N ha^{-1} was similar to that at 0 kg N ha^{-1}, but there was a significant increase at 112 kg N ha^{-1}. Based on the results thus far, optimum N fertilization rate in South Dakota is around 56 kg N ha^{-1}. No N application resulted in lower biomass production while a higher rate (112 kg N ha^{-1}) exacerbated adverse environmental effects.

Keywords: Switchgrass, Nitrogen fertilizer, Bioenergy, Nitrous oxide, Nitrate, Nitrogen recovery, Nitrogen removal

Introduction
Switchgrass (Panicum virgatum L.) has been extensively studied because it has proven production across a range of environments, it is suitable for marginal and erosive land, and it has relatively low water and nutrient requirements (Sanderson and Adler, 2008; Parrish and Fike, 2005). Although switchgrass tolerates low soil fertility, optimizing biomass and maintaining quality stands requires nitrogen (N) fertilizer inputs and proper management.

The amount of N removed in biomass is important in determining fertilizer needs and usefulness as a feedstock. Matching the N application rate with N removal has obvious agronomic, economic, and environmental advantages. Recovery of applied N under average field conditions is often no greater than 50-60% (Allison, 1966). A common range is between 30 and 70% recovery of applied N during the year of application, with 10-40% incorporated into organic matter, 5-10% lost by leaching, and 10-30% lost in gaseous form (Kundler, 1970). In previous work in South Dakota, switchgrass biomass yield increased with N rates up to 56 kg ha^{-1}, but no further yield improvement was noted above this rate (Mulkey et al., 2006). Excessive N application rates above plant requirements may cause increases in N losses through denitrification as nitrous oxide (N_2O) or nitrate (NO_3) leaching.

Excess N in rivers, lakes, and groundwater can be toxic to humans and cause water quality problems in

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natural water systems (Hallberg and Keeney, 1993; Dinnes et al., 2002; Keeney, 2002; Townsend et al., 2003; Foley et al., 2005). The Environmental Protection Agency has set the maximum contaminant level of nitrogen as nitrate (NO_3-N) at 10 mg L^{-1} for the safety of drinking water (EPA, 1974). Nitrate is a negatively charged ion that is repelled by, rather than attracted to the negative charged clay mineral surface in soil (i.e., the CEC). It is the primary form of N leached into groundwater, is totally soluble at concentrations found in soil, and moves freely through most soils. Leaching of nitrate is accelerated when N rates from synthetic fertilizer or animal waste exceed plant nutrient requirements.

Nitrous oxide in the atmosphere coming from N fertilizer contributes to global warming. While the major greenhouse gas (GHG) issue for the total economy is CO_2, for agriculture the most important is N_2O, mainly from soils and N inputs to crop and soil systems (U.S. EPA, 2007). Even though N_2O is a small part of the overall GHG issue, it becomes a major issue because agriculture is considered to be its major source, and it is linked to soil management and fertilizer use. Nitrogen application rate is one of the most important factors affecting N_2O emission from fertilized fields (IFA/FAO, 2001). Eichner (1990) reported that fertilizer type, application rate, application technique, and application timing were management related factors affecting fertilizer-derived N_2O emissions. These management factors can be utilized to reduce N_2O emissions from arable land.

From an agronomic and environmental viewpoint, a desirable management strategy for switchgrass is to increase yield of biomass while decreasing potential adverse environmental effects. One way to do this is to decrease synthetic fertilizer inputs, particularly N fertilizer. Therefore, understanding N losses with N fertilization is critical as we evaluate field scale switchgrass production techniques. The objective of this study was to determine N losses during growing season from switchgrass field affected by different N fertilizer rates in South Dakota.

Materials and Methods

This study was conducted in South Dakota for 1 year in 2010. Experiment site was near Bristol, SD USA (45°16' 8.274"N; 97°50'8.9694"W) on a Nutley-Sinai (silty clay, mixed, Chromic Hapluderts) with 2-20% slope. Sunburst was planted on 17 May 2008. Experimental designs were plots in randomized complete blocks with four replications across landscape. Individual plot size 0.8 ha to allow for using conventional agricultural equipment. Three levels of N fertilization (0, 56, and 112 kg N ha-1) were applied from 2009 to 2012 every year in spring. Switchgrass was harvested once each year after a killing frost.

Yield was determined by harvesting a windrow through the center of each plot with discbine at a height of 10 to 15 cm. Biomass from each windrow was packaged in large round bales and weighed. Three hundred g subsamples were collected from each bale using a Yankton Hay Probe for further analyses. The subsamples were weighed, dried at 60 °C for 48 h in a forced-air oven, reweighed to determine dry matter yield, and ground in preparation for N analysis. All subsamples were ground in a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA) to pass a 1-mm screen and reground to uniformity in a Udy-cyclone impact mill (Udy Co., Ft. Collins, CO) with a 1-mm screen. Concentrations of total N were quantified using a Vario Max CNS elemental analyzer (Elementar Instrument, Mt. Laurel, NJ).

Cumulative amount of NO_3 in leachates from each plot during the growing season was measured. Cumulative loss of nitrogen through leaching was calculated based on leaching volumes and concentration of NO_3 in leachate. Daily precipitation and evapotranspiration data from an on-site weather station was used to determine annual leaching volume for the year of application. Suction lysimeters were placed at a 1 m depth in the center of each plot to prevent interference between treatments. Water samples were collected monthly from the lysimeters during the growing season. Samples were analyzed for nitrate-N using a NO_3 analyzer (Latchat Quick Chem Flow Injection Analysis).

PVC static flux chambers (25 cm diameter x 15 cm height) [Mosier and Hutchinson, 1981] were used to obtain gas flux measurements from the research plots according to the methods of Parkin and Venterea (2010). Duplicate chambers located at three landscape positions (crest, mid-slope, and toe) per plot were sampled at two-week intervals during the growing season of the year. At each sampling event, a time-series of ten-ml chamber headspace samples were collected with a syringe via a chamber septum and added to 10-ml, nitrogen-filled serum vials. N_2O was quantified by gas chromatography [Shimadzu 14B with a CombiPal AOC-5000 autosampler, 2-ml injection loop, Porapack Q precolumns, a 1/8" stainless steel Porapack Q (80/100 mesh) column, nitrogen carrier gas, and an electron capture detector at 260°C]. Calibration was routinely performed using dilutions of a certified gas standard mix (Scotty, Plumsteadville, PA) handled in the same manner as biomass samples. Gas fluxes were calculated using linear regression and the average of six chambers at three landscapes used to represent each plot for further analysis. Cumulative annual gas fluxes were calculated by linear interpolation among the individual measurements.

Total N removal was calculated annually by multiplying biomass yield by the N concentration in dry matter. Fertilizar N removal was calculated annually as the difference between total N removal from the unfertilized treatment (0 kg ha-1) and total N removal from the fertilized treatment (56 or 112 kg ha-1).

Nitrogen use in relation to dry matter yield was determined using two different metrics: nitrogen-use efficiency (NUE) and apparent nitrogen recovery (ANR). Nitrogen-use efficiency (kg biomass per kg N) for each harvest was calculated as (Zemenchick and Albrecht, 2002; Nova and Loomis, 1981):
NUE = (yield at Nx − yield at N0)/kg of N applied
Where Nx = N rate >0, and N0 = no N applied

Apparent N recovery (%) for each harvest was calculated using the difference method (Crasswell and Godwin, 1984):
ANR = [(kg N removed at Nx − kg N removed at N0)/kg N applied at Nx] × 100
Where Nx = N rate >0, and N removal was calculated by multiplying biomass yield times the concentration of N in the biomass.

Data were analyzed by ANOVA using SAS software v9.2 (SAS, 2008). Mean difference was separated using Fisher's protected LSD.

Result and Discussion

Biomass yield

Switchgrass biomass yield significantly increased with increasing N application rate up to 56 kg N ha^{-1}, but no more increase in biomass yield was observed above this rate (Fig. 1). Switchgrass biomass response to N fertilization varies with regional environment and soil fertility. A similar result was found in a switchgrass trial conducted previously in South Dakota (Mulkey et al., 2006). Int hat study, yields of switchgrass harvested around a killing frost on Conservation Reserve Program (CRP) land increased with N application up to 56 kg ha^{-1} but there was no benefit to additional N. However, switchgrass biomass increased with increasing N rates up to 168 kg ha^{-1} in low organic matter and low fertility soils in Texas USA (Muir et al., 2001), and Vogel et al. (2002) reported that each Mg of switghrass biomass required 10 to 12 kg N ha^{-1} in the Midwestern USA.

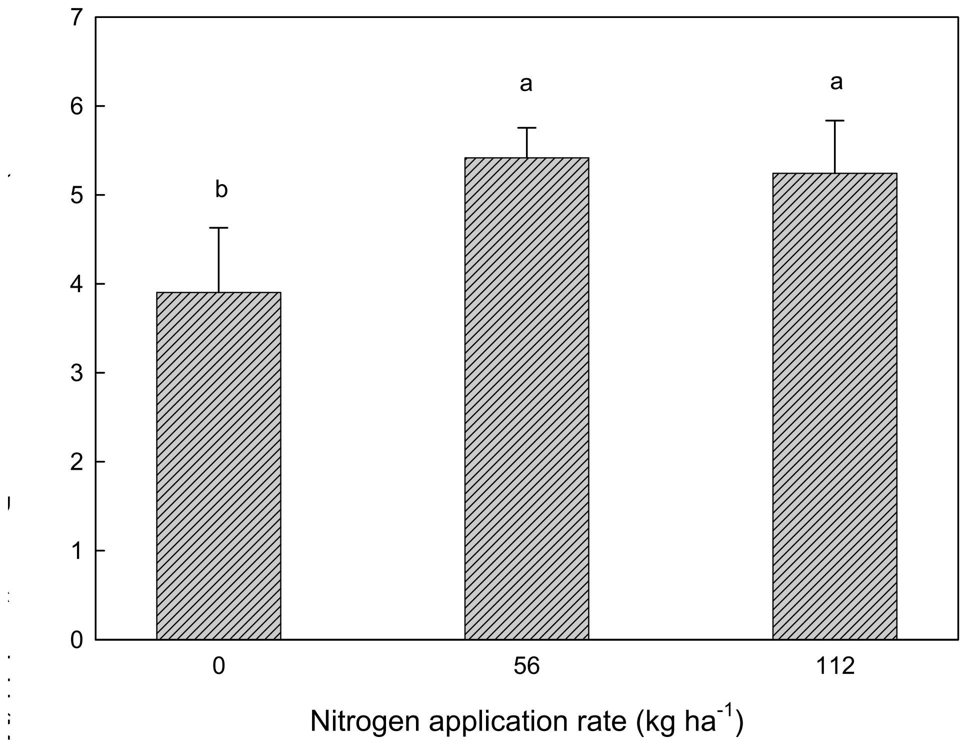

Figure 1. Biomass yield as affected by N rate in switchgrass harvested in South Dakota the second year after establishment (2010).

Nitrogen use

Total N removal increased with increasing N application rate, even though mean value was not significantly different among N rates (Table 1). Higher total N removal at 112 kg N ha^{-1} compared to that at 56 kg N ha^{-1} resulted from higher N concentration in switchgrass at the rate of 112 kg N ha^{-1}. However, NUE at 56 kg N ha^{-1} was much higher than that at 112 kg N ha^{-1}, even though mean value was not significantly different among the N rates. This high NUE at 56 kg N ha^{-1} might cause no difference of biomass yield between these N rates. Apparent fertilizer N recoveries were 3.8 and 5.1 % at 56 and 112 kg N ha^{-1}, respectively. These recovery rates are much lower than found by other researchers who reported that N recovery was about 31% following switchgrass fertilization at 90 kg N ha^{-1} per year in Pennsylvania USA (Stout and Jung, 1995), but similar to annualized values of about 10% at 90 kg N ha^{-1} in Virginia USA (Lemus et al., 2008). At this recovery rate, more than 90% of total fertilizer N is unaccounted for, and may be susceptible to loss through leaching, denitrification, and volatilization, or some portion of it may have become sequestered in belowground storage pools, i.e., roots, microbial biomass, and/or soil organic matter. In addition to wasting money on unnecessary fertilizer, excessive N application beyond plant requirements could cause adverse environmental effects through N_2O gas emission and NO_3 leaching.

Table 1. N concentration, total N removal, fertilizer N removal, apparent N recovery (ANR), and N use efficiency (NUE) as affected by N rate in switchgrass harvested in South Dakota the second year after establishment (2010)

N metrics	N rate (kg N ha^{-1})			$LSD_{0.05}$
	0	56	112	
N concentration (g N kg^{-1})	1.42	1.36	2.20	NS
Total N removal (kg N ha^{-1})	5.29	7.43	11.05	NS
Fertilized N removal (kg N ha^{-1})	-	2.13	5.76	NS
ANR (%)	-	3.81	5.14	NS
NUE (kg biomass kg N^{-1})	-	27.02	11.96	NS

Nitrogen losses

Cumulative N_2O flux during the growing season increased with increasing N application rate (Fig. 2). Mean values of cumulative N_2O flux were 204, 334, and 451 g N_2O-N ha^{-1} at N rates of 0, 56, and 112 kg N ha^{-1}, respectively. In this study, portions of fertilizer N loss through N_2O emission ranged from 0.4-0.6%. Cumulative N_2O flux in the 0 N control (204 g N_2O-N ha^{-1}) was similar with that reported by Garland et al. (2011) (190 g N_2O-N ha^{-1}) in a grape vineyard with no-nitrogen and no-tillage practice.

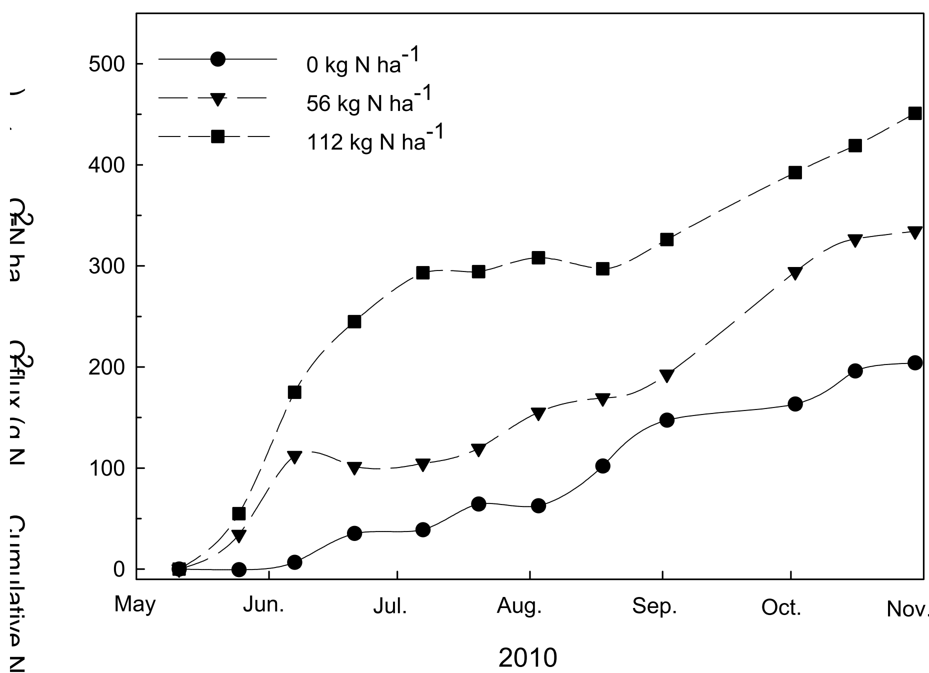

Figure 2. N rate effect on cumulative N_2O flux during 2010 switchgrass growing season.

Cumulative NO_3 leaching with N fertilization at 56 kg N ha^{-1} was similar to that at 0 kg N ha^{-1}, but there was a substantial increase at 112 kg N ha^{-1}, even though the mean value was not significantly different among N rates (Fig. 3). Cumulative NO_3 leaching at 112 kg N ha^{-1} (6.21 kg NO_3-N ha^{-1}) was lower than that (8.43 kg NO_3-N ha^{-1}) reported in other work conducted on maize (*Zea mays*) silage with 142 kg N ha^{-1} application (Gheysari et al., 2009). Differences in the root system of annuals and perennials is likely to affect NO_3 leaching. An existing root system in perennials at the time of N application could reduce NO_3 leaching whereas the root system may be limited in annual species at the time of N application (Bransby et al., 1998). High NUE at 56 kg N ha^{-1} might account for lower cumulative NO_3 leaching at 56 kg N ha^{-1} compared to 112 kg N ha^{-1} (Table 1). 2.13 kg N ha^{-1} and 5.76 kg N ha^{-1} of fertilizer N were removed by switchgrass at 56 kg N ha^{-1} and 112 kg N ha^{-1} N application, respectively. Therefore, 53.87 kg N ha^{-1} and 106.24 kg N ha^{-1} of N fertilizer were not used by switchgrass, respectively. While switchgrass biomass yield was not significantly different between the two N rates (56 and 112 kg N ha^{-1}), much greater N fertilizer at 112 kg N ha^{-1} N may have beenthrough NO_3 leaching and N_2O emission.

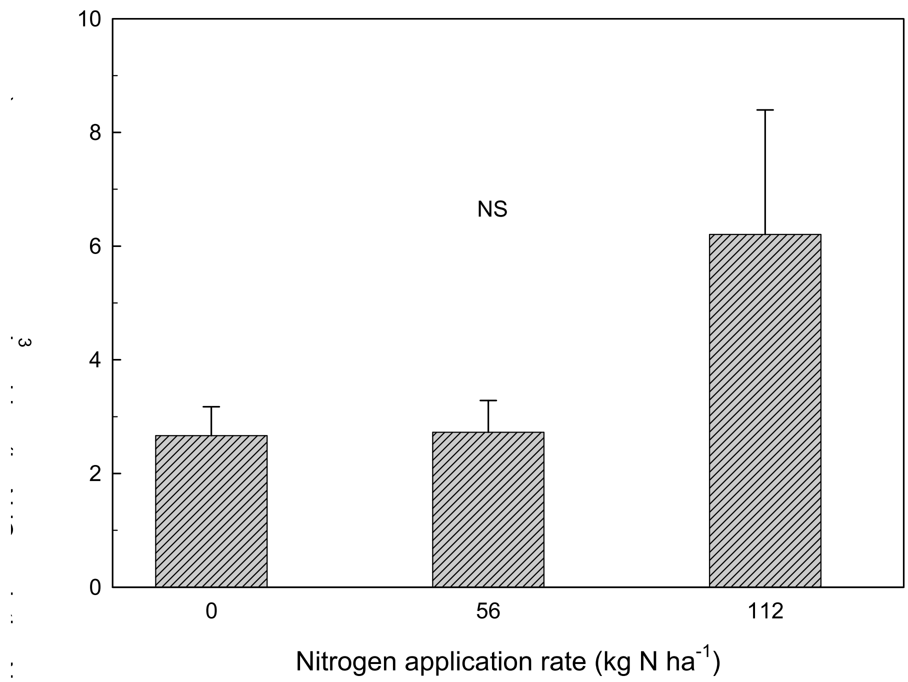

Figure 3. N rate effect on cumulative NO_3 leaching during 2010 switchgrass growing season.

Conclusion

Biomass yield significantly increased with N application up to 56 kg N ha^{-1} but there was no benefit to 112 kg N ha^{-1}. Apparent fertilizer N recoveries were 3.8 and 5.1 % at 56 and 112 kg N ha^{-1} year^{-1}, respectively. However, nitrogen use efficiency was higher with N fertilization at 56 kg N ha^{-1} than that at 112 kg N ha^{-1}. Cumulative N_2O emissions during the growing season increased with N fertilization. Cumulative NO_3 leaching with N fertilization at 56 kg N ha^{-1} was similar to that at 0 kg N ha^{-1}, but there was a significant increase at 112 kg N ha^{-1}. Based on the results thus far, optimum N fertilization rate in South Dakota is around 56 kg N ha^{-1}. No N application resulted in lower biomass production while a higher rate (112 kg N ha^{-1}) exacerbated adverse environmental effects.

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3.10
SWITCHGRASS RESPONSE TO N FERTILIZER ACROSS DIVERSE ENVIRONMENTS IN THE USA: A REGIONAL FEEDSTOCK PARTNERSHIP REPORT

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Abstract
The Regional Feedstock Partnership is a collaborative effort between the Sun Grant Initiative (through Land Grant Universities), the US Department of Energy, and the US Department of Agriculture. One segment of this partnership is the field-scale evaluation of switchgrass (*Panicum virgatum* L.) in diverse sites across the USA. Switchgrass was planted (11.2 kg PLS ha^{-1}) in replicated plots in NY, OK, SD, and VA in 2008 and in IA in 2009. Planting occurred in AL in 2010 following unsuccessful attempts in 2008 and 2009. Adapted switchgrass cultivars were selected for each location and baseline soil samples collected before planting. Nitrogen fertilizer (0, 56, and 112 kg N ha^{-1}) was applied each spring beginning the year after planting, and switchgrass was harvested once annually after senescence. Establishment, management, and harvest operations were completed using field-scale equipment. Switchgrass production ranged from 2 to 11.5 Mg ha^{-1} across locations and years. With the exception of the IA location, yields were lowest the year after planting. Yield increased with 56 kg N ha^{-1} at SD and VA but did not increase further at the high N rate. There was no effect of N at OK or IA, and a negative response at NY. Initial soil N levels were lowest in SD and VA (significant N response) and highest at the other three locations (no N response). These results demonstrate the importance of proper N management in order to reduce unnecessary expense and potential environmental impacts of switchgrass grown for bioenergy across the USA.

Keywords: Switchgrass, Biomass, Fertilizer, Regional Feedstock Partnership

Introduction
Switchgrass has been extensively studied for its value as a forage, conservation, and bioenergy crop (Casler and Boe, 2003; McLaughlin and Walsh, 1998; Muir et al., 2001; Sanderson et al., 1999; Vogel et al., 2002). It offers a number of distinct benefits including broad adaptation, improved soil conservation and quality (Lee et al., 2007; McLaughlin and Walsh, 1998), reduced greenhouse gas emissions (McLaughlin et al., 1996), and carbon sequestration (Garten and Wallschleger, 2000; Lee et al., 2007; McLaughlin et al., 2002; Liebig et al., 2008). In particular, it has high yield potential on land marginal to row crop production (Mulkey et al., 2006). In previous work in South Dakota USA, Mulkey et al. (2006) found that switchgrass grown in marginal soil was well suited for sustainable biomass energy production.

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Although switchgrass tolerates low soil fertility, optimizing biomass and maintaining quality stands requires nitrogen (N) fertilizer inputs and proper management. Switchgrass responds positively to N fertilization, but its response varies with regional environment and soil fertility. Switchgrass biomass increased with increasing N rates up to 168 kg ha^{-1} in low organic matter and low fertility soils in Texas USA (Muir et al., 2001), and Vogel et al. (2002) reported that each Mg of switchgrass biomass required 10 to 12 kg N ha^{-1} in the Midwestern USA. However, Mulkey et al. (2006) reported no benefit with N application rates above 56 kg ha^{-1} on Conservation Reserve Program lands in South Dakota. A major question regarding switchgrass management as a bioenergy crop is optimizing N application rate since excessive N fertilization may result in adverse environmental and economic effects.

Direct comparisons of N fertilization in replicated switchgrass field trials across the USA are limited. This study is one segment of the Regional Feedstock Partnership, a program funded by the US Department of Energy and coordinated by the Sun Grant Initiative, which was designed to evaluate dedicated herbaceous energy crops and CRP land across environmental gradients in the USA. Specifically, the objective of this research was to assess the yield potential and quality parameters of switchgrass grown in different environments using standard agricultural practices.

Materials and Methods

Site description

This study was conducted at six locations across the USA including South Dakota (SD), New York (NY), Iowa (IA), Oklahoma (OK), Virginia (VA), and Alabama (AL). The SD location was near Bristol, SD USA (45°16' 8.274"N; 97°50'8.9694"W) on a Nutley-Sinai (silty clay, mixed, Chromic Hapluderts) with 2-20% slope; the NY location was near Tompkins, NY USA (42° 27' 44.5896" N; 76° 27' 38.1882" W) on an Erie channery (fine-loamy, mixed, mesic Aeric Fragiaquepts) with 2-8% slope; the IA location was near Ames, IA USA (41° 58' 59.001" N; 93° 41' 50.0346" W) on a Clarion-Nicolette (fine-loamy, mixed, mesic Typic Hapludolls) with 0-9% slope; the OK location was near Muskogee, OK USA (35° 44' 32.9994" N; 95° 38' 21.12" W) on a Parsons-Carytown (fine, mixed, thermic Mollic Albaqualfs-Albic Natraqualfs) with 0-3% slope; and the VA location was near Pittsylvania, VA USA (36° 55' 56.2656" N; 79° 11' 23.8842" W) on a Mayodan (fine sandy loam, mixed, thermic Typic Hapludults) with 2-15% slope. Data from AL is not included in these results due to establishment failures in 2008 and 2009 and excessive weed competition prohibiting application of N treatments to the established stand in 2011.

Experimental design and switchgrass management

A locally adapted switchgrass cultivar was planted at each location at a rate of 10 lbs pure live seed (PLS) a^{-1} (10 kg PLS ha^{-1}). Cultivar, planting date, total field size, and harvest dates from 2009-2011 are listed for each location in Table 1. All trials reported here were planted in 2008 except IA which was planted in 2009. Experimental design at each location was a randomized complete block with four replications across the landscape. Individual plot size ranged from 1 to 2 acres (0.4 to 0.8 ha) to allow use of conventional agricultural equipment. Nitrogen was applied annually during spring beginning the year after planting at 0, 50, and 100 lbs a^{-1} (0, 56, and 112 kg N ha^{-1}) at all locations. Switchgrass was harvested once annually after a killing frost beginning the year after establishment. Wet conditions precluded fall harvest in VA; therefore, switchgrass was harvested in January of the following year when soil was dry enough to allow equipment access.

Table 1. Location, cultivar, planting date, field size, and 2009-2011 harvest dates for switchgrass field trials.

Location	Cultivar	Planting Date	Field size Acre (ha)	Harvest Date 2009	2010	2011
OK	Blackwell	2 Sep. 2008	18 (7.3)	13 Nov.	28 Oct.	16 Dec.
NY	Cave-In-Rock	29 May 2008	12 (4.9)	22 Oct.	2 Nov.	3 Nov.
SD	Sunburst	17 May 2008	24 (9.7)	28 Oct.	5 Nov.	3 Nov.
VA	Alamo	1 July 2008	15 (6.0)	10 Jan. 2010	Jan. 2011	18 Jan. 2012
IA	Cave-In-Rock	8 May 2009	18 (7.3)	NA	18 Nov.	7 Nov.

Yield was determined by harvesting a windrow through the center of each plot with locally available harvest equipment (typically a sickle bar mower or disc mower) at a height of 4 to 6 inches (10 to 15 cm). Biomass from this windrow was baled in large round or large square packages and bales were weighed. Subsamples (approximately 0.7 lbs or 300 g) were collected with a hay probe attached to an electric drill from the center of bales for further analyses. Subsamples were weighed, dried at 140°F (60°C) for 48 h in a forced-air oven, reweighed to determine dry matter yield, and ground in preparation for chemical analysis. All subsamples were ground in a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA) to pass a 0.039 in. (1 mm) screen and reground to uniformity in an Udy-cyclone impact mill (Udy Co., Ft. Collins, CO) with a).039 in. (1 mm) screen. Subsamples were not collected at VA in 2010.

Soil sampling

A hydraulic soil probe with a 2.6-in (6.6 cm) internal diameter was used to collect soil samples at initiation of the research at each location. Four random cores were collected from each plot to a depth of 39.4 in. (1 m). Each core was subdivided into depth increments of 0 to 2, 2 to 6, 6 to 12, 12 to 24, and 24 to 39 in. (0 to 5, 5 to 15, 15 to 30, 30 to 60, and 60 to 100 cm) after which soil from each of the four cores at each depth was composited for analysis. Surface residue was removed before sampling. Soil samples were initially sieved to pass a 0.31 in. (8 mm) screen and dried in a forced-air oven at 104°F (40°C) until consistent mass was attained. Visible plant residue and roots were removed before drying. Dried soil samples were ground to pass a 0.08 in. (2 mm) screen for chemical analysis.

Results and Discussion

Switchgrass yield ranged from around 1.0 to 4.9 tons acre^{-1} (2 to 11 Mg ha^{-1}) across locations and years (Fig. 1).. Stand frequency was assessed using the method of Vogel and Masters (2001) at all locations during the seeding year, and was above 40% at NY, IA, and OK, but was below 40% at VA and SD (data not shown). By the second year after establishment, stand frequency was above 40% at all locations. Schmer et al. (2005) found that establishment year frequencies below 40% often led to led to lower yields in subsequent years while those with frequencies greater than 40% in the establishment year were better able to optimize yields later. However, while not analyzed directly, yield was higher the year after planting at all locations except IA, regardless of stand frequency the planting year.

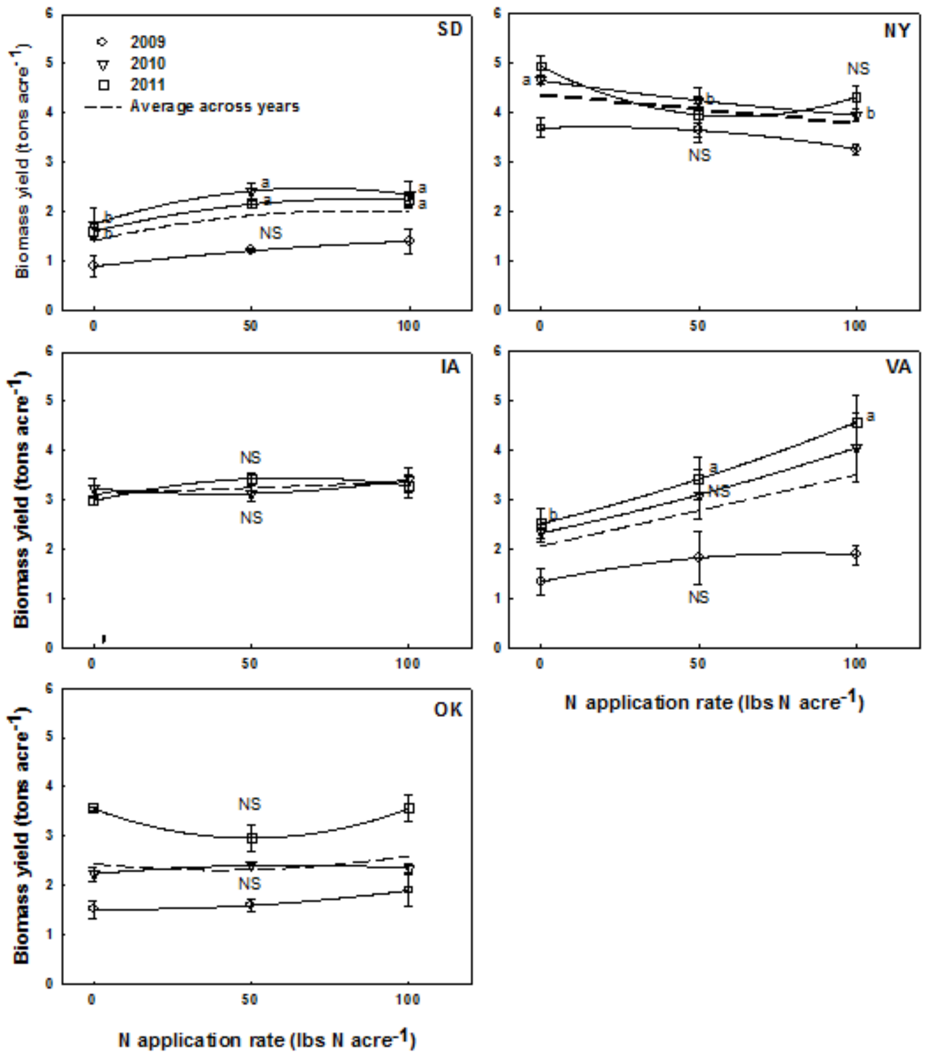

Figure 1. Switchgrass yield response to N at five locations in the US.

Switchgrass responded positively to N in 3 of 14 location/year combinations and there was one location/ year combination (NY in 2010) where a negative response was noted (Fig. 1). Nitrogen rate had no effect on switchgrass biomass production the year after planting at any location. South Dakota and VA were the two states with relatively consistent positive relationship between switchgrass yield and N rate. In SD, switchgrass yield increased with 50 lbs N acre^{-1} (56 kg N ha^{-1}) in 2010 and 2011. This is similar to results of Mulkey et al. (2006) in SD using switchgrass on CRP lands. At OK and IA, the response curve was essentially flat, and in NY yield decreased with increasing N. Based on visual evaluation of the NY location, there appeared to be a problem with lodging of high yielding switchgrass, a problem which was exacerbated at the higher N rates. Thus, we suspect that difficulties associated with efficiently harvesting lodged biomass may have contributed to the negative response to N in 2011 in NY (H. Mayton, personal communication). Interestingly, the two locations (SD and VA) where positive responses to N were found had the lowest soil N concentrations at the beginning of the research (Table 2).

Table 2. Percent soil N in the top 12 inches (30 cm) at five locations before switchgrass planting.

	Soil Depth		
Location	0-2 in (0-5 cm)	2-6 in (5-15 cm)	6-12 in (15-30 cm)
SD	0.19 (1.93)*	0.16 (1.55)	0.10 (1.04)
NY	0.30 (3.02)	0.23 (2.29)	0.18 (1.80)
IA	0.25 (2.53)	0.23 (2.26)	0.19 (1.86)
OK	0.24 (2.39)	0.17 (1.68)	0.12 (1.17)
VA	0.11 (1.12)	0.06 (0.55)	0.04 (0.41)

* Values in parentheses are g N kg^{-1} soil.

Conclusions

Nitrogen application rate did not consistently increase switchgrass biomass production at the field scale in trials conducted across the US. However, N was particularly important for switchgrass production on fields (SD and VA) with low initial soil N tests. Yields were lowest the year after establishment in all locations except IA despite the fact that OK, IA, and NY had greater than 40% stand frequencies in the seeding year. Harvest will be done in 2012, allowing us to better ascertain the viability of switchgrass as a bioenergy crop in several regions of the US.

Funding Acknowledgement

This research was supported by funding from the North Central Regional Sun Grant Center at South Dakota State University through a grant provided by the US Department of Energy Office of Biomass Programs under award number DE-FG36-08GO88073.

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3.11 ACCUMULATION OF BIOMASS AND COMPOSITIONAL CHANGE OVER THE GROWTH SEASON FOR SIX PHOTOPERIOD SORGHUM LINES

Leo Hoffmann Jr.[1,], William L. Rooney[1]*

Abstract

Biomass sorghums [*Sorghum bicolor* (L.) Moench] are short-day photoperiod sensitive (PS) types meaning that the crop will grow vegetatively until the fall season in subtropical and temperate environments. This feature results in high biomass yield potential and mitigates drought susceptibility. The objective of this study is to assess compositional changes and biomass growth patterns over a growing season for PS sorghum. The experiment had a randomized complete block design with two reps, six genotypes, and 13 harvest dates. Harvest started at 60 days after planting (DAP) and continued every 15 days thereafter in both College Station (CS) and Corpus Christ (CC) in Texas, 2010. At each harvest, dry biomass yield, height and biomass composition (lignin and cellulose) were measured. The biomass accumulation followed a standard growth pattern and were maximum between 180 and 225 DAP where the best genotype produced a dry biomass yield of approximately 21000 lb*ac^{-1}. Height increased up to 13 feet cm between 180 and 225 DAP. Height and biomass yield patterns were similar, indicating that height is important to increase yield. Lignin and cellulose content increased with maturity with maximum lignin content between 14.5 to 15.5% and maximum cellulose at 31 to 32%; both occurred between 180 and 195 DAP at CS. The results indicate that maximum sorghum biomass accumulation occurs between 120 and 210 days and is genotype dependent. Biomass production plans for sorghum should identify different sorghum genotypes to maximize yield within these harvest times.

Keywords: Biomass yield, Lignin, Cellulose, Near infrared spectroscopy

Introduction

The need for reliable, available and efficient sources of energy is critical to the long-term sustainability of modern society. Several biofuel crops have been proposed by the scientific community, among them is sorghum [Sorghum bicolor (L.) Moench]. As a crop, sorghum can be divided in five categories; grain sorghum, forage sorghum, broomcorn, sweet sorghum and high biomass sorghum (Rooney et al. 2007, 147).

Biomass sorghums are highly productive because they are very photoperiod sensitive (PS) which allows them to grow in a vegetative stage for long periods. Vegetative growth allows increased biomass accumulation and enhanced drought tolerance. These PS lines/hybrids are capable of long periods of vegetative growth before reproductive growth is initiated in response to a reduction in daylength, usually below 12.5 hours (Rooney and Aydin, 1999, 397). Thus, in temperate and some subtropical climates, the initiation of reproductive growth does not occur until the fall season and post-anthesis growth is limited by cold weather.

Because these biomass sorghums do not produce grain, the primary carbohydrate production is the structural carbohydrates of lignin, cellulose and hemicellulose in the forms of roots, leafs, and most important stems. Additionally, non-structural carbohydrates (sugars), protein and minerals make up the rest of the tissue. Lignin is a primary component of the biomass and acts as a matrix in the cell wall that

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integrates and surrounds cellulose and hemicelluloses compounds. Lignin is antagonist to biofuel conversion and it binds to enzymes intended to cellulose and hemicellulose breakdown. For this reason, biomass composition becomes an important component in the development of energy crops.

Together with productivity, the quality of the biomass is important to biofuel conversion production systems. Biomass quality and its stability could be a limiting factor for profitability of the production system. In numerous studies plant biomass composition was found to be variable according to genotype, environment and stage of plant growth effects (Reeves, 1987, 1583; Pordesimo et al. 2005, 366). Pordesimo et al., (2005, 366), during their growth stage study of corn stover composition found that lignin concentration increased with crop maturation, implicating decreased quality. If biomass sorghum is to become established as a dependable feedstock for biofuel conversion, an understanding of biomass accumulation and biomass composition evolution over the growing season is key to increasing efficiency and profitability of this operation.

With these factors in consideration, the objectives of this study was to assess the biomass yield accumulation and composition patterns of six photoperiod sensitive sorghum lines over the growing season in College Station and Corpus Christi, Texas.

Materials and Methods

A randomized complete block design with two replications was used. The variables in the design were maturity and genotype. For genotypes, six different sorghum lines (R.09072, R.09093, R.09106, R.09084, R.09098 and R.09110) were selected based on desirable characteristics from previous evaluations. Harvest was initiated at 60 days after planting (DAP) and occurred every 15 days until the end of the growing season. Planting date was April 16, in College Station (CS) and April 25 in Corpus Christi (CC). The harvest size for the experimental units was approximately 16 ft^2. At each harvest date, plant height (ft) and dry biomass yield ($lb*ac^{-1}$) were recorded. In addition, composition (lignin % and cellulose %) analyses based on samples of grounded dry biomass were estimated using near infrared spectroscopy (NIR) methods.

Results

In the combined analysis, genotypes varied for plant height, dry biomass yield and lignin content (Table 1). Location had a significant effect for the composition traits lignin and cellulose content. As expected date of harvesting represented by DAP, which represents crop maturity, accounted for the largest proportion of variation and influenced all four traits. Interactions among main sources of variation were observed. Hence, the effects above described reflect different levels of influence on agronomic productivity and quality of composition of the crop.

Table 1. Summary of ANOVA, means square values for the field and composition (12 harvest dates) data collected from six different photoperiod sensitive sorghum lines, in two locations, Corpus Christi and College Station, in Texas, 2010.

		Field			Composition	
Source	**DF**	**Height**	**Dry Yield**	**DF**	**Lignin**	**Cellulose**
Error	130	1320.90	34.64	132	0.7	2.25
Genotype	5	4774.50*	159.97*	5	1.78*	2.79
Location	1	1463.50	134.84	1	3.10*	46.04*
DAP	10‡	197679.90*	803.40*	11	19.07*	16.62*
GxL	5	2997.00	18.43	5	1.50	4.12
GxDAP	50	1642.50	33.68	55	1.01*	3.19
LxDAP	10	1596.50	140.85*	11	6.40*	23.99*
GxLxDAP	50	1590.50	21.04	55	0.43	2.71
Rep	1	1154.60	373.62*	1	5.53*	12.50*
Mean		311.98 cm	17.46		14.31	31.13
R^2		0.93	0.76		0.81	0.75
CV%		11.65	33.71		5.85	4.82

* Significantly different at level of 0.05 of probability.
† Mean of yield data expressed in Mg*ha^{-1}.
‡ reduction on harvest dates were done due missing data.

A standard growth curve shape was recorded for the traits plant height and dry biomass yield with log and lag phase (Figure 1). For both traits a log phase with exponential growth was observed from the beginning of harvest up to 120 DAP. At this stage plants were growing 1.30 feet per week and producing 1700 to 2600 lb*ac^{-1} per week (Figure 1). The line R.09098 was the most productive during the log phase and at the mark of 120 DAP reached approximately 19000 lb*ac^{-1} of dry biomass. After 120 DAP, variation due to genotype and harvest date was minimal and in some cases, there was reduction in the rate of gain or actual gain of dry matter and height.

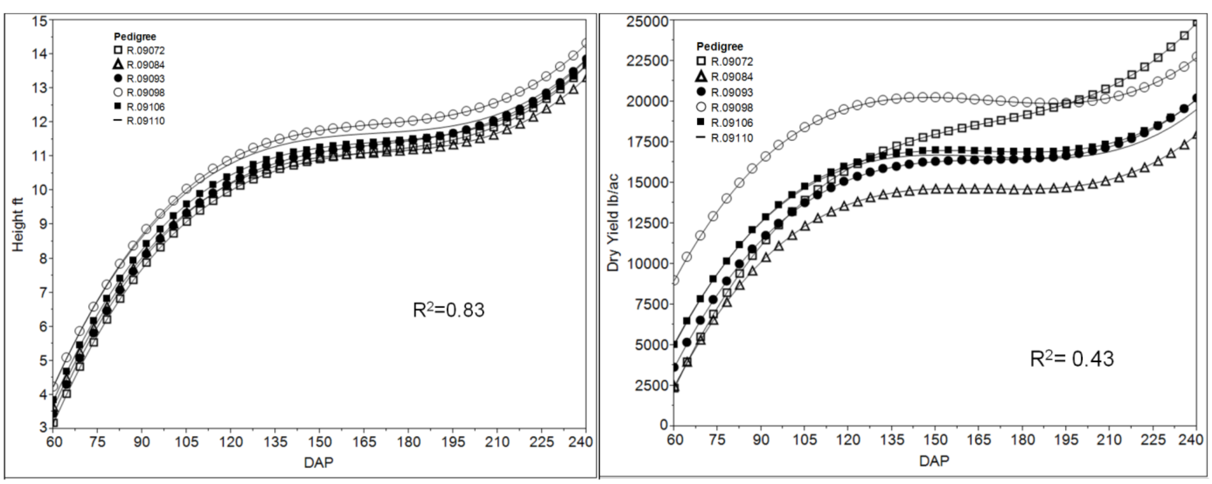

Figure 1. Cubic regression for plant height (left) and total dry biomass yield (right) of six genotypes of biomass PS sorghum in combined CC and CS locations, in Texas, 2010.

The lignin content varied across locations, but lignin concentrations peaked concomitant with maximum yields. In CC, lignin concentration peaked (15.12% on R.09072 to 16.25% on R.09106) at 210 DAP while in CS, it peaked (14.62% on R.09106 to 15.25% on R.09098) slightly earlier at 195 DAP. In both locations, there was a slight drop in lignin concentration in the last one or two harvests, possibly due to plant degradation as growing conditions deteriorated (Figure 2). Cellulose concentration in the plant had a different pattern for each location. At CC, a drop in cellulose concentration (33 at 90 DAP to 30.5 % at 225 DAP) was observed at the mark of 150 DAP but by the end of season 225 cellulose it recovered to 31.5% in average (data not shown). This change in composition as the growing season ends is characteristic of grasses species and it is known as lignifications which usually reflects a drop in quality of the forages/biomass.

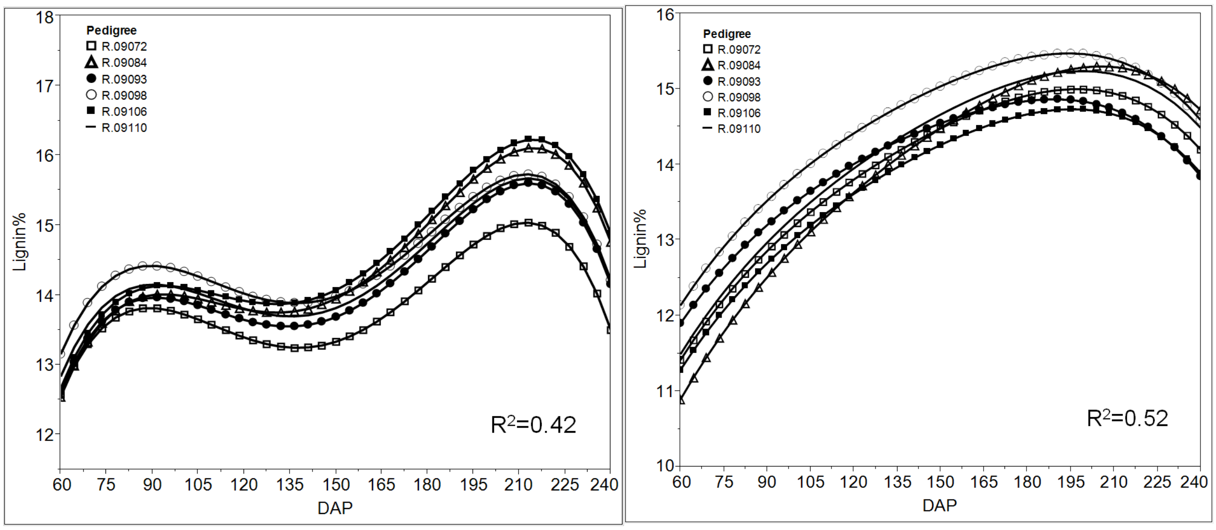

Figure 2. Quartic regression for lignin percentage of dry biomass of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010.

Together agronomic and compositional data presented a harvest window from fourth to seventh month. Over this harvest window there are compromises that must be made. For example, earlier harvest results in more leaf material, higher moisture content and higher ash content per unit of biomass (data no shown). Later in the season, harvested biomass sorghum may have less water, lower ash content and leaf material, but the lignin concentration are likely higher.

Conclusions

In summary, the data indicate that harvest can start as early as 120 days after planting and continue until at least 240 days after planting. Although, monitoring the quality of sorghum biomass during this period will help the crop system managers to have a best biofuel conversion ratio possible for the given harvesting period.

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3.12
GREEN-CANE HARVEST OF SUGARCANE EFFECTS ON BIOMASS AND ENERGY YIELDS AND NUTRIENT REMOVAL

Paul M. White, Jr.[1,], Ryan P. Viator[1], Edward P. Richard, Jr.[1], Michael P. Grisham[1]*

Sugarcane yields in Louisiana can approach 40 dry Mg ha^{-1}, making sugarcane an attractive biofuel feedstock as well as a profitable sugar crop. Existing technology used in green-cane harvesting can be used to allow chopper harvester extractor fans to remove variable amounts of extraneous leaf material from the cane stalk and deposit it on the soil surface during harvest. The objectives were to (1) evaluate biomass and energy yields of selected sugarcanes, (2) estimate residue nutrient losses, and (3) evaluate logistics of harvesting at different extractor fan speed settings simulating sugar or biomass harvest. A commercial sugarcane variety HoCP 96-540 and a high-fiber "energycane" variety L 79-1002 were planted in 2009 and the plant cane was harvested in Nov. 2010 and first stubble in Oct. 2011. Three fan speeds were used: (1) Optimal range of 750 rpm for sugar harvest; (2) Fans turned off to simulate biomass harvest; and (3) mid-range of 375 rpm to deposit some crop residue. For plant cane, L 79-1002 (38 dry Mg ha^{-1}) produced more biomass than HoCP 96-540 (31-32 dry Mg ha^{-1}). For first ratoon, the varieties produced the same biomass (32 dry Mg ha^{-1}). Slowing or stopping extractor fans increased dry biomass yield by up to 13 Mg ha^{-1}. However, turning off extractor fans increased nutrient removal from field by 9.6, 2.5, and 14 kg of N, P_2O_5, and K_2O ha^{-1}, respectively, when compared to the 750 rpm fan speed. The biomass harvest strategy with the harvester's extractor fans turned off, designed to remove as much total biomass as possible, increased the number of cane wagon loads by a factor of 1.6, due to lower density of crop residue. Adoption of biomass harvest strategy using existing sugarcane harvest technology produced dry feedstock yields of 39 Mg ha^{-1}, but the nutrient losses associated with residue removal may make lower yields more sustainable.

Keywords: Sugarcane, energycane, feedstock, nutrients, soil quality

Introduction

Sugarcane yields in Louisiana can approach 40 dry Mg ha^{-1}, and hybrids of sugarcane and wild germplasm can double that number (Legendre and Burner, 1995). making it an attractive biofuel feedstock source as well as a profitable sugar crop. Existing technology used in green cane harvesting can be used to allow chopper harvester extractor fans to remove variable amounts of extraneous leaf material. Lower fan speeds (650 rpm) removed less leafy material and led to more fiber, soluble solids, and mud, and less sucrose purity and extraction efficiency, as compared to higher fan speeds (Eggleston et al., 2012). Fan speeds as high as 1050 rpm led to a 13% reduction in cane yield when cut under wet field conditions, as cane billets were blown away with the leaf material (Viator et al., 2007). Thus an optimal fan speed for sugar harvest would minimize leafy trash and optimize retention of cane billets. In Louisiana, green cane harvesting typically deposits between 6-24 Mg ha^{-1} of leafy material on the soil surface (Viator et al., 2007). For a biomass harvest, this additional leafy material will be collected and sent to a biorefinery or other processing center.

Complete removal of aboveground biomass for cellulosic feedstock harvesting operations may affect crop sustainability by negatively impacting soil health. Varvel et al. (2008) reported that 51% removal of corn stover resulted in significant reductions of corn grain and corn stover yield over a six-year period. Similarly, Wilhelm et al. (1986) reported a 0.1 Mg ha^{-1} grain yield reduction and a 0.3 Mg ha^{-1} reduction in stover residue produced for each Mg ha^{-1} of stover removed. Karlen et al. (1994) reported a loss of 4.5

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and 0.28 Mg ha^{-1} of soil carbon (C) and nitrogen (N) after 10 years of corn stover removal, when compared to non-removal. However, with complete removal of the extraneous leaf material, producers of perennial crops may avoid negative impacts of the crop residue mulch layer on the emerging crop, including cooler, wetter soil conditions during spring emergence, leached autotoxic chemicals, and reduced weed management options (Viator et al., 2006; Viator et al., 2008).

This research was initiated to (1) evaluate biomass and energy yields of sugarcane, (2) estimate residue nutrient losses, and (3) evaluate logistics of harvesting at different extractor fan speed settings simulating sugar or biomass harvest.

Materials and Methods

The field experiment was conducted at the USDA-ARS Sugarcane Research Unit's Ardoyne Farm near Schriever, LA (29°38'11" N, 90°50'25" W). Two sugarcane varieties, HoCP 96-540, and L 79-1002 were hand-planted in 2009 in Cancienne silt loam (Fine-silty, mixed, nonacid, thermic, aeric fluvaquent) after four-months of fallow. Sugarcane was planted in raised beds at a spacing of 1.8 m at a rate of three mature stalks with a 10% overlap. Planted stalks, 1.5 m in length, were covered with 8 cm of packed soil. A tank-mix containing metribuzin (4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) at 3.4 kg a.i. ha^{-1} and pendimethalin (*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine) at 2.2 kg a.i. ha^{-1} was broadcast immediately after planting for weed control. Nitrogen [UAN 32% - consists of urea (35%), ammonium nitrate (45%), and water (20%)], phosphorus (diammonium phosphate), and potassium (potash) were applied in mid-April at 135, 34, and 68 kg ha^{-1}, respectively, as an injected band on both sides of the planted cane. Tebufenozide (*N*-*tert*-butyl-*N'*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide) at 0.1 kg a.i. ha^{-1} was applied in the summer for sugarcane borer (*Diatraea saccharalis* F.) control when infestations reached thresholds defined by Louisiana State University Cooperative Extension Service (Legendre et al., 2001).

Whole plot size was 11 m wide X 122 m in length and consisted of six rows. The whole plots were divided into 4 replications of each variety. Splitplots were 5.5 m wide x 30 m in length and consisted of three rows. Three chopper harvester extractor fan speeds were evaluated: (1) Optimal range of 750 rpm for sugar harvest; (2) Fans turned off to simulate biomass harvest; and (3) Mid-range of 375 rpm to deposit some crop residue. Harvest was conducted in November 2010 (plant cane) and October 2011 (first ratoon). Cane yield (Mg ha^{-1}) was determined using a modified tipper-wagon equipped with electronic load cells and fitted with a billet sampling basket that allowed an operator to catch a sample of billeted cane as it is being transferred from the harvester to the wagon. Juice was removed from cane using the prebreaker and core press method (Johnson and Richard, 2005; Meade and Chen, 1977). Brix was measured on cane juice using a refractometer. The resulting fiber cake was dried at 65°C for three days. Percent fiber was the weight of dry fiber cake divided by 1 kg original cane sample. Dry matter was the sum of brix and fiber. Plant cane and first ratoon data were analyzed using PROC MIXED (SAS Institute, 2008) with variety and fan speed as fixed variables and replication as a random variable using SAS version 9 software (SAS Institute, Cary, NC). Means of significant effects were separated using the PDIFF option with the SAXTON macro at the P=0.05 level (Saxton, 1998).

Results and Discussion

For plant cane, both variety and fan speed significantly affected dry biomass yields (Table 1). The high fiber L 79-1002 out yielded HoCP 96-540 by 5 Mg ha^{-1}, and slowing (375 rpm) or turning off extractor fans increased dry biomass yields by 32% and 39%, respectively, as compared to the typical sugarcane harvest fan speed (750 rpm). Dry biomass yields differences were consistent between L 79-1002 (66 Mg ha^{-1}) and the commercial sugarcane variety CP 65-357 (13 Mg ha^{-1}) harvested with extractor fans turned off (Bischoff et al., 2008). For first ratoon, both varieties produced the same dry biomass yields (Table 1). However, similar to plant cane, slowing or stopping extractor fans increased dry biomass yields by as much as 52%.

Table 1. Dry biomass yields for plant cane and first ratoon for HoCP 96-540 and L 79-1002 at three fan speeds.

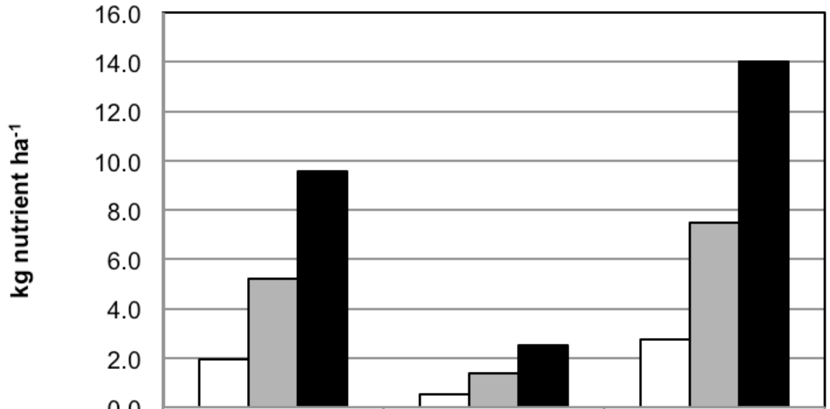
	Plant cane	**First ratoon**
Variety	----------- Mg dry biomass ha^{-1}-----------	
HoCP 96-540	**32 Ba***	**31 Aa**
L 79-1002	**38 Aa**	**32 Ab**
Fan speed (r.p.m.)		
750	**28 Ba**	**25 Cb**
375	**37 Aa**	**33 Bb**
Off	**39 Aa**	**38 Aa**

*Means followed by the same uppercase letter in each column and same lowercase letter in each row are not significantly different at the $p<0.05$ level.

Sugarcane HoCP 96-540 dry matter yields were consistent between plant cane and first ratoon, averaging 32 Mg ha^{-1}. In contrast, dry biomass yield of L 79-1002 decreased 19% between crops. L 79-1002 was considered as moderately susceptible to smut (Bischoff et al., 2008) which may account for the yield decline as visible smut whips were prevalent in the crop (White, personal observance). Both the 750 and 375 rpm fan speeds resulted in different yields between plant cane and first ratoon. The 'fans off' treatment did not. A possible explanation is lower stalk yield in the ratoon crop, when averaged across both varieties, which would account for a greater percentage of dry biomass as fan speed increased.

Samples of commercially released high fiber sugarcane (Ho 02-113) contained 16.6 GJ Mg^{-1} of combustible dry matter (P. White and G. Aita, unpublished data). This is similar to reported values for switchgrass (*Panicum virgatum* L.) of 16.4 GJ Mg^{-1}, giant reed (*Arundo donax* L.) of 17.6 GJ Mg^{-1}, and miscanthus (*Miscanthus x giganteus)* of 17.1 GJ Mg^{-1} (Angelini et al., 2009; Lemus et al., 2002). Using 16.6 GJ Mg^{-1} as a conversion factor the dry matter yields would produce a gross energy yield range of 420 – 650 GJ ha^{-1}, with an average for plant cane and first ratoon of 570 and 520 GJ ha^{-1}, respectively. Switching extractor fans off and removing the maximum amount of leafy material resulted in a per hectare increase of up to 180 GJ ha^{-1} for plant cane and 210 GJ ha^{-1} for first stubble.

Cane leaves sampled in 2009 and 2010 (n=288) contained 6.1, 0.7, and 7.3 g N, P, and K per kg^{-1} dry material, respectively (P. White, unpublished data). Leaf material collected per fan speed are estimated to be 3.3 Mg ha^{-1} (750 rpm), 9.4 Mg ha^{-1} (375 rpm), and 18 Mg ha^{-1} (fans off) based on a 7% leafy material harvest under optimal conditions (R. Viator, personal communication, 2012) and measured total dry biomass yield (Table 1). Combining these data resulted in estimated nutrient export increases for each fan speed setting (Figure 1). The biomass harvest scenario (fans off) would result in a loss of 9.6, 2.5, and 14 kg N, P_2O_5, and K_2O ha^{-1}. This is 7.6, 2.0, and 11.2 kg N, P_2O_5, and K_2O ha^{-1} higher than the optimal fan speed for sugarcane harvest (750 rpm), or \$10.49, \$1.43, and \$12.72 in 2012 fertilizer costs for N, P_2O_5, and K_2O, respectively (USDA-NASS, 2012). The increased leaf residue harvested also increased cane wagon dumps by a factor of 1.6 due to decreased density. Averaged across varieties, mean cane wagon dump weights were 750, 420, and 440 kg, for the 750 rpm, 350 rpm, and fans off treatments, respectively. Each dump occupied the same volume, but the 750 rpm fan speed resulted in significantly heavier loads than the 350 rpm or fans off treatments, which were not significantly different.



	N	P2O5	K2O
750 r.p.m.	1.9	0.5	2.8
375 r.p.m.	5.2	1.4	7.5
Fans off	9.6	2.5	14

Figure 1. Estimated nutrient exports from field under different harvest fan speed settings for HoCP 96-540 and L 79-1002.

Conclusions

Adoption of a biomass harvest strategy using existing sugarcane harvest technology produced dry feedstock yield of up to 39 Mg ha^{-1}, or a gross energy yield of 650 GJ ha^{-1}. However, this greater yield would increase nutrient (N, P, and K) export compared to the sugarcane harvest scenario (750 rpm). Replacement costs for these nutrients would be \$24.55 ha^{-1}. Additional fuel inputs are also likely due to decreased density of biomass which contains leaf material, and concomitant in-field travel time. It may be more sustainable to return a portion of the biomass to the field when nutrient losses associated with residue removal are taken into account. Higher income from greater yields may offset increased inputs (nutrients and fuel) to make “energycane” production economical.

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3.13 CONSERVATION RESERVE PROGRAM (CRP) GRASSLAND FOR SUSTAINABLE BIOMASS FEEDSTOCK PRODUCTION

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Abstract

The 2005 Billion Ton Study proposed that up to 10 million ha of Conservation Reserve Program (CRP) grassland could be dedicated to produce approximately 110 million dry metric tons of bioenergy feedstock annually. The Biomass Regional Feedstock Partnership has identified grasslands planted under the CRP as a potential source for herbaceous bioenergy feedstock. The goal of this project is to assess the yield potential of the CRP grasslands across diverse regions and the significance of management practices on sustainable biomass production. Consistent with that goal, the objective of this project was to evaluate the biomass production potential for CRP land dominated by either warm- or cool-season grass mixtures across the regions of national CRP land distribution during 2008–2011. Standard field-scale agricultural practices were used as management guidelines at each location. Maximum biomass yields required N fertilization; maximal yields ranged from 2.0–3.9 Mg ha^{-1} for the three warm-season CRP sites and 3.6–5.8 Mg ha^{-1} for the three cool-season CRP sites. In addition to N fertilization, precipitation during the growing season was one of the major limiting factors of biomass production. However, harvest timing did not have any consistent impact on biomass yield. In 2011, biomass production at the OK and KS sites was limited by extreme drought conditions. The results of this study demonstrated that CRP grasslands have the potential for biomass production. However, sustainable management practices, including N and harvest management, are required to maximize the biomass production.

Keywords: Conservation Reserve Program (CRP), warm-season grass, cool-season grass, feedstock, N fertilization, harvest management

Introduction

Ideal bioenergy feedstocks should produce less life-cycle greenhouse gases and not compete with food crop production (Tilman et al., 2009). Perennial grasses have been suggested as a cellulosic bioenergy feedstock because of they do not compete with food crops for acreage, sequester carbon, and efficiently use water and nutrients (Tilman et al., 2009). However, perennial grasses grown on productive farmland could cause indirect land use changes and, ultimately, could have an impact on food production and greenhouse gas emissions (Campbell et al., 2008; Searchinger et al., 2008). With these concerns, Conservation Reserve Program (CRP) grasslands have been identified as a sustainable bioenergy feedstock resource, since CRP land would require minimal land use changes for biofuel production

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(Walsh et al., 2003). The Food Security Act of 1985 established the CRP as a land retirement program to reduce soil erosion, enhance water supplies, and improve water quality. This program encourages farmers to convert their highly erodible farmland or other environmentally sensitive lands to a permanent vegetative cover. As of July 2012, 11.98 million ha were enrolled in the CRP, which was significantly reduced from 13.64 million ha in 2007 (USDA-NRCS, 2012). As a result of recent high commodity prices, CRP enrollment acres are expected to continue to decline if farmers cannot find a way to increase their farm income from this land.

The land enrolled in the CRP was identified as one resource for sustainable bioenergy feedstock production by the Sun Grant Initiative/USDOE Regional Biomass Feedstock Partnership. Accordingly, this program has performed replicated field trials on CRP land using field-scale agricultural practices in order to assess the yield potential and suitability of CRP grasslands as a bioenergy feedstock source, across logical regions of adaptation. The specific objectives of this study were to determine the effect of N fertilization and harvest timing on biomass yield over the four years management period across the regions of the national CRP land distribution.

Materials and Methods

Six test locations (Figure 1) were identified based on the CRP grassland distribution in the United States. The established CRP stands were located at the following sites: Foster County, North Dakota (ND, 47.5°N 99.2°W); Ellis County, Kansas (KS, 38.8°N 99.4°W); Jackson County, Oklahoma (OK, 34.7°N 99.3°W); Chouteau County, Montana (MT, 47.1°N 110°W); Boone County, Missouri (MO, 39°N 92.2°W); and Oconee County, Georgia (GA, 33.8°N 83.4°W).

The predominate species varied among the six locations: C_4, warm-season grasses at the ND, KS, and OK sites; and C_3, cool-season grasses at the MT, MO and GA sites. In addition to the C_3 grasses, alfalfa was also a predominate species at the MT site. All locations had been managed in accordance with the CRP regulations, including no N fertilization and/or above-ground biomass harvest since the start of the CRP contract. All field sites were selected in the spring of 2008 and mowed to a height of 10–15 cm before applying fertilization treatments.

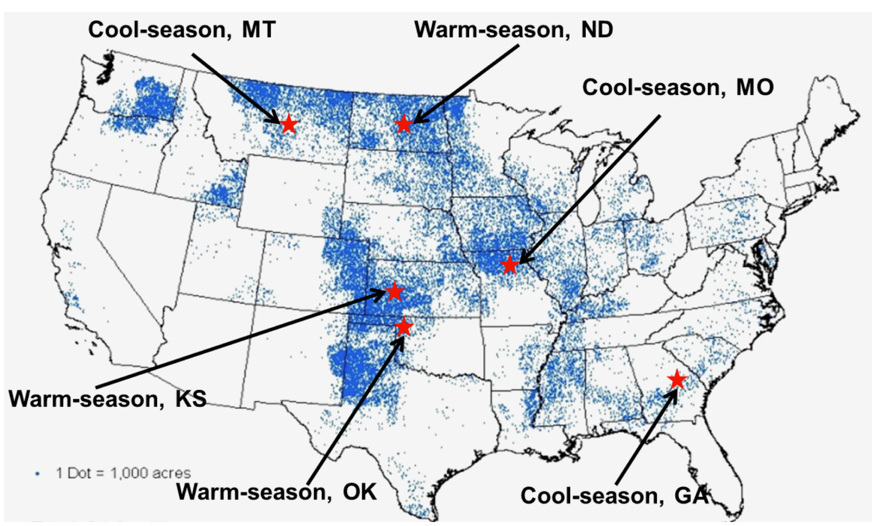

Figure 1. US Map of 2010 Conservation Reserve Program (CRP) enrollment in 2010 and the warm- and cool-season mixtures research site locations.

The experimental design was a factorial arrangement of three N rates and two harvest dates within a randomized complete block with three replicates at each location. The plot size for the treatments was approximately 0.5 ha. Urea N fertilizer was annually broadcasted at 0, 56, and 112 kg N ha^{-1} using a farm-scale fertilizer spreader before spring green-up. No other fertilizer was applied as a treatment; however, phosphorus was added to some of the sites early in the experiment to increase deficient levels.

Biomass yield was determined from a whole plot harvest with a farm-scale harvester at a cutting height of 10–15 cm. For the warm-season CRP sites, biomass was harvested annually either at the anthesis (peak standing crop, PSC) or at the end of the growing season (EGS). For the cool-season CRP sites, biomass was harvested annually either at the anthesis (peak standing crop, PSC) and/or at the end of the growing season (EGS) depending on the location. PSC harvest timing was determined at each location by the predominant species reaching anthesis. Above-ground biomass for each plot was baled with a large round baler, weighed, and then sub-sampled to determine the dry matter concentration. All other detailed harvest timing and frequency information has been described previously (Lee et al., 2012). The effect of harvest timing and N fertilization, as well as their interaction effect on yield, were analyzed using the JMP statistical package (SAS Institute, Cary, NC, USA).

Results and Discussion

Annual precipitation at all sites during this research period were within 30% of the long-term average (30 years) except the OK and KS sites in 2011. Total precipitation during 2011 at the OK and KS sites were 31% and 58% of the long-term average, respectively. Because of extreme drought conditions in 2011, no biomass was harvested at the OK site.

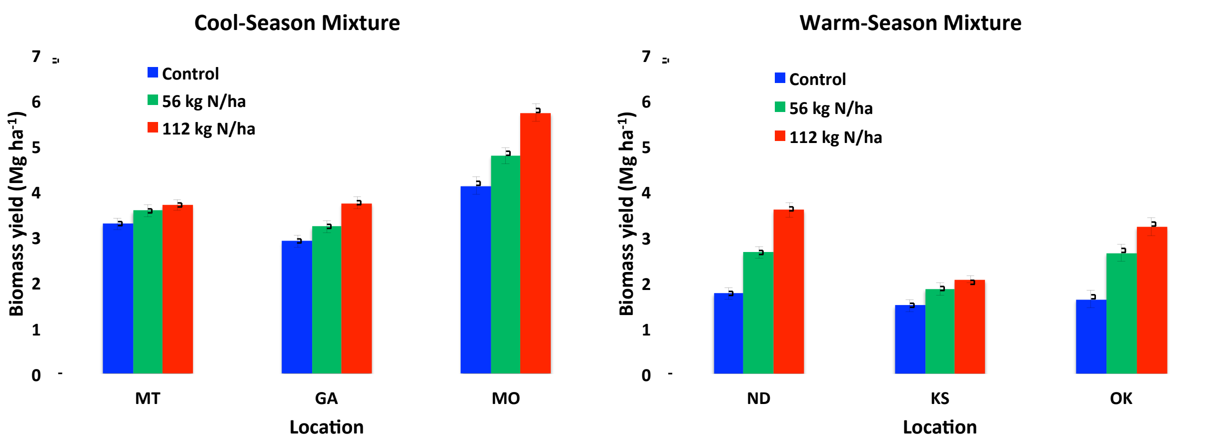

Figure 2. Biomass yields on warm- and cool-season mixtures and responses to nitrogen fertilization at Conservation Reserve Program (CRP) grasslands during the 2008–2011 growing seasons. Biomass yields were averaged across years and harvest timing.

Overall biomass yields were higher in cool-season mixtures than warm-season mixtures (Fig. 2). Nitrogen fertilization had a significant effect on the biomass yield and the maximum biomass yields were achieved with the N rate of 112 kg ha^{-1} in all locations except MT. The sites with legume species (MT, MO, and KS) were less responsive to N fertilization than the sites without legume species (GA, ND, and OK) (Fig. 2). Maximum biomass yields were achieved with N fertilization ranging from 2.0–3.9 Mg ha^{-1} for the three warm-season CRP sites and 3.6–5.8 Mg ha^{-1} for the three cool-season CRP sites.

There was no clear pattern of biomass yield response to harvest timing and its carryover effects across locations during the study period, even though a significant interaction between harvest timing and year was present (Fig. 3) The seasonal and yearly variations in biomass yield were correlated with the seasonal patterns of precipitation rather than harvest timing and its carryover effects as reported in other monoculture systems (Mulkey et al., 2006; Vogel et al., 2002).

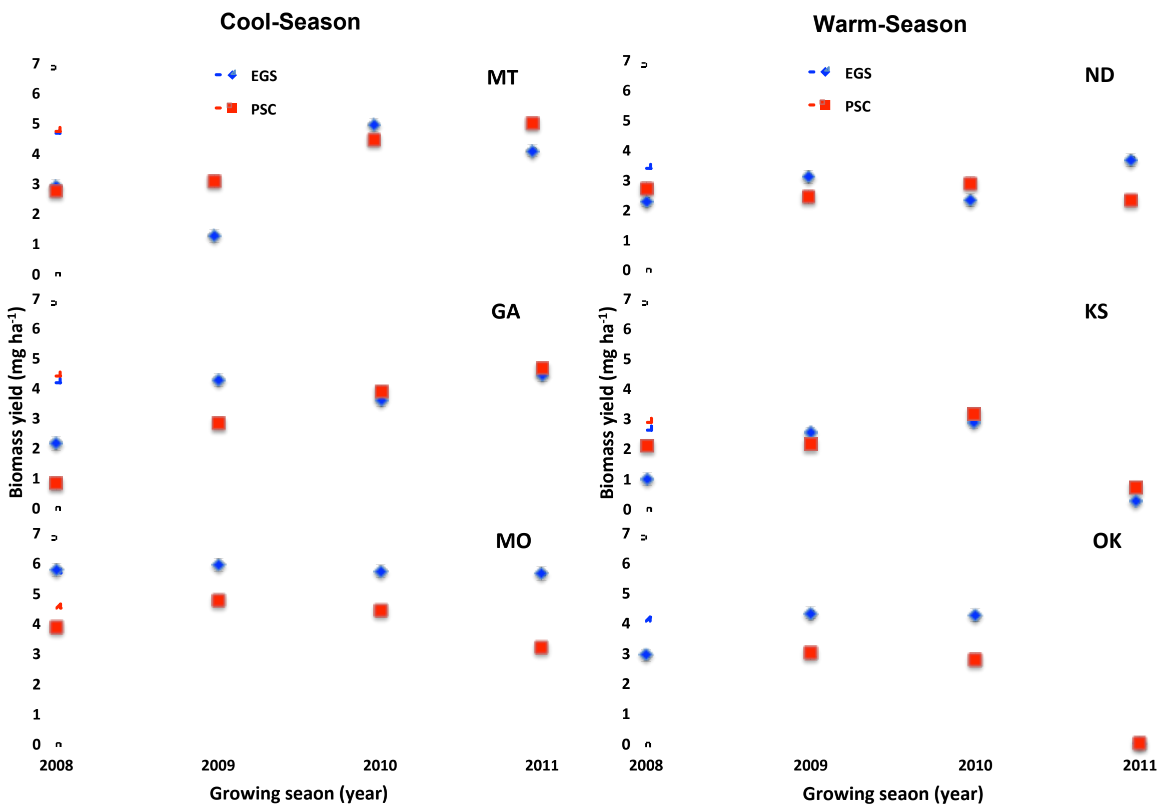

Figure 3. The effect of harvest timing (peak standing crop, PSC; end of the growing season, EGS) on biomass yield of cool- and warm-season mixtures at Conservation Reserve Program (CRP) grasslands during the 2008–2011 growing seasons. Biomass yields were averaged across N rates.

Conclusion

Conservation Reserve Program land has a potential for biomass production. This 4-year study indicated that CRP grasslands could produce cellulosic biomass feedstock with an annual yield of 4.25 Mg ha^{-1} as projected in the 2005 Billion Ton study (Perlack et al., 2005). However, sustainable management practices, especially N fertilization, are required to maximize biomass production. To monitor stand persistence for sustainable biomass production, field trials should be continued at the same locations, even though harvest timing did not have a significant impact on biomass yield and stand longevity. Precipitation during the growing season was one of the major limiting factors for biomass production. The extreme drought in the Great Plains region in 2011 significantly reduced the average biomass yields. Therefore, for an accurate assessment of the biomass feedstock production potential on CRP grasslands, a study conducted over a longer period of time is necessary.

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3.14
NITROGEN APPLICATION AND HARVEST TIMING AFFECT BIOMASS YIELD AND COMPOSITION ON CRP GRASSLAND

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Abstract
Conservative Reservation Program (CRP) grassland has potential to be used for biomass feedstock production. However, management strategies for soil fertility and harvest timing are needed for sustainable production. A replicated study was conducted from 2009-2011 to investigate the biomass yields and compositions of CRP grassland with mixed alfalfa and grass vegetation in central Montana, as affected by nitrogen (N) application rates and harvest timing. Nitrogen was applied in the spring of each year at 0, 56, and 112 kg N/ha and biomass was harvested at peak production and after frost kill. Biomass yields varied from year to year. Nitrogen and harvest timing had significant effects on biomass yields. Averaged over three years, biomass yields were 3479, 3762, and 3998 kg/ha at 0, 56, and 112 kg/ha N rates, respectively. The biomass yield was 4105 kg/ha at the peak production stage, compared with 3387 kg/ha at the frost kill. Nitrogen application and harvest timing also significantly affected the species compositions. The proportion of alfalfa significantly decreased from 49% to 35% and the grass significantly increased from 51% to 65% when the N rate increased from 0 to 56 kg/ha, and the species compositions did not change when N rate further increased from 56 to 112 kg/ha. At the peak production stage, the proportion of alfalfa and grass was 52% and 48%, respectively, compared to 29% and 71% at frost kill. Less alfalfa proportion in the biomass at frost kill was due to the senescence and drop of alfalfa leaves.

Key Words: *Biomass, Biofuel, Cell wall components, CRP, Nitrogen*

Introduction
Conservation Reserve Program (CRP) was initiated and designed nationwide to safeguard environmentally sensitive lands from degradation. It is a voluntary program for agricultural landowners or operators that provides rental payments and cost share assistance to control soil erosion, improve water quality, and enhance wildlife habitat (FSA, USDA, 2011). News release from USDA Farm Service Agency indicated that CRP prevents the erosion of 325 million tons of soil each year and carbon sequestration equal to taking almost 10 million cars off the road (USDA, 2012). Conservation Reserve Program enrollment across the United States as of October 2010 was approximately about 12.5 million hectares, mostly dedicated to grasses. From these lands, it was estimated to get about 17 to 28 million tons of dry biomass for bioenergy production (DOE, 2003; Perlack et al., 2005).

One of the CRP fields near Moccasin, Montana, where we carried out the experiment is mainly covered with alfalfa and grass mixture. Legumes were interseeded into grass to increase soil nitrogen thus reducing fertilizer costs, improved forage yield and quality (Bates, 1995; George et al., 1995). However, it is not clear if addition of nitrogen fertilizer and harvesting at different times will increase biomass yield, species composition and improve feedstock quality. Therefore, an experiment was carried out to investigate the effect of applying nitrogen fertilizer rates and harvesting times on biomass yield, species composition and biomass qualities for biofuel feedstock on a grass and alfalfa mixture CRP land in central Montana.

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Materials and Methods

The experiment was carried out on producer's field close to Central Agricultural Research Center ($47^0$03'30" N; $109^0$57'30" W; 1400 m elevation) of Montana State University from 2009 to 2011. The soil in this area is classified as a Judith clay loam (fine-loamy, carbonatic, frigid Typic Calciborolls). This location received an average annual rainfall of about 373 mm with 7^0C annual average air temperature. Treatments were applied in split plot design with three replications. Main plot treatments were nitrogen fertilizer rates (0, 56 and 112 kg ha^{-1}) and harvesting times (July and October) were assigned to subplots. The dominant plant species of the CRP pasture are intermediate wheatgrass (*Agropyron intermedium*), pubescent wheatgrass (*Thinopyrum trichophorum*), tall wheatgrass (*Thinopyrum ponticum*) and alfafa (*Medicago sativa*). Urea was applied in April every year as source of nitrogen fertilizer. Acid detergent fiber, neutral detergent fiber and acid detergent lignin were determined following Ankom technology procedure (Ankom Technology, MacEdon, NY) (Goering and Van Soest, 1970). Then, cellulose, hemicellulose and lignin content were calculated as specified by Lemus et al. (2002). Analysis of variance was performed using SAS computer software (SAS Ver 9.3) and protected LSD at 5% probability level was used to differentiate treatments' effect when appropriate.

Results and Discussion

Maximum grass biomass (3371 kg ha^{-1}) was recorded in year 2010 (Table 1) due to optimum rainfall (Fig. 1) and an increase in grass density during the second growing season. This result is in agreement with Johnson et al. (2001) finding. The percentage of alfalfa was 60% higher than grass in 2009 but reduced to the extent of 30% in subsequent years (Table 1). Maximum biomass of alfalfa (1654 kg ha^{-1}) was recorded at 0 kg N ha^{-1} (Table 1). This result suggests that nitrogen fertilization is not important for alfalfa production. However, grass biomass and its percentage composition and total biomass (sum of alfalfa and grass) increased linearly with increase of nitrogen fertilizer application rates (Table 1). Harvesting alfalfa July resulted in greater dry biomass yield (2038 kg ha^{-1}) than harvesting October (709 kg ha^{-1}). This low yield of alfalfa in October is associated with senescence of leaves as observed in the field. Harvesting grass in October resulted in 29% more biomass yield than July (Table 1). The interaction treatment means indicated that the maximum grass (2858 kg ha^{-1}) and total biomass were recorded with the application of 56 kg N ha^{-1} and harvested in October compared to other treatments (data not shown).

Table 1. Means for biomass yield and species composition for the different years, nitrogen rates and harvesting times from 2009-2011 at CRP grassland field, Central Agricultural Research Center, Montana.

Main factors	Levels	Biomass yield (kg ha^{-1})			Proportion (%)	
		Alfalfa	Grass	Total	Alfalfa	Grass
Year	2009	1353a	700b	2053b	60a	40b
	2010	1373a	3371a	4744a	30b	70a
	2011	1395a	3054a	4449a	31b	69a
Nitrogen rate (kg ha^{-1})	0	1654a	1826b	3481b	49a	51b
	56	1173b	2591a	3764ab	35b	65a
	112	1293b	2707a	4001a	37b	63a
Harvesting time	July	2038a	2069b	4108a	52a	48b
	October	709b	2681a	3390b	29b	71a
Mean		1374	2375	3749	40	60
CV (%)		39.69	20.37	15.69	25.18	17.10
Means followed by a common letter in a column for the same main factor are not significantly different from each other at 5% LSD.						

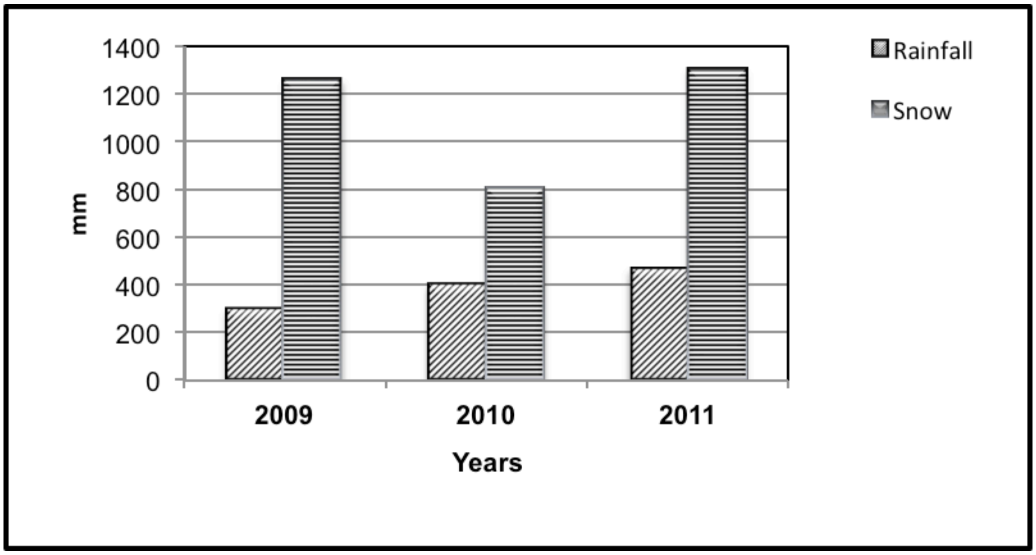

Fig. 1. Total rainfall and snow depth from January to October for the different years at Central Agricultural Research Center, MT.

Application of different nitrogen fertilizer rates did not affect cellulose, hemicellulose and lignin content (Table 2). Similarly, previous research showed that increased N fertilization had little to no effect on these parameters for native warm-season grasses (Cuomo and Anderson, 1996; Rogers et al., 1996). As indicated in Table 2, October harvesting of alfalfa increased cellulose, hemicellulose and lignin content by 80, 79 and 84%, respectively, compared to July. This is due to a decrease in leaf : stem ratio in October than July as observed in the field. As shown in Table 2, the energy content of grass and alfalfa was not affected by nitrogen application rates but it was significantly increased when biomass was harvested in October than July.

Table 2. Means for main cell wall components in 2009 and 2010 and energy content (calorie per gram) in 2011 for grass and alfalfa as affected by nitrogen rates and harvesting times at CRP grassland field, Central Agricultural Research Center, Montana.

Factors	Levels	Cellulose (%)		Hemicellulose (%)		Lignin (%)		Energy content (Cal/g)	
		Grass	Alfalfa	Grass	Alfalfa	Grass	Alfalfa	Grass	Alfalfa
Year	2009	31.13a	33.61a	30.17a	14.12a	4.39a	10.25a	NA	NA
	2010	36.25a	35.11a	29.35a	13.32a	3.41a	9.80a	NA	NA
N (kg/ha)	0	33.46a	34.51a	29.62a	14.09a	4.18a	10.25a	4156a	4166a
	56	32.82a	34.16a	29.23a	14.12a	4.00a	10.15a	4161a	4161a
	112	32.55a	33.86a	29.26a	13.77a	3.93a	10.26a	4165a	4174a
Harvesting time	July	30.84b	24.43b	27.82b	10.04b	2.54b	7.21b	4102b	4098b
	October	34.98a	43.91a	30.92a	17.95a	5.48a	13.23a	4219a	4236a
Mean		32.95	29.37	4.04	34.17	13.99	10.22	4161	4167
CV (%)		4.18	2.98	13.84	4.43	4.19	7.09	0.71	0.51

Means followed by a common letter in a column for the same main factor are not significantly different from each other at 5% LSD. NA=Not available

Conclusion

Application of nitrogen fertilizer was not required to maximize alfalfa biomass yield but 56 kg N ha^{-1} and October harvesting is recommended for maximum grass and total biomass yield. Following these management practices increased cellulose and energy content of the biomass, important quality parameter for biofuel feedstock.

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4 SYSTEM SUSTAINABILITY

4.1
INFLUENCE OF CORN STOVER HARVEST ON SOIL QUALITY ASSESSMENTS AT MULTIPLE LOCATIONS ACROSS THE U.S.

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Abstract
Corn stover has been identified as a biofuel feedstock due to its abundance and a perception that the residues are unused trash material. However, corn stover and other plant residues play a role in maintaining soil quality (health) and enhancing productivity, thus use of this abundant material as feedstock must be balanced with the need to protect the vital soil resource. Plant residues provide physical protection against erosion by wind and water, contribute to soil structure, nutrient cycling, and help sustain the soil microbiota. Replicated plots were established on productive soils at several locations (IA, IN, MN, NE, PA, SD, and SC) and a multi-year study was carried out to determine the amount of corn stover that can be removed while maintaining the current level of soil quality for each soil. These sites represented a range of soil types and climatic conditions, and have been ongoing for and least five years with some much longer studies. All sites had at least three levels of stover harvest: grain only (control), maximum removal (90-100%) and a mid-range removal rate (~50%). Data from 4 sites are presented (IA, IN, MN, and NE). The Soil Management Assessment Framework (SMAF) was used to score and assess changes in selected soil quality indicators. Data shows that removal at the highest rates resulted in some loss in soil quality with respect to soil organic carbon and bulk density. These sites were converted to no-till when the experiments were initiated, thus SOC accrual because of the shift in tillage management appeared to balance any losses due to feedstock harvest.

Keywords: Soil organic carbon, bulk density, soil quality index

Introduction
Due to its plentiful supply, corn stover has been identified as an important feedstock for cellulosic ethanol production (Perlack et al. 2005), which is needed to offset a portion of 14 million barrels of oil consumed daily in the U.S. as transportation fuel (National Academy of Sciences (NAS) 2009). While corn (*Zea mays* L.) stover is abundantly available, with about 35 million ha of corn planted each year, there is a

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perception that it is trash, an unused material left behind after harvest. The Billion Ton Report (BTR) (Perlack et al. 2005) did recognize the need for leave sufficient crop residues on the soil surface to protect against wind and water erosion. Soil scientists have raised the concern that excessive feedstock harvest could reduce crop yields, either directly or indirectly, through reductions soil organic carbon (SOC) and other nutrients concentrations as well as decreasing overall soil quality (Wilhelm et al. 2010). Soil organic carbon has been reduced by 30-50% of pre-cultivation levels due to tile drainage, intensive tillage systems, use of monoculture or simple two crop rotations such as corn/soybean (Schlesinger 1985). Declining SOC levels negatively impacts a number of vital soil ecosystem functions, including plant productivity, nutrient cycling, soil structure, and water infiltration and storage.

Cropland soil quality can be impacted by the interaction of a number of factors such as climate, soil type, and management practices such as soil tillage and crop rotations. To evaluate the impact of these factors on critical soil ecosystem functions, soil quality assessment tools are needed. These assessment tools need to be sensitive to changes in management as well as taking into account differences in inherent soil attributes (Stott et al. 2011). Monitoring changes in soil quality should give indications of the long term impact of stover removal on soil quality.

One such assessment tool is the Soil Management Assessment Framework (Andrews et al. 2004), which is available in Excel format, and focuses on how well the soil is functioning. It uses measured data for dynamic soil indicators to assess management effects on soil ecosystem services using a three-step process that includes indicator selection, indicator interpretation, and integration into an index. The SMAF uses soil taxonomy as a foundation for assessment, allowing for the modification of many of the scoring indicator values to be based on soil suborder properties. Soil quality and its assessment are soil and site specific, and depend on a variety of factors such as inherent soil characteristics, landscape and climatic influences, and human values such as intended land use, management goals, and environmental protection. The SMAF includes soil physical, chemical, and biological indicators that are management-sensitive, and therefore dynamic. Currently, SMAF includes 13 indicators with scoring curves consisting of interpretation algorithms. Those used in this study included bulk density (BD), electrical conductivity, pH, extractable P and K, SOC, and β-glucosidase (BG) activity (Andrews et al. 2004; Stott et al. 2010; Wienhold et al. 2009).

The objective of this paper is to examine changes in selected soil quality indicators that have occurred over time from experiments that included as a component the impact of corn stover removal on soil ecosystem services.

Methods

Four study sites from across the U.S. were used in this study (Tables 1-4). All sites included a minimum of two corn stover levels, 0 and 50% removal, with 3 sites having additional levels. No-till practices were included at all sites. Crop rotations varied from site to site, and included continuous corn, corn-soybean (*Glycine max* L.), and corn-soybean-winter wheat (*Triticum aestivum* L.). Data were derived from both published and unpublished sources. The soil analytical data presented were gathered using standard methodologies that were the same across all the sites. Soil quality indicator scores were calculated with SMAF. Changes (Δ values) were calculated by subtracting the initial value from the latest value available. For example, the change in SOC would be calculated as $\Delta SOC = SOC_{final} - SOC_{initial}$. Changes in the SMAF indicator scores were calculated in a similar manner.

Results and Discussion

A study in Ames, IA (Karlen, 2011;Table 1) observed significant decreases in the SMAF SOC indicator scores in the near-surface (0-15 cm) soil, but all treatments suffered a similar drop, hinting that there were other factors at play other than the removal of corn stover for biofuel feedstock. The SMAF bulk density indicator scores were improved. Nutrition-related indicators, which are easily managed through amendments, remained substantially unchanged, regardless of the level of feedstock harvest.

Table 1. Response of selected SMAF soil indicator scores after 5 years of corn stover harvest near Ames IA. The dominant soils were a Canisteo silty clay loam for the continuous corn and a Clarion loam for the rotational corn system, all under no-till management. Sampling depth was 0-15 cm. The change in indicator scores were calculated as the difference between the score first and last years. A difference ≥ 0.10 was significant. Data and SMAF calculations from Karlen et al. (2011).

	Change in SMAF Indicator Scores after 5 Years						ΔIndex
Continuous Corn	**SOC[1]**	**BD**	**pH**	**EC**	**P**	**K**	**SQI**
Whole Plant[2]	-0.34	0.23	0.01	0.00	0.00	0.01	-0.01
Upper half	-0.31	0.19	0.00	0.00	0.00	0.08	0.00
Lower half	-0.29	0.18	0.01	0.00	0.00	0.12	0.01
No removal	-0.33	0.10	0.01	0.00	0.00	0.06	-0.02
Corn in Rotation with Soybean							
Whole Plant	-0.03	0.06	0.00	-0.05	0.00	0.04	0.01
Upper half	0.01	0.11	-0.01	0.00	0.02	0.22	0.06
Lower half	0.08	0.15	0.00	0.00	-0.02	-0.01	0.03
No removal	-0.17	0.13	0.00	-0.08	0.00	0.20	0.02

[1]Abbreviations: SOC, soil organic carbon; BD, bulk density; EC, electrical density; ΔSQI, change in soil quality index, calculated using the Soil Management Assessment Framework.
[2]Portions of the corn stalk that were harvested for biofuel feedstock.

In West Lafayette, IN (DE Stott, unpublished data; Table 2), the ΔSOC concentrations increased in the 0-30 cm layer, probably due to the conversion to no-till, even so, the increase SOC for the 100% stover removal rate was significantly lower than the other treatments, and the increases overall were much lower in the 30-60 cm layer. The changes in the SMAF SOC indicator scores were not significantly different between treatments. Bulk density increased in the top layer, but the changes in the SMAF bulk density indicator scores were negligible. However, in the 30-60 cm depth, the indicator scores were significantly decreased, with the 75 and 100% removal rates significantly lower than the 0% removal rate.

Table 2. Changes in selected soil indicators after 6 years of corn stover removal from continuous corn sites in West Lafayette, IN. The dominant soil was a Chalmers silt loam with no-till management initiated at the beginning of the experiment. The values represent changes in soil indicator values and SMAF soil indicator scores after 6 years of corn stover removal. A change in the SMAF score ≥0.1 is significant. Data from Stott, DE, unpublished.

Stover Removal Rate	ΔSOC[1] (g kg^{-1})	ΔSOC Score[2]	ΔExt. P (mg kg^{-1})	ΔExt. P Score	ΔBulk Density (g cm^{-3})	ΔBulk Density Score
<u>0-30 cm sampling depth</u>						
0%	2.3	0.07	17.67	0.10	0.25	-0.04
50%	2.6	0.10	43.00	0.03	0.27	-0.06
75%	2.1	0.06	18.33	0.22	0.20	-0.01
100%	1.5	0.04	29.17	0.17	0.19	-0.02
<u>30-60 cm sampling depth</u>						
0%	0.1	0.00	-0.33	0.00	0.21	-0.23
50%	0.3	0.01	-1.33	-0.04	0.35	-0.34
75%	0.5	0.01	1.00	-0.12	0.23	-0.40
100%	0.6	0.02	-3.00	0.02	0.22	-0.41

[1] Abbreviations: SOC, soil organic carbon, PNP, *p*-nitrophenol released; Δ=amount of change, calculated by subtracting the initial value from the value after 6 years.
[2] Indicator scores calculated using the Soil Management Assessment Framework.

For sites near Mead, NE (Follett, 2012), there were decreases in SOC concentrations in the surfaces layers over 9 years, translating into a significant drop in the SMAF SOC indicator score, however there were slight increases in the lower layers. Both treatments had a weighted mean 0.13 increase in SOC indicator score over the 150-cm profile during the 9-yr period. Bulk density scores also dropped for both treatments. The 50% removal treatment had a slightly greater drop in bulk density over the 150-cm profile.

The Morris, MN study (JMF Johnson, unpublished data; Table 3) was interesting due to the inclusion of a corn-soybean-wheat rotation and in the use of established no-till. Changes in the SMAF SOC, bulk density, and β-glucosidase activity (an enzyme involved in cellulose degradation) indicator scores were minor. Extractable P and K indicator scores (data not presented) showed no significant differences.

Table 3. Changes in selected soil indicators after 9 years of corn stover removal from continuous corn sites near Mead, NE. The dominant soil was a Yutan clay loam - Tomek silt loam mix. The values represent soil indicator values and SMAF soil indicator scores measured initially and after 9 years of no-till management and corn stover removal. A change in the SMAF score ≥0.1 is significant. Data from Follett, 2012.

Removal Rate	Depth of sampling (cm) 0-5	5-10	10-30	30-60	60-90	90-120	120-150
	Soil Organic Carbon (g kg^{-1})						
Initial	28.6	31.2	2.3	1.4	1.9	3.0	5.0
0%	21.8	30.3	8.5	7.2	10.7	15.8	24.2
50%	24.4	30.4	8.9	7.3	11.0	15.7	24.1
	SMAF[2] SOC Indicator Score						
Initial	0.62	0.71	0.03	0.02	0.03	0.03	0.04
0%	0.37	0.68	0.07	0.06	0.09	0.19	0.46
50%	0.46	0.69	0.07	0.06	0.10	0.18	0.45
	ΔSMAF SOC Indicator Score						
0%	-0.26	-0.03	0.04	0.03	0.07	0.15	0.42
50%	-0.16	-0.03	0.05	0.03	0.07	0.15	0.41
	Soil Bulk Density (g cm^{-3})						
Initial	1.20	1.34	1.36	1.29	1.32	1.35	1.39
0%	1.33	1.45	1.45	1.32	1.35	1.37	1.71
50%	1.32	1.45	1.43	1.35	1.40	1.44	1.45
	SMAF Bulk Density Indicator Score						
Initial	0.80	0.49	0.46	0.59	0.53	0.48	0.41
0%	0.51	0.35	0.35	0.53	0.48	0.44	0.23
50%	0.53	0.35	0.37	0.48	0.40	0.36	0.35
	ΔSMAF Bulk Density Indicator Score						
0%	-0.29	-0.15	-0.11	-0.06	-0.05	-0.03	-0.19
50%	-0.27	-0.15	-0.09	-0.12	-0.13	-0.12	-0.07

[1] Abbreviations: SOC, soil organic carbon; Δ=amount of change, calculated by subtracting the initial value from the value after 6 years.
[2] Indicator scores calculated using the Soil Management Assessment Framework (SMAF).

Shifting from conventional to no-till before the start of corn stover harvest for biofuel feedstock was helpful in maintaining near-surface SOC at levels that has not degraded the soil ecosystem functions in which SOC is involved, however SOC levels in lower depths were lower with sustained feedstock harvest. In addition, bulk density indicator scores have decreased. Nutrient and chemical scores, such as extractable P and K, and pH, saw very little change, which would be expected as these can be modified by managing inputs. There were some indications (data not presented) that concentrations of trace elements such as copper and zinc, were becoming a concern with continued feedstock harvest, and may need to be addressed in the future to maintain comparable yields.

Table 4. Changes in selected after 6 years in Morris, MN. The dominant soils were Barnes loam and Aastad clay loam. For the SMAF indicator scores, a change ≥0.10 was significant. Data from Johnson, JMJ, unpublished.

			Depth of Sampling (cm)									
			0-20	0-60	0-20	0-60	0-20	0-60	0-20	0-60	0-20	0-20
Removal Rate	Crop[1]	Tillage[2]	Δ SOC(%)[3]		Δ SOC SMAF Score[4]		Δ Bulk Density (g cm^{-3})		Δ Bulk Density SMAF Score		Δ β-gluc.	Δ β-gluc. SMAF Score
0	**C**S	Chisel	0.01	-0.05	0.00	-0.02	0.07	0.05	-0.07	-0.08	-125	-0.08
50			0.08	0.13	0.03	0.02	0.12	0.04	-0.11	-0.04		
75			0.16	0.09	0.06	0.00	0.08	0.01	-0.10	0.01		
100			0.07	0.09	0.03	-0.01	0.01	0.04	-0.03	-0.06	-139	-0.09
0	**C**S	Est.NT	0.11	0.04	0.04	0.00	0.01	-0.12	0.01	0.07	-183	-0.20
50			-0.04	0.10	0.00	0.01	-0.04	0.03	0.03	-0.07		
75			0.10	0.15	0.04	0.02	-0.13	0.02	0.18	-0.01		
100			0.18	0.24	0.07	0.04	-0.09	-0.10	0.18	0.15	-157	-0.14
0	C**S**	Chisel	-0.01	0.13	-0.01	0.01	-0.05	-0.08	0.10	0.06	-134	-0.09
50			0.04	0.20	0.01	0.01	-0.04	0.01	0.06	-0.04		
75			0.12	0.20	0.05	0.03	-0.05	0.21	0.03	-0.08		
100			0.08	0.20	0.03	0.02	0.04	0.01	-0.02	-0.03	-130	-0.09
0	C**S**	Est. NT	0.21	0.16	0.08	0.03	-0.11	-0.04	0.08	0.06	-193	-0.21
50			0.03	0.23	0.01	0.03	-0.04	0.04	0.08	0.00		
75			0.11	0.05	0.04	-0.01	-0.02	0.09	-0.01	-0.15		
100			-0.06	0.07	-0.03	-0.01	-0.06	0.06	0.08	-0.08	-168	-0.17
0	CS**W**	New NT	0.22	0.26	-0.10	0.00	-0.36	-0.01	-0.21	-0.14	-64	-0.03
50			1.25	0.49	0.02	0.02	-0.01	0.03	0.01	-0.02	17	0.04
75			1.97	0.62	0.02	0.02	-0.02	0.01	0.01	0.01	16	0.04
100			1.85	0.61	0.04	0.02	0.01	0.08	-0.01	-0.07	-69	-0.04

[1] Crop Rotation, crop at time of sampling is in bold, C=corn, S=soybean, W=wheat.

[2] Tillage, primary tillage, Est. NT=no-till established for 11 yr before experiment; New-NT, no-till established at beginning of experiment.

[3] Abbreviations: SOC, soil organic carbon; SMAF, soil management assessment framework, β-gluc, β-glucosidase activity reported as mg *p*-nitrophenol released g^{-1} soil hr^{-1} incubation; Δ=amount of change, calculated by subtracting the initial value from the value after 6 years.

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4.2 CORN STOVER REMOVAL IMPACTS ON N2O EMISSION AND SOIL RESPIRATION: LESSONS FROM AUTOMATED CHAMBER MEASUREMENTS

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Abstract

Corn stover removal for bioenergy production could have a variety of indirect consequences through its effect on soil processes, including N_2O production and soil respiration. Because these effects may be episodic in nature, weekly 30 minute snapshots with static chambers may not provide an accurate picture. We adapted automated soil respiration chambers by incorporating a portable N_2O analyzer, allowing us to measure both CO_2 and N_2O fluxes on an hourly basis through the growing season in a corn field in southern Minnesota in 2011 and 2012 that has been part of the Sun Grant/REAP project for the past four years, with three levels of stover removal: zero, full, and intermediate. Four chambers were installed on zero removal plots and four on full removal plots. At the Iowa Sun Grant site, another set of automated chambers was used to monitor soil respiration in 2012 for two tillage types (chisel and no-till) and two residue removal levels (50% and no removal). Results at this site showed highest cumulative soil respiration from the no-till, 50% removal and lowest totals from the no-till, no-removal, with intermediate values for the chisel plow treatments. The MN data revealed higher N_2O emission from the full removal plots, possibly a consequence of enhanced mineralization of soil organic matter. CO_2 loss from the full removal plots was slightly lower, but the difference between treatments was much smaller than the amount of C removed in the residue, implying loss of soil carbon from the full removal plots.

Keywords: stover removal, greenhouse gases, N_2O, respiration

Introduction

An overarching goal of the USDA-ARS REAP Project, with the support of the USDOE Sun Grant Program, has been to assess the total environmental impact of harvesting crop residues for bioenergy production. Since a primary purpose of bioenergy production is to reduce projected changes in global climate, any associated effects on the production of greenhouse gases are particularly important to understand (Carter et al., 2012). It is plausible to expect that removal of crop residue might affect not only soil respiration, but also N2O production, and that there might be interactive effects of tillage (Mutegi et al., 2010). Two of the sites where corn stover removal treatments have been established are a private farm in Rice County, MN and the Iowa State University Agronomy and Biosystems Engineering Farm in Boone County, IA. Treatments include zero removal, partial (~50%) removal, and maximum (~100 %) removal, and they have been in place since 2007 at both sites.

Most research on greenhouse gas emissions from soils has been conducted with static chambers. Small (typically 20 cm diameter) rings are pushed into the soil, and emission rates are periodically measured by placing a cap over the ring and sampling the interior gas at intervals with a syringe over a period of 30-60 minutes (Venterea et al., 2009). Samples are subsequently analyzed on a gas chromatograph, and the change in internal concentration of the gas of interest (CO_2, N_2O, or CH_4) is then regressed against time to estimate the flux. This method is labor-intensive, so the resulting data set typically provides an hourly flux measurement for each treatment once every 1-2 weeks. If the metric of interest is the cumulative

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seasonal or annual emission, it thus is necessary to integrate an uncomfortably sparse data set, in which the actual measurements cover less than 1 % of the total time period. This can result in substantial uncertainty, particularly for N_2O, since its production can be highly episodic (Wagner-Riddle et al., 2007). Much of the annual loss may occur in a few bursts, e.g.- during spring thaw and in the hours following a heavy rain. These are generally times at which it may be difficult or impossible to make a static chamber measurement. While soil respiration is generally less episodic than N_2O, it is still sufficiently variable to induce large uncertainties in cumulative estimates derived from typical static chamber sampling schedules (Parkin, 2008). For these reasons, we deemed it advisable to deploy automated sampling chambers in this project. A variety of automated systems have been developed for such applications, but until recently there were no commercially available options. However, such systems have recently become available for CO_2. One such system has been used for automated soil respiration measurements at the Iowa site. Another has been modified to include an N_2O analyzer, so that both N_2O and CO_2 could be measured at the Minnesota site (Fassbinder et al. , submitted).

Methods

Boone Co. IA site- Field plots to determine the effect or corn (*Zea mays*, L.) residue removal on soil properties were initiated on the Iowa State University Agronomy/Agricultural and Biosystems Engineering farm in 2007. Deep tillage was completed on the entire site followed by seeding to oats (*Avena sativa* L.). In the spring of 2008 four replications of 22 treatments were established in a randomized complete block design with 12.2 by 91.4 m plots. Soils at the site are mapped as Webster silty clay loam, Clarion loam, and Canisteo silty clay loam. Hourly soil CO_2 flux measurements from four treatments (chisel plow and no-till with no surface residue removal and approximately 50% surface residue removal) were initiated on March 26, 2012. All monitored treatments were in continuous corn with 76 cm row spacing. Three 20 cm-diameter PVC collars were inserted in each plot at in-row, mid-row (center of collar 38.1 cm from row), and 1/3 mid-row (center of collar 25.4 cm from nearest row) positions. Two Li-Cor LI-8100A automated soil CO_2 flux systems (Li-Cor Biosciences, Lincoln, NE) were used to monitor soil CO_2 flux. These systems consisted of a LI-8100 gas analyzer, LI-8150 multiplexer, and 8100-401 long term chamber. Soil water content (0-6 cm, ML2, Delta-T Devices, Burwell, United Kingdon) and soil temperature (5 cm.,8100-203, Li-Cor Biosciences) were measured every hour adjacent to each collar. All sensors were removed during planting (day of year 111 to 117) and sidedress application of nitrogen fertilizer (day of year 153 to 158).

Rice Co. MN site-The farm on which these plots were established in fall 2008 is located 11 km SSE of Northfield, MN on a Garwin silt loam, with an organic carbon content of 2.1%. The plots are part of the REAP stover removal project. The field has been in continuous corn since 2007. The experiment is a randomized complete block design with 4 replications. Treatments include full (~100%), partial (~50%) and 0 stover removal, under both conventional (moldboard plow) and reduced (strip-till) tillage. Plots are 6 m wide x 91.4 m in length. Automated chambers have been used for the past three years on selected plots in the reduced tillage treatments. In 2010, six chambers were installed, with 2 each on the 0, 50%, and 100% removal plots. In 2011 and 2012, eight chambers were deployed, with 4 each on the 0 and 100% removal plots. The system uses the same chambers as the IA study (LI8100-104, Licor Lincoln, NE), but with a multiplexer of our own design that uses a set of solenoids mounted on a manifold and controlled by a datalogger. When a given chamber is actuated (once each hour for 450 s), air drawn from it is routed first to an infrared gas analyzer (Model 840, Li-Cor Inc., Lincoln, NE, USA) for CO_2 and H_2O analyses, then scrubbed to remove CO_2 and H2O before entering an N_2O analyzer (Model M320EU2, Teledyne Instruments API, San Diego, CA, USA). Within the analyzer, a Nafion column removes water vapor prior to the sample cell. Hourly flux measurements were averaged for each treatment and aggregated into daily totals.

Results

IA site- The 2012 measurement period included a warmer than normal March, a cooler than normal April, and much warmer than normal May through July. There were extended periods of no or low rainfall from day 128 to 146 and day 172 to 210. CO_2 flux followed a typical seasonal pattern, increasing with increasing soil temperature (Fig. 1).

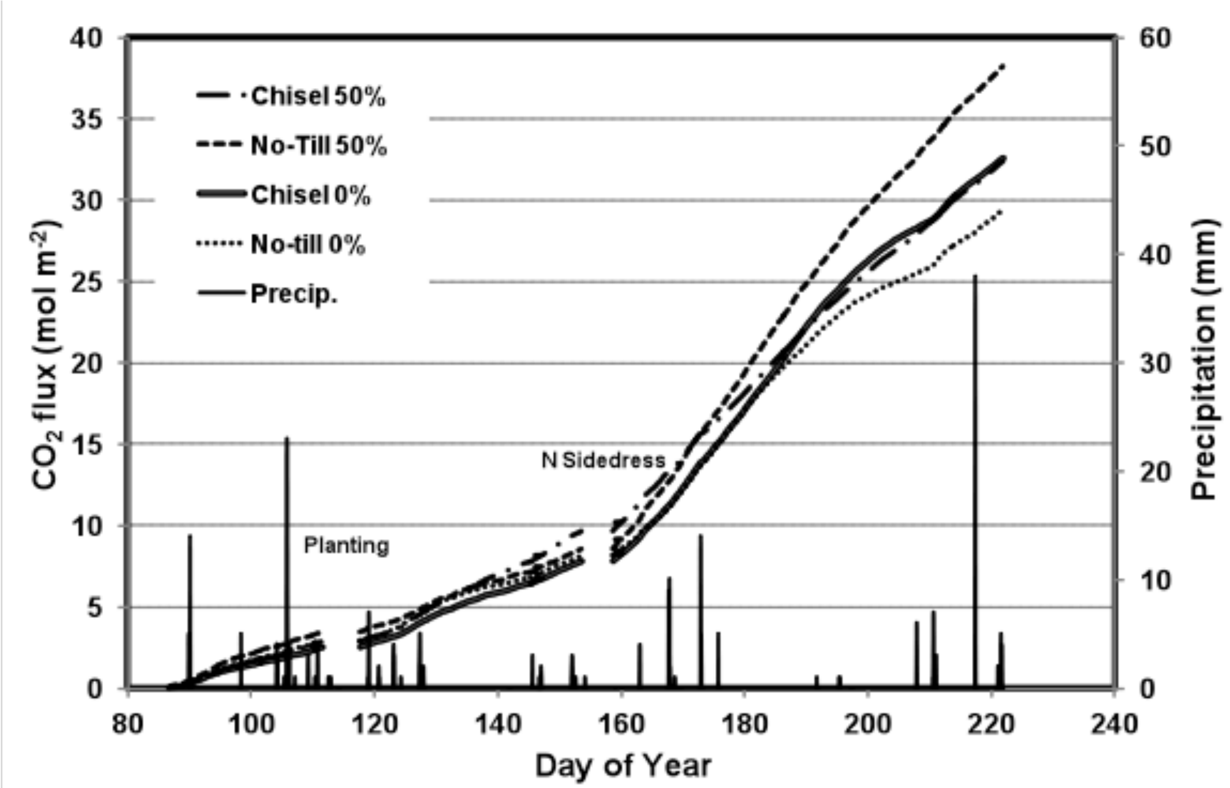

Fig 1. Cumulative soil CO_2 flux and precipitation at Ames location in 2012.

During the extended dry periods, CO_2 fluxes declined for most treatments, as measured volumetric water contents were decreasing from > 0.3 to as low as 0.1 m^3 m^{-3}. The no-till with 50% residue removal treatment showed only a small decline in CO_2 flux during the second dry interval, which resulted in an increasing separation in cumulative flux between this treatment and all others. By contrast, the chisel with 50% residue removal, which had the greatest early season cumulative flux, displayed decreasing fluxes from day 175 relative to the no-till 50% removal treatment.

None of these trends in CO_2 flux can be readily explained from the soil temperature or water content data, which show no consistent patterns or significant trends among treatments. Removal of 50% of the surface residue did not result in lower soil water content (due to great soil water evaporation) or higher soil temperatures for either chisel or no-till treatments. Expression of these effects would be expected early in the growing season when the corn canopy did not yet shade a significant portion of the soil surface.

MN Site- An example of the cumulative N_2O flux data is shown in Fig. 2.

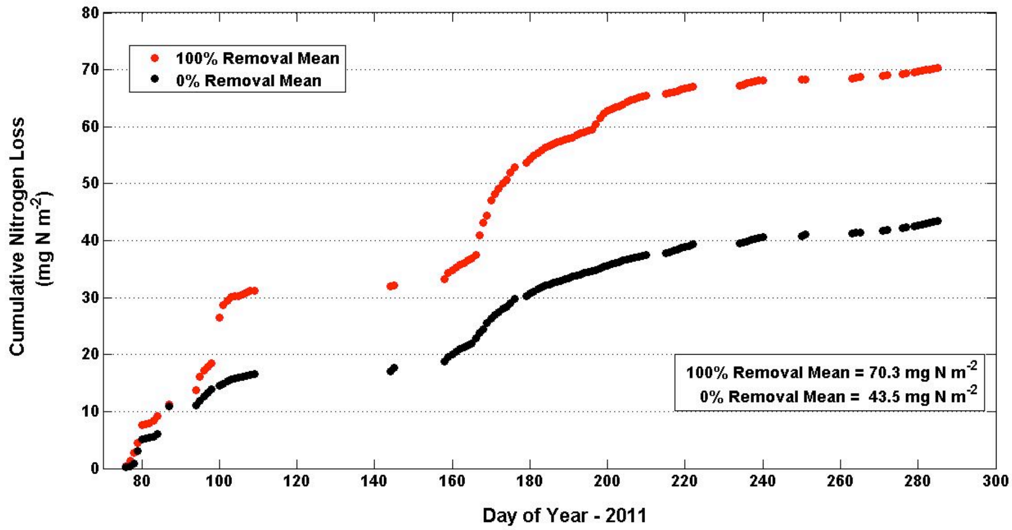

Fig. 2. Cumulative N_2O emissions in 2011, Rice Co. MN.

Gaps indicate power outages or times when the chambers were removed for field operations. Cumulative totals (not gap-filled) for the periods of operation in each year are listed in Table 1.

Table 1. Cumulative measured emissions (no gap filling done) of N_2O and CO_2 for Rice Co., MN site.

Year	N_2O, mmol m^{-2}		CO_2, mol m^{-2}	
	0 removal	Full removal	0 removal	Full removal
2011	0.99	1.60	8.2	7.43
2012	2.80	3.07	6.93	6.59

In both 2011 and 2012, cumulative N_2O emissions were higher in the 100% removal plots than the 0 removal plots. CO2 loss (mean daily values shown in Fig. 3) was surprisingly similar for the two treatments, despite the large difference (~5 Mg ha^{-1}) in residue return.

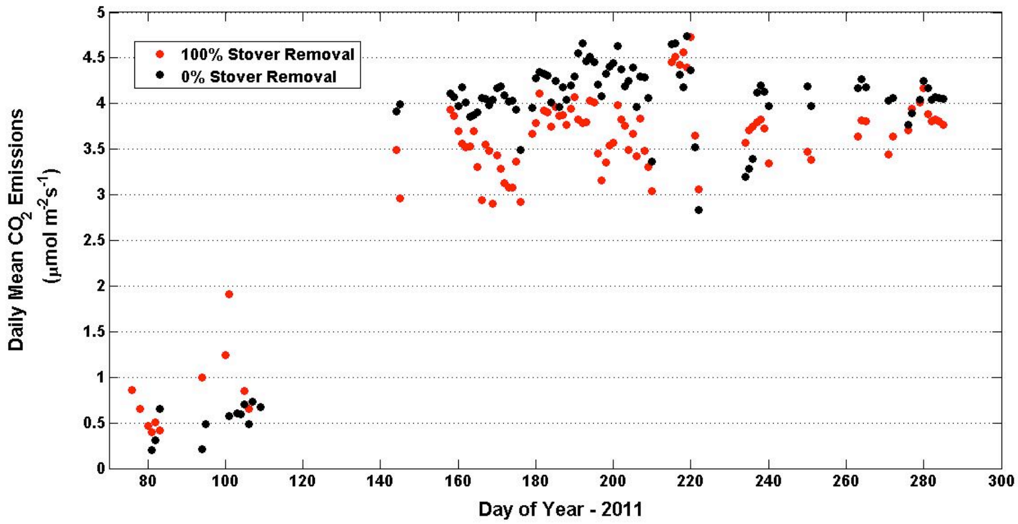

Fig. 3 Mean daily CO2 emissions in 2011. Rice Co. MN

The latter result suggests substantial mineralization of soil organic matter in the 100% removal plots, which is consistent with results from soil sampling. This may also explain the contrast in N_2O losses – increased availability of NO_3 in the full removal plots due to greater mineralization of soil organic matter, abetted by higher near-surface soil temperatures in the spring.

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4.3
CORN STOVER MANAGEMENT EFFECTS ON SOIL ORGANIC CARBON CONTENTS FROM SEVERAL U.S. LOCATIONS

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Abstract
Corn stover is becoming a major bioenergy feedstock, the supply amount dependent on high quality soil. Thus, the soil resource provides the foundation for building a sustainable biofuel economy. As a bioenergy foundation, this resource must be safeguarded from overzealous residue harvest, which can exacerbate erosion and a loss of soil organic carbon (SOC). Furthermore, greater amounts of residue return may be needed on some soils and agricultural systems to maintain SOC than control erosion. Replicated plots established on productive soils were included in a multi-location (six states, 16 sites) and a multi-year study to determine the amount of corn stover needed to maintain SOC contents. A related objective assessed how management, climate and initial soil parameters influence SOC response to stover harvest. Sites in these agricultural systems represented a range of soil types, climatic conditions, and study duration. Sites had at least three levels of stover harvest: grain only (control), maximum possible residue removal and an intermediate rate. Regression analyses were used at each site to estimate the relationship between the actual amount of stover returned and the change in SOC (ΔSOC). These sites provided SOC and corn stover data to facilitate calculation of a minimum biomass that needs to remain on the field to avoid a SOC loss. Synthesis of these results will assist refinement for stover harvest recommendations that should safeguard the soil resource and maintain future productivity.

Key words: bioenergy, sustainable, renewable energy, soil organic carbon

Introduction
Corn stover (*Zea Maize* L.) was identified as potential herbaceous bioenergy feedstock based upon the vast acreage and yield potential (US DOE 2011). A vital aspect of utilizing stover for energy is to establish harvest criteria that avoid exacerbating soil erosion or degrading SOC. Conservation planning for erosion control has a long history, whereas, managing to maintain SOC is a relatively recent event (USDA-NRCS 2006). The amount of residue that needs to be kept on a soil to maintain SOC can exceed the amount needed for erosion control (Wilhelm *et al.* 2007). A literature review including several crops and managements estimated 2.5 ± 1.7 Mg C ha^{-1} yr^{-1} (n = 28) [6.3 Mg ha^{-1} yr^{-1} aboveground residue], was needed to maintain SOC contents (Johnson *et al.* 2010). While these crude estimates are conceptually

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useful, verification and refinement are necessary if they to be used for site-specific recommendations, especially under different climatic and soil and agricultural systems (Wilhelm *et al.* 2010). A goal of this partnership is it improve and verify the amount of retained stover needed to sustain SOC content (Karlen 2010). Data were gathered from a range of locations with divergent climatic conditions, and soil properties in anticipation of the results providing key determinates of SOC responses to stover harvest.

Material and Methods

A description of the climate, soil and management characteristics is summarized in Table 1. Each partner provided an average amount of stover returned and a corresponding ΔSOC (0-15 or 0-30 cm). The amount of aboveground biomass needed to maintain SOC was estimated independently at each site (Table 2). The ΔSOC (g kg^{-1}) was calculated (ΔSOC = $SOC_n - SOC_i$), where SOC_n is the concentration at some time after treatment application and SOC_i is initial SOC. Consistent with others (e.g., Halvorson and Schlegel 2012; Larson *et al.* 1972; Wilhelm *et al.* 2007) the average ΔSOC was regressed against the average amount of stover left in the field. A positive slope indicates SOC accrual increases as the amount of aboveground stover biomass is returned.

Table 1. Regional Partnership Corn-Stover Sites, Mean Annual Precipitation and Mean Annual Temperature, Soil Type, and Primary Tillage (listing more than one tillage indicates multiple fields), Study Duration, and Crop Rotation.

Site	State	City/site	Elev[I]	Lat	Long	MAP	MAT	Soil type[II]	Tillage	Dur	Crop[IV]
#			m	°N	-°W	cm	°C			yr	
1	IN	West Lafayette	213	40	97	95	10	SL	NT	5	MSo
2	IA	Ames/Boyd	340	42	94	92	10	L	CP	4	MSo
3	IA	Ames/ Bruner	340	42	94	92	10	CL	CP	5	M
4	IA	Ames/7071	340	42	94	92	10	SiCL, L	CP/NT	3	M
5	MN	Lamberton	350	44	95	71	6.1	CL	CP/ST	2	M
6	MN	Morris	350	45	96	65	5.8	CL, L	CP/NT	4	M
7	MN	Northfield	290	44	93	71	6.1	SiC, SiL,	ST/MBP	2	M
8	NE	Ithaca[V]	1166	41	96	74	9.8	SiCL, SiL	NT	5	M
9	SC	Florence [VI]	140	34	79	130	17	LS	PS	4	MSo
10	SD	Brookings[VII]	490	44	96	58	6.1	SiCL	NT	5	MSo

[I]Abbrevations: Dur=Duration; Elev=elevation, Lat,= latitude, Long=longitude west, MAP=mean annual precipitation, MAT = mean annual temperature.

[II]Soil type: C=clay, L=loam, S = sand, Si = silt.

[III] Primary tillage: CP = chisel plow, D=disk, MBP = moldboard plow, NT= no tillage, PS, Planter with subsoiler, ST= strip tillage.

[IV]Crops: M=maize, So=soybean, cc=cover crop.

[V](Follett *et al.* 2012)

[VI](Novak *et al.* 2010)

[VII](Hammerbeck *et al.* 2012; Stetson *et al.* 2012)

Table 2. Initial Surface Properties, Tillage Depth at Vary Sites Established to Assess the Minimum Biomass Needed to Maintain SOC (X-intercept).

Site	BD[I]	pH	Sand	Clay	P	K	SOC	Sample depth	Tillage depth	Slope	X-intercept[II]
#	g cm^{-3}		g kg^{-1}		mg kg^{-1}		g kg^{-1}	cm		ΔSOC Mg^{-1} biomass	Mg ha^{-1} yr^{-1}
1	1.3	5.8	180	100	14	112	16.9	30	0	0.48	-2.97
2	1.4	6.7	400	200	22	95	19.0	15	20	0.22	5.85
3	1.2	7.7	350	350	32	134	39.4	15	20	1.32	3.98
4a	1.3	6.3	300	325	33	176	23.7	15	20	0.11	56.1
4b	1.3	6.4	300	325	32	164	23.7	15	0	0.27	28.6
5a	1.8	6.4	350	350	12	167	16.3	30	20	-0.16	12.3
5b	1.8	6.0	350	350	12	167	17.2	30	20	0.10	-23.8
6a	1.3	6.8	350	260	17	163	22.9	30	20	-0.06	16.6
6b	1.4	6.2	370	250	18	172	21.0	30	0	-0.02	64.5
6c	1.4	6.4	410	230	19	146	37.0	30	0	0.21	4.18
7a	1.0	6.0	210	300	45	306	37.0	30	20	1.03	7.29
7b	1.0	6.4	210	300	45	306	36.7	30	25	-1.63	1.23
8	1.3	6.6	150	300	15	NA	13.1	30	0	0.33	2.65
9	1.3	4.8	730	20	26	74	18.0	15	30	0.12	6.48
10a	1.4	6.3	60	470	50	195	27.1	30	0	0.17	0.47
10b	1.4	6.3	60	470	50	195	27.1	30	0	0.22	-4.07

[I]Abbreviations: BD=bulk density, SOC=soil organic carbon

[II]Bold indicates that ΔSOC as a function of stover returned had a positive slope and a negative Y-intercept; thus, the resulting X-intercept approximates the annual amount of stover needed to maintain SOC in that system.

To bolster the prediction of corn stover vs. ΔSOC, results from published studies room corn-based systems (Table 3) (Allmaras *et al.* 2004; Benjamin *et al.* 2010; Clapp *et al.* 2000; Clay *et al.* 2006; Clay *et al.* 2001; Crookston *et al.* 1991; Halvorson and Schlegel 2012; Huggins *et al.* 1998; Larson *et al.* 1972; Linden *et al.* 2000; Pikul *et al.* 2008; Reicosky *et al.* 2002; Vanotti *et al.* 1997; Varvel and Wilhelm 2010; Vitosh *et al.* 1997; Wilts *et al.* 2004) were included in the multiple regression analyses. The duration of study among these published works describing 15 sites ranged from seven to 29 years; were located between latitudes 38 to 45 ^{o}N and longitude -89 to -103 ^{o}W, sand content ranged from 80 to 850 g kg^{-1}, clay content ranged from 65 to 420 g kg^{-1}, SOC_i ranged from 8 to 38 g kg^{-1}, soil pH ranged from 6.4 to 7.1, initial bulk density ranged from 1.3 to 1.7 g cm^{-3}, sample depth varied from 15 to 30 cm, and tillage depth ranged from zero to 30 cm. The estimated minimum amount of corn stover to return to maintain SOC content in these published works ranged from 2.9 to 8.25 Mg ha^{-1} yr^{-1}.

Table 3. Published Data Metadata Mean Annual Precipitation and Mean Annual Temperature, Bulk Density, Study Duration, Crop Rotation, pH, Soil Texture, Initial SOC, Properties, Sample and Tillage Depth, Slope and Minimum Biomass Need to Maintain SOC.

Pub[I]	Elev[II]	Lat	Long	MAP	MAT	BD	Dur			Sand	Clay	SOC	Sample depth	Tillage depth	Slope	Minimum Residue
#	m	°N	-°W	cm	°C	g cm^{-3}	yr	Crop[III]	pH	g kg^{-1}			cm		ΔSOC Mg^{-1} biomass yr^{-1}	Mg ha^{-1} yr^{-1}
1	290	45	93	82	7.2	1.3	13	M	6.4	200	150	20	30	0	0.153	6.87
1	290	45	93	82	7.2	1.3	13	M	6.4	200	150	20	30	25	0.135	6.60
1	290	45	93	82	7.2	1.3	13	M	6.4	200	150	18	30	17	0.229	8.12
2	1000	41	95	93.4	104	1.3	11	M	6.5	80	360	18	30	25	2.5	6.00
3	1380	48	103	40	10	1.5	7	MR	7.0	250	420	8	30	35	-0.013	2.90
3	1380	48	103	40	10	1.5	7	MR	7.0	250	420	8	30	0	1.03	5.56
4	2020	41	100	47.5	8.9	1.4	14	M	6.5	NA	NA	9.5	15	10	NA	6.00
5	1090	38	101	44	10.7	1.4	9	MR	7.1	190	256	10	30	0	0.133	6.80
6	1750	44	96	60	6.3	1.3	8	MSo	7.0	135	280	26	15	20	NA	4.00
7	260	42	85	78	8.6	1.7	19	M	6.5	850	65	9.4	23	30	1.44	6.40
8	330	43	89	79	7.6	1.4	25	M	6.7	100	300	19	20	25	NA	5.10
9	350	44	95	63	6.2	1.3	10	M	7.0	322	331	38	15	30	NA	7.20
10	350	45	95	61	5.7	1.2	29	M	7.5	370	250	26	20	25	NA	8.25
11	500	44	96	58	7.2	1.4	10	M	6.4	420	185	18	15	25	3.19	8.00
11	500	44	96	58	7.2	1.4	10	MSo	6.4	420	185	18	15	25	3.86	6.20

[I] 1=(Allmaras *et al.* 2004; Clapp *et al.* 2000; Linden *et al.* 2000); 2=(Larson *et al.* 1972); 3=(Benjamin *et al.* 2010); 4=(Varvel and Wilhelm 2010); 5=(Halvorson and Schlegel 2012); 6=(Clay *et al.* 2006; Clay *et al.* 2001); 7=(Vitosh *et al.* 1997); 8=(Vanotti *et al.* 1997); 9=(Crookston *et al.* 1991; Huggins *et al.* 1998); 10=(Reicosky *et al.* 2002; Wilts *et al.* 2004); 11=(Pikul *et al.* 2008)

[II] Abbreviations: Dur=Study duration; Elev=elevation; Lat,= latitude, Long=longitude west, MAP=mean annual precipitation, MAT = mean annual temperature; Pub=publication number; NA-not available, SOC=soil organic carbon

[III] C=corn, S-soybean, R=longer rotations included

Current data (n=16; Table 2) plus previously published data (n=15; Table 3) provided a substantial number of observations (n=31) that represent a range in conditions for a viable metadata analysis of how stover harvest impacts SOC. Therefore, a forward-selection, multiple regression analysis (SAS Institute 2009) was used to identify properties that define variables that refine SOC response to residue harvest.

Results and Discussion

Understanding how stover harvest impacts SOC is crucial to making sustainable harvest recommendations. Provided the rate of humification and mineralization remain constant over the course of a study, the annual rate of biomass input should proportional ΔSOC (Johnson *et al.* 2010). Thus, when ΔSOC as a function of stover returned has a positive slope and a negative Y-intercept; the resulting X-intercept approximates the annual amount of stover needed to maintain SOC in that system. Based on these criteria, minimum biomass requirements ranged from 0.46 to 56.1 Mg ha^{-1} yr^{-1} at site 10a and 4a, respectively, with a mean of 11.7 Mg ha^{-1} yr^{-1}. Excluding the site 4a and 4b, the mean is 4.0 Mg ha^{-1} yr^{-1}, which is within the range of published previously. The sites (Table 1, 2) represent a range of soils, climates and management practices that may explain the divergence in soil response to stover harvest.

Including 17 variables from all site-field data (Table 2) in a forward-selection multiple-regression resulted in a model ($R^2 = 0.86$) that included seven variables [sampling depth, (partial $R^2 = 0.23$; P=0.08); initial K concentration, SOC_i (partial $R^2 = 0.19$; P=0.06), tillage depth (partial $R^2 = 0.12$; P=0.09), years, initial bulk density and mean annual temperature)]. The other variables had P values >0.1. Inclusion of previously published data failed to identify statistically significant regression models that predicted minimum biomass requirements. Multiple regression of published and current data resulted in a 10 variable model $R^2 = 0.7$. The variables included were study duration (partial $R^2 = 0.18$; P=0.03), sample depth (partial $R^2 = 0.16$; P=0.02), longitude (partial $R^2 = 0.07$; P=0.11), pH (partial $R^2 = 0.08$; P=0.11), clay (partial $R^2 = 0.06$; P=0.12) and tillage depth, SOC_i, latitude, sand content and rotation, which collectively only increased the model R^2 by 0.14; P>0.1). Regression data only from no tillage management tillage resulted in an eight variable model ($R^2 = 0.99$), significant contributions detected for sand content, elevation and mean annual precipitation with partial $R^2 = 0.38$; 0.22 and 0.04, respectively, P>0.05. In contrast, multiple regression among the tilled studies failed to identify any significant partial R^2 among the 11 variables included in the model ($R^2 = 0.67$). Measurable ΔSOC can take many years to detect. Additionally, soil profile depth is also crucial, as tillage or lack thereof changes the distribution of C as well as influence soil processes involved with residue decomposition. This analysis reinforces the importance of including climate, soil and management parameters to more fully understand soil response to management changes such as stover harvest.

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4.4
USING DAYCENT TO MODEL THE SOIL IMPACTS OF HARVESTING CORN STOVER FOR BIOENERGY

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Abstract
Minimizing GHG emissions and avoiding soil carbon (C) depletion associated with feedstock production is a key concern with the development of corn stover for biofuel, in order to prevent reductions in soil fertility and negative climate impacts from residue removal. While experimental data are crucial to understand site-specific corn stover removal impacts on soil C and GHG flux, models are needed to project how corn stover removal may impact the land on greater temporal and spatial scales. We used grain yield, soil C, and N_2O flux data collected from published literature as well as multiple Corn Stover Removal Team experimental sites to test DAYCENT performance modeling the impacts of corn stover removal. We aggregated measured data across residue removal treatments and compared measured trends to DAYCENT modeled results. DAYCENT performed well simulating SOC change, reasonably well simulating average grain yields, but represented interannual grain yield poorly. In this analysis, residue removal did not significantly affect average soil C change or yields. DAYCENT underestimated N_2O flux, which is an area for further model parameterization to better predict impacts of residue removal on GHG flux.

Keywords: DAYCENT, soil carbon change, corn stover, bioenergy

Introduction
As an agricultural byproduct of corn (*Zea mays*) grain production, corn stover, the plant residue remaining after harvest, is an attractive source of biomass for bioenergy in the United States (US). The US Department of Energy Billion Ton Study Update (2011) considers agricultural residues such as corn stover to be among the best sources of raw material to support bioenergy industry, citing their immediate large-scale availability, potential low costs, and widespread distribution in predominant crop production regions (U.S. Department of Energy 2011).

Economic viability and environmental sustainability are two major concerns with the expansion of agricultural residue harvest for bioenergy production. Crop residues can be an important source of organic matter to maintain soil fertility and promote favorable soil structure. Also by changing the input of plant material into soils, crop residue harvests may impact emissions of greenhouse gases (GHGs) such as N_2O and CH_4 from decomposition as well as change the total C stored in soil. However these impacts are not easily generalized across regions; they depend on multiple factors including climate, soil type, prior land use, and crop management. The development of residue use for bioenergy production is best informed by regional analyses to determine where residues can be harvested sustainably, in sufficient quantities to support a viable bioenergy industry. A tool suited to these analyses is DAYCENT, a process-based ecosystem model.

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The DAYCENT model, developed at Colorado State University, simulates GHG gas fluxes as well as plant/soil C dynamics and many other ecosystem variables (Parton 1987, 1988). DAYCENT and its predecessor CENTURY have been applied and tested in many agricultural systems both in the US and globally (e.g. Del Grosso et al. 2008; Galdos et al. 2009; Abdalla et al. 2010), and have been used to evaluate several types of bioenergy production systems (e.g. Adler et al. 2007; Davis et al. 2010). While DAYCENT modeling of corn production has been widely tested, its performance in evaluating the impacts of corn stover harvest remains unknown.

This project evaluated DAYCENT performance in simulating soil C change, corn yields, and N_2O emission impacts of varying levels of corn stover removal. This analysis will allow a better understanding of DAYCENT performance in modeling corn stover removal for bioenergy, to better inform evaluations of management scenarios as corn stover use for bioenergy is developed.

Methods

DAYCENT includes routines for simulating the movement of soil nutrients through different pools, the movement of water through soil layers, physiology-based plant growth, and many other ecosystem components that are described in greater detail elsewhere (e.g. Del Grosso et al. 2008). The key drivers of DAYCENT include maximum and minimum daily temperature, daily precipitation, soil texture, and land management (including specific plant types grown and soil management such as tillage and nutrient additions).

To test the DAYCENT model, a series of data were assembled from published literature evaluating two sites for the soil and crop production impacts of long-term corn stover removal (Clapp et al. 2000; Linden et al. 2000; Reicosky et al. 2002; Wilts et al. 2004). Data were also assembled from two Corn Stover Regional Partnership sites established as a subset of the US Agricultural Research Service Renewable Energy Assessment Project (REAP) to evaluate the sustainability of corn stover harvest (Karlen 2010). The REAP sites offer a more complete dataset, including grain and stover yields, soil C change from 0 – 20cm, and N_2O emissions (Table 1).

Table 1. Summary of study locations and treatments.

Source	Location	Lat/Long	Management Description	Grain yield	Stover yield	Soil C	N_2O
Reicosky et al, 2002; Wilts et al. 2004	Morris, MN	45.6/-95.9	29 years continuous corn; moldboard tillage; low fert. (83 kg N/ha), high fert. (166 kg N/ha), & control (0 kg N/ha); 0 & 100% stover removal	X	X	X	-
Clapp et al., 2000; Linden et al., 2000	Rosemount, MN	44.7/-93.1	13 years continuous corn; chisel, moldboard & no till tillage; 0 & 200 kg N/ha; 0 & 100% stover removal	X	X	X	-
REAP: Swan Lake Exper. Site	Morris, MN	45.7/-95.8	7 years corn/soy rotation; chisel & no till tillage; 130 kg N/ha; 0, 50, 100% stover removed	X	X	X	X
REAP: U of N Ag. Research & Develop. Center	Ithaca, NE	41.2/-96.4	13 years continuous corn; no till tillage; 60, 120, & 180 kg N/ha; 0 & 100% stover removed	X	X	X	X

Statistical analyses were completed using R-2.15.1 software (R Core Team 2012). Regression analyses were applied to compare measured versus modeled grain yield, SOC change, and annual N_2O flux. The specific interaction of data generation method (measured versus modeled) as well as the effect of stover harvest were assessed using a two-way analysis of variance (ANOVA). Welsh two-sample t-tests were applied to compare measured to modeled N_2O flux by individual treatment effects.

Results and Discussion

Modeled and measured SOC change exhibited a significant correlation and high level of accuracy when compared to a linear 1:1 relationship (Figure 1, A).

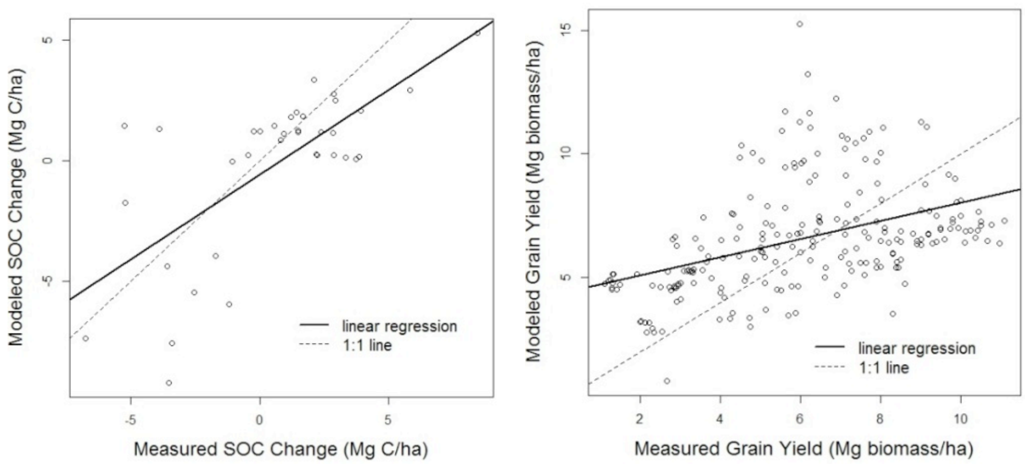

Figure 1. Measured versus modeled change in soil C (A) for three sites and grain yield (B) for four sites showing a significant relationship (adjusted R^2 = 0.46, p<<0.001 and adjusted R^2 = 0.18, p<<0.001, respectively). One site was excluded from the soil C regression due to irregular measurement data.

Both measured and modeled data suggested increased soil C with residue retention compared with removal (Figure 2, A). However, neither modeled nor measured average SOC change was significant across residue removal treatments, and there was no significant interaction between data generation (modeled versus measured) and residue removal rate (removed versus returned) (Figure 2, A, p>0.05). Soil C is highly heterogeneous and changes slowly through time, which may account for the lack of significant differences over the experiment duration. These data suggest that corn stover removal for bioenergy should proceed carefully, in the context of protecting soil C and soil fertility, with opportunities for both increases and declines. The measured data are aggregated across only three sites; data from more varied locations and with longer timelines would help determine specific residue removal rates that maintain soil C.

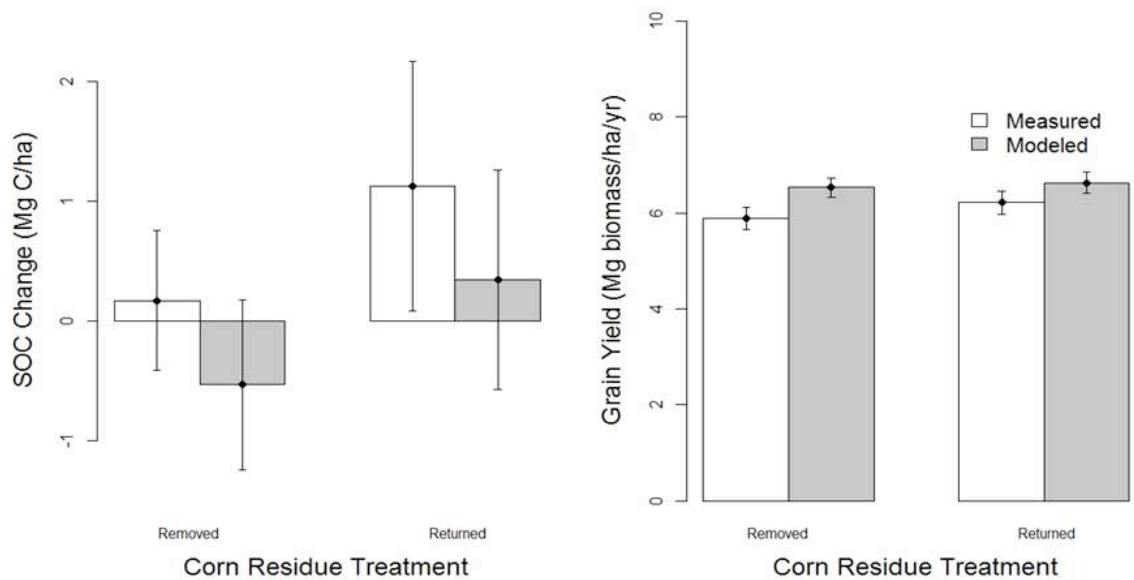

Figure 2. Modeled versus measured average change in SOC (A) and annual grain yield (B) when corn stover residue is removed (>0% removal) versus returned (0% removal), showing standard error (n = 21 and 15 for SOC change, n = 122 and 99 for grain yield, respectively).

DAYCENT simulated average grain yields with reasonable accuracy and neither measured nor modeled data showed any significant effect of residue removal on average yields; modeled and measured average yields were not significantly different across residue removal, and there was no significant interaction effect between data generation (modeled versus measured) and residue removal rate (removed versus returned) (Figure 2, B, $p>0.05$). However modeled and measured grain yield exhibited a significant correlation, but a lower level of accuracy relative to a linear 1:1 relationship when individual treatments were compared interannually (Figure 1, B). At low measured values the model tended to overestimate yields (grouped data above the 1:1 line, Figure 1, B), while at high measured values the model tended to underestimate yields (grouped data below the 1:1 line, Figure 1, B). Of particular interest is the group of high modeled yields (> 12 Mg biomass/ha) where measured yields were only around 6 Mg biomass/ha (Figure 1, B). These data were all from one site at that experiment's highest fertilizer treatment. It suggests that the true production system is limited by some other factor than N application, and that this other factor is not captured accurately in DAYCENT. DAYCENT yield simulations with residue removal could likely be improved with better parameterization of N interactions with crop growth.

DAYCENT performed poorly in the comparison of modeled and measured annual N_2O flux, with no significant correlation (adjusted $R^2 = -0.007$, $p = 0.4$). Overall modeled data were significantly lower than measured data (Figure 3, A). The model only performed well at Site A, one of the two sites used in this analysis, with no significant difference between measured and modeled data (Figure 3, B, $p = 0.0998$).

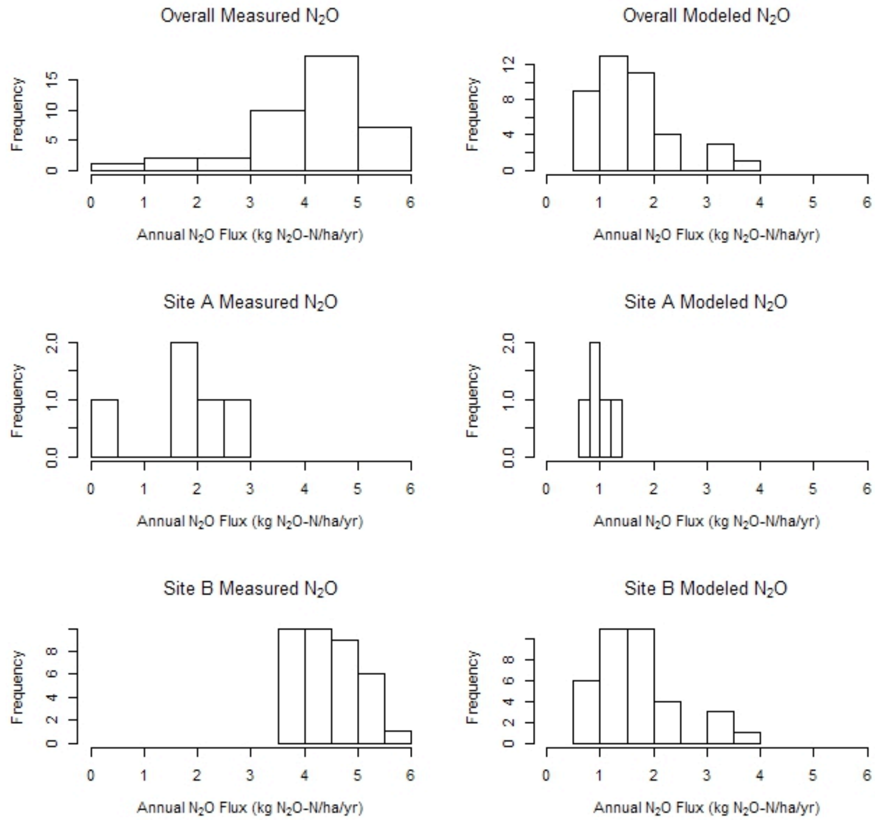

Figure 3. Histograms of overall (A) and by site (B & C) annual N_2O flux, comparing measured (column 1) to modeled (column 2) data. Significant differences between measured and modeled data (by row) are indicated by **, where p<<0.001.

Expansion of DAYCENT N_2O flux testing across more sites will help determine the mechanisms behind DAYCENT's under predictions. N_2O flux is highly variable, and is an area where site-specific modeling with DAYCENT could have high value in directing management decisions as corn stover use for bioenergy is expanded.

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4.5
CORN GRAIN, STOVER YIELD AND NUTRIENT REMOVAL VALIDATIONS AT REGIONAL PARTNERSHIP SITES

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Abstract
Corn (*Zea mays*, L.) stover has been identified as a major feedstock for cellulosic bioenergy. This report summarizes grain and stover yield as well as N, P, and K removal at several Sun Grant Regional Partnership (SGRP) sites. National Agricultural Statistical Service (NASS) grain yields were used to assess the relevancy of plot-scale yields with county averages. Seasonal variation in weather patterns caused yields to differ substantially among sites and years. Nutrient removal estimates were significantly influenced by the sampling method (*i.e.* analysis of hand samples between physiologic maturity and grain [15]harvest versus stover collected during the harvest operation). Based on ancillary studies that indicate corn stover should not be harvested if average grain yields are less than 175 bu ac^{-1} (11 Mg ha^{-1}), these studies show that non-irrigated SGRP sites with the highest potential for sustainable corn stover harvest were located between -91º and -93º west longitude. The more eastern (-78º w longitude) and western (-96º w longitude) sites did not have sufficient yield for sustainable routine stover harvest, although with good management, corn could still be part of an overall landscape approach for sustainable feedstock production in those areas. For producers with consistently high yields (*i.e.* > 200 bu ac^{-1}) and where residue management may actually be a major problem (*e.g.* in irrigated areas), moderate stover harvest may actually decrease fuel use and save additional energy by reducing the amount of tillage needed to

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prepare subsequent seedbeds. Less intensive tillage could also preserve rhizosphere carbon and/or soil structure benefits often attributed to no-till systems.

Keywords: bioenergy, sustainable feedstock production, nutrient removal

Introduction

The U.S. EPA has identified corn stover, the aboveground material left in fields after grain harvest, as "the most economical agricultural feedstock … to meet the 16 billion gallon cellulosic biofuel requirement" (Schroeder, 2011). They estimated that 7.8 billion gallons of ethanol would come from 82 million tons of corn stover by 2022, which is consistent with conclusions reached by the U.S. Department of Energy (2011). A major reason stover was identified as an important feedstock is because of the vast area upon which corn is grown in the Midwestern U.S.A.

Harvesting corn stover was envisioned as a "win-win" management practice because as a perceived, underutilized resource (Nelson et al., 2002; Perlack et al., 2005; BRDB, 2008) it could provide cellulosic feedstock for bioenergy production and reduce the cost encountered by producers (Duffy, 2012) for residue management (\$20 to \$30 ac^{-1} or \$45 to \$65 ha^{-1}). However, corn stover provides many ecosystem services (Johnson et al., 2010; Wilhelm et al., 2007; 2010) and its harvest will increase annual nutrient removal (Karlen et al., 2011a, 2011b), so care must be taken to identify the locations and quantity of stover that can be harvested in a sustainable manner. The objective for this report is to summarize grain and stover yield as well as N, P, and K removal at several SGRP sites.

Materials and Methods

A regional research partnership among the North Central Sun Grant Association, the ARS Renewable Energy Assessment Project (REAP) team, and the Idaho National Laboratory (INL) was initiated in 2008 to quantify the quantity of corn stover produced and effects of moderate- or high-rates of harvest. A core experiment consisting of no-tillage (or the least amount of tillage necessary to establish a corn crop), three rates of stover harvest (none, moderate, and high), and four replications was agreed upon for each of the SGRP sites. On-going, long-term ARS and/or university field trials assessing effects of crop residue harvest were leveraged when feasible to build a more robust dataset.

Results and Discussion

This report focuses on crop yields and nutrient removal from field studies at the SGRP sites. Soil organic carbon, GHG, economic assessments, soil quality, stover composition and energy content assessments from these SGRP sites are addressed elsewhere in this Proceedings document. Table 1 lists the location and provides basic soil, climate, and management information for the various sites. Table 2 shows that with the exception of sites 5, 9, 13, and 14, crop yields at these SGRP locations were at or above the NASS county averages for those locations. Site 5 was a long-term no-tillage site in MN where K deficiency has been documented as a limiting factor. Except for one year, sites 9 in NE and 13 and 14 in SD were generally limited by inadequate rainfall. Sites 5, 6, (MN) and 12 (PA) showed a slight yield decrease where the maximum amount of stover was removed, but short-term effects at other locations were either negligible or even positive (presumably due to less N immobilization and slightly warmer early-season soil temperatures). Overall, sites between located between -91º and -93º west longitude showed the highest potential for sustainable stover harvest.

Table 1. Location, primary tillage (multiple "sites" if more than one), cropping system, elevation, mean annual precipitation, mean annual temperature, soil type, and duration of regional partnership field studies summarized for this report.

Site	State	County	Tillage† / Crop‡	Elev.	Lat	Long	MAP	MAT	Soil type§	Age
				m	°N	-°W	cm	°C		yr
1/2	IA	Boone	CP & NT / MM	340	42	94	92	10	SiCL, L	4
3/4	IL	Warren	CP & NT / MM	229	41	91	100	11	SiL	4
5	MN	Stevens	NT/95 / MS	350	45	96	65	5.8	CL, L	17
6	MN	Stevens	NT/05 / MS	350	45	96	65	5.8	CL, L	7
7/8	MN	Rice	MbP & ST / MM	290	44	93	71	6.1	SiC, SiL,	4
9	NE	Saunders	NT/NI¶ / MM	1166	41	96	74	9.8	SiCL, SiL	14
10	NE	Saunders	NT/Irr¶ / MM	1166	41	96	74	9.8	SiCL, SiL	11
11/12	PA	Centre	NT / MM/MS	352	41	78	97	9.7	SiCL, SiL	4
13	SD	Brookings	NT / MS	490	44	96	58	6.1	SiCL	8
14	SD	Brookings	NT / MS+CC	490	44	96	58	6.1	SiCL	8
15	SC	Darlington	NT/IRSS / MM	140	34	79	130	17	LS	4

† Tillage – CP = chisel plow; NT – no-tillage; ST – strip tillage; MbP – moldboard plow; IRSS – in-row subsoiling (done beneath each row at planting)
‡ Crop -- MM – continuous maize; MS – maize/soybean; CC – cover crop;
¶ Water management (NE location only) -- NI – non-irrigated; Irr – irrigated
§ Soil type – L = loam; CL = clay loam; SiC = silty clay; SiL = silt loam; SiCl = silty clay loam; LS = loamy sand

Table 2. Four-year NASS and measured corn grain yields for various stover harvest strategies at fifteen regional partnership sites.

Site	NASS Yields†		No Removal		Partial Rem.		High Removal	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	---------- bu acre^{-1} at 15.5% moisture content ----------							
1	166 –182	174	202--142	171	166--208	188	171--220	188
2	166 --182	174	135--187	168	161--200	183	175--222	195
3	153 --207	183	148 --268	218	152 -- 72	212	150 --262	215
4	153 --207	183	90 -- 254	177	127 --257	200	126 -- 262	205
5	140 --180	159	119 -- 193	143	111 – 193	146	82 -- 192	137
6	140 --180	159	127 -- 204	164	119 – 198	160	131 – 189	157
7	160 --191	177	178 --203	199	180 --203	198	178 -- 211	199
8	160 --191	177	191 –198	199	180 --201	198	178 -- 203	196
9	136 --167	149	45 --162	96	--	--	37 – 185	109
10	179 --214	193	147 --275	190	115 --329	209	135 – 302	209
11	93 -- 136	121	44 --217	147	69 -- 219	153	47 -- 217	147
12	93 -- 136	121	64 -- 215	152	88 -- 219	152	57 -- 211	143
13	141 --159	154	84 -- 181	123	96 -- 192	126	92 -- 187	125
14	141 --159	154	73 -- 181	119	97 -- 193	126	87 -- 187	123
15	47 -- 121	77	21 -- 181	83	9 -- 156	81	13 --159	84

† NASS averages across all management practices except irrigated vs non-irrigated for 2008 -- 2010

Table 3 shows the measured or estimated (sites 3 and 4) quantities of stover harvested at the various sites. Estimates were made for sites 3 and 4 because that location was added three years after the SGRP study was initiated and actual residue weights were not collected. Full removal at sites 3 and 4 was accomplished by chopping stalks and raking them off the plots. This technique left a considerable amount of fine material, which was estimated to be less than 10% of the residue dry weight. Partial removal (estimated at 50 to 60%) was done by raking without chopping stalks. Actual quantities of stover harvested at all other sites using a variety of collection strategies were measured and found to be much lower than the estimated quantities. Based on other studies (e.g. Karlen et al., 2012), the harvest strategy used at sites 3 and 4 was probably much more aggressive than the other mechanical operations. Seasonal and location differences were quite large reflecting different growing conditions and in some years lodging caused by severe wind storms. Overall, stover harvest averaged 1.6 and 3.0 tons acre^{-1} (3.6 and 6.7 Mg ha^{-1}) for the moderate and maximum harvest treatments, respectively.

Table 4 shows N, P, and K removals associated with the moderate- and high-removal treatments. Values for sites 1 and 2 were measurements made on stover samples collected after single-pass harvesting with a commercial scale combine (Karlen et al., 2011a), while those from other sites were based on hand samples collected between physiological maturity and combine harvest. Nutrient removal estimates based on the hand samples were much higher for the hand samples reflecting the nutrient translocation from the stalks that occurs after the crop matures. This emphasizes the importance of documenting the time of sampling when estimating nutrient removal as part of the subsequent year's soil fertility/fertilizer program.

Table 3. Stover harvest rates for moderate- and high-removal treatments at fifteen regional partnership sites.

Site	Moderate Harvest				Maximum Harvest			
	2008	2009	2010	2011	2008	2009	2010	2011
	tons acre^{-1} at 0% moisture content							
1	2.4	1.9	1.2	1.7	2.8	3.3	1.9	3.2
2	--	2.1	0.8	1.5	--	3.6	2.3	2.8
3†	3.0	2.5	2.5	2.0	5.4	4.7	4.5	3.8
4†	2.9	2.5	2.4	1.8	5.2	4.6	4.3	3.4
5	1.9	2.1	0.8	2.0	1.6	2.6	2.7	2.8
6	2.0	2.6	2.5	1.7	2.2	3.0	3.0	3.2
7	--	1.7	1.8	1.7	--	3.6	3.5	3.4
8	--	1.6	1.8	1.8	--	3.5	3.5	3.5
9	--	--	--	--	1.8	2.0	1.2	4.5
10	0.8	1.7	2.1	4.5	1.5	3.4	4.0	8.7
11	1.3	1.5	1.3	0.8	2.7	3.7	2.8	1.8
12	1.3	1.5	1.3	0.7	2.8	3.5	2.5	1.7
13‡	1.6	3.0	0.4	0.9	1.8	2.7	0.8	2.5
14‡	1.3	3.0	0.4	1.0	2.1	2.8	0.9	1.8
15	1.2	1.8	1.5	1.1	2.1	3.7	2.9	2.2

† Harvested amounts estimated based on a harvest index of 0.5 and either 50% or 90% removal rates

‡ Maximum stover harvest was calculated by subtracting grain yield from moderate harvest from weights obtained when the entire plant was harvested using a commercial silage chopper.

Table 4. Average N, P, and K removal rates (lb/acre) associated with moderate- and high-removal treatments at eleven regional partnership sites.

Site	Moderate Harvest			Maximum Harvest		
	N	P	K	N	P	K
	---------- lb acre^{-1} ----------					
1	17.6	2.5	29.7	28.5	3.5	45.3
2	15.4	1.4	28.0	27.0	2.8	52.9
5	22.6	4.9	37.3	60.1	11.2	85.1
6	23.9	4.2	46.3	56.4	10.0	119.2
7	21.4	2.3	41.5	44.7	10.9	84.2
8	21.8	2.5	39.6	44.6	4.4	75.3
9	--	--	--	38.6	4.4	56.9
10	45.8	6.3	65.7	82.3	11.2	113.4
11	20.7	2.2	31.7	44.7	4.7	68.8
12	21.3	2.4	31.0	44.1	4.8	61.5
15	24.7	3.2	31.4	48.5	5.3	61.5

Summary and Conclusions

This report documents grain and stover yield for several SGRP field research sites as well as estimates of nutrient removal. The most favorable area for sustainable stover harvest appears to lie between -91º and -93º west longitude. Hand samples and harvest index calculations and hand samples appear to overestimate stover quantities and nutrient removal.

Acknowledgment

USDA-Agricultural Research Service as part of the USDA-ARS-REAP project. Additional funding the North Central Regional SunGrant Center at South Dakota State University through a grant provided by the USDOE – Office of Biomass Programs under award number DE-FC36-05GO85041

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4.6
A MULTI-FACTOR ANALYSIS OF SUSTAINABLE AGRICULTURAL RESIDUE REMOVAL POTENTIAL

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Abstract
Agricultural residues have significant potential as a near term source of cellulosic biomass for bioenergy production, but sustainable removal of agricultural residues requires consideration of the critical roles that residues play in the agronomic system. Previous work has developed an integrated model to evaluate sustainable agricultural residue removal potential considering soil erosion, soil organic carbon, greenhouse gas emission, and long-term yield impacts of residue removal practices. The integrated model couples the environmental process models WEPS, RUSLE2, SCI, and DAYCENT. This study uses the integrated model to investigate the impact of interval removal practices in Boone County, Iowa, US. Residue removal of 4.5 Mg/ha was performed annually, bi-annually, and tri-annually and were compared to no residue removal. The study is performed at the soil type scale using a national soil survey database assuming a continuous corn rotation with reduced tillage. Results are aggregated across soil types to provide county level estimates of soil organic carbon changes and individual soil type soil organic matter content if interval residue removal were implemented. Results show interval residue removal is possible while improving soil organic matter. Implementation of interval removal practices provide greater increases in soil organic matter while still providing substantial residue for bioenergy production.

Keywords: residue removal, model integration framework, agricultural residues, soil organic matter

Introduction
Agricultural residues are the plant material other than grain (cob, stalk, leaves, etc.) produced when commodity grain crops such as corn (*Zea mays L*.), wheat (*Triticum aestivum L.),* and barley (*Hordeum vulgar L*.) are grown. Removing agricultural residues for energy production has received significant attention over the past few decades. However, determining the quantity of agricultural residues that can be sustainably removed from a field is challenging because of the diverse functions that agricultural residues provide within the agroecosystem. Considering this range of functions Wilhelm et al. (2010) identified six environmental factors that can potentially limit the quantity of agricultural residues that can be removed sustainably: soil organic carbon, wind and water erosion, plant nutrient balances, soil water and temperature dynamics, soil compaction, and off-site environmental impacts. The impact of residue removal on soil organic matter has received considerable attention among these six limiting factors because soil organic matter is critical to long term soil productivity. Soil organic matter enables nutrient cycles, helps stabilize soil structure, and facilitates water retention resulting in increased crop productivity. Soil organic matter also helps mitigate soil erosion losses due to wind and water (Johnson et al., 2010). Because of this it is essential to understand how residue removal impacts soil organic matter to determine the long term sustainability of a specific residue removal practice.

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This paper utilizes a model integration framework to quantitatively analyze how residue removal practices impact long term soil organic matter levels. There are a wide range of potential residue removal practices and each can impact soil organic matter levels differently. Previous efforts have qualitatively considered how changing the removal rates, i.e. the percentage of residue biomass taken off the field, can impact soil organic matter levels (Muth et al., 2012). The challenge with implementing lower removal rates to address soil organic matter constraints, and broader sustainability concerns, is that lower removal rates are often not economically viable (Hess et al., 2009). One way to address concerns about soil organic matter losses and still remove residues at rates which are economically viable is to implement interval removal practices. These practices will remove residues one out of every two or three years that a residue producing crop is grown on a field. Interval removal practices have significant potential but need further analysis. To provide this analysis, this paper extends the previous integrated model developed by Muth and Bryden (2012) to provide quantitative soil organic matter analyses using the DAYCENT model (Parton et al., 1998) to assess the sustainability of interval residue removal practices.

Muth and Bryden (2012) developed an integrated model for determining sustainable agricultural residue removal using the model integration framework VE-Suite (McCorkle and Bryden 2007). The integrated model performs sustainable residue removal assessments considering water erosion, wind erosion, and soil organic matter constraints. Three models were integrated into the framework: The Revised Universal Soil Loss Equation, Version 2 (RUSLE2) (McCool et al. 2004); the Wind Erosion Prediction System (WEPS) (Wagner 1996); and the Soil Conditioning Index (SCI) (USDA-NRCS 2012). In 2012, Muth et al. (2012) performed a county-level national assessment of sustainable residue removal using the model integration framework.

Extension of the integrated model to include DAYCENT provides the ability to quantify the impacts of sustainable residue removal on the soil and environment. DAYCENT is a biogeochemical model that simulates daily fluxes of carbon and nitrogen between the atmosphere, vegetation, and soil (Del Grosso et al., 2001). DAYCENT is comprised of several submodels that together enable assessments of nitrogen-gas fluxes, carbon dioxide flux from soil respiration, soil organic carbon and nitrogen, new primary production, and daily water and nitrate leaching. This study investigates four interval removal practices in Boone County, Iowa, US: no residue, annual, bi-annual, and tri-annual removal. Results were aggregated over 30 soils under a continuous corn rotation and reduced tillage management to provide a county level assessment of soil organic matter impacts.

Methodology

The integrated model is comprised of disparate models and databases that together provide the capability to quantitatively assess the impact of residue removal on soil organic carbon. Figure 1 shows the flow of information within the model integration framework.

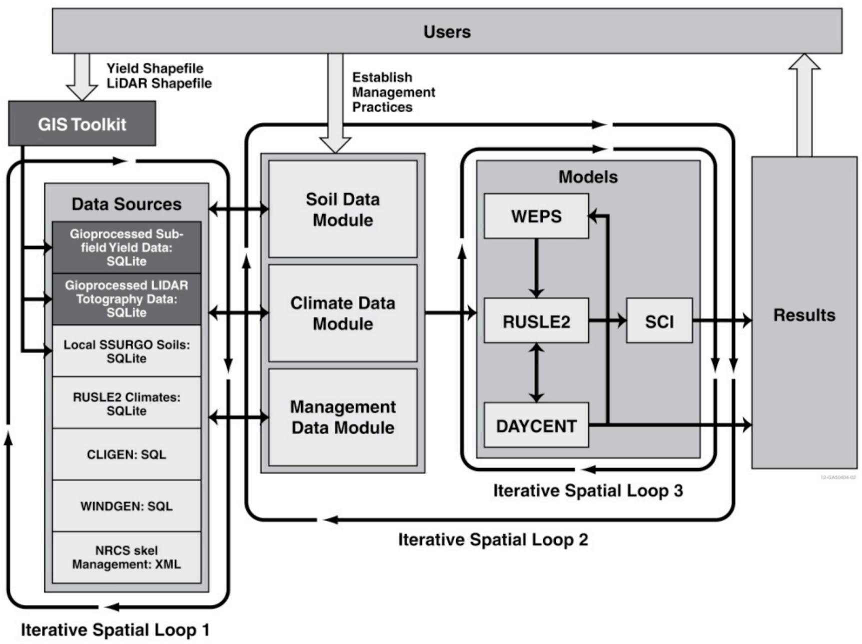

Figure 1. Data flow through the integrated model

Users select the area of interest, crop rotation, land management practices, and residue removal scenario. Soil and climate data are dynamically acquired based upon the selected area. Because the integrated models were developed and maintained by different organizations, file formats, data acquisition, and model execution are different. To address this issue, databases and file converters have been developed to enable consistent data flow across the integrated models.

The model simulation is a two-part process: calibration of the integrated model and scenario simulation. First, model inputs are initialized for a defined scenario. Spatial selection defines the climates and soils for the scenario. Climate and soil databases are loaded and queried to create the respective input files. Management and simulation files are built for WEPS and then the model is executed. Wind erosion results are passed into RUSLE2. RUSLE2 is executed and results are passed into SCI. SCI uses organic matter, field operation, and water erosion results from RUSLE2 and wind erosion results from WEPS to determine the soil conditioning index. Sustainability is defined as total erosion being less than the t-factor for the soil and a SCI greater than or equal to zero. If sustainable, DAYCENT is then initialized with soil, climate, and management data consistent with previous models. Erosion results are passed to DAYCENT and the crop is calibrated against the yield input. Once the calibration process is completed, the scenario is simulated considering soil, climate, and land management practices using the same sequence of execution.

The integrated model is utilized to assess an interval residue removal scenario for a continuous corn rotation under reduced tillage land management practices in Boone County, Iowa, US. The 2010 Billion Ton Update (US DOE, 2011) yield scenario is the basis for calibration of the integrated model. The climate inputs utilize stochastically generated data for a single year allowing the simulation to run without considering any climatic stress events that may actually occur over a 20-year weather cycle. Interval residue removal practices are run across each soil to determine the impacts on soil organic matter for the major soil types used in row crop production in Boone County.

Results and Discussion

This study investigated the long-term impact of interval removal practices on soil organic carbon under a continuous corn rotation and reduced tillage management practices (Figure 2).

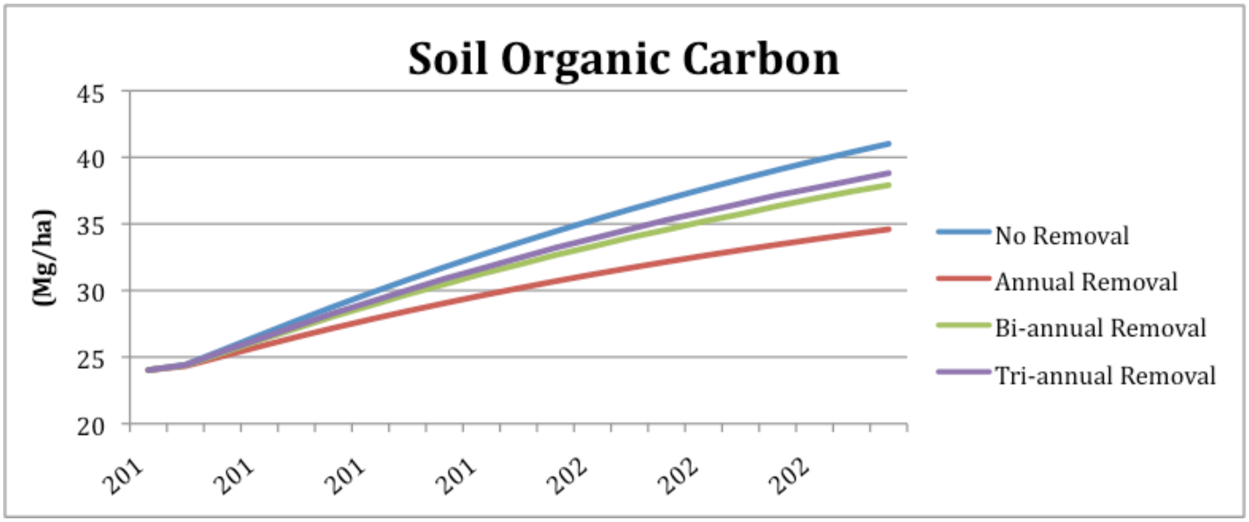

Figure 2. Changes to soil organic carbon per interval harvesting scenario

Annual removal of nearly 4.5 Mg/ha increases soil organic carbon under each scenario but over the 20 year simulation soil organic carbon is reduced more than 7 Mg/ha relative to the no removal scenario. Table 1 shows the predicted changes in soil organic matter.

Table 1. Predicted changes to soil organic matter in the top 6 soils in Boone County considering interval residue removal practices.

Soil Type	Map Unit Acres (%)	% Organic Matter (SSURGO)	Predicted levels of SOM (%)			
			No Residue Removal	Annual Residue Removal	Bi-annual Removal	Tri-annual Removal
Canisteo silty clay loam, 0 to 2 percent slopes	24.54	6.5	9.22	8.52	8.92	9.01
Clarion loam, 2 to 5 percent slopes	19.74	3.5	4.96	4.59	4.80	4.85
Nicollet loam, 1 to 3 percent slopes	14.2	5.5	7.81	7.21	7.55	7.62
Webster silty clay loam, 0 to 2 percent slopes	9.64	6.5	9.14	8.44	8.81	8.94
Harps loam, 0 to 2 percent slopes	4.49	5.0	7.11	6.57	6.87	6.94
Clarion loam, 5 to 9 percent slopes, moderately eroded	4.49	2.7	3.81	3.50	3.67	3.70

The results show that some soils could potentially reach over 9% soil organic matter under no residue removal management. Two key conclusions result from this analysis. First, for each of these soils and residue removal practices the long term soil organic carbon increases. This is a very positive conclusion suggesting that residue removal at the modeled rates will be sustainable for energy production. Second, the interval removal schemas provide an agronomic strategy which will result in less soil organic matter stress than annual removal. This is important for considering best management practices for residue removal on land which is potentially at risk.

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4.7
SITE-SPECIFIC TRADE-OFFS OF HARVESTING CEREAL RESIDUES AS BIOFUEL FEEDSTOCKS

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Abstract
Cereal residues are considered an important feedstock for future biofuel production. Harvesting residues, however, could lead to serious soil degradation and impaired agroecosystem services. We evaluate trade-offs of harvesting residues including impacts on soil erosion and quality, soil organic C (SOC) and nutrient removal. Agricultural data from 369 geo-referenced points on the 37-ha Washington State University Cook Agronomy Farm were used to develop straw harvest scenarios for conventional tillage (CT) and no-tillage (NT) and both two- and three-year crop rotations. Site-specific estimates of ethanol production from two- and three-year rotation scenarios ranged from 681 to 1541 L ha^{-1} yr^{-1} indicating that both crop rotation and site-specific targeting of residue harvest are important factors. Harvesting straw reduced residue C inputs by 46% and resulted in levels below that required to maintain SOC under CT. This occurred as a function of both straw harvest and low residue producing crops in rotation. Harvesting straw under CT was predicted to reduce soil quality as Soil Conditioning Indices (SCI) were negative throughout the field. In contrast, SCI's under NT were positive despite straw harvest. Replacement value of nutrients (N, P, K, S) removed in harvested winter wheat straw was \$14.54 Mg^{-1} straw. Fertilizer replacement costs of harvested winter wheat straw ranged from \$36.04 to \$80.30 ha^{-1} and averaged \$61.04 ha^{-1} for the field. Harvesting straw involves trade-offs that will vary on a site-specific basis. Support practices such as crop rotation, reduced tillage and site-specific nutrient management need to be considered if straw harvest is to be sustainable.

Keywords: wheat residue, biofuels, cereal straw, ethanol, soil quality, soil carbon

Introduction
Biofuels are targeted to replace 30% of current U.S. petroleum gasoline by 2030 (Perlack et al., 2005; USDOE-EIA, 2009). Second generation feedstocks such as corn (*Zea maize* **L.**) stover and wheat (*Triticum aestivum* **L.**) straw are considered to be significant future sources of bioenergy (Hill et al., 2006; Graham et al., 2007). Harvesting crop residues as biofuel feedstocks will involve trade-offs between energy production and agroecosystem services. While residues can interfere with crop planting and harbor disease and insect pests, the beneficial aspects of returning crop residues to soil are numerous including reduction of erosion, drought resistance and maintenance of soil organic matter (SOM) (Blanco-Canqui and Lal, 2009; Huggins et al., 2011). The importance of evaluating site-specific, landscape scale trade-offs will increase as agricultural expectations broaden to include food, feed, energy and agroecosystem services and as agricultural technologies facilitate site-specific decisions (Dale et al., 2011). Our objectives are to determine site-specific trade-offs associated with harvesting cereal straw in farming systems typical of the dryland annual cropping region of the PNW. Specifically, our analysis evaluates tillage, crop rotation and residue harvest effects on site-specific field locations to: (1) quantify potential ligno-cellulosic ethanol production; (2) evaluate the hazard of soil erosion; (3) assess impacts on soil quality; and (4) determine nutrients removed in straw harvest and their fertilizer replacement value.

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Materials and Methods

The study was conducted on a 37-ha field at the Washington State University Cook Agronomy Farm (CAF) (46° 47' N, 117° 5' W) near Pullman, WA. The field has terrain and silt loam soils that developed in loessial deposits typical of the Palouse soil association (USDA SCS, 1980) (Fig. 1). A systematic, non-aligned grid of 369 geo-referenced sampling locations was established in 1999 across the 37-ha (Fig. 1). Grain yield was hand harvested on 2 m^2 areas at each of the 369 locations and aboveground biomass (both crop yield and residue) on 1/3 of the locations from 1999 through 2003 representing crop rotations with winter wheat, spring wheat, barley (*Hordeum vulgare* L.) and pea (*Pisum arvense* L.). Relative yields for a given location were averaged across the five year (1999-2003) time period (Fig. 1).The relative yield map and associated harvest indices were used to distribute whole-field (37-ha) crop yield and residue variability for three simulated rotations consisting of: (1) two-year rotation of winter wheat-spring pea; (2) three-year rotation of winter wheat-spring pea-spring wheat; and (3) three-year rotation of winter wheat-spring barley-spring wheat. Whole-field average yields typical for the area were targeted: winter wheat, 6480 kg ha^{-1} (90 bu ac^{-1}), spring pea, 2464 kg ha^{-1} (2200 lbs ac^{-1}); spring wheat, 4370 kg ha^{-1} (65 bu ac^{-1}) and spring barley, 5040 kg ha^{-1} (4500 lbs ac^{-1}). Site-specific yield and harvest index estimates were then used as input for calculating cereal residues that could be harvested as biofuel feedstocks and for assessing residue C and nutrient (N, P_2O_5, K_2O and S) removal. For calculating the site-specific potential for biofuel production from harvested residues, we assumed a 50% residue harvest (baling) efficiency (Perlack et al., 2005), a minimal cereal grain yield of 3300 kg ha^{-1} considered necessary for residue baling operations (Duft and Pray, 2002), and a lignocellulosic ethanol conversion of 77 gallons of ethanol per dry ton of cereal residue (Kadam and McMillan, 2003). Residue C and nutrient (N, P_2O_5, K_2O and S) removal were based on average sample results for organic C, N and S from dry combustion (Leco C/N/S analyzer) and P and K by inductively coupled argon plasma–atomic emission spectroscopy (ICP–AES; Jarrell-Ash, Thermo Fisher Scientific, Waltham, MA). Straw nutrient and C concentrations used in the analyses were: residue C: 450 g kg^{-1}; residue N: 4.5 g kg^{-1}, residue P: 0.35 g kg^{-1}; residue K: 5 g kg^{-1}; and residue S: 0.6 g kg^{-1}. Average fertilizer prices ($ kg^{-1} of nutrient) for 2007 through 2011 were derived from the Idaho Input Cost publication series (2011) and used to calculate the fertilizer replacement value of the harvested cereal residue. Fertilizer application costs were not included. The fertilizer costs were: $1.33 kg^{-1} N; $1.37 kg^{-1} P_2O_5; $1.17 kg^{-1} K_2O; and $0.75 kg^{-1} S. The price of N is based on a simple average of anhydrous, liquid (Uran, 32-0-0-0) and dry (as urea, 46-0-0-0) and the price of P is an average of liquid (10-34-0-0) and dry (11-52-0-0) P formulations. For other fertilizers containing N (e.g. diammonium phosphate, ammonium sulfate), the nitrogen price is based on the appropriate form (i.e., liquid or dry) for the type of fertilizer.

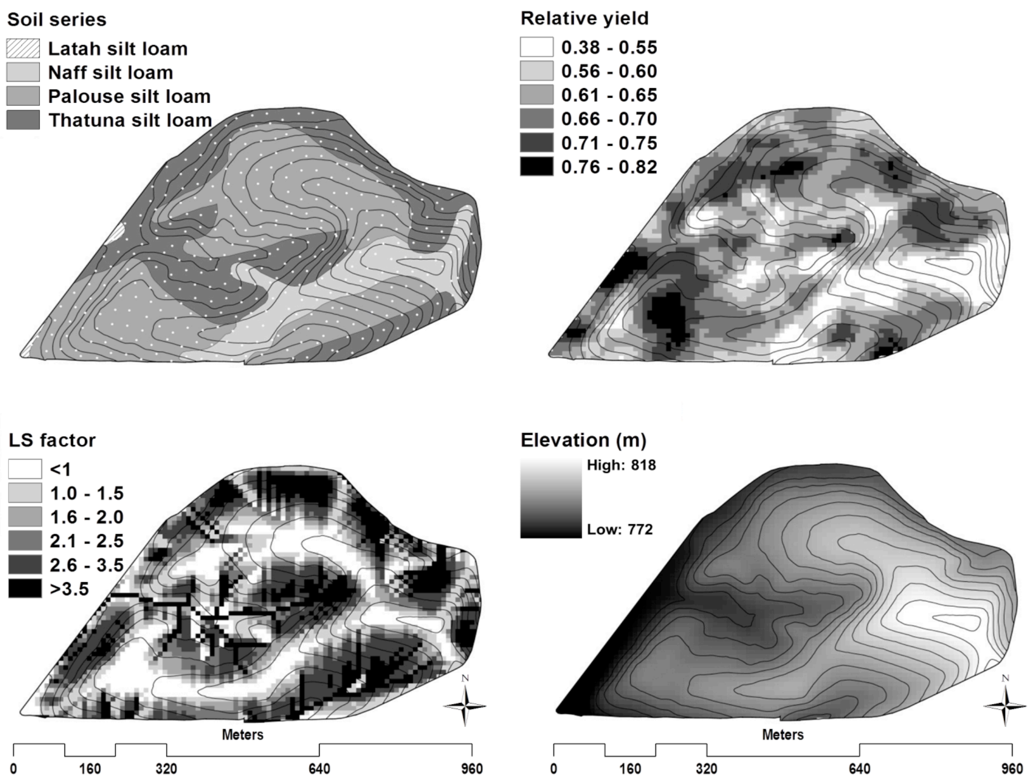

Figure 1. Spatial distribution of soil series with georeferenced sample locations, relative yield, RUSLE LS factor and elevation at the 37-ha Cook Agronomy Farm.

Spatial estimates of average annual soil erosion were calculated using Revised Universal Soil Loss Equation (RUSLE) (USDA, 2011) factorss (R_{eq}, K, L, S, C and P) for the 37-ha field based on a 10-m^2 grid for five different crop rotation and tillage scenarios with and without cereal residue harvest (Desmet and Glovers, 1996; Mitasova et al, 1996 and McCool et al., 1989). Conventional tillage (CT) scenarios consisted of moldboard plowing following winter wheat harvest; chisel plowing with twisted shanks following spring cereal harvest; and offset disking following spring pea harvest. Secondary tillage under CT consisted of various field cultivation and harrow operations followed by conventional seeding. On average, CT scenarios had 7 field operations per crop year including tillage, seeding, fertilizing, spraying and harvest. Only the three-year crop rotation scenarios were considered for no-tillage (NT) as increases in weed and disease pressure make two-year NT rotations problematic in the study region. No-tillage operations consisted of NT seed drills equipped with double-disk and fertilizer openers as well as spraying and harvesting operations. On average, NT scenarios for the three-year rotations had 5 operations per crop year.

The Soil Conditioning Index (SCI) was estimated on a 10-m^2 grid basis across the field using: (1) site-specific crop yields to assess the organic matter (OM) subfactor; (2) specific field operations for each crop rotation-tillage scenario to calculate the field operations (FO) disturbance ratings subfactor; and (3) RUSLE estimates. The SCI was then calculated for each 10-m^2 grid where: SCI = OM·0.4 + FO·0.4 + ER·0.2.

Results and Discussion

Estimated ethanol yields

Estimated annual ethanol yields from harvesting cereal straw for the 37-ha field were greatest for WW-SB-SW (1180 L ha^{-1}) and least for WW-SP (681 L ha^{-1}) with WW-SP-SW yielding intermediate values (Fig. 2). Assuming a price of \$0.69 L^{-1} (\$2.59 gal^{-1}) for ethanol (Chicago Mercantile Exchange, 2012), the retail value of ethanol derived from this field averaged \$470 to \$814 ha^{-1} depending on rotation. Due to the wide range in cereal straw yields and the contiguous nature of field areas with similar yields, some areas would be more economical to harvest than others and site-specific data on crop residue yields could prove valuable (Long et al., 2010).

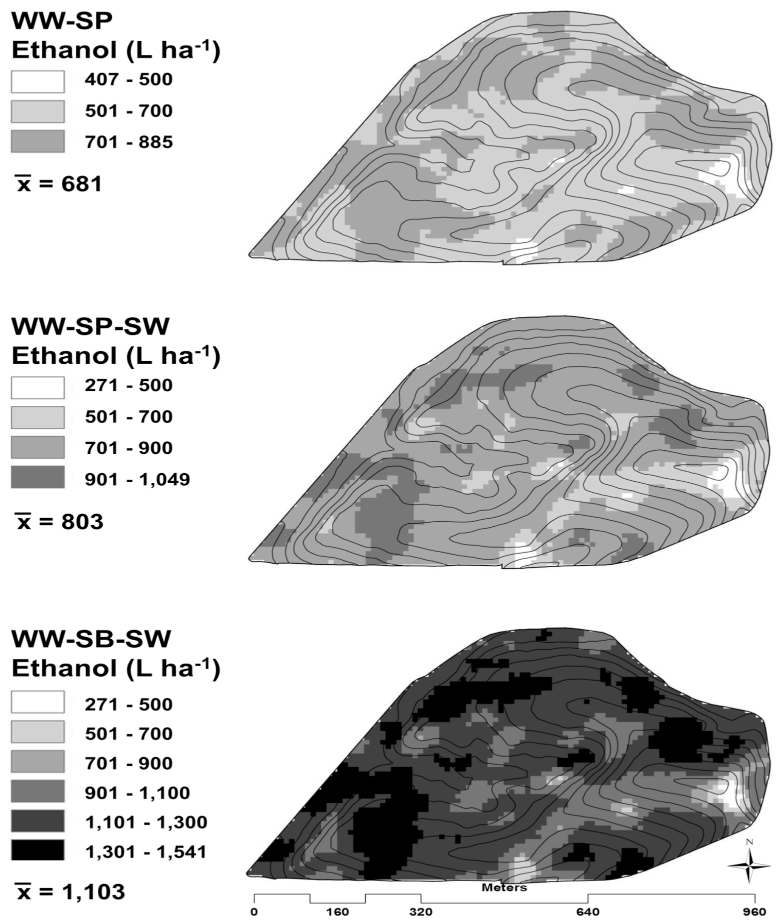

Figure 2. Potential ethanol production (L ha^{-1} yr^{-1}) from harvesting cereal straw at site-specific field locations for three crop rotation scenarios at the 37-ha Cook Agronomy Farm.

Crop Residue Carbon Inputs

The average annual production of crop residue C generally increased from 2477 kg C ha^{-1} to 3029 kg C ha^{-1} as the proportion of crop rotation in cereals increased (Fig. 3). This occurred as spring pea produced the lowest amount of residue C (950 kg C ha^{-1}) as compared to spring barley (2084 kg C ha^{-1}), spring wheat (2556 kg C ha^{-1}) and winter wheat (3473 kg C ha^{-1}). Annual returns of C required for maintaining soil organic C (SOC) and to meet soil conservation needs in dryland wheat producing areas of the Pacific Northwest have been estimated as 1500 to 2500 kg C ha^{-1} (Rasmussen and Collins, 1991; Kerstetter and

Lyons, 2001; Banowetz et al., 2008). On the 37-ha CAF, residue C production had a 2-fold range and a few small, unproductive areas had average annual C inputs of less than 2000 kg C ha^{-1} when spring peas were included in rotation (Fig. 3). Harvesting cereal residues, however, significantly reduced annual C inputs from straw to less than 2000 kg C ha^{-1} at all field locations (Fig. 3) despite 50% harvesting efficiencies and crop yield constraints for baling residue. Therefore, residue C returned to soil following residue harvest in these scenarios is marginal at best for maintaining SOC. Technologies that increase residue harvest efficiencies (Shinners et al., 2009) could exacerbate this situation.

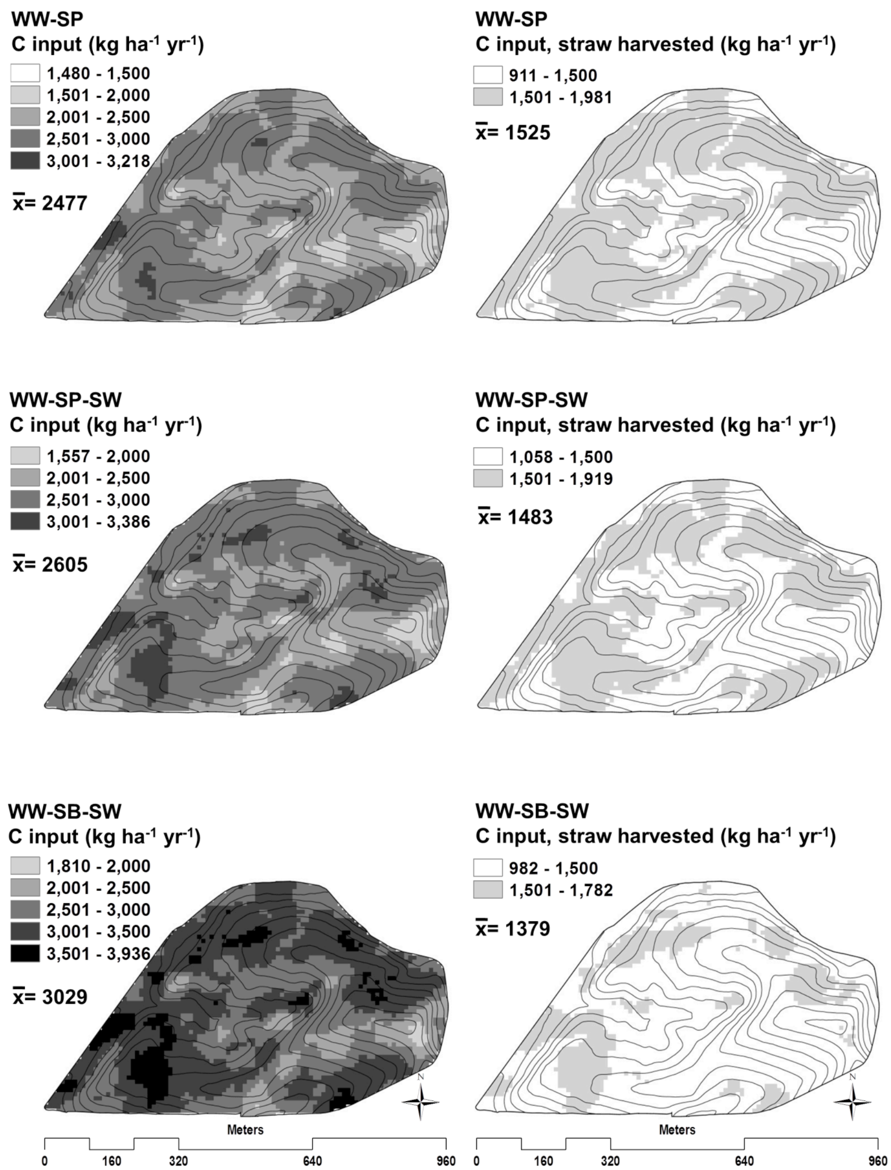

Figure 3. Estimated amounts of pre- and post-harvest cereal straw C (kg ha^{-1}yr^{-1}) at site-specific field locations for three crop rotation scenarios at the 37-ha Cook Agronomy Farm.

Soil Erosion and Soil Conditioning Index

The two-year CT had the greatest estimated soil erosion and the most negative SCI as compared to all other scenarios (Table 1, Fig. 4). The average soil erosion estimates for the field were over three-times the soil erosion tolerance levels of 11.2 Mg ha^{-1} yr^{-1}. The negative SCI values indicate that soil quality will decline due to soil erosion and lack of sufficient C inputs. The greatest soil erosion and most negative SCI's occurred on steeper field slopes. Shifting from a two- to three-year rotation under CT decreased soil erosion estimates and improved SCI, although many field locations still had soil erosion above tolerance levels and negative SCI's (Fig. 4). The NT scenarios reduced soil erosion estimates to levels well below soil tolerance and all field locations exhibited a positive SCI indicating that harvesting cereal residues may be a sustainable option when combined with NT, but not under CT (Table 1, Fig. 4).

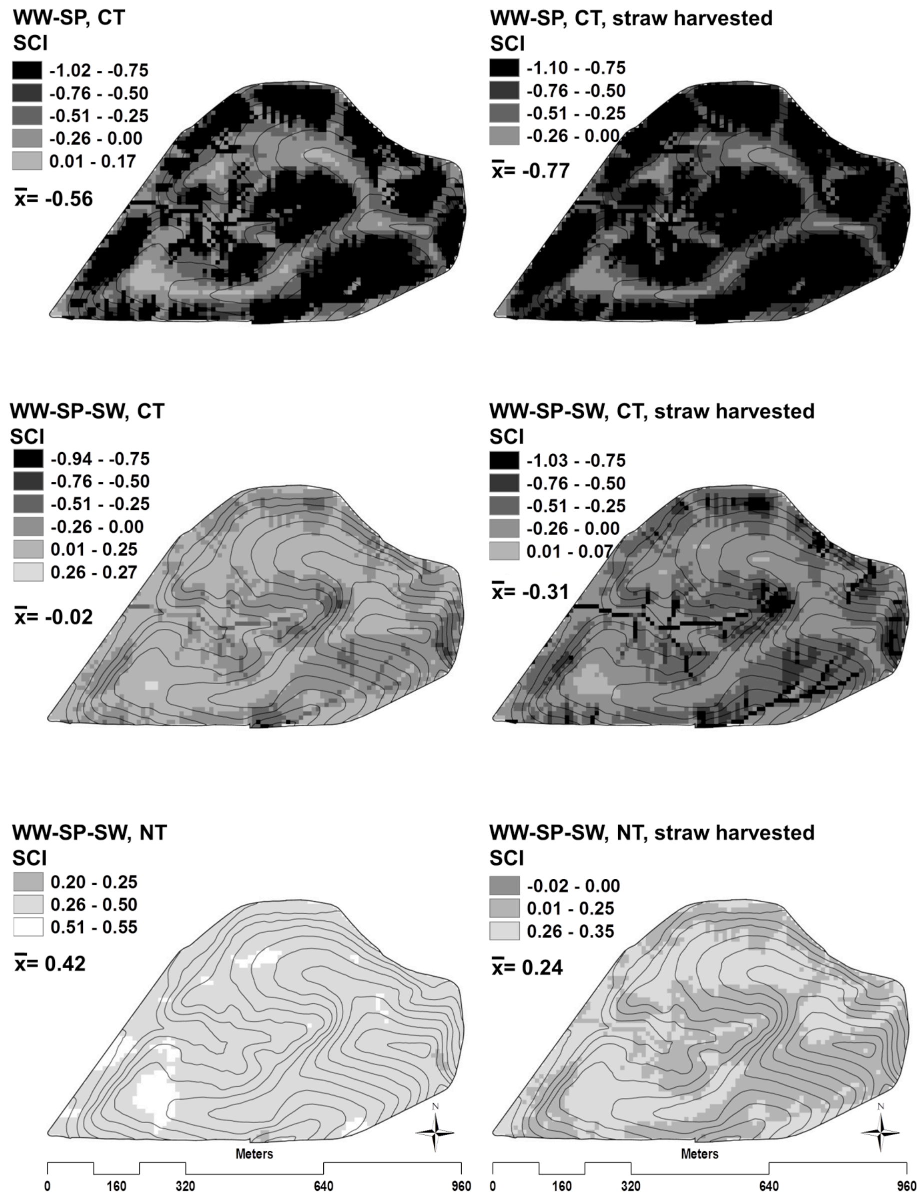

Figure 4. Estimated Soil Conditioning Index (SCI) for three crop rotation-tillage scenarios with and without cereal straw harvest at the 37-ha Cook Agronomy Farm.

Table 1. Crop rotation, tillage and residue harvest effects on soil erosion estimates and soil conditioning indices at the 37-ha CAF.

Properties[c]	WW-SP[a]		WW-SP-SW				WW-SB-SW			
	CT[b]		CT		NT		CT		NT	
	x[d]	Range	x	Range	x	Range	x	Range	x	Range
Erosion $Mg\ ha^{-1}\ yr^{-1}$	42	0.6 to 167	10	0.1 to 47	1.0	0.01 to 5.89	5.6	0.04 to 38	0.5	0.01 to 3.1
Erosion RH $Mg\ ha^{-1}\ yr^{-1}$	56	0.8 to 222	18	0.3 to 71	2.7	0.04 to 12.9	22	0.3 to 86	2.3	0.03 to 9.8
SCI	-0.56	-1.02 to 0.17	-0.02	-0.94 to 0.27	0.42	0.20 to 0.55	0.17	-0.51 to 0.42	0.52	0.29 to 0.67
SCI RH	-0.77	-1.10 to 0.00	-0.31	-1.03 to 0.07	0.24	-0.02 to 0.35	-0.34	-1.00 to 0.11	0.27	0.05 to 0.38

[a]WW-SP = two-year winter wheat (WW), spring pea (SP) rotation;
WW-SP-SW = three-year winter wheat, spring pea, spring wheat (SW) rotation;
WW-SB-SW = three-year winter wheat, spring barley (SB), spring wheat rotation.
[b]CT = conventional tillage; NT = no-tillage.
[c]RH = residue harvested; SCI = soil conditioning index.
[d]x = field mean; Range = field range.

Nutrient removal

Harvesting cereal residues removes essential crop nutrients including N, P_2O_5, K_2O and S (Fig. 5) that will eventually need to be replaced, often with additional applications of synthetic fertilizers. Harvesting the straw from all cereals over the course of a three-yr WW-SB-SW rotation resulted in an average annual removal of 16 kg N ha^{-1}, 2.9 kg P_2O_5 ha^{-1}, 22 kg K_2O ha^{-1} and 2.2 kg S ha^{-1} (Table 2, Fig. 5) with an overall estimated fertilizer replacement cost of \$53.54 ha^{-1} (Table 2). Site-specific nutrient removal estimates with straw harvest over the WW-SB-SW rotation ranged over five-fold for the field (Table 2, Fig. 5) and estimated annual costs for fertilizer replacement were from \$12.01 to \$69.2 ha^{-1} (Table 2). On a nutrient per dry cereal straw mass basis, harvested straw would export 4.5 kg N Mg^{-1}; 0.8 kg P_2O_5 Mg^{-1}; 6.0 kg K_2O Mg^{-1}; and 0.6 kg S Mg^{-1}, which in fertilizer replacement value would be worth \$14.54 Mg^{-1} dry straw. These estimates demonstrate that nutrient export in harvested straw represents a significant cost to producers if not recaptured in the sale of the straw. The large spatial range of nutrients removed in harvested straw will lead to greater within field variation in soil nutrient supplies with regular straw harvest and site-specific nutrient management will be necessary to achieve soil fertility and crop nutritional requirements as well as nutrient use efficiency goals.

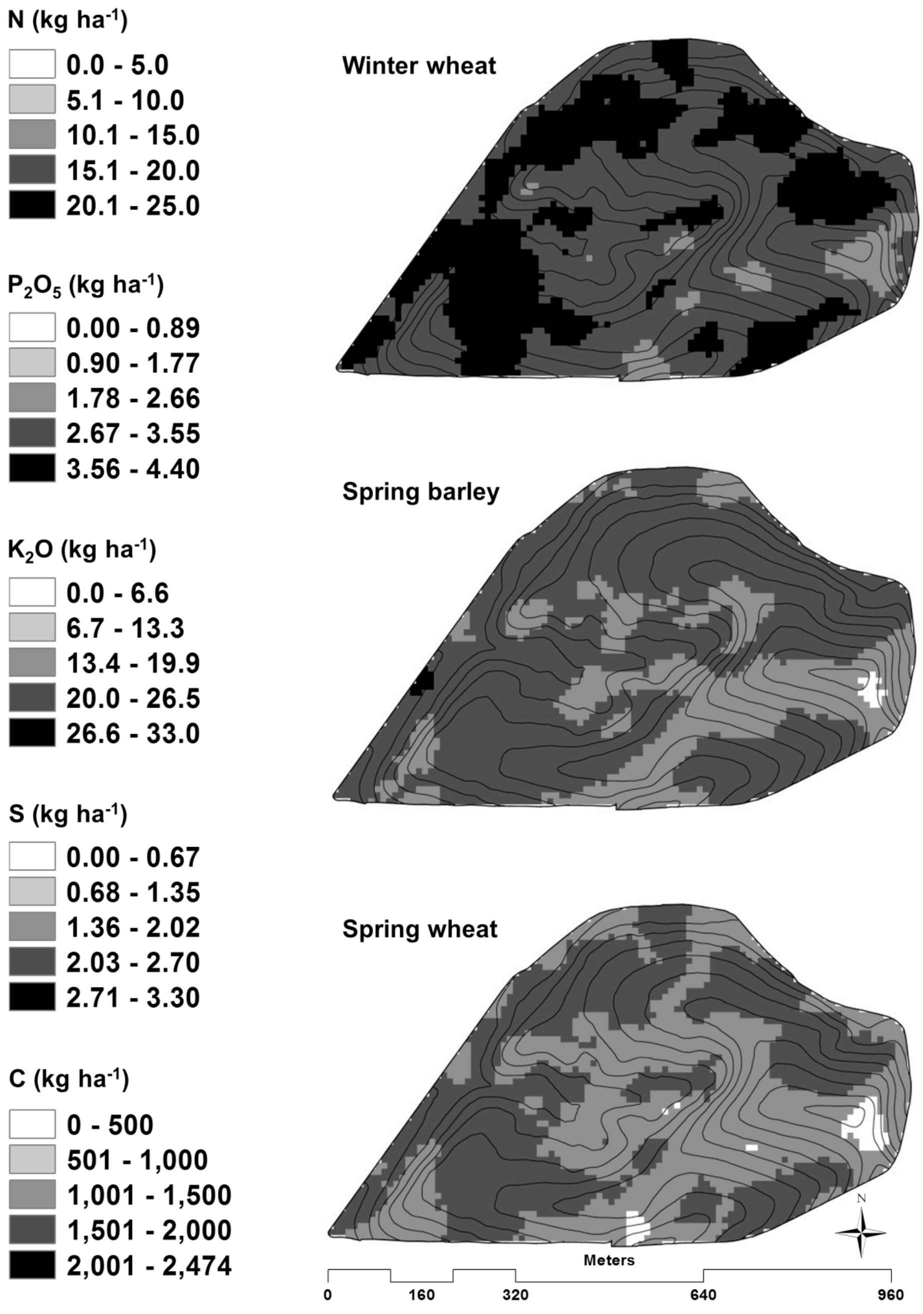

Figure 5. Estimated export of nitrogen (N), phosphorus (P_2O_5), potassium (K_2O), sulfur (S) and carbon (C) in harvested cereal straw at the 37-ha Cook Agronomy Farm.

Table 2. Nutrients (N, P, K and S) and C exported with cereal residue harvest and their fertilizer replacement value for the 37-ha CAF.

Properties	Winter wheat		Spring wheat		Spring barley	
	x[a]	Range	x	Range	x	Range
N harvested, kg ha^{-1}	19	11 to 25	15	0 to 19	16	0 to 21
N value, dollars ha^{-1}	25.20	14.59 to 33.16	19.90	0 to 25.20	21.22	0 to 27.86
P_2O_5 harvested, kg ha^{-1}	3.4	2.0 to 4.4	2.6	0 to 3.4	2.8	0 to 3.7
P_2O_5 value, dollars ha^{-1}	4.67	2.75 to 6.04	3.57	0 to 4.67	3.84	0 to 5.08
K_2O harvested, kg ha^{-1}	25	15 to 33	20	0 to 26	21	0 to 27
K_2O value, dollars ha^{-1}	29.26	17.56 to 38.62	23.41	0 to 30.43	24.58	0 to 31.60
S harvested, kg ha^{-1}	2.54	1.52 to 3.30	1.95	0 to 2.57	2.11	0 to 2.75
S value, dollars ha^{-1}	1.91	1.14 to 2.48	1.47	0 to 1.93	1.59	0 to 2.07
C harvested, kg ha^{-1}	1904	1138 to 2474	1463	0 to 1924	1582	0 to 2062

[a]x = field mean; Range = field range.

Summary and Conclusions

Trade-offs regarding residue harvest were evaluated for crop rotation and tillage scenarios relevant to the annual dryland cropping region of the PNW. Significant field variability with greater than 2-fold differences in cereal straw production occurred and may lead to the development of site-specific harvesting strategies. Determining levels of straw that can be sustainably harvested will need to evaluate crop rotation and tillage factors. In our scenarios, longer three-year rotations coupled with NT minimized adverse effects of residue harvest and resulted in tolerable levels of soil erosion and positive SCI's throughout the field. In contrast, scenarios where straw harvest was combined with CT, unsustainable levels of soil erosion and negative SCI's were predicted. Evaluating annual returns of C required for maintaining soil organic matter will need to be considered on a site-specific and crop rotational basis as C inputs from residues are significantly affected by crop rotation and field location. Estimates of nutrient removal in harvested residues were substantial and varied up to five-fold throughout the field. Repeated straw harvest would increase nutrient variability in the field and require precision nutrient management to address site-specific conditions. Other important trade-offs that were not evaluated include improved plant-available water and drought resistance as well as wildlife habitat that are derived from maintaining surface residues. Overall, harvesting cereal residues as biofuel feedstocks should carefully consider the site-specific nature of trade-offs and tailor practices such as variable residue harvest, crop rotation, reduced tillage and site-specific nutrient management to meet conservation and production goals.

Acknowledgements

This study was conducted as part of the Climate Friendly Farming Project with funding provided by the Paul G. Allen Family Foundation. Additional support was provided through the USDA Solutions to Economic and Environmental Problems (STEEP) project.

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4.8
EFFECT OF SOIL MOISTURE ON ECOSYSTEM RESPIRATION AND ITS RELATIONSHIP WITH SOIL TEMPERATURE IN SWITCHGRASS

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Abstract
Understanding the response of ecosystem respiration (ER) to major environmental drivers is critical for estimating carbon sequestration and large-scale modeling research. Temperature effect on ER is modified by other environmental factors, mainly soil moisture, and such information is lacking for switchgrass (*Panicum virgatum* L.) ecosystem. The objective of this study was to examine seasonal variation in ER and its relationships with soil temperature and moisture in a switchgrass field. Ecosystem respiration from the nighttime net ecosystem CO_2 exchange measurements by eddy covariance system was analyzed. Nighttime ER ranged from 2 (early May) to 10 µmol m^{-2} s^{-1} (mid-August) and showed a clear seasonality with low rates during warm (> 30 °C) and dry periods (< 0.20 m^3 m^{-3} of soil water content). No single temperature or moisture function described the variability in ER over the whole season. However, exponential temperature model accounted for 59% of the seasonal variation in ER at adequate soil moisture (> 0.20 m^3 m^{-3}), indicating that soil moisture < 0.20 m^3 m^{-3} started to limit ER. Temperature sensitivity (Q_{10}) for ER showed linear relationship (R^2 = 0.99) with soil moisture up to 0.30 m^3 m^{-3} and then decreased due to limitation of soil-atmosphere gas exchange in wet soil. The Q_{10} values were 1.45, 1.91, 2.57, and 1.81 for soil moisture classes of 0.14 – 0.19, 0.20 – 0.24, 0.25 – 0.29, and 0.30 – 0.54 m^3 m^{-3}, respectively. These results suggest that soil moisture greatly influences the dynamics of ER and its relationship with soil temperature in drought-stressed switchgrass ecosystem.

Keywords: Ecosystem respiration, eddy covariance, Q10 value, soil moisture, soil temperature, switchgrass

Introduction
Ecosystem respiration (ER) is the CO_2 efflux from ecosystem to the atmosphere due to autotrophic (vegetation respiration) and heterotrophic (soil respiration) activities. Small changes in ER, due to global warming, may alter the source-sink status of an ecosystem because ER is the second most important flux in the global carbon cycle after photosynthesis (Davidson et al., 2006). Determining the response of ER to environmental perturbations is critical to predict past and future climates (Cox et al., 2000). Ecosystem respiration is influenced by both environmental and biological factors (Flanagan and Johnson, 2005). Many researchers have used soil temperature (Ts) as the main environmental driver to explain plant and soil respiration (Gifford, 2003; Lloyd and Taylor, 1994). Respiration modeling by simple temperature dependence equations was well established in the late 19th century (for example Arrhenius expression in 1889 and van't Hoff equation in 1898). However, the model is largely empirical and has not been modified much till now, and the mechanistic understanding of the influence of environmental factors on ER is still unclear.

Currently, the exponential temperature-respiration response function for nighttime turbulent periods has been a well-established convention to fill the nighttime data gaps and to replace ER values measured during low turbulence conditions for eddy covariance scientific communities. Temperature effect may be inconsistent during diverse environmental conditions (Davidson et al., 1998). Soil water content (SWC) is

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the second most important abiotic variable for explaining ER in semi-arid and arid regions (Qi and Xu, 2001) since it strongly affects the physiology of vegetation and soil microbial activities. Temperature dependency of Q_{10} is well understood (Lloyd and Taylor, 1994). But the effect of SWC on Q_{10} is relatively little understood. A detailed understanding of the influence of soil moisture on the ER-Ts relationship can provide accurate predictions of changes in net ecosystem CO_2 exchange (NEE) and ER in response to future climate change. It will also be of great significance in reducing biases in partitioning of eddy covariance measurements of NEE into gross ecosystem productivity (GEP) and ER. This study takes an advantage of unusual drought experienced by switchgrass during the 2011 growing season (a wide range of volumetric SWC ranged from 0.11 to 0.55 m^3 m^{-3} was observed) which provided us a unique opportunity to investigate the influence of soil moisture on temperature response of ER in switchgrass.

Materials and Methods

Site Description, Flux, Temperature, and Water Measurements

Eddy covariance measurements of CO_2, H_2O, and energy fluxes were performed at 10 Hz frequency (10 samples sec^{-1}) in a switchgrass (cv. Alamo) field at South Central Research Station, Chickasha, OK (35.04° N latitude, 97.95°W longitude, and 330 m above sea level altitude) during the 2011 growing season. The experiment was rainfed in McLain silt loam soil (fine, mixed, superactive, thermic Pachic Argiustolls). The site received only 58% rainfall in 2011 when compared to the 30-year mean (896 mm). Very low amounts of rainfall (52% of the 30-year mean (319 mm)) were recorded during the active growing period (June to September). Half hourly measurements of Ts and SWC near soil surface were recorded using water content reflectometers (CS616, Campbell Scientific Inc., Logan, UT, USA) and averaging soil temperature probes (TCAV-L, Campbell Scientific Inc., Logan, UT, USA).

Ecosystem respiration estimation

During nighttime, NEE is equivalent to ER whereas GEP is zero (NEE = GEP - ER) since no photosynthesis occurs at night (Barr et al., 2002). Only measured quality nighttime data was used for this study. Six hours nighttime NEE values recorded between 10 PM – 4 AM were used to avoid the period of formation and breakup of the air temperature inversion. Data was constricted to high turbulence periods (> 0.20 ms^{-1}) to avoid underestimation of NEE measurements by the eddy covariance system.

Soil temperature dependent exponential model and Q_{10} calculations

The exponential function of Ts to ER was fitted using the following formula in SigmaPlot software (Systat Software Inc.):

$$ER = b_0 \exp^{(bTs)} \qquad (1)$$

where Ts is mean near surface soil temperature, and b_0 and b are empirical coefficients.

The Q_{10} value (the factor by which ER increases with a 10° C rise in temperature) was calculated as follows:

$$Q_{10} = \exp^{(10b)} \qquad (2)$$

Influence of soil moisture on temperature dependence of ecosystem respiration

Temperature dependence of ER was evaluated for four soil moisture classes (volumetric water content of 0.14 – 0.19, 0.20 – 0.24, 0.25 – 0.29, and > 0.30 m^3 m^{-3}) to examine the influence of soil moisture on the ER-Ts relationship. For each moisture class, Q_{10} value was estimated independently from exponential models based on Ts as in equation 2.

Results and Discussion

Seasonal variability in ecosystem respiration

Nighttime ER ranged from 2 (early May) to 10 μmol m^{-2} s^{-1} (mid-August) and showed a clear seasonality with low rates during warm (> 30^{o} C) and dry periods (< 0.20 m^3 m^{-3} of SWC) (Fig. 1). The effect of the mid-season drought became apparent as volumetric SWC fell below 0.12 m^3 m^{-3} and ER rates declined rapidly mainly in July at around DOY 180-210. It showed that the combined effects of high temperature and low soil moisture decreased ER. Drought reduces soil respiration by suppressing activities of roots and soil microorganisms (Wen et al., 2006). We observed a notable increase in ER "pulse effect" after rainfall events as reported by some previous studies (Flanagan et al., 2002; Liu et al., 2002; Xu and Baldocchi, 2004). Respiration pulse after rain events may have been caused by multiple factors, which is relatively little understood. Some possible reasons might be the enhanced microbial metabolism due to wetting of dry soil (Xu et al., 2004), rapid decomposition of soil organic matter (Gao et al., 2012), increased access to microbial substrate (Huxman et al., 2004), and pulse effect associated with soil degassing right after rainfall (Liu et al., 2002).

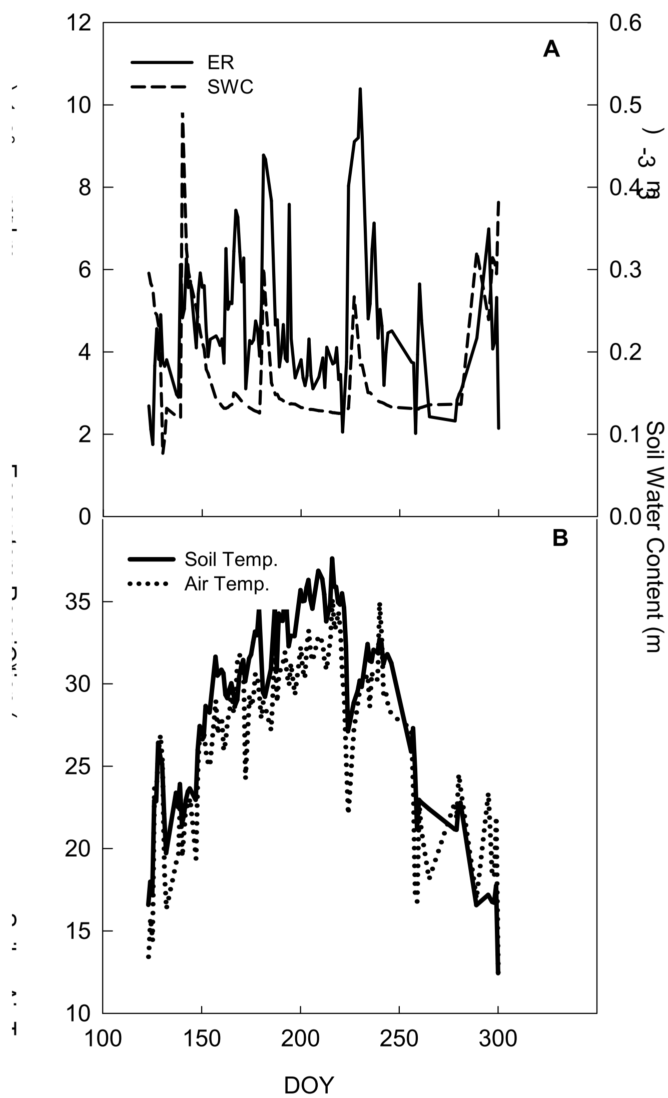

Fig. 1. Seasonal patterns of ecosystem respiration and soil water content (A); soil and air temperature (B). The figure shows great variations in ER rates with lower rates during warm and dry periods.

Dependency of ecosystem respiration on soil temperature and moisture

We first plotted soil moisture and temperature separately against ER for the whole growing season (May to October) to evaluate moisture and temperature dependency of ER, respectively. Single temperature or soil moisture function failed to describe the seasonal variability in ER. However, exponential model based on Ts accounted for 59% of the variations in ER from May to September at relatively higher level of soil moisture (> 0.20 m^3 m^{-3} of volumetric SWC) [ER = 0.7205*exp (0.0814*Ts), P < 0.0001]. The model explained 50% of the seasonal variability in ER [ER = 1.2752*exp (0.0606*Ts), P < 0.0001] when October data was included. This reduction in model capability to describe ER variability was most likely attributed to reduction in ER rates in October due to the beginning of crop senescence and decrease in temperature. Similarly, seasonal Q_{10} value based on Ts when SWC was > 0.20 m^3 m^{-3} was 2.26 for May to September and it decreased to 1.83 for May to October.

Response of ecosystem respiration to soil temperature

To investigate the influence of Ts on ER, mean ER rates were calculated for 15, 20, 25, 30, and 35° C for the active growing periods (May to August). Mean ER rates were 1.8, 4, 4.8, 5.7, and 3.7 μmol m^{-2} s^{-1} at 15, 20, 25, 30, and 35° C, respectively. It showed that ER rate at 15° C was 2.2 times smaller than when Ts was 20° C. The ER rate increased by 1.4 times at 30° C as compared at 20° C. The result showed that ER rates increased with increasing temperature up to 30° C and declined thereafter. Figure 2A also corroborates this result that ER increased rapidly at lower temperatures, higher ER rates were observed between 20 and 30° C, and ER rates declined as temperature increased over 30° C. Due to this reason, the exponential temperature function gave better fit at temperature less than 30° C (Fig. 2B).

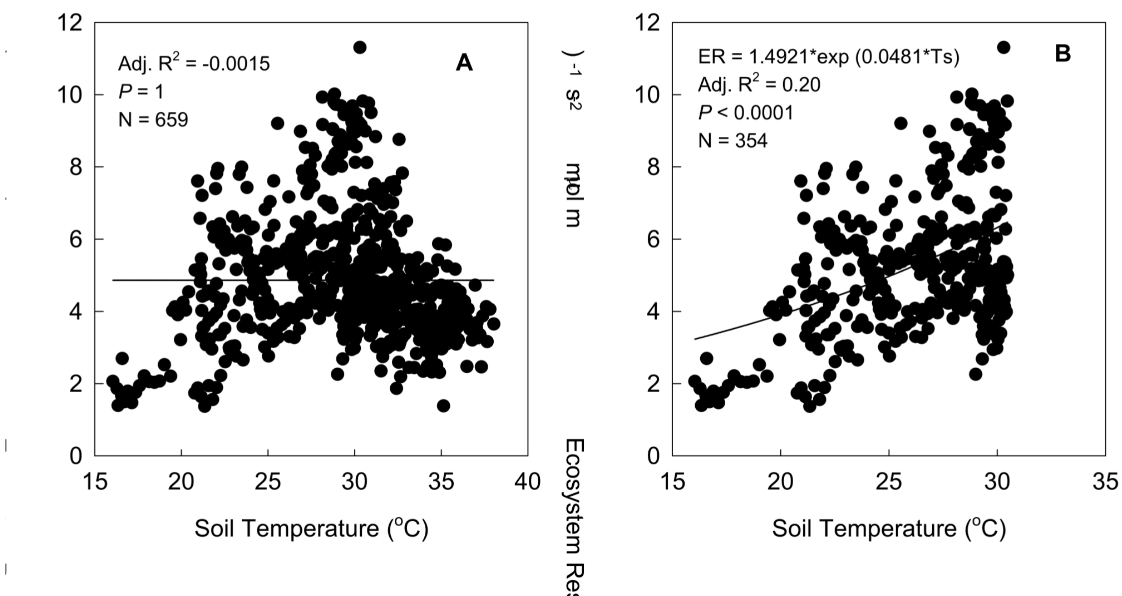

Fig. 2. Temperature dependence of ecosystem respiration (ER) for the periods of May to August across all Ts ranges (A) and only for Ts < 30° C (B). The ER rates declined beyond 30° C and the exponential model failed to provide better fit.

Effect of soil moisture on relationship between ecosystem respiration and soil temperature

When data was sorted into different moisture classes, the better fits for temperature response functions were observed at volumetric water content of 0.20 m^3 m^{-3} or more suggesting that volumetric water content below 0.20 m^3 m^{-3} limited ER in switchgrass. Xu et al. (2004) observed that volumetric water content below 0.15 m^3 m^{-3} limited ER in a grassland. However, these values are often site-specific.

Evaluation of a relationship between half hourly values of Ts and SWC showed negative relationship between them on seasonal scale (May to October) (Ts = 35.06 – 45.26*SWC, R^2 = 0.31, P < 0.0001). The negative correlation between SWC and Ts exerts confounding effect on determining the control of SWC on ER (Davidson et al., 1998). Evaluation of correlation between ER and Ts at different water levels showed a stronger correlation at higher water levels (adj. R^2 = 0.73 for SWC of 0.25-0.29 m^3 m^{-3} vs. adj. R^2 = 0.18 for SWC of 0.14-0.19 m^3 m^{-3}). Gao et al. (2012) also reported similar results that the correlation was stronger (R^2 = 0.23) at higher SWC (≥3%) than when SWC was ≤ 3% (R^2 = 0.08). Due to this reason, empirical functions provided better fits at higher levels of water contents when data was sorted into different moisture classes (Fig. 3).

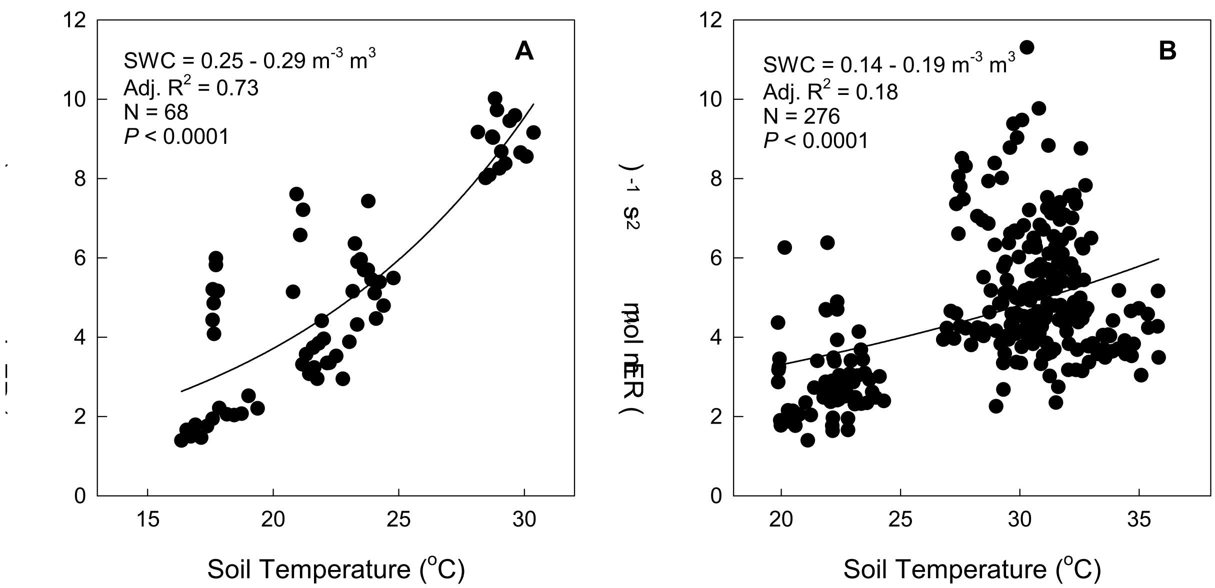

Fig. 3. Responses of the relationship between ecosystem respiration (ER) and soil temperature (Ts) to soil water content (SWC). The model provided better fits at higher available soil moisture.

Moisture sensitivity of Q_{10}

The influence of soil moisture on Q_{10} of ER was evaluated at four soil moisture classes. The Q_{10} of ER showed linear relation (R^2 = 0.99) with SWC up to 0.30 m^3 m^{-3} and then decreased due to limitation of soil-atmosphere gas exchange in wet soil. The Q_{10} values were 1.45, 1.91, 2.57, and 1.81 for soil moisture levels of 0.14 – 0.19, 0.20 – 0.24, 0.25 – 0.29, and > 0.30 m^3 m^{-3}, respectively. A few previous studies have also reported that seasonal Q_{10} values are positively related to SWC for a limited range of SWC (Janssens and Pilegaard, 2003; Xu and Qi, 2001). The result showed that soil moisture affected Q_{10}, indicating the confounding effects of soil moisture and temperature on ER. Wen et al. (2006) also reported similar results that both dry and wet soil conditions lower Q_{10} values. The ER would be less sensitive to temperature (smaller Q_{10} value) in wet soil due to reduction in rates of decomposition and microbial production of CO_2, and the CO_2 efflux would be high at intermediate SWC due to increased microbial and root respiration (Davidson et al., 1998). Lower values of Q_{10} during dry periods attributed to low temperature sensitivity of ER since major portions of ER come from the more recalcitrant carbon material (Liski et al., 1999).

Conclusion

Single soil temperature (Ts) or moisture function failed to describe the variability in ER over the whole season. However, Ts exerted dominant control over seasonal variation in ER at adequate soil moisture. The relationship between Ts and ER was greatly influenced by soil moisture. The ER rate and Ts showed stronger correlation at higher soil moisture. The Q_{10} for ER was higher at moderate SWC and relatively smaller under dry and wet conditions. The result indicated that soil moisture alters the temperature sensitivity of ER by influencing the physiological activity of vegetation and root microbes.

Acknowledgements

This study was funded by USDA-NIFA through Biomass Research and Development Initiative (BRDI), a joint effort between the U.S. Department of Agriculture (USDA) andthe U.S. Department of Energy (DOE). This paper is a contribution of the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK.

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4.9
EVALUATING THE IMPACT OF SWITCHGRASS INTERCROPPING IN LOBLOLLY PINE PLANTATIONS ON LONG-TERM SOIL PRODUCTIVITY

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Abstract
Switchgrass (*Panicum virgatum* L.) intercropping in loblolly pine (*Pinus taeda* L.) plantations is a novel approach to simultaneously grow biomass for biofuel production while still managing for high quality wood products. However, it is critical to understand potential effects this intensive management system could have on long-term soil productivity. As a result of this gap in scientific knowledge, a study has been established by Catchlight Energy LLC (a Chevron|Weyerhaeuser joint venture) on land owned and managed by Weyerhaeuser Company to investigate effects of intercropping and biomass management on site productivity and sustainability. The Lenoir 1 Intercropping Sustainability Study is a collaborative-based experiment with industry, university and government partners including Weyerhaeuser Company, Catchlight Energy LLC (CLE), North Carolina State University, Virginia Tech University, Duke University, Yale University, University of North Carolina-Greensboro, Roanoke College, and the U.S. Forest Service.Treatments range from pine only to pine intercropped with switchgrass with and without biomass removal. Site preparation for each treatment varied in intensity and number of entries with heavy equipment. Furthermore, treatments with switchgrass require annual entries to cut, rake, and bale this feedstock. Impacts of these non-traditional forest management approaches on soil sustainability and productivity were evaluated.

Keywords: intercropping, switchgrass, loblolly pine, biofuels, bioenergy, soil sustainability

Introduction
The Lenoir 1 Intercropping Sustainability Study is a collaborative-based experiment with industry, university and government partners including Weyerhaeuser Company, Catchlight Energy LLC (CLE), North Carolina State University, Virginia Tech University, Duke University, Yale University, University of North Carolina-Greensboro, Roanoke College, and the U.S. Forest Service. This study was established, and is being maintained, by CLE a joint venture between Chevron and Weyerhaeuser Company. While CLE continues to provide critical financial support for this research, several grants have been received by CLE scientists and collaborators to complement these funds.

The study was established in 2008 and located in Lenoir County, NC. The previous stand was a 109 ha (270 ac) 1974 loblolly pine (*Pinus taeda*) plantation. As is typical for the region, a series of linear drainage ditches which improve the hydrologic conditions for pine growth and survival in plantations, occurred parallel to one another through the study area. Pre-harvest sampling occurred during summer 2008 including baseline soil nutrients, soil strength, plant diversity, aboveground productivity, and foliar nutrient analysis of crop trees. These baseline data were essential to evaluate long-term sustainability of the various biomass management regimes examined. Treatments were installed between winter 2008 and summer 2009 with loblolly pine seedlings planted in the winter of 2008 and switchgrass (*Panicum virgatum*) planted in summer 2009. Similar soil and productivity data have been collected annually (pine and switchgrass) and will continue at least through canopy closure. Pine trees were established using

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standard Weyerhaeuser methods including clearcut harvest of the existing stand followed by site preparation and planting (approx. 1,100 trees ha^{-1}/435 trees ac^{-1}), vegetation management, and fertilization.

The overall objective of this long-term study is to examine effects of intercropping and/or biomass management on sustainability and site productivity in a loblolly pine plantation. The study is a complete randomized block design with seven treatments (Figure 1) replicated across four blocks (n =28) on approximately 0.8 ha (2 ac) treatment plots with a permanent 0.4 ha (1 ac) measurement plot established in each experimental unit. The seven treatments were:

1. Traditional pine establishment with biomass left in place (non-merchantable material left on site) – serves as the control for this study as it represents traditional operational practices (PB+)
2. Traditional pine establishment with biomass removed (material that could potentially be used for biofuel production was removed) (PB-)
3. Intercrop pine-switchgrass with biomass left in place (P×SB+)
4. Intercrop pine-switchgrass establishment with biomass removed (P×SB-)
5. Pine establishment with a row of biomass trees flat-planted in between crop tree beds with biomass left in place (PxPB+)
6. Pine establishment with a row of biomass trees flat-planted in between crop tree beds with biomass removed (PxPB-)
7. Switchgrass only (S)

Figure 1. Schematic diagram of seven treatments installed at Lenoir 1 Sustainability Study.
(- Biomass = Biomass Removed; + Biomass = Biomass in place)

Site preparation varied by treatment. Treatments with pine were V-sheared and bedded using a bulldozer with required attachments to create a raised planting surface for pines. Weyerhaeuser's liquid suspension based fertilizer with 3% nitrogen (N), 6.2% phosphorus (P), 2.5% potassium (K), 4.5% magnesium (Mg) and 2% calcium (Ca) was incorporated into beds to promote seedling root development and establishment. For biomass removed treatments (B-), an excavator removed residual woody debris after clearcut harvesting. Although using an excavator is not standard practice and is not operationally or economically feasible, this experimental treatment was used to remove as much biomass as possible.

Intercropped switchgrass treatments (P×SB+, P×SB-) incurred additional V-shearing to prepare a 3 m (10 ft) strip between crop tree rows to plant switchgrass. The entire plot was V-sheared and root raked for the switchgrass only (S) plots.

An average of 9.4 Mg ha^{-1} (4.2 tons ac^{-1}) of biomass remained on "biomass in place" treatments and 1.5 Mg ha^{-1} (0.67 tons ac^{-1}) of biomass remained on "biomass removed" treatments (Beauvais 2010). Imazapyr (pre-emergent herbicide) was used to control competition before planting pine seedlings. Crop trees were planted at 6.1 m × 1.5 m (20ft x 5ft) spacing and switchgrass (Alamo cultivar) was planted at 9 kg ha^{-1} (8 lbs ac^{-1}) of pure live seed (PLS) per hectare in six rows spaced 40 cm (15.75 in) apart to a depth of 0.6 cm (0.25 in) and covered with soil using a modified corn planter. Simultaneous with planting, the aforementioned liquid suspension fertilizer was applied to beds to foster rapid root development and switchgrass establishment. Switchgrass was fertilized with Weyerhaeuser's coated Arborite® fertilizer at a rate of 65 kg N (58 lb), 6.6 kg P (5.9 lbs) and 0.24 kg B (0.21 lbs) per hectare (acre) during second growing season (June 2010). Also during this period, switchgrass plots were sprayed with 2,4 – D and a post-emergent herbicide (Basagran) to control competing vegetation.

Switchgrass was mowed only following the first growing season (March 2010). Every year thereafter switchgrass was mowed, raked and baled using standard agricultural equipment. Furthermore, an annual nitrogen based fertilization regime [approximately 65 kg N per grass hectare (50 lbs per grass acre)] was applied provided the right climatic conditions were present. For example, switchgrass was not fertilized during the third growing season (2011) due to an extreme drought. Switchgrass was fertilized in 2010 and 2012.

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4.10
INCORPORATING UNCERTAINTY INTO LIFE-CYCLE ANALYSIS (LCA) OF SHORT-ROTATION WILLOW (SALIX SPP.) CROPS

Jesse Caputo[1,], Steve Balogh[1], Timothy A. Volk[1], Leonard Johnson[2], Maureen Puettman[3], Bruce R. Lippke[4], Elaine Oneil[4]*

Abstract
Life-cycle analysis (LCA) methodologies are popular tools for quantifying the energy demand, materials usage, and environmental emissions of biofuels over their entire life cycle. In order to estimate fossil fuel demand and greenhouse gas emissions associated with short-rotation willow (*Salix* spp.) crops in New York State, we constructed an LCA model capable of estimating point estimates and measures of variability for a number of key processes across 8 management scenarios. Our analysis identified a small number of variables driving the performance of the entire system. The largest fraction of the energy demand across all scenarios was driven by the use of diesel fuels, the largest proportion of which was associated with final harvesting and delivery of willow chips. Similar patterns were found for greenhouse gas emissions across all scenarios, as fossil fuel use served as the biggest source of emissions in the system. Carbon sequestration in the belowground portion of the willow system provided a large carbon sink that more than compensated for carbon emissions across 8 scenarios. The subsequent uncertainty analyses revealed that variability associated with willow yield, leaf decay, and belowground carbon sequestration resulted in large variability in system-wide performance. Standard deviations for both energy demand and greenhouse gas emissions overlapped widely across all scenarios. We conclude from this analysis that a better understanding of what drives variability in the biological portions of the system is necessary to produce reliable estimates of the emissions and energy performance of short-rotation woody crops.

Keywords: Bioenergy, biomass, short rotation woody crops, life-cycle analysis, uncertainty analysis

Introduction
Short-rotation woody crops (SRWC) are being widely promoted as renewable, sustainable sources of low-carbon energy (Volk et al. 2011a). In order to better understand their full potential, tools for quantifying their environmental performance are necessary. Life-cycle analysis (LCA) is a widely accepted methodology for quantifying the energy demand, materials usage, and environmental impacts of processes over their entire life cycle – from "cradle-to-grave" (Fantozzi and Buratti 2010, Buonocore et al. 2011, Neupane et al. 2011). Our objective for this study was to build on a previous life cycle analysis of the willow biomass cropping (*Salix* spp.) system in New York State (Heller et al. 2003), by updating with data that has been collected in the past decade and incorporating the uncertainty associated with a number of key parameters that can now be quantified. As opposed to models parameterized solely using mean values, models that include parameter uncertainty allows us to better understand how biological and geographic variation may affect overall system performance (Johnson et al. 2011).

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Methods

We created a model of the willow biomass crop production system using SimaPro 7.0. This commercial software package is used to create comprehensive models of complex processes; to tabulate the energy demand, materials usage, and emissions generated over the entire extent of the process (the life-cycle inventory or LCI), and to run a number of analyses comparing different processes according to a wide range of criteria. Output from these analyses can include point estimates as well as measures of uncertainty, using Monte Carlo analysis.

The software system integrates a number of global databases (including EcoInvent and USLCI) of standardized LCI data for a wide range of standard chemicals, materials, machinery, and energy applications. We obtained additional data pertaining specifically to the willow production system from a number of sources. The basic structure of the model as well as many parameter values do not differ significantly from the analysis of Heller et al. (2003), but have been updated to reflect changes in harvesting machinery and fuel consumption rates, chemical and equipment usage, and crop management practices. This information was derived from a number of publications (see Volk et al. 2011a for review) as well as discussion among the operators and staff involved in the production process. In addition to updating machinery, fuel and chemical use in the model, we incorporated the most current willow biomass yield estimates from Volk et al. (2011b). We built a planting stock nursery module based on interviews with the owner and operator of a commercial willow nursery in Western NY (Rak, *personal communication*). We also incorporated greenhouse gas emissions data associated with belowground carbon sequestration (based on Pacaldo et al. *in review*) and nitrous oxide emissions associated with leaf litter (Adegbidi 1994). Most parameters included in the model are point estimates. However, we included measures of variability associated with three key biological parameters - willow yield, belowground carbon sequestration, and leaf litter for use in uncertainty analysis. The boundaries of the model contain all processes within the field, the nursery, and delivery of the final willow chips. Conversion processes were not included. Carbon sequestered within the above-ground portion of the willow shrubs was not included.

We ran the model according to eight scenarios, based on the permutations of three variables. First, we ran the model both with fertilizer (F) and without fertilization (NF), based on a study that found no effect of fertilizer on yield (Quaye and Volk 2011). Second, we ran the model using two haul distances, 195 and 71 km. These values were based on average round trip haul distances from field to large (>340 mgy) centralized (C) or smaller (<227 mgy) distributed (D) biorefineries, as published in the New York Renewable Fuels Roadmap (Wojnar 2010). Lastly, we included two yield scenarios, one based on the mean yield of all clones across the nine yield trials reviewed in Volk et al. (2011b) (AC, 9.2 odt ha^{-1} yr^{-1}), as well as one based on the mean yield of the top performing three clones at each of the trials (T3, 11.8 odt ha^{-1} yr^{-1}).

We used SimaPro to assess point estimates for both energy demand (MJ, nonrenewable fossil fuels) and greenhouse gas emissions (kg C02-eq, IPCC 2007 GWP 100a) for the eight scenarios listed above. We also ran uncertainty analyses for each of the eight scenarios to determine the variability in system-wide performance due to the variability of the three key biological parameters identified above.

Results and Conclusions

Cumulative energy demand ranged from 445.0 to 1052.4 MJ odt^{-1} among the 8 scenarios (Table 1). Assuming an energy content of 20 GJ per odt of chips, this equates to an approximate energy ratio of between 1:19 and 1:45 for willow biomass delivered to an end user. The largest fraction of energy demand across all scenarios was the use of diesel fuels, 48-77% of which was utilized in the delivery of willow chips from the field gate to the end user. Harvesting operations had a greater energy demand than other field processes. Similar patterns were found for greenhouse gas emissions across all scenarios, for the simple reason that fossil fuel use served as the biggest source of emissions in the system (Table 2). Among the 8 scenarios, baseline emissions ranged from 94 to 124 kg CO_2-eq odt^{-1} (~4.7 to 6.2 g CO_2-eq MJ^{-1}). However, carbon sequestration in the belowground portion of the willow system provided a large carbon sink that more than compensated for carbon emissions across all eight scenarios. Including this carbon storage resulted in net emissions from -138.4 to -52.9 kg CO_2-eq odt^{-1} (~-6.9 to -2.7 g CO_2-eq MJ^{-1}).

Table 1: Energy demand (MJ odt^{-1}) for willow chips delivered to end user, under eight scenarios (AC = mean yield from all clones, T3 = mean yield from top three clones, F/NF = fertilizer or no fertilizer, C = delivered to large centralized users [haul distance = 195 km, to end user and back], D = delivered to distributed facilities [haul distance = 71 km, to end user and back]).

Scenario	Total (MJ odt^{-1})	Willow Production (MJ odt^{-1})	Delivery (MJ odt^{-1})	Diesel (MJ odt^{-1})
AC-F-C	1052.4	393.1	659.2	917.5
AC-F-D	633.2	393.1	240.0	498.2
AC-NF-C	928.3	269.1	659.2	914.1
AC-NF-D	509.1	269.1	240.0	494.9
T3-F-C	958.6	299.4	659.2	855.9
T3-F-D	539.4	299.4	240.0	436.7
T3-NF-C	864.2	204.9	659.2	853.3
T3-NF-D	445.0	204.9	240.0	434.1

Note: Columns are not cumulative; energy demand associated with diesel use is also incorporated into production, harvest and delivery.

Table 2: Net greenhouse gas emissions (kg CO_2-eq odt^{-1}) associated with the production of willow chips delivered to end user, under eight scenarios (AC = mean yield from all clones, T3 = mean yield from top three clones, F/NF = fertilizer or no fertilizer, C = delivered to large centralized users [haul distance = 195 km, to end user and back], D = delivered to distributed facilities [haul distance = 71 km, to end user and back]).

Scenario	Total (kg CO_2-eq odt^{-1})	Willow Production (kg CO_2-eq odt^{-1})	Delivery (kg CO_2-eq odt^{-1})	Diesel (kg CO_2-eq odt^{-1})
AC-F-C	-84.1	-130.9	46.8	65.1
AC-F-D	-113.8	-130.9	17.0	35.3
AC-NF-C	-108.6	-155.4	46.8	64.8
AC-NF-D	-138.4	-155.4	17.0	35.1
T3-F-C	-52.9	-99.7	46.8	60.7
T3-F-D	-82.6	-99.7	17.0	31.0
T3-NF-C	-71.6	-118.3	46.8	60.5
T3-NF-D	-101.3	-118.3	17.0	30.8

Note: Columns are not cumulative; emissions associated with diesel use also are incorporated into chip production and delivery. For all AC scenarios, chip production includes -203 kg CO_2-eq odt^{-1} from belowground carbon sequestration and 29.9 kg CO_2-eq odt^{-1} from biogenic production of N_2O. For all T3 scenarios, chip production includes -155 kg CO_2-eq odt^{-1} from belowground carbon sequestration and 22 kg CO_2-eq odt^{-1} from biogenic production of N_2O.

The subsequent uncertainty analyses revealed that variability associated with willow yield, leaf decay, and belowground carbon sequestration resulted in large variability in system-wide performance (Table 3). Standard deviations for both energy demand and greenhouse gas emissions overlapped widely across all scenarios.

Table 3: Energy demand and greenhouse gas emissions associated with the production of willow chips delivered to end user under eight scenarios (AC = yields from all clones, T3 = yields from top three clones, F/NF = fertilizer or no fertilizer, C = delivered to large centralized users [haul distance = 195 km, to end user and back], D = delivered to distributed facilities [haul distance = 71 km, to end user and back]).

	Cumulative energy demand (MJ odt^{-1})			Net greenhouse gas emissions (kg $C0_2$-eq odt^{-1})		
Scenario	Mean	Median	St.Dev.	Mean	Median	St.Dev.
AC-F-C	1055.2	1049.8	42.9	-86.5	-85.2	21.1
AC-F-D	634.0	630.2	43.1	-115.0	-114.0	21.5
AC-NF-C	929.5	927.6	21.7	-109.6	-108.8	23.1
AC-NF-D	510.4	507.9	21.6	-138.3	-137.6	22.5
T3-F-C	959.7	956.8	28.8	-52.7	-51.9	14.7
T3-F-D	539.1	536.2	27.9	-83.1	-83.3	14.8
T3-NF-C	864.8	864.1	12.1	-72.0	-71.9	15.6
T3-NF-D	446.5	445.7	12.3	-101.5	-101.2	16.0

In a review of 26 LCA analyses of short rotation willow and poplar systems, Djomo et al. (2011) found that net energy ratio ranged from 1:13 to 1:79 at the farm gate and 1:3 to 1:16 delivered to an end user. The authors also found that greenhouse gas emissions ranged from 0.6 to 10.6 g CO^2-eq MJ^{-1}, although most studies did not incorporate sequestration in belowground biomass. Our results indicate a higher net energy ratio for delivered willow biomass in New York. Greenhouse gas emissions were lower than the range of studies reviewed because of our inclusion of belowground biomass data. If sequestration is not included, the emissions associated with the current study fall within the range found by Djomo et al. (2011).

We conclude from this analysis that short-rotation willow continues to be a promising energy source in central New York when considered from an energy and greenhouse gas perspective. Furthermore, reduction in diesel use, whether by more efficient harvesting activities or through promotion of decentralized biorefineries that result in shorter haul distances, remains the most efficient means of improving system-wide performance. In terms of the crop production system, yield has a significant impact on the energy and greenhouse gas balances. Finally, a better understanding of the drivers of the variability in the biological portions of the system is necessary to produce reliable estimates of the greenhouse gas emissions and energy performance of short-rotation woody crops.

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4.11
EXTENT AND DISTRIBUTION OF SUSTAINABLE INTENSIVE FOREST BIOFUEL PRACTICES

Nettles, Jami E[1, *], *Zakiya H. Leggett*[2]

Abstract
While attempts are being made to understand effects of biofuel feedstock growth and harvesting on the environment, distribution and extent of planting is difficult to quantify. The Billion Ton Study estimated magnitude and location of possible forest sources of feedstock, but there is still much inherent uncertainty in such predictions, particularly at operational scales. Some sustainability and environmental models have included production and economic drivers to estimate land use conversion from timber to biofuel but other analyses have started with the premise of high conversion and biomass removal. To provide some estimation of distribution and extent of biofuel production in an operational forestry setting, we used an existing research platform, developed and maintained by Catchlight Energy LLC (CLE), to examine potential use of commercial forests for more intense biofuel cultivation within site limitations due to erosion potential and water quality concerns.

The CLE platform is examining biodiversity, water quality and quantity, and soil productivity responses from operational intercropping of a perennial grass (*Panicum virgatum*) between tree rows in existing loblolly pine (*Pinus taeda*) plantations. Current forestry Best Management Practices were used during establishment to protect water quality and aquatic life. Early results indicate that carefully intercropped sites maintain many of the environmental benefits of a forest, but research sites also included those of marginal slope and soil stability to bound establishment conditions.

Sediment monitoring, operational feedback, and logistical concerns were used to map sites suitable for biofuel operations while protecting water quality. Harvest residual measurements were added to estimate maximum biomass removal within existing pine plantations and at a landscape level. Results and methods will be presented.

Keywords: Intensive forestry, intercropping, switchgrass, *Panicum virgatum,* erosion, water yield, water quality, biomass, residual removal, Alabama, Mississippi

Introduction
While many attempts are being made to understand effects of biofuel feedstock growth and harvesting on sustainability , distribution and extent of planting is difficult to estimate. Some sustainability and environmental models have included production and economic drivers to estimate land use conversion from timber to biofuel, but other analyses have started with simpler premises of high conversion and biomass removal. This study examines a more realistic conversion of forest to agriculture and agro-forestry sites, specifically, limitations due to existing forestry Best Management Practices (BMPs) and site-specific protection, and resultant effects on water volume and biomass removal. This study does not anticipate market forces, economic or timber-biofuel strategies, or other landowner decisions, but uses simple tools to predict and model the maximum extent of biofuel operations. The scenarios were biased to represent these maximum operations and effects, without regard to economic feasibility, to provide realistic model bounds.

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Catchlight Energy, LLC (CLE), a Chevron|Weyerhaeuser joint venture (http://www.catchlightenergy.com/Sustainability.aspx), is conducting both research and operational scale biofuel plantings in Mississippi and Alabama Switchgrass (*Panicum virgatum*), a perennial grass, has been intercropped in existing pine plantations, and a robust set of studies is being used to measure biodiversity, water quality and quantity, and soil productivity, effects of intercropping with an overall goal of understanding sustainability of an intercropping system. The research studies are designed to not only describe this particular scenario, but to provide data, analysis, and models to predict effects of a variety of intensive forestry and biofuel practices.

In this study, we use early results from sediment monitoring and operational analyses combined with detailed mapping to estimate maximum feasible area for intensive biofuel operations within existing pine plantations.

Sites

Large watershed studies are being conducted in North Carolina, Mississippi, and Alabama, shown in Figure 1. The sediment survey and simulation models were conducted on land owned and managed by Weyerhaeuser Company in Mississippi and Alabama.

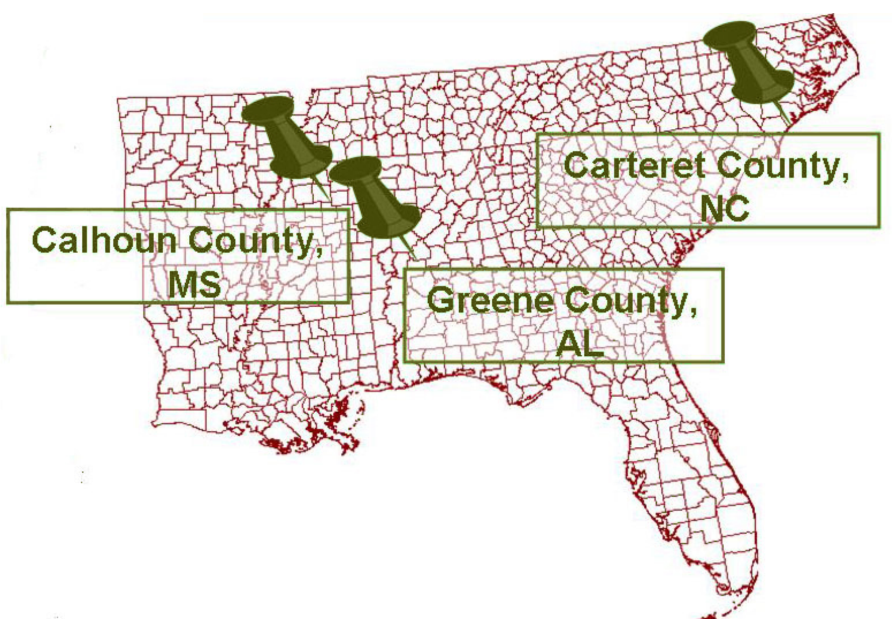

Figure 1. Large Watershed Sites

Sediment Survey

A field survey was conducted to estimate sediment impact of intercropping at an operational scale (Nettles et al, 2011). The results quantified amount of additional riparian protection given when operating under these intensive practices on forest soils and moderate slopes and verified efficacy of management practices in minimizing sediment movement into riparian areas. The study was conducted on operational tracts with the highest erosion potential, under high rainfall and early vegetative growth when maximum movement would be seen.

Nine intercropped sites totaling almost 280 hectares were surveyed, and the switchgrass rows closest to each riparian buffer checked for sediment incursions due to biofuel operations. Location, estimated sediment incursion length, sediment depth, soil texture, sediment barriers, and stream connectivity were recorded for each sediment incursion point. Each point was mapped, the delivery pathway delineated, and delivery potential determined. Sites had primary riparian buffers established under forestry Best Management Practices, and secondary buffers left in pine but not intercropped. Secondary buffers are not prescribed, but placement and width were determined on site by operators during switchgrass site preparation and planting. They were mapped and found to average 21m, in addition to the 18m primary buffer.

We found 4 incursions with sediment delivery potential although none posed a significant threat to water quality. The incursions could have been prevented with more operational experience and were not an inevitable outcome of intensive practices in forests. In addition to the riparian buffers, berms formed during the intercropping process, forest planting techniques, and site layout kept sediment movement localized and protected water quality.

Biomass Removal

In preparation for installation of biofuel research, a study of maximum possible biomass removal was implemented in a Weyerhaeuser pine plantation in eastern North Carolina as part of the CLE research platform (Lenoir 1 site; Beauvais, 2010). Two-acre blocks of flat, coastal plain forest were harvested and raked, and biomass removal quantified. This treatment does not represent an economically feasible option, and is only operationally feasible in very flat, uniform forests, but was designed to quantify the maximum possible residual removal. Operators were able to remove 85% of the above ground biomass on these research plots. Coarse roots in this managed forest with sparsely planted, large crop trees was estimated to be 19% before harvest (Miller et al., 2006) for a total forest biomass removal of 69%.

Operational Simulations

Weyerhaeuser Company's north and central Mississippi and Alabama ownership boundaries, riparian buffers, and rotation ages were used for the biomass simulation. Simulations were carried out at a HUC-12 watershed level, with each watershed averaging just over 10,000 ha.

Water yield

Average annual potential evapotranspiration (PET) was taken from the CGIAR Consortium for Spatial Information database (Trabucco and Zomer, 2009), based on the Hargreaves (Hargreaves et al., 1985) model. Average annual precipitation was based on 1971-2000 data from the PRISM Climate Group at Oregon State University (Daly et al., 2002).

Evapotranspiration was computed from the 2-parameter Zhang model (Zhang et al., 2001). Although this is a broad simplification of complex physical phenomena, it is appropriate for use in bounding long term changes in water yield due to vegetation changes under the many simplifying conditions made in this simulation (Zhang et al., 2005). Evapotranspiration was multiplied by a rotation factor to give a spatio-temporal average of less than 1 in operable areas and 1 in riparian buffers. Crop trees were assumed to go from 0 to full ET capacity in 4 years, return to half capacity with thinning, and back to full ET within 2 years.

Simulations included switchgrass intercropping and the intercropping of a hypothetical plant able to use 100% of PET. These were assumed to be planted at the same time as the pine plantation and be viable until pine canopy closure. Figure 2 shows an example of the ET changes modeled with intercropping.

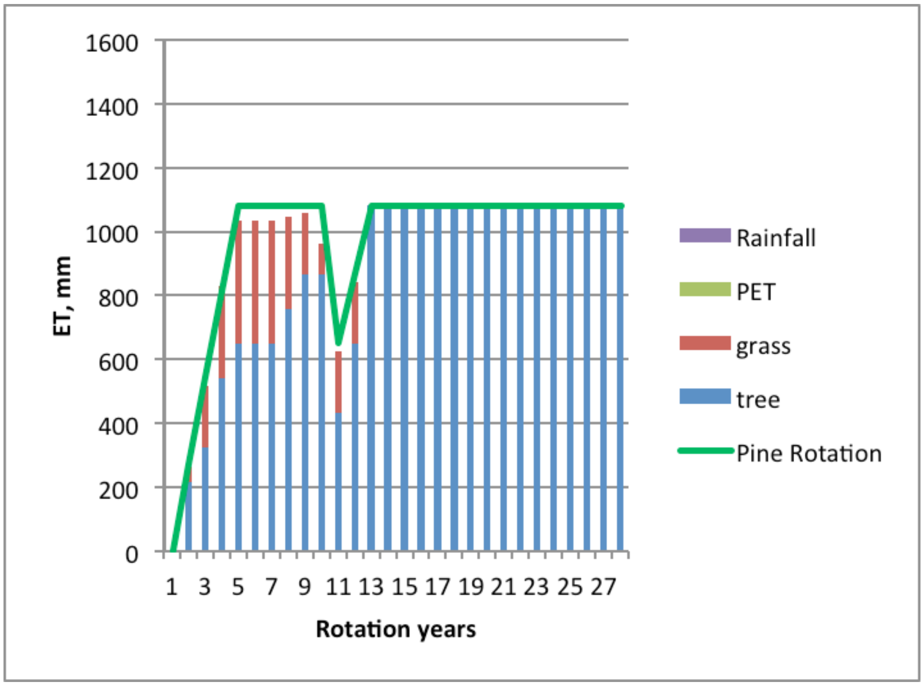

Figure 2. Example of ET model

For the intercropping scenario, water use decreased by an average of 2% per watershed, with one watershed predicted to show a 3% decrease. For the maximum change scenario – full site PET use – water use increased by an average of 15% per watershed, with a maximum of 25%. There was no attempt to route or upscale local runoff changes. Although changes in management tract scale water use would be greatly, if not completely, attenuated in nature, the change in water balance across the watershed gives a meaningful reference number.

Biomass removal

Biomass removal was modeled over Weyerhaeuser's Mississippi/Alabama ownership, also aggregated at the HUC-12 level. The model does not include any other land use, but management units were grouped by watershed to include the actual variability of riparian buffers and ownership patterns. Assuming that every management tract at maturity was harvested with this intense level of biomass removal and a conservative estimate of remaining standing biomass, removal would average 3.5% of the aboveground biomass on Weyerhaeuser forests, or 2.8% of the total biomass. The maximum was 4.6%.

Conclusions

These simulations place bounds on the extent of forest biomass practices, both in biomass removal and water yield changes. Using a large industrial forest ownership across two states gives realistic values of maximum effects. Although these conditions are not economically feasible, they are useful in building scenarios of possible future conditions.

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4.12
CONVENTIONAL LIFECYCLE ANALYSIS METHODS ARE INADEQUATE FOR EVALUATING CLIMATE MITIGATION OPTIONS

Antonio M. Bento[1] and Richard Klotz[1]*

Policy makers are increasingly relying on lifecycle analysis (LCA) metrics of greenhouse gas emissions to establish which technologies should be supported for climate mitigation purposes. Our concern is that conventional LCA methods are not designed to measure the emissions impact of a large-scale *policy induced* change in a technology. Therefore, conventional LCA methods do not consider the policy driving the expansion of a technology and potential interactions with pre-existing policies, and fail to account for a complete set of market adjustments, particularly that rate at which competing technologies are displaced. We propose a policy-based LCA method that overcomes these critical limitations of conventional LCA methods and demonstrate the advantages of the policy-based method using US biofuel policies. Our conclusion is that conventional LCA methods are inadequate for policy evaluation.

There are two commonly used technology specific methods for calculating lifecycle emissions: attributional and consequential (Table 1). Attributional LCA methods aggregate emissions across sectors using the flow of materials and energy to the production of an average unit of the technology (*1*–*7*). Consequential LCA methods evaluate how an exogenous unit expansion of a technology, ignoring policy drivers, impacts emissions in sectors associated with the production of the of that technology (*8*), and incorporate selected sources of emissions induced by market adjustments, such as land use change (LUC) due to expanded biofuel production (*9*, *10*). Lifecycle emissions savings of a technology are calculated as the difference between the lifecycle emissions of the technology of interest, and the lifecycle emissions of an equivalent unit of an alternative technology.

We propose a novel policy-based LCA method for calculating lifecycle emissions savings that addresses four major concerns with using conventional LCA methods as a policy evaluation tool. First, conventional LCA methods ignore the policy instrument that triggers the expansion of the technology being evaluated. Yet, prior literature relying on economic modeling shows that the costs of emissions reductions depend on economic adjustments in related input and output markets and therefore vary across policies (*11*–*13*). This implies that for the same expansion in a technology, policies may have different implications for emissions. Second, emissions resulting from a policy intervention are critically influenced by the presence of pre-existing policies in related markets (*14*, *15*). The policy-based LCA method addresses these two concerns by evaluating the impacts of a policy-driven unit expansion of a technology on economy-wide emissions, relative to a counterfactual baseline that incorporates pre-existing policies.

Third, conventional LCA methods evaluate the emissions of competing technologies in isolation and therefore do account for the markets that determine the rate at which the technology being evaluated displaces alternative technologies. Instead, conventional LCA methods rely on an implicit assumption that this rate of displacement is equal to one. Finally, conventional LCA methods fail to consider the complete set of policy induced multi-market adjustments (*16*). Policy-based LCA simultaneously considers adjustments in all major sectors and markets impacted by the policy, including the input markets for the technology supported by the policy and the input markets for the alternative technology.

Given the recent focus on the lifecycle emissions savings of liquid biofuels (*1*, *2*, *5*, *8*, *9*), we demonstrate the superiority of the policy-based method over widely accepted conventional LCA methods using two

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current US corn ethanol policies: the Renewable Fuel Standard (RFS), which operates as a mandate for corn ethanol, and the Volumetric Ethanol Excise Tax Credit (VEETC), which serves as a subsidy for ethanol used to produce blended fuel (see Supplementary Text for details). We show that conventional LCA methods routinely generate inaccurate estimates of the predicted impact of corn ethanol policies on emissions. By not accounting for the full set of policy induced market adjustments, conventional LCA methods typically overestimate emissions savings relative to the policy-based method, but can underestimate emissions savings in certain policy configurations. Ultimately, our conclusion is that widely accepted conventional LCA methods are inadequate for policy evaluation and should be replaced by the proposed policy-based LCA methods.

Our analysis relies on an integrated economic and emissions model (see Methods and Materials). The economic model is a multi-market equilibrium model of the US economy that links fuel, food and land markets and considers trade in crops and crude oil. The emissions sources accounted for include: gasoline and ethanol production and combustion, rest of world crude oil use, domestic agricultural production (including farm input production) and the expansion of cropland domestically and in the rest of the world.

The impact of a policy on ethanol and emissions is calculated by comparing the model outcomes with the policy in place to a baseline simulation. The baseline simulations establish the counterfactual level of emissions and ethanol without the policy of interest imposed. Policy-based lifecycle emissions savings are the total change in emissions divided by the quantity of ethanol added. To provide a clear comparison with other lifecycle studies, we decompose the total change in emissions by source.

We calculate the policy-based lifecycle emissions savings of ethanol under four policy cases. First, we evaluate the RFS relative to a baseline with the VEETC in place. Although the VEETC expired in 2011, including the VEETC in the baseline replicates the historical policy context in which the RFS and VEETC coexisted. To analyze the interactions between the VEETC and the RFS, we consider the impact of the RFS when the VEETC is renewed and when the VEETC is phased out. To isolate the impacts of the RFS and the VEETC we evaluate each relative to an alternative baseline in which the VEETC was never been imposed.

4.13
CONVERSION OF PASTURE TO ENERGY CANE FOR BIOENERGY IS PREDICTED TO ALTER GREENHOUSE GAS EXCHANGE AND SOIL CARBON

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Abstract
Bioenergy related land use change stands to alter biogeochemical cycles and global greenhouse gas budgets. Energy cane (*Saccharum officinarum* L.) is an emerging biofuel feedstock for cellulosic bio-ethanol production with potential for high yields and limited competition with food production. The DAYCENT biogeochemical model was parameterized to infer potential yields of energy cane and how changing land from grazed pasture to energy cane would affect greenhouse gas (CO_2, CH_4 and N_2O) fluxes and soil C pools. The model was used to simulate energy cane production on sandy, nutrient poor Spodosols and organic Histosols in south-central Florida. Energy cane was productive on both soil types (46-76 Mg dry mass yield); yields were maintained through three annual cropping cycles on Histosols but declined with each harvest on Spodosols. Overall, conversion from pasture to energy cane created a sink for GHGs on Spodosols and reduced the size of the GHG source on Histosols. However, the change from pasture to energy cane caused Histosols to lose 4493 gCO_2 eq · m^{-2} over 15 years of energy cane production. Cultivation of energy cane on former pasture on Spodosol soils in the southeast US has the potential for high biomass yield, GHG sequestration, and at these yields could spare significant areas of land in meeting the US cellulosic ethanol mandate.

Keywords: Biofuel, energy cane, greenhouse gases, land use change, soil carbon

Introduction
Land use and management changes associated with the emerging bioenergy industry are likely to have substantial impacts on global GHG budgets Searchinger et al., 2008). A change from fossil fuels to an energy economy more reliant on plant-derived biofuels has the potential to reduce GHG emissions. The prospect of lowering emissions is one factor leading to the United States' mandate to produce 136 billion liters of bio-ethanol by 2022 (US Congress, 2007). However, meeting this mandate will require substantial land area, which implies potentially major changes to regional biogeochemical cycling.

The Southeastern United States holds particular potential for cultivation of second-generation biofuel crops (Sladden et al., 1991). In comparison with the Midwestern US, this region's longer growing season, high precipitation and relatively lower land costs make it attractive for biofuel crop production.

Energy cane is a high-yield, cold-tolerant, but low sucrose variety of commercially produced sugar cane (*Saccharum officinarum*; Sladden et al.,1991), and a promising crop for ligno-cellulosic fuel production. Because of its low sugar concentration, it has received little attention as a commercial crop, but has been

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kept in seed banks for the purpose of introducing cold-tolerant genes into higher sucrose sugarcane strains (Sladden et al., 1991). With the development of ligno-cellulosic ethanol conversion technologies, sucrose concentration is less important for ethanol production, and energy cane could become an important biofuel feedstock as it can yield 25-74 $Mg \cdot ha^{-1} \cdot yr^{-1}$ dry mass (Table 1).

Previous modeling studies predicted that the conversion of land in the rain-fed Midwest currently used to produce corn for ethanol to perennial biofuel feedstocks such as switchgrass or *Miscanthus* (a close relative of sugarcane) would greatly reduce or reverse the emission of GHG to the atmosphere and rebuild depleted carbon stocks in the soil (Davis et al., 2011). There have been no comparable studies that address how changing a landscape to cultivate energy cane will impact GHG emissions and soil C stocks. This is addressed here by utilizing a process based biogeochemical model (DAYCENT) to run *in silico* experiments to test the general hypotheses that land use change from pasture to energy cane production changes ecosystem GHG flux and soil C storage, and that soil type is an important driver of this change.

Methods

The effect of conversion of pasture to energy cane on either spodosol or histosol soils were simulated with the DAYCENT model. Plant characteristics for sugarcane from Vallis et al. (1996) were used as a starting point to build appropriate energy cane features into the DayCent model, and literature values of aboveground production (dry mass) for sugarcane and energy cane were used to validate the model.

The DayCent model was developed to simulate ecosystem dynamics for agricultural, forest, grassland and savanna ecosystems (Parton et al., 2010). The model is a daily time step version of the Century model (Parton et al., 1994), using the same soil carbon and nutrient cycling submodels to simulate soil organic matter dynamics (C and N) and nitrogen mineralization. DAYCENT uses more mechanistic submodels than Century to simulate daily plant production, plant nutrient uptake, trace gas fluxes (N_2O, CH_4, and N_2), NO_3 leaching, and soil water and temperature (Del Grosso et al. 2005).

The Century model has been used to simulate sugarcane production in Brazil (Galdos et al., 2009) and Australia (Vallis et al., 1996). These authors show that the Century/DayCent soil organic matter sub-model can correctly simulate the impacts of burning, fertilizer, and organic matter additions on soil carbon levels and surface litter decay.

Results and Discussion

Before land use change, pasture on both soil types was a net source of GHGs to the atmosphere (Table 1). The model estimated that pastures were sinks for CO_2, with total C uptake of 1159 g CO_2 m^{-2} and 1367 g CO_2 m^{-2} over 15 years on Spodosols and Histosols, respectively (Table 1). In the absence of cattle, both soil types were CH_4 sinks (112 and 135 g CO_2eq, respectively), but we know that this is an unrealistic scenario because of the land use history of the site. Thus, including reasonable estimates of CH_4 efflux from cattle and N_2O efflux from soils resulted in net GHG emission to the atmosphere on both pasture soils (Table 1). Following conversion to energy cane, the production of N_2O on Spodosols increased, but this increase was offset by uptake of CO_2 and the change from a source to a sink for CH_4 , with the net effect that Spodosols became a net GHG sink (Table 1). Indeed, over 15 years energy cane on Spodosol was a GHG sink of > 40 Mg CO_2eq per hectare (Table 1). On Histosols, eliminating grazing following the conversion of pasture to energy cane caused a similar decrease in CH_4 efflux to the atmosphere but, unlike Spodosols, this land use change reduced N_2O emissions (Table 1). However, this system switched from a net CO_2 sink to a source, and this change in total system C prevented energy cane on Histosols from becoming a net sink for GHGs. The driver for GHG production on Histosol was higher R_H, and significant losses of soil organic matter that resulted in total C efflux from these soils (Table 1).

Table 1: Modeled ecosystem carbon, nitrogen and greenhouse gas fluxes after converting pasture to energy cane on nutrient poor Spodosols and organic matter rich Histosols. Greenhouse gas and N mineralization values are the sum of values from pasture 15 years prior to conversion to energy cane and the sum values for 15 years following the conversion to energy cane. Positive values indicate a flux to the atmosphere and negative values indicate uptake from the atmosphere by the ecosystem. Soil organic matter values are the differences between the last year of energy cane production and the last year of pasture. Total GHG values are the sums of CH_4, N_2O and total system C flux (calculated in DayCent as the difference between all C uptake and storage versus efflux from respiration) expressed as CO_2e. Differences (Δ) represent the values for energy cane minus pasture.

	Spodosols			Histosols		
	Pasture	Energy cane	Δ	Pasture	Energy cane	Δ
SOC (g C · m^{-2})	2736	2513	-224	16087	10373	-5715
Nitrogen Mineralization (g N · m^{-2})	134	203	69	216	293	77
Heterotrophic Respiration (g C · m^{-2})	3130	2913	-218	2413	5715	3302
Total Soil CO_2 Efflux (g C · m^{-2})	8148	8993	845	8111	11540	3429
CH_4 (g CO_2eq · m^{-2})	2980	-33	-3013	2958	-46	-3004
N_2O (g CO_2eq · m^{-2})	214	649	435	6713	1742	-4970
Total System C Flux (g CO_2eq · m^{-2})	-1159	-2812	-1653	-1367	924	2291
Total Greenhouse Gas Flux (g CO_2eq · m^{-2})	2035	-2196	-4231	8304	2620	-5684

The environmental impacts of changing land use from pasture to energy cane were highly dependent on the soil type. Whereas the cultivation of Histosols results in high CO_2 efflux and the reduction of soil carbon, the model predicted that energy cane crops on Spodosols would act as a net C and GHG sink. From both a biofuel and biogeochemical perspective, these results suggest that energy cane grown on nutrient poor soils, as opposed to organic soils, has the potential to be a high-yielding bio-ethanol feedstock while creating GHG sink in the Southeastern United States.

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4.14
PROJECTING GHG EMISSIONS FROM AGRICULTURE: EFFECTS OF SHIFTING TRENDS IN CELLULOSIC FEEDSTOCK DATA

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Abstract
Recent updates of biomass feedstock data indicate that achieving US bioenergy mandates will rely more heavily upon crop residues and forest biomass than previously thought, and less upon herbaceous grasses. Considering the changes in estimated feedstock mix, we estimate total GHG emissions from the agricultural sector will increase if ethanol mandates are met by 2025. Our earlier work projected that GHG emissions from agriculture would decline as a result of meeting mandates. Under the newest projection and using new data reported in the DOE's *Billion Ton Update*, emissions will increase from agriculture. New projections raise GHG emissions by 12 million metric tons carbon per year above old projections by 2030. The increase in projected GHG emissions is due to a decline in herbaceous grass plantings (which sequester soil carbon), an increase in crop residue harvesting (which inhibits soil carbon increases), and an increase in forestland harvesting (where carbon is not accounted for yet in our model, but will likely raise emissions further). If reducing GHGs from agriculture is one objective of biofuel mandates, we suggest that policies should be adopted to either incentivize herbaceous grasses over other biomass feedstocks, or restrict harvesting of crop residues and forest biomass.

Keywords*:* 1st generation ethanol, 2nd generation ethanol, carbon sequestration, carbon flux

Introduction
The 2007 expansion of the Renewable Fuels Standard mandates the steady increase in use of 2nd generation biofuels to 20 billion gallons by 2022. Second generation biofuels are made from lignocellulosic materials such as corn stover, wheat straw, switchgrass, and wood. Ideally, if 2nd generation biofuels can be developed, they would simultaneously contribute to offsetting dependence on foreign oil and reducing carbon levels while not competing directly with food uses.

Over the past 10 years, we have used current data on available types of feedstocks, feedstock production costs, and feedstock yields to estimate the economically viable feedstock mix that would be required to meet the mandates, the land use impacts, and the GHG impacts of the agricultural sector as a whole. Updating our data for the recent Department of Energy's *Billion Ton Update* has significantly changed our earlier estimates in the feedstock mix to meet the mandate and GHG implications of meeting the mandate. In this paper we compare most recent estimates with earlier estimates (reported in the 25x25 working group paper, *Analysis of the Implications of Climate Change and Energy Legislation on the Agricultural Sector*)

Methodology
The analytical tool used to conduct this analysis is an integrated socioeconomic-biogeophysical model. The integrated model is driven by data on economics, soil attributes, crop rotation, land management, and energy consumption. The economic core of the model is a modified version of the University of

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Tennessee's Policy Analysis System model (POLYSYS), which is a partial equilibrium displacement model that iterates annually and simulates results until the year 2030 (Ray et al.; De La Torre Ugarte et al. 1998; De La Torre Ugarte et al. 2008).

POLYSYS is structured as a system of interdependent modules simulating (a) crop supply for the continental US, which is disaggregated into 3110 production regions; (b) national crop demands and prices; (c) national livestock supply and demand; and (d) agricultural income. Variables that drive the modules include planted and harvested area, production inputs, yield, exports, costs of production, demand by use, farm price, government program outlays, and net realized income. Management practices currently considered in POLYSYS include corn, grain sorghum, oats, barley, wheat, soybeans, cotton, rice, hay, herbaceous and woody cellulosic feedstocks, afforestation, and pastureland. Three levels of tillage management are included for each crop. Conventional-tillage, reduced-tillage, and conservation tillage are defined, respectively, as leaving less than 15% of the ground covered by crop residue, between 15-30% ground cover, and greater than 30% ground cover (CTIC).

The model makes use of over 3,500 unique regional crop budgets, which are based on regional differences in crop production operations. These "operation budgets" list a daily schedule of all machinery and production inputs used to produce each crop. Both direct and indirect energy and carbon emissions have been tied to each input of the operation budgets (Nelson). 'Direct carbon' includes emissions from the use of fuel on farms, dissolution of agricultural lime, changes in soil carbon, and carbon equivalent emissions of N_2O. 'Indirect carbon' includes carbon dioxide emissions from fossil fuels used in the production, transport, and application of all agricultural inputs have been calculated by West and Marland (2002) for cultivated lands. By tying emissions to operations and inputs applied, the model can estimate changes in production emissions under assumptions of land use changes.

Several layers of biogeophysical data were integrated to develop a model capable of estimating changes in soil organic carbon (SOC) at the county level. Regional carbon management response curves (West et al. 2003), STATSGO soils data (USDA) and Landsat land cover data (Homer) were integrated to determine potential changes in SOC associated with each unique combination of soil type, crop type and crop management (West et al 2008).

Data Changes

There have been significant changes in crop residue data and woody material data. Earlier residue data on the quantities of stover and straw that can be sustainably removed were obtained through the DOE Oak Ridge National Laboratory (ORNL) (Walsh et al, 2003), which limited the amount of residues removed to keep soil loss due to rain and wind erosion at the' tolerable' level (Nelson, 2002). In addition to the rain and wind erosion estimates, we also applied residue retention estimates that maintain steady soil carbon levels based on the results of Wilhelm et al. (2007).

Most recent data on harvestable residue quantities that keep soil loss below tolerable levels and also maintains soil carbon levels was obtained from ORNL and based on work from Muth et al. (2011). The major change between the old and the new data, is that the old data was derived using the RUSLE equation to estimate retention coefficients, and the new data was derived using the RUSLE2 equation. The new data indicates that significantly more residues can be removed.

There have also been significant changes in the sources and amount of woody material that can be collected. Earlier data uses estimates of forest residues, mill wastes, fuel treatments and forestland thinning derived from the US Forest Service Inventory. The new data also includes estimates of standing wood available for sustainable harvesting after other uses have been met. The new data indicates that significantly more woody material can be sourced.

We analyze the significance of these recent data changes by running a scenario to meet ethanol mandates by 2025 using both the old and new data, and then compare the resulting feedstock mix and discuss its implications on US agricultural sector GHG emissions.

Results

Results indicate that under the new data assumptions, large increases in crop residue harvesting and woody material harvesting occur at lower prices, therefore significantly less herbaceous grasses come into production (table 1).

Table 1. Quantity of harvested feedstocks under old and new data assumptions

	Old data	New data
Crop Residues (mil dt)	29.7	116.9
Herbaceous grasses (mil dt)	179.5	15.9
Woody (mil dt)	13.3	90.8
Total	222.5	223.6

Units: Million dry tons

Year: 2030

When crop residues and woody material are harvested there is little or no below-ground carbon accumulation occurring. Whereas when herbaceous grasses are grown and harvested, the below-ground biomass 'dies back' and acts to pulse carbon into the soil. Figure 1 indicates that as a large amount of herbaceous grasses are planted and harvested for biomass under the old data assumptions, soil carbon is increased, and net carbon emissions from the agricultural sector fall by 8 million metric tons over 20 years. Yet under the new data assumptions, more crop residue and woody material harvesting occurs, less herbaceous grasses are planted, and total GHG emissions increase over the 20 years instead of decrease.

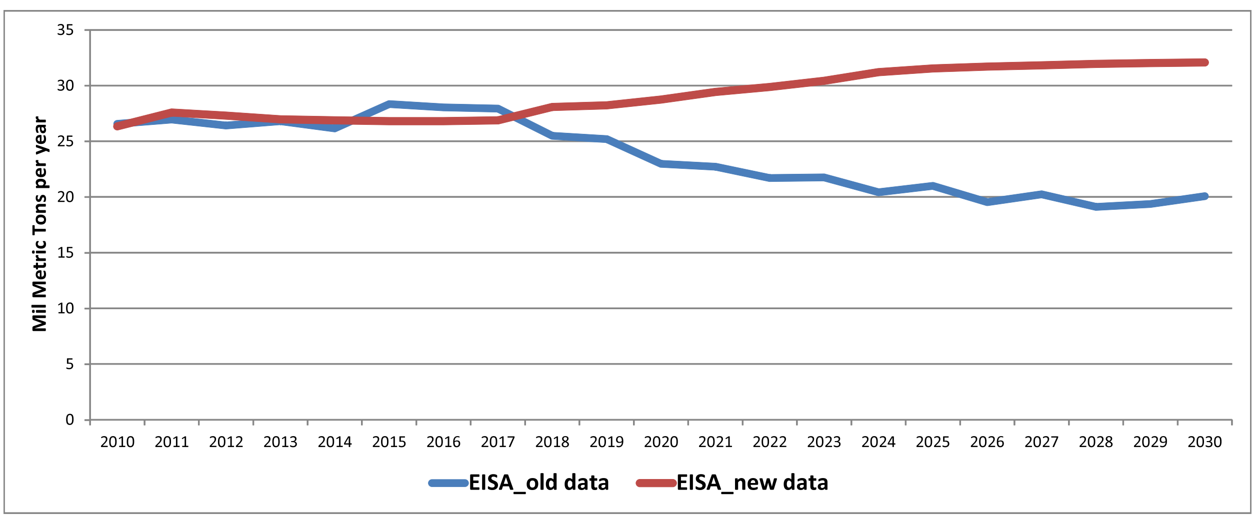

Figure 1. Net GHG emissions over 20 years from the agricultural sector under old and new data assumptions.

Table 2 shows that although there is a net drop in GHG emissions from farm inputs from 50 MMtCeq under the old data assumptions to 41 MMtCeq under the new data assumptions, total emissions increase by 12 MMtCeq as a result of what occurs in the soil – Soil sequestration declines from 30 MMtC under the old data assumptions to only 8.5 MMtC under the new data assumptions (negative numbers in table 2 indicate carbon accumulation).

Table 2. GHG emissions under old and new data assumptions

	Old data	New data
Emissions from inputs (MMtCeq)	49.9	40.64
Sequestration in soils (MMt C)	-29.83	-8.57
Total net flux	20.07	32.07

Units: Million metric tons carbon equivalent
Year: 2030

Discussion

New data indicates that standing wood harvesting and crop residue harvesting are capable of being comparatively more extensive at lower prices than previously thought. The previous benefits of herbaceous grasses evident in the older estimates are not realized in the newer estimates. Herbaceous grasses put half their growth belowground every year, and are harvested annually, resulting in root zone die-back and an annual pulsing of carbon into the soil. If carbon is an important policy goal of biofuel production, additional incentives/restrictions may be necessary to increase carbon benefits.

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4.15
GREENHOUSE GAS FLUXES IN RESPONSE TO CORN STOVER HARVEST

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Abstract
Agricultural soils play a critical role in the mitigation of increasing levels of atmospheric greenhouse gases (GHGs) such as carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). Identifying management strategies (fertilization, tillage, irrigation) that optimize corn stover removal rates for livestock feed and for cellulosic bioenergy is key to enhancing the long-term sustainability of yield and biomass, increasing soil carbon storage, minimizing erosion, and reducing agricultural GHG emissions. In-field measurements of management effects on GHG emissions provide critical data for quantifying the net energy efficiency and economic feasibility of bioenergy production systems. This assessment reviews GHG fluxes in corn stover removal systems under different management regimes for nine corn stover team sites in the Sun Grant Regional Partnership and the USDA-ARS's Renewable Energy Assessment Project (REAP). Cumulative GHG emissions varied widely across locations, by management, and by year. Despite this high variability, maximum stover removal averaged across all sites, years, and management resulted in lower total emissions of CO_2 (-15 ± 4%), N_2O (-19 ± 9%), and CH_4 (-16 ± 10%) compared to no stover removal. Decreases in GHG emissions in stover removal treatments were attributed to decreased availability of stover-derived C and N inputs into soils, as well as possible microclimatic differences. Exceptions to these trends occurred for all GHGs, highlighting the importance of site-specific management and environmental conditions on GHG fluxes in agricultural soils.

Funding agency: USDA-Agricultural Research Service as part of the USDA-ARS-REAP project. Additional funding the North Central Regional Sun Grant Center at South Dakota State University through a grant provided by the USDOE – Office of Biomass Programs under award number DE-FC36-05GO85041

Introduction
The central U.S. Corn Belt is expected to be a major contributor of crop residues such as corn stover for the Nation's developing renewable biomass energy industry (Johnson *et al.* 2007). For renewable biomass sources to provide C-neutral or C-negative energy, management practices that enhance soil organic carbon (SOC) storage and decrease direct emissions of greenhouse gases (GHGs) are needed to maximize the displacement of fossil fuel use (IPCC 2007). Plant C inputs provide the energy for

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[4] USDA-ARS, IA

[5] USDA-ARS, SD

[6] USDA-ARS, IN

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microbially-driven soil processes that control both SOC storage and emissions of various GHGs (carbon dioxide, CO_2; nitrous oxide, N_2O; methane, CH_4). Other management practices (*i.e.,* tillage, fertilization, irrigation, crop rotation) also can affect direct GHG emissions from agricultural ecosystems (Johnson *et al.* 2010; Cavigelli and Parkin 2012). The sustainability of a corn stover-based bioenergy feedstock, therefore, depends on identifying optimal stover harvest rates and site-specific management practices that maintain or improve SOC levels, minimize the risks of soil erosion or loss in soil productivity, and reduce direct GHG emissions.

A major challenge to determining optimal site-specific crop, soil, and residue management practices has been the paucity of field studies examining stover removal effects that also include measurements of direct GHG emissions. Currently, the USDA-Agricultural Research Service (ARS) is conducting a multi-location field assessment on the effects of corn stover removal and other management practices that includes quantification of direct GHG emissions in states across the Corn Belt (IN, IA, MN, NE, SD). This research is part of the USDA-ARS's Renewable Energy Assessment Project (REAP) and a broad integrated study funded through USDA and DOE (Karlen 2010; Wilhelm *et al.* 2010). The objective of this report is to provide a regional summary of how corn stover removal in conservation and conventional tillage systems affect direct GHG emissions in the U.S. Corn Belt.

Materials and Methods

Site Descriptions

The nine study sites used in this report span seven locations in the central U.S. (Table 1; Figure 1). All locations test a minimum of two corn stover removal treatments: no removal or maximum removal. Although stover removal methods and equipment vary from site to site, maximum removal rates are approximately 40% to 90% of stover biomass at all sites. Other management practices (*i.e.*, N fertilizer type and rate; crop rotation; irrigation; biochar application) and management timing vary by site, as appropriate to local soil and climate conditions. Results presented here are limited to comparing cumulative annual GHG emissions in no removal vs maximum removal treatments in conservation tillage systems (*i.e.,* reduced tillage, no-tillage; n = 9 sites) or conventional tillage systems (*i.e.,* disk tillage, chisel plow, moldboard plow; n = 3 sites).

Table 1. General descriptions of study locations and treatments.

Location Name	Lat[1] (°N)	Long (°W)	Elev (m)	MAT (°C)	MAP (mm)	Establ. Year	Trt. Reps	Other Management Treatments[2]
Morris, MN	45.7	-95.8	357	5.8	645	1995	4	Crop rotation
Morris, MN	45.7	-95.8	357	5.8	645	2005	4	Crop rotation
Brookings, SD	44.3	-96.8	502	6.1	580	2000	3	Crop rotation
Rosemount, MN	44.7	-93.1	294	6.4	879	2005	3	Crop rotation, N, Tillage
Ames, IA	42.0	-93.6	291	9.2	865	2007	3	Biochar, Tillage
Mead, NE	41.2	-96.5	363	9.8	704	1997	3	N
Mead, NE	41.2	-96.5	363	9.8	704	2000	4	Irrigation, Tillage
Clay Center, NE	40.5	-98.1	544	10.1	721	2010	4	Irrigation, N, Cover crop
West Lafayette, IN	40.5	-87.0	213	10.3	946	2006	3	None

[1]Lat=Latitude; Long=Longitude; Elev=Elevation; MAT=mean annual temperature; MAP=Mean annual precipitation; Establ. Year=year established; Trt. Reps=treatment replicates; [2]Only tillage reported here.

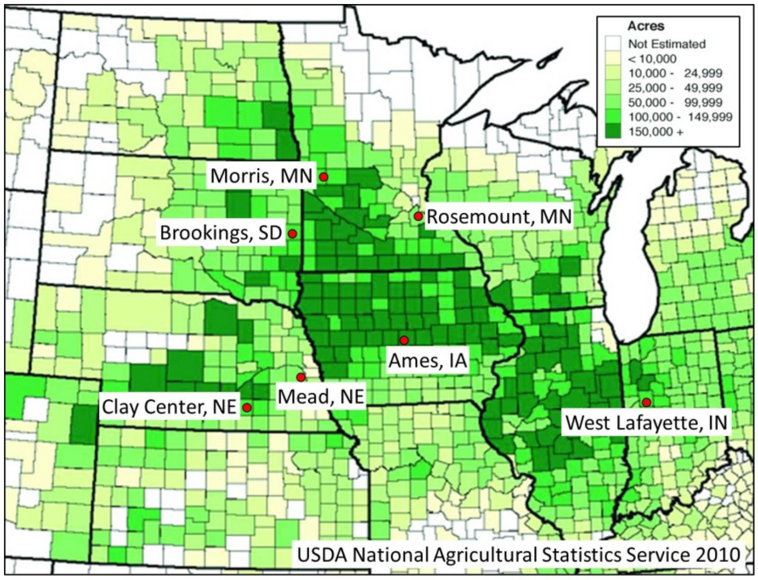

Fig. 1. Study site locations in the U.S. Corn Belt where greenhouse gas fluxes are measured. Map shows corn production acreage per county (USDA National Agricultural Statistics Service 2010).

Gas Sampling and Analyses

Greenhouse gas fluxes were measured and calculated at all sites using sampling designs and protocols standardized by the USDA-ARS's Greenhouse Reduction through Agricultural Carbon Enhancement Network (GRACEnet) (Parkin and Venterea 2010). Gas measurements were taken with static vented chambers using a stratified sampling design, and collected a minimum of every 14-days during the growing season and monthly during the non-growing season at all sites. Concentrations of CO_2, N_2O and/or CH_4 were measured using gas chromatography within 10 days of sample collection. Gas flux rates were calculated as concentration change over time within the closed chamber volume. Cumulative GHG emissions were estimated by linear interpolation of flux rates between sampling dates then summing daily rates over each year. To account for site-specific management, the effect of maximum corn stover removal was calculated relative to no stover removal at each location [i.e. (maximum removal – no removal)/(no removal)*100; %]. Positive percentage values indicate that maximum residue removal increased GHG emissions relative to no stover removal, and negative values indicate decreased emissions relative to no stover removal. Values reported as means ± standard errors.

Results and Discussion

Cumulative gas emissions from corn production systems across the nine team sites varied widely by location, year, and residue removal treatment. In general, stover removal decreased total emissions of all GHGs: -15 ± 4% for CO_2, -19 ± 9% for N_2O, and -16 ± 10% for CH_4 (Table 2, Figure 2). The nine sites covered a large region spanning geographic gradients in both mean annual temperature (MAT; increasing from north to south) and mean annual precipitation (MAP; increasing from west to east), but no correlations between MAT or MAP with cumulative emissions of any measured gas were apparent.

Table 2. Estimated minimum (Min), maximum (Max), and mean (± se) values for cumulative GHG emissions across all study locations and years under conservation or conventional tillage management.

Tillage Type (No. of Sites)	Residue Removal	kg CO_2-C ha^{-1} y^{-1}			g N_2O-N ha^{-1} y^{-1}			g CH_4-C ha^{-1} y^{-1}		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Conservation	None	815	6197	3812 (511)	0.6	5.1	2.9 (0.6)	-0.42	1.09	0.02 (0.18)
(n = 9)	Maximum	789	6296	3311 (498)	0.4	5.0	2.3 (0.5)	-0.31	0.84	0.06 (0.14)
Conventional	None	2655	5768	4745 (725)	0.5	2.0	1.3 (0.8)	nd[1]	nd	-0.35 (-)
(n = 3)	Maximum	1771	5375	4190 (819)	0.2	2.2	1.2 (1.0)	nd	nd	-0.19 (-)
All Treatments	None	815	6197	4061 (423)	0.5	5.1	2.6 (0.5)	-0.42	1.09	-0.02 (0.16)
(n = 9)	Maximum	789	6296	3545 (423)	0.2	5.1	2.1 (0.5)	-0.31	0.84	0.04 (0.13)

[1]nd = no data

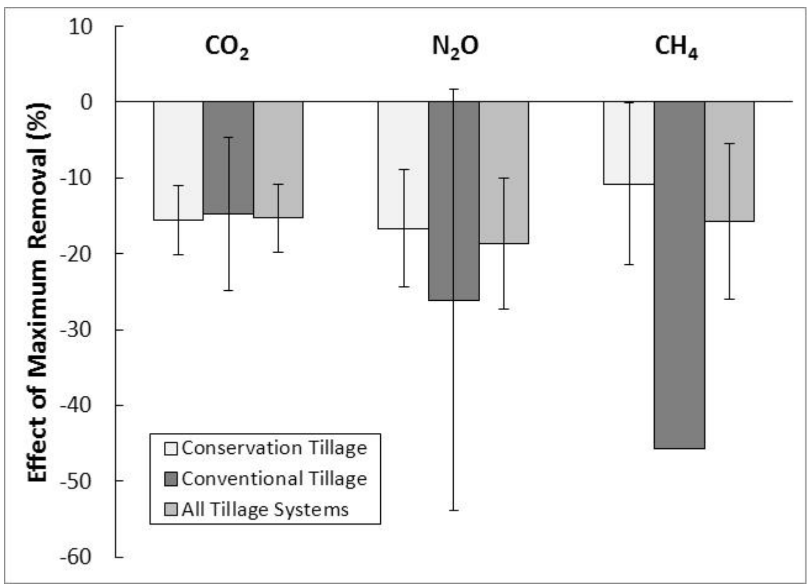

Figure 2. Relative effect of maximum corn stover removal (%; mean ± se) on cumulative greenhouse gas fluxes compared to no stover removal under conservation tillage, conventional tillage, and for all systems. No standard error is shown for CH_4 under conventional tillage because only one site measured this GHG.

Overall, conservation tillage management (*i.e.,* reduced tillage, no-tillage) tended to decrease cumulative CO_2 emissions and increase cumulative N_2O emissions compared to conventional tillage management (*i.e.,* disk tillage, chisel plow, moldboard plow) (Table 2). Conservation tillage is a key strategy for mitigating CO_2 emissions from agriculture (Morgan *et al*. 2010, Cambardella *et al.* 2012) and has been shown to build SOC in surface soils in the central U. S. Corn Belt (Johnson *et al.* 2005). Accumulation of SOC and reduced soil disturbance in conservation tillage systems could provide more favorable microsite conditions for denitrification, a microbially-mediated process that contributes significantly to agricultural N_2O emissions (Mosier *et al.* 1996). Regardless of tillage management practice, maximum residue removal tended to decrease GHG emissions overall relative to no residue removal. Stover removal likely created drier soil microsites as well as decreased the availability of residue-derived energy sources, decreasing overall soil microbial activity and subsequent release of GHGs.

Agricultural management plays an important role in the mitigation of rising atmospheric greenhouse gas levels by promoting C sequestration in cropland soils and biomass and by potentially reducing GHG emissions. These preliminary results from multi-location, field-measured GHG fluxes demonstrate that maximum corn stover removal generally lead to reduced GHG emissions. Optimal stover removal rates, however, will depend on site-specific management decisions and environmental conditions and is the subject of ongoing research.

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