SWITCHGRASS SOLUTION: ENHANCING
ABOVE- AND BELOWGROUND ECOSYSTEM
SERVICES THROUGH LOW-INPUT
HIGH-DIVERSITY BIOFUELS

By

MORGAN ANN NOLAND

Bachelor of Science in Natural Resource
Ecology and Management

Oklahoma State University

Stillwater, Oklahoma

2010

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2013
SWITCHGRASS SOLUTION: ENHANCING
ABOVE- AND BELOWGROUND ECOSYSTEM
SERVICES THROUGH LOW-INPUT
HIGH-DIVERSITY BIOFUELS

Thesis Approved:

Dr. Gail Wilson

Thesis Adviser

Dr. Karen Hickman

Dr. Yanqi Wu
ACKNOWLEDGEMENTS

I would first like to thank my graduate advisor, Dr. Gail Wilson, for her guidance, encouragement, and patience over the years. She has not only helped me grow into a professional and confidant person, but her passion and excitement for research and ecology is wonderfully contagious. She taught me to understand nature through research and I am forever grateful for everything I have learned from her.

I would also like to thank Dr. Karen Hickman, for not only serving on my graduate committee but also for all of her guidance and support over the years. Also, thank you for converting me into a plant person (she said she would, and she definitely succeeded)! Thank you to my other committee member, Dr. Yanqi Wu. His support, ideas, and input were extremely helpful in my research and the writing of my thesis.

A very big thanks to the many undergraduate and graduate students who have helped me on this project, both in the lab and in the field! Also thank you to Tim Vugteveen and Jeremy Letethouse for always being such a big help during fieldwork and for always taking us to Portillo’s! Thank you to Mike Miller for all of his input and guidance on my project and also for his hospitality while staying in Illinois!

Thank you to my family who has always been my biggest support system throughout my life. All their love, encouragement, honesty, and laughter have made me the person I am today! We are a goofy bunch, but I would never trade any of us for the world!

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.
Thank you Dad for teaching me the value of hard work and determination. I am so grateful for the many years he spent coaching me in softball. Those were some great years and I am so happy we were able to share that! Thank you Mom for being so loving and caring, and always taking the time for us kids, even now! Thank you for teaching me the importance of selflessness and family! Thank you to Mom and Dad for supporting me through school; I am so fortunate to have had all their love and encouragement over the years. Thank you to all my grandparents! Thank you Dada for all of his love and encouragement through both of my degrees! I miss Momma, Mom, and Popo, but I will always cherish my memories of them all. Thank you to Alex and Taylor for being the best brother and sister I could ever ask for. I love how we can always be ourselves with each other and our ability to make each other laugh! No matter how far apart we are from one another we will always be a family and I am so thankful for that!

Thank you to all my friends for always taking the time to listen and forever making me laugh. Also, a special thank you to Sam Porec, for opening my eyes to the fascinating world of ecology…. if it was not for her I would have never transferred into Wildlife Ecology! We have been on many adventures together and I know there will be many more to come. She is an incredible friend and I am forever thankful she is in my life.

Lastly, I would like to thank my wonderful fiancé Mitch Greer. He kept me as close to sane as possible through this entire process. His love and encouragement kept me going when I needed it most. I also appreciate all his patience and help with my statistics! Thank you for always loving me, no matter how big of a pain in the ass I can be! You bring such joy and laughter to my life and I will love you always!
Name: MORGAN ANN NOLAND

Date of Degree: DECEMBER, 2013

Title of Study: SWITCHGRASS SOLUTION: ENHANCING ABOVE- AND BELOWGROUND ECOSYSTEM SERVICES THROUGH LOW-INPUT HIGH-DIVERSITY BIOFUELS

Major Field: NATURAL RESOURCE ECOLOGY AND MANAGEMENT

Abstract: It has been suggested that native prairie species such as switchgrass (*Panicum virgatum*) can provide a sustainable, low-input biofuel feedstock, while at the same time sequestering large amounts of soil carbon. Perhaps the most sustainable approach in producing carbon-negative bioenergy utilizes mixed-species perennial ecosystems. Previous studies on restored prairie communities indicate that long-term yields can be greater with higher plant diversity than with low diversity communities or monocultures. In addition, diverse grassland plantings can provide habitat for beneficial invertebrates and wildlife. We tested our hypothesis that low-input cultivation utilizing intra- and inter-specific diversity reduces fertilizer input and nutrient leaching while providing above- and belowground ecosystem services such as wildlife habitat, aggregate stability, and increased soil carbon. Specifically, our study assessed arbuscular mycorrhizal (AM) hyphal abundance, soil carbon, aggregate stability, and above- and belowground biomass production in established plots at Argonne National Laboratory, Batavia, Illinois. Our study included monocultures of three different switchgrass cultivars, inter-cropping of these three cultivars (intra-specific diversity), and combinations of switchgrass and native prairie species (inter-specific diversity). Annual productivity of extra-radical AM hyphae was assessed using hyphal in-growth bags. Phospholipid fatty acid analyses determined total soil microbial community composition and total AM fungal biomass. Our data indicate aboveground biomass production of the switchgrass cultivar cave-in-rock, was significantly greater than southlow, big bluestem/switchgrass mix, or the diverse prairie mix. Soil from the prairie mix had significantly greater aggregate stability than all other treatments, except switchgrass mix or kanlow monocultures. However, no differences were found among extra-radical hyphal production, total microbial biomass, total AM fungal biomass, or belowground biomass. Overall, our study indicates that intra- or inter-specific feedstock production can decrease fertilizer inputs while improving aboveground ecosystem services, with no loss in belowground services.
TABLE OF CONTENTS

CHAPTER 1 – SWITCHGRASS SOLUTION: ENHANCING ABOVE- AND BELOWGROUND ECOSYSTEM SERVICES THROUGH LOW-INPUT HIGH-DIVERSITY BIOFUELS

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>II. INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>III. GOALS AND OBJECTIVES</td>
<td>9</td>
</tr>
<tr>
<td>IV. OBJECTIVES AND HYPOTHESES</td>
<td>10</td>
</tr>
<tr>
<td>V. METHODOLOGY</td>
<td>11</td>
</tr>
<tr>
<td>Plant Components</td>
<td>12</td>
</tr>
<tr>
<td>Soil</td>
<td>12</td>
</tr>
<tr>
<td>Nutrient Analysis</td>
<td>12</td>
</tr>
<tr>
<td>Soil Aggregate Stability</td>
<td>12</td>
</tr>
<tr>
<td>Soil Biological Components</td>
<td>13</td>
</tr>
<tr>
<td>External Mycorrhizal Mycelia</td>
<td>13</td>
</tr>
<tr>
<td>Microbial Community</td>
<td>13</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>14</td>
</tr>
</tbody>
</table>
VI. RESULTS .............................................................................................................. 15

   Aboveground Biomass ............................................................................................ 15
   Plant Species Diversity .......................................................................................... 15
   Belowground Components ..................................................................................... 15
   Soil Microbial Communities ............................................................................... 16

VII. DISCUSSION .......................................................................................................... 16

LITERATURE CITED .................................................................................................... 25

TABLES ....................................................................................................................... 46

FIGURES ................................................................................................................... 48
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Established feedstock production plots with monocultures of three cultivars, and intra- and inter-specific diversity plots established by Argonne National Laboratory located in Batavia, Illinois in 2008. Cultivars and/or prairie grass or forb species planted into each treatment................................................................. 46</td>
<td></td>
</tr>
<tr>
<td>2. Shannon-Weiner diversity indices (H') calculated for each diversity treatment..47</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Annual aboveground biomass production of monoculture and intra- and inter-</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>specific diversity plots. No year x treatment interactions were observed;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>therefore year 2011 and 2012 were combined. Error bars show ± 1 standard</td>
<td></td>
</tr>
<tr>
<td></td>
<td>error. Bars with the same letter are not significantly different (P &lt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Belowground ecosystem services (a) Belowground biomass (roots + rhizomes)</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>production (g/m²); (b) Soil organic carbon (mg C/g soil); (c) Soil aggregate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stability (geometric mean diameter) of monoculture and intra- and inter-specific</td>
<td></td>
</tr>
<tr>
<td></td>
<td>diversity plots. No year x treatment interactions were observed; therefore</td>
<td></td>
</tr>
<tr>
<td></td>
<td>year 2011 and 2012 were combined. Error bars show ± 1 standard error. Bars</td>
<td></td>
</tr>
<tr>
<td></td>
<td>with the same letter are not significantly different (P &lt; 0.05)................</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Soil biological components: (a) Production of extra-radical hyphae; (b)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Total soil microbial biomass; (c) Total arbuscular mycorrhizal fungal biomass</td>
<td></td>
</tr>
<tr>
<td></td>
<td>based on phospholipid fatty acid analysis of monoculture and intra- and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inter-specific diversity plots. No year x treatment interactions were</td>
<td></td>
</tr>
<tr>
<td></td>
<td>observed; therefore year 2011 and 2012 were combined. Error bars show ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>standard error. No statistical differences among treatments were observed...</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER I

SWITCHGRASS SOLUTION: ENHANCING ABOVE- AND BELOWGROUND ECOSYSTEM SERVICES THROUGH LOW-INPUT HIGH-DIVERSITY BIOFUELS

ABSTRACT

It has been suggested that native prairie species such as switchgrass (*Panicum virgatum*) can provide a sustainable, low-input biofuel feedstock, while at the same time sequestering large amounts of soil carbon. Perhaps the most sustainable approach in producing carbon-negative bioenergy utilizes mixed-species perennial ecosystems. Previous studies on restored prairie communities indicate that long-term yields can be greater with higher plant diversity than with low diversity communities or monocultures. In addition, diverse grassland plantings can provide habitat for beneficial invertebrates and wildlife. We tested our hypothesis that low-input cultivation utilizing intra- and inter-specific diversity reduces fertilizer input and nutrient leaching while providing above- and belowground ecosystem services such as wildlife habitat, aggregate stability, and increased soil carbon. Specifically, our study assessed arbuscular mycorrhizal (AM)
hyphal abundance, soil carbon, aggregate stability, and above- and belowground biomass production in established plots at Argonne National Laboratory, Batavia, Illinois. Our study included monocultures of three different switchgrass cultivars, inter-cropping of these three cultivars (intra-specific diversity), and combinations of switchgrass and native prairie species (inter-specific diversity). Annual productivity of extra-radical AM hyphae was assessed using hyphal in-growth bags. Phospholipid fatty acid analyses determined total soil microbial community composition and total AM fungal biomass. Our data indicate aboveground biomass production of the switchgrass cultivar cave-in-rock, was significantly greater than southlow, big bluestem/switchgrass mix, or the diverse prairie mix. Soil from the prairie mix had significantly greater aggregate stability than all other treatments, except switchgrass mix or kanlow monocultures. However, no differences were found among extra-radical hyphal production, total microbial biomass, total AM fungal biomass, or belowground biomass. Overall, our study indicates that intra- or inter-specific feedstock production can decrease fertilizer inputs while improving aboveground ecosystem services, with no loss in belowground services.

**INTRODUCTION**

Global environmental changes occur as a number of interacting components that alter structure and function of ecosystems. Industrial and agricultural activities may be contributing substantially to these changes (Vitousek 1994). The growing human population has created a larger need for energy use per capita in many of the populous countries worldwide (Johansson et al. 1993). One of the many global changes that have occurred is the increasing concentration of carbon dioxide in the atmosphere potentially linked to the increase of fossil fuel combustion (Vitousek 1994). The climatic
consequences of increasing atmospheric carbon dioxide concentrations have received large amounts of attention, as these increases may be responsible for more than half of the anticipated global climate change over the next century (Lashof and Ahuja 1990; Rodhe 1990; Cherubini 2011).

The Energy Independence and Security Act (EISA) of 2007 mandates increased reliance on biofuels to reduce our dependency on foreign oil. The US Departments of Agriculture (USDA) and Energy (DOE) estimated that the US would require one billion mega-grams (Mg) of biomass annually to displace 30% of current US petroleum demand with biofuels (Perlack et al. 2005; Johnson et al. 2010). This has led to large debates over how to best produce the needed biomass, how to determine optimal biofuel species, cropping systems, and appropriate land to be used for biofuel production, as well as whether biofuel mandates should exist at all (Tilman et al. 2009; Robertson et al. 2008; Mosnier 2013).

Central to this debate is the concern that increasing our agricultural footprint through biofuel crops produced with traditional agricultural approaches will not only compete with food production but could potentially take conservation lands out of production (Secchi and Babcock 2007; Tilman et al. 2006). Another issue is that biofuel feedstocks produced from annual food crops such as corn and soybean offer only marginal improvements in net energy production because their cultivation requires large quantities of nitrogen and phosphorus fertilizers for optimum yields and energy input. Production of nitrogen fertilizer accounts for a major portion of agricultural fossil fuel use (Vitousek et al. 1997). Also, fertilizer usage is a barrier to achieving carbon neutrality because it reduces the functioning of mycorrhizas and other plant-soil-microbe
interactions that generate biological soil fertility and increase soil carbon sequestration (Zhu and Miller 2003, Welbaum et al 2004; Martinez 2010).

Corn and soybean produce very little in terms of fuel; if all US corn and soybean production were dedicated to biofuel production, only 12% of gasoline and 6% of diesel needs would be met (Hill et al. 2006; Ajanovic 2013). Furthermore, because production of these feedstocks competes with food production on prime agricultural lands, their use will stimulate land conversion and generate a ‘carbon debt’ because soil carbon sinks are diminished when unmanaged land is converted to production agriculture (Fargione et al. 2008; Duval et al. 2013). Conversion of large acreage in the US from diverse native plant communities or restored prairie to monocultures for biofuel production also raises concerns about sustainability, such as loss of wildlife habitat, dependence on ecologically and economically costly fertilizer applications, decrease in soil health, loss of biocontrol and pollinator species, and disruption of biogeochemical cycles (Tilman et al. 2006; Foley et al 2005; Fargione et al. 2009; Duval et al. 2013). Globally, conversion of land to agriculture to meet food demand is expected to create the loss of one billion hectares (Bha) of ecosystem area and associated services by 2050 (Tilman et al. 2001). At the same time, land devoted to biofuel production could increase to around 1.5 Bha by 2050 (Field et al. 2007; Duval et al. 2013).

In order to reduce the issues from corn and soybean biofuels, it has recently been suggested that prairie grasses could provide a sustainable, low-input biofuel feedstock, while at the same time sequestering large amounts of soil carbon (Garten et al. 2010; Aguirre et al. 2012; Dale et al. 2013). Temperate grasslands have a enormous capacity to capture carbon from the atmosphere, due largely in part to the extensive root systems of
long-lived prairie plants that facilitate belowground carbon sequestration (Pacala and Socolow 2004; Aguirre et al. 2012). The central United States, once home to vast expanses of tallgrass prairie, offers the soils and climate needed to sustainably grow next-generation biofuel feedstocks.

The US Department of Energy Herbaceous Energy Crops Program (HECP) screened more than 30 herbaceous species for their potential as biofuel feedstocks (Parrish and Fike 2005; Aguirre et al. 2012). Native switchgrass (*Panicum virgatum* L.) was selected as a model herbaceous species because of its perennial growth habit, high yielding potential on marginal lands, wide environmental tolerance, compatibility with conventional farming practices, and its potential to revegetate drastically disturbed sites for reconstructed grasslands (Parrish and Fike 2005; Aguirre et al. 2012). This is an ideal alternative to fossil fuels because when burned directly or converted to ethanol, prairie grasses can have a negative carbon balance, compared to fossil fuels which usually have positive carbon balances (Garten et al. 2010; Aguirre et al. 2012; Dale et al. 2013). Using established technology, prairie hay can be pelletized and burned for distillation heat in corn ethanol production or co-fired in coal power plants for electricity. It can also be converted to next-generation liquid biofuel using emerging technology for cellulosic ethanol or synthetic fuel (Tilman et al. 2006; Aguirre et al. 2012). Additionally, there are potentially significant indirect benefits from the ecological services generated from sustainable use of reconstructed prairie systems, including ground water purification, reduced soil erosion, improved soil health and structure (i.e. aggregate stability), pasture-based animal production, endangered species and wildlife habitat, recreation, and
additional carbon sequestration abilities attributed to its associations with arbuscular mycorrhizal (AM) fungi.

Warm-season prairie grasses are highly dependent upon symbiotic associations with AM fungi (Wilson and Hartnett 1998; Wilson et al. 2009). Mycorrhizas are a key mechanism for the high nutrient and water-use efficiencies and large biomass yields of native prairie grasses in low-input systems. Mycorrhizal fungi have come to be viewed not only as a plant symbiont, but also as a critical piece to both the plant and soil relationship, that serves as an essential connection in the plant-soil continuum (Wilson et al. 2009; Johnson & Graham 2013). Mycorrhizal function is largely based on reciprocal transfer of photosynthate from the plant and phosphorus, nitrogen, and other nutrients from the fungus. AM fungi can exert a sizable carbon sink that cause plants to increase their rates of photosynthesis (Miller et al. 2002; Fitter 2006; Kiers et al. 2011; Johnson & Graham 2013). In addition, AM fungal hyphae can replace root hairs and root epidermal cell surfaces as sites of phosphorus and nitrogen uptake (Smith et al. 2011). AM fungi form a large network of hyphae outside of the root called extra-radical hyphae (ERH), which play a critical role in carbon translocation into the soil and provide a key link in the terrestrial carbon cycle (Zhu and Miller 2003; Fitter et al. 2006; Kiers et al. 2011).

AM fungi not only play a critical role in the sequestration of soil carbon but also in the formation and maintenance of soil aggregates. Soil aggregation is an ecosystem variable that influences virtually all nutrient cycling processes and soil biota (Diaz-Zorita et al. 2002; Wilson et al. 2009). Soil health and structure can benefit because microaggregates can be formed into macroaggregates (>250 µm in diameter) by binding agents such as decomposable organic material, small diameter roots, and associated AM
hyphae (Jastrow & Miller 2000; Wilson et al. 2009). Because soil aggregation protects carbon rich detritus from microbial degradation, an increase in aggregation proves to be an important mechanism in increasing sequestration of carbon (Rillig et al 2007; Wilson et al. 2009).

Mycorrhizal symbioses also confer other plant benefits including improved plant-water relations, tolerance to soil contaminants including herbicides, and resistance to pathogens (Miller and Jastrow 2000; Smith & Smith 2011). At the ecosystem level, AM fungi play a critical role in the formation of soil structure (Miller and Jastrow 2000; Wilson et al. 2009) and regulating carbon flux from plants to the soil (Zhu and Miller 2003; Smith & Smith 2011). Mycorrhizas enhance soil carbon sequestration because they transfer carbon away from root surfaces where microbial metabolism is greatest into the soil matrix, including aggregates (Zhu and Miller 2003; Wilson et al. 2009). AM fungi directly contribute to soil aggregation through the physical entanglement of soil particles with external hyphae (Tisdall and Oades 1982; Wilson et al. 2009). This large network may consist of 20-30% of soil microbial biomass and as much as 15% of soil organic carbon pool (Leake et al. 2004; Wilson et al. 2009). The soil aggregation formation is extremely important because it is an ecosystem variable that influences virtually all nutrient cycling processes and soil biota (Diaz-Zorita et al. 2002). Soil aggregation protects carbon rich detritus from microbial degradation, and it is considered to be an important mechanism in increasing carbon sequestration (Rillig 2004; Rillig et al. 2007; Wilson et al. 2009). Some studies have even shown that these AM fungi symbionts can greatly influence plant and fungus productivity as well as play an
important role in regulating plant community structure and diversity (Hartnett and Wilson 2002; Johnson et al. 2010; Fitzsimons and Miller 2010).

It is proposed that producing perennial biofuel crops on degraded agricultural land, otherwise “marginal” land, will reduce competition with food crops, maintain wildlife habitat, and minimize effects on carbon storage (Tilman et al. 2006; Fargione et al. 2008; Robertson et al. 2012). However, there are few studies that have been conducted to test the long-term sustainability of harvesting biomass on such lands. There have also been few studies on how genetic diversity, the use of multiple cultivars, (intra-specific diversity) will influence aboveground production as well as belowground interactions. Cultivars are cultivated varieties of plants often developed through hybridization, and selected for morphological, physiological, and/or reproductive traits (Judd et al. 2002; Aguirre et al. 2012; Nageswara-Rao et al. 2013) such as high productivity, seed quality, pest resistance, enhanced photoperiod response, and stress tolerance (Fehr 1987; Aguirre et al. 2012; Nageswara-Rao et al. 2013).

Another promising approach in producing carbon negative bioenergy is the use of low-input high-diversity (LIHD) cultivation because it is capable of maximizing production while sequestering carbon. LIHD utilizes high diversity of perennial species within biofuel feedstock cultivation. Ecological research suggests that LIHD systems on degraded prairie and abandoned agricultural land have higher annual aboveground net primary productivity (ANPP) than do unmanaged monocultures grown on the same lands (Tilman 2006; Campbell et al. 2008; Johnson et al. 2010). ANPP is defined as the aboveground component of the net amount of carbon assimilated by a defined area of vegetation over a defined period of time (Haberl et al. 2007; Johnson et al. 2010).
added diversity in LIHD cultivation allows communities to be better equipped to deal with annual variations in precipitation and typically have fewer problems with pathogen buildup than monocultures (Fornara and Tilman 2008; Harrison & Berenbaum 2013). Also, including legumes in the mixture is a low-input alternative to nitrogen fertilizer, which could help achieve carbon neutrality (Wang et al. 2010; Johnson et al. 2010). LIHD cultivation can provide numerous indirect benefits such as habitat for wildlife and pollinators, large reductions in agricultural inputs (i.e. fertilizers and pesticides), no soil tillage, and low water demand (Palmer and Wallace 2007; Johnson et al. 2010). Multiple studies have shown conversion of monoculture cropland to Conservation Reserve Program (CRP) land has significant positive impacts on grassland wildlife, such as nesting birds and mammals (Reynolds 2005 and Niemuth et al 2007). Herkert (2007) reported population trends for Henslow’s sparrow (*Ammodramus henslowii*) in Illinois counties were directly related to the amount of CRP land, and attributed the recovery of this species primarily to the increase in diverse perennial grasslands created by the CRP. Niemuth et al. (2007) reported almost two million birds of five grassland nesting bird species would be lost without the CRP in North and South Dakota.

**GOALS AND OBJECTIVES**

Our study will provide information to help develop sustainable regional feedstock production practices through increasing carbon sequestration in soil systems by selection of feedstock genotypes. The major goal of this study is to develop low-input high-diversity cultivation that will produce high biomass per unit area without increased cultural inputs, which will ultimately reduce the cost per unit biofuel. Since biofuel feedstock is comprised of shoots, it is understandable that the focus of previous studies
have focused aboveground. However, an important but overlooked plant trait that
directly influences sustainability is the degree to which switchgrass and other prairie
species rely on the mycorrhizal symbiosis for optimal growth and production. By
assessing intra- and inter-specific diversity in conjunction with cultivar associations with
mycorrhizal fungi, this study may help serve as a basis for selecting cultivars that will
improve the symbiotic contributions to feedstock production systems, which will
optimize sustainability of the biofuels feedstock production.

OBJECTIVES AND HYPOTHESES

Overall Objective:

By understanding the relationships between the characteristics of switchgrass genotypes
and high species diversity and their effects on mycorrhizal symbioses, we can better
inform resource managers of ways to maximize biomass production without
compromising belowground ecosystem services.

Objective 1: Assess the influence of switchgrass cultivars on mycorrhizal fungal
production, soil carbon inputs, and aggregate stability.

Hypothesis: Genotypic differences among switchgrass cultivars and species
diversity will influence AM fungal biomass and consequently soil carbon and
aggregation.

Objective 2: Assess the influence of plant diversity on mycorrhizal fungal production,
soil carbon inputs, and aggregate stability.

Hypothesis: Due to complementarity of traits, increasing species diversity (inter-
specific) and genetic diversity (intra-specific) will increase biomass yield and
feedstock quality, as well as increase AM fungal biomass and carbon inputs.
METHODOLGOY

To accomplish our objectives, we studied plant-soil-microbial processes in cultivars of switchgrass and other prairie grasses in established feedstock production trials located in Batavia, Illinois in field diversity plots established by Argonne National Laboratory (ANL) in 2008. The feedstock production plots are located across 13.5 acres (5.5 hectares) with 42 total plots of 20m X 36m each. The plots are separated by alleys planted with short-stature bunchgrasses. To assess the influence of plant diversity (inter- and intra-specific) on mycorrhizal fungal production, soil aggregate stability, and soil carbon sequestration, we established five sub-plots within each of these established plots. We compared four different levels of intra-specific diversity with monocultures of three different switchgrass cultivars (Kanlow; lowland cultivar originating from central Oklahoma, Cave-in-Rock; intermediate cultivar from southern Illinois, and Southlow; upland cultivar from southwest Michigan) and a mixture of the three switchgrass varieties, as well as three levels of inter-specific diversity with combinations of switchgrass and big bluestem (*Andropogon gerardii*) varieties and other prairie grasses and forbs (Table 1). Therefore, this study consisted of 7 diversity treatments x 3 replications x 3 sample dates for a total of 63 samples. To assess belowground microbial communities, soil aggregate stability, soil carbon, and above- and belowground biomass production, transects were positioned across each plot and the five sub-plots were established. We sampled multiple times throughout the study; April 2011, October 2011, October 2012, and July 2013.
Plant Components

Both above- and belowground plant biomass were sampled at the end of each growing season following plant senescence (Fall 2011 and 2012). Aboveground biomass was clipped from a 0.25m² quadrat; biomass was separated by species. Roots were collected using a soil core (10 cm deep x 15 cm diameter) and washed free of soil. Above- and belowground biomass were dried and then weighed to calculate total plant biomass. In summer 2013 (July), we sampled for species composition by using a 1m² frame in each sub-plot, to estimate percent cover using the modified Daubenmire cover class method (Daubenmire 1959). Using the species composition cover class data, we calculated the Shannon-Weiner diversity index.

Soil

Nutrient Analysis

Soil samples from the first, third, and fifth sub-plot of each whole plot were collected and analyzed during the April and October 2011 sampling periods. We tested for soil organic carbon (SOC), and total C and N content determined by direct combustion using a C/N analyzer (Carlo Erba Instruments). Plant-available PO₄-P (Melich III), and extractable NH₄-N, and NO₃--N were also quantified. The Oklahoma State University Soil/Water/Forage Analytical Laboratory conducted these soil tests.

Soil Aggregate Stability

Soil was collected fall 2011 and 2012 from each of the 5 sub-plots of each whole plot and homogenized prior to analysis. Soil aggregation was assessed according to the methods of Mikha et al. (2005). Soil fractions were separated by slaking air-dry soil
followed by wet-sieving (Elliott 1986) though a series of four sieves (2000, 250, 53, and 20 µm). Samples were then dried and weighed.

**Soil Biological Components**

*Quantification of External Mycorrhizal Mycelia (Extra-radical hyphae)*

Extra-radical hyphal (ERH) production was quantified by using mesh in-growth bags (Schweiger and Jakobsen 1999; Wallander et al. 2001). We adapted this procedure and used hyphal in-growth bags made from nylon mesh (50-µm nylon mesh, 10 x 5 x 2 cm) that allowed mycelia to grow into the bag but excluded the plant roots. The bags were filled with previously sieved red flint sand and gravel (.80-1.20mm) and sealed. The in-growth bags were placed at a depth of 0-10 cm in April 2011 (beginning of growing season). At the end of the growing season (October) 2011, the bags were collected and the sand was carefully extracted for ERH. This was accomplished by using 53-µm- and 38-µm-diameter nested sieves in sequence to collect the mycelia. The collected mycelia were freeze-dried and weighed.

*Microbial Community/Quantification of the Mycorrhizal Fungus*

One of the primary limitations in predicting the contributions of AM fungi to switchgrass and other prairie species carbon inputs, has been then ability to quantify the mycorrhizal fungus accurately (Graham and Miller 2005). A major advancement in quantifying AM fungi association is the use of maker phospholipids (PLFA) (Olsson 1999; Allison and Miller 2004). PLFAs are constituents of biological membranes that can be used to estimate the biomass of fungi, because biovolume and cell surface area are well correlated (Tunlid and White 1992). PLFA 16:1ω5c; 20:1ω9; and 22:1ω13 can be used to quantify AM fungi biomass and energy reserve status, respectively (Olsson 1999).
18:2ω6,9; 18:2ω9,12c; and 18:1ω9c can be used quantitatively for saprophytic fungi. PLFA will be extracted from the soil (collected during both sampling periods) using a modification of the Bligh and Dyer (1959) extraction (White and Ringelberg 1998). The total lipid extract will be separated into PLFA using silicic acid chromatography; the fatty acids are then cleaved from the glycerol backbone using KOH saponification; and the harvested fatty acids are methylated to form fatty acid methyl esters (FAME) (Allison and Miller 2005; White and Ringelberg 1998). The FAMEs were analyzed by gas chromatography and mass selection detection (Hewlett Packard). In addition to measuring AM fungal biomass, the PLFA and NLFA allowed for a simple measure of microbial diversity by determining the differences in evenness between treatments and provided valuable data related to carbon processing and storage (Olsson and Johnson 2005, Ziegler et al. 2005).

Statistical Analysis

Using the species composition cover class data, we calculated the average Shannon-Weiner diversity index for each treatment. Data were analyzed using a two-way analysis of variance (ANOVA) to assess differences in treatment x year. There were no interactions between year and any treatment, thus data from 2011 and 2012 were combined and reanalyzed using a one-way analysis of variance (ANOVA) to test for treatment differences. All analyses were conducted using Sigma Plot (Version 12.5, Systat Software, San Jose, CA) and an alpha = 0.05 (Version 12.5, Systat Software, San Jose, CA).
RESULTS

Aboveground Biomass

The aboveground biomass production of the switchgrass cultivar Cave-in-Rock was significantly greater than Southlow, and big bluestem/switchgrass mix, or the prairie mix (Figure 1). The Canada wildrye/switchgrass mix also produced significantly greater biomass than Southlow and the prairie mix (Figure 1). The Kanlow cultivar, switchgrass/Canada wildrye mix, and switchgrass cultivar mix were characterized by an intermediate production of aboveground biomass, compared to the other treatments (Figure 1).

Plant Species Diversity

Plant species diversity was assessed based on species composition cover class data collected mid-summer (peak growing season). Shannon-Weiner diversity index indicates the prairie mix had the higher average $H'$, compared to all other treatments (Table 2).

Belowground Components

Belowground biomass production (roots and rhizome production) was not statistically different among treatments (Figure 2a). Few significant differences in soil organic carbon were observed. The big bluestem/switchgrass mix was significantly greater in soil carbon compared to the cultivar Kanlow, however, all other treatments were not significantly different from one another (Figure 2b). The soil from prairie mix plots had significantly greater aggregate stability than all monoculture treatments and switchgrass mix treatments except Kanlow or the switchgrass cultivar mix (Figure 2c).
No other differences were observed in soil aggregate stability between treatments (Figure 2c).

**Soil Microbial Communities**

Extra-radical hyphal production did not statistically differ among treatments (Figure 3a). Total microbial biomass was assessed using phospholipid fatty acid (PLFA) analyses and no statistically significant differences were observed between treatments (Figure 3b). Total arbuscular mycorrhizal fungal biomass was assessed using PLFA analyses and no significant differences were found between treatments (Figure 3a).

**DISCUSSION**

Together land-use change and agricultural intensification, such as fertilizer production and use, are currently regarded as the greatest current global threat to maintaining biodiversity and above- and belowground ecosystem services (Green et al. 2005; Robertson et al. 2012). With land-use changes attributed to the expansion of bioenergy crops, such as corn and soybean, reduction of biodiversity is inevitable (Fletcher et al. 2011; Meehan et al. 2010; Robertson et al. 2012). Since biofuel feedstock is comprised of shoots, it is understandable that the focus of previous studies has been aboveground. However, belowground interactions that influence soil quality, such as stable macroaggregation and soil carbon sequestration, are important to also consider, as well. Therefore, in this study, we assessed above- and belowground traits of three widely used cultivars (Cave-in-Rock, Kanlow, and Southlow) planted as monocultures and planted in combination (intra-specific plantings; all three cultivars planted as a mix). Furthermore, low-input, high diversity (LIHD) cultivation, such as diverse plantings of switchgrass cultivars, diverse warm-season prairie grasses, or diverse prairie grasses and
forbs show great potential of suitable habitat for wildlife as well as pollinators and beneficial arthropods (Dale et al. 2010; Landis & Werling 2010; Robertson et al 2012). Therefore, we also assessed above- and belowground components of inter-specific plantings.

Aboveground production is the overall key aspect of biofuel feedstock management, because the aboveground yield is ultimately the sought after commodity. Our study found that both Cave-in-Rock (as a monoculture) and the Canada wildrye/switchgrass mix produced greater aboveground biomass, compared to the prairie mix or Southlow cultivar (grown as a monoculture). A possible explanation that the cultivar Cave-in-Rock produced greater biomass than the prairie mix and Southlow could be due to the “cultivar vigor hypothesis” (Wilsey 2010). This hypothesis suggests human selection for increased vigor ultimately leads to increased resource capture and aboveground biomass production in released cultivars, compared to native genotypes (Lesica and Allendorf 1999; Wilsey 2010; Lambert et al 2011). Gustafson et al. (2004) showed that the *Andropogon gerardii* (Big Bluestem) cultivar, roundtree, produced greater biomass and heights than did plants from local seed. Baer et al. (2005) also reported that a cultivar of a switchgrass lowland species attained very high dominance and suppressed overall ecosystem diversity.

We hypothesized the switchgrass mix (intra-specific diversity) or the prairie mix (intra-specific diversity) would produce greater aboveground biomass, as compared to monocultures, because an incorporation of multiple cultivars or multiple species allows for roots to fill different spatial niches. Ecological theory predicts that productivity will be greatest in mixtures of species that are able to use resources differently in time and
space (i.e., have greater niche partitioning) (Tilman et al. 1997; Wilsey 2010; Lambert et al. 2011). Diverse grassland plantings exploit complementarity of plant species, and thus, are better equipped to deal with annual variations in climate and typically have fewer problems with pathogen buildup than monocultures (Fornara and Tilman 2008). Studies have been conducted to determine the minimum number of species required to maximize aboveground biomass yield. An experiment with prairie species diversity ranging from 1 to 15 species indicated that maximum forage yield and stability will likely be achieved with two to three species that are well matched to the environment, as opposed to planting a random assemblage of many species (Tracy and Sanderson 2004; DaHann et al. 2010). In contrast, plots with 11 grasses and 28 forbs consistently produced higher yields than plots with seven grasses, with a 43% greater hay yield in the diverse plots throughout the eight-year study (Bullock et al. 2007; DaHann et al. 2010), with the presence of the legume species, L. perennis, to be very important in maximizing aboveground biomass production (DeHaan et al. 2010). Multiple studies have documented the importance of discovering a key species for obtaining maximum biomass yield (Piscasso et al. 2008; Frankow-Lindberg et al. 2009; DeHaan et al. 2010). In our study, it appeared the selection of Cave-in-Rock or the mixture of switchgrass and Canada wildrye were the optimum selection for biomass production in the spatial and temporal factors of the 2011 and 2012 growing seasons. However, we did not observe greater aboveground production in the switchgrass mix (intra-specific diversity) or the prairie mix (intra-specific diversity), as we hypothesized.

There are multiple factors that may have influenced the slight decrease in aboveground biomass production of the intra-specific or inter-specific diversity mixes.
Not only do species and cultivars interact, but planting densities, harvest dates, locations, precipitation, and years all interact with biomass production (DeHaan et al. 2010). In our study, planting densities, location, and harvest dates were kept constant and, therefore, were not factors in biomass production differences among treatments. However, timing since establishment could be playing a role in aboveground biomass production, with a resultant increase in biomass production of Cave-in-Rock monoculture compared to the prairie mix. In a meta-analysis of 44 different experiments examining plant species diversity on biomass production, Cardinale et al. (2007) reported the likelihood of transgressive over-yielding increased through time with an average of 5 years for the most diverse polyculture to have unambiguous evidence of over-yielding. The plots of our study were established in 2008 and had been in production for three (2011) or four (2102) years prior to our harvest. Therefore, in terms of Cardinale et al. (2007) production predictions, our study site is in mid- to late-stages of establishing; especially important for slower establishing forb species. Previous studies suggest minimal to no initial C$_4$ grass in the seed mix leads to significantly greater forb recruitment (Dickson and Busby 2008; Wilsey 2010), as compared to highly diverse initial mixes, which was planted in our study. Wilsey (2010) suggests low initial C$_4$ grasses with additions of this functional group seeded in later years, after the forbs have had time to establish.

Another possible factor influencing production of forb species, resulting in lower biomass production of the prairie mix was a lack of precipitation that occurred during both 2011 and 2012 growing seasons. The high diversity prairie mix produced similar or greater aboveground biomass, compared to the monocultures, in the two years prior to our study (2009 and 2010) (personal communication R. M. Miller; Argonne National
Laboratories). The robust grass species, specifically the switchgrass cultivars, are considerably more adapted, and have been bred to increase drought tolerance, as compared to non-cultivated forb species (Judd et al. 2002; Lambert et al. 2011).

While the prairie mix did not produce greater aboveground biomass, as compared to the Cave-in-Rock monoculture, our diversity plots contained a greater number of forb species, and a significantly greater diversity (H'). High diversity cultivation shows great promise as a more sustainable biofuel feedstock, providing key aboveground services such as habitat for wildlife and pollinators (Bianchi et al 2006; Gardiner et al 2010; Harrison & Berenbaum 2013). Corn-based ethanol production may negatively affects birds of high conservation concern more than any other agricultural and natural land-use change (Fletcher et al. 2011). Annual crops, like corn, do not provide useful habitat, because these fields are typically bare of vegetation during much of the year. Roberson et al (2011) found that grassland bird species richness in monoculture switchgrass fields was significantly greater, compared to that of cornfields. Although when grown for bioenergy, switchgrass is typically grown as a monoculture, it was still capable of providing migratory stopover habitat for species such as the Northern Harrier (*Circus cyaneus*), Sedge Wren (*Cistothorus platensis*), Bobolink (*Dolichonyx oryzivorus*), Dickcissel (*Spiza americana*), Henslow’s Sparrow (*Ammodramus henslowii*), and Grasshopper Sparrow (*A. savanarum*) (Roberson et al. 2011). However, plant species diversity and structure still plays an important role in grassland bird habitat selection, as overall species richness of grassland birds in switchgrass monocultures during breeding season and migration was lower than native grasslands with high plant species diversity (Bakker & Higgins 2009).
Pollinators have a key function in the maintenance of terrestrial ecosystem integrity through their role in plant reproduction, the products of which support a wide range of invertebrates, birds, and mammals (Wilson et al. 1999; Woodcock et al. 2007). Pollinators also provide goods and services to society, with many of the world’s crops being dependent upon pollinators for their productivity (Klein et al. 2007). Experimental manipulations of biodiversity show insect diversity are positively correlated with plant diversity (Haddad et al. 2001; Ries et al. 2001), and floral abundance or percent forb cover are strong predictors of butterfly abundance (Myers et al. 2012; Reeder et al. 2005; Vogal et al. 2010). Beneficial arthropods have been observed to be more diverse and abundant in diverse native prairie and in switchgrass monocultures, as compared to monocultures of corn (Gardiner et al. 2010; Harrison and Berenbaum 2013).

It can be argued that aboveground production is what is important in biofuel feedstocks because the aboveground yield is the commodity. However, if sustainable feedstock production is a goal, then breeders and managers also need to consider belowground traits as well. In addition to the potentially significant indirect aboveground ecosystem services from sustainable use of reconstructed prairie systems, such as habitat for threatened bird species, wildlife, and pollinators, belowground services such as reduced soil erosion, improved soil tilth, and carbon sequestration may also be improved.

In our study, soil aggregate stability was greater in soils associated with the high diversity mix (inter-specific diversity), compared to monocultures of Cave-in-Rock or Southlow, or the inter-specific diversity plots planted to switchgrass cultivars and big bluestem or Canada wildrye. Soil aggregate stability is extremely important because it is an ecosystem variable that influences virtually all nutrient cycling processes and soil
biota (Diaz-Zorita et al. 2002; Parent et al. 2012). The stability of soil aggregates to exposure of the disruptive force of wetting reflects positively on the permeability of the soil, the resistance of the soil to erosion, and the potential for the soil to sequester carbon (Rillig et al. 2010).

When considering soil aggregate stability, the effects of primary producers and their root growth are important to consider. Plant species diversity affects soil processes in many ways (Niklaus et al. 2007, Xavier et al. 2013). Vegetation effects on aggregation can occur through biomass allocation, litter inputs, the architecture and biomass of roots, alterations in AM fungal selectivity and, of course, the allocation of photosynthetically derived carbon to the AMF hyphal network (Treseder & Turner 2007; Coleman 2008). Although root biomass production did not differ between treatments, the prairie mix treatment may have had relatively stable soil aggregation due to the high variation of roots present. The suite of different plant species representing varying functional groups presumably lead to roots filling different spatial sites within the soil matrix. Furthermore, host-specific selection in AM fungal community composition has been reported (Eom et al. 2000; Ji et al. 2013), and it is clear that not all AM fungi are equal in their effects on plants or soils (van der Heijden et al. 1998; Klironomos 2003). Plant roots appear to host multiple species of AM fungi, cultivating particular combinations of fungi in their roots so that the diversity of fungi is enhanced (Maherali & Klironomos 2007). Host plants are able to shape distinctive AM fungal communities even when inoculated with the same AM fungal species (Bever et al. 1997; Uibopuu et al. 2009), with evidence that plants actively select fungal taxa that are most beneficial for uptake of limiting nutrients (Bever et al. 2009; Kiers et al. 2011). Therefore, high diversity plant communities may also lead
to highly diverse AM fungal communities, increasing the likelihood of hyphal production.

In our current study, annual production of AM hyphae or total AM hyphae present in soils was not significantly different in response to intra- or interspecific diversity in either year, therefore there was no strong relationship between aggregate stability and AM fungal production. However, fungal species diversity was not assessed in our study, and increased plant diversity may result in greater fungal diversity. There may be a greater quantity of fungal species that are specific to aggregate formation and stability associating with the high diversity plantings, compared to the lower diversity plots, with no alteration in total fungal biomass production.

Additionally, while our monocultures (Cave-in-Rock or Southlow) and low diversity treatments (switchgrass and big bluestem, or switchgrass and Canada wildrye) did not increase soil aggregate stability or soil carbon storage, these treatments also did not compromise belowground characteristics and presumably provided key aboveground services such as wildlife and pollinators habitat. Previous studies have seen high input practices supporting feedstock species such as corn or soybean, or exotic plant species such as *Arundo donax* (giant reed), *Phalaris arundinacea* (reed canary grass), or Asian miscanthus hybrids (*Miscanthus x giganteus*) decrease soil aggregate formation and soil carbon storage (Lewandowski et al. 2003).

Harvest management of biomass fields will play a large role in determining vegetation structure, and thus the field’s value for wildlife and pollinator habitat. Harvest management considerations include the seasonal timing of harvest, the height at which vegetation is harvested, and the proportion of available grassland that is harvested and
left intact. The ideal harvest scenario is likely to be one that produces a mosaic of harvested and intact patches, but further research is needed to determine the appropriate scale of these patches (Fargione et al. 2009). Establishing LIHD cultivation is relatively expensive, compared to high input monocultures, in part because of high seed costs. While these costs may initially hinder the large-scale establishment of diverse prairie grasses for bioenergy production, these practices should be encouraged as the use of LIHD cultivation and their associated wildlife benefits will be most effective in meeting long-term goals of sustainable feedstock production. Local and federal government agencies, bioenergy industries, and conservation communities need to work together to increase supply and lower seed prices, or otherwise offset higher costs of high diverse seed mixes (Fargione et al. 2009). Cost-share programs could share establishment costs for projects resulting in quantifiable benefits for targeted wildlife populations. Our research supports that intra- and inter-specific diversity for feedstock cultivation may be an important management tool; developing a more sustainable biofuel feedstock, maintaining belowground ecosystem services such as soil aggregate stability and carbon sequestration, while also providing key wildlife and pollinator habitat.
LITERATURE CITED


Table 1. Established feedstock production plots with monocultures of three cultivars, and intra- and inter-specific diversity plots established by Argonne National Laboratory (ANL) located in Batavia, Illinois in 2008. Cultivars and/or prairie grass or forb species planted into each treatment.

<table>
<thead>
<tr>
<th>Species/Cultivar</th>
<th>Cave-in-Rock</th>
<th>Kanlow</th>
<th>Southlow</th>
<th>Switchgrass Mix</th>
<th>Big Bluestem Mix</th>
<th>Canada Wildrye Mix</th>
<th>Prairie Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switchgrass (Cave-in-Rock)</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Switchgrass (Kanlow)</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Switchgrass (Southlow)</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Big Bluestem (Southlow)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Big Bluestem (Rountree)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Big Bluestem (Epic)</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Canada Wildrye (Elymus Canadensis)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Indiangrass (Sorghastrum nutans)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Showy tick trefoil (Desmodium canadense)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Round-headed bush clover (Lespedeza capitata)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Purple prairie clover (Dalea purpurea)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Tall tickseed (Coreopsis tripteris)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Smooth oxeye (Heliopsis helianthoides)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Yellow coneflower (Ratibida pinnata)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>New England aster (Aster nova-angliae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Culver’s root (Veronicastrum virginicum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Table 2. Shannon-Weiner diversity indices (H') calculated for each diversity treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H' Average</th>
<th>H' Minimum</th>
<th>H' Maximum</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cave-in-Rock</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kanlow</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Southlow</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Switchgrass Mix</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Big Bluestem Mix</td>
<td>0.6697</td>
<td>0.6248</td>
<td>0.6923</td>
<td>0.0224</td>
</tr>
<tr>
<td>Canada Wildrye Mix</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prairie Mix</td>
<td>1.7967</td>
<td>1.7159</td>
<td>1.8912</td>
<td>0.0511</td>
</tr>
</tbody>
</table>
Figure 1. Annual aboveground biomass production of monoculture and intra- and inter-specific diversity plots. No year x treatment interactions were observed; therefore year 2011 and 2012 were combined. Error bars show ± 1 standard error. Bars with the same letter are not significantly different (P ≤ 0.05)
Figure 2. Belowground ecosystem services (a) Belowground biomass (roots + rhizomes) production (g/m²); (b) Soil organic carbon (mg C/g soil); (c) Soil aggregate stability (geometric mean diameter) of monoculture and intra- and inter-specific diversity plots. No year x treatment interactions were observed; therefore year 2011 and 2012 were combined. Error bars show ± 1 standard error. Bars with the same letter are not significantly different (P ≤ 0.05)
Figure 3. Soil biological components: (a) Production of extra-radical hyphae; (b) Total soil microbial biomass; (c) Total arbuscular mycorrhizal fungal biomass based on phospholipid fatty acid analysis of monoculture and intra- and inter-specific diversity plots. No year x treatment interactions were observed; therefore year 2011 and 2012 were combined. Error bars show ± 1 standard error. No statistical differences among treatments were observed
VITA

Morgan Ann Noland

Candidate for the Degree of

Master of Science

Thesis:  SWITCHGRASS SOLUTION: ENHANCING ABOVE- AND 
BELOWGROUND ECOSYSTEM SERVICES THROUGH LOW-INPUT 
HIGH-DIVERSITY BIOFUELS

Major Field:  Natural Resource Ecology and Management

Biographical:

Education:

Completed the requirements for the Master of Science in Natural Resource Ecology and Management at Oklahoma State University, Stillwater, Oklahoma in December 2013.

Completed the requirements for the Bachelor of Science in Natural Resource Ecology and Management at Oklahoma State University, Stillwater, Oklahoma in 2010.

Experience:

Sr. Research Specialist, Department of Natural Resource Ecology and Management, Oklahoma State University 2013-2014

Research Technician in Grassland Ecology, Department of Natural Resource Ecology and Management, Oklahoma State University, May 2007-December 2010

Research Technician in Wildlife Ecology, Department of Natural Resource Ecology and Management, Oklahoma State University, 2010-2011

Professional Memberships:

Soil Ecological Society
Ecological Society of America
Oklahoma State Chapter of the Wildlife Society
Oklahoma Invasive Plant Council