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Switchgrass cultivation within loblolly pine plantations influences invertebrate community composition and resource use

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ABSTRACT

Global interest in biofuels has resulted in the development of novel land-use practices for the production of cellulosic biomass. One novel land-use practice that has recently been developed is intercropping switchgrass (*Panicum virgatum*) between rows of planted loblolly pines (*Pinus taeda*). However, our understanding of how intercropping switchgrass influences loblolly pine flora and fauna is limited. Therefore, we evaluated the influence of switchgrass cultivation within loblolly pine stands on invertebrate communities. We detected 2,913 individuals (n = 1,172 and 1,741 in 2014 and 2015, respectively), encompassing 13 orders. To examine invertebrate community composition among treatments, we conducted metric multidimensional scaling (MDS) in R. Multivariate analysis determined that treatment had a significant (Pr(>r) = 0.01) influence on invertebrate communities. Furthermore, stable isotope analysis suggests that Orthopterans are not assimilating cultivated switchgrass (C_4 species), but are instead assimilating C_3 plant species, such as *Rubus argutus* (sawtooth blackberry). Results indicate that switchgrass intercropping may be a viable land-use practice for the co-production of cellulosic biomass and forest products and the maintenance of invertebrate communities associated with loblolly pine plantations.

Key words: Loblolly pine (*Pinus taeda*), switchgrass (*Panicum virgatum*), intercropping, invertebrates, intensive forestry

INTRODUCTION

Concerns regarding finite reserves of fossil fuels, environmental costs associated with fossil fuels, and energy security has increased global interest in alternative renewable energy sources (McLaughlin et al. 1999, Koh and Ghazoul 2008). For example, biofuels produced from cellulosic biomass from plants, including willows (*Salix* spp.), giant miscanthus (*Miscanthus* x *giganteus*), switchgrass (*Panicum virgatum*), and agricultural residues, have garnered considerable attention (Sanderson et al. 2006, Heaton et al. 2008). Depending on how primary materials supporting biofuel production are cultivated and refined, biofuels can serve as an alternative energy source to fossil fuels, reducing greenhouse gas emissions and helping to conserve biological diversity (Fargione et al. 2008, Koh and Ghazoul 2008, Fletcher et al. 2011). However, raw materials involved in biofuel production require either a significant redirection of land used for agricultural production or conversion of land currently in a natural state, potentially affecting biological diversity (Hoekman 2008, Broch et al. 2013). Therefore, it is vital to understand how the cultivation and production of cellulosic biomass for biofuel influences existing ecological communities.

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As interest in biofuels increases, novel land use practices and techniques, such as producing biofuels from biomass grown on marginal land or from waste biomass, must be developed to significantly contribute to current and future energy needs (Fargione et al. 2008). One such novel land use is the intercropping of switchgrass within loblolly pine plantations (Riffell et al. 2012). Switchgrass intercropping is the process in which switchgrass is seeded between rows of planted loblolly pines and then grown and harvested annually (or semiannually) until shade from adjacent pines excludes switchgrass (Riffell et al. 2012). This practice reduces the amount of land required for cellulosic biomass production, while creating a system that produces an annual source of switchgrass biomass during early rotation and marketable forest products from loblolly pines (Riffell et al. 2012).

The benefits derived from switchgrass intercropping, such as minimal land required for cultivation and simultaneous production of cellulosic biomass and forest products, can aid in developing a renewable alternative energy source. However, alterations to stand establishment practices aimed at facilitating switchgrass cultivation may have unforeseen consequences on ecological communities. Switchgrass intercropping requires extensive removal of coarse woody debris and application of herbicide to facilitate establishment (Loman et al. 2013, Wheat 2015). Congregation of, or loss of coarse woody debris within pine stands to support establishment of switchgrass may affect species associated with these microhabitat structures (Ulyshen and Hanula 2009, Riffell et al. 2011, Loman et al. 2013). Furthermore, suppression of non-switchgrass plant species through herbicide application may alter the plant community structure and composition, influencing biological diversity (MacArthur and McArthur 1961, Tews et al. 2004, Iglay et al. 2012, Wheat 2015).

Some research has focused on assessing the influence of switchgrass intercropping on loblolly pine communities. Prior studies have revealed variable responses across taxa and temporal scales (e.g. Homyack et al. 2013, Loman et al. 2014, Wheat 2015). For example, plant communities exhibited a reduction in richness and diversity following switchgrass intercropped stand establishment (Wheat 2015). Whereas, the plant community diversified in switchgrass intercropped stands ≥ 5 years post establishment (Iglay et al. 2012). Moreover, herpetofaunal diversity and richness was determined to not be influenced by intercropping switchgrass, whereas, avian communities experienced an initial lag in abundance compared to reference stands (Homyack et al. 2013, Loman et al. 2014). However, no study has yet assessed the influence of intercropping switchgrass on invertebrate communities within industrial pine plantations.

Therefore, our objective was to assess the response of invertebrate communities to intercropping switchgrass within loblolly pine stands. We hypothesized that invertebrate communities would differ across treatment stands (switchgrass intercropped, switchgrass monoculture, and control). We predicted that switchgrass cultivation would result in unique communities that differed in composition from control treatments. Furthermore, we were interested in determining if cultivated switchgrass, a plant that uses the C_4 photosynthetic pathway, was assimilated by Orthopterans. We hypothesized that the introduction of switchgrass into the system would alter Orthopteran diet. We predicted that Orthopteran diet would shift from one consisting primarily of C_3 plant species to a combination of C_3 and C_4 plant species.

METHODS

Study Area — We collected invertebrate data in Kemper County, Mississippi, USA (32 51’ N, 88 33’ W) within the Interior Flatwoods Area of the Upper Coastal Plain (Petty 1977). Research was conducted on property owned and managed by Weyerhaeuser, within research sites established and maintained by Weyerhaeuser and Catchlight Energy LLC (CLE), a joint venture between Chevron and Weyerhaeuser. The climate was subtropical with mean annual temperatures of 16 – 18° C (minimum and maximum) and a mean annual precipitation of 140 cm (National Oceanic and Atmospheric Administration 2013). The study area consisted of 9,600 ha of loblolly pine stands of various ages (70%), mature pine-hardwoods (17%), hardwoods (10%), and non-forested areas (3%; Iglay 2010).

Experimental Design — Our study followed a complete randomized block design consisting of five sampling blocks. There were three 10-ha experimental plots with randomly assigned treatments (control, switchgrass intercropped, and switchgrass monoculture) within each block. Each block was previously a mature loblolly pine stand that was clearcut harvested during 2009 – 2010. Control treatments used Weyerhaeuser standards for site preparation, competition control, fertilization, tree planting, and tree spacing. Site preparation included a V-blade plow, bedding plow, and subsoil ripper to establish pine beds. Pine seedlings were spaced 1.5×6.1 m, resulting in pine beds and inter-bed rows having widths of 1.2 m and 4.9 m, respectively. A banded application of imazapyr (0.29 L/ha; Arsenal® AC, BASF Corp., Research Triangle Park, NC) and sulfometuron-methyl (0.15 L/ha; Oust®, E.I. du Pont de Nemours and Company, Wilmington, DE) was applied during the first growing season to pine beds to temporarily reduce woody and herbaceous competition.

Switchgrass intercropped treatments had similar site preparation as control stands, with the addition of more extensive coarse woody debris removal. Once pine beds were established, a V-blade plow was used to remove coarse woody debris from inter-bed rows into pine bed edges. Upon clearing inter-bed rows, a banded application of glyphosate (2.34 – 4.68 L/ha; Accord®XRT, Dow AgroSciences, Indianapolis, IN) was applied to inter-bed rows. Inter-bed rows were then disked and broadcast seeded with switchgrass. Switchgrass intercropped stands were seeded in summer 2011 and again in 2012, due to poor establishment after initial seeding. Inter-bed rows were sprayed with a banded application of glyphosate and disked a second time during the reseeding event in 2012. Site preparation for switchgrass monoculture treatments included complete removal of coarse woody debris using a V-blade plow and broadcast application of glyphosate (2.34 – 4.68 L/ha; Accord®XRT, Dow AgroSciences, Indianapolis, IN). Glyphosate was applied to reduce plant competition prior to disking and broadcast seeding of switchgrass. Switchgrass was fertilized (Arborite) and treated with herbicide (banded treatment of triclopyr [Garlon 4 Ultra®], metsulfuron methyl, and chlorsulfuron [Cimmaron Plus®]) during 2014 and fertilized only in 2015.

Invertebrate Sampling — Three permanent sampling points were established along the southeastern to northwestern corners of experimental plots, with end points ≥ 50 m from edges. Permanent points were also arranged to maximize distances from streamside management zones (pine/hardwood and hardwood corridors maintained along waterways). We randomly generated four paired points within a 50 m radius of the three permanent sampling points per treatment (24 points per treatment). Paired points allowed us to stratify sampling points by beds (planted pines) and inter-bed rows (either shrub vegetation or switchgrass). Monocultures lacked beds and inter-bed rows with sampling conducted at four unpaired points (12 points per treatment).

We sampled invertebrates three times annually from late May-2014 – 2015 to late July-2014 – 2015, with one sampling event in May and two in July, a period selected to coincide with breeding and nesting period of avian species. We sampled invertebrates using heavy duty, 38.1 cm diameter sweep-nets with a 91.4 cm handle (BioQuip Products, Rancho Dominguez, California, USA), as this is the optimal method for collecting invertebrates associated with medium height vegetation (e.g. shrubs and saplings), typical of our study sites (Ozanne 2005). We collected invertebrate samples when the vegetation was dry and wind speeds were < 20km/h, as these are optimal conditions for effective capture of invertebrates using sweep-nets (Doxon et al. 2011). Only one person collected samples during each sampling event to reduce bias associated with using multiple observers (Buffington and Redak 1998). We ensured that net sweeps were consistent among treatment types, with nets reaching a maximum height of 3 m with the arc (approx. 2 m wide) ending at ground level. At the end of each sweep, we twisted nets 180° to prevent escapes (Doxon et al. 2011). We placed samples from each sampling location into individual sealable plastic bags until storage by freezing was available (Doxon et al. 2011). We identified all invertebrates to order using the dichotomous key of Triplehorn and Johnson (2005).

Stable Isotope — Orthopteran, switchgrass, and *Rubus argutus* (sawtooth blackberry) samples were collected and used in stable isotope analysis. We collected all plant samples at locations where invertebrates were sampled. Analysis for the stable isotope ratios of $^{13/12}C$ were carried out at the Chemical Tracer Laboratory, University of Windsor (Windsor, Ontario). Stable isotope abundances are expressed in d notation as the deviation from standards in parts per thousand (%) according to the following equation:

$$\delta X \sim [(R_{sample} / R_{standard}) - 1] * 1000,$$

where X is ^{13}C, and R is the corresponding ratio $^{13}C/^{12}C$.

We selected *Rubus argutus* as the focal C_3 species because of its prevalence and dominance across the sampled landscape. All samples were freeze dried for 48 hours and then homogenized using a mortar and pestle. Samples were then analyzed to determine ratios of heavier to lighter isotopes of carbon ($^{13}C/^{12}C$) using a Delta V Advantage isotope ration mass spectrometer (Thermo Electron Corporation, Bremen, Germany) coupled with a 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA). As least three different lab standards and one NIST (8414) reference standard were also analyzed. Reference standard material was Pee Dee Belemnite carbonate.

Statistical Analysis — Metric Multidimensional Scaling: To examine invertebrate community level dissimilarity among treatments, we conducted metric multidimensional scaling (MDS) implemented in the Community Ecology Package (package "vegan") in R. We selected the Bray-Curtis distance measure as this a distance measure commonly used for ordinations involving ecological data. The invertebrate species matrix consisted of the maximum number of observations of each order within each treatment. The site matrix consisted of treatment as an environmental variable. Prior to analysis, we eliminated observations of Acari, Decapoda, Mantodea, Opiliones, and Phasmatodea as these orders had minimal observations across treatments. Removal of minimal observations was conducted as it aids in reducing the dimensionality of the ordination. An unconstrained ordination was then performed to determine if the first two axes explained a majority of the variation within the model. Explaining a majority of the variation within the first two axes allows for easier visualization and, in turn, greater interpretability of the ordination. An ordination was considered acceptable if at least 70% of the variation could be explained within the first two axes. Function "envfit" within the vegan package was then used to test the significance of the environmental variable (Treatment) using permutation tests. Tests were considered significant at alpha = 0.05.

Stable Isotope — To compare carbon fractionation values across samples, we conducted an analysis of variance and then used a Tukey post-hoc comparison test to determine the mean difference between sample types (switchgrass, *Rubus argutus*, and Orthoptera). C_3 plants, such as *Rubus argutus*, have $\delta^{13}C$ values ranging from -21 to -33‰ while C_4 plants have $\delta^{13}C$ values that range from -9 to -17‰. Animals do not appreciably change the carbon isotope ratios of their carbon sources (DeNiro and Epstein 1978). Thus, we anticipated that Orthoptera would have $\delta^{13}C$ values most similar to their carbon sources (Button et al. 1980).

RESULTS

We detected 2,913 individuals (n = 1,172 and 1,741 in 2014 and 2015, respectively), encompassing 13 orders over the 2-year sampling period (2014 – 2015). The metric multidimensional scaling attained a convergent two-dimensional solution with 72% of the variation explained within the first two axes. Therefore, the ordination was considered an adequate representation of the underlying data. The environmental variable of treatment was significant ($Pr(>r) = 0.01$) and there was an adequate correlation between the variable (treatment) and the ordination ($R^2 = 0.44$). Results of the ordination indicate that Araneae, Hemiptera, and Orthoptera were most abundant in monoculture treatments, whereas, Coleoptera were most abundant in switchgrass intercropped treatments (Figure 1). Diptera and Hymenoptera fell between the control and intercrop treatments, suggesting that abundance of these orders did not differ much between switchgrass intercropped and control treatments (Figure 1). Finally, Neuroptera and Lepidoptera were most abundant in the control treatment (Figure 1).

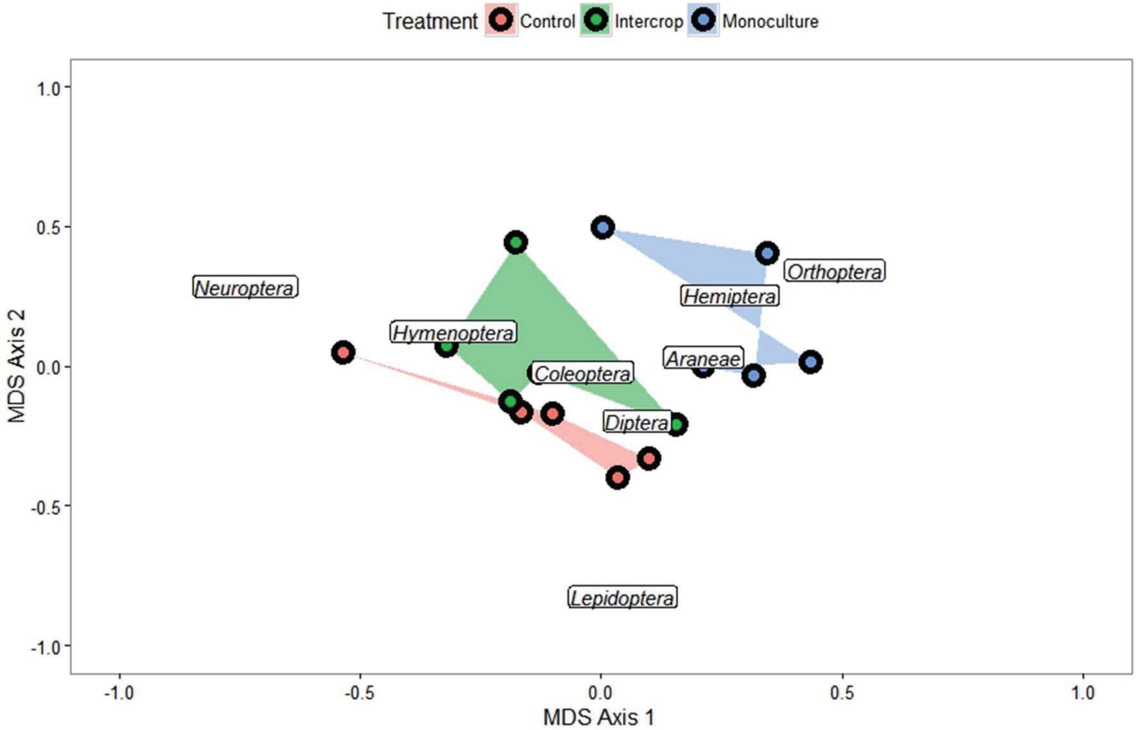

Figure 1: First two axes of metric multidimensional scaling of invertebrate community response to switchgrass cultivation within a loblolly pine plantation in Kemper County, Mississippi, USA, 2014 – 2015.

Mean and standard deviation of $\delta^{13}C$ of switchgrass, *Rubus argutus*, and Orthoptera samples where -12.43 ± 0.52, -30.12 ± 0.50, and -26.13 ± 2.13, respectively. Comparisons of mean $\delta^{13}C$ indicate that switchgrass and *Rubus argutus* had distinct isotopic values, which was expected based on the photosynthetic pathways of these species. Our post-hoc comparison test revealed significant differences between all comparisons of samples (Table 1). Mean $\delta^{13}C$ values of Orthopteran were within range of *Rubus argutus*, suggesting that Orthopterans are not extensively assimilating this species, but are assimilating it and other C_3 plant species in some capacity. Furthermore, mean $\delta^{13}C$ values of Orthopteran were not within range of switchgrass, suggesting that Orthopterans are not assimilating this C_4 plant species.

DISCUSSION

Results of our study indicate that invertebrate community composition differs with the cultivation of switchgrass within loblolly pine plantations. Switchgrass monoculture treatments promoted a unique invertebrate community mainly, but not entirely, composed of Orthopteran, Hemipteran, and Araneae orders. This unique composition of invertebrate orders stems from the replacement of a majority of the vegetation associated with managed pine stands (e.g. woody shrub-loblolly pine) with switchgrass. Switchgrass intercropped and control treatments promoted a similar invertebrate community, with some variation in composition. For example, Neuropteran and Lepidopteran orders were associated with control treatments, whereas, Coleopterans, Dipterans, and Hymenopterans were associated with switchgrass intercropped and control treatments. The overlap of invertebrate communities between these treatments suggests that switchgrass intercropped and control treatments support a similar invertebrate community. This overlap in invertebrate communities may parallel similar plant communities existing between pine beds of switchgrass intercropped treatments (e.g. woody shrub-loblolly pine) and control treatment plots.

Orthopterans were found to be more closely allied with monoculture treatments than any other treatment. However, results of the stable isotope analysis suggest that Orthopterans do not extensively assimilate switchgrass into their bodies, but instead likely largely acquire *Rubus argutus* or other C_3 plants found within treatment stands. Orthopterans may use monocultures for accessibility to forage (C_3 plant species), or as cover reducing exposure to predators (Joern 1982). The structure of plant communities within the monoculture treatment of our study may not restrict Orthopteran movement, granting access to greater total area within stands and increasing foraging ability. Furthermore, switchgrass plantings may allow for greater abundances of Orthopteran forage, as monocultures were not dominated by a woody shrub-loblolly pine plant community (e.g. Quinn and Walgenbach 1990). Orthopteran forage may not be out competed by woody shrubs and loblolly pines in switchgrass monocultures as they may be in portions of intercropped and throughout control treatments.

Although gaining important insight regarding the influence of switchgrass cultivation on invertebrates, our study focused on a subset of the entire invertebrate community associated with loblolly pine plantations. Our chosen sampling technique (sweep-nets) limited us to assessing invertebrates associated with grasses and small shrubs (Ozanne 2005). Therefore, to better elucidate the response of a larger portion of the invertebrate community, multiple sampling techniques should be employed. For example, coupling sweep-nets, pit-fall traps, and branch clipping would capture fossorial, ground-dwelling, switchgrass, and woody shrub-pine associated invertebrates, greatly improving inferences that could be drawn (Ozanne 2005, Woodcock 2005).

Invertebrate sampling should encompass a larger portion of the year, as our sampling was limited to May – July. Increasing the temporal scope within years would allow for the evaluation of the effect of switchgrass cultivation on the seasonal patterns exhibited by some invertebrates (Pinheiro et al. 2002, Danks 2007). Moreover, identification of samples should be taken to a lower taxonomic level than order. Identification of samples to a lower taxonomic level would allow for greater delineation of invertebrate functional roles and habitat requirement. Building on our research, future studies need to investigate the influence of switchgrass cultivation on invertebrate communities across a broad temporal scale (stand establishment to pine harvest [~30 years]), within different plantation types (loblolly vs. slash pine [*Pinus elliottii*]), and across different geographic regions.

Based on our findings, planting switchgrass monocultures shifts invertebrate community composition away from what is typically found in loblolly pine stands (e.g. control treatments) of a similar age. Therefore, it is recommended that switchgrass monocultures not be used as a means of cultivating cellulosic biomass for biofuel production within loblolly pine plantations. However, the land-use practice of switchgrass intercropping has the potential to both produce cellulosic biomass for biofuel production and maintain invertebrate communities associated with typically managed loblolly pine stands. Results of our study suggest that switchgrass intercropping maintains an invertebrate community similar to that found in typically managed loblolly pine stands of a similar age. However, further assessment of switchgrass intercropping and its influence on invertebrate communities is warranted to formulate a more complete management recommendation.

Our study illuminates the relationships among invertebrate communities and the cultivation of switchgrass within a loblolly pine plantation. It provides much needed baseline information on invertebrate community response to the cultivation of switchgrass within plantations. However, continued assessment of invertebrate communities is warranted across multiple temporal and spatial scales, especially considering the ecosystem services and food resources provided by invertebrate species (Hamilton 1941, Holmes and Schultz 1988, Lousey and Vaughan 2006, Horn and Hanula 2008). Furthermore, continued assessment of invertebrate communities is warranted as global interest in biofuel production continues, and means of alternative land use practices within loblolly pine plantations are explored.

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Table 1: Results of post-hoc comparison test of mean difference in d^{13}C between samples of switchgrass (PAVI), *Rubus argutus* (RUAR), and Orthoptera obtained within a loblolly pine plantation in Kemper County, Mississippi, USA, 2014 – 2015.

Comparison	Mean Difference	Lower	Upper	P-value
PAVI-Orthoptera	13.70	11.26	16.14	<0.01
RUAR-Orthoptera	-3.99	-6.06	-1.92	<0.01
RUAR-PAVI	-17.69	-20.59	-14.78	<0.01