SWITCHGRASS RHIZOBACTERIAL COMMUNITY STRUCTURE AS A FUNCTION OF CULTIVAR AND NITROGEN FERTILITY AT TWO SITES IN MINNESOTA

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Abstract

Biofuel production using native perennial grasses such as switchgrass (*Panicum virgatum)* has potential to reduce U.S. dependence on foreign oil and curtail greenhouse gas emissions. Switchgrass can also provide significant ecosystem services, such as nutrient loss reduction and carbon sequestration. Best management practices (BMPs) for switchgrass fertility are being established on a regional basis, yet little work has been done to examine the intersection of soil fertility with soil biology in switchgrass-for-biofuel plantings. This study examines the effect of nitrogen and cultivar on biomass production and switchgrass rhizobacterial community structure at two locations in Minnesota. Metagenomic analyses of the switchgrass rhizosphere will provide new insight into changes in soil ecology as a result of biofuel crop management.

Introduction

Relative to annual crops, placing marginal lands into switchgrass (*Panicum virgatum*) production for biomass can minimize topsoil erosion, improve water quality, increase carbon sequestration and provide wildlife habitat, leaving prime agricultural land to be used for food production (Hill et al., 2006; Mitchell et al., 2008; Parrish and Fike, 2005). Before large-scale production of perennial grass biofuel feedstock can be economically feasible, however, producers must demonstrate a capacity to grow, harvest, and transport a consistent supply of feedstock (Mitchell et al., 2008; Perrin et al., 2008). Best management practices (BMPs) for switchgrass and other native perennials grown as bioenergy feedstocks are being established on a regional basis. Much of the regional BMP and extension demonstration work is part of CenUSA Bioenergy (www.cenusa.iastate.edu, USDA NIFA-AFRI Competitive Grant No. 2011-68005-30411), a multi-year, multi-institution project aimed at building a sustainable production and distribution scheme for bioenergy in the Central USA. CenUSA's regional trials include nitrogen fertility and harvest management studies of several feedstocks, including a new biomass switchgrass variety, 'Liberty', on marginal soils.

The CenUSA fertility trials in Minnesota provide an ideal opportunity for work at the intersection of soil fertility and biology, examining the switchgrass rhizosphere bacterial community structure as a function of cultivar and nitrogen fertility. There is very little information regarding the community composition of rhizobacteria in switchgrass, plant-microbe interactions in the rhizosphere, or how switchgrass-for-biomass plantings alter microflora relative to native landscapes or agricultural crops (Chaudhary et al., 2012; Ker et al., 2012;

Kleczewski et al., 2012). No studies have examined the rhizosphere microflora of 'Liberty', nor have switchgrass rhizosphere observations been made on Minnesota soils.

Therefore, this work seeks to address the following questions: Does microbial community composition relate to biomass production and nutrient uptake? Does the switchgrass rhizosphere community composition change with soil fertility levels? Does 'Liberty', an F1 hybrid, support different rhizobacterial populations than a locally-derived cultivar, such as 'Sunburst', which was selected from native seed originating near Yankton, SD (Tober et al., 2007)? Even though 'Liberty' was bred for characteristics such as biomass quantity and fuel conversion quality (Vogel et al., 2014), might there be unintended consequences of the breeding that help or hurt the plant's nutrient acquisition in light of the microbial community? Answering any of these questions will not only inform us of the community composition of microflora in the switchgrass rhizosphere, but will add to the growing body of research aimed at leveraging microflora to promote more sustainable production practices (Kim et al., 2012; Kleczewski et al., 2012) and/or enhance bioremediation of contaminated soils using native grasses (Lin et al., 2005, M. Sadowsky, personal communication, 2014).

Methods

Our field trials are being conducted at two marginal sites in Minnesota: the Sand Plain Research Farm (SPRF) near Becker, MN (45°32'19''N, 93°52'32''W) and the Southwest Research and Outreach Center (SWROC) located near Lamberton, MN (44°14'24''N, 95°19'00''W). While much of the SWROC has highly productive loam soils, our two-acre site lies on eroded Ves loam with a 3-6% slope (class 2e). Our two-acre SPRF site is located on the Hubbard-Mosford complex of excessively drained loamy sand (class 4s).

The SPRF site was established in spring of 2012 and the SWROC site was established in spring of 2013. Establishment and herbicide application were after Mitchell et al. (2012), and the experimental design largely follows CenUSA protocol (Moore, 2012). The experimental design is a split-split plot, randomized complete block with four replications. Main plots are harvest regime: harvest at post-anthesis stage, harvest within two weeks after a killing frost, and alternate early and late harvests. Subplots are feedstock, including 'Shawnee' switchgrass, a hardy, productive forage-type (Vogel et al., 1996), 'Liberty' switchgrass, a new, highly-productive, bioenergy-type F1 hybrid (Vogel et al., 2014), and 'Sunburst' switchgrass, a locally-derived ecotype bred for seedling vigor and productivity (Boe and Ross, 1998). Sub-subplots are nitrogen fertilizer: 0, 56, 112 kg N ha^{-1} (0, 50, 100 lbs ac^{-1}) applied as Agrotain-coated urea at the start of the second and subsequent growing seasons. Pre-emergent atrazine was applied at Lamberton, and 2,4-D was used for broadleaf weed control at both sites, in combination with hand-weeding, as needed. While all plots are used for biomass determination, only the 0 and 112 kg N ha^{-1} plots harvested in August were used for the rhizosphere study to capture extremes in fertility and collect microbial populations at peak biomass production near the post-anthesis stage. The SWROC site was harvested on August 14, 2014 and the SPRF site was harvested on August 19, 2014.

To determine biomass, hand samples were collected from each subplot and the center swath of each subplot was cut using a Carter harvester (Carter Mfg. Co., Inc., Brookston, IN). The

mechanically harvested biomass was weighed wet in the field, and subsamples were taken back to the lab, separated to determine grass or weed biomass, weighed wet, dried, and reweighed to determined dry matter (DM) concentration. The average DM concentration of subsamples were used to adjust the field-harvested biomass weight to oven-dry weights. After drying, the samples were ground and analyzed for tissue nitrogen concentration, although the nitrogen data were not available at the time of this report.

Switchgrass root samples were collected for metagenomic analysis using a tractor-mounted hydraulic probe (Giddings Machine Co., Windsor, CO). Samples at the SWROC were collected on August 18, 2014, and at the SPRF on August 20, 2014. Three plant samples were taken per subplot, and crown of each plant was cut to the ground and extracted to a depth of 15.24 centimeters (6 inches), with a diameter of 6.7 centimeters (2 5/8 inches). Plastic soil tube liners with vinyl caps (Giddings Machine Co., Windsor, CO) were used for each sample and auger bits were cleaned with 70% ethanol between samples to minimize cross-contamination. Samples were transported to the laboratory on ice and refrigerated at 4°C until processing. Several roots from each sample were picked out with tweezers, briefly shaken to remove non-adhering soil, placed into a 50 mL Falcon tube with a 0.1M diammonium phosphate buffer solution and shaken for 30 minutes to remove rhizosphere soil. After shaking, roots were removed, weighed, dried, and re-weighed for dry matter determination. Each rhizosphere soil sample was frozen at -80°C until further processing. Upon thawing, samples were centrifuged at 7500 RPM for 20 minutes and DNA extraction was done using PowerSoil® DNA Isolation Kits (MO BIO Laboratories, Inc., Carlsbad, CA). The DNA samples were sent to the University of Minnesota Biomedical Genomics Center for sequencing. Gravimetric soil water content and root biomass were also evaluated to describe the microbial environment in each subplot at the time of sampling. The metagenomic data, however, were not available at the writing this report.

Preliminary results

Dry matter (DM) yields on a per-hectare basis are shown in figures 1 and 2 for the SPRF and SWROC sites, respectively. The SPRF site exhibits considerably lower yield than the SWROC site, likely due to the excessively-drained loamy sand soil. The differences in DM yield as a function of applied nitrogen are also more pronounced at the SPRF site, likely due to low soil organic matter. The differences in DM yield as a function of nitrogen rate are less pronounced at the SWROC site, likely because of greater soil organic matter and loamy texture. At Becker, ‘Liberty’ appears less productive, likely due to winterkill and stand reduction. Some winterkill in ‘Liberty’ was also observed at Lamberton. While much work remains to be done, results from the microbial metagenomics may reveal more information about the differences in growing conditions at these sites. Whether or not a relationship exists between biomass production and microbial community structure remains to be seen. The results from the metagenomics will also indicate whether or not differences exist in the community structure of rhizobacteria as a function of cultivar and nitrogen rate. Regardless of whether or not these differences exist, a preliminary survey of rhizobacteria in switchgrass has potential to inform future work with beneficial microbes, leading to more sustainable production of switchgrass for biomass, particularly on marginal soils in Minnesota.

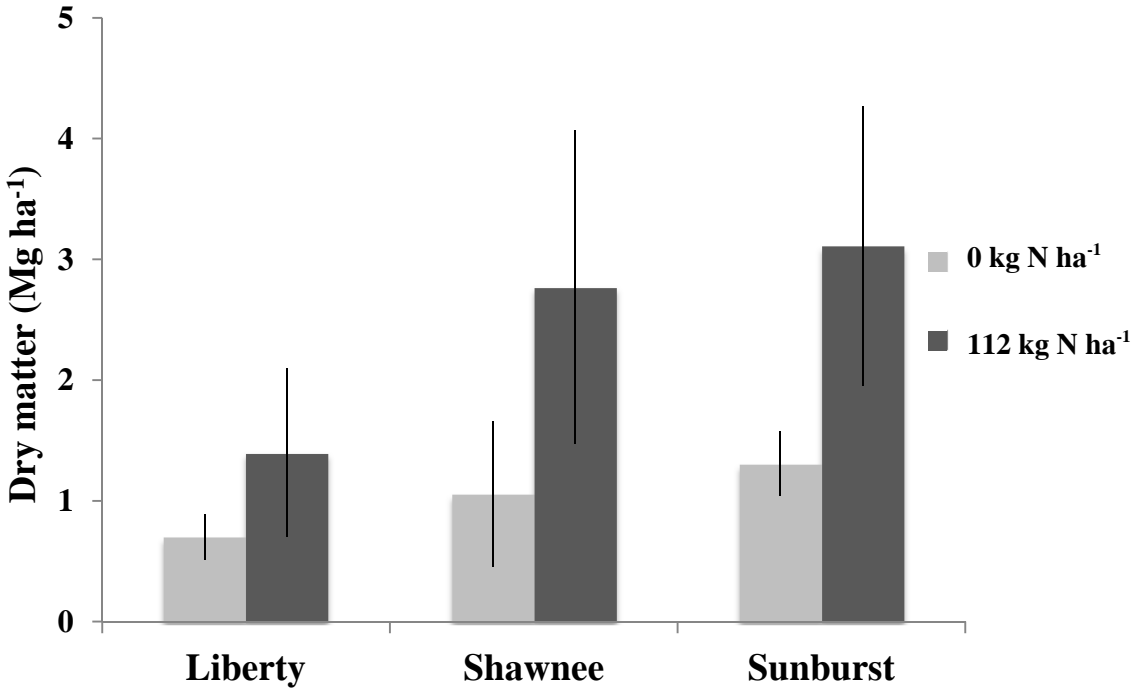

Figure 1. Dry matter yield of switchgrass harvested on August 18, 2014 at the Sand Plain Research Farm near Becker, MN.

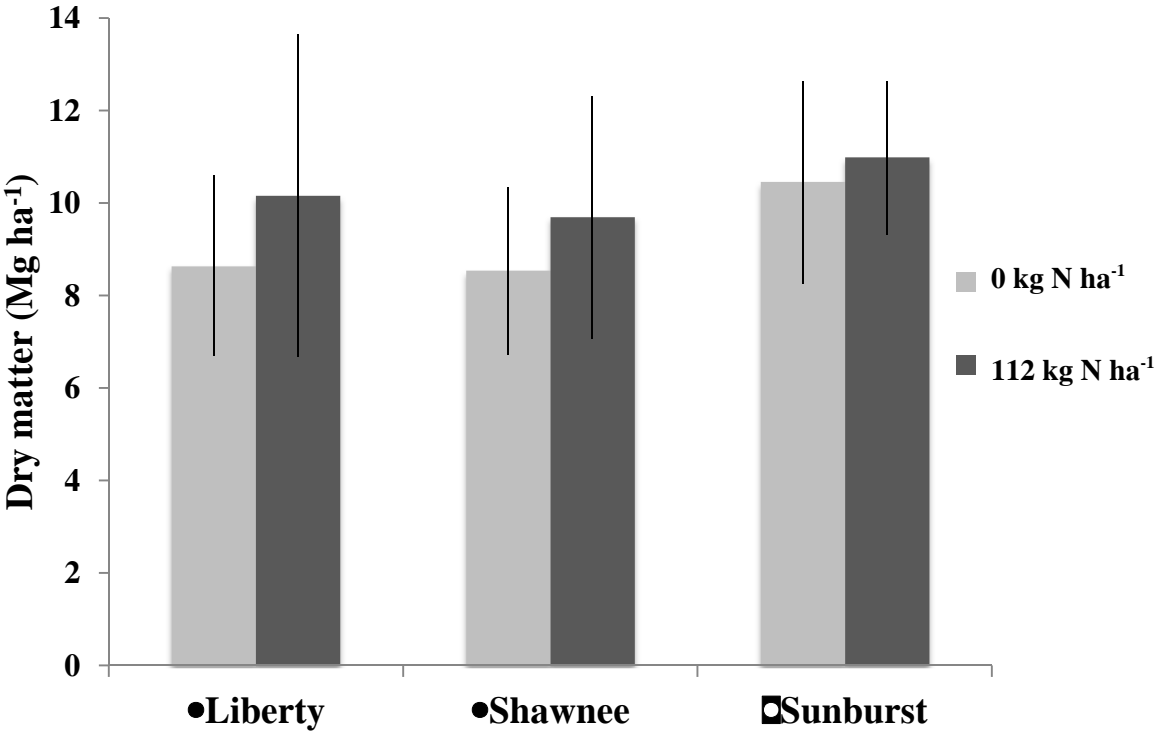

Figure 2. Dry matter yield of switchgrass harvested on August 14, 2014 at the Southwest Research and Outreach Center near Lamberton, MN.

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