

Switchgrass Rhizosphere Microflora and the Effects of Nitrogen and Cultivar on Community Structure

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Introduction

Switchgrass (Panicum virgatum), a native warm-season perennial, is a promising bioenergy crop in the US. Switchgrass can be grown on marginal lands, reserving prime lands for food production.

Marginal soils often have low plantavailable nutrients relative to prime lands. Therefore, might there be potential for microbes to assist with production on marginal soils? Before microbes can be enlisted to help, switchgrass rhizosphere microflora must first be identified.

Research questions

Who's there?

 What is the community structure of bacteria and fungi in the switchgrass rhizosphere?

Does structure change with...

- Cultivar?
- Nitrogen fertilization?

Methods

- Establish trial plots.
- Apply nitrogen
- in year 2. Post-frost harvest, determine biomass.
- Sample roots with 2.5-cm probe.
- Obtain DNA from rhizosphere soil with PowerSoil extraction kit.

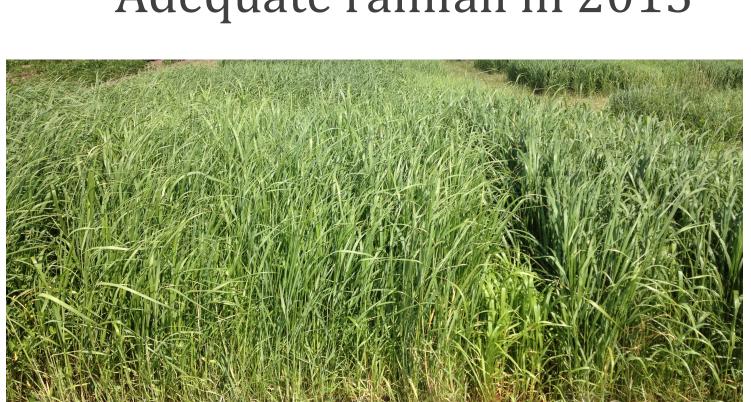


- Send DNA to U of M Genomics Center.
- Group taxonomy using 'mothur'.

Considering cultivars and conditions

LAMBERTON

- Established in 2013
- Well to somewhat poorlydrained loam
- Adequate rainfall in 2013



'Liberty'

New lowland-type F1 hybrid bred for biomass production and hardiness

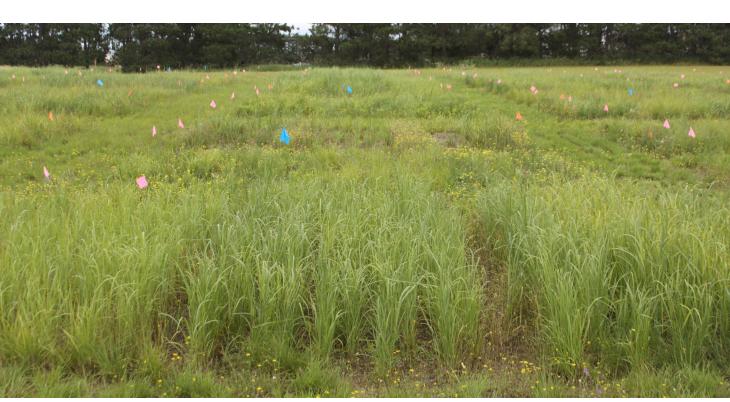
'Sunburst'

Very hardy upland ecotype bred for seedling vigor and forage yield

BECKER

- Established in 2012
- Excessively-drained loamy sand
- Drought conditions in 2013





Preliminary results: Biomass

	Liber	ty	Sunburst			
Site	Mean DM	Std. Dev.	Mean DM	Std. Dev.	t-test (df)	p-value
Lamberton	6.68	2.28	3.88	1.09	3.83 (22)	<0.001
Becker	0.34	0.12	0.43	0.02	1.49 (6)	0.185

Table 1. 2013 post-frost dry matter (DM) yield (Mg ha⁻¹) at Lamberton and Becker, N = 0 kg ha⁻¹

- At Lamberton, establishment year yield of 'Liberty' was significantly greater than that of 'Sunburst'.
- No significant difference in yield was observed in year two at Becker.

Preliminary results: Bacterial metagenomics 100% Less abundant orders Bacillales Xanthomonadales Pseudomonadales Clostridiales Rhodocyclales Rhodospirillales Reads 50% Solirubrobacterales Caulobacterales | Acidobacteria – Group 3 Planctomycetales Sequence 30% Rhodocyclales Acidobacteria – Group 6 | Acidobacteria – Group 1 Myxococcales Sphingomonadales Burkholderiales Rhizobiales **Sphingobacteriales** Actinomycetales Sunburst Liberty Sunburst Becker Lamberton

Figure 1. Distribution of the most abundant orders identified at Lamberton and Becker for N=0 kg ha⁻¹, following 2013 post-frost harvest. Three samples summed per cultivar, one cultivar per site.

	Site	Liberty		Sunburst				
	(# orders)	OTUs	S.I.	OTUs	S.I.			
	Lamberton	8828	7.49	10898	7.89			
	(152)	+/- 3887	+/- 0.55	+/- 335	+/- 0.10			
	Becker	11586	8.11	9685	7.84			
	(155)	+/- 481	+/- 0.15	+/- 4323	+/- 0.77			
Table 2. Number of operational taxonomic units (OTUs) and mean Shannon index (SI) per cultivar								

- Species richness (Shannon index) is similar at both sites.
- Community difference between cultivars, based on phylogenetic lineage of OTUs, may be significant at Becker (unweighted UniFrac P<0.1), but not at Lamberton*.

*At both sites, weighted UniFrac (unique fraction) P<0.001. 'Weighted' accounts for relative abundance of OTUs ('unweighted' does not) and is usually significant.

Discussion

- Drought conditions at Becker contributed to very poor yield.
- 'Liberty' may be susceptible to winterkill in post-establishment years, resulting in lower yields.
- Difference in community structure between sites is likely a function of environment, e.g. soil type and previous crops.
- Early results may indicate greater bacterial community variability between cultivars at Becker.

Next steps

- Characterize bacterial community for remaining 2013 samples.
- Identify rhizosphere fungi (U of M Genomics Center).
- Complete 2014 biomass harvest.

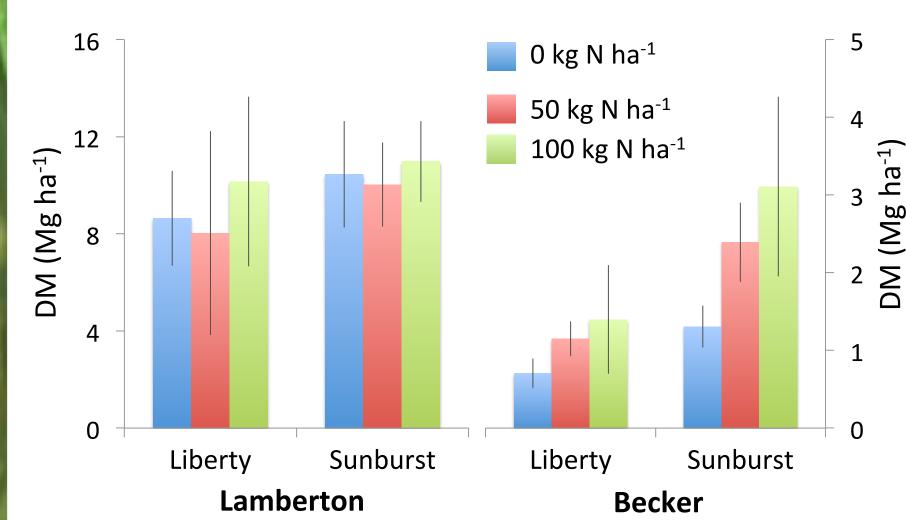


Figure 2. Preliminary dry matter (DM) yield at Lamberton and Becker, August, 2014

- Extract DNA from 2014 samples at 0 and 100 kg N ha⁻¹.
- Compare results from 2014 to those from 2013 (baseline).

Selected references

Boe and Ross, 1998. Crop Science, 38:540 Brejda et al., 1998. Agron. J., 758:753 Lozupone and Knight, 2005. Appl. Env. Microb., 71:8228 Mitchell et al., 2008. Biof., Biop., Bioref., 2:530 Moore (PI), 2012. CenUSA Factor Analysis Plot Protocol Parrish and Fike, 2005. Crit. Rev. Plant Sci., 24:423 Schloss et al., 2009. Appl. Environ. Microbiol., 75:7537 Staley et al., 2014. Front. Microbiol., 5:524 Vogel et al., 2014. J. Plant Reg. doi:10.3198/jpr2013.12.0076crc

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