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Productivity and community composition change in prairie biomass feedstocks

Kathleen Loretta Madsen
University of Northern Iowa

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PRODUCTIVITY AND COMMUNITY COMPOSITION CHANGE IN PRAIRIE BIOMASS FEEDSTOCKS

An Abstract of a Thesis
Submitted
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Kathleen Loretta Madsen
University of Northern Iowa
May 2020

ABSTRACT

With increasing concerns of climate change and resource usage, there is a growing interest in diverse prairie biomass as a form of alternative energy. In this study, we look at four potential biomass feedstock treatments that were chosen specifically to target high biomass yields and consist of native Iowa tallgrass prairie species. The four feedstocks consist of a one-species monoculture of C_4 switchgrass (*Panicum virgatum* L.), a 5-species mixture of C_4-grasses, a 16-species mixture of C3 and C_4-grasses, forbs, and legumes, and a 32-species mixture of C3 and C_4-grasses, sedges, forbs, and legumes. Four replicate plots (0.33-0.56 ha each) of each feedstock were planted on three different soil types; the Flagler sandy loam (sand), Waukee loam (loam), and the Spillville-Coland complex (clay) for a total of 48 plots. We assessed productivity by harvesting all plant material in ten randomly located 0.3 m^2 quadrats within each plot and sorted the biomass into five functional groups (C_4-grasses, C3, forb, legume, and unseeded species). All four feedstock mixes produced similar biomass yields and are viable mixes for a high-diversity feedstock. The 32-species treatment produced significantly more biomass than the 5-species treatment. Soil type was correlated to both productivity and community composition. Each feedstock had variable yields depending on soil type. The 16- and 32-species community composition was affected by year. The 16- and 32-species treatments shifted from forb and legume dominant to C_4-grasses and unseeded species dominant across the duration of the study. The Switchgrass monoculture had a higher percentage of unseeded species than the other three treatments. These results indicate that feedstocks

should be selected based on site specific parameters in order to maximize productivity yield.

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This Study by: Kathleen Loretta Madsen

Entitled: Productivity and Community Composition Change in Prairie Biomass Feedstocks

has been approved as meeting the thesis requirement for the

Degree of Master of Science

Date Dr. Ai Wen, Chair Thesis Committee

Date Dr. Kenneth Elgersma, Thesis Committee Member

Date Dr. Laura Jackson, Thesis Committee Member

Date Dr. Jennifer Waldron, Dean of Graduate College

DEDICATION

I’d like to dedicate this paper to my husband and best friend, Grant Brinkmeyer, for always being by my side and encouraging me to further my education.

ACKNOWLEDGEMENTS

I would like to thank Dr. Ai Wen, Dr. Kenneth Elgersma, Dr. Laura Jackson, and Dr. Mark Sherrard for all their input and advice for this study. Field work was made possible with the help of undergraduates Sarah Madsen, Mason Severson, Thomas Beckett, and Selicia Parker. Special thanks to my fellow graduate students M. Corinne Myers, MJ Lashbrook, and Alec Glidden for their help in field work and support throughout this study. I would also like to thank my husband, family, and friends for the constant support and encouragement. Finally, thank you to the Tallgrass Prairie Center and the University of Northern Iowa's Biology Department for supporting this project, and the Iowa Nutrient Research Center for funding.

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CHAPTER ONE

PRODUCTIVITY OF HIGH-DIVERSITY PRAIRIE BIOMASS FEEDSTOCK

Introduction

In 2007, the United States (US) Congress passed the Energy Independence and Security Act to enhance production of clean renewable fuels. The Act mandated an increase in biofuel production from approximately 4.7 billion gallons in 2007 to 36 billion gallons in 2022. Corn is the main feedstock used for producing ethanol fuel in the US, but there are several environmental disadvantages to producing corn for ethanol. The first is land use competition. Land used to produce ethanol cannot be used to produce food or provide any other ecosystem services (Tilman *et al*., 2006; Robertson *et al*., 2017; Lautala *et al*., 2015). The second is the high input requirements associated with growing corn (e.g., water, fertilizer, herbicides, and pesticides), which can deplete groundwater reserves and reduce water quality (Robertson *et al.*, 2017). These shortcomings have sparked interest in alternative biofuel feedstocks, such as switchgrass (*Panicum virgatum* L.) and *Miscanthus* (*Miscanthus x giganteus*). Switchgrass and *Miscanthus* are highly productive, require fewer inputs than corn, and can be grown on marginal agricultural land (Sanderson & Adler, 2008; Lewandowski & Schmidt, 2006). Another viable biofuel feedstock, particularly in the Midwestern United States, is a high-diversity mixture of native perennial prairie plants (Tilman *et al.* 2006; Zhang *et al*., 2018). High-diversity perennial mixtures are also highly productive but should enhance other ecosystem services to a greater extent than perennial monoculture feedstocks (Tilman *et al.,* 2006*;* Zilverberg *et al*., 2014; Abernathy *et al*., 2016).

In addition to biofuel production, high-diversity perennial mixtures provide numerous ecosystem services on the landscape (Skevas *et al*., 2016; Robertson *et al*., 2017). Perennial plantings sequester high amounts of carbon in their roots and soil, reducing the carbon debt of establishing or maintaining these plantings (Zilverberg *et al.*, 2014; Yang *et al.*, 2018). Their dense root systems reduce runoff and erosion, which increases water quality and decreases nutrient leaching (Randall *et al.*, 1997; Yang *et al.*, 2018; Oates *et al.*, 2016). While perennial monoculture feedstocks can struggle in highly degraded soils (Tilman *et al.*, 2006), high-diversity perennial mixtures can actually improve degraded soil by increasing soil organic carbon stocks (McLauchlan *et al.*, 2006). High-diversity perennial mixtures also increase microbial genetic diversity to a greater extent than switchgrass (Watrud *et al.*, 2013; Robertson *et al*., 2017). High-diversity perennial mixtures also provide superior habitat for birds (Myers *et al.*, 2015) and resources for pollinators (Myers *et al*., 2012) than perennial monocultures. These ecosystem services enhance the overall value of high-diversity mixtures as a biofuel feedstock.

The best location for high-diversity prairie biofuel feedstock would likely be on marginal agricultural land. Strategically installing diverse perennial feedstocks on marginal agricultural land would reduce competition with food production (Tilman *et al*., 2006; Robertson *et al*., 2017; Zhang *et al.*, 2018) and reestablish highly endangered tallgrass prairie on the landscape. Since 1990, 30% of abandoned US cropland is considered marginal agricultural land. This could be ideal land for high-diversity biofuel production provided the landowner is willing and located reasonably close to a lignocellulosic bioethanol facility (Robertson *et al.*, 2017). Greater production of high-diversity bioenergy would alleviate pressure on bioethanol facilities in years with low monoculture feedstock productivity. Further, by varying harvest times, the use of different bioenergy crops would reduce transportation and storage costs (Maung *et al.*, 2013). Delaying harvest of high-diversity feedstocks would also optimize their value as wildlife and pollinator habitat (Myers *et al.*, 2012, 2015; Zilverberg *et al.*, 2014; Yang *et al.,* 2018), and further reduce fertilizer requirements for the feedstock (Heaton *et al.,* 2009, Glover *et al.,* 2010).

Much of the evidence supporting the use of high-diversity perennial mixtures for bioenergy comes from diversity-ecosystem function studies. These studies have consistently demonstrated that high-diversity mixtures produce more biomass than low-diversity mixtures (Cardinale *et al.*, 2007*;* Tilman *et al.*, 2006; Fornara & Tilman, 2009). One mechanism for this pattern is that high-diversity mixtures have greater niche differentiation (mechanisms summarized in Fornara & Tilman, 2009). High-diversity mixtures display greater phenological, physiological, and morphological (e.g., plant height, root depth and density) variability than monocultures. This variability reduces resource competition and increases nutrient acquisition and uptake (Postma & Lynch, 2012). Another mechanism for this pattern is that high-diversity mixtures have greater facilitation. The inclusion of legumes in high-diversity mixtures increases nitrogen (N) availability for C_4-grasses and other functional groups within the community (Fornara & Tilman, 2009), thereby reducing the need for N fertilizer (Jarchow & Liebman, 2013; Sanderson & Adler, 2008). Legumes also increase soil water retention, which further enhances uptake of ammonium (NH_4^+) and nitrate (NO_3^-) within the community (Fornara & Tilman, 2009).

There may be issues, however, with relating the results of diversity ecosystem function studies to a bioenergy context. The design of most diversity-ecosystem function studies consists of randomized species assemblages grown in small, hand-weeded plots (e.g., Cedar Creek Ecosystem Science Reserve and Jena biodiversity experiment). More research is needed to determine whether the results of diversity-ecosystem function studies translate to realistic bioenergy feedstock assemblages grown at a production-level scale (Zilverberg *et al.*, 2014; Abernathy *et al.*, 2016; Lee *et al.,* 2018). Many of these studies are conducted for only a small time frame. A meta-analysis conducted by Cardinale *et al*. (2007) summarized 44 experiments that looked at the relationship between species diversity and production. The overarching conclusion was that diversity affects change over long periods of time, and the variability in observed results may be explained by differing experimental timelines. On average, polycultures had higher yields than monocultures, but it can take between two and five growing seasons for polycultures to begin out-producing monocultures. This was attributed to complementarity between species and functional groups that increased over time (Cardinale *et al.* 2007; Fornara & Tilman, 2009).

We present data from years six to ten of a long-term diversity-productivity experiment. In the study, we compared biomass production of four potential perennial biomass feedstocks (a one-species switchgrass monoculture, a 5-species mixture of C_4-grasses, a 16-species mixture of C_3-grasses, C_4-grasses, forbs, and legumes, and a 32-

species mixture of C_3-grasses, C_4-grasses, forbs, legumes, and sedges; see Methods for details). Productivity was compared on three different soil types (Flagler sandy loam, Waukee loam, Spillville-Coland complex) (Soil Survey Staff, 2013). In years one to five of this study, we found differences in productivity between feedstocks (the switchgrass monoculture was more productive than the 5-species feedstock but not more productive than the 16- and 32-species feedstocks) and soil types (the Waukee loam soil was more productive than the Spilleville-Coland soil, which was more productive than the Flagler sandy loam soil) (Abernathy *et al.*, 2016). In this study, we predicted similar differences in productivity between feedstocks and soil types as Abernathy *et al.*, 2016 but also, that the complementarity effects of the 16- and 32- species feedstocks will lead to increased biomass production than there was in years one to five.

Methods

Site History

This study was conducted at the Cedar River Ecological Research Site (CRERS) in Black Hawk County, Iowa, USA (N 43.3861, W -92.22241). The site is owned by the Black Hawk County Conservation Board and was leased out for soybean and corn production when the 40 ha plot was first purchased. In 2009, the research site was established by the University of Northern Iowa's Tallgrass Prairie Center to study the ecosystem services of prairie biomass feedstocks. Four different feedstocks of varying diversity were chosen specifically to target high yields ideal within production plots. These consisted of only native Iowa tallgrass prairie species. The four feedstocks are a one-species monoculture of C_4 switchgrass (*Panicum virgatum* L.), a 5-species mixture of C_4-grasses, a 16-species mixture of C_3- and C_4-grasses, forbs, and legumes, and a 32-species mixture of C_3- and C_4-grasses, sedges, forbs, and legumes (Table 1). The site consists of three different soil types; the Flagler sandy loam (sand), Waukee loam (loam), and the Spillville-Coland complex (clay) (Table 2). Four replicate plots (0.33-0.56 ha each) of each feedstock were planted on each of the three soil types for a total of 48 plots (Figure 1).

Table 1: Species list of each diversity treatment. (Modified from Abernathy, 2015)

Scientific Name	**Common Name**	**FG**	**1**	**5**	**16**	**32**
Panicum virgatum	switchgrass	C_4 grass	X	X	X	X
Andropogon gerardii	big bluestem	C_4 grass		X	X	X
Bouteloua curtipendula	side-oats grama	C_4 grass		X	X	X
Schizachyrium scoparium	little bluestem	C_4 grass		X	X	X
Sorghastrum nutans	indian grass	C_4 grass		X	X	X
Elymus canadensis	canada wildrye	C_3 grass			X	X
Elymus virginicus	virginia wildrye	C_3 grass			X	X
Agropyron smithii	western wheatgrass	C_3 grass			X	X
Sporobolus compositus	tall dropseed	C_4 grass				X
Carex bicknellii	copper shouldered oval sedge	sedge				X
Carex brevior	plains oval sedge	sedge				X
Carex gravida	long-awned bracted sedge	sedge				X
Dalea purpurea	purple prairie clover	legume				X
Desmodium canadense	showy tick-trefoil	legume			X	X
Helianthus grosseserratus	sawtooth sunflower	forb			X	X
Heliopsis helianthoides	oxe-eye sunflower	forb			X	X
Lespedeza capitata	round-headed bush clover	legume			X	X
Oligoneuron rigidum	stiff goldenrod	forb			X	X
Phlox pilosa	prairie phlox	forb				X
Ratibida pinnata	grey headed coneflower	forb			X	X
Astragalus canadensis	milk vetch	legume			X	X
Silphium laciniatum	compass plant	forb			X	X
Amorpha canescens	leadplant	legume				X
Artemisia ludoviciana	prairie sage	forb				X
Baptisia leucantha	white wild indigo	legume				X
Echinacea pallida	pale purple coneflower	forb				X
Erynigium yuccifolium	rattlesnake master	forb				X
Monarda fistulosa	wild bergamot	forb				X
Symphyotrichum laevae	smooth blue aster	forb				X
Symphyotrichum novae angliae	new england aster	forb				X
Tradescantia bracteata	prairie spiderwort	forb				X
Zizia aurea	golden alexander	forb				X

FG = functional group; ‘X’ indicates inclusion

Table 2: Chemical and physical properties of the three soil types. (Soil Survey Staff 2013). SOC, TN, P, K, and C/N are courtesy of Cynthia Cambardella (2008). (Modified from Abernathy, 2015)

Soil Type	Percent Clay	Percent Sand	Percent Silt	TN	SOC	C/N	P	K	CSR
sand	9.6	72.5	17.9	1.44b	14.24b	9.88b	99.4a	153a	50
loam	11.8	70.2	18.0	2.14a	23.37a	10.93a	57.9b	146a	79
clay	21.7	41.2	37.1	2.29a	24.90a	10.86a	85.0a	154a	60

TN = Total Nitrogen (g kg^{-1}); SOC = Soil Organic Carbon (g kg^{-1}); C/N = Carbon:Nitrogen; P = Phosphorous (mg kg-1); K = Potassium (mg kg-1); CSR = Corn Suitability Rating

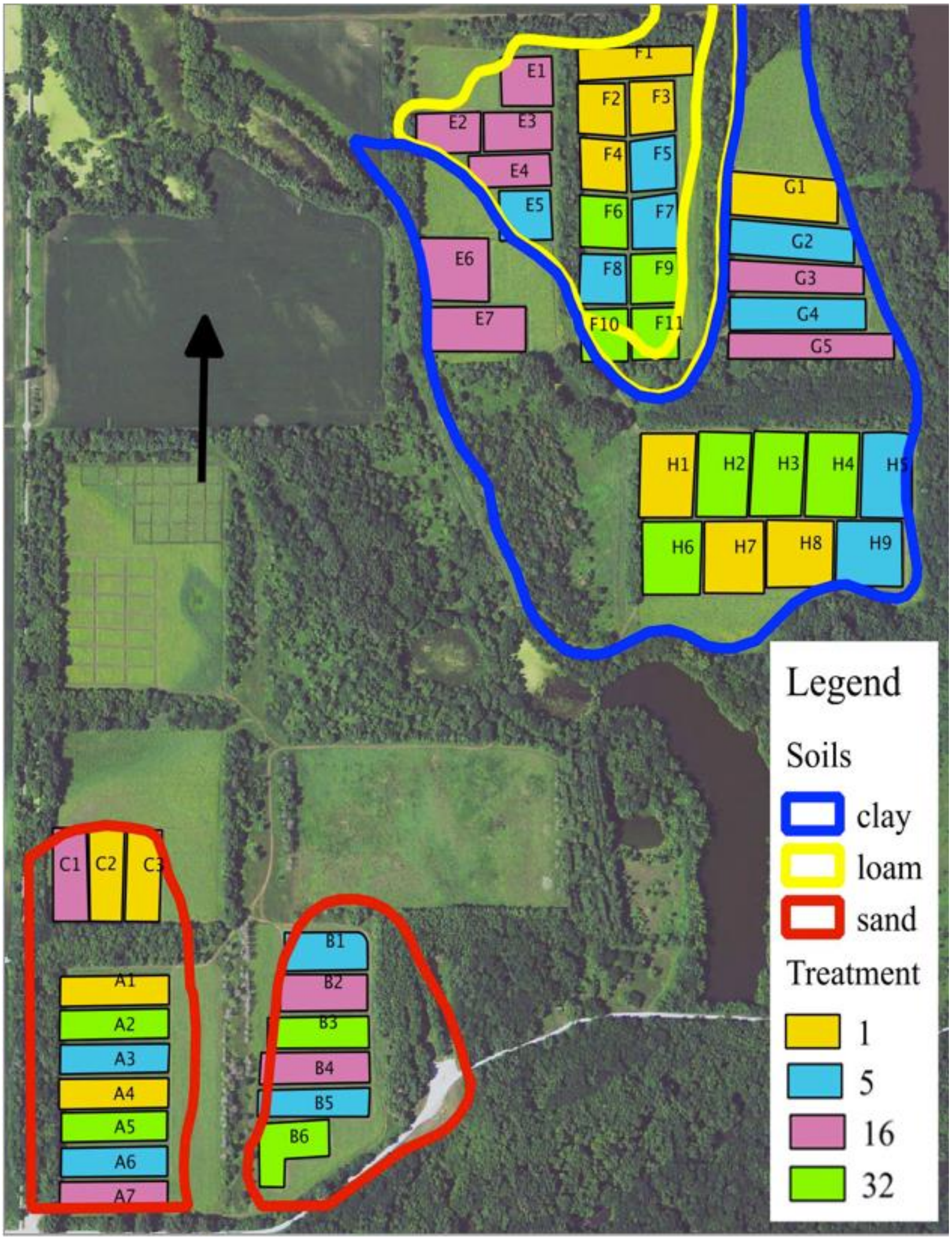

Figure 1: Site map located in Black Hawk County, IA. Fields are labeled by letter and the plots within each field are labeled by number sequentially from north to south. Soil data was acquired from Soil Survey Staff (2013) and the base map from Iowa State University Geographic Information Systems Support & Research Facility (2014). (Modified from Abernathy, 2015)

During the study period, this site went through a series of disturbances, both intentional (burning and haying) and unintentional (flooding) (Table 3). Burning and haying cycles were determined using suggestions listed in Smith *et al.*, (2010). Plots were burned in years 2011 and 2014. Plots were hayed every three years (2012, 2015, and 2018) to model regular management that could occur at a Conservation Reserve Program (CRP) site if contracted before 2010 (USDA-FSA, 2011). Due to site location being in proximity to the Cedar River, flooding was a common occurrence. Flooding can hinder prairie establishment and increase unseeded species abundance in many prairie plantings (McIndoe *et al.*, 2008). This became an issue for years 2013 and 2014 in the northern clay and loam plots. Flooding occurred from May to June during the growing season of each year for these plots, as well as the fall of 2016 and 2018.

Table 3: Site management and sampling modified from Abernathy, 2015. Establishment mowing and burning are consistent with prairie reconstruction management (Smith *et al*. 2010).

Year	**Month**	**Management, disturbance, and sampling**
2009		
	May	Treatments planted
	June	Establishment mowing
2010		
		No management
2011		
	Spring	All plots burned
	Fall	A7 hayed
2012		
	Spring	All plots hayed except A7
	July	Start of drought
2013		
	May – June	Clay and loam soil flooded
2014		
	Spring	All plots burned
	June – July	Clay and loam soils flooded
2015		
	Spring	All plots hayed
2016		
		No management
	September	Clay and loam flooded
2017		
		No management
2018		
	September	Clay and loam flood
	Fall	All plots hayed
2019		
		No management

Average temperature during the growing season (April to October) was calculated using data from the nearest recording weather station, Waterloo Municipal Airport, IA

US (Elevation: 264.6 m, 42.5544 N, -92.4011 W), using NOAA's website (NCDC, 2020). Average temperature across all ten years in this area was 17.1˚ C. Precipitation data was downloaded from the nearest recording weather station Waterloo 1.9 SSE, IA US1IABH0006 (elevation: 278 m, 42.4650 N, -92.3410 W). Average rainfall during the ten years of this study was 811.5 mm (Figure 2). The year 2012 was a drought year for this site and both 2013 and 2014 experienced flooding in the northern plots (Figure 1, loam and clay soils).

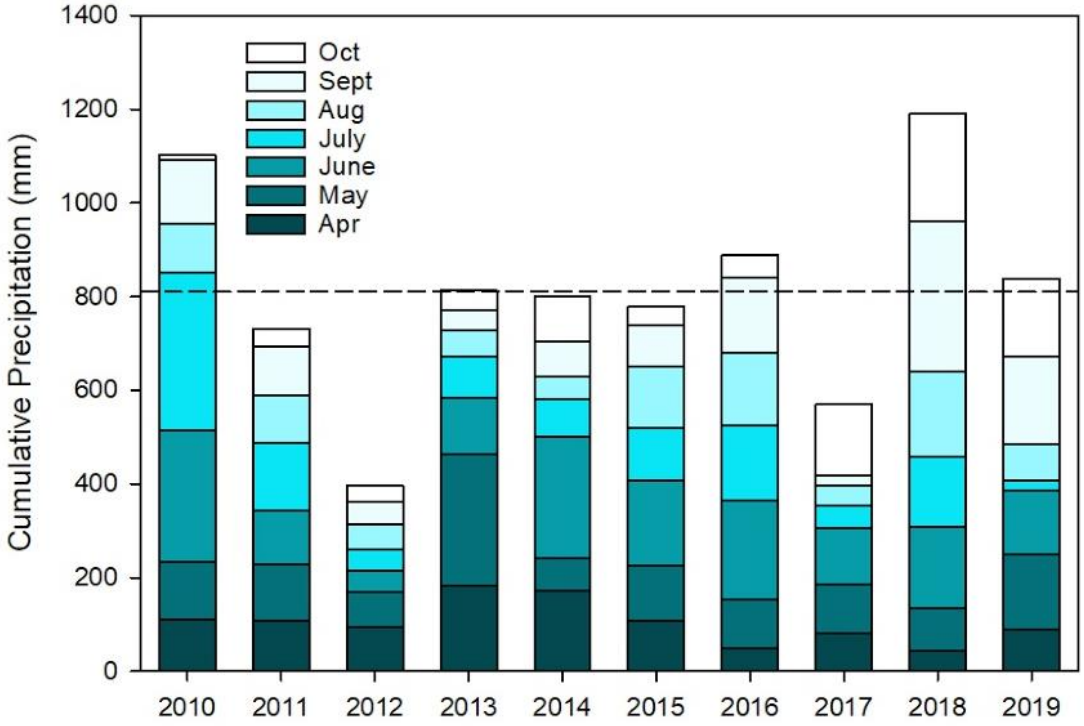

Figure 2: Cumulative precipitation of the growing season (April-October) for each of the ten years of this study. Average cumulative precipitation over ten years is shown by the dashed line at 811.5 mm.

Experimental Design

Sampling was completed for all 48 plots between August and November from 2010 to 2019. Ten random quadrats were sampled within each plot and all standing biomass was cut to ground level. In 2018, only 5 quadrats were sampled in each plot due to reduced manual labor during harvest season. During the first three years (2010-2012) quadrat size was 0.1 m^2 and was increased to 0.3 m^2 for all following years. This increased the amount of total biomass harvested during the collection process. Each quadrat was dried to a constant mass at a minimum of 65°C for 72 hours and then weighed in grams. Biomass within each quadrat was separated into functional groups for the years 2010-2016 and 2019. These functional groups consisted of C_4-grasses, C_3-grasses, forbs, legumes, and unseeded species. The unseeded species functional group included all forb, legume, C_3-grasses, etc. collected during harvest that were not a part of a feedstocks seed mix. Biomass weighed for each quadrat was summed for each plot and converted to megagrams per hectare within each year. All functional groups, including unseeded species, were included in total biomass calculations, simulating actual harvest of biomass feedstocks.

Statistical Analysis

To assess the overall effect of the predictor variables (year, soil, and feedstock) on productivity within the experiment a repeated measures Analysis of Variance (ANOVA) was used. Treatment and soil were set as factors and year was set as the repeated measures. Plot was nested in year as a random effect. An ANOVA was used to test the significance of interactions between two or more independent variables. All plots were treated independently of each other in this analysis. Packages "nlme" and "multicomp" were used during statistical calculations in R (version 1.2.5033). A Q-Q plot was constructed to assess normality for the total productivity across all ten years. Data that were not normally distributed were log-transformed to meet the assumption of normality. Model residuals were assessed by constructing a plot with the residuals from this ANOVA. A Tukey HSD post hoc test was run to assess what specific groups were causing the overall significance of the ANOVA. Cook's Distance was used to identify any highly influential points within the data and none were found.

Variance was calculated for each plot over the ten year study. A two-way ANOVA was run to assess the effects of treatment and soil type and their interaction on feedstock resilience. Soil and treatment were set as factors. Boxplots were constructed and a Tukey HSD post hoc test was run to assess what specific groups within treatment and soil were causing the overall significance.

Results

We predicted similar differences in productivity between feedstocks and soil types as Abernathy *et al.*, 2016; we also predicted the complementarity effects of the 16- and 32- species feedstocks would be more apparent, producing more biomass, than they were in years one to five. Feedstock, soil type, and year were found to be significant (Table 4). Differences between the three soil types were driven by both the loam and clay soil types. Loam (7.46 Mg/ha/yr) and clay (7.41 Mg/ha/yr) had significantly higher productivity than sand soil (6.38 Mg/ha/yr) (Figure 3). The time factor (year) was significant due to the early establishment years (first six years of the study 2010-2015) with higher biomass yield (Figure 4). Year 2011 (10.96 Mg/ha) had significantly higher productivity than every year other than 2013 (8.64 MG/ha) and 2015 (8.39 Mg/ha). Both 2013 and 2015 had significantly higher productivity than 2012 (5.81 Mg/ha), 2016 (5.90 Mg/ha), 2017 (6.14 Mg/ha), 2018 (4.78 Mg/ha), and 2019 (6.38 Mg/ha), but were not significantly different from each other. Year 2018 (4.78 Mg/ha) produced significantly less productivity than all other years except 2017 (6.14 Mg/ha). The significant feedstock treatment effect was due to the 5- and 32-species treatments (Figure 5). The 32-species mix (7.47 Mg/ha/yr) was significantly more productive than the 5-species mix (6.4 Mg/ha/yr). The 16-species mix (7.32 Mg/ha/yr) was marginally more productive than the 5-species mix (6.4 Mg/ha/yr). The switchgrass monoculture (7.13 Mg/ha/yr) was not significantly different from any other feedstock.

Table 4: ANOVA table reporting differences in productivity (Mg/ha) between three factors (treatments, soil types, and years) and their interactions. Year was used as the repeated measure.

	d.f.	denDF	MS	F
Year	9	323	51.171	<0.0001***
Feedstock	3	36	9.575	<0.0001***
Soil	2	36	16.154	0.0001**
Year × Feedstock	27	323	3.146	<0.0001***
Year × Soil	18	323	11.746	<0.0001***
Feedstock × Soil	6	36	5.253	0.0006**
Feedstock × Soil × Year	54	323	1.073	0.3481

***p<0.001;**p<0.01;*p<0.05

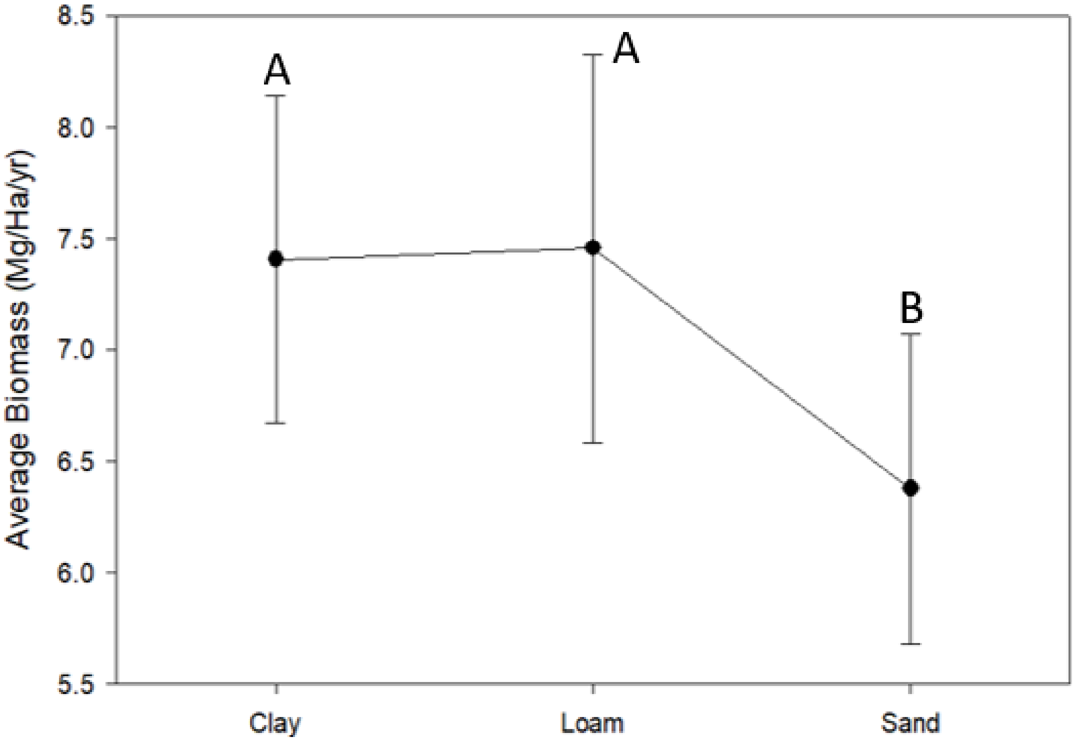

Figure 3: Average biomass of each soil type (means +/- 1SE). Different letters indicate significant difference between soil types.

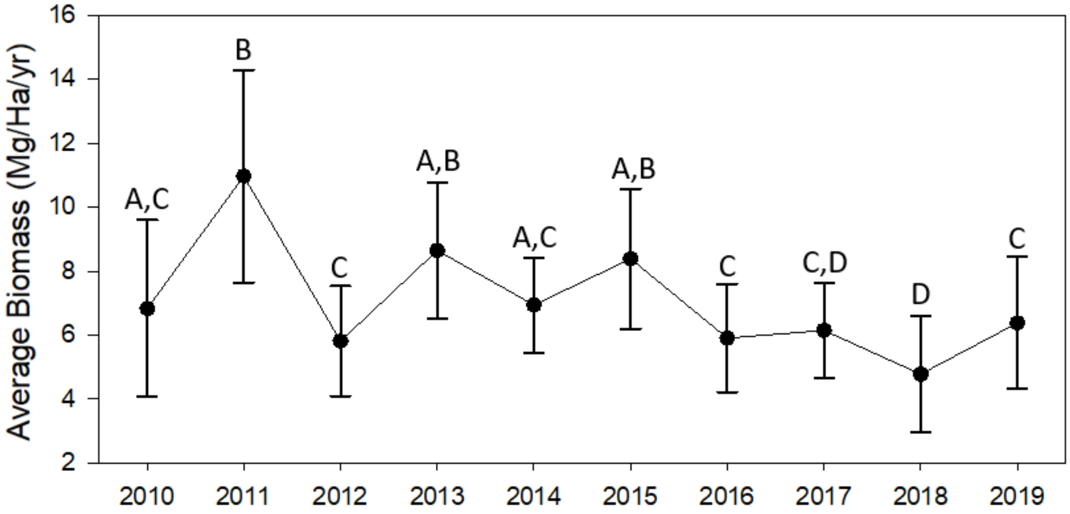

Figure 4: Average yearly biomass at CRERS (means +/- 1SE). Different letters indicate significant difference between years.

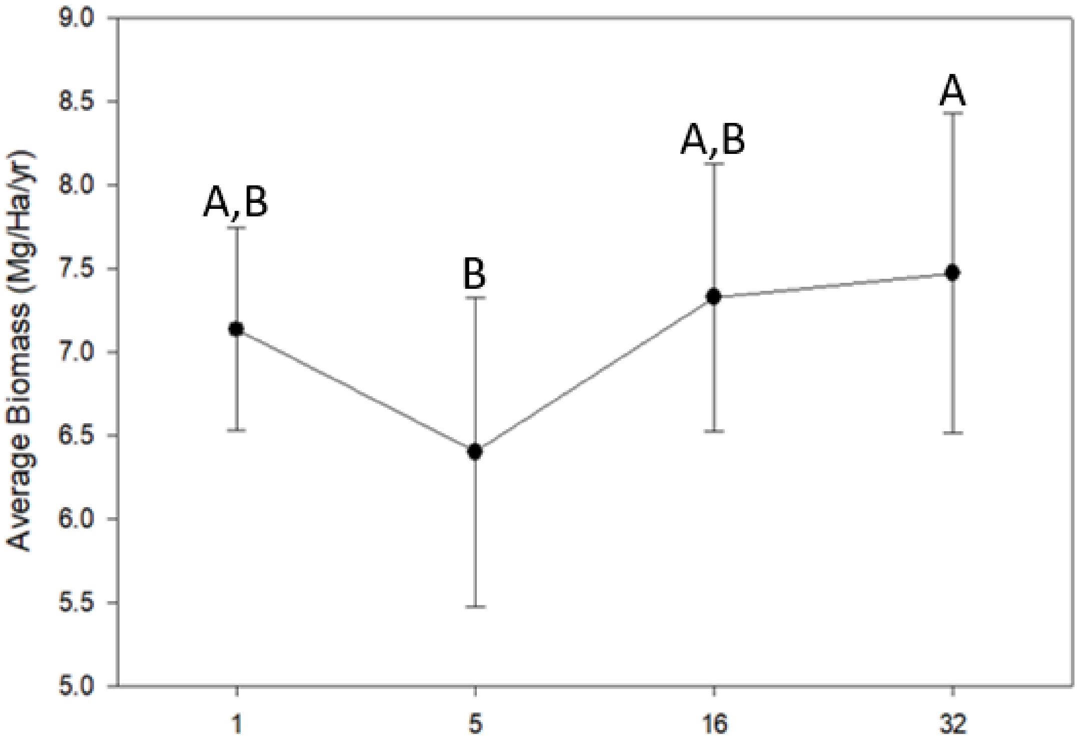

Figure 5: Average biomass of each feedstock type (means +/- 1SE). Different letters indicate significant difference between feedstocks.

We predicted to see an increase in complementarity effects within the 16- and 32-species feedstocks by observing an increase in biomass yield in the last five years of the study. We observed the opposite, a decrease in biomass yield for three of the four treatments (Figure 6 (a-c)). The 32-species feedstock had relatively similar yearly average biomass yield across all ten years (excluding years 2011 which had a very high average in all four treatments). We observed the highest average biomass yields in year 2011 and the lowest average biomass yields in year 2018 for all four feedstocks.

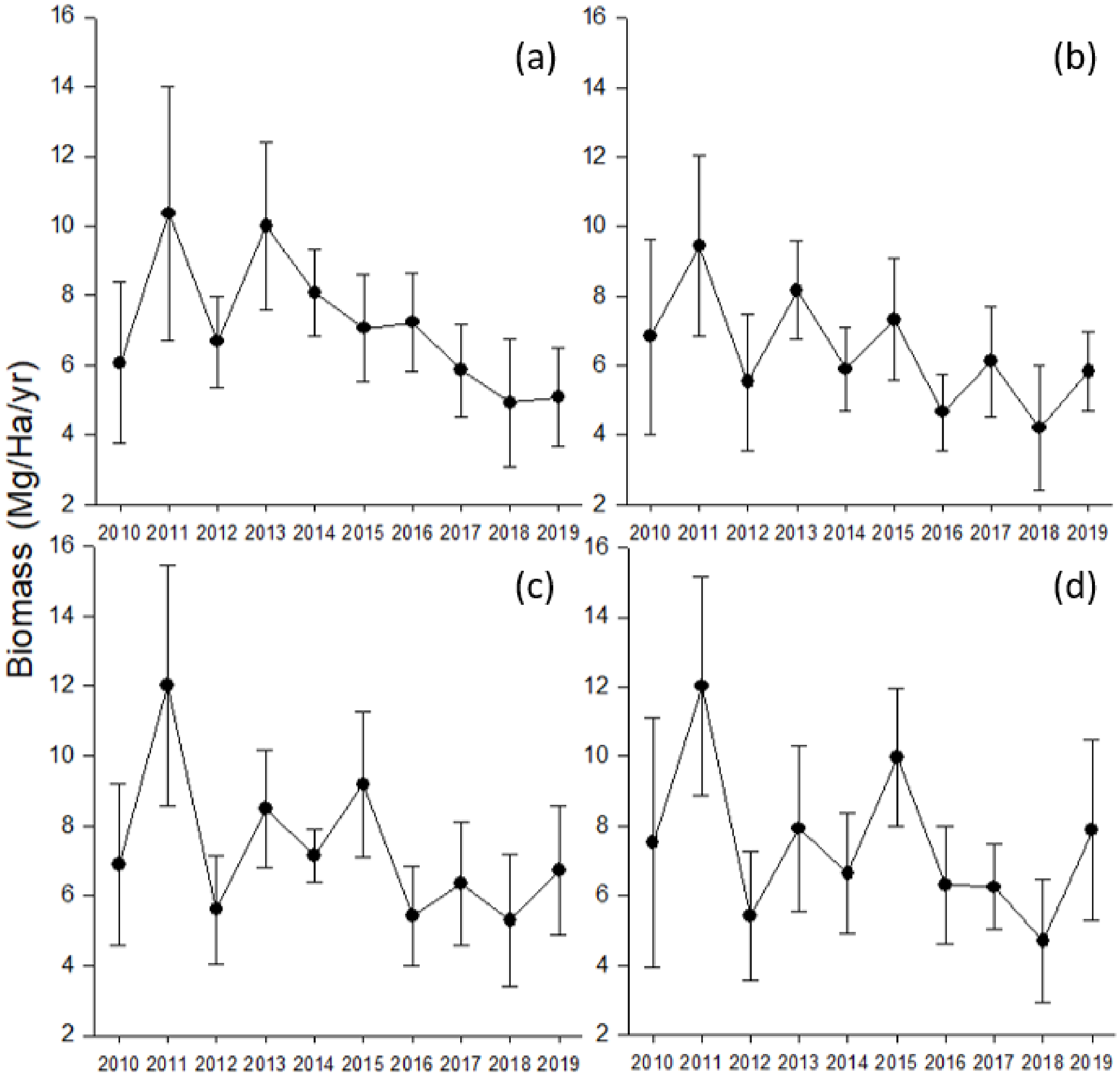

Figure 6: Average yearly biomass of each of the four treatments (means +/- 1SE). (a), (b), (c), and (d) correspond to switchgrass, 5-species, 16-species, and 32- species treatments respectively.

Figure 7 (a-d) compares all biomass collected over ten years of each treatment on each of the three soil types. Switchgrass had the highest productivity on the clay soil (54.74 Mg/ha) but less on both the loam and sand (Figure 7(a), 50.10 Mg/ha and 47.70

Mg/ha respectively). The 5-species treatment produced very similar biomass yields for both loam and clay (49.33 Mg/ha and 49.76 Mg/ha respectively) but produced significantly less biomass on the sandy soil (Figure 7(b), 37.5 Mg/ha). The 16-species mix produced similar biomass amounts for both loam and sand (46.68 Mg/ha and 48.18 Mg/ha respectively), but had significantly higher productivity on the clay soil (Figure 7(c), 54.06 Mg/ha). Finally, the 32-species mix produced more biomass on the loam and clay soils (57.00 Mg/ha and 52.74 Mg/ha respectively), but was significantly less productive on the sandy soil (Figure 7(d), 47.80 Mg/ha). Looking at the cumulative productivity of each soil type (Figure 8(a-c)), we see that the sandy soil produced similar biomass for the switchgrass, 16-species, and 32-species (47.70 Mg/ha, 48.18 Mg/ha, and 47.80 Mg/ha respectively), but significantly lower productivity from the 5-species treatment (Figure 8(a), 37.50 Mg/ha). The loam soil has higher productivity in the 32-species treatment (57.00 Mg/ha) than the other three treatments (Figure 8(b), 50.10 Mg/ha, 49.33 Mg/ha, and 46.68 Mg/ha for the switchgrass, 5-species, and 16-species treatment respectively). Finally, the clay soil produced relatively similar amounts of biomass for all four feedstocks (Figure 8(c), 54.74 Mg/ha, 49.76 Mg/ha, 54.06 Mg/ha, and 52.74 Mg/ha for the switchgrass, 5-species, 16-species, and 32-species mix respectively).

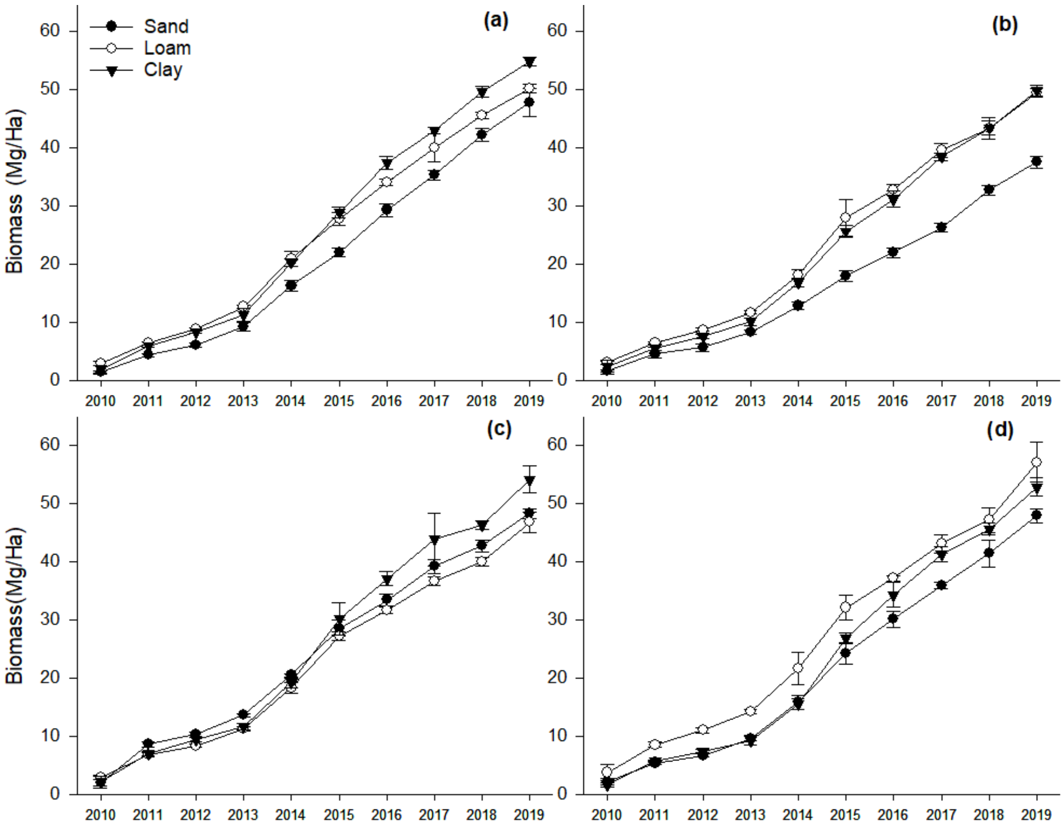

Figure 7: Total cumulative biomass values of each soil type between the four treatments (means +/- 1SE). (a), (b), (c), and (d) correspond to switchgrass, 5-species, 16-species, and 32- species treatments respectively.

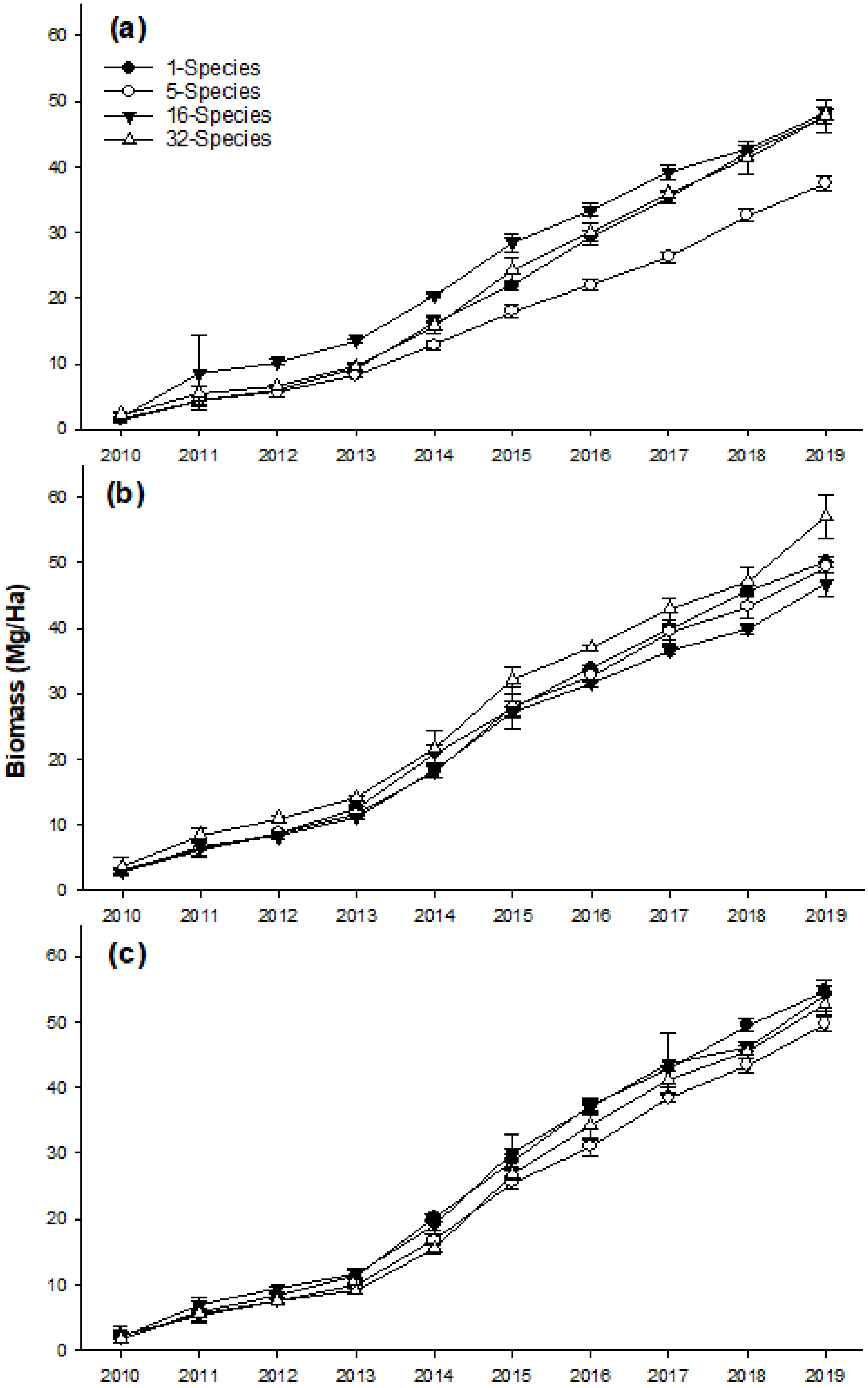

Figure 8: Total cumulative biomass values of each soil type between the three soil types (means +/- 1SE). (a), (b), and (c) correspond to Sand, Loam, and Clay respectively.

Soil and feedstock treatment had a significant effect on the variance within each plot (Table 5). The variance among 32-species treatment plots was significantly higher than both the switchgrass monoculture and 5-species treatment, but not significantly different from the 16-species treatment (Figure 9). The variance among 16-species treatment plots was only significantly higher than the 5-species treatment. The 5-species and switchgrass monoculture were not significantly different from each other. The sand soil was significantly different from both the loam and clay soils, which were not significantly different from each other.

Table 5: ANOVA table reporting differences in variance (Mg/ha/yr^2) between two factors (treatments and soil types) and their interactions.

	d.f.	SS	MS	PR(>F)
Soil	2	3.272	1.0905	<0.0001***
Feedstock	3	4.092	2.0459	<0.0001***
Soil × Feedstock	6	2.302	0.3837	0.0036**
Residuals	36	3.453	0.0959	

***p<0.001;**p<0.01;*p<0.05

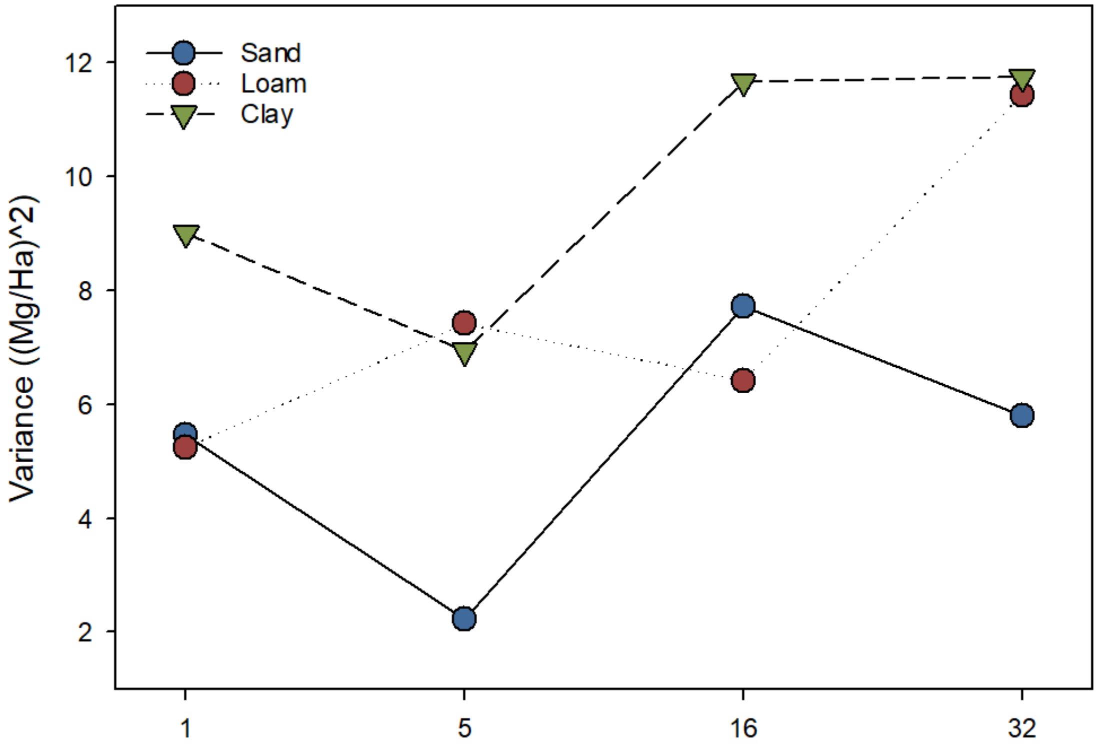

Figure 9: Variance of the four treatments on each soil type.

Discussion

We analyzed the effects of species diversity on productivity, and did not find any significant correlation between the two, except a significant difference in productivity between the 32- and 5-species feedstock. No other feedstocks were significantly different from each other. Previous studies using plot-scale randomized plant assemblages have shown a strong correlation between productivity and diversity; however, our findings using operational-scale designed feedstocks did not replicate these results (Cardinale *et al.*, 2007*;* Tilman *et al.*, 2006; Fornara & Tilman, 2009). Observing a significantly higher productivity yield in the 32- and 16-species feedstocks than in the switchgrass monoculture would have more closely followed the findings of these previous studies. One explanation for why we may not have seen a positive correlation between productivity and diversity is the difference in methods in our study when compared to other diversity/productivity studies. In those other studies, species were chosen for their increased productivity potential at harvest rather than at random (Tilman *et al.*, 2006). Our results are more in line with previous experiments that looked at productivity and diversity using realistic feedstock mixes (Zilverberg *et al.*, 2014; Robertson *et al*., 2017; Abernathy *et al.*, 2016). These studies found that there was no correlation between diversity and productivity. Another explanation for why we may not have seen a positive correlation between productivity and diversity could be the abundance of unseeded species in the switchgrass monoculture (see chapter two: Figure 15 and Figure 16). With such a high unseeded species abundance, we hypothesize that the switchgrass treatment may be behaving more like a polyculture than a monoculture and benefitting from services provided by invading C_3-grasses, forbs, and legumes. Another explanation may be the flooding that occurred in 2013 and 2014. Switchgrass is highly resistant to pressure from flooding and low nutrients (Sanderson & Reed, 2000). This would allow the switchgrass monocultures plots to remain productive in these years, when other treatments may have not been allowing for greater biomass production.

We did not observe any complementarity effects in any of the four treatments. Both the 16- and 32-species treatments did not have an increase in biomass yield in the last five years of the study. Previous studies have found that polycultures had higher biomass yields than monocultures after 5 years of growing. The studies have attributed this increase to complementarity effects between species and functional groups within the polycultures (Cardinale *et al.* 2007; Fornara & Tilman, 2009). One reason why we may not have observed complementarity within these high-diversity mixtures may be the irregularity of disturbance at the site. The drop in biomass yield that occurs in the last five years of this study coincides with the decrease in disturbances at the site after year 2014. This may indicate a larger influence of disturbance on productivity than complementarity.

The 5-species treatment had significantly lower productivity than the 32-species treatment. On average it had the lowest productivity of all other feedstocks. One explanation may be the species composition of this feedstock. This treatment consists of five C_4-grasses which deplete the nitrogen, phosphorus, and potassium in the soil faster than the other three feedstock mixes (Sherrard *et al*., 2019). Both the 16- and 32-species treatments have additional functional groups, including legumes. Legumes help retain carbon, nitrogen, and water in the ground for other species to absorb (Fornara & Tilman, 2009). The 5-species and the switchgrass feedstocks lack this added cross species relationship. So why then did the switchgrass monoculture do so much better on average than the 5-species feedstock? One benefit of the multi-species feedstocks may be increased invasion resistance (Fargione & Tilman, 2005; Picasso *et al.*, 2008; Abernathy *et al.*, 2016). Nearly 20.5% of the average biomass of the switchgrass monoculture was unseeded species, which is high when compared to the 5-species mix (2.7% unseeded species) and the other feedstocks (32-species: 5.6%, 16-species: 5.5%). The nearly 10-fold greater unseeded species biomass in the switchgrass monoculture may help explain its high productivity relative to the 5-species mixture. This hypothesis will be explored further in chapter two. Though we did not observe any significant increase in productivity with a high diversity feedstock, similar to the findings of Robertson *et al.*, (2017), there was no observed penalty either. Growing high-diversity feedstock still produces similar yields as a currently used alternative biofuel feedstock, switchgrass. A high-diversity feedstock would have the added benefits of enhanced ecosystem services and resilience (Tilman *et al.*, 2006*;* Zilverberg *et al*., 2014; Abernathy *et al*., 2016).

The sand soil was found to have a lower variance than the other two soil types. This soil type was also never flooded like the clay and loam. The flooding that occurred on the clay and loam soil may explain the lower resilience of these plots. We observed lower resilience in the 16- and 32-species treatments compared to the switchgrass monoculture and 5-species treatment, which was demonstrated by the relatively larger variance in the 16- and 32-species treatments (Figure 9). This suggests, surprisingly, a greater resistance to change of yearly biomass production in the 5-species and switchgrass treatments. Other studies have found the opposite trend of increased resilience as diversity increases and more functional groups are present (Loreau *et al.*, 2001; Berg *et al.*, 2012; Isbell *et al*., 2015). One reason for our results may be the irregularity of disturbance at the site or the time frame of the experiment. The 5-species treatment and switchgrass treatment had lower variance the two high-diversity mixes. These findings mirror several other studies results, concluding diversity does not increase resilience (Kreyling *et al.*, 2008; Grime, 1998). We concluded that resilience cannot be explained alone by the species diversity in the treatment, but also needs to include disturbance in consideration (Loreau & Behera, 1999; Craine *et al.*, 2013).

Differences were not only observed between feedstocks, but also between the three soil types. The 5-species and the 32-species treatments accumulated the least amount of biomass on the sand soil type. The 16-species and switchgrass treatments had increased productivity on the clay soil. These results suggest that tailoring feedstock mixes to sites may be the best method to maximize productivity. For instance, if a biofuel production site had a similar soil composition to the loam soil of this study, selecting the 32-species treatment would potentially bring about the highest biomass yield. Increased yields may be caused by initial soil fertility (Fornara &Tilman, 2009). However, the effect of initial soil fertility is known to lessen throughout time. Another reasoning behind the observed yield differences between feedstocks may be the composition of the soil. A previous study, Myers *et al.*, 2015, found that the loam and clay soil at this site have the highest amounts of both macro- and micronutrients. These two soil types also produced taller vegetation and had increased forb cover. In contrast, the sand soil contained less nutrients and produced shorter, patchier vegetation (Myers *et al.*, 2015). The texture of the sand soil allows for faster leaching of soil nutrients causing strain on these treatments, especially the 5-species treatments that lack legumes to help replenish lost nitrogen (Scherer-Lorenzen *et al.*, 2003; Fornara & Tilman, 2009). The soil texture would also lead to lower soil moisture in the sand soil compared to the other two soil types. Overall, we found that feedstock productivity was highly influenced by soil type. Our findings suggest that feedstock mixes should be tailored to site specific characteristics and species could promote high productivity and complementary relationships to increase biomass yield while minimizing unseeded species invasion.

CHAPTER TWO

COMMUNITY COMPOSITION CHANGE OF HIGH-DIVERSITY PRAIRIE BIOMASS FEEDSTOCK

Introduction

Loss of biodiversity is a main driver of the rapid change in ecosystems today (Hooper *et al.,* 2012). North American tallgrass prairie is one of the most endangered ecosystems in the world, with less than 0.1% of pre-settlement prairie remaining (Smith, 1998). With such alarming amounts of habitat loss within this ecosystem, prairie restorations have become increasingly important. One form of restoration, high-diversity biomass feedstock, has become a large area of interest due to its biofuel potential and ecosystem services (Tilman *et al.,* 2006*;* Zilverberg *et al*., 2014; Abernathy *et al*., 2016). Biomass feedstocks are plantings of native cellulosic or lignocellulosic crop that can be used to make biofuel. Current biofuel production comes from two main sources; monoculture crops (corn, soybean, switchgrass, etc.) and waste biomass (straw, corn stover, and waste wood) (Kim & Dale, 2004). However, there have been many recent studies looking at the production and ecosystem services of a high-diversity prairie biomass feedstock (Tilman *et al.,* 2006*;* Zilverberg *et al*., 2014; Abernathy *et al*., 2016; Myers *et al.,* 2015). Currently, Switchgrass (*Panicum virgatum* L.) is the leading lignocellulosic biofuel feedstock due to its high productivity in monoculture plantings and its wide range of tolerance for environmental conditions. These plantings have some ecological values, but fall short when compared to the environmental benefits of high-diversity prairie polycultures (Robertson *et al*., 2011). Current studies on high-diversity feedstock are focused on productivity outcomes, and very few studies to date have examined compositional shifts in these polycultures over time (Stahlheber *et al*., 2016). Compositional shifts may be important as feedstock crops can be in operation for long periods of time. In addition, some studies have found a positive correlation between aboveground productivity and species diversity within a polyculture (Lambers *et al.*, 2004; Isbell *et al.*, 2011; Isbell *et al*., 2015; Tilman *et al.*, 2006; Fornara & Tilman, 2009). Furthermore, plant functional groups play a key role in the productivity of a polyculture (Polley *et al.*, 2013).

The tallgrass prairie is normally characterized by an abundance of dominant perennial grasses and sparsely distributed satellite species that fill the remaining space (Gotelli & Simberloff, 1987). In both remnant and restored prairies, succession is constantly occurring through time. Dornbush, (2004) looked at a remnant prairie located in Pocahontas County, Iowa, U.S. This prairie reserve had a reduction of unseeded species and shifts in species abundance of forbs over a fifty year time span. Schramm, (1990) described succession within recently established prairie restorations. Succession was split into four stages (Schramm, 1990), detailing the change in composition and diversity of established prairies while time progresses. Unseeded species reduction and shifts in forb diversity were observed and perennial grass dominance increased after the second or third year of establishment. The ratio of grass:forb is very important in a restoration seed mix, with increased grass cover leading to lower weed invasion and decreased erosion (Meissen *et al.*, 2019). Overall, prairie restorations are able to restore soil processes and other ecological services, but may not fully capture the diversity and community composition of a remnant prairie (Baer *et al*., 2003; Polley *et al*., 2005). In general, prairie composition within restorations increase in perennial grass cover and decrease in unseeded species dominance (Camill *et al*., 2004; Gibson *et al*., 2013; Olechnowski *et al*., 2009; Wagle & Gowda, 2018). Legume dominance should increase as time passes and promote growth of C_4-grasses (Piper *et al*., 2007). These shifts in community composition through time affect the homogeneity of prairie restoration and similar patterns should be seen in a high-diversity prairie feedstock. Homogeneity of these feedstocks is important for processing the biomass at crop specific bioethanol facilities (Zhang *et al.,* 2018). Changes in diversity within high-diversity prairie feedstocks are most notably attributed to fertilization and haying management regimes. Fertilization has been found to increase productivity, but decrease the overall diversity of a feedstock (Wilson & Tilman, 1993; Wagle & Gowda, 2018). Haying has varying effects on community composition depending on time and frequency of occurrence (Stahlheber *et al.*, 2016). In general, haying increases species diversity and decreases the dominance of perennial grasses (Wagle & Gowda, 2018).

A common issue for both prairie restorations and biomass feedstocks is unseeded species invasion. With increased unseeded species dominance, there is an increase in management requirements that can pose an economic risk for growers (Miesel *et al.*, 2012). Miesel *et al.*, 2012 found that increasing unseeded species dominance within feedstocks has a negative correlation to biomass yield. This supports the use of high-diversity feedstocks due to a reduction in labor cost for unseeded species removal, when compared to monocultures (Zhang *et al.*, 2018). As stated above, the current lignocelluslosic biofuel commonly used is switchgrass. Switchgrass requires a focus on unseeded species control and preemptive management (Sanderson *et al.,* 2006). This is especially true for the first few years of establishment (Minelli & Venturi, 2004). Furthermore, biofuel facilities process crops by species classification (softwood, grass, etc.), and variability can cause a negative impact on ethanol yield if not originally accounted for (Zhang *et al.,* 2018). This means unseeded species in a switchgrass biofuel crop could cause a reduction in ethanol yield. In comparison, polycultures have an increased invasion resistance to unseeded species when compared to monocultures like switchgrass (Fargione & Tilman, 2005; Picasso *et al.,* 2008; Abernathy *et al.,* 2016). This has been attributed to increased spatial use, resource use, and increased biomass production making it more difficult for invaders to establish (Kennedy *et al.,* 2002; Abernathy *et al.,* 2016; Fargione & Tilman, 2005). In particular, the presence of diverse functional groups has been found to enhance invasion resistance within a community (Pokorny *et al.,* 2005). However, these results have been disputed with some studies finding the opposite, species-poor communities resisting invasion better than more diverse ones (Robinson *et al.,* 1995; Stohlgren *et al.,* 1999).

We present the results of this decade-long study analyzing community composition change of two high-diversity feedstock treatments (a 16-species mixture of C_3-grasses, C_4-grasses, forbs, and legumes, and a 32-species mixture of C_3-grasses, C_4-grasses, forbs, legumes, and sedges) across three soil types (Flagler sandy loam, Waukee loam, Spillville-Coland complex) (Soil Survey Staff, 2013). We calculated unseeded species composition in all four high-diversity treatments. Unseeded species are defined as species that were not planted, regardless of whether it is a forb or a grass, or whether native or exotic. A previous analysis found that within the first five years of this study, the composition of the two treatments changed with time. The 16- and 32-species treatments differed between soil types as well (Abernathy *et al.*, 2016). The switchgrass monoculture had the highest percentage of unseeded species in comparison to the other three treatments. We predict to see the same trends as Abernathy *et al.*, 2016 regarding soil influence on community composition. We predict to see a compositional change in functional groups from year one to year ten, with an increase in both perennial grasses and legumes. Switchgrass will continue to produce large unseeded species biomass yields compared to the other three treatments.

Methods

For experimental setup and site history, refer to the Site History and Experimental Design sections in Chapter one of this thesis. The switchgrass monoculture and 5-species plots were excluded from this portion of the analysis due to the presence of only two functional groups, C4-grasses and unseeded species, within these plots. A total of 24 plots was analyzed with the two treatments (16- and 32- species mix) replicated four times on each of the three soil types (sand, loam, and clay) within each year. After harvesting, the plants were sorted into functional groups-- C_3-grass, C_4-grass, forb, legume and unseeded species for years 2010-2016 and year 2019. Biomass was not sorted in 2017 and 2018 due to reduced manual labor during harvest season. As in Chapter one, the ten quadrats sampled within each plot were summed and converted to megagrams per hectare.

Statistical Analysis

Non-metric Multidimensional Scaling (NMDS) was used to assess community composition change in both the 16- and 32-species plots over ten years. A Permutational Multivariate Analysis of Variance (PERMANOVA) was run for both the 16- and 32-species feedstocks; year and soil were set as factors. A PERMANOVA was used to fit linear models to distance matrices; and here the PERMANOVA model examined whether the functional group composition varied among years and soil types using the Adonis function in the "vegan" package for R (Oksanen *et al.,* 2019). I utilized the NMDS plots of both the 16- and 32-species mixes separated by soil type (sand, loam, and clay) to visualize the ten year succession. The number of dimensions for each NMDS model was selected by using the stress levels. Stress is the measure of difference between the observed dissimilarity and the true dissimilarity. This measures how well the model matches the ranks of the actual data (Kruskal, 1964). Stress < 10 is considered excellent while 10 < stress < 20 is considered good (Kruskal, 1964). Stress calculated below 20 were considered acceptable and two dimensions were used for all NMDS models. The function "metaMDS" in the R package "vegan" (version 2.5-6) was used to run these models and 200 iterations were used to select the best NMDS result (Oksanen *et al.,* 2019).

Results

We predicted to observe similar trends as Abernathy *et al.*, 2016 regarding soil influence on community composition. We expected to see a change in functional group composition from year one to year ten, and a continued increase in both perennial grasses and legumes. The PERMANOVA analysis revealed that functional group composition changed overtime in both the 16- and 32-species treatments (Table 6 and Table 7). The 16-species treatment's functional group composition significantly changed across year, soil type, and with the interaction of both these factor (Table 6). The interaction between soil and year (R^2= 0.14346) had a greater influence on community composition change than the soil interaction by itself (R^2= 0.03459), but year had a stronger influence than both of these (R^2= 0.61043). Compositional change over time was driven by increased planted forb and legume species in the early establishment years that declined in the remaining seven years. Years 2014 to 2019 shifted towards C_4-grasses, unseeded species, and C_3-grasses (Figure 10). NMDS analyses for the 16-species treatment subset by soil type show the difference in community compositional shifts across the three soil types (Figure 11). All three soil types were strongly influenced by the interaction with year, with clear directional change in community composition through time and very little overlap. The sand soil had less change through time than the other two soil types (Figure 11). The 32-species treatment's functional group composition significantly changed across year, soil type, and with the interaction of both of these factor (Table 6). The interaction between soil and year (R^2= 0.10433) had a greater influence on community composition change than the soil interaction by itself (R^2= 0.02592), but year had a stronger influence then both of these (R^2= 0.63631). In the 32-species plots the significance for year was driven by the community shift from forb, C_3-grasses, and legume to C_4-grasses and unseeded species (Figure 12). NMDS analysis for the 32-species treatment subset by soil type shows the difference in community compositional shifts across the three soil types. Both the loam and clay soils were strongly influenced by the interaction with year, with clear directional change in community composition through time and very little overlap. The sand soil had less of a visible trajectory, meaning the interaction between year and soil was less apparent on this soil type (Figure 13).

Table 6: PERMANOVA analysis shows significant changes in community composition of the 16-species feedstock of both factors (soil types and years) and their interactions.

	d.f.	Sum of Sqs	R^2	F	Pr(>F)
Soil	2	0.4344	0.03459	5.8873	0.001***
Year	7	7.6664	0.61043	29.6840	0.001***
Soil × Year	14	1.8017	0.14346	3.4880	0.001***
Residual	72	2.6565	0.21152		
Total	95	12.5589	1.00000		

***$p<0.001$;**$p<0.01$;*$p<0.05$

Table 7: PERMANOVA analysis shows significant changes in community composition of the 32-species feedstock of both factors (soil types and years) and their interactions.

	d.f.	Sum of Sqs	R^2	F	Pr(>F)
Soil	2	0.3371	0.02592	3.9978	0.004**
Year	7	8.2737	0.63631	28.0363	0.001***
Soil × Year	14	1.3566	0.10433	2.2984	0.001***
Residual	72	3.0354	0.23344		
Total	95	13.0027	1.00000		

***$p<0.001$;**$p<0.01$;*$p<0.05$

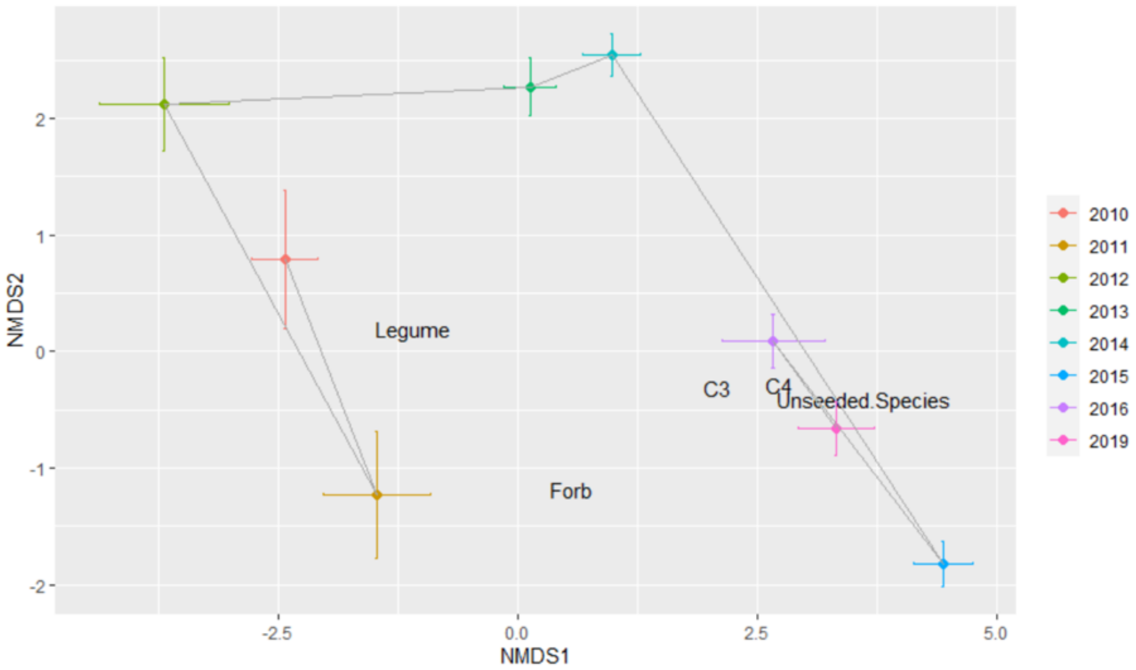

Figure 10: Community composition of the 16-species treatment averaged across soil types. Similarity is displayed by proximity of points (shorter distances between points equate to greater similarity). Each point represents yearly plot averages of NMDS scores in the 16-species treatment (means +/- 1SE). Black texts indicate the contribution of each functional group along NMDS axes; NMDS1 is positively correlated with C_4-grasses, C_3-grasses, and unseeded species, which is also positively correlated to the community composition of later years' measurements (2014-2019).

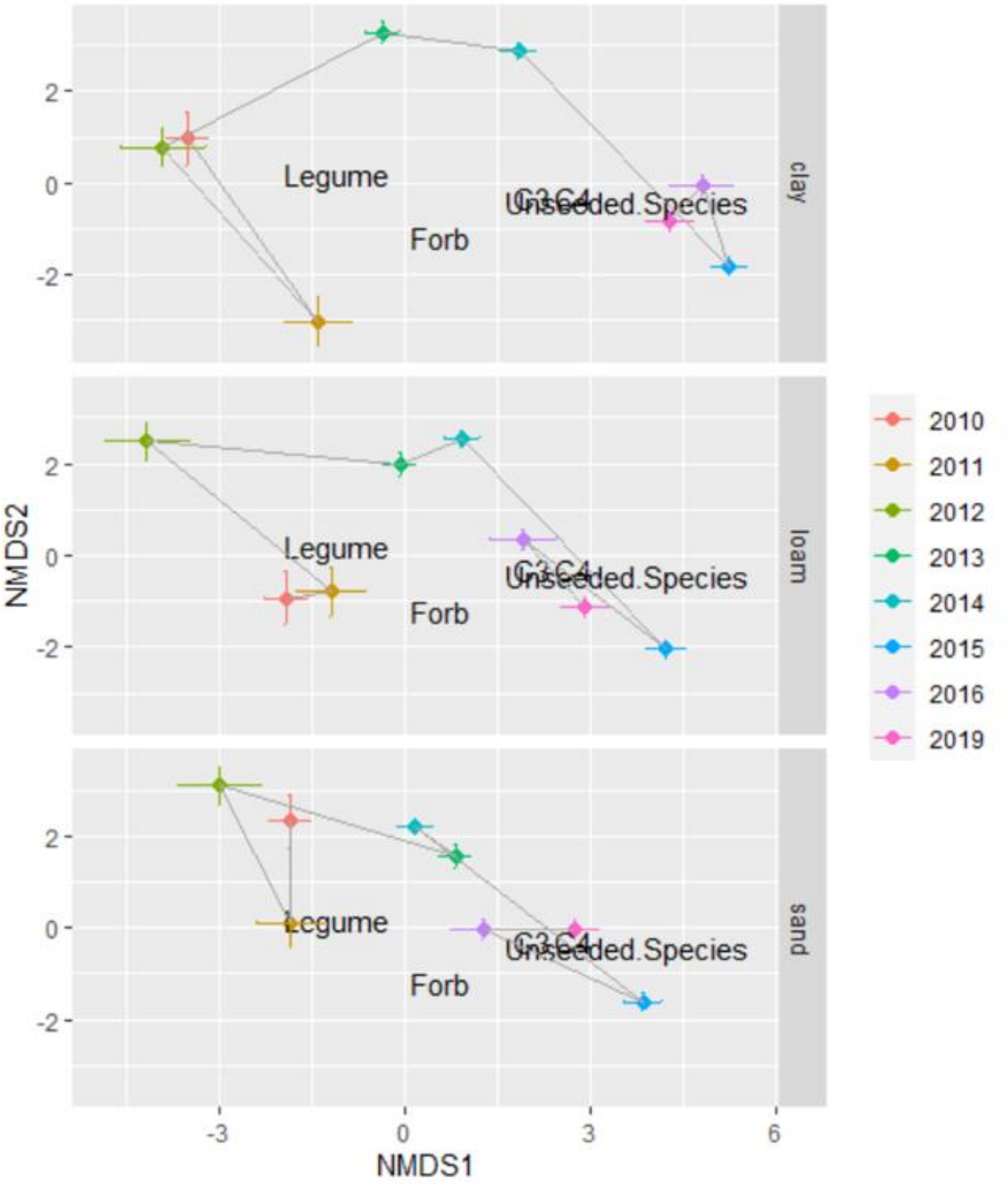

Figure 11: Community composition of the 16-species treatment subset by soil type sand, clay, and loam. Similarity is displayed by proximity of points (shorter distances between points equate to greater similarity). Each point represents yearly plot averages of NMDS scores in the 16-species treatment for each soil type (means +/- 1SE). Black texts indicate the contribution of each functional group along NMDS axes.

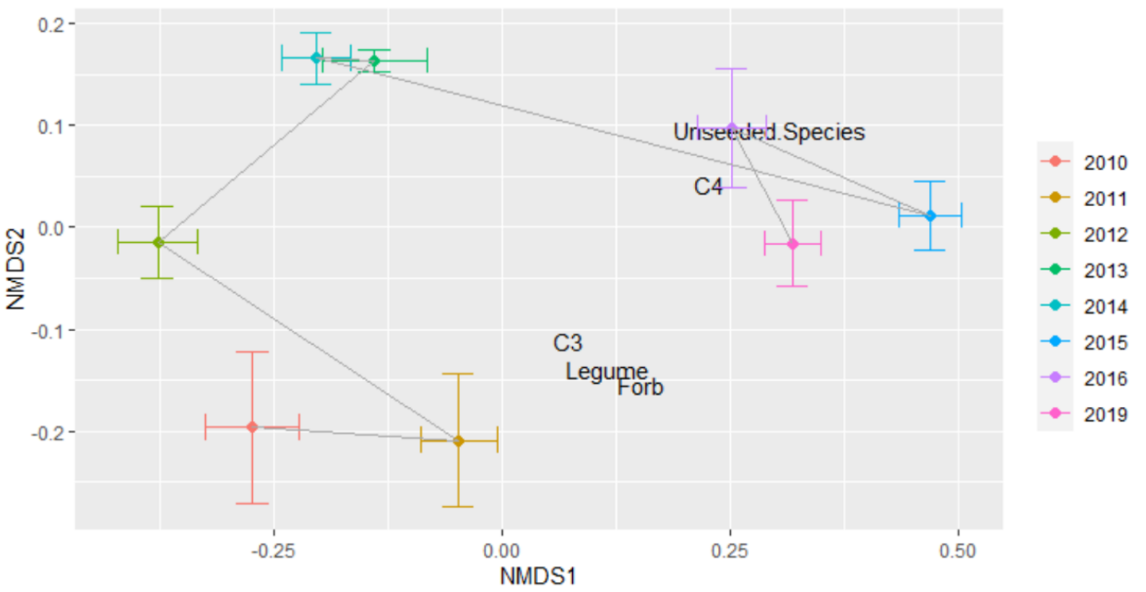

Figure 12: Community composition of the 32-species treatment averaged across soil types. Similarity is displayed by proximity of points (shorter distances between points equate to greater similarity). Each point represents yearly plot averages of NMDS scores in the 32-species treatment (means +/- 1SE). Black texts indicate the contribution of each functional group along NMDS axes; NMDS1 is positively correlated with C_4-grasses and unseeded species, which is also positively correlated to the community composition of later years' measurements (2014-2019).

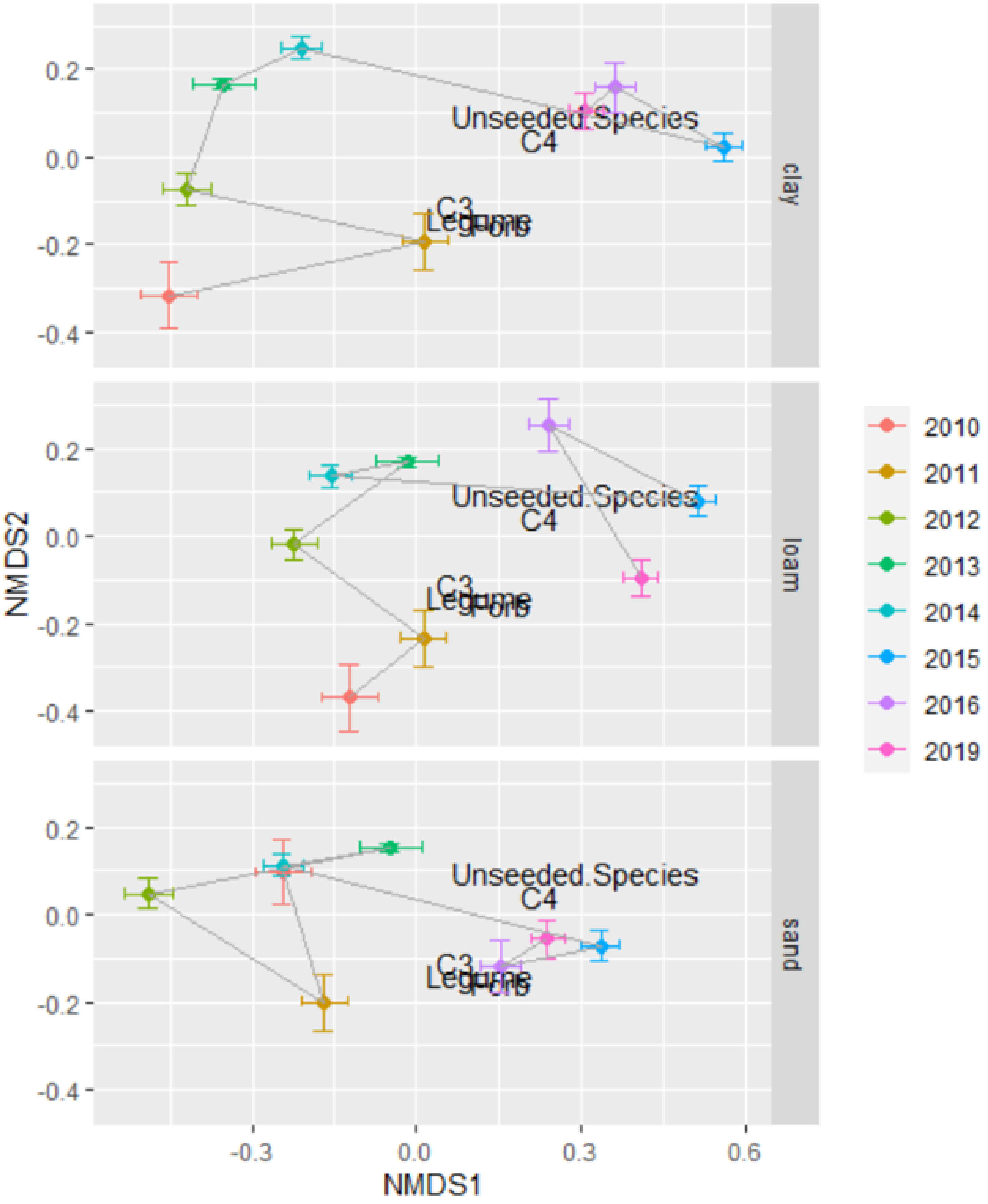

Figure 13: Community composition of the 32-species treatment subset by soil type sand, clay, and loam. Similarity is displayed by proximity of points (shorter distances between points equate to greater similarity). Each point represents yearly plot averages of NMDS scores in the 32-species treatment for each soil type (means +/- 1SE). Black text indicates the contribution of each functional group along the NMDS axes.

We expected to observe higher unseeded species biomass yields in the switchgrass monoculture than the other three treatments. Both the 16- and 32-species treatments were invasion resistant containing only 5.5% and 5.6% of unseeded species respectively within their total average biomass across all ten years. To compare their invasion resistance across all biofuel treatments, we also evaluated the percentage of unseeded species in the monoculture and 5-species treatment. Invasion resistance was highest in the 5-species treatment; only 2.7% of its total biomass was unseeded species. Alternatively, the switchgrass monoculture had a very large percentage of unseeded species, around 20.5% of the total biomass (Figure 14 and Figure 15). When unseeded species were excluded from the biomass calculation, the switchgrass monoculture had the lowest average productivity when all soil types were summed (Figure 14). When each soil type was evaluated separately, the switchgrass monoculture had the lowest productivity in loam soil. In sandy soil, the 5-species treatment had a smaller average than the switchgrass. However, in clay soil, the switchgrass monocultures had the highest biomass average (Figure 14). When the unseeded species group was included in the biomass calculation, in all four feedstock treatments, the unseeded species group had a lower productivity on the sand soil than the loam and the clay (Figure 15). Unseeded species biomass increased with time through this experiment (Figure 16). A relative increase in unseeded species dominance for the switchgrass monoculture occurred in year 2014 and continued for the remaining five years. In the switchgrass monoculture, the average unseeded species biomass exceeded the average C_4-grass biomass in year 2019 (Figure 16 (a)).

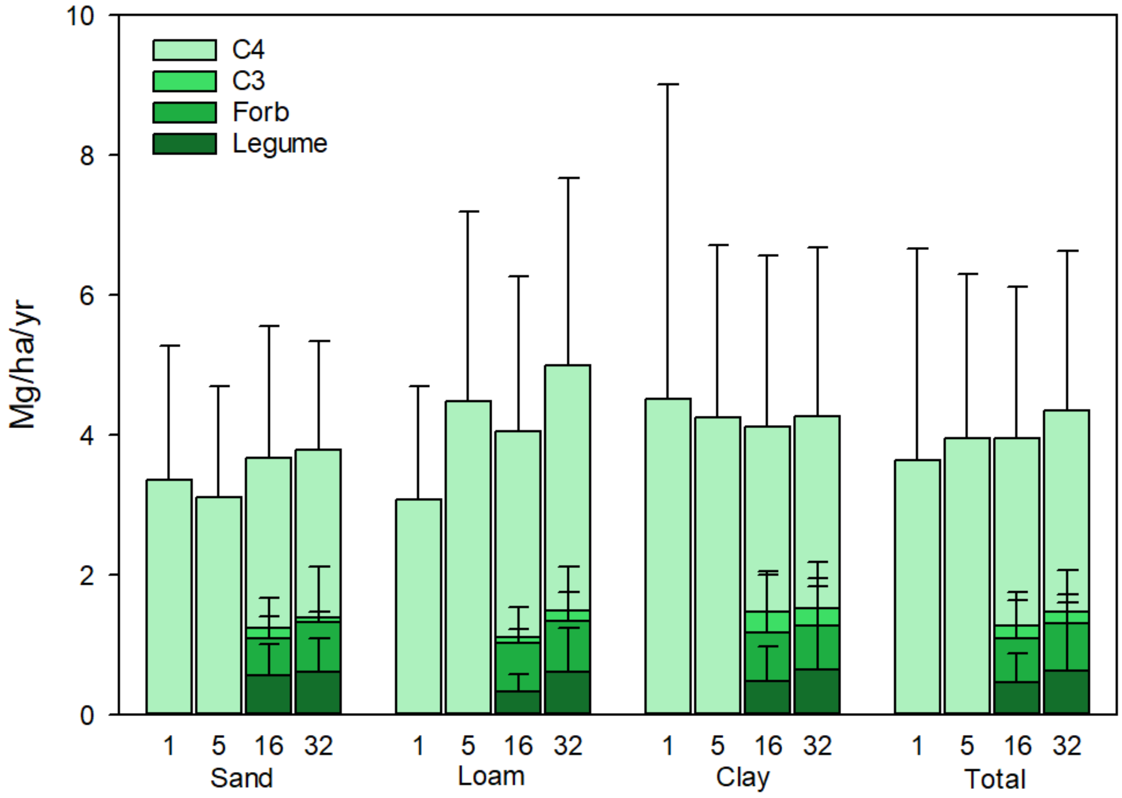

Figure 14: Average biomass of the four feedstock treatments on individual soil type and in total across ten years; unseeded species functional group was excluded from the measurement (ten year means +/- 1SE).

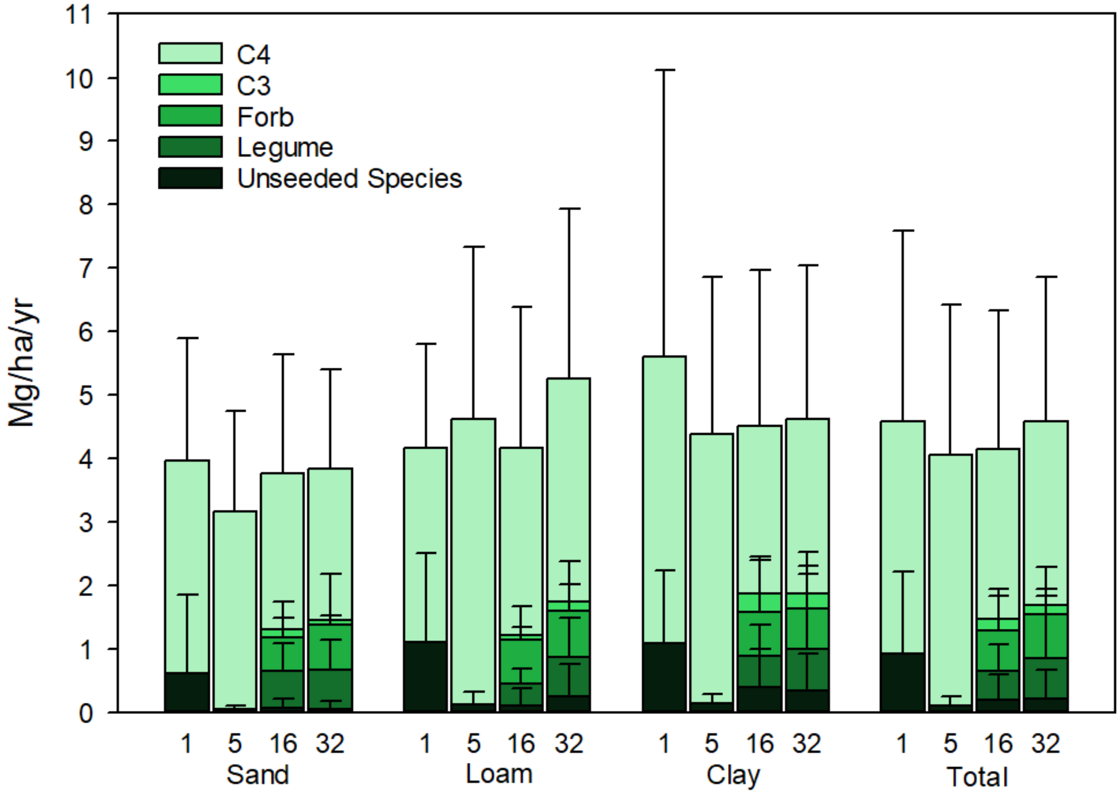

Figure 15: Average biomass of the four feedstock treatments on individual soil type and in total across ten years; all functional groups are included (ten year means +/- 1SE). Across all four feedstock treatments, the unseeded species functional group had the lowest biomass in sand soil compared to loam and clay.

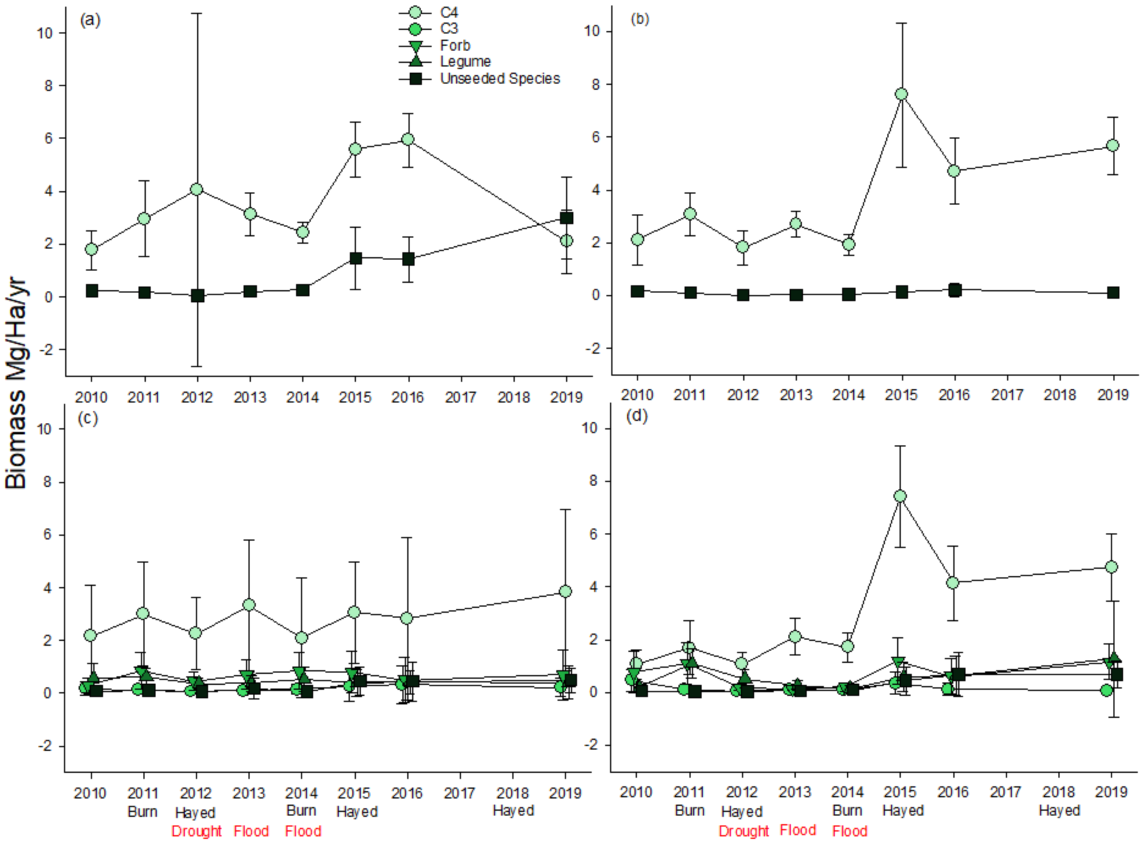

Figure 16: Productivity of the four feedstock treatments, with the five functional groups separated over ten years (ten year means +/- 1SE). Treatments include the switchgrass monoculture (a), 5- (b), 16- (c), and 32-species treatments (d). Management practices are listed below the x-axis, unplanned disturbances are indicated in red.

Discussion

Our results indicate a change in community composition over time in both the 16- and 32-species treatments. The legume and forb functional groups decreased with time, shifting towards the unseeded species and C_4-grass functional groups in the 16- and 32-species treatment. The C_3-grass functional group was higher in the later years (2014-2019) in the 16-species mixture and higher in the establishment years (2010-2013) of the 32-species mixture. Past studies have looked at community composition change of both disturbed and undisturbed plantings. Our site has a history of both planned and unplanned disturbances. We would expect to see a decrease of C_4-grasses and an increase in forb dominance through time due to prescribed burns (Gibson &Hulbert, 1987). The opposite, increasing C_4-grasses and decreasing forb dominance, is a trend we would expect to see in non-disturbed plantings (Gibson *et al.*, 2013; Olechnowski *et al.*, 2009). Strangely enough, we observed similar trends to a non-disturbed prairie in the 16- and 32-species treatments (Camill *et al*., 2004; Gibson *et al.*, 2013; Olechnowski *et al.*, 2009, Lambers *et al.*, 2004). One explanation for this may be the general irregularity and low frequency of disturbance regimes. Other studies focus on one disturbance at a time (i.e. burning would be the only disturbance for one growing season), over the length of our study three different disturbances occurred at varying times and intensities. In 2012 a drought and haying occurred in the same growing season and in 2014 the plots were both burned and flooded. After 2014, disturbances occurring at the site greatly decreased to only two haying events for the last five years.

Soil had an effect on community composition change in both the 16- and 32-species mixes. However, the biggest effect of soil was its interaction with year, meaning the soil's biggest influence is because it changes the trajectory of community composition through time. In general, both mixes decreased in forb and legume functional groups while shifting towards the C_4-grass and unseeded species functional groups across each of the three soil types. Successional changes over time were less dramatic on the sand soil for both mixes. One explanation for this would be the nutrients of these soil types. As stated above, Myers *et al.*, 2015, found that the loam and clay soil have the highest amounts of both macro- and micronutrients. These two soil types also produced taller vegetation, whereas the sandy soil produced shorter, patchier vegetation (Myers *et al.*, 2015). Soil fertility influences prairie species diversity and succession (Bauer *et al.*, 2015; Baer *et al.*, 2003; Baer *et al.*, 2005). This could explain the succession in the sand soil which had very little change in functional group dominance and the loam and clay which had a more apparent change.

The switchgrass monoculture had produced significantly more unseeded species than the other three treatments. The treatment with the highest invasion resistance was the 5-species mix, closely followed by the 16- and 32-species treatment. As stated in chapter one, the 5-species treatment is composed of C_4-grasses which deplete the nitrogen, phosphorus, and potassium in the soil faster than the other three feedstock mixes (Sherrard *et al.*, 2019). This creates a nutrient poor environment that may be harder for unseeded species to invade. The 16-and 32-species treatments have high invasion resistance compared to the switchgrass monoculture. This is in line with previous findings attributing spatial and resource use within polycultures as a deterrent for unseeded species establishment (Kennedy *et al.*, 2002; Abernathy *et al.*, 2016; Fargione & Tilman, 2005). There is an apparent increase in unseeded species biomass after 2014 for the switchgrass monoculture. This is most likely a result of the disturbance irregularity at our site. Though burns are normally healthy for a prairie, flooding can be detrimental to species establishment and increase unseeded species abundance (McIndoe *et al.*, 2008). This may attribute to the increased dominance of unseeded species after 2014 which was a flood year. After 2014, only two more planned disturbances, haying, occurred at the site for the remaining five years. Haying and burning help reduce unseeded species growth in prairie restorations (Harmon-Threatt & Chin, 2016). This quick decrease in disturbance may contribute to the increased unseeded species dominance as well. Many of the unseeded species collected from the switchgrass plots were either non-native species like *Cirsium arvense* or seeded species in the other treatments like *Sorghastrum nutans*. In 2019, the average biomass of unseeded species was higher than the C_4-grass in these plots. However the switchgrass monoculture sill produced similar biomass to the high-diversity treatments. This is most likely attributed to the highly productive species, like *Sorghastum nutans*, that invaded from other high-diversity plots. Our findings suggest a shift towards C4-grass and unseeded species functional groups in high-diversity feedstocks through time and that weed dominance increases as switchgrass monocultures age.

CHAPTER THREE

CONCLUSION

Future Research

Though our studies showed a need for site specific biomass feedstocks, future research still needs to be conducted in this area. For one, a better understanding of soil and feedstock relationships needs to be formed in order to better plan for actual implementation. At the CRERS site specifically, testing nutrient depletion in the soil would help show the long term effects of diversity on soil fertility. This would help detect any positive effects from soil fertility on productivity and what long-term effects may have resulted or still exist (Fornara &Tilman, 2009). Paring this with our community compositional results may help shed light on functional group shifts within each treatment. This would help highlight how functional groups behave on varying soil compositions and fertility (Miles & Knops, 2009).

This study was done on marginal land, with variable disturbances. The site was hayed in 2012, 2015, and 2018 to model the management of biomass productivity sites. However, most biofuel production sites would be hayed once or twice a year. The management of our site could be more comparable to management of a CRP site. Contracts for CRP approved before 2010 can hay once every three years and can be sold as biomass, which is similar to our site's haying regime (USDA-FSA, 2011). This study looked at high diversity feedstocks solely on marginal land. Corn/Ethanol is commonly produced on productive farmland that could be used for food production. Looking at these high diversity feedstocks on productive farmland used for ethanol production could exhibit benefits of planting high-diversity prairie on more than just marginal lands. This would allow a comparison between ethanol and high diversity feedstock productivity on similar production scales.

Conclusion

All four feedstock mixes produced similar biomass yields and are viable mixes for a biomass feedstock. Switchgrass monocultures have a higher percentage of unseeded speciess than the other three feedstocks. Soil type plays an important role in both productivity and community composition. Switchgrass and the 5-species feedstocks had higher resilience than the high-diversity mixtures. This indicates that feedstocks should be selected based on site specific parameters in order to maximize productivity yield.

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