Species composition influences soil nutrient depletion and plantphysiology in prairie agroenergy feedstocks

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Species composition influences soil nutrient depletion and plant physiology in prairie agroenergy feedstocks

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Abstract. High-diversity mixtures of perennial tallgrass prairie vegetation could be useful biomass feedstocks for marginal farmland in the Midwestern United States. These agroenergy crops can help meet cellulosic agrofuel targets while also enhancing other ecosystem services on the landscape. One proposed advantage of high-diversity prairie biomass feedstocks is that they should become nutrient limited at a slower rate than monoculture feedstocks. In this study, we examine rates of soil nutrient depletion and the physiology and performance of a focal species (switchgrass, Panicum virgatum L.) in four prairie agroenergy feedstocks with different species composition and diversity. The feedstocks in this study were a 1-species switchgrass monoculture, 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, forbs, legumes, and sedges. To assess feedstock effects on soil, we measured changes in soil N/P/K over a five-year period. We also performed a greenhouse study, in which we grew switchgrass plants in field soil conditioned by each feedstock. To assess feedstock effects on plant function, we measured four physiological traits (photosynthetic rate, chlorophyll concentration, leaf florescence, leaf N concentration) on switchgrass plants within each feedstock in the field. In the soil analysis, we found that the 5-species feedstock displayed higher rates of soil N/P/K depletion than other feedstocks. In the greenhouse analysis, we found that switchgrass plants grown in soil conditioned by the 5-species feedstock were smaller than plants grown in soil conditioned by other feedstocks. In the physiological analysis, we found that switchgrass plants in the 5-species feedstock had lower leaf N, photosynthesis, chlorophyll concentration, and higher florescence than switchgrass plants growing in other feedstocks. Collectively, our results show that prairie agroenergy feedstocks with different species composition and diversity have different rates of soil nutrient depletion, which influences the physiology and performance of plants within the feedstock. These differences would ultimately impact the ecosystem services (e.g., biomass production, need for fertilizer) that these prairie agroenergy feedstocks provide.

Key words: chlorophyll concentration; leaf N; photosynthesis; prairie agroenergy feedstocks; soil nutrients; switchgrass.

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INTRODUCTION

High-diversity mixtures of perennial tallgrass prairie vegetation could be useful agroenergy feedstocks for marginal farmland in the Midwestern United States (Tilman et al. 2006). Biodiversity–ecosystem function studies suggest that high-diversity perennial feedstocks have higher energy output per unit fossil fuel input than corn grain ethanol and produce more biomass than most perennial monoculture feedstocks (e.g., switchgrass) on marginal farmland (Tilman et al. 2006, Cardinale et al. 2007). Biodiversity–ecosystem function studies also suggest that high-diversity feedstocks retain more nutrients than low-diversity feedstocks, which could potentially lead to slower rates of soil nutrient depletion (Dybziniski et al. 2008, Fornara and Tilman 2009). Rates of soil nutrient depletion have important ecological and economic implications for agroenergy; specifically, feedstocks with higher rates of nutrient depletion will require more fertilizer. However, our understanding of the relationship between diversity, species composition, and soil nutrient dynamics is largely based on experiments involving random species assemblages grown in small, hand-weeded plots. More data are needed to determine whether these results translate to production-scale agroenergy feedstocks with non-random species assemblages.

Diversity could influence rates of soil nutrient depletion in agroenergy feedstocks via complementarity effects (e.g., the extent of niche differentiation and the inclusion/exclusion of legumes; Cardinale et al. 2007, Fargione et al. 2007, Loreau and Hector 2001; mechanisms reviewed in Fornara and Tilman 2009). In brief, high-diversity mixtures are more morphologically (e.g., root depth and architecture) and phenologically variable than low-diversity mixtures (Hooper and Vitousek 1997). This variability can expand the timeframe, depth, and form of mineral nutrient uptake during the growing season and ultimately lead to greater nutrient uptake and lower nutrient losses (McKane et al. 1990, Hooper and Vitousek 1997, Scherer-Lorenzen et al. 2003, Postma and Lynch 2012, Cong et al. 2014). Further, the inclusion of legumes (Bertness and Callaway 1994, Callaway et al. 2003, Hooper and Dukes 2004, Fargione et al. 2007, Temperton et al. 2007) and even certain non-legumes (James and Baldani 2012) in high-diversity mixtures can increase soil N availability for the plant community. If these ecological mechanisms operate in prairie agroenergy crops, then a high-diversity feedstock that includes legumes should deplete soil nutrients at a slower rate than a low-diversity feedstock that lacks legumes.

Examining the physiology and performance of focal plant species within prairie agroenergy feedstocks can provide insight into differences in soil nutrient availability between feedstocks. This approach, known as the phytometer method (Clements and Goldsmith 1924), has been used in several diversity–ecosystem function studies to examine the effects of individual species, or functional groups, on soil properties and plant function. These studies have shown, for example, that the presence of legumes in a community tends to increase the photosynthetic rate, tissue N concentration, total N content, and performance of neighboring non-legumes (Spehn et al. 2002, Temperton et al. 2007, Postma and Lynch 2012). Similarly, the presence of grasses within a community tends to decrease the performance of neighboring non-grasses (Schmidtke et al. 2010). These phytometer studies suggest that the species composition and diversity of a prairie agroenergy feedstock will influence the physiology of plants within those feedstocks, in a manner that reflects the underlying soil impacts of the feedstock.

Since 2010, we have been studying productivity and other ecosystem services in four prairie agroenergy feedstocks with different species composition and diversity at Cedar River Ecological Research Site (CRERS) in Black Hawk County, Iowa, USA. The four feedstocks at CRERS are (1) a switchgrass (Panicum virgatum L.) monoculture; (2) a 5-species mixture of C_{4} grasses; (3) a 16-species mixture of C_{3} and C_{4} grasses, forbs, and legumes; and (4) a 32-species mixture of C_{3} and C_{4} grasses, forbs, legumes, and sedges. Previous research at CRERS has shown that the feedstocks differ in productivity; specifically, the 5-sp feedstock is less productive than the 1-, 16-, and 32-sp feedstocks (Abernathy et al. 2016). In the current study, our goal was to examine rates of soil nutrient depletion in these
feedstocks, and their effect on plant physiology and performance, as a potential reason for the observed productivity differences.

To examine feedstock effects on soil, we used three approaches. First, we measured changes in soil N/P/K in each feedstock between 2010 and 2014 to assess feedstock effects on soil nutrients. Second, we measured four traits associated with plant N (chlorophyll concentration, leaf N, light-saturated photosynthetic rate [$A_{sat}$], and leaf fluorescence [$F_{vF_m}$]) on switchgrass plants within each feedstock to assess whether feedstock effects on soil influence plant physiology. Third, we conducted a greenhouse study in which we grew switchgrass plants in field soil conditioned by each feedstock to assess whether feedstock effects on soil influence above- and belowground biomass production. For the soil analysis, we predicted that rates of soil nutrient depletion would be higher in the low-diversity (1- and 5-sp) feedstocks without legumes than in the high-diversity (16- and 32-sp) feedstocks containing legumes. For the physiological analysis, we predicted that switchgrass plants in the low-diversity feedstocks would have lower chlorophyll concentration, leaf N, $A_{sat}$, and $F_{vF_m}$ than switchgrass plants in the high-diversity feedstocks. In the greenhouse analysis, we predicted that switchgrass plants would produce less biomass in field soil conditioned by the low-diversity feedstocks than in field soil conditioned by the high-diversity feedstocks. We used the results of these three analyses to assess potential mechanisms (e.g., niche differentiation, legume facilitation) of feedstock effects on soil properties.

**Methods**

**Study site**

This experiment was conducted at CRERS (site map available in Appendix S1: Fig. S1): a 40-ha site, located on marginal former agricultural land in Black Hawk County, Iowa, USA (42°23' N, 92°13' W). There are three soil types at CRERS: (1) a Flagler sandy loam; (2) a Waukee loam; and (3) a Spillville–Coland alluvial complex (Natural Resources Conservation Service 2014). The current study was conducted on the Flagler sandy loam soil only, which has the highest percentage of sand (73.8% sand, 17.0% silt, and 9.2% clay), the lowest corn suitability rating (50; an index [0–100] that ranks soils in Iowa based on potential row crop productivity), the lowest nutrient availability, and lowest water holding capacity of the three soil types (Myers et al. 2012, 2015, Natural Resources Conservation Service 2014, Sherrard et al. 2015).

There are four prairie agroenergy feedstocks with different diversity and species composition at CRERS. The feedstocks are a 1-species switchgrass monoculture, 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, forbs, legumes, and sedges (species list and seeding rates available in Appendix S1: Table S1). Each feedstock contains all species from feedstocks of lesser diversity. Perennial agroenergy crops are not yet widely produced commercially in the United States, so the species mixture of each feedstock was specifically designed as a potential prairie agroenergy feedstock (for selection criteria, see Abernathy et al. 2016). Four replicate plots (0.33–0.56 ha each) of each feedstock were randomly established on each soil type for a total of 48 research plots (four replicates × four feedstocks × three soil types). The current study was conducted on all 16 plots in the Flagler sandy loam soil.

The site was established in 2009. Before site establishment, the land was used to grow corn and soybeans (late-1980s–2007). All plots were seeded with Roundup ready soybeans in July 2008, and glyphosate was applied in July/August 2008. Research plots were seeded with the perennial feedstocks in 2009 from least to most diverse using a Truax native seed drill. Additional site management has included the following: establishment mowing (June 2009) to reduce competition with annual weeds, burning (April 2011 and April 2014), and haying (March 2012 and March 2015). A small patch of crown vetch and reed canary grass was treated with glyphosate in 2014 to prevent spread; otherwise, no fertilizers, herbicides, pesticides, weeding, or irrigation have been applied to the plots.

**Soil analysis**

In May 2009, we established four sampling locations in each plot. Sampling locations were randomly selected at ~20-m intervals along the long axis of each plot and each location was
recorded using a handheld GPS unit. At each sampling location, we removed three soil cores to a depth of 15 cm using a 3.2 cm diameter handheld soil probe. We cut the cores into two depth increments (0–7.5 and 7.5–15 cm) and combined soil from each depth increment to produce one composite sample of each depth increment from each coring location. In May 2014, we returned to the same sampling locations and repeated this protocol. Soil samples were stored at −20°C and thawed at 4°C immediately prior to processing.

Total soil N and Mehlich-III extractable P and K were quantified for all soil samples. Field-moist soil samples were pushed through an 8 mm diameter sieve, and a portion of the 8-mm sieved soil was pushed through a 2-mm sieve and air-dried. Soil water content was determined gravimetrically after oven-drying overnight at 105°C. P and K were quantified using inductively coupled plasma-optical emission spectroscopy (Tran and Simard 1993). A sub-sample of air-dried, 2-mm sieved soil was pulverized prior to quantification of total soil N using dry combustion in a Fison NA 15000 Elemental Analyzer (ThermoQuest, Austin, Texas, USA).

Physiological analysis

To assess whether feedstock diversity and species composition influence plant physiology and performance, we used *Panicum virgatum* L. (switchgrass). We focused on switchgrass because it is recommended for agroenergy by the U.S. Department of Energy (McLaughlin et al. 1999, McLaughlin and Kszos 2005), it is a central component of the tallgrass prairie ecosystem (Beaty et al. 1978), and it is the only species present in every feedstock at CRERS. Like other warm-season C₄ grasses, switchgrass tolerates low soil fertility (Owens et al. 2013, Zhu et al. 2014), has high water-use efficiency and nitrogen-use efficiency (McLaughlin et al. 1999, Parrish and Fike 2005, Lemus et al. 2008, Gianoulis and Danalatos 2014, Zhu et al. 2014), has high belowground productivity (Sladden et al. 1991, Ma et al. 2010), and has high rates of photosynthesis (Wullschleger et al. 1996). Switchgrass requires minimal inputs for high biomass production but responds positively to N addition (Heaton et al. 2009, Owens et al. 2013). In comparison with some other prairie C₄ grasses (e.g., *Andropogon gerardii* [big bluestem], *Schizachyrium scoparium* [little bluestem], *Sorghastrum nutans* [Indian grass]), switchgrass has relatively low root length density, low tissue N concentration, and is less efficient at extracting soil nitrate (Fargione and Tilman 2006). As an agroenergy crop, switchgrass is highly productive (Beale and Long 1997) with consistent annual yields (Wright and Turhollow 2010, Abernathy et al. 2016), but fertilization is required for long-term annual harvest (Lemus et al. 2008, Muir et al. 2001, Parrish and Fike 2005) as yields tend to decline with stand age (Arundale et al. 2014).

In 2014 and 2016, we systematically sampled 200 switchgrass plants in each feedstock (50 plants per plot × four plots per feedstock × four feedstocks = 800 plants per year). In 2014, we established five 30 m transects in each plot. Transects were placed along the longest axis of each plot and were evenly distributed (5–10 m apart) across the plot width. To minimize edge effects, we did not place transects within 5 m of any plot edge. We systematically selected the closest switchgrass tiller at 3-m intervals along the length of each transect (10 plants per transect). The purpose of the 3-m interval was to minimize the possibility of selecting two tillers from the same genet (Beaty et al. 1978). In 2016, we employed a similar sampling scheme, but used two instead of five transects, with 25 plants per transect instead of 10.

To assess plant investment in the light-harvesting complex of photosynthesis, we measured apparent chlorophyll concentration on all switchgrass plants using a portable chlorophyll meter (SPAD 502; Minolta, Ramsey, New Jersey, USA). Measurements were made at three different positions along the length of the youngest fully expanded leaf on the culm and the average value was reported. Measurements were made between 1000 and 1400 CST, on 16–17 July 2014 and on 28–29 June 2016.

Leaf fluorescence (\(F_{v}/F_{m}\)) was measured on all plants using the Pocket PEA chlorophyll fluorometer (Hansatech Instruments, Norfolk, UK). \(F_{v}/F_{m}\) provides an estimate of the maximum quantum efficiency of photosystem II. Leaves were dark-adapted with leaf clips for 15 min prior to measurement. Plants with low \(F_{v}/F_{m}\) values (<0.55) were re-measured. Plants with low \(F_{v}/F_{m}\) values (<0.55) on the second round of
measurements were excluded from analyses. Measurements were made from 1000 to 1300 CST on 21–29 July 2014 and 5–13 July 2016.

We measured light-saturated photosynthesis ($A_{\text{sat}}$) on a subset of plants using an open path gas-exchange system (LI-6400; Li-Cor, Lincoln, Nebraska, USA). The subset consisted of all 50 plants in one plot of each feedstock (200 plants total). To minimize environmental differences between feedstocks, we specifically selected four adjacent plots for this analysis (plots A4, A5, A6, and A7; for site map; see Appendix S1: Fig. S1). Measurements were made on the youngest fully expanded leaf on the culm at a controlled cuvette temperature of 26°C, a reference CO₂ concentration of 400 μmol/mol, a vapor pressure deficit of 1.8–2.0 kPa, and a saturating irradiance of 2000 μmol m⁻² s⁻¹. Plots, and rows within plots, were measured in random order. All measurements were made between 900 and 1300 CST on 23–26 July 2014 and 14–18 July 2016. Leaf area was measured using a digital caliper. We measured percent leaf N on all plants via elemental combustion. For this analysis, we harvested a 5- to 10-cm section of the same leaf used to measure the other physiological traits. Leaves were harvested on 28–29 July 2014 and 18 July 2016 (after all other field-based physiological measurements were completed) and dried to a constant mass at 70°C (min. 48 h). To account for variation in leaf carbohydrate levels (Garnier et al. 2001), all leaves were harvested between 1000 and 1300 CST. Leaves were ground using the Retsch MM400 ball mill with stainless steel jar and balls (Retsch, Haan, Germany). Ground tissue was analyzed for percent N via dry combustion GC analysis. The 2014 samples were analyzed using the COSTECH Analytical Elemental Combustion System 4010 (Costech Analytical Technologies, Valencia, California, USA) at the Ecosystem Analysis Laboratory, University of Nebraska-Lincoln (Lincoln, Nebraska, USA). The 2016 samples were analyzed using the Flash 2000 Elemental Analyzer (Thermo Fisher Scientific, Cambridge, UK) at University of Northern Iowa (Cedar Falls, Iowa, USA).

Greenhouse analysis

To test whether feedstock effects on soil influence plant growth, we performed a greenhouse analysis in which we grew switchgrass plants in field soil conditioned under each feedstock. Field soil was collected on 8 June 2016. We collected soil to a depth of 15 cm from three random sampling locations in each plot on the Flagler sandy loam soil. All soil from the same feedstock soil source was combined and homogenized. We pushed the soil through a 10-mm sieve to remove rocks and roots. Forty plants were grown from seed in each feedstock soil source (40 plants × four feedstock soil sources = 160 plants total). Plants were grown individually in 950-mL conainers (Model MT38; Stuewe and Sons, Tangent, Oregon, USA). Seeds used for this analysis were harvested from four randomly selected switchgrass plants (one per feedstock) at CRERS in 2015 (hereafter referred to as seed families). The 40 plants grown in each feedstock soil source consisted of ten plants from each seed family. To germinate seeds, we placed the seeds in darkness on moist filter paper at 4°C for six days (1–6 June 2016) and then at 20°C for three additional days (6–9 June 2016). We placed one germinated seed into each conainer on 9 June 2016. Seedlings that had not emerged from the soil by 13 June 2016 were excluded from analysis. We harvested the plants after 12 weeks on 1 September 2016. The harvested tissue was sorted into above- and belowground biomass, dried to a constant mass at 70°C (min. 48 h), and weighed.

Statistical analysis

Soil analysis.—We compared total N and extractable P and K for each soil depth increment between agroenergy feedstocks within a year and between years within a feedstock using a mixed model analysis of variance (ANOVA). We also tested whether the change in soil nutrient availability differed between feedstocks using one-way ANOVA with feedstock as a fixed factor. Mean separations were carried out using the Tukey-Kramer HSD test with a critical value of $\alpha = 0.05$.

Physiological analysis.—We compared switchgrass physiology between feedstocks using one-way ANOVA with feedstock as a fixed factor. We tested the assumption of homogeneous residual variance and normally distributed residual variance by visually inspecting the model residuals. For both the 2014 and 2016 data, we log-transformed percent leaf N and $x^4$-transformed $F_{a}F_{m}$.
to improve normality. The Box–Cox procedure was used to determine optimal transformation. Transformation did not qualitatively alter the significance of any statistical tests; therefore, we report F-ratios and P-values based on the transformed data. To further examine significant differences between feedstocks, we used Tukey’s HSD tests.

**Greenhouse analysis.**—We compared above- and belowground biomass production between feedstocks using ANOVA with feedstock soil source as a fixed factor and seed family as a random factor. We tested the assumption of homogeneous, normally distributed residual variance by visually inspecting the model residuals. To further examine significant differences between feedstock soil sources, we used Tukey’s HSD tests.

**Sown vs. actual diversity.**—To examine the relationship between sown diversity in 2009 and actual diversity in 2014, we estimated basal area coverage of all sown and unsown species in ten 0.1-m² quadrats (20 × 50 cm) in each plot on the Flagler sandy loam soil in July 2014. Two 10-m transects were randomly placed in each plot (one running north to south, one running east to west). To avoid edge effects, no transects were placed within 2 m of any plot edge. Five quadrats were surveyed at 2-m intervals along the length of each transect. Basal area coverage was estimated at 2.5 cm above the ground by comparing the total area of live material to 0.006-cm² standardized squares. We used basal area coverage to compute Shannon diversity indices for each plot. We found that sown diversity was strongly correlated with actual diversity in 2014 (r = 0.869, R² = 0.755, p < 0.0001); as a result, we used sown diversity as an explanatory variable in all subsequent analyses.

Statistics for the soil analysis were performed in SAS v9.4 (SAS Institute, Cary, North Carolina, USA). All other statistics were performed using the nlme package (Pinheiro et al. 2015) of R (v. 3.2.2; R Core Team 2015).

**RESULTS**

**Soil analysis**

There was significant depletion of soil nutrients in the 1-, 5-, and 32-sp feedstocks (Table 1). In the 5-sp feedstock, subsurface N, surface P, and surface and subsurface K decreased from 2009 to 2014. In the 1- and 32-sp feedstocks, subsurface N decreased from 2009 to 2014. The rate of soil nutrient depletion differed between feedstocks. Depletion of surface N was higher in the 5-sp feedstocks than in the 32-sp feedstock, depletion of surface P was higher in the 5- and 32-sp feedstocks than in the 16-sp feedstock, and depletion of surface K was higher in the 5-sp feedstock than in the 16-sp feedstock.

**Physiological analysis**

In general, switchgrass plants in the 5-sp feedstock displayed significantly lower values of the four measured physiological traits than switchgrass plants in other feedstocks (Fig. 1, Table 2). In 2014, switchgrass plants in the 5-sp feedstock had the lowest leaf N, A_sat, chlorophyll concentration, and F_v/F_m (Fig. 1a–d). Plants in the 1- and 32-sp feedstocks had the highest leaf N (Fig. 1a).

**Table 1. Changes in soil N/P/K in the four prairie agroenergy feedstocks (FS) from 2009 to 2014.**

<table>
<thead>
<tr>
<th>Depth</th>
<th>FS</th>
<th>N (g/kg) 2009</th>
<th>N (g/kg) 2014</th>
<th>Δ</th>
<th>P (mg/kg) 2009</th>
<th>P (mg/kg) 2014</th>
<th>Δ</th>
<th>K (mg/kg) 2009</th>
<th>K (mg/kg) 2014</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–7.5 cm</td>
<td>1</td>
<td>1.47ab</td>
<td>1.41ab</td>
<td>−0.06ab</td>
<td>84</td>
<td>66</td>
<td>−18ab</td>
<td>148</td>
<td>120</td>
<td>−28ab</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.33b</td>
<td>1.25b</td>
<td>−0.09b</td>
<td>108</td>
<td>77</td>
<td>−32a</td>
<td>168</td>
<td>127</td>
<td>−41a</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.43ab</td>
<td>1.50a</td>
<td>+0.07ab</td>
<td>96</td>
<td>84</td>
<td>−12b</td>
<td>133</td>
<td>158</td>
<td>+25b</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>1.56a</td>
<td>1.57a</td>
<td>+0.01a</td>
<td>95</td>
<td>65</td>
<td>−29a</td>
<td>167</td>
<td>137</td>
<td>−30ab</td>
</tr>
<tr>
<td>7.5–15 cm</td>
<td>1</td>
<td>1.24</td>
<td>1.09</td>
<td>−0.15</td>
<td>43</td>
<td>42</td>
<td>−1</td>
<td>102</td>
<td>64</td>
<td>−38</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.19</td>
<td>0.98</td>
<td>−0.21</td>
<td>60</td>
<td>55</td>
<td>−4</td>
<td>146</td>
<td>60</td>
<td>−86</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.14</td>
<td>1.02</td>
<td>−0.13</td>
<td>62</td>
<td>64</td>
<td>−2</td>
<td>127</td>
<td>67</td>
<td>−61</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>1.25</td>
<td>1.05</td>
<td>−0.20</td>
<td>45</td>
<td>46</td>
<td>+1</td>
<td>86</td>
<td>54</td>
<td>−32</td>
</tr>
</tbody>
</table>

**Notes:** Significant changes (Δ) in nutrient concentration over time within a given feedstock and depth increment are indicated in bold. Significant differences between feedstocks in initial nutrient concentration (2009), final nutrient concentration (2014), and change in nutrient concentration (Δ) within a given depth increment are indicated with letters.
Plants in the 1-sp feedstock had the highest \( A_{\text{sat}} \) and chlorophyll concentration (Fig. 1b, c). Plants in the 16-sp feedstock had the highest \( F_{\text{v}}F_{\text{m}} \) (Fig. 1d). Results in 2016 were very similar to results in 2014. In 2016, switchgrass plants in the 5-sp feedstock again had the lowest leaf N, \( A_{\text{sat}} \), chlorophyll concentration, and \( F_{\text{v}}F_{\text{m}} \) (Fig. 1a-d).

Greenhouse analysis

Switchgrass plants grown in field soil conditioned by the 5-sp feedstock produced significantly less above- and belowground biomass.

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Fig. 1. Differences in switchgrass physiology between the four prairie agroenergy feedstocks at Cedar River Ecological Research Site. Four physiological traits (leaf N [a], light-saturated photosynthetic rate [\( A_{\text{sat}} \), b], chlorophyll concentration [c], and \( F_{\text{v}}F_{\text{m}} \) [d]) were measured on switchgrass plants within each feedstock in two nonconsecutive years: 2014 (gray bars) and 2016 (white bars). Bars represent means (±1 standard error) within each feedstock. One-way ANOVA and Tukey’s HSD test were used to assess whether switchgrass physiology differed between feedstocks within a given year and significant differences are indicated with letters.
than switchgrass plants grown in field soil conditioned by the other three feedstocks (Fig. 2).

**Discussion**

In this study, we examined rates of soil nutrient depletion and the physiology and performance of a focal species (switchgrass) in four prairie agroenergy feedstocks with different species diversity and composition. Collectively, our three studies suggest that an agroenergy crop composed of five C₄ grasses depletes soil nutrients at a faster rate than a switchgrass monoculture or a high-diversity prairie mixture that contains legumes (i.e., the 16- and 32-sp mixtures). These results suggest that nutrient depletion could be a mechanism for the low productivity of the 5-sp feedstock reported in a previous study (Abernathy et al. 2016).

Soil nutrient depletion may have been higher in the 5-sp feedstock than in other feedstocks because of lower niche differentiation (relative to the 16- and 32-sp feedstocks) and higher rates of nutrient uptake (relative to the switchgrass monoculture). The 5-sp feedstock, which consists of five C₄ grass species, has less variability in root morphology, rooting depth, and phenology than the 16- and 32-sp feedstocks, which could result in greater leaching losses and faster nutrient depletion in the 5-sp feedstock (Fornara and Tilman 2009). Our results provide less support for the interpretation that the presence/absence of legumes is responsible for differences in soil nutrient depletion because depletion of all nutrients (N, P, and K) was higher in the 5-sp feedstock than in the high-diversity feedstocks, and because the presence of legumes did little to slow the rate of soil N depletion in the high-diversity feedstocks. In contrast to the 5-sp feedstock, we did not detect depletion of P or K in the switchgrass monoculture and the rate of subsurface N depletion in the switchgrass monoculture was comparable to the high-diversity feedstocks. Switchgrass has lower root length density and is less efficient at extracting soil nutrients than the other C₄ grasses in the 5-sp feedstock (Andropogon gerardii [big bluestem], Schizachyrium scoparium [little bluestem], and Sorghastrum nutans [Indian grass]; Fargione and Tilman 2006). Lower nutrient uptake in switchgrass monocultures could translate to lower nutrient loss when feedstocks are harvested or burned.

Differences in switchgrass physiology also support the interpretation that soil nutrient depletion was higher in the 5-sp feedstock than in other feedstocks. Our results show that switchgrass plants in a 5-sp feedstock have lower leaf N, lower $A_{sat}$, lower chlorophyll concentration, and higher leaf florescence (lower $F_{sat}$/$F_{m}$) than switchgrass plants in other feedstocks. These results suggest that soil nutrient depletion in the 5-sp feedstock negatively impacts traits associated with plant N. Interestingly, we found that switchgrass plants in switchgrass monocultures have higher leaf N, higher $A_{sat}$, and higher chlorophyll concentration than switchgrass plants in other feedstocks. This suggests that switchgrass is influenced by the identity of neighboring species. Previous studies have shown that switchgrass is a poor competitor in polyculture (Fargione and Tilman 2006) and our results suggest that reduced tissue N and photosynthesis could be a mechanism for this performance difference. On average, switchgrass plants in the 5-sp feedstock were more likely to be surrounded by other C₄-grasses, which are effective N extractors/scavengers (Ranells and Wagger

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**Table 2. Analysis of variance (ANOVA) table reporting differences in switchgrass physiology between the four agroenergy feedstocks at Cedar River Ecological Research Site.**

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf N</td>
<td>3</td>
<td>3,783</td>
<td>6.056</td>
<td>2.019</td>
</tr>
<tr>
<td>$A_{sat}$</td>
<td>3</td>
<td>3,195</td>
<td>3016.6</td>
<td>1005.5</td>
</tr>
<tr>
<td>Chlorophyll concentration</td>
<td>3</td>
<td>3,796</td>
<td>2654.4</td>
<td>884.8</td>
</tr>
<tr>
<td>$F_{sat}$/$F_{m}$</td>
<td>3</td>
<td>3,770</td>
<td>0.103</td>
<td>0.034</td>
</tr>
<tr>
<td>2016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf N</td>
<td>3</td>
<td>3,794</td>
<td>4.021</td>
<td>1.340</td>
</tr>
<tr>
<td>$A_{sat}$</td>
<td>3</td>
<td>3,196</td>
<td>2553.2</td>
<td>851.1</td>
</tr>
<tr>
<td>Chlorophyll concentration</td>
<td>3</td>
<td>3,796</td>
<td>5510.3</td>
<td>1836.8</td>
</tr>
<tr>
<td>$F_{sat}$/$F_{m}$</td>
<td>3</td>
<td>3,762</td>
<td>0.188</td>
<td>0.063</td>
</tr>
</tbody>
</table>

**Notes:** Feedstock was a fixed factor in the ANOVA. Reported values are as follows: numerator and denominator degrees of freedom (df), sum of squares (SS), mean squares (MS), and F-statistics (F), and significance is indicated with asterisks (**P < 0.001). Leaf N data were log-transformed in 2014 and 2016, and $F_{sat}$/$F_{m}$ data were x²-transformed in 2014 and 2016 to improve normality. Abbreviations are $A_{sat}$ is light-saturated photosynthetic rate, and $F_{sat}$/$F_{m}$ is ratio of variable florescence to maximum florescence (a measure of the maximum quantum efficiency of photosystem II).
Fig. 2. Above- and belowground biomass of switchgrass plants grown in field soil collected from each agroenergy feedstock at Cedar River Ecological Research Site. Plants were grown for 12 weeks in a greenhouse. Bars represent mean (±1 standard error) aboveground (white portion of bar) and belowground (gray portion of bar) biomass production in each feedstock soil source. Tukey’s HSD test was used to assess whether above- and belowground biomass production differed between feedstock soil sources, and significant differences are indicated with letters.

1997, Temperton et al. 2007, Carlsson et al. 2009). Conversely, switchgrass plants in the 16- and 32-sp feedstocks were more likely to be surrounded by a combination of C₄-grasses, forbs, and legumes. While the presence of legumes can increase the photosynthetic rate, tissue N concentration, total N content, and performance of neighboring non-legumes (Spehn et al. 2002, Lee et al. 2003, Temperton et al. 2007, Postma and Lynch 2012), our results suggest that their presence did not fully mitigate the negative impact of neighboring C₄-grasses. Another potential mechanism for the superior performance of switchgrass plants in the 1-sp feedstock is that switchgrass produces higher quality litter (tissue with lower biomass-to-N ratio) than other C₄-grasses (e.g., big bluestem and little bluestem), which could reduce N immobilization (Wedin and Tilman 1990). Switchgrass also has high N use efficiency (Parrish and Fike 2005, Giannoulis and Danalatos 2014, Zhu et al. 2014) and high N retranslocation (44–66%; Dohleman et al. 2012, Heaton et al. 2009, Pedroso et al. 2014) and may harbor effective rhizosphere populations for nutrient uptake and growth (Brevda et al. 1998, Bahulikar et al. 2014).

Results from our greenhouse study also support the interpretation that soil nutrient depletion was higher in the 5-sp feedstock than in other feedstocks, though biotic plant–soil feedbacks may also play a role. Switchgrass plants produced less above- and belowground biomass in soil conditioned by the 5-sp feedstock than in soil conditioned by other feedstocks. This result suggests that the low photosynthesis and tissue N of switchgrass plants in the 5-sp feedstock are at least partly due to feedstock effects on soil, and not solely due to an inability to compete with other C₄ grasses for soil nutrients in this mixture. Feedstock effects on soil might be abiotic (i.e., nutrient depletion) and/or biotic (i.e., an accumulation of species-specific soil biota), but separating these two experimentally is challenging (Brinkman et al. 2010, van der Putten et al. 2013). Our soil sampling demonstrates nutrient depletion in the 5-sp feedstock soil where switchgrass performed poorly, suggesting that abiotic factors play an important role. Biotic factors, if species-specific, should be strongest in the 1-sp feedstock soil (Schlatter et al. 2015, van der Putten et al. 2016), yet we found that switchgrass plants performed most poorly in soil from the 5-sp feedstock in our greenhouse analysis. McKenna et al. (2018) also investigated plant–soil feedbacks in common prairie species and found marginal evidence for plant–soil feedbacks in switchgrass. By comparing unsterilized soil from switchgrass monocultures to sterilized soil and re inoculated sterilized soil, they showed a negative biotic feedback in switchgrass, though
the overall soil effect was only marginally significant. Our experiment did not experimentally separate biotic and abiotic soil effects, but poor switchgrass performance in the 5-sp feedstock is highly consistent with abiotic effects of nutrient depletion, and less consistent with species-specific biotic feedbacks.

From an agroenergy perspective, the results of our three analyses suggest that a prairie agroenergy feedstock composed of five C_4 grasses will require more fertilizer than a high-diversity feedstock containing legumes. By contrast, our results do not support the hypothesis that a switchgrass monoculture will require more fertilizer than a high-diversity feedstock containing legumes. This finding highlights one limitation of extrapolating from biodiversity-ecosystem function studies with randomized plant assemblages to a biofuel context, where plant assemblages are non-random.

The management strategy of prairie agroenergy feedstocks will ultimately influence rates of soil nutrient depletion (Guretzky et al. 2011, Wilson et al. 2013). Cedar River Ecological Research Site has been managed in a way that balances the provisioning of ecosystem services (e.g., wildlife habitat, soil and water conservation, and recreation) with economic return (e.g., biomass production). Our management rotation (no management/burn/hay) and other management tactics (establishment mowing, spring biomass harvest) have helped minimize competition with annual weeds (Abernathy et al. 2016) and foster productivity (Smith et al. 2010), while still maintaining high habitat value for wildlife (e.g., fall and winter habitat for birds; Fargione et al. 2009). However, nutrient depletion would occur at a faster rate in annually harvested feedstocks. Low harvest frequency may have limited our ability to detect nutrient depletion in the 1-sp feedstock and/or the capacity of legumes to slow rates of soil N depletion in the high-diversity feedstocks. Nevertheless, the higher rate of soil nutrient depletion observed in the 5-sp feedstock would likely persist under alternative management scenarios and be amplified by greater harvest frequency.

Our inability to detect differences in the rate of nutrient loss between the 1-sp feedstock and high-diversity feedstocks might also have been influenced by the initial nutrient content of our soil and the age of our experiment/research site. The Flagler sandy loam soil at CRERS has higher N content than many of the research sites used in diversity–productivity experiments (Lambers et al. 2004, Tilman et al. 2006, 2012, Fornara and Tilman 2009, Isbell and Wilsey 2011, Jungers et al. 2013). As a result, it may take longer to detect the benefits of species interactions (e.g., niche differentiation and legume facilitation) on soil nutrients (Dybzinski et al. 2008). Consistent with this interpretation, long-term diversity–productivity studies have shown that the superior yields of high-diversity vs. low-diversity mixtures often become more pronounced with time (Cardinale et al. 2007) and that the effect of diversity on N-cycling changes with time (Oelmann et al. 2011).

In conclusion, our results indicate that a five-species mixture of C_4 grasses depletes soil nutrients at a faster rate than other potential prairie agroenergy feedstocks, which influences the physiology and performance of plants within this mixture. Faster nutrient depletion could be a mechanism for the low productivity of the 5-species feedstock reported in previous research from CRERS (Abernathy et al. 2016). Conservation Reserve Program (CRP) land in the Midwestern United States is often seeded with a mixture of C_4 grasses that is comparable to the five-species feedstock in our study. Previous research has suggested that this CRP land has the potential to be a large existing source of biomass for agroenergy (Adler et al. 2009, Jungers et al. 2013). Our results, however, suggest that a 1-sp switchgrass monoculture or a high-diversity mixture (with legumes) would be better choices for future CRP installations because they deplete soil nutrients at a slower rate and foster higher rates of plant photosynthesis.

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