

Cup plant biases prairie nitrogen composition in response to increasing community diversity

Jacob Hill

Iowa State University Honors Program Thesis

April 21, 2017

Introduction

Biodiversity and ecosystem function (BDEF) studies illuminate positive relationships between species diversity and ecosystem functions such as nutrient uptake and biomass production (Tilman, Isbell & Cowles 2014). Oppositely, they also illuminate the negative consequences of local species losses for human valued ecosystem processes which scale up to the landscape and global level (Hooper, *et al* 2012). Of particular importance to the agricultural landscape of Iowa is the functional role of biodiversity in water quality improvement by way of complementary nutrient uptake between species in high diversity ecosystems (Cardinale 2011). To this effect, the strategic reintroduction of diverse, native perennial ecosystems such as prairie into and at the edge of row-crop fields can reduce nitrate ($\text{NO}_3\text{-N}$), phosphorus, and soil losses from agricultural fields to surface waters via runoff (Zhou, *et al* 2014). These ecosystems, known as prairie buffers, act to mitigate the negative effects of nutrient enrichment and soil deposition on aquatic life and downstream human water use. Buffers are areas planted in permanent vegetation at the edge of or integrated within agricultural landscapes designed to slow water runoff, and intercept and remove soils and pollutants from farm field runoff (Zhou, *et al.* 2014). As such, I use *prairie buffer community* to refer to the mixture of plant species occupying a given buffer.

Nitrogen (N) is typically the resource limiting plant growth in grassland ecosystems (Wedin 1996). Nitrogen use efficiency (NUE), or the ratio of aboveground biomass to aboveground N, can be measured to detect limitation by N of individual species and whole communities in varying diversity contexts (van Ruijven & Berendse 2005). NUE is a reasonable indicator of N limitation because it reflects the plastic capacity of plants to allocate biomass to aboveground structures (i.e., stems and leaves) more efficiently when N is limiting (Fornara & Tilman 2009; van Ruijven & Berendse 2005). To a similar effect as NUE, the ratio of carbon:nitrogen (C:N) in plant tissue, a common metric for gauging the degree of N availability and thus limitation of plants (Sterner & Elser 2002), may be employed.

Under conditions of N enrichment, which occur in prairie buffer communities, high diversity systems are expected to take up and allocate N to aboveground tissue more efficiently (increased NUE and C:N) than low diversity systems due to increased niche partitioning of N by plant species, which results in complementary or synergistic resource use at community scales (Leimer *et al* 2016). However, the “hidden” disproportionate effect of individual species on community wide ecosystem functions, known as selection effects, may also be at play (Huston 1997; Tilman, Isbell & Cowles 2014). Therefore, in addition to plant diversity, the species composition of prairie buffer communities may be important to their functional efficiency in N removal from surface runoff.

Previous work in BDEF has focused more on the functional response of communities to increasing diversity rather than the functional response of individual species to increasing diversity (De Deyn *et al* 2009). However, focus has shifted to investigating how the ecological functions of individual species or functional groups change under varying levels of diversity (Novotny *et al* 2007; van Ruijven & Berendse 2005; Borer *et al* 2015). Still, however, ecosystem function responses to increasing diversity under conditions of nutrient enrichment, which is particularly important for prairie buffer communities, is less well understood (but see Craven *et al* 2016). In central Iowa prairie communities planted to maximize biomass production for biofuel feedstocks, for example, diversity unexpectedly increased in addition to productivity with fertilization in comparison to unfertilized controls, which the authors suggest may result from the annual harvest of aboveground plant material

This project aimed to determine if the species composition and diversity of prairie buffer communities subject to agricultural nitrogen (N) enrichment were important factors in determining the efficiency with which these communities remove N. This was accomplished indirectly by measuring the N composition of leaves (C:N) and aboveground biomass (NUE) of cup plant (*Silphium perfoliatum*), a highly productive prairie forb in the aster family (Gansberger, Montgomery & Liebhard 2015), and comparing cup plant's response to the community in whole. Gauging the degree of N limitation of cup plant in comparison to N limitation of the whole community in response to increasing diversity allowed me to determine if species composition is an important factor for reducing N losses from row crop systems.

Objectives

I sought to understand if and how a focal species, the highly aggressive prairie forb cup plant (*Silphium perfoliatum*), changes its aboveground nitrogen composition in comparison to neighboring species during the growing season and in response to increasing diversity in restored prairie communities. Ecological stoichiometry, a simple tool for studying resource allocation and competitive strategies between and among species as well as across whole communities, was employed to elucidate mechanistic processes underlying nitrogen composition patterns. If plant tissue composition of a limiting nutrient such as nitrogen remains constant despite changes in nutrient composition of the resource or changes in growth stage, then that plant or community is said to exhibit strict stoichiometric homeostasis (Sternner & Elser 2002). If, however, tissue nutrient composition deviates from homeostasis, then assuming the influence of intraspecific genetic variation is small, an ecological intervention may be inferred. The intervention of interest here is that of increased competition and partitioning of nitrogen resources conferred by an expected increase in physiological, phenological, and morphological complexity of high diversity systems.

To assess patterns in plant composition and investigate their underlying ecological processes, I proposed the following three primary hypotheses to guide my research:

- Hypothesis 1 (H₁): Foliar (C:N) will increase as cup plant progresses from the pre-flowering to post-flowering stages as more N is allocated to apical and reproductive growth toward the end of the season;

- Hypothesis 2 (H₂): As a plastic response to increased competition for and limitation by N, foliar C:N will increase as community species diversity increases; and
- Hypothesis 3 (H₃): Total aboveground tissue N concentration of (a) cup plant and (b) neighboring species will decrease (proportional to a C:N increase) with increasing community species diversity as individuals increase in NUE..

Testing these hypotheses allowed for a comparison of resource use patterns between highly productive individual species and less productive neighbors, which are important for prairie buffer community functional efficiency.

Methods

Study Site

In 2009, nine treatments randomly arranged across 36 plots (2 m x 2 m) were established for an experiment studying buffer diversity and pollinator benefits at ISU's Field Extension and Education Laboratory in Boone, Iowa (42° 00.318' N, 93° 47.272' W) (Gill *et al* 2014). If certain plant species were eliminated over time, Replacement plugs were planted in 2014 for species excluded from the plots between 2009 and 2014.

I capitalized on the design of an ongoing experiment on these same plots investigating the abilities of native perennial plantings varying in diversity and composition to reduce NO₃-N losses from agricultural surface runoff (Zimmerman & Schulte, Iowa State University, unpublished data). Simulated agricultural runoff events, administered monthly, delivered equal loads of NO₃-N in equal concentrations to each plot during the growing season. No plots observed in this study included N-fixing legumes, although the larger study does include plots with legumes. Therefore, only exogenous N was added to the system. N inputs were standardized and realistic for a native perennial system located within an agricultural context in Iowa.

I used four of the treatments arranged across 16 (four replicates) of the plots in this study (N = 16), with varying number of prairie species in a conserved sequence of species addition. Plots including four combinations (four replicates each) of non-leguminous forbs, C4, and C3 grasses varying in richness from two, three, five, to 12 species each included cup plant. Plots of planted richness levels two, three, and five included only forbs while 12 species plots were planted with forbs and grasses (Table 1).

Table 1. Species included in two, three, five, and 12 species treatments. The abbreviations WS and CS represent warm season, and cool season, respectively. Adapted from Gill *et al* (2014).

| Treatment (Planted richness) | Common Name | Scientific Name | Functional group |
|-------------------------------------|-------------------------------|--|-------------------------|
| 2, 3, 5, 12 | Cup plant | <i>Silphium perfoliatum</i> | Forb |
| 2, 3, 5, 12 | Meadow zizia | <i>Zizia aptera</i> | Forb |
| 3, 5, 12 | Swamp milkweed | <i>Asclepias incarnata</i> | Forb |
| 5, 12 | Pinnate prairie coneflower | <i>Ratibida pinnata</i> | Forb |
| 5, 12 | New England aster | <i>Symphyotrichum novae- angliae</i> | Forb |

| | | | |
|----|-------------------|--------------------------------|------------|
| 12 | Canada anemone | <i>Anemone canadensis</i> | Forb |
| 12 | Prairie ironweed | <i>Vernonia fasciculata</i> | Forb |
| 12 | Common boneset | <i>Eupatorium perfoliatum</i> | Forb |
| 12 | Smooth blue aster | <i>Symphotrichum laeve</i> | Forb |
| 12 | Switchgrass | <i>Panicum virgatum</i> | Grass (WS) |
| 12 | Little bluestem | <i>Schizachyrium scoparium</i> | Grass (WS) |
| 12 | Canada wildrye | <i>Elymus canadensis</i> | Grass (CS) |

Sampling Methods

Because plots of the same treatments did not necessarily maintain abundance or richness of species over time, observed diversity was assessed at a sub-plot scale. Each plot was sub-sampled to species in four random stratified quadrats (0.25 m²) within each plot for number of stems (forbs) and percent cover (grasses) at the flowering sampling period (July 21-25). One quadrat was placed in each of four 1 m² quarters of each plot in a randomized fashion. The random placement of quadrats was conserved across all plots in order to make diversity estimations equitable among plots. Stem counts and cover estimates in each sub-sample (1 m²) were composited by species and summed in each plot (N = 16) so that diversity could be assessed using Simpson's Diversity Index (SDI; Gurevitch, Scheiner & Fox 2006), which accounts for both richness and the relative abundance of species, calculated as follows:

$$SDI = 1 - (\sum n(n-1) / T(T-1)),$$

where n is the total number of individuals of a particular species, and T is the total number of individuals of all species. A high value of SDI corresponds to a high level of community diversity, which is itself cooperatively and positively influenced by species richness and species evenness (Gurevitch, Scheiner & Fox 2006).

All species, including species not planted but which invaded the plots, were included in the above sampling and calculation procedure. I counted the number of stems of each present forb species and estimated the percent cover of each present grass species in each 0.25 m² sub-plot quadrat. These values were summed by plot to obtain n , and all stem and percent cover counts were summed to obtain T in the above equation. I sampled during the flowering period as it was assumed to be representative of the average diversity of each plot over the project's total observation period. Additionally, as a density effect covariate, the total number of cup plant stems in each plot was counted at the beginning of the growing season.

To test for variation in foliar C:N due to growth stage and community diversity, I sampled leaves at three different points during the 2016 growing season: 1) pre-flowering period (June 9); 2) flowering period (July 21); and 3) post-flowering (senescence) period (September 16). I harvested leaves from each of the top, bottom, and mid-stalk locations of three randomly selected cup plant shoots in each of the treatments. Leaf samples were composited by plot. The height (cm) of each stalk from which a leaf was removed along with six other randomly selected stalks in each plot was measured to obtain an average stem height measure. Leaf samples were dried, grounded, weighed, and analyzed for percent total N (%TN) and percent total C (%TC) at the

Iowa State University Plant and Soil Analysis Lab (For detailed TN and TC methods, see Horneck & Miller 1998).

I destructively sampled above-ground plant material from each plot at the end of the growing season on November 17, 2016, and separated into cup plant and other species aggregates for analysis. Total aboveground tissue samples were dried, weighed, and analyzed for %TN at the ISU Plant and Soil Analysis Lab. Aboveground N composition measures included %TN and NUE, calculated as the following: aboveground biomass (g) / unit aboveground nitrogen mass (g) (van Ruijven & Berendse 2005).

Statistical Methods

Changes in C:N across growth stage and in response to diversity in cup plant foliar C:N (H_1 , H_2) was analyzed using a two-way repeated measures ANOVA with plot as random effect and sampling date and planted richness level as fixed effects. This model assumed that the level of variation in outcomes was the same at each sampling date and the correlation between observations from the same plot on different sampling dates was the same for any pair of sampling dates. Because profile plot lines appeared to deviate slightly from parallel in initial graphical analysis, a date by richness interaction was also included in the model. If F tests were significant, post-hoc pair-wise comparisons were conducted using Tukey's HSD.

Because %TC data were not gathered for total aboveground biomass, a similar approximation of nitrogen composition and NUE was estimated as total aboveground biomass (g) / aboveground TN (g) as in van Ruijven and Berendse (2005). Raw %TN was also included in analyses, but its correlation with NUE for both cup plant ($r = -0.99$; $p = 2.4e-12$) and neighboring species aggregates ($r = -0.98$; $p = 3.7e-10$) warranted its dismissal in favor of the latter approximation.

Initial graphical analysis of biomass data revealed an outlying observation at the three species planted richness level with regard to total biomass production (994.3 g) and neighboring species NUE (80.2 g biomass / g N). Further investigation justified the plot's exclusion from analysis as this plot's soil surface was severely disturbed during the observation period, preventing regular vegetative growth and resulting in extremely low production relative to other plots.

The response of aboveground N composition to community diversity (H_3) was assessed using one-way MANOVA with cup plant and neighboring species NUE as response variables and planted richness as explanatory variable. Because initial cup plant stem density was found to be significantly correlated with community-wide NUE, a MANCOVA to test for planted richness effects after adjusting for initial stem density was also performed.

Lastly, to determine the significance, influence, and importance of diversity, initial stem density, stem height and NUE of cup plant and neighboring species to the community-wide NUE, a multiple linear regression model was selected based on minimization of the Bayesian Information Criterion (BIC) and Mallows' C_p (Ramsey & Schafer, 2013) Other constraints included the requirement that models including interaction effects must also include corresponding main effects.

Results

Diversity

Species planted in each study plot persisted through time, though rather sparsely and variably in some cases as indicated by large variance around mean stem or cover counts (Table 2). Cup plant dominated plots in each treatment, but was less dominant in plots of higher planted richness. Despite periodic weeding to remove unwanted species and maintain the species of interest, plant species from nearby plots or in the surrounding environment invaded nearby plots. Prairie ironweed and smooth blue aster colonized the three and five species treatments, respectively, while New England aster colonized the three species treatments. Pinnate prairie coneflower colonized the two and three species treatments. Smooth brome (*Bromus inermis*), a cool-season pasture grass very common in Iowa but not purposely planted as a part of the experiment, was very abundant on average in 12 species plots (31% cover \pm 29.68% m⁻²), but this was due to its rampant invasion of one plot. As each treatment was replicated only four times, this plot had a large influence on mean abundance. Other invading plant species included three forbs native to Iowa: Canada goldenrod, stiff goldenrod, and smooth ox-eye sunflower. Each of these, excluding Canada goldenrod, were planted in other plots of the larger study. These species colonized sparsely in comparison to smooth brome, but were in many cases, as or more abundant than intentionally planted species particularly in the 12 species treatment. As noted above, I included all unplanted colonizers in SDI.

Table 2. Abundance estimates of species averaged by planted richness treatment. Mean abundance (number of stems in the case of forbs and percent cover in the case of grasses) is followed by standard error (SE).

| Common Name | Abundance Estimates per m ² (mean ± SE) | | | |
|--|--|------------------|------------------|-------------------|
| | Plot Planted Richness | | | |
| | <u>2 Species</u> | <u>3 Species</u> | <u>5 Species</u> | <u>12 Species</u> |
| Cup plant ^{2,3,5,12} | 38.75 ± 3.20 | 32.33 ± 1.20 | 29.25 ± 7.69 | 26.5 ± 5.07 |
| Meadow zizia ^{2,3,5,12} | 23 ± 8.76 | 20.67 ± 6.57 | 24.25 ± 10.87 | 6 ± 10.87 |
| Swamp milkweed ^{2,3} | 0 ± 0 | 1.33 ± 0.33 | 0.25 ± 0.25 | 0.25 ± 0.25 |
| Pinnate prairie coneflower ^{5,12} | 3 ± 2.38 | 8.67 ± 7.69 | 15.75 ± 3.75 | 17.5 ± 2.50 |
| New England aster ^{5,12} | 0 ± 0 | 1.33 ± 1.33 | 5.5 ± 3.10 | 11 ± 2.27 |
| Canada anemone ¹² | 0 ± 0 | 0 ± 0 | 0 ± 0 | 21.5 ± 9.26 |
| Prairie ironweed ¹² | 0 ± 0 | 0.33 ± 0.33 | 0 ± 0 | 1 ± 0.71 |
| Common boneset ¹² | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1.25 ± 1.25 |
| Smooth blue aster ¹² | 0 ± 0 | 0 ± 0 | 4.5 ± 3.57 | 1.25 ± 0.48 |
| Switchgrass ¹² | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1.25 ± 1.25 |
| Little bluestem ¹² | 0 ± 0 | 0 ± 0 | 0 ± 0 | 2.5 ± 2.50 |
| Canada wildrye ¹² | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1 ± 1.00 |
| Canada goldenrod* | 0 ± 0 | 0 ± 0 | 2 ± 2.00 | 3.5 ± 3.18 |
| Stiff goldenrod* | 0.5 ± 0.50 | 2.33 ± 1.86 | 1.25 ± 0.95 | 1.75 ± 1.75 |
| Smooth ox-eye sunflower* | 0 ± 0 | 0 ± 0 | 0 ± 0 | 2.5 ± 2.50 |
| Smooth brome* | 0 ± 0 | 0 ± 0 | 0 ± 0 | 31 ± 29.68 |

Cup plant stem height

At the senescence sampling stage, cup plant was assumed to be at its maximum stem height. Average cup plant stem height at this stage was significantly, negatively associated with SDI ($r = -0.58$, $p = 0.023$), indicating that as community diversity increased, cup plant maximum heights were shorter at the end of the growing season (Figure 1).

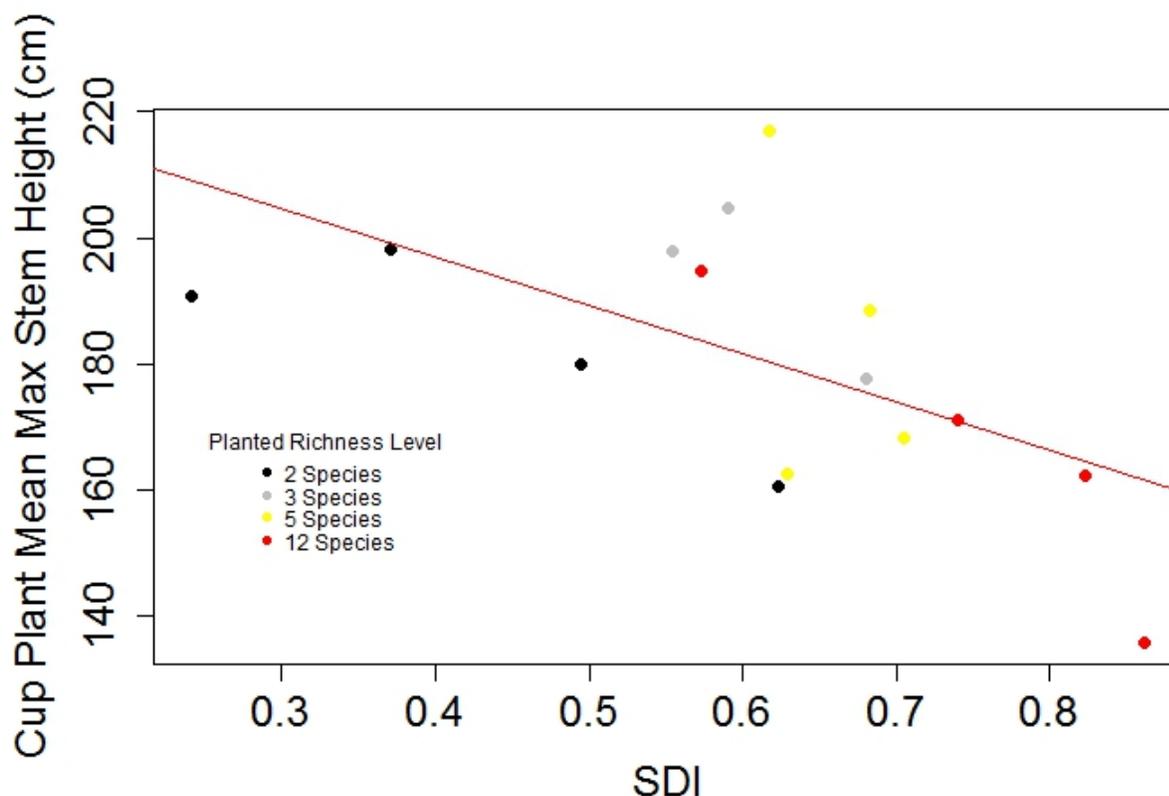


Figure 1. Cup plant mean maximum stem height (cm) measured at the senescence sampling period regressed against Simpson's Diversity Index. A significantly, negative association between mean maximum stem height and SDI was observed ($r = -0.58$; $p = 0.023$).

Cup plant foliar C:N

Repeated measures ANOVA suggested that cup plant mean average foliar C:N changed across sampling period ($F_{2,24} = 34.58$, $p = 8.5e^{-8}$). Averaging across planted richness, Tukey HSD tests specified that C:N of leaves sampled during flowering ($p = 2.9e^{-5}$) and senescence ($p = 2.2e^{-5}$) differed significantly from leaf C:N of the pre-flowering period. This finding partially supports the hypothesis that foliar C:N would increase with progressing growth stage, but a significant difference was not observed between flowering and senescence C:N ($p = 0.99$). Ignoring sampling date, C:N did not differ significantly between planted richness levels ($F_{3,24} = 0.50$, $p = 0.68$), which rejects the hypothesis that foliar C:N would increase with increasing planted

richness. Furthermore, no significant interaction between growth stage and planted richness was observed ($F_{3,24} = 0.62$, $p = 0.71$; Figure 2).

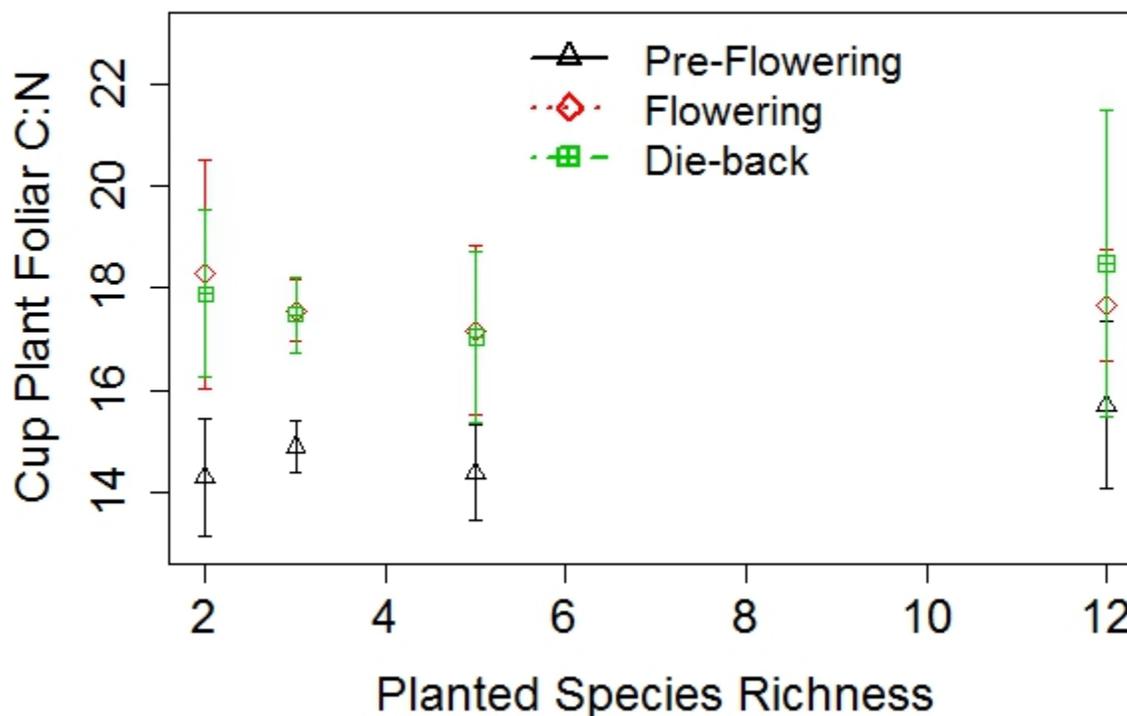


Figure 2. Cup plant foliar carbon:nitrogen ratio (C:N) in varying levels of planted species richness across growth stage from pre-flowering (triangle) to flowering (diamond) to senescence (square), here deemed die-back. Cup plant foliar C:N changed across growth stage from pre-flowering to die-back ($p = 2.2e^{-5}$) and from pre-flowering to flowering ($p = 2.9e^{-5}$). C:N did not vary significantly among planted richness treatments.

Stoichiometric homeostasis was thus maintained regardless of planted richness. As hypothesized, foliar C:N increased from pre-flowering to flowering and post-flowering stages, but in opposition, did not increase in between the latter two stages. In contrast to my hypothesis, foliar C:N did not increase significantly with increasing community diversity.

Total aboveground nitrogen composition

Initially, all available data, including the 12 species richness level were investigated, which suggested that there was no significant effect of planted richness on aboveground nitrogen composition ($F_{6,20} = 1.72$, $p = 0.17$). However, after excluding the 12 species level of responses from analysis as well as an outlier from the three species level (Figure 3), MANOVA revealed a significant effect of planted richness ($F_{4,12} = 7.92$, $p = 0.0023$). It should also be noted that overall, cup plant was a more efficient user of N than the neighboring species aggregate ($p = 9.7e^{-5}$, $df = 12$; Figure 3).

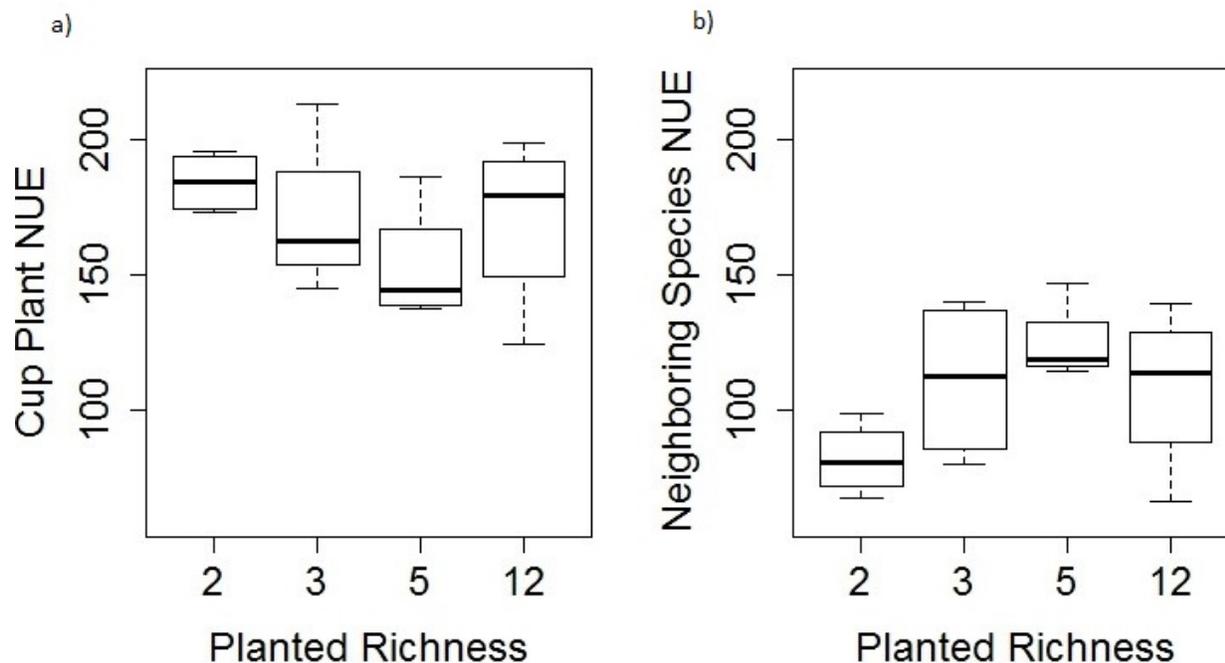


Figure 3. Nitrogen use efficiency (NUE) for (a) cup plant and (b) its neighboring species in aggregate measured from plots with varying planted species richness. No significant differences between richness levels were detected in cup plant NUE. Only neighboring species NUE at richness levels two and five differed significantly from each other ($p = 0.042$), although richness levels three and five were nearly significantly different from each other ($p = 0.065$). Overall, cup plant (a) had a higher NUE than neighboring species (b) ($p = 9.7e^{-5}$).

In forb-only plots stoichiometric homeostasis was not wholly maintained across diversity levels for the neighboring (non-cup plant) species aggregate, but homeostasis was not statistically violated in cup plant tissue. These positive and negative relationships as well as their statistical relevance were bolstered by linear relationships between NUE and observed plot-wise community diversity, here measured using SDI (Figure 4).

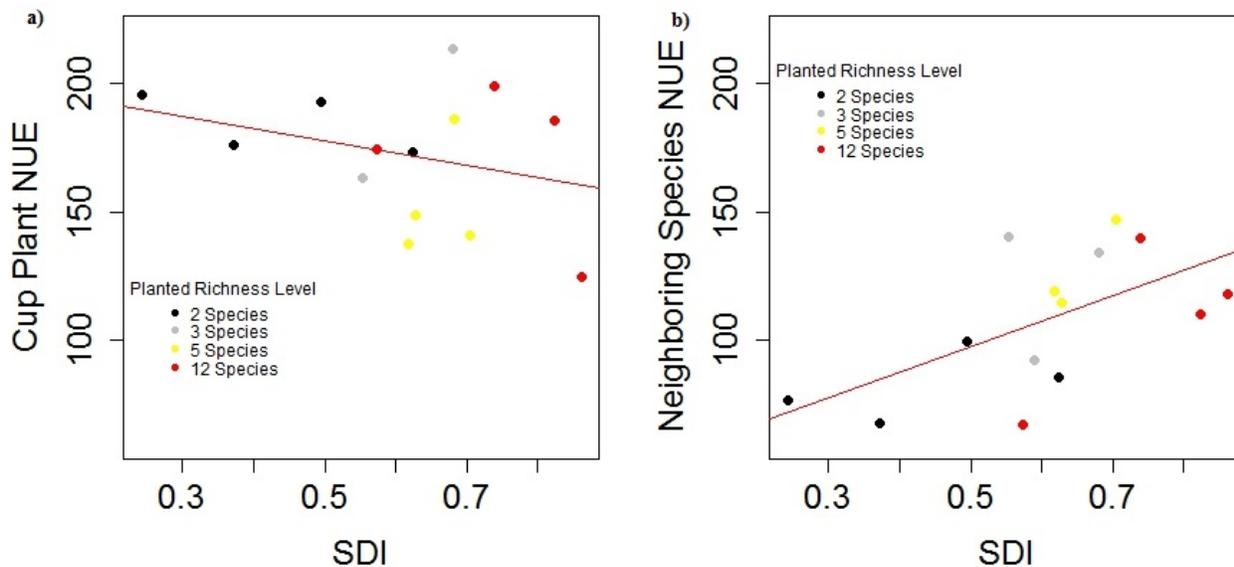


Figure 4. Cup plant nitrogen use efficiency (NUE) (a) was not significantly negatively associated with SDI ($r = -0.30$, $p = 0.30$). Neighboring species NUE (b) was positively linearly associated with Simpson's Diversity Index (SDI, $r = 0.60$, $p = 0.024$).

Neighboring species NUE was significantly positively associated with community diversity while cup plant NUE was not. Therefore, stoichiometric homeostasis was not maintained along a diversity gradient for the neighboring species aggregate while homeostasis was maintained in cup plant. To understand these results in the context of community-wide patterns in NUE and productivity, community biomass and NUE were regressed against Simpson's Diversity (Figure 5).

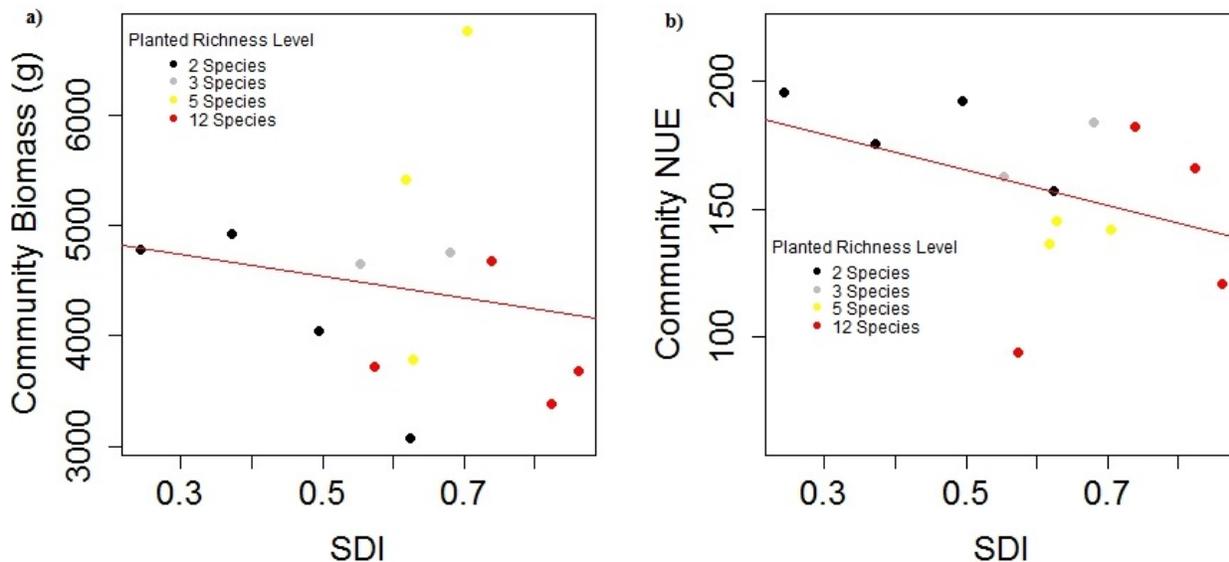


Figure 5. Community aboveground biomass production (a) was not significantly linearly associated with Simpson's Diversity Index (SDI, $r = -0.17$, $p = 0.58$) while community NUE (b) was also not significantly linearly associated with SDI ($r = -0.40$, $p = 0.18$).

Neither community biomass ($p = 0.58$) nor community NUE ($p = 0.18$) were significantly linearly associated with SDI, indicating that productivity did not increase nor vary linearly with biodiversity in this specific sequence of increasing species diversity and that community NUE did not increase with increasing diversity as hypothesized. Furthermore, the latter relationship would suggest that cup plant NUE exerted considerable bias on community-wide NUE as neighboring species NUE was significantly positively associated with diversity while cup plant NUE was not (Figure 3).

A multiple linear regression model explaining variation in community-wide NUE of the following form was selected according to the criteria noted above:

$$\text{Community NUE} \sim \text{Cup Plant NUE} + \text{Cup Plant Initial Stem Density}$$

A summary of the coefficients and significance of the model terms are found below.

Table 3. Summary of selected multiple linear regression model parameters for explaining variation in community NUE. The model explained 66.7% of the variation in community NUE and was significant at a level $\alpha = 0.05$ ($p = 0.00163$).

| Coefficient | Model Estimate | SE | <i>t</i> value | <i>p</i>-value |
|------------------------|-----------------------|-----------|-----------------------|-----------------------|
| Intercept | 18.72 | 32.02 | 0.59 | 0.5718 |
| Cup Plant NUE | 0.57 | 0.20 | 2.90 | 0.0160 |
| Cup Plant Stem Density | 1.65 | 0.55 | 2.98 | 0.0137 |

According to this model ($p = 0.00163$), cup plant NUE and initial stem density explained 66.7% of the variation in community-wide NUE, indicating a biased effect of cup plant density and N composition on the community response to increasing species diversity. Adjusting for the effect of stem density, a 1 g/g increase in cup plant NUE was associated with a 0.57 g/g increase in community NUE. Adjusting for the effect of cup plant NUE, a 1 stem/m² increase in stem density was associated with a 1.65 g/g increase in community NUE. Therefore, community NUE appears to be most influenced by cup plant NUE and its initial stem density.

Discussion

In agreement with my hypothesis (H_1), foliar N content (C:N) increased as cup plant progressed from the vegetative (pre-flowering phase) into the reproductive and senescence phases of the growing season (Figure 2). This finding is expected based upon general principles of plant resource economics. The allocation of biomass to one plant organ or process (e.g., leaves and photosynthesis) necessarily involves de-allocation to a competing organ and interest such as roots or flowers (N foraging and storage, and reproduction, respectively) (Tilman 1988; Olff 1992). N allocation to the reproductive structures of a flower, particularly to N-rich proteins in pollen, can be subsidized by non-foliar plant parts or withdrawn from leaf N pools (Elser & Sterner 2002), which explains seasonal variation in foliar C:N.

Aside from purely physiological interest, this result is also pertinent to discussions of phenological complementarity in N use, as cup plant's seasonal window of rapid vegetative growth preceding reproduction was detected by lower C:N ratios during the pre-flowering phase due to increased N allocation to light foraging. Perhaps, for example, species assemblages featuring a variety of windows of rapid vegetative growth, flowering, and senescence stages would generate complementary relationships in N uptake and biomass production or promote coexistence (Hooper & Vitousek 1997; Tilman, Isbell & Cowles 2014). The lack of positive diversity-productivity and NUE-diversity relationships in community aboveground biomass herein suggest that cup plant's resource use was not complementary to neighboring species. More rigorous study of cup plant's seasonal variation in foliar C:N relative to that of neighboring species along a larger diversity gradient might reveal more complementary community resource use.

In contrast to my hypothesis (H₂), no significant relationship between cup plant foliar C:N and species diversity was observed. This result is surprising considering that leaves are expected to be more responsive to changes in fertility than are stems and roots (Elser & Sterner 2002). Further, as a nutrient, in this case N, becomes more limiting, its concentration relative to C generally tends to decrease in autotroph biomass (Elser & Sterner 2002). Borer *et al* (2015) found an unexpected result in which foliar C:N of four focal prairie species (two grasses and two legumes) decreased with increasing plant community species diversity in addition to increasing consumer diversity, though consumer diversity was a far stronger influence on C:N than plant diversity. Perhaps consumer diversity is an important environmental component untested herein responsible for the lack of association between C:N and diversity.

The above results suggest either that 1) N was not limiting cup plant at intra-seasonal sampling times and thus the community was not foraging for N at maximum efficiency, or 2) that cup plant allocated more biomass to roots for optimal N foraging as a response to decreased N:light resource ratios (Tilman 1988). I provide no conclusive evidence for either mechanism as plant-available soil N and light availability were not directly measured at these time steps, and were only assumed to decrease with increasing species diversity. A significant negative relationship between mean cup plant stem height and SDI at maximum shoot height (Figure 1) suggests that the second mechanism may have been operating, as optimal foraging theory also predicts that root:shoot ratios (shoot height or girth decreases relative to root biomass) should increase with decreasing nutrient:light ratios (Elser & Sterner 2002; Tilman 1988). If cup plant individuals were more limited by N than light as diversity increased, then they may have allocated more biomass to roots to forage for N. However, the monthly addition of NO₃-N may have overwhelmed any positive relationships between diversity and foliar C:N as the internal N content of communities is expected to increase with N addition (Bracken *et al* 2015). As observed in leaf tissue, then, cup plant was not more limited by N as diversity increased in these low diversity contexts. Further study of cup plant foliar C:N in higher diversity community settings than 12 species mixtures might reveal such a positive relationship.

In contrast to my hypotheses and the findings of previous studies (Fargione *et al* 2009; Fornara & Tilman 2009; van Ruijven & Berendse 2005), aboveground NUE of neither the focal species

(H_{3a}; Figures 3a, 4a) nor whole communities (Figure 5a) exhibited a significant positive relationship with community species diversity, whether this relationship was explored using planted species richness or SDI. More diverse species mixtures were expected to use N more efficiently when it is limiting due to increased spatial or temporal niche differentiation (Leimer, *et al* 2016). However, the positive relationships between NUE and diversity observed in these studies occurred in concert with positive diversity-productivity relationships, which were not observed herein (Figure 5b), and additionally, these studies did not include N addition. Neighboring species NUE (H_{3b}; Figures 3b, 4b), however, was positively associated with increasing species diversity from the two to five species richness level as well as on a continuous gradient derived from Simpson's Diversity Index. I offer an explanation for and implications of this differential response of co-occurring species in prairie buffers below.

Cup plant, on average, was a far more efficient user of N than neighboring species in this study. Therefore, N limitation for cup plant is likely met at a higher threshold than N limitation for neighboring species, which could explain why positive relationships between community and cup plant NUE and diversity did not occur while neighboring species NUE was positively associated with diversity (Tilman 1988). Because N was applied monthly to all plots to simulate agricultural runoff inputs, N limitation thresholds for cup plant and thus the community at large may have only been met at much higher levels of diversity, or, as indicated by the selected explanatory model, very high densities of cup plant. Additionally, the suggestive but insignificant jump in cup plant NUE at the 12 species richness level, which featured grasses, would indicate that grasses may remove much available N from the system (Abbas *et al* 2013), thus creating a more limiting N environment for cup plant.

Overall, community NUE was best explained by individual characteristics of cup plant (Table 3), indicating that species composition of prairie buffer communities is indeed an important factor for maximizing N removal from agricultural runoff. Community NUE increased with cup plant NUE and cup plant stem density, suggesting that for these prairie buffer communities, community NUE is highly dependent on highly productive component species such as cup plant. However, because the communities investigated herein were of low richness and evenness relative to remnant prairie communities, these relationships cannot be generalized. Therefore, further research of the N use of highly productive species like cup plant nested within the response of whole communities to a larger range of species richness levels is necessary to determine how important species composition is.

Conclusion

In this project, I sought to determine if species composition in addition to diversity was important for the capacity of prairie buffer communities to efficiently remove N from agricultural runoff. To this end, I compared the N composition of a highly productive prairie forb cup plant to the N composition of neighboring species in response to increasing species diversity. Major findings are summarized below.

- Community N composition was best explained by cup plant density and cup plant N composition.

- As community species diversity increased, cup plant and whole communities did not use N more efficiently as hypothesized, indicating that cup plant individuals and whole communities were not more limited by N.
- Neighboring species did use N more efficiently as community species diversity increased, indicating that species other than cup plant were more limited by N as community diversity increased.

My observations suggest that the N content (C:N and NUE) of prairie buffer communities featuring cup plant and perhaps other highly productive and efficient species, is not dependent only on diversity as such, but also upon the species composition and densities of highly productive species within the community. Cup plant appeared to override the expected positive influence of diversity on nitrogen use efficiency of prairie buffer communities by means of its high density and high NUE relative to neighboring species. Therefore, as far as aboveground N content may be linked to efficiency of N uptake from $\text{NO}_3\text{-N}$ enriched runoff, further research is necessary to assess the NUE of individual species nested within prairie buffer communities to improve these systems' capacity for N removal from agricultural runoff. Determining optimal mixtures and relative densities of species for maximizing N removal may be critical to improving the functional efficiency and efficacy of prairie buffer communities.

References

- Abbas, M., Ebeling, A., Oelmann, Y., Ptacnik, R., Roscher, C., Weigelt, A., Weisser, W.W., Wilcke, W. & Hillebrand, H. (2013) Biodiversity Effects on Plant Stoichiometry. *PLoS ONE*, **8(3)**, 1-11.
- Borer, E.T., Lind, E.M., Ogdahl, E.J., Seabloom, E.W., Tilman, D., Montgomery, R.A. & Kinkel, L.L. (2015) Food-web composition and plant diversity control foliar nutrient content and stoichiometry. *Journal of Ecology*, **103**, 1432-1441.
- Bracken, M.E.S., Hillebrand, H., Borer, E.T., Seabloom, E.W., Cebrian, J., Cleland, E.E., Elser, J.J., Gruner, D.S., Harpole, W.S., Ngai, J.T. & Smith, J.E. (2015) Signatures of nutrient limitation and co-limitation: Responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos*, **124**, 113-121.
- Cardinale, B.J. (2011) Biodiversity improves water quality through niche partitioning. *Nature*, **472**, 86-89.
- Craven, D., Isbell, F., Manning, P., Connolly, J., Bruelheide, H., Ebeling, A., Roscher, C., Van Ruijven, J., Weigelt, A., Wilsey, B., Thakur, M.P., Tilman, D., Vogel, A. & Eisenhauer, N. (2017) Plant diversity effects on grassland productivity are robust to both nutrient enrichment and drought. *Phil. Trans. R. Soc. B*, **371**.
- De Deyn, G.B., Quirk, H., Yi, Z., Oakley, S., Ostle, N.J. & Bardgett, R.D. (2009) Vegetation composition promotes carbon and nitrogen storage in model grassland communities of

- contrasting soil fertility. *Journal of Ecology*, **97**, 864-875.
- Fargione, J., Tilman, D., Dybzinski, R., Hille, J., Lambers, R., Clark, C., Harpole, W.S., Knops, J.M.H., Reich, P.B. & Loreau, M. (2009) From selection to complementarity: shifts in the causes of biodiversity–productivity relationships in a long-term biodiversity experiment. *Proc. R. Soc. B*, **274**, 871-876.
- Fornara, D.A. & Tilman, D. (2009) Ecological mechanisms associated with the positive diversity-productivity relationship in an N-limited grassland. *Ecology*, **90**(2), 408-418.
- Gansberger, M., Montgomery, L.F.R. & Liebhard, P. (2015) Botanical characteristics, crop management and potential of *Silphium perfoliatum* L. as a renewable resource for biogas production: A review. *Industrial Crops and Products*, **63**, 362-372.
- Gurevitch, J., S.M. Scheiner, & G.L. Fox, G.L. (2006). *The Ecology of Plants, 2nd Ed.* Sinauer Associates, Sunderland, MA, USA.
- Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L. & O'connor, M.I. (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, **486**, 105-108.
- Hooper, D.U., & Vitousek, M.I. (1997) The effects of plant competition and diversity on ecosystem processes. *Science*, **277**, 1302-1305.
- Horneck, D.A., & Miller, R.O., (1998) Determination of Total Nitrogen in Plant Tissue. p. 75-84 in *Handbook of Methods for Plant Analysis*. Ed.: Kalra, Y.P.
- Huston, M.A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**: 449–60.
- Leimer, Y., Oelmann, N., Eisenhauer, A., Milcu, C., Roscher, S., Scheu Alexandra, Weigel, C., Wirth, & Wilcke, W. (2016) Mechanisms behind plant diversity effects on inorganic and organic N leaching from temperate grassland. *Biogeochemistry*, **131**, 339–353.
- Novotny, A.M., Schade, J.D., Hobbie, S.E., Kay, A.D., Kyle, M., Reich, P.B. & Elser, J.J. (2007) Stoichiometric response of nitrogen-fixing and non-fixing dicots to manipulations of CO₂, nitrogen, and diversity. *Oecologia*, **151**, 687-696.
- Olf, H. (1992) Effects of light and nutrient availability on dry matter and N allocation in six successional grassland species: Testing for resource ratio effects. *Oecologia*, **89**, 412–421.
- Ramsey, F., & Schafer, D., (2013) *The Statistical Sleuth: A Course in Methods of Data Analysis*, 3rd Edition.
- Sterner, R.W., J.J. Elser. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to Biosphere*. Princeton University Press. Princeton, N.J., USA.
- van Ruijven, J., F. Berendse. 2005. Positive effects of plant species diversity on productivity in the absence of legumes. *Ecol. Lett.* **6**, 170–75.
- Tilman D. 1988. *Plant Strategies and the Dynamics and Structure of Plant Communities*. Princeton University Press. Princeton, N.J., USA.

Tilman D., F. Isbell, J.M. Cowles (2014) Biodiversity and Ecosystem Functioning. *The Annual Review of Ecology, Evolution, and Systematics*, **45**, 471-493.

Wedin, D., (1996) Nutrient cycling in grasslands: An ecologist's perspective. p. 29-44 in *Nutrient Cycling in Forage Systems*. Eds: Joost, R.E., Roberts, C.A. Potash and Phosphate Institute, Manhattan, KS, USA.

Zhou, X., Helmers, M., Asbjornsen, H., Kolka, R. & Cruse, R. (2014) Nutrient removal by prairie filter strips in agricultural landscapes. *Journal of Soil and Water Conservation*, **69(1)**, 54-64.