

SPECIAL FEATURE – STANDARD PAPER

WHETHER IN LIFE OR IN DEATH: FRESH PERSPECTIVES ON HOW PLANTS AFFECT BIOGEOCHEMICAL CYCLING

Food-web composition and plant diversity control foliar nutrient content and stoichiometry**Elizabeth T. Borer^{1*}, Eric M. Lind¹, Eric J. Ogdahl^{1,2}, Eric W. Seabloom¹, David Tilman¹, Rebecca A. Montgomery³ and Linda L. Kinkel⁴**

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Summary

1. The content and ratio of nutrients in plants can be constrained by a wide array of factors, including nutrient supply, light intensity, herbivory, infection or intrinsic growth rate and can, in turn, affect many ecosystem processes including photosynthesis, decomposition, resource limitation and nutrient cycling. Studies of plant stoichiometry and stoichiometric homeostasis have focused primarily on the role of nutrient supply as a constraint on plant tissue chemistry, yet recent work suggests that local plant diversity, plant species composition and consumers may change the nutrient composition of whole plant communities.

2. By experimentally removing insects, foliar fungi and soil fungi from a long-term experiment manipulating plant diversity, we found that the foliar stoichiometry of individual plant species depends on biotic context.

3. Local plant diversity and the composition of the consumer community each altered foliar tissue carbon and nutrient chemistry of four different grassland species. The greatest impacts of consumers on foliar chemistry occurred at low plant diversity, and these changes induced by altering the food web were of a similar magnitude to the effects of fertilization or drought found in previous work. Consumers and plant diversity acted primarily on foliar carbon and nitrogen, whereas changes in foliar phosphorus were associated with the productivity of the local plant community. Thus, changes in whole-community stoichiometry that have been documented in response to alterations of the consumer food web or plant community are underlain by stoichiometric changes in individual species as well as plant species compositional changes.

4. *Synthesis.* These results suggest a new pathway by which loss of consumer or plant diversity may significantly impact the wide variety of ecosystem processes that depend on foliar nutrient content.

Key-words: carbon, foliar fungi, functional group, grassland, insects, legumes, nitrogen, phosphorus, plant population and community dynamics, productivity

Introduction

Leaf elemental content [e.g. nitrogen (N), phosphorus (P) or carbon (C)] and ratios can be used to predict rates such as

herbivore and detritivore growth and reproduction, disease dynamics, ecosystem productivity, nutrient cycling, decomposition and resource limitation in populations and communities (Mattson 1980; Cebrian 1999; Elser *et al.* 2000; Perez-Harguindeguy *et al.* 2000; Moe *et al.* 2005; Smith 2007). The ratio of foliar N:P may reflect species-specific functional

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traits, as it generally scales with the nutrient content of other plant tissues (Kerkhoff *et al.* 2006), and N:P can constrain population growth rates and relative competitive abilities of plants in shared environments (Hillebrand & Sommer 1999; Güsewell 2004). Among autotrophs, the N:P ratio often indicates the nature of nutrient limitation, as these macronutrients constrain growth rates for most autotrophic species (Elser *et al.* 2000), but the relative requirements for and content of macronutrients differ among autotroph species (Tessier & Raynal 2003; Ågren 2004).

Although it is well-established that the nutrient content of autotroph communities can change with nutrient supply (e.g. Ordonez *et al.* 2009; Bracken *et al.* 2015), there is growing evidence that nutrient composition at the plant community scale also may be influenced by the diversity and composition of the plants and consumers within the community (Abbas *et al.* 2013, 2014; Ebeling *et al.* 2014; Striebel, Behl & Stibor 2009; Zhang, Han & Elser 2011; De Deyn *et al.* 2009). For example, recent work has demonstrated that both vertebrate and invertebrate consumers can alter plant stoichiometry (Zhang, Han & Elser 2011; Zheng *et al.* 2012). In addition, when grassland plant diversity is manipulated, the aggregate above-ground C:P and N:P ratios increase with both plant functional group richness and species diversity, and the composition (e.g. legume or grass abundance) also can substantially alter community level plant tissue chemistry (Abbas *et al.* 2013). The community-scale chemistry can, in turn, determine the stoichiometric composition of consumers (Abbas *et al.* 2014) and control community-wide herbivory and decomposition rates (Ebeling *et al.* 2014). However, these studies focus on aggregate plant community nutrient stoichiometry rather than individual species responses. Thus, it is unclear how much of the observed community-scale change arises from a shift in the relative abundance of species with differing C, N and P content and how much arises from variation in the tissue chemistry of individual species (Abbas *et al.* 2013).

Stoichiometric homeostasis is the ability of an organism to maintain its internal elemental composition regardless of the elemental composition of its resource supply (Sterner & Elser 2002). Whereas vascular plants are generally assumed to maintain a higher degree of homeostasis than autotrophs such as algae (Sterner & Elser 2002), there is considerable interspecific variation in tissue C:N:P ratios and the homeostasis of these ratios (Güsewell 2004; Novotny *et al.* 2007; Yu *et al.* 2011; Borer *et al.* 2013; Bracken *et al.* 2015). Variability in the content of nutrients and carbon among individuals of the same species reflects many concurrent environmental and physiological processes supporting the acquisition of these elements (Aerts & Chapin 2000; Sterner & Elser 2002), but the resulting plant tissue elemental balance can determine the rate of many ecological processes, including carbon uptake and growth, loss to herbivory, infection and decomposition, and the fate of organic matter (Mattson 1980; Cebrian 1999; Smith 2007; Schmitz 2008; Hillebrand *et al.* 2009). Species' homeostasis can scale up to predict community characteristics and ecosystem carbon capture and loss: recent work demonstrates that communities composed primarily of

homeostatic species are more productive and stable (Yu *et al.* 2010). However, changes in biomass C:N:P ratios observed in vascular plant communities (e.g. De Deyn *et al.* 2009; Abbas *et al.* 2013) likely reflect a combination of changes in individual physiology and nutrient chemistry (Novotny *et al.* 2007) as well as turnover in the relative abundance or identity of species present in a community (Elser *et al.* 2010).

In addition to community-scale stoichiometric changes with plant diversity, evidence also is emerging that consumers can alter individual (Zhang, Han & Elser 2011) and community-scale (Zheng *et al.* 2012) nutrient ratios in plants via selective consumption of nutritious plant tissues and plant species. However, the effects of trophic interactions on plant stoichiometry and homeostasis have received little attention, to date (Elser *et al.* 2007, 2010). Here, we examine the degree to which foliar stoichiometry of grassland plants changes within individual species growing in communities of differing plant species richness and food-web composition. We examine the role of food-web complexity in controlling foliar stoichiometry by determining the role of different consumer groups on species-level nutrient content and stoichiometry in four grassland species, two warm season (C4) grasses and two legumes. We use long-term experimental manipulations of plant and food-web diversity to quantify whether plant and consumer community diversity and composition independently or interactively control species-level stoichiometry. We also examine the role of longer-term effects of plot-scale plant functional group composition (i.e. mass of legumes, forbs, C3 grasses and C4 grasses) and productivity (i.e. peak annual biomass) on foliar nutrient content.

Materials and methods

The 9 × 9 m biodiversity treatments used in the current experiment were planted in 1994 with 1, 4 and 16 perennial prairie species. Species in each treatment were randomly chosen from a pool of 18 species, spanning four functional groups: legumes, forbs, C3 grasses and C4 grasses (for methods details, Tilman, Reich & Knops 2006).

In 2008, we established experimental food-web manipulation subplots nested within 41 plant diversity experimental plots to examine the role of different components of the grassland food web in modifying ecosystem functions. In these 1.5 × 2 m subplots, we manipulated the presence of insects, soil fungi and foliar fungi by applying fungicides and insecticides alone and in combination on a fortnightly basis. The food-web manipulation treatments were crossed with plant community diversity for a total of five food-web manipulation treatment subplots (control, soil fungicide, foliar fungicide, insecticide and all combined) that were randomly assigned to locations within each of the 41 plant diversity plots (5 × 41 = 205 food-web manipulation subplots).

The grassland food web was manipulated using commonly applied agricultural pesticides. To control foliar fungi, we applied Quilt (Syngenta Crop Protection, Inc., Greensboro, NC, USA), a combination of azoxystrobin (7.5%) and propiconazole (12.5%), every other week from the beginning of the growing season through mid-September. Azoxystrobin inhibits fungal mitochondrial respiration, stopping spore production, germination and mycelial growth of fungi; propiconazole inhibits fungal demethylation, stopping cellular growth. Because these

chemicals differ in action, their joint application reduces the likelihood of fungal adaptive resistance. Soil fungi were controlled via monthly application throughout the growing season (late-April until mid-September) of Ridomil Gold SL (Syngenta Crop Protection, Inc.), a soil drench fungicide containing mefenoxam (45.3%). Mefenoxam inhibits ribosomal RNA synthesis in many soil-inhabiting fungi, stopping mycelial growth and spore formation. We reduced insects in the subplots via application of the insecticide Marathon II (OHP, Inc., Mainland, PA, USA; 21.4% imidacloprid), every other week through the growing season. One to two times each growing season, malathion was applied instead of Marathon II to reduce the possibility for adaptation by the local insect populations to insecticide. Both of these chemicals are broad-spectrum insect neurotoxins; imidacloprid is a neonicotinoid compound, whereas malathion is an organophosphate insecticide. The class of fungicidal compounds used in this study (Triazoles) has been shown not to induce changes in the growth, height or leaf area of wild grasses (Paul, Ayres & Wyness 1989); less is known of their effects on non-agricultural legumes. Similarly, work examining the insecticides used in this study suggests minimal direct effects on plant growth (Brown, Leijn & Stinson 1987) or foliar nitrogen (Syvertsen & Dunlop 2009), and there is no evidence for non-target effects of pesticide treatments on other components of the food web (e.g. fungicides on insect communities, Sotherton & Moreby 1988).

To examine the role of food-web composition and plant diversity on plant traits, in August 2011, we collected leaves from four focal species in two functional groups, *Andropogon gerardii* and *Schizochyrium scoparium* (C4 grasses), and *Lespedeza capitata* and *Lupinus perennis* (legumes), in the treated subplots nested in the 1, 4 and 16 species diversity plots. These species were selected to represent a broad range of the foliar chemistry and other traits represented by the planted species in this experiment (Craine *et al.* 2002). For each species, leaves of 2–4 individual plants per subplot were collected between 1 August 2011 and 15 August 2011. Because every species did not occur in every subplot, between 31 and 43 of the 205 food-web manipulation subplots contained, and were sampled for, the focal species (*A. gerardii* from 31; *L. capitata* from 43; *L. perennis* from 33; *S. scoparium* from 34) for a total of 498 individual plants sampled from the food-web manipulation subplots for this study. Across the planted diversity gradient, each species was found in a single monoculture plot, 1–3 four-species mixtures and 3–5 plots planted with 16 species. For *L. perennis*, *S. scoparium* and *A. gerardii*, we

collected the entire above-ground tiller closest to a randomly determined location within each subplot; for *L. capitata*, we collected the top one-third of the stem.

Samples were brought back to the laboratory, and we determined the effect of the food-web manipulation treatments on each individual by measuring foliar fungal infection severity (following James 1971) and recording the proportion of leaves with herbivory damage (Siemann *et al.* 2004; Schowalter 2011). Leaves and petioles from each species were then dried and homogenized by sample subplot. A subsample of the dried and homogenized tissue of each plant species was assayed for %C and %N using dry combustion GC analysis (Ecosystems Analysis Laboratory at University of Nebraska, using a COST-ECH ESC 4010 Element Analyzer). Another subsample from each subplot was ashed (following Miller 1998) and assayed for %P via sulphuric acid digestion followed by spectrophotometric analysis (for methods details, Clesceri, Greenberg & Eaton 1998).

We assessed the changes in each of the measured plant nutrients and nutrient ratios in response to manipulated plant diversity and food-web composition by developing individual regression models for each nutrient and ratio. We included the 9 × 9 m plant diversity plots and 1.5 × 2 m food-web manipulation subplots as random effects in our regression models to account for the nested study design. This approach effectively accounts for the nested structure of the data and uses the correct degrees of freedom for each test (Pinheiro & Bates 2000). Using planned contrasts, we tested for differing responses of foliar chemistry between grasses and legumes and between the species pairs within each functional group in response to the experimental treatments, plot productivity and plot composition. To assess whether the food web has a changing role in determining foliar chemistry at low vs. high plant diversity, we analysed means across the four focal species to provide replicated estimates of foliar responses at all levels of plant diversity. Because the experiment lacked replicated plot-scale monocultures for each focal species, we did not test for interactions among the four different focal species' responses to food-web treatments as a function of plant diversity. Plant diversity was natural log-transformed in all analyses, and all analyses were performed in R (v. 3.1.1; R Foundation for Statistical Computing, Vienna, Austria).

We developed individual response models using model averaging (Grueber *et al.* 2011) to assess the relative importance of many potentially covarying factors and to recognize explicitly that there could be a suite of similar models describing the observed changes in plant carbon and nutrient chemistry of our focal species. We used the lmer

Table 1. Model selection for tissue nutrient and carbon content in all diversity plots. Model shows only parameters with high estimated importance (> 0.75, see Materials and methods). All parameters that were included in any model within four AIC units of the best model, regardless of importance or *z*-value, are shown in Table S1. There were 706 total models fit for each parameter (max of 10 parameters for each model). Importance is single value for species contrasts

Category	Source	Per cent N (<i>N</i> = 13 models)			Per cent P (<i>N</i> = 29 models)			Per cent C (<i>N</i> = 12 models)		
		Import.	Est.	<i>z</i>	Import.	Est.	<i>z</i>	Import.	Est.	<i>z</i>
Intercept	Intercept		1.637	45.5		0.188	29.7		45.004	299.3
Plant diversity	Plot richness	1.00	0.047	0.8				1.00	0.274	1.1
Species	Grasses vs. legumes	1.00	−0.470	31.2	1.00	0.034	13.1	1.00	0.244	2.7
	Among grasses	1.00	0.044	2.1	1.00	0.026	7.3	1.00	−0.258	2.0
	Among legumes	1.00	0.045	2.3	1.00	0.008	2.4	1.00	2.814	23.5
Food web manipulation	Insecticide	0.93	0.054	1.6						
	Foliar fungicide	1.00	−0.055	1.6				1.00	−0.040	0.2
Diversity by food web	Diversity by insecticide	0.88	−0.147	2.5						
	Diversity by foliar fung.	1.00	0.216	3.3				1.00	1.152	3.4

Table 2. Model selection for ratios of carbon and nutrients in tissues in all diversity plots. Model shows only parameters with high estimated importance (> 0.75 , see Materials and methods). All parameters that were included in any model within four AIC units of the best model, regardless of importance or significance, are shown in Table S2. There are 706 total models fit for each parameter (max of 10 parameters for each model). Importance is single value for species contrasts

Category	Source	C:N ($N = 24$ models)			C:P ($N = 23$ models)			N:P ($N = 23$ models)		
		Import.	Est.	z	Import.	Est.	z	Import.	Est.	z
Intercept	Intercept		30.559	51.0		253.455	31.0		9.570	40.5
Plant diversity	Plot richness	1.00	-1.724	1.7						
Species	Grasses vs. legumes	1.00	8.904	30.9	1.00	-41.430	13.0	1.00	-4.179	39.5
	Among grasses	1.00	-1.636	4.1	1.00	-26.484	6.0	1.00	-0.388	2.6
	Among legumes	1.00	0.874	2.3	1.00	1.785	0.4	1.00	-0.526	3.8
Food-web manipulation	Foliar fungicide	1.00	1.191	1.8						
Diversity by food web	Diversity by foliar fungicide	1.00	-4.899	3.3						

function in the lme4 R library and dredge and model.avg functions in the MuMIn R library to assess models and estimate parameter values. In brief, across all diversity levels (Tables 1 and 2), we fit all possible subsets of the following model to test for effects of the food web and plant diversity treatments on plant tissue chemistry:

Tissue Chemistry \sim Species + Plant Diversity
 + Food-web Treatment + Species * Food-web Treatment
 + Plant Diversity * Food-web Treatment

To examine the role of the local functional group composition and productivity in indirectly altering foliar stoichiometry of our focal species, we performed an additional analysis in the 16-species plots; we focus on these because in monoculture and 4-species plots, plot functional group composition was partially or fully confounded with the focal species. We determined the relationship between the foliar chemistry of our focal species and the legume, forb and C4 grass functional group composition of plots by estimating the biomass of these groups in subplots of the 16-species plots. Total above-ground productivity

also can affect nutrient cycling (Craine *et al.* 2002) potentially altering foliar nutrient content (Jarrell & Beverly 1981), so we included this factor in our models, as well. In 2011, all above-ground biomass was clipped as close to the ground as possible in a 2×0.1 m strip in each subplot using a 0.1-m wide handheld clipper. This biomass was sorted to species, dried to constant mass and weighed to the nearest 0.001 g. We summed across the mass of all species within each functional group in each subplot to determine the total functional group biomass. We examined the effect of the local functional group composition and productivity on the tissue chemistry of our focal species in the 16-species plots using the following model (Table 3):

Tissue Chemistry \sim Species + Legume Mass + Forb Mass
 + C4 Grass Mass + Total Plant Mass
 + Food-web Treatment + Species
 * Food-web Treatment

For both analyses, we calculated the AIC for all nested models then selected the set of models that were within four AIC units of the best (lowest AIC) model (Tables 1–3). Because all models are

Table 3. Model selection for tissue carbon, nutrients and their ratios in 16-species plots. Model shows only parameters with high estimated importance (> 0.75 , see Materials and methods). All parameters that were included in any model within four AIC units of the best model, regardless of importance or significance, are shown in Table S3 and S4. There are 387 total models fit for each parameter (max of 10 parameters for each model). Importance is single value for species contrasts

Category	Source	Per cent N ($N = 40$ models)			Per cent P ($N = 27$ models)			Per cent C ($N = 43$ models)		
		Import.	Est.	z	Import.	Est.	z	Import.	Est.	z
Intercept	Intercept		1.647	40.5		0.188	49.8		45.039	302.1
Plant community	Log Total Plot Mass				0.93	0.015	2.6			
Species	Grasses vs. legumes	1.00	-0.456	26.3	1.00	0.033	12.0	1.00	0.330	2.8
	Among grasses	1.00	0.038	1.5	1.00	0.033	8.4	1.00	-0.317	1.8
	Among legumes	1.00	0.030	1.3	1.00	0.008	2.2	1.00	2.593	16.0

Category	Source	C:N ($N = 58$ models)			C:P ($N = 32$ models)			N:P ($N = 69$ models)		
		Import.	Est.	z	Import.	Est.	z	Import.	Est.	z
Intercept	Intercept		30.156	44.1		253.362	42.4		9.572	60.1
Plant community	Log Total Plot Mass				0.96	-22.107	2.3			
Species	Grasses vs. legumes	1.00	8.686	27.3	1.00	-40.004	11.3	1.00	-4.076	38.2
	Among grasses	1.00	-1.694	3.6	1.00	-32.952	6.3	1.00	-0.614	3.9
	Among legumes	1.00	0.900	2.1	1.00	1.138	0.2	1.00	-0.532	3.7

subsets of the full model, they do not exceed allowable degrees of freedom in the total data set. Model parameter estimates were made only for models in which the parameter was included (natural average method, Grueber *et al.* 2011). The AIC-weighted 'importance' represents the relativized sum of the Akaike weights summed across all models in which the parameter appears that are within four AIC units of the model with the lowest AIC value. Thus, parameters without estimates were never included in models within four AIC units of the top model. In all models presented here, parameter importance is strongly correlated with the proportion of final models containing each parameter, so we only present importance in the model tables. We present estimates for parameters with high importance (> 0.75) in the main text and all importance, parameter estimates and z -values for models in the supplemental tables (Tables S1–S4 in Supporting Information).

Results

The food-web treatments substantially altered infection and damage on the focal plants. The foliar fungicide treatment reduced per cent foliar fungal infection severity approximately threefold from 10.2% (SEM = 1.4) in control plots to 3.0% (SEM = 0.3), where fungicide was applied ($P < 0.01$). Similarly, the insecticide treatment reduced the proportion of insect-damaged leaves approximately twofold from 9.2% (SEM = 2.4) in control plots to 5.0% (SEM = 0.9), where insecticide was applied ($P < 0.01$). Although we do not have a comparable measure for soil fungi, this treatment substantially altered plant composition (Fig. S1), demonstrating sig-

nificant plot-scale effects of this treatment, as well. Thus, the intensity of food-web interactions (fungal infection and insect herbivory) was substantially altered by the food-web manipulation treatments.

Overall, plant tissue chemistry differed strongly among the focal species (Fig. 1), but species' chemistry also changed in response to interactions between food-web composition and plant diversity (Fig. 2, Tables 1 and 2) and with plant productivity (Table 3). Across treatments, foliar %N was higher in legume species, whereas foliar %P was higher in grass species (Fig. 1, Table 1). Foliar %C was similar in the two grass species, but was strongly divergent between the legumes (Fig. 1, Table 1). Grass %C was intermediate to the foliar %C of the two legumes, with foliar %C higher in *L. capitata* and lower in *L. perennis* compared to the grasses (Figs 1 and 2). Foliar CN ratios were much lower in legumes than grasses (Fig. 1, Table 2), and CN also varied with the composition of the food web (Table 2). Because of their relatively low foliar P, legumes had higher foliar NP and CP ratios than grasses (Fig. 1, Table 2).

With increasing diversity of the local plant community, foliar %C and %N increased in the focal species (Table 1). However, because %N increased more rapidly than %C, foliar CN ratios in the focal plant species declined with increasing plant diversity (Table 2). Although foliar %P did not vary as a function of plot-scale plant diversity (Table 1), %P of the focal species increased with total plot productivity in 16-species plots (Table 3). In contrast to the effects of plant diversity and productivity, the functional group composition of the

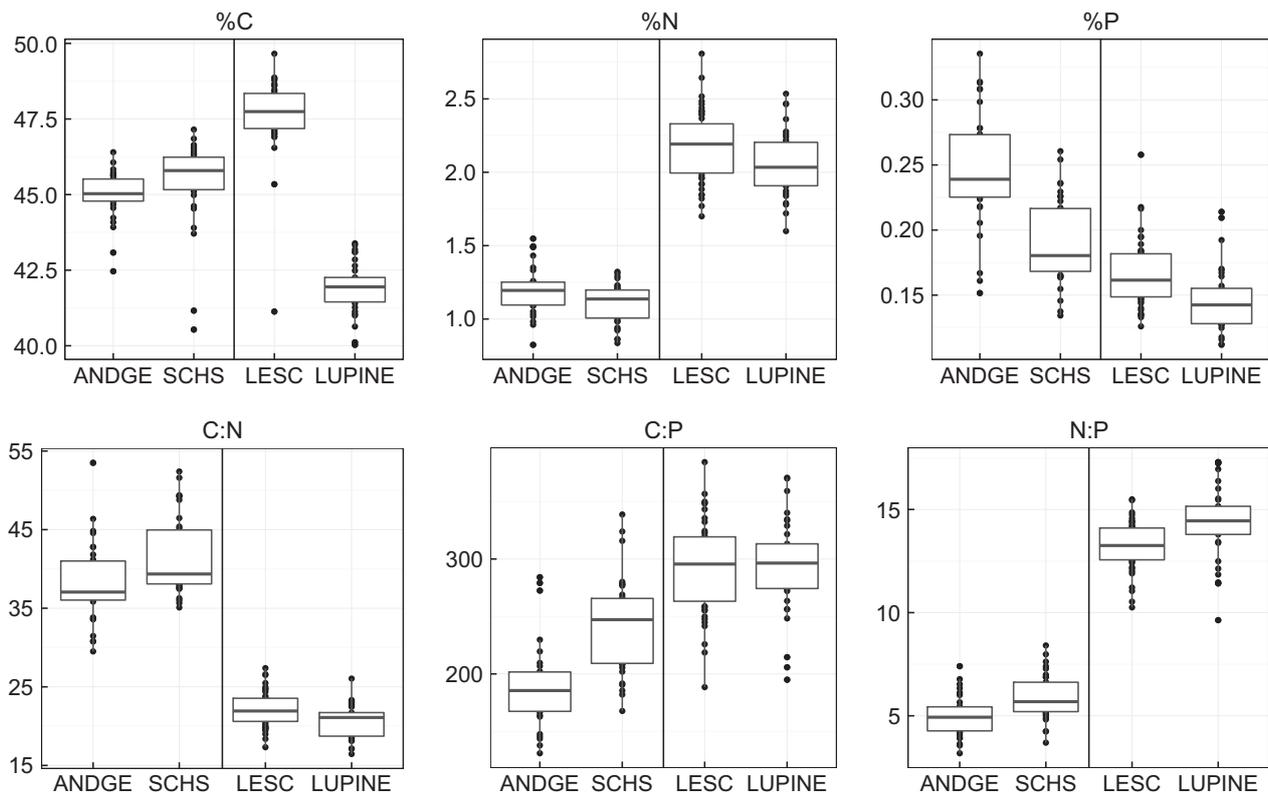


Fig. 1. Foliar carbon and nutrient percentages and ratios for C4 grasses *A. gerardii* (ANDGE) and *S. scoparium* (SCHS) and legumes *L. capitata* (LESC) and *L. perennis* (LUPINE) across all study plants.

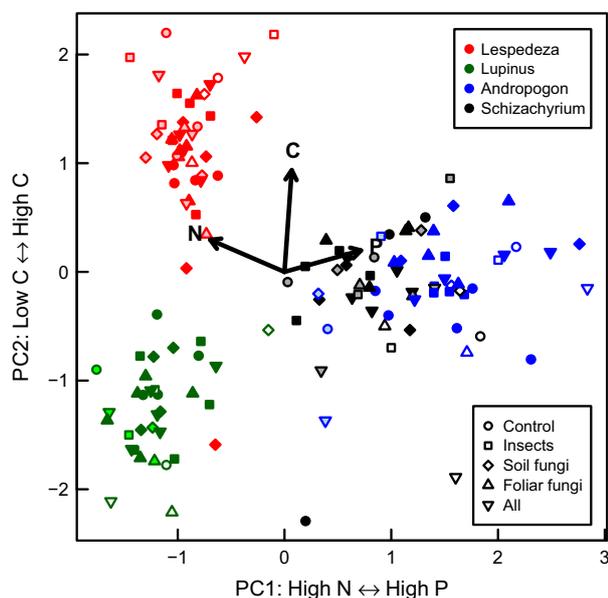


Fig. 2. Principle components analysis illustrates relationships among %C, %N and %P for C4 grasses *A. gerardii* and *S. scoparium* and legumes *L. capitata*, and *L. perennis* in response to each of the food-web manipulation treatments. The first two axes accounted for 84% of the variance (PC1 = 48%, PC2 = 36%). Arrows represent the raw loadings of the original carbon and nutrient variables on the first two axes. Symbols represent each treatment; colour density is greatest in 16-species plots and least in monocultures.

local community did not strongly alter the C, N or P chemistry of any individual species (Table 3).

Food-web composition and gradients of species richness induced variation in plant tissue C and N chemistry across all study species and were included in all of the top models describing these responses. Although foliar P increased with plot-scale productivity, %P did not vary predictably with consumer manipulations or plot diversity. Functional groups differed in their responses to changes in the food web: the grass foliar CN ratio was higher and far more variable than the legume CN ratio in response to the food-web treatments (Table 2). Removal of foliar fungi had the strongest and most consistent effects on foliar chemistry across the focal species, causing approximately a 20% change in foliar %N across the range of plant diversity treatments (Figs 3 and S3). For all species, foliar fungicide reduced foliar %N and %C in low diversity plots (Table 1, Figs S2 and S3); however, in the absence of foliar fungi, %N (Fig. 3) and %C increased with plant diversity across all species (Table 1, Figs S3 and S4). As a result, foliar CN was elevated in the absence of foliar fungi at low diversity relative to the control subplots, and CN increased with decreasing diversity (Table 2, Fig. S5). Foliar CP and NP ratios were not strongly or consistently affected by foliar fungicide (Table 2). In contrast, insecticide increased foliar %N, particularly at low plot diversity (Fig. 3, Table 1, Fig. S3), but did not induce changes in foliar %P, %C or any ratios for any of the focal species (Table 1, Figs S2 and S4–S7). Soil drench fungicide did not affect the foliar chemistry of any of the focal plants.

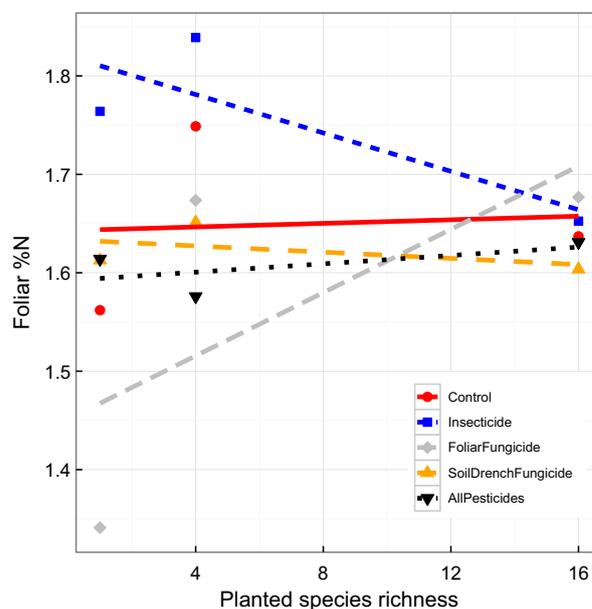


Fig. 3. Mean effect of food-web manipulation treatments and plant diversity on subplot-scale foliar %N across the four study species. The solid line represents control subplot means, dashed lines are the three individual food-web manipulation treatments, and the dotted line shows the plot-scale response when all three food-web manipulation treatments are combined. Fig. S3 shows trends of individual species.

Insecticide and foliar fungicide induced opposing effects of nearly identical magnitude overall and across a gradient of diversity, thus apparently counteracting each other when in combination (Fig. 3, Table 1). In particular, subplots where foliar fungi, soil fungi and insects were concurrently removed, there were no significant changes in plant chemistry at any diversity level (Fig. 3, Tables 1 and 2). Thus, in spite of the individual effects of foliar fungicide and insecticide on plant chemistry, their combined effect did not differ from control subplots with both groups present because of their nearly equal and opposite effects on tissue chemistry.

Discussion

The local plant community diversity and the relative abundance of consumer groups in the community each induced independent effects on the tissue carbon and nutrient chemistry of four different grassland species. Consumer manipulations thus join plant community characteristics such as diversity, productivity (Craine *et al.* 2002) and the abundance of legumes or forbs (Fornara, Tilman & Hobbie 2009) as forces that can alter nutrient dynamics in plant communities and induce changes in foliar chemistry of individual species. In particular, food-web composition primarily altered plant tissue C and N in our focal species, and these effects increased as diversity declined. In contrast, plant tissue P and the C:P ratio varied most strongly with productivity, not consumers. The within-species changes in foliar chemistry induced by changes in the food web and local plant diversity were of a similar magnitude to effects induced by experimental fertilization and drought treatments (e.g. Heckathorn &

Delucia 1995; Joern & Mole 2005), suggesting that consumers also can induce meaningful changes in plant chemistry. Although the rate and ratio of nutrient supply have been shown to be important determinants of vascular plant species' stoichiometry (e.g. Ordonez *et al.* 2009; Bracken *et al.* 2015), this experiment demonstrates that the nutrient content and ratios in plant tissues also vary among species in response to the local food-web composition. Given the role of plant chemistry in processes spanning individual physiology up to ecosystem processes (Elser 2006; Schmitz 2008), the importance of community context for determining the content and ratio of nutrients in grassland species suggests an important, reciprocal role of species' competitive and trophic interactions in these processes.

Growing recognition of the importance of ecological stoichiometry in understanding ecological systems has led to an increasing number of studies of homeostasis of nutrient content and ratios in a wide range of organisms (Persson *et al.* 2010). Although stoichiometric homeostasis has been found in wetland plants and aquatic macrophytes (Demars & Edwards 2007), other studies have found a wide range of tissue nutrient content within species under differing environmental nutrient supply (Persson *et al.* 2010; Yu *et al.* 2011). The current study provides insights into the unexplained variation in plant chemistry that has been observed within and among vascular plant taxa (Yu *et al.* 2011). Whereas work to date has demonstrated that factors such as light, nutrient supply, growth rate or phylogenetic constraints can control plant nutrient content and stoichiometry (Güsewell 2004; Kerkhoff *et al.* 2006; Elser *et al.* 2010), the current work clarifies that the plant diversity and food-web context also can shape the tissue nutrient content of individual species.

Our results build on previous work examining the effects of consumers on foliar nutrients and the intensity of consumer–plant interactions across diversity gradients. For example, the severity of infection by species-specific foliar fungi declines with increasing plant diversity (Mitchell, Tilman & Groth 2002; Rottstock *et al.* 2014), suggesting that foliar fungi have greatest effects on their host plants at low plant diversity. The decline in foliar N in subplots where fungi were removed in the current experiment, particularly at low diversity, is consistent with past studies which have shown that foliar fungal diseases can cause N retention in diseased leaves (Bastiaans 1993; Garry *et al.* 1996; Gooding *et al.* 2005). Taken together, these results suggest that the magnitude of the effect of foliar fungi on host foliar chemistry depends on the intensity of the fungal–host interaction. Similarly, insect herbivores tend to occur in greatest density (Root 1973) and cause greatest damage (e.g. Stein *et al.* 2010), where plant species are present in monoculture stands. Where we removed insects, particularly at low plant diversity, foliar N increased which is consistent with recent work demonstrating an increase in the CN ratio of food plants with high grasshopper density due to selective removal of the most nutritious plants and tissues (Zhang, Han & Elser 2011). While translocation of nutrients to below-ground

tissues in response to herbivory could lead to the patterns we observed, the effects observed here are in contrast to earlier work on *A. gerardii* that found increased foliar %N with hand pruning (Seastedt, Ramundo & Hayes 1988), further substantiating the likelihood that these patterns were generated via selective herbivory. Thus, our results extend previous work by demonstrating that these documented effects of consumers on foliar chemistry are general across all four of the species studied. The opposing effects of insects and fungi on plant C and N chemistry, particularly at low diversity, additionally demonstrate that different members of the consumer food web can induce counteractive, stabilizing effects on foliar chemistry.

These results also suggest a new pathway by which consumers may play a role in feedbacks to plant tissue chemistry that determine net competitive ability and relative abundance within communities. Models of resource competition among plant species often assume that individuals only alter nutrient availability via consumption and predict that the species that can persist on the lowest supply of a shared resource will competitively exclude others (e.g. Tilman 1982); however, inclusion of nutrient recycling can induce long-term species cycling or successional processes (Daufresne & Hedin 2005). In experimental grasslands, above-ground nutrient content and stoichiometry at the community level also can change with plant composition and diversity (De Deyn *et al.* 2009; Abbas *et al.* 2013). Our results suggest that predictable changes in the chemistry of whole communities with changes in the consumer community (Zheng *et al.* 2012) may be partially a function of changes in species-level foliar chemistry, but are likely a combination of changes in individual chemistry and in the relative abundance of species.

In spite of the compositional change induced in the plant community by the soil fungicide treatment, we found no net effect of soil fungicide on the foliar chemistry of our focal species. Soil fungi include a diverse array of species spanning beneficial, neutral and antagonistic interactions with plant species (Raaijmakers *et al.* 2009). For example, mycorrhizal fungi in the soil are well known for their key role in the phosphorus nutrition of plants (Bolan 1991), suggesting that this treatment should have led to reduced foliar phosphorus, particularly in those species most dependent on mycorrhizal associations. However, soil fungi also include pathogens that can suppress plant nutrient uptake and growth, often with the most extreme effects at low plant diversity (Maron *et al.* 2011). Thus, a counterbalancing effect among the many groups represented in the soil food web may have led to the absence of an effect of this treatment on foliar chemistry (Raaijmakers *et al.* 2009).

Past work has demonstrated that the current study's species affect soil nutrients via species-level differences in tissue chemistry (Wedin & Tilman 1990) and fine root turnover (Fornara, Tilman & Hobbie 2009), and even apparently small changes in soil C, N and C:N due to these species-level differences can significantly alter the long-term trajectory of soil N-mineralization (Wedin & Pastor 1993). Further, the within-

species changes in foliar C and N chemistry induced by altering the food web were similar in magnitude to within-species changes induced by manipulations such as drought or fertilization (Heckathorn & Delucia 1995; Joern & Mole 2005; Novotny *et al.* 2007) and to differences among grassland species (Tjoelker *et al.* 2005), suggesting that the magnitude of within-species foliar nutrient changes induced by foliar fungal infection and insect damage could be large enough to alter nutrient cycling rates. Past work has shown that foliar fungi can influence litter decomposition and nutrient cycling in both forest trees (Osono 2006) and grasslands (Lemons, Clay & Rudgers 2005; Purahong & Hyde 2011). Grasshopper consumption and excretion rates also can impact grassland plant stoichiometry (Zhang, Han & Elser 2011) and nitrogen recycling, ultimately influencing plant community composition (Belovsky & Slade 2000). The results of the current work suggest that declines in plant diversity (Hooper *et al.* 2012) and changes in grassland composition may lead to greater variation in individual plant stoichiometry, possibly altering ecosystem process rates, in response to the local food-web composition.

Foliar tissue stoichiometry represents an aggregate measure of plant physiology which can be constrained by factors including edaphic nutrient supply, light availability, growth rate, competition, infection and herbivory (Elser *et al.* 2010). Yet the primary focus of studies of ecological stoichiometry of individuals in a community context has been on the degree of homeostasis of plants subjected to different nutrient environments (e.g. Yu *et al.* 2011). In addition, the role of plant foliar carbon and nutrient stoichiometry for consumer nutrition and feeding rates has received far more attention, to date, than the effects of consumers on plant stoichiometry (e.g. Hillebrand *et al.* 2009; Elser *et al.* 2010). In grasslands, both community-scale and individual-scale stoichiometry can vary with plant diversity (Novotny *et al.* 2007; De Deyn *et al.* 2009; Abbas *et al.* 2013). Here, we have demonstrated that the stoichiometry of individual plant species also varies in response to the composition of the consumer community. In particular, the greatest impact of consumers on foliar carbon and nitrogen in both grasses and legumes occurred at low plant diversity. Foliar tissue phosphorus, in contrast, varied most strongly with local plant productivity, but not plant diversity or food-web composition. These findings highlight the potential for feedbacks between the diversity and composition of plant and consumer communities and the foliar chemistry of individuals and have significant implications for the wide variety of ecosystem processes that depend on foliar nutrient content (Cebrian 1999; Elser *et al.* 2000; Moe *et al.* 2005; Smith 2007; Schmitz 2008).

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Author contributions

ETB and RM conceived of the tissue sampling. DT, LK and Helene Muller-Landau conceived of the diversity and consumer manipulation experiments. ETB, EML and EO did the laboratory processing of samples, and ETB, EML, EO and EWS analysed the data. ETB wrote the initial draft of the manuscript, and all other authors contributed to subsequent drafts.

Data accessibility

These data were collected within the Cedar Creek Ecosystem Science Reserve Long-Term Ecological Research program and are publically available at <http://www.cedarcreek.umn.edu/research/data>, under the e244 experiment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Model selection for nutrients and carbon in all diversity plots.

Table S2. Model selection for ratios of carbon and nutrients in tissues in all diversity plots.

Table S3. Model selection for tissue carbon and nutrients in 16-species plots.

Table S4. Model selection for tissue carbon and nutrient ratios in 16-species plots.

Figure S1. Soil fungicide effects on plant composition within treatment plots.

Figure S2. Effect of experimental treatments on plot-scale foliar %C of the four study species.

Figure S3. Effect of experimental treatments on plot-scale foliar %N of the four study species.

Figure S4. Effect of experimental treatments on plot-scale foliar %P of the four study species.

Figure S5. Effect of experimental treatments on plot-scale foliar C:N of the four study species.

Figure S6. Effect of experimental treatments on plot-scale foliar C:P of the four study species.

Figure S7. Effect of experimental treatments on plot-scale foliar N:P of the four study species.