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Plant community and tissue chemistry responses to fertilizer and litter nutrient manipulations in a temperate grassland

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Abstract Human-mediated nutrient amendments have widespread effects on plant communities. One of the major consequences has been the loss of species diversity under increased nutrient inputs. The loss of species can be functional group dependent with certain functional groups being more prone to decline than others. We present results from the sixth year of a long-term fertilization and litter manipulation study in an old-field grassland. We measured plant tissue chemistry (C:N ratio) to understand the role of plant physiological responses in the increase or decline of functional groups under nutrient manipulations. Fertilized plots had significantly more total aboveground biomass and live biomass than unfertilized plots, which was largely due to greater productivity by exotic C_3 grasses. We found that both fertilization and litter treatments affected plant species richness. Species richness was lower on plots that were fertilized or had litter intact; species losses were primarily from forbs and non-Poaceae graminoids. C_3 grasses and forbs had lower C:N ratios under fertilization with forbs having marginally greater %N responses to fertilization than grasses. Tissue chemistry in the C_3 grasses also varied depending on tissue type with reproductive tillers having higher C:N ratios than vegetative tillers. Although forbs had greater tissue chemistry responses to fertilization, they did not have a similar positive response in productivity and the number of forb species is decreasing on our experimental plots. Overall, differential nutrient uptake and use among functional groups influenced biomass production and species interactions, favoring exotic C_3 grasses and leading to their dominance. These data suggest functional groups may differ in their responses to anthropogenic nutrient amendments, ultimately influencing plant community composition.

Keywords Plant functional group · Plant tissue chemistry · Fertilization · Litter · Nutrient amendments

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Introduction

Anthropogenic addition or loss of nutrients from ecosystems can have widespread effects on terrestrial plant communities (Vitousek et al. 1997; Sala et al. 2000; Suding et al. 2005; Phoenix et al. 2006). Many human activities have resulted in increased nutrient inputs and greater primary productivity, consequently leading to declines in plant species diversity, particularly in native species (Wedin and Tilman 1996; Vitousek et al. 1997; Phoenix et al. 2006; Patrick

et al. 2008). For instance, nitrogen deposition is considered one of the leading threats to biodiversity, next to habitat loss and global climate change (Sala et al. 2000). Increasing nitrogen deposition, leading to excess nitrogen, may cause communities to become more invasible (Davis et al. 2000), resulting in the loss of existing species. However, certain species and functional groups are more prone to decline under resource additions than others (Mountford et al. 1993; Reich et al. 2001; Suding et al. 2005). This may occur because particular species or functional groups are able to quickly take advantage of any changes in resource availability and come to dominate (Suding et al. 2005; Vinton and Goergen 2006). Resource additions may lead to a shift in the intensity of competitive interactions from belowground (soil resources) to aboveground (light: Tilman 1988; Cahill 1999), thus, favoring species or functional groups that are better competitors for light. Understanding the changes in plant tissue chemistry associated with altered resource availability can help us to predict how nutrient uptake and allocation strategies may influence which functional groups will dominate and which will decline.

The effects of anthropogenic amendments on communities and ecosystems will depend on whether the amendments represent inputs or removals. Most studies on resources inputs focus on nutrient inputs, specifically the addition of nitrogen or phosphorus (DiTommaso and Aarssen 1989). Communities that receive nutrient inputs generally have greater overall productivity, reduced species diversity, and a change in species composition (Wedin and Tilman 1996; Vitousek et al. 1997; Phoenix et al. 2006; Patrick et al. 2008). Human manipulations of litter, including mowing and the removal of biomass through harvesting or logging, have led to the removal of substantial amounts of nutrients that are locked in plant tissues, potentially affecting plant community composition through lower soil fertility and nutrient availability (Rizand et al. 1989).

Changes in environmental nitrogen, via fertilization or N deposition, can affect resource allocation and tissue chemistry of individual plants (Reich et al. 2003; Harapiak et al. 2004; Vinton and Goergen 2006). Fertilized plants often have greater allocation to reproduction and produce more flowers (Pieters and Baruch 1997). Fertilization has variable effects on plant tissue chemistry, increasing tissue nitrogen for some species and having no effect on other species (Wedin and Tilman 1996; Padgett and Allen 1999; Aber et al. 2003; Vinton and Goergen 2006), which may reflect different abilities to take up or allocate N. For example, Padgett and Allen (1999) found exotic annuals from the coastal sage scrub had greater leaf nitrogen concentrations with fertilization, while fertilization did not have a significant effect on nitrogen concentrations in native shrubs. Currently, coastal sage scrub communities are experiencing a loss of native shrubs and a concomitant increase in exotic annuals. Taken together, these data suggest that nitrogen inputs may lead to plant communities that are dominated by species or functional groups that have greater growth or abundances under increased nitrogen inputs (Phoenix et al. 2006).

An important consequence of nutrient inputs is increased invasion by exotic species (Hobbs and Huenneke 1992; Davis et al. 2000). In many grasslands, grass functional groups dominate under fertilization as most other functional groups decline. Often, exotic grass species comprise all or a substantial proportion of the successful grass functional group (Suding et al. 2005; Patrick et al. 2008). The success of exotic species has been attributed to high soil nitrogen availability in invaded areas (Averett et al. 2004; Stohlgren et al. 1999) or specific traits that make exotic species more competitive than native species (Cahill 1999; Vinton and Goergen 2006). Once established, an exotic species can have important ecosystem effects that lead to positive feedbacks for itself or for other species that have similar growth, competitive, or ecophysiological characteristics (Wedin and Tilman 1990; Drenovsky and Batten 2007).

Plants primarily influence nutrient cycling through litter (Wedin and Tilman 1990; Hobbie 1992; Eviner et al. 2006; Vivanco and Austin 2006). Plants that live in nutrient rich habitats generally produce high quality tissues that are rich in nitrogen. The amount of nitrogen in litter affects both decomposition rates and soil N mineralization. High quality litter (i.e., low C:N ratio, or low lignin:N) is more rapidly decomposed than low quality litter, resulting in more rapid nutrient cycling (Melillo et al. 1982; Taylor et al. 1989; Facelli and Pickett 1991; Hobbie 1992). Wedin and Tilman (1990) found that N mineralization rates in soils under different grass species varied by up to 10-fold, which they attributed to differences in

belowground litter quality. The effect of litter quantity on nutrient cycling will depend on the species present and their productivity. Vinton and Goergen (2006) found that *Bromus inermis* produced comparatively more biomass than *Panicum virgatum* under the same nitrogen addition treatments. However, *B. inermis* had more rapid decay rates than *P. virgatum* due to higher litter quality. Thus, nutrient cycling was dominated by *B. inermis* litter quantity and quality in this system.

Here, we utilize a long-term field experiment to examine the effects of two resource manipulation treatments, fertilization and litter removal, on tissue chemistry responses of two functional groups that have been differentially impacted by resource additions. In the first four years of the experiment, only C_3 grass species richness was not altered by fertilization or litter removal (Patrick et al. 2008); all other functional groups, including the most speciose group, forbs, decreased under fertilization. However, with fertilization, the C_3 grass functional group, comprised primarily of exotic species, produced over double the biomass of unfertilized plots, indicating that although grass species were not lost from either treatment, the C_3 grasses became dominant. We present results on plant productivity and species richness from the sixth year of the experiment and consider these results in light of our tissue chemistry results in order to understand changes in the plant community.

We hypothesize that C_3 grasses would have a greater response in tissue nutrient concentrations under fertilization and litter manipulations than forbs (i.e., there will be a strong functional group by treatment interaction). As exotic C_3 grasses previously exhibited a strong biomass response to nutrient manipulations (Patrick et al. 2008), we predicted that C_3 grasses will also have larger differences in tissue C:N ratios when fertilized and with litter intact, indicating greater N uptake, compared to when they are unfertilized and litter is removed. We also predicted that plants from both functional groups would have the lowest C:N ratios from plots that were fertilized and had litter intact; conversely, plants from plots that were unfertilized and had litter removed would have the highest C:N ratio. As changes in resource availability may also affect plant resource allocation, we examined whether fertilization and litter treatments had different effects on reproductive and vegetative tissues. We predicted that reproductive tissues would have a lower C:N ratio because flowers would be a strong nitrogen sink.

Materials and methods

Study site and experimental design

Plant community samples and tissues for chemical analyses were collected in 2007 from a long-term, old-field grassland experiment at the University of Akron Field Station-Bath Nature Preserve, Bath Township, Summit County, Ohio, USA (see Patrick et al. 2008 for details). Briefly, the field site was used as a hay meadow until 1996, after which it was maintained as a grassland through annual mowing by the township. The vegetation was a mix of grasses and herbaceous plants, dominated by exotic C_3 grasses, such as *B. inermis, Poa pratensis,* and *Phleum pratense*. The forbs and woody species were a mix of native and exotic species (Patrick et al. 2008), while the non-Poaceae graminoids (species belonging to the Cyperaceae and Juncaceae) consisted primarily of native species.

Twenty-four 20 m diameter circular plots were established in 2001. Plots were separated by at least 20 m and split into two groups of 12 by a dirt road running through the field. Each group of 12 plots was divided into three blocks of four plots with each block containing all four treatments. Each plot within a block was randomly assigned to one of four treatments: fertilizer addition only, litter removal only, fertilizer addition with litter removal, and control (no fertilizer and no litter removal). 20 g N/m^2 of Osmocote 8–9 month Slow Release Fertilizer (18:16:12 NPK; Scotts, Marysville, OH, USA) was applied to each of the fertilized plots every April, well above the ambient nitrogen deposition rates in this area of $\sim$1 g N/m^2/year (Patrick et al. 2008). In 2007, the amount of fertilizer was reduced to 15 g N/m^2 per plot. Unfertilized plots were treated in the same manner as fertilized plots except that no fertilizer was applied.

The effects of mowing (i.e., physical disturbance) and the removal of litter are often confounded in mowing manipulations (e.g., Collins et al. 1998; Fynn et al. 2004). In this study, they were decoupled because all plots were first mowed (litter left in situ) and then litter was removed from selected plots.

Therefore, any observed litter effects were due directly to the presence or absence of litter. Mowing occurred in late August to early September each year, after peak flowering and seed set for most of the species on our plots. After the township mowed the field site with a large tractor and brush hog mower, litter was removed from the litter removal plots using a 23 hp lawn tractor with a pull-behind 8 hp Agri-Fab Mow-N-Vac trailer attachment (Agri-Fab, Sullivan, IL, USA). In order to control for the disturbance from litter removal, we drove the lawn tractor and Mow-N-Vac trailer attachment over litter intact plots, weather permitting (i.e., if it was not raining).

Plant community sampling

Aboveground plant productivity and species richness were determined from three replicate quadrats in each plot for a total of 72 quadrats (24 plots $\times$ 3 quadrats). Each 0.25 m by 0.25 m quadrat was randomly located within a circular plot with the exception that the current year's quadrats did not overlap with quadrats that were sampled in previous years. In the middle of August, 2007, before annual mowing, all plant materials within quadrats were clipped to ground level and all aboveground biomasses within quadrats were collected. Harvested materials were separated into litter (litter from the growing season and completely senesced, brown tissues) and live tissues, which were sorted by species. Sorted materials were then oven dried at 65°C for at least 48 h before weighing for dry biomass.

Tissue collection and C:N analysis

In order to assess the effects of fertilization and litter removal on plant tissue chemistry of functional groups, we chose two C_3 grass species and three forb species that were found on a majority of the plots (R.J. Mitchell, unpublished data; J. Pan, unpublished data). The grasses, *B. inermis* and *P. pratense* (hereafter, *Bromus* and *Phleum*, respectively), averaged $\sim 34.5 \pm 0.6\%$ SE of the total C_3 grass biomass and the forbs *Glechoma hederacea, Plantago lanceolata,* and *Prunella vulgaris* (hereafter, *Glechoma, Plantago,* and *Prunella*, respectively) were $\sim 36.7 \pm 0.8\%$ of the total forb biomass per plot in 2007. C_3 grasses were collected when plants were flowering in June and July, 2007. One reproductive and one vegetative tiller was collected from three plants in each plot, for a total of 144 samples across all plots (2 tiller types $\times$ 3 plants $\times$ 24 plots). For two of the forbs, *Glechoma* and *Prunella,* tissues were collected when plants were flowering in August. For *Glechoma,* we collected sprigs of stolons, which included any flowers that were present on stolons. For *Prunella,* we collected the whole flowering stem, including all leaves. Tissues for *Plantago* were collected after flowering in August; two leaves were collected per plant. We attempted to sample three plants per plot for each of the forb species, but were unable to find enough plants on some of the plots. After tissues were collected, they were kept on ice or refrigerated until drying. Tissues were dried at 60°C for a minimum of 48 h. Dried tissues were then rinsed three times with deionized water, dried again at 60°C for 48 h, and kept at room temperature until analysis.

All dried tissue samples were finely ground before analysis. For the grasses, vegetative and reproductive tillers were ground and analyzed separately. All tissues were combined and ground together for forbs. Total C and N were analyzed for each sample by micro Dumas combustion using a CN Analyzer (Costech Analytical Technologies, Ventura, CA, USA).

Statistical analysis

Plant biomass and species richness data were analyzed using Proc GLM in SAS Version 9.1 (SAS Institute, Inc. Cary, NC, USA). In all biomass and richness analyses, we tested the model of fertilization (fertilized vs. unfertilized), litter (litter intact vs. removed), and the interaction between fertilization and litter on functional group data. Since preliminary analyses showed that the block effect was not statistically significant, it was not included in the final analyses for biomass and richness. For the biomass data, we took the mean of the total biomass found for each of the three quadrats as an estimate of the plot biomass. In order to determine the number of species per plot, we counted the total number of unique species found in all three of the replicate quadrats; any species that appeared in at least one of the quadrats was considered to be present on the plot. For both the biomass and species richness data, we had a total sample size of 24 plots.

Species were then categorized into five functional groups, C_3 grasses, non-Poaceae graminoids (e.g., Cyperaceae, Juncaceae), forbs, woody plants, and "other" (e.g., mosses); the same functional groups used in Patrick et al. (2008), with the exception of "other". We used a MANOVA to analyze the effects of fertilization and litter on live plant biomass and species richness by functional group (Stevens 1996). Five response variables were used for the live plant biomass analysis: C_3 grass biomass, non-Poaceae graminoid biomass, forb biomass, woody plant biomass, and "other" biomass. Five response variables were used for the species richness analysis: the number of species for each of the five functional groups. Pillai's Trace was interpreted for all MANOVAs. In order to determine which response variables differed between treatment groups, univariate ANOVAs were conducted on the response variables used in the MANOVA. In order to understand overall differences in total productivity, we also analyzed total biomass (live biomass plus litter) and live biomass with ANOVAs. Data were visually inspected to determine if they met statistical assumptions. Results for untransformed data are presented for ease of interpretation.

For the %N, %C, and C:N ratio data, we calculated a mean value for each species by plot, using all plants sampled per species for each plot. We grouped species by functional groups, C_3 grasses (*Bromus, Phleum*), and forbs (*Glechoma, Plantago, Prunella*), for all analyses. In order to determine whether there were differences in tissue chemistry, as measured by %N, %C, and C:N, between the different treatments, we tested the model of block, functional group (grass vs. forb), fertilization (fertilized vs. unfertilized), litter (litter intact vs. removed), and all interactions between functional group, fertilization, and litter. Data from the vegetative tillers of grasses were used for this analysis.

We also examined whether fertilization and litter treatments had different effects on the tissue chemistry of vegetative and reproductive tissues. As distinct vegetative and reproductive tissues were only available for *Bromus* and *Phleum,* we could only examine treatment effects on different tissue types in the grasses. We used a split-plot analysis to test block, the main plot factors of fertilization and litter, the subplot factor of tissue type (vegetative vs. reproductive tiller), and the interactions between tissue type, fertilization, and litter for each species. Due to unequal sample sizes, type III sums of squares were interpreted for these analyses.

Results

Plant productivity and species richness by functional group

Fertilized plots produced over double the amount of total aboveground biomass ($F_{1,23} = 46.65$, $P < 0.0001$) and had significantly more live tissue at the end of the season than unfertilized plots ($F_{1,23} = 43.30, P < 0.0001$; Fig. 1a). The relative live biomass contribution of the different functional groups also varied by fertilization treatment (Pillai's Trace, $F_{5,16} = 14.40, P < 0.0001$). The biomass on fertilized plots consisted of >80% C_3 grasses with forbs being the

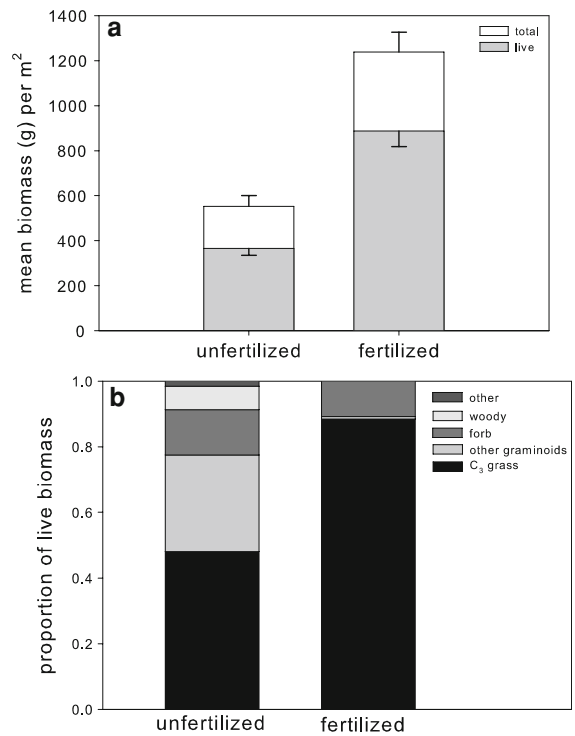

Fig. 1 **a** Mean live (−SE) and total (+SE) biomass and **b** proportion of total biomass from each functional group by fertilization treatment. Total biomass is composed of live (*green*) biomass and litter (*brown biomass*). "Other graminoids" refers to non-Poaceae graminoids (e.g., Cyperaceae, Juncaceae) and "other" to species that are not part of the other four functional groups

next most prevalent group (Fig. 1b). Although almost half of the live biomass on unfertilized plots was produced by C_3 grasses (~48%), the other four functional groups had a greater overall contribution on unfertilized plots, particularly non-Poaceae graminoids, which were almost 30% of the live biomass (Fig. 1b). The relative contribution of forbs to live biomass was not significantly different between the fertilization treatments. The litter treatment did not have any significant effects on plant biomass.

Both the fertilization and litter treatments had significant effects on species richness (Pillai's Trace, $F_{5,16} = 4.88$, $P = 0.007$ and $F_{5,16} = 3.20$, $P = 0.034$, respectively). Fertilized plots had fewer overall species, fewer functional groups, and fewer species per functional group than unfertilized plots (Fig. 2a). The differences in species richness were primarily due to differences in the non-Poaceae graminoids ($F_{1,23} = 5.042$, $P = 0.026$) and forb functional groups ($F_{1,23} = 13.10$, $P = 0.002$) with fertilized plots having fewer species of both of these functional groups than unfertilized plots. Plots with litter intact had fewer species than plots that had litter removed (Fig. 2b). Species richness differences were due to differences in the number of forb species ($F_{1,23} = 4.50$, $P = 0.047$) with litter removed plots having a greater number of forb species.

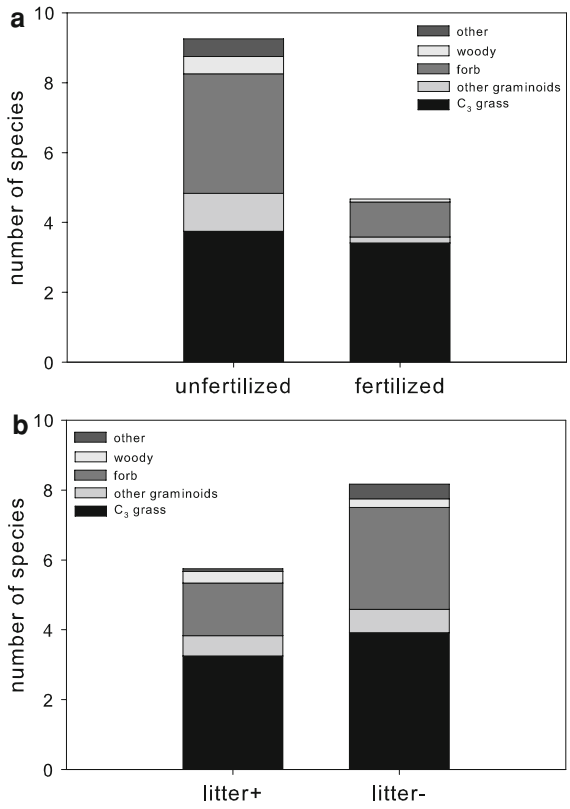

Fig. 2 Mean number of species stacked by functional group for **a** fertilization treatments and **b** litter treatments. Differences in the overall number of species for unfertilized versus fertilized and litter intact (litter +) versus litter removal (litter−) plots were statistically significant (Pillai's Trace, $F_{5,16} = 4.88$, $P = 0.007$ and $F_{5,16} = 3.20$, $P = 0.034$, respectively). See Fig. 1 for key to functional groups

Effects on tissue chemistry by functional group

Fertilization by itself had significant effects on overall plant tissue chemistry (Table 1). Plants from fertilized plots had lower C:N ratios than plants from unfertilized plots (36.89 ± 2.01 vs. 43.78 ± 1.69, respectively). Differences in the C:N ratio were due to significant differences in %N (1.43 ± 0.098 vs. 1.083 ± 0.045 for fertilized and unfertilized plots, respectively), as %C was not different in fertilized and unfertilized plots (Table 1).

Tissue chemistry was significantly different by functional group (Table 1). Forbs had lower C:N ratios than C_3 grasses (34.95 ± 1.61 vs. 46.42 ± 1.73, respectively), which was due to greater %N in the forbs (1.34 ± 0.09 vs. 1.07 ± 0.04 for forbs and C_3 grasses, respectively). C_3 grasses had significantly greater %C than forbs (44.02 ± 0.19 vs. 42.57 ± 0.20, respectively). We found that fertilization had slightly different effects on the functional groups. There was a marginal interaction between plant functional group and fertilization for %N with fertilization having a larger effect on the %N of forbs than C_3 grasses (Fig. 3), although this difference did not lead to a significant functional group by fertilization effect for C:N ratios.

Analysis of vegetative versus reproductive tillers in the C_3 grasses

We found that reproductive and vegetative tillers had different tissue chemistry in both grass species (Table 2). For both *Bromus* and *Phleum*, %C was not significantly different between tiller types, although tissue type approached significance for *Bromus* (Table 2). Vegetative tillers were richer in nitrogen than reproductive tillers, which led to lower C:N ratios in vegetative tillers than reproductive tillers (Fig. 4a).

Table 1 ANOVAs for %C, %N, and C:N testing differences between plant functional groups (C_3 grass vs. forb)

Effect	%C				%N			C:N		
	df	MS	F	*P*	MS	F	P	MS	F	*P*
B	**5**	**6.71**	**6.23**	**<0.001**	0.18	1.14	0.3487	44.46	0.47	0.7967
F	1	0.11	0.11	0.7451	**3.91**	**24.37**	**<0.001**	**1743.24**	**18.46**	**<0.001**
L	1	0.64	0.60	0.4420	0.00	0.00	0.9972	82.72	0.88	0.3528
F * L	1	0.65	0.61	0.4380	0.09	0.55	0.4620	32.25	0.34	0.5610
GF	**1**	**31.57**	**29.32**	**<0.001**	**3.12**	**19.42**	**<0.001**	**3280.37**	**34.73**	**<0.001**
GF * F	1	1.79	1.66	0.2024	*0.55*	*3.45*	*0.0677*	15.42	0.16	0.6875
GF * L	1	0.23	0.21	0.6452	0.00	0.03	0.8725	192.23	2.04	0.1584
GF * F * L	1	0.55	0.51	0.4780	0.00	0.00	0.9632	0.43	0.00	0.9463

B Block; *F* Fertilization; *L* Litter; *GF* Grass or forb; *MS* Type III mean square; *F* F-ratio, *P* *P*-value. For grasses, data from vegetative tillers were used. Significant effects ($P < 0.05$) are in bold and marginally significant effects ($0.05 < P < 0.10$) are in italics

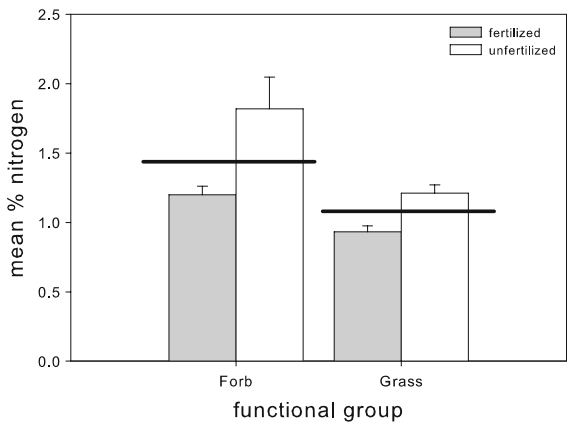

Fig. 3 Mean (+SE) %N for functional groups by fertilization treatment. The horizontal lines represent the mean %N for forbs and grasses, regardless of fertilization treatment. The grass data are from vegetative tillers

Fertilization had significant effects on the %N and C:N of both grass species (Table 2). Plants from fertilized plots of both species had greater %N and lower C:N ratios than plants from unfertilized plots (%N ± SE: 1.12 ± 0.07 vs. 0.88 ± 0.05 for fertilized and unfertilized *Bromus*, respectively; 1.003 ± 0.05 vs. 0.76 ± 0.05 for fertilized and unfertilized *Phleum*, respectively; C:N ± SE: 44.44 ± 2.59 vs. 53.80 ± 2.78 for fertilized and unfertilized *Bromus*, respectively; 51.42 ± 2.59 vs. 63.24 ± 3.57 for fertilized and unfertilized *Phleum*, respectively). %C was also significantly affected by fertilization for *Phleum* (44.16 ± 0.27 vs. 43.68 ± 0.38 for fertilized and unfertilized, respectively). The interaction between fertilization and tissue type was significant for C:N in *Phleum*. Although vegetative tillers had lower C:N ratios than reproductive tillers overall, reproductive tillers had a stronger response to fertilization, as indicated by the greater difference in C:N ratio between fertilized and unfertilized plants (Fig. 4b).

Litter had significant effects on the C:N ratio and approached significance for %N in *Bromus* (Table 2). Plants from plots with litter intact had greater %N in their tissues (1.09 ± 0.06) and lower C:N ratios (44.20 ± 2.47) than plants from plots where litter was removed (0.92 ± 0.07 and 53.58 ± 2.89, respectively); differences in C:N were mainly due to changes in %N because litter did not have a significant effect on %C.

Discussion

We found that both of our nutrient amendments, fertilization and litter removal (nutrient inputs and removals, respectively), influenced plant community composition and tissue chemistry. Our plant community results, from the sixth year of a long-term study, were mostly consistent with earlier results (Patrick et al. 2008). Functional groups that were previously successful under nutrient manipulations, C_3 grasses, continued to be successful, while other functional groups, non-Poaceae graminoids and forbs, continued to decline. Plant productivity continued to be higher on fertilized plots, largely due to greater productivity by members of the C_3 grass functional group and lower relative productivity by the non-Poaceae

Table 2 ANOVAs for %C, %N, and C:N for *Bromus inermis* and *P. pratense*

			%C			%N			C:N		
Species	Effect	df	MS	F	*P*	MS	F	*P*	MS	F	*P*
Bromus inermis	B	5	**11.17**	**4.81**	**0.0104**	0.09	1.12	0.3977	132.92	0.83	0.5518
	F	1	0.68	0.30	0.5962	**0.7**	**8.81**	**0.0109**	**1269.10**	**7.85**	**0.0150**
	L	1	2.17	0.94	0.3511	*0.34*	*4.26*	*0.0596*	**882.75**	**5.50**	**0.0355**
	F*L	1	0.26	0.11	0.7411	0.05	0.61	0.4475	33.27	0.21	0.6564
	T	1	*2.46*	*3.81*	*0.07*	**0.39**	**15.69**	**0.0013**	**520.85**	**7.18**	**0.0172**
	F*T	1	0.7	1.08	0.3147	**0.12**	**4.95**	**0.0419**	196.73	2.71	0.1205
	L*T	1	0.36	0.56	0.4646	0.04	1.59	0.2269	34.95	0.48	0.4983
	F*L*T	1	0.40	0.62	0.4448	0.05	2.19	0.1595	161.46	2.22	0.1566
Phleum pratense	B	5	**13.38**	**22.59**	**<0.0001**	0.02	0.38	0.8531	135.73	0.79	0.5742
	F	1	**3.21**	**5.42**	**0.0344**	**0.66**	**13.71**	**0.0021**	**1490.94**	**8.66**	**0.0101**
	L	1	0.69	1.16	0.2989	0.004	0.09	0.771	277.79	1.61	0.2234
	F*L	1	0.22	0.33	0.5484	0.07	1.36	0.262	183.06	1.06	0.3189
	T	1	0.07	0.11	0.7421	**1.03**	**27.99**	**<0.0001**	**4030.78**	**44.49**	**<.0001**
	F*T	1	0.18	0.30	0.5913	0.00	0.00	0.9752	**409.52**	**4.52**	**0.0476**
	L*T	1	0.57	0.97	0.3382	0.04	1.10	0.3071	65.37	0.72	0.4068
	F*L*T	1	0.85	1.44	0.2451	0.00	0.06	0.8017	76.09	0.84	0.3715

Significant effects ($P < 0.05$) are in bold and marginally significant effects ($0.05 < P < 0.10$) are in italics

Effects are *B* Block; *F* Fertilization; *L* Litter; *T* Tissue type (reproductive or vegetative tiller)

graminoids, woody, and "other" functional groups (Fig. 1b). Lower biomass production by non-Poaceae graminoids on fertilized plots was likely the result of high species losses in that group; these species virtually disappeared from fertilized plots (Fig. 2a). All functional groups had a decrease in species richness under fertilization, but statistically significant losses were seen only in the non-Poaceae graminoids and forbs. Interestingly, the relative proportion of live biomass from forbs was not significantly different on fertilized and unfertilized plots, even though there were significantly fewer forb species on fertilized plots. This suggests that the remaining forb species on fertilized plots had increased productivity.

Two of the forb species that made up particularly large proportions of the live biomass on the fertilized plots they were found on were *Galium mollugo* and *G. hederacea*. One trait that both of these exotic species had in common was lateral spread by clonal growth; *G. mollugo* by rhizomes and *Glechoma* by stolons. Clonal species in other systems have had similarly large growth responses to fertilization relative to non-clonal species (Reynolds et al. 2007). Under fertilization, the success of clonal plants may be attributed to physiological integration (i.e., resource sharing among ramets *via* clonal connections) of soil resources (Reynolds et al. 2007). However, clonal plants are also known to share carbon (Pan and Clay 2004 and references therein) and this ability to share carbon may be especially important in environments where light competition is intense, such as fertilized plots. Patrick et al. (2008) found that photosynthetically-active radiation (PAR), measured in August (pre-mowing), was significantly lower on fertilized plots than unfertilized plots. Under greater aboveground competition, the number of clonal plant species may increase because they can escape light competition by lateral spread and translocate fixed carbon to shaded parts of the plant, unlike non-clonal plants that have limited strategies for competing for light (e.g., growing taller).

The success or decline of particular functional groups may also depend on whether members are native or exotic. The interactions between species in communities consisting of entirely exotic or native species are different and can affect productivity and

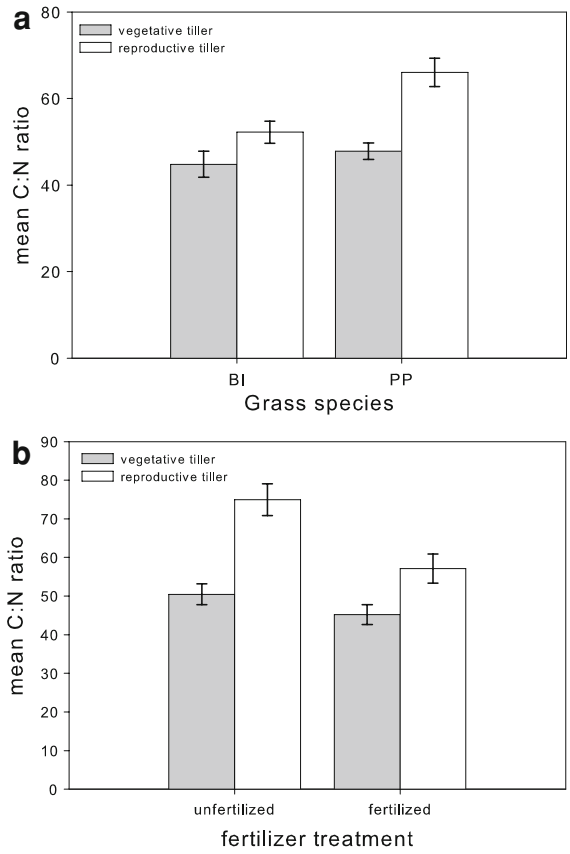

Fig. 4 Mean (± SE) C:N ratio for **a** the two grass species by tissue type and **b** for *P. pratense* by fertilizer treatment and tissue type. In both species, reproductive tillers had higher C:N ratios. BI is for *B. inermis* and PP for *P. pratense*

species diversity (Wilsey et al. 2009). Native and exotic C_4 grasses have been found to allocate growth differently with native species producing greater belowground growth and exotic species greater aboveground growth; they also differ in their ability to take up nutrients with exotic species having greater nutrient uptake (Wilsey and Polley 2006). Exotic species also commonly have greater relative growth rates than native species, allowing them to successfully compete for light (Grotkopp and Rejmanek 2007; James and Drenovsky 2007). The abundance of native and exotic species varied in each of our functional groups (Patrick et al. 2008, J.J. Pan, unpublished data) and may have played a role in whether a particular functional group declined. Both of the functional groups that had statistically significant declines in our study contained native species and the group that experienced the greatest decline, non-Poaceae graminoids, had the largest proportion of native species. Within forbs, both native and exotic species were lost on fertilized plots. The dominant functional group on fertilized plots, C_3 grasses, consisted primarily of exotic species. As the study continues, we expect to see greater numbers of exotic species on fertilized plots, where there is an excess of resources (Davis et al. 2000).

Nitrogen inputs may have had a number of effects that led to the dominance of C_3 grasses and the concomitant decline of the other functional groups. Nitrogen inputs may favor plant species or functional groups that have high relative growth rates and are able to rapidly take advantage of changes in nutrient availabilities (Reich et al. 2003; Suding et al. 2005; Vinton and Goergen 2006). In our study, this would imply that the dominant C_3 grass functional group should have higher relative growth rates than the declining forbs. Although we did not measure relative growth rates, the tissue chemistry results, percent cover data (J.J. Pan, unpublished data), and overwhelming dominance of the C_3 grasses in the total biomass support high relative growth rates by C_3 grasses under fertilization. Furthermore, the active growth of C_3 grasses early in the season and their standing biomass later in the season could lead to the suppression of shorter stature functional groups that are light limited (Foster and Gross 1998; Reich et al. 2001; Vinton and Goergen 2006). This may prevent short-statured forbs from converting the greater available nitrogen into biomass (but see above).

Litter manipulation had significant effects on species richness, primarily through effects on forbs, where species richness was significantly greater when litter was removed compared to when litter was left intact. Litter removal may favor forb species by decreasing aboveground competition for light, reducing inhibitory effects of litter on plant growth, or increasing seed recruitment. It was unlikely that litter removal had substantial effects on light competition in this study because litter removal occurred at the end of summer and PAR was not significantly affected by the litter treatments (Patrick et al. 2008). Litter can negatively affect the growth of already established plants, particularly when there are few living neighbors (Foster and Gross 1997). We did not see any significant effects of litter removal on forb biomass, suggesting that, while there were a greater number of forb species when litter was

removed, litter removal did not lead to greater growth by forbs. The presence of litter can also inhibit seed germination and seedling growth (Foster and Gross 1998; Fynn et al. 2004). A number of forb species at our study site are biennials (e.g., *Daucus carota*) or short-lived perennial species (e.g., *P. lanceolata*). The removal of plant litter could increase recruitment of forb species that primarily rely on seed recruitment on our plots, leading to greater species richness.

We found that both C_3 grasses and forbs had higher tissue N concentrations and lower C:N ratios with fertilization than without fertilization. In contrast to what we predicted, the functional group by fertilization interaction was marginally significant for %N and forbs actually had greater tissue N than C_3 grasses and a larger difference in tissue N between fertilized and unfertilized plots. Despite these differences, forbs did not have a similar positive productivity response as was observed in the C_3 grasses. Results from other studies have also found C_3 grasses to have a positive biomass response to nitrogen fertilization and forbs having no biomass response to fertilization (e.g., Mountford et al. 1993) but greater tissue nitrogen (Reich et al. 2001). The observed differences in tissue chemistry between forbs and C_3 grasses may reflect different strategies of N uptake or allocation (Wilsey and Polley 2006; Wilsey et al. 2009). C_3 grasses on our plots contained a much larger pool of N (mean functional group biomass/m^2 × mean %N per treatment) than forbs; ~9.3 g N/m^2 was in aboveground C_3 grass biomass versus ~2 g N/m^2 for forbs in fertilized plots and ~1.5 g N/m^2 for grasses versus ~0.6 g N/m^2 for forbs on unfertilized plots. C_3 grasses had approximately 6x more N per m^2 on fertilized compared to unfertilized plots, while forbs had only ~3.4x more N, suggesting that C_3 grasses had greater uptake and put more N into producing biomass.

In contrast to fertilization, litter manipulation had no significant effects on tissue N or C:N ratios either by itself or in conjunction with fertilization, with the exception of tiller differences in *Bromus* (see below). The litter removal treatments removed a majority of the current year's standing aboveground biomass, both living and brown, but some aboveground and all belowground biomass was left behind. If plant growth was primarily belowground, then removal of aboveground litter will have limited effects on nutrient availability and plant tissue chemistry (Rizand et al. 1989; Vivanco and Austin 2006). Wilsey and Polley (2006) found that exotic C_4 grasses had lower belowground biomass than either native forbs or native C_4 grasses. If our functional groups exhibit similar exotic versus native patterns in belowground biomass, then we would expect to see greater effects of litter manipulation on tissue chemistry as the community becomes increasingly dominated by exotic species and more nutrients are removed through aboveground litter. We saw a trend for plants on plots with litter intact to have lower C:N ratios compared to plants on litter removal plots (e.g., *Bromus*).

Tissue chemistry in *Bromus* and *Phleum* was greatly affected by tissue type, as both species had higher nitrogen and lower C:N ratios in vegetative tillers than reproductive tillers, contrary to our predictions that reproductive tillers would be a larger N sink. This difference in tissue chemistry may be driven by structural differences between tillers rather than by nitrogen sink strength. Reproductive tillers for both grasses were much taller (~ 2x taller) than vegetative tillers (J.J. Pan, personal observation). In addition, at the time of collection, most of the reproductive tillers were still flowering and may not have been as large of a nitrogen sink as when seeds are being filled. If nutrient additions led to the production of more reproductive tillers by grass plants (Pieters and Baruch 1997), then the observed differences in reproductive versus vegetative tiller tissue chemistry may have important effects on nutrient cycling, i.e., slower nutrient cycling on plots with more reproductive tissues (higher C:N ratios) compared to vegetative tissues. Moreover, these differences between tissue types can lead to a mosaic of tissue chemistry habitats and have widespread indirect effects on other species (e.g., herbivores and microbes) and on multiple trophic levels.

The results from this study support that human-mediated nutrient amendments can have widespread effects on plant communities. Overall, we found that fertilization had stronger effects than litter treatments. The exotic C_3 grass functional group dominated under fertilization while the mostly native non-Poaceae graminoid functional group exhibited the greatest decline. Although forbs had greater tissue chemistry responses to fertilization, they also continued to decline on fertilized plots. Conversely, forbs were the most impacted by litter treatments, with

increasing species richness under litter removal. Anthropogenic changes that shift the balance between existing species and functional groups can greatly change the face of terrestrial communities.

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