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Large-scale manipulation of plant litter and fertilizer in a managed successional temperate grassland

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Abstract Plant litter may play an important role in herbaceous plant communities by limiting primary production and influencing plant species richness. However, it is not known how the effect of litter interacts with fertilization. We tested for the role of litter and fertilization in a large-scale experiment to investigate effects on diversity and biomass of plant species, growth forms, native vs. non-native groups, and abiotic ecosystem components (e.g., soil moisture, PAR). We manipulated plant litter (removed vs. left in situ) and nutrient availability (NPK-fertilized vs. unfertilized) for 4 years in 314-m^2 plots, replicated six times, in an old-field grassland. While many of our species-level results supported previously published studies and theory, our plant group results generally did not. Specifically, grass species richness and forb biomass was not affected by either fertilization or plant litter. Moreover, plant litter removal significantly increased non-native plant species richness. Relative to native plant species, all of our experimental manipulations significantly increased both the biomass and the species richness of non-native plant species. Thus, this grassland system was sensitive to management treatments through the facilitation of non-native plant species. We coupled biotic and abiotic components within a nonmetric multidimensional scaling (NMS) analysis to investigate treatment effects, which revealed that specific treatments altered ecosystem development. These results suggest that fertilization and plant litter may have larger impacts on plant communities and on ecosystem properties than previously understood, underscoring the need for larger-scale and longer-term experiments.

Keywords Disturbance · Nitrogen · NMS ordination · Non-native species · Plant functional group · Species diversity

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Introduction

Plant litter is a fundamental factor affecting plant community structure (Facelli and Pickett 1991; Bosy and Reader 1995; Xiong and Nilsson 1999), and its effects have been investigated in a variety of habitats (for a summary of habitats, see Weltzin et al. 2005). Plant litter influences soil moisture (Hamrick and Lee 1987), acts as a mechanical impediment to germinating seedlings (Facelli 1994), alters light attenuation at the soil surface which affects germination and establishment (Goldberg and Werner 1983; Facelli and Pickett 1991; Weltzin et al. 2005), and provides

cover for seed and seedling predators (Hulme 1996, Edwards and Crawley 1999). In unproductive communities, plant litter may facilitate colonization and ameliorate abiotic conditions (e.g., moisture, soil temperature) necessary for germination and establishment (Hamrick and Lee 1987). In highly productive plant communities, plant litter can increase the dominant plant competitor biomass and litter production, thereby decreasing plant species richness (Foster and Gross 1997, 1998; Long et al. 2003), whereas removal of plant litter in highly productive communities can moderately increase species richness (Long et al. 2003). Additionally, plant litter production increases as a result of nitrogen (N) enrichment (Foster and Gross 1998), which can cause a further reduction in plant species richness (Carson and Peterson 1990; Foster and Gross 1998; Xiong and Nilsson 1999; Long et al. 2003).

A major consequence of human activity has been ecosystem eutrophication via anthropogenically induced increases in N fertilization and atmospheric nitrogen deposition (Vitousek et al. 1997; Fenn et al. 2003; Galloway et al. 2003), resulting in significant biodiversity loss and ecosystem function (McCann 2000; Larsen et al. 2005). The effects of increased N on terrestrial plant communities are well documented, showing declines in plant species richness while augmenting plant biomass across a variety of natural and semi-natural habitats (Bobbink et al. 1998) and experimentally manipulated habitats and microcosms (Hector et al. 1999; Tilman et al. 2002a). The resulting species thinning often leads to the rarest species disappearing first and an increase in non-native species (Hector et al. 1999; Tilman et al. 2002a; Suding et al. 2005). A common approach to plant biodiversity studies is to segregate plant species into plant groups (e.g., forbs and graminoids, native and non-native) to test for aggregate responses to treatments (Wilsey and Polley 2006). The rarest species in grassland systems are generally in the functional groups of forbs, non-Poaceae graminoids, and woody plants. Conventional theory (Suding et al. 2005) would predict that these functional groups are then likely to lose the most species as a result of fertilization, though the interacting effects of plant litter and fertilization on plant groups within a naturalized plant community remain largely unexplored. Since litter removal should result in greater light availability at the soil surface, a reduction in mechanical impediment, and fewer seed predators, these conditions may promote seedling emergence and increased plant species richness.

Here we report the results of the first 4 years of an ongoing field study to investigate the effects of the experimental manipulation of NPK fertilization and plant litter on plant community structure, plant group responses, and abiotic factors; as well as the coupling of biotic (e.g., plant biomass, plant species richness) and abiotic (e.g., PAR, soil moisture, and soil organic content) factors. We explore three hypotheses: (1) plant biomass will increase and plant diversity will be reduced (even within growth forms and native vs. non-native groups), (2) plant litter will negatively affect plant species diversity irrespective of fertilization, though the effect will be greater in fertilized plots, and (3) plant litter and fertilization will interact to alter plant community structure and ecosystem properties.

Materials and methods

Study site and experimental design

The study was conducted at the 163.5 ha Bath Nature Preserve (BNP; 41° 10′ 36.2″ N, 81° 38′ 58.7″ W), Bath Township, Summit County, Ohio, USA, in a 16 ha section of an upland former hay meadow. Until 1996, the study site was a hay meadow, harvested one or many times per year. From 1997 through the present, the area has been mown annually by the local township in late August–early September, near or at the end of the growing season, and the mown vegetation has been left on the field. The vegetation is an herbaceous, graminoid community largely dominated by cool-season grasses, e.g., *Bromus inermis* Leyss., *Festuca arundinacea* Schreb., *Phleum pratense* L., and *Anthoxanthum odoratum* L (Gleason and Cronquist 1991). Aboveground biomass samples taken before the start of experimental manipulations ranged from approximately 500–1000 g m^{-2} (unpublished data, L.B.P.; mean = 702 g m^{-2}, $n = 24$, SD = 127), placing it as moderately productive relative to other grassland sites across the US (Sala et al. 1988) and within the upper Midwest (Foster and Gross 1998). The dominant soil type is Ellsworth silt loam (ElB), which consists of moderately well drained, moderately deep, to deep soils formed in

silty clay loam or clay loam glacial till of the Wisconsin Age (Ritchie and Steiger 1974). Soil nutrient analyses conducted before the start of the experiment placed total nitrogen at 0.19 ± 0.02% (n = 24) and total phosphorus at 11.2 ± 8.3 kg/ha (n = 24), indicating moderate productivity.

In August 2001, twenty-four 20-m diameter circular plots (314 m^2) were established. These experimental plots were separated by at least 20 m and were at least 30 m away from any other habitat (e.g., roads, forest). Due to the size of the plots, proximity constraints, and the size of the fields available, plots covered two adjacent fields separated by a rarely used, restricted access single-lane dirt road, resulting in 12 plots in each field. Treatments were applied in a 2 × 2 factorial design of fertilizer (+F = fertilizer added, –F = no fertilizer) and plant litter (–L = litter removed, +L = litter left in situ after yearly mowing) with the control plots characterized as no fertilization and plant litter left in situ (+L/–F), resulting in six replicates per treatment. In April 2002 and continuing each April through 2005, Scotts brand Osmocote 8–9 month Slow Release Fertilizer 19–6–12 (NPK; Scotts, Marysville, OH, USA) was applied at 200 kg N ha^{-1} (20 g N m^{-2}) in fertilized plots, well above the Köchy and Wilson (2005) 15 g N m^{-2} yr^{-1} threshold necessary to induce a eutrophication effect in grasslands and other habitats. We could not exclude ambient wet/dry atmospheric N deposition, though deposition rates from 1990 to 2005 were relatively low at approximately 10.1 kg N ha^{-1} yr^{-1} (1.01 g N m^{-2} yr^{-1}) at a nearby monitoring site in Lykens (162 km west of our study site), OH, USA, and approximately 9.3 kg N ha^{-1} yr^{-1} (0.93 g N m^{-2} yr^{-1}) at another nearby monitoring site in Mercer Co. (G. K. Goddard site; 96 km east of our study site), PA, USA (US EPA 2005). Within two days of annual mowing of the whole site by the local township with a large tractor and brush hog mower (autumn 2001–2004), litter was removed from litter removal treatments using a small 23 hp lawn tractor with a pull-behind 8 hp Agri-Fab Mow-N-Vac trailer attachment (Agri-Fab, Sullivan, IL, USA).

Plant community sampling

During the second week of August 2002, total plant biomass was sampled in 0.25 m^2 quadrats from three randomly chosen locations within each plot, one sample per third of each circular plot (n = 72). Hereafter, "total biomass" refers to standing crop biomass and plant litter biomass together, where all references to "litter" refer to the previous year's mown vegetation and any vegetation senesced and found within the sampling quadrat after standing crop removal. During the second week of August 2003, total plant biomass samples were again taken in a 0.25 m^2 quadrat, though only one randomly chosen sample per plot was taken (n = 24 biomass samples). For 2004 and 2005, standing crop biomass (also referred to as "living biomass") samples were sorted to species in the field as samples were clipped, with plant litter (also referred to as "litter biomass") also collected in each quadrat after the standing crop biomass was removed. Sampling started near the end of the growing season in mid–late July and was completed in early August. For 2004, one standing crop biomass sample was collected from a randomly chosen location within each plot (n = 24). For 2005, three standing crop biomass samples were collected from randomly chosen locations with each plot, one sample per third of each circular plot (n = 72). Each year, collected plant biomass samples were returned to the lab and stored at approximately 6°C until they could be dried at 70°C for 72 h, then biomass determined.

Abiotic sampling

On 9 August 2005, five measurements of photosynthetically active radiation (PAR) were taken at the soil surface every 3 m along a north-south transect through the center of each plot (n = 120 PAR measurements) with a LI-COR LI-190SA Quantum Sensor averaging μmol s^{-1} m^{-2} for 30 s and logged to a LI-COR model LI-1400 data logger. Four days after the last rain event, on 10 August 2005, 2-cm diameter soil plugs were taken to a depth of approximately 20 cm for the same locations at which PAR was measured (n = 120 soil plugs). The wet mass of each soil plug was recorded, and then soil plugs were frozen until they could be dried at 70°C for 10 days. Dry mass was recorded for each soil plug to determine percent soil moisture, then each soil plug was heated at 550°C for 4 h, cooled in a desiccator to room temperature, and then mass was recorded to determine percent soil organic content.

Statistical analyses

To analyze trends in all biotic and abiotic response variables among treatments and between years, we used SAS software Version 8.01 (SAS Institute Inc. 1999). We calculated maximum likelihood to generate approximate *F*-tests in PROC MIXED with Type III effects based upon the covariance structure of compound symmetry. The various models used the different response variables (total biomass, Shannon's H diversity, PAR, soil moisture, soil organic content), and predictors used fertilized vs. unfertilized, litter removed vs. litter left in situ, year, and their fully factorial interactions, with year as the repeated predictor. To account for uneven sample sizes among years, the response variables were averaged for each plot each year ($n = 24$)

Species were classified by growth form: forbs, grasses (Poaceae), non-Poaceae graminoids (i.e., Juncaceae, Cyperaceae), and woody plants. Plants were also classified native and non-native. The classification into "native" or "non-native" was made with Andreas et al. (2004) and refers to plant species that are native or non-native to Ohio, USA. To calculate species richness for each group within a plot, each species within a plot was counted only once, even if that species was sampled more than once within a plot, then we summed the total number of species within each group within a plot, yielding $n = 24$ samples in each group. To calculate the biomass of each group within a plot, we averaged the total biomass of each species across each sampling replicate within a plot (including zeroes for species not sampled in all sampling locations within a plot), then we summed the total biomass of species within each group within a plot, yielding $n = 24$ samples in each group. For each of the six groups, we used SAS (SAS Institute Inc. 1999) to calculate maximum likelihood in PROC MIXED with Type III effect. We first used the species richness, then the biomass of each of the six groups as response variables with fertilized vs. unfertilized, and litter removed vs. litter left in situ as the predictor variables. The 2004 plant sampling data showed similar trends in species abundances and distributions relative to the 2005 plant sampling data, therefore we chose to include only analyses for the 2005 plant sampling period for brevity.

We applied canonical correspondence analysis (CCA) using PC-ORD Version 4.37 (McCune and Mefford 1999) to assess treatment effects in species distributions for 2005. The biotic data used in the analysis were the average biomass of each species within each of the 24 plots, while the environmental data in the analysis were the four treatments as dummy variables. The resulting matrix had the average individual species biomass within a plot for the columns and 24 rows (plots). The CCA used Biplot scaling optimized to species.

To assess treatment effects on aggregate ecosystem properties (properties with both the biotic and abiotic components), we applied nonmetric multidimensional scaling (NMS; Kruskal 1964) using PC-ORD (McCune and Mefford 1999). For 2005, variables used for each of the 24 plots were average species richness per plot, average standing crop biomass, average litter biomass, average PAR per plot, average percent soil moisture per plot, and average percent soil organic content per plot, resulting in a matrix with six columns and 24 rows (plots). Because (1) NMS is scale sensitive, (2) these variables are on radically different measurement scales, and (3) variables have an enormous range of values between variables, data were transformed to proportions relative to the highest value for each variable (i.e., each value in a column was divided by the largest value in that column, creating a range from 0 to 1 for each column). The NMS analysis was run with Sørensen distance, time as the random seed for the starting configuration, 9999 runs stepping down from 6 to 1 dimensions with the real data, 999 Monte Carlo runs to assess the probability of a similar final stress obtained by chance, and a 0.005 stability criterion. Additionally for 2005, we used PC-ORD (McCune and Mefford 1999) to run the multi-response permutation procedure (MRPP; Mielke 1984) to test for the hypothesis of no difference among treatments. The MRPP used Sørensen distance with the four treatments as the a priori groupings, resulting in a matrix with six columns (biotic and abiotic variables) and 24 rows (plots) and was calculated with all four treatments together, and for pairwise comparisons between treatments to test for the strength of difference between individual treatments.

Results

General trends

Fertilization and, to a lesser extent, the presence of plant litter significantly increased total plant biomass (Fig. 1a). The repeated PROC MIXED (Table 1) indicated a significant time effect (Year) interacting with fertilization, with fertilization steadily increasing total plant biomass through time (Fig. 1a), though Year by itself had no significant effect. However, the non-significant interaction between fertilization and plant litter (Table 1) indicated that fertilization and plant litter had independent effects on total biomass production. Similarly, fertilization had a significant effect on Shannon's H (Fig. 1b), with litter treatment marginally significant (Fig. 1b). In contrast to total plant biomass, Year interactions did not affect Shannon's H (Table 1).

As expected, 2005 plant litter as a response variable (i.e., litter biomass) was significantly affected by the experimental treatments ($F_{3,68}$ = 19.10; $P < 0.0001$), with mean values and standard errors in parentheses as follows: +litter/–fertilizer 44.26 (4.04), –litter/–fertilizer 14.04 (1.22), +litter/+fertilizer 60.18 (9.18), and –litter/+fertilizer 15.58 (2.14).

Percent soil moisture (Fig. 1c) was decreased by fertilization ($F_{1,20}$ = 17.13, P = 0.0005), but was not significantly affected by litter ($F_{1,20}$ = 1.88, P = 0.1860) and the interaction between litter and fertilization ($F_{1,20}$ = 1.84, P = 0.1901). Percent soil organic content (Fig. 1c) was not significantly affected by fertilization ($F_{1,20}$ = 1.59, P = 0.2224) nor litter ($F_{1,20}$ = 1.01, P = 0.3273), and there was no significant interaction between litter and fertilization ($F_{1,20}$ = 2.15, P = 0.1578). PAR (Fig. 1d) was strongly decreased by fertilization ($F_{1,20}$ = 86.13, $P < 0.0001$), but without significant effects of either litter ($F_{1,20}$ = 0.56, P = 0.4628) or the interaction between litter and fertilization ($F_{1,20}$ = 0.16, P = 0.6905).

Species-level analyses

Biomass samples for 45 species were collected during 2005 (Table 2) and used for the CCA analysis. The first axis of the CCA (Fig. 2) accounted for 12.4% of the variance in plant species biomass and separated fertilized from unfertilized plots, suggesting that plant species differentially respond to fertilization, with the majority of species in unfertilized plots. This first axis generally separated two of the most

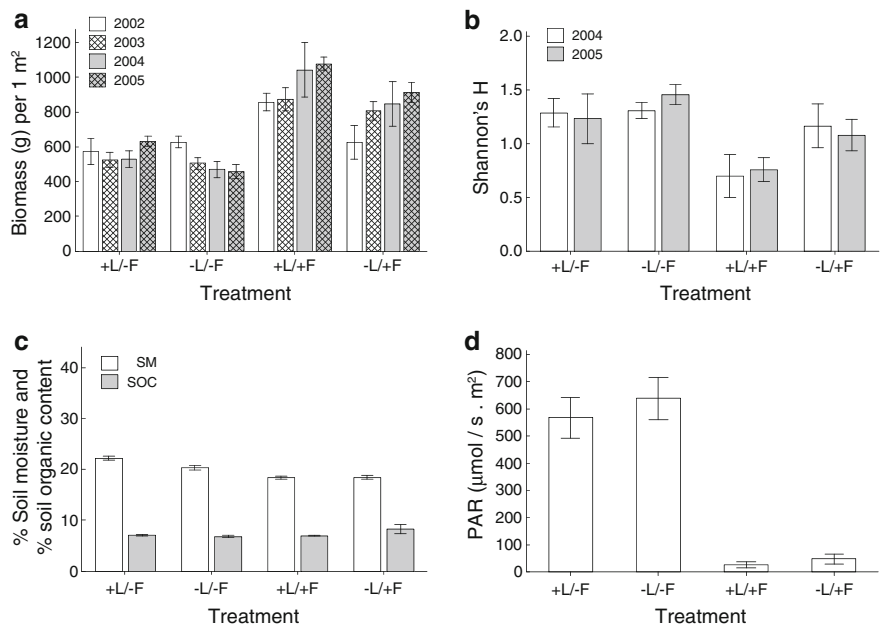

Fig. 1 (**a**) Average total biomass (g) per 1 m^2 within each treatment in each year (2002–2005). (**b**) Average Shannon's H per 0.25 m^2 within each treatment in each year (2004–2005). (**c**) Percent soil moisture (SM) and percent soil organic content (SOC) within each treatment for 2005. (**d**) PAR in each treatment for 2005. Full results of the repeated-measures PROC MIXED for (**a**) and (**b**) are in Table 1. All error bars are ±1 SE. +L indicates litter left in situ, –L indicates litter removed, +F indicates fertilization, –F indicates no fertilization

Table 1 Results of repeated-measures ANOVA for total biomass and Shannon's H

	df numerator	*df* denominator	*F*	*P*
Total biomass				
Fertilized	1	20	63.3	**<0.0001**
Litter	1	20	6.24	**0.0213**
Year	3	60	1.92	0.1356
Fertilized*litter	1	20	1.83	0.1910
Year*fertilized	3	60	4.82	**0.0045**
Year*litter	3	60	0.65	0.5830
Year* fertilized*litter	3	60	0.94	0.4268
Shannon's H				
Fertilized	1	20	9.93	**0.0050**
Litter	1	20	4.24	**0.0526**
Year	1	20	0.03	0.8852
Fertilized*litter	1	20	1.18	0.2896
Year*fertilized	1	20	0.08	0.7748
Year*litter	1	20	0.02	0.8873
Year* fertilized*litter	1	20	0.81	0.3787

A significant "Fertilized" effect indicates a significant difference in plots either fertilized (+F; 20 g N m^{-2} added) or unfertilized (–F), a significant "Litter" effect indicates a significant difference in plots where plant litter was either removed (–L) or left in situ (+L), and a significant "Year" effect indicates a significant difference among sampling years, and Fertilized*litter, Year*fertilized, Year*litter, and Year*fertilized*litter represent their fully factorial interactions. *P*-values significant at $\alpha = 0.05$ are denoted by bolding

abundant graminoids, *B. inermis* and *Dactylis glomerata*, and the occasionally abundant forbs (e.g., *Galium mollugo*, *Cirsium arvense*) from two of the other abundant graminoids, *F. arundinacea* and *A. odoratum*, and from the rarer and lower biomass plant species (e.g., *Achillea millefolium*, *Hieracium aurantiacum*, and *Potentilla recta*). The second axis (Fig. 2) accounted for 7.9% of the variance in plant species biomass and separated litter removed from litter left in situ plots, suggesting that plant species also respond to differential litter loads. The greatest species responses to plant litter clustered around the fertilized and litter removed (–L/+F) pole. The species-environment Pearson correlations (R^2) were 0.882 for the first axis and 0.890 for the second axis.

Plant group responses

Fertilization decreased species richness within three of the plant growth-form groups, i.e., forbs, non-Poaceae graminoids, and woody plant species (Table 3, Fig. 3a). The grasses showed no significant responses to any of the predictor variables for species richness (Table 3, Fig. 3a). Forbs were the only growth-form plant group that was significantly affected by litter as species richness increased with litter removal (Table 3, Fig. 3a). Fertilization increased the biomass of grasses, but decreased the non-Poaceae graminoids (Table 3, Fig. 3b). Thus, while the species richness of grasses was unaffected by either fertilization or litter, the biomass of the grasses were strongly affected by fertilization (Fig. 3b).

Fertilization decreased the species richness for both the native and non-native plant groups (Table 3, Fig. 4a). Litter had no effect on native plant species richness, but the removal of litter increased non-native plant species richness (Table 3, Fig. 4a). Fertilization increased the biomass of non-native and native plant species, but the effect was much higher for non-native with biomass approximately doubling in fertilized plots (Table 3, Fig. 4b).

Ecosystem response

NMS ordination showed tight clustering of plots into treatments (Fig. 5). The ordination axes explained 50.2% of the variance, with the first axis explaining 40.8% of the variance, and the second axis explaining 9.3% of the variance. The final stress = 5.83 with a final instability = 0.086, and results of the Monte Carlo simulation indicated that this stress was less than expected by chance ($P = 0.001$). Following Clarke (1993), a final stress between 5 and 10 was a very good ordination and did not present any real risk of misinterpretation. The first axis separated fertilized and unfertilized plots with high correlations to living biomass ($R^2 = 0.829$) in the direction of fertilized plots, and correlations to species richness ($R^2 = 0.582$), PAR ($R^2 = 0.750$), and percent soil moisture ($R^2 = 0.479$) in the direction of unfertilized plots, while litter biomass and percent soil organic content were not well correlated ($R^2 = 0.008$ and $R^2 = 0.053$, respectively). The second axis separated

Table 2 Proportion of total living biomass for each plant species in 2005

Species	Code	Growth form	Native/ non	% Biomass
Achillea millefolium L.	ACHMI	F	Non	0.0007
Agropyron repens (L.)	AGRRE	G	Non	0.0216
***Anthoxanthum odoratum* L.**	**ANTOD**	**G**	**Non**	**0.0439**
Apocynum cannabinum L.	APOCA	F	Native	0.0015
Aster pilosus Willd.	ASTPI	F	Native	0.0052
Aster prealtus Poir.	ASTPR	F	Native	0.0080
***Bromus inermis* Leyss.**	**BROIN**	**G**	**Non**	**0.2766**
Bromus japonicus Thunb. Ex Murr.	BROJA	G	Non	0.0001
Calystegia sepium (L.)	CALSE	F	Native	0.0001
Carex ssp. L.	CARSP	S	Native	0.0094
Cerastium nutans Raf.	CERNU	F	Native	0.0003
Chrysanthemum leucanthemum L.	CHRLE	F	Non	0.0020
Cirsium arvense (L.) Scop.	CIRAR	F	Non	0.0062
Cornus racemosa Lam.	CORRA	W	Native	0.0004
Crataegus ssp. L.	CRASP	W	Native	0.0116
***Dactylis glomerata* L.**	**DACGL**	**G**	**Non**	**0.0454**
Daucus carota L.	DAUCA	F	Non	0.0005
Euthamia graminifolia (L.)	EUTGR	F	Native	0.0001
***Festuca arundinacea* Schreb.**	**FESAR**	**G**	**Non**	**0.2088**
Frangula alnus P. Mill.	FRAAL	W	Non	0.0003
Fraxinus americana L.	FRAAM	W	Native	0.0005
Galium mollugo L.	GALMO	F	Non	0.0080
Geum ssp. L.	GEUSP	F	Native	0.0001
Glechoma hederacea L.	GLEHE	F	Non	0.0012
Hieracium aurantiacum L.	HIEAU	F	Non	0.0044
Holcus lanatus L.	HOLLA	G	Non	0.0190
Hypericum punctatum Lam.	HYPPU	F	Native	0.0001
Juncus tenuis Willd.	JUNTE	S	Native	0.0050
Oxailis stricta L.	OXAST	F	Native	0.0003
Panicum ssp. L.	PANSP	G	Native	0.0003
***Phleum pratense* L.**	**PHLPR**	**G**	**Non**	**0.0769**
Plantago lanceolata L.	PLALA	F	Non	0.0109
***Poa pratensis* L.**	**POAPR**	**G**	**Non**	**0.1889**
***Poa trivialis* L.**	**POATR**	**G**	**Native**	**0.0252**
Potentilla canadensis L.	POTCA	F	Native	0.0023
Potentilla recta L.	POTRE	F	Non	<0.0001
Prunella vulgaris L.	PRUVU	F	Native	0.0077
Prunus serotina Ehrh.	PRUSE	W	Native	<0.0001
Ranunculus acris L.	RANAC	F	Non	0.0013
Scirpus georgianus Harper	SCIGE	S	Native	0.0034
Taraxacum officinale Weber	TAROF	F	Non	0.0003
Toxicodendron radicans (L.) Kuntze	TOXRA	W	Native	<0.0001
Trifolium campestre Schreb.	TRICA	F	Non	0.0002
Trifolium pratense L.	TRIPR	F	Non	<0.0001
Viburnum rafinesquianum J.A. Schultes	VIBRA	W	Native	0.0013

"Code" refers to the five letter species abbreviation used in CCA ordinations in Fig. 4. "F" indicates forbs, "G" indicates grasses (Poaceae), "S" indicates graminoids other than Poaceae, and "W" indicates woody plant species. "Native" refers to plant species indigenous to Ohio, USA, whereas "Non" refers to plant species not native to Ohio, USA. Bold text indicates the seven most abundant grass species

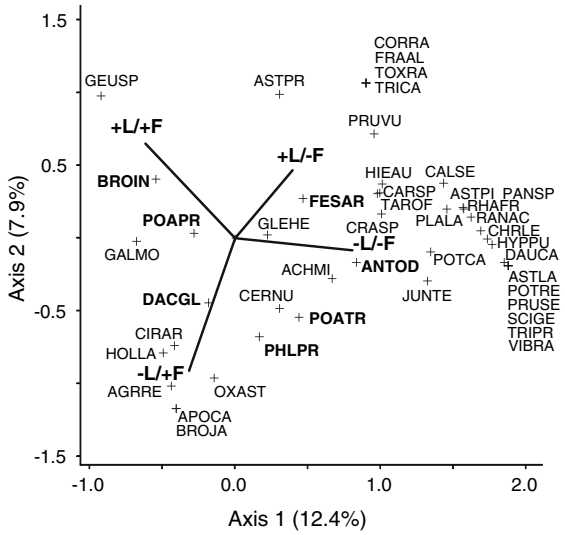

Fig. 2 Ordination diagrams of the first two axes of CCA with the four treatments as environmental dummy variables. Vectors indicate the direction and strength of correlations between axes scores and the environmental dummy variables, and the percent of variance explained by each axis is noted next to the axis title. Biomass of each of the 45 plant species collected in 2005 with R^2 correlations of axis to environment variables for axis 1: +L/–F = 0.080, –L/–F = 0.634, +L/+F = 0.457, and –L/+F = 0.162, and for axis 2: +L/–F = 0.314, –L/–F = 0.051, +L/+F = 0.258, and –L/+F = 0.709. See Fig. 1 for key to treatment symbols. The five letter species codes are defined in Table 2 symbols

litter removed from litter left in situ plots with a good correlation to litter biomass ($R^2 = 0.551$) and only very weak or no correlations to the other five variables: living biomass $R^2 = 0.001$, species richness $R^2 = 0.015$, PAR $R^2 = 0.007$, percent soil moisture $R^2 = 0.066$, and percent soil organic content $R^2 = 0.028$.

This strong separation of plots into treatment clusters was supported by MRPP (Table 4). When all four treatments were run together, the null hypothesis of no difference between treatments was rejected with high within-group agreement and very strong separation between groups. Pairwise comparisons of treatments showed that fertilized plots, while still significantly distinct, were more similar to each other than fertilized treatment plots are to any of the unfertilized treatment plots. The same pattern existed for unfertilized plots, with strong separation of unfertilized plots, yet with lower dissimilarity than when unfertilized plots were compared to fertilized plots. As expected, the maximal differences occurred when extremes of treatments were paired, as in –L/–F vs. +L/+F, and +L/–F vs. –L/+F, indicating that "opposite" treatments radically alter biotic and abiotic components of the local habitat.

Table 3 Results of factorial ANOVA for each of the six plant groups

	Species richness		Biomass	
	F	***P***	***F***	***P***
Forbs				
Fertilized	84.44	**<0.0001**	2.01	0.1712
Litter	9.88	**0.0051**	0	0.9694
Fertilized*litter	0.53	0.4766	0	0.9746
Grasses (Poaceae)				
Fertilized	0.31	0.5853	109.56	**<0.0001**
Litter	0.31	0.5853	0.59	0.4498
Fertilized*litter	0.31	0.5853	1.12	0.3023
Non-Poaceae Graminoids				
Fertilized	9.62	**0.0056**	7.07	**0.0151**
Litter	0.38	0.5421	0.50	0.4857
Fertilized*litter	3.46	0.0776	0.02	0.9011
Woody				
Fertilized	5.07	**0.0357**	0.89	0.3555
Litter	0.56	0.4616	0.61	0.4442
Fertilized*litter	0.14	0.7114	1.07	0.3139
Native				
Fertilized	23.75	**<0.0001**	4.06	0.0575
Litter	0.01	0.9427	1.03	0.3212
Fertilized*litter	2.8	0.1099	0.08	0.7837
Non-native				
Fertilized	14.15	**0.0012**	206.69	**<0.0001**
Litter	8.42	**0.0088**	2.15	0.1586
Fertilized*litter	0.29	0.5970	1.48	0.2374

"Fertilized" treatment is either fertilized (20 g N m^{-2} added) or unfertilized; Litter treatment is either plant litter removed or left in situ. "Fertilized*Litter" indicates their factorial interaction. For all analyses, *df* numerator = 1, and *df* denominator = 20. *P*-values significant at $\alpha = 0.05$ are denoted by bolding

Discussion

Our results clearly show that plant litter and fertilization alter plant community biomass, plant community structure, and ecosystem properties. The spatial and temporal scale of our study allowed us to assess species-level responses to fertilization and

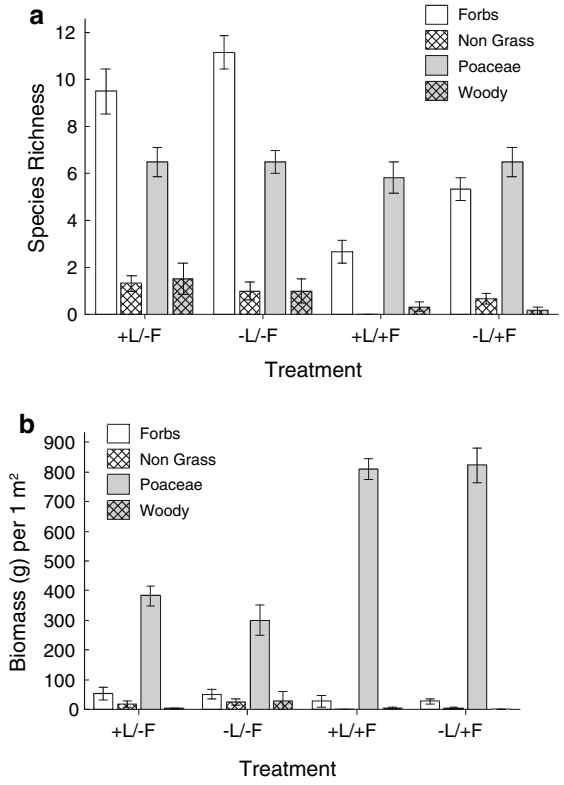

Fig. 3 (**a**) Average species richness of each growth form plant group within each treatment, and (**b**) average biomass of each growth form plant group within each treatment. Non-grass refers to non-Poaceae graminoids, i.e., Juncaceae and Cyperaceae. Full results of the factorial ANOVAs are in Table 3. See Fig. 1 for key to treatment

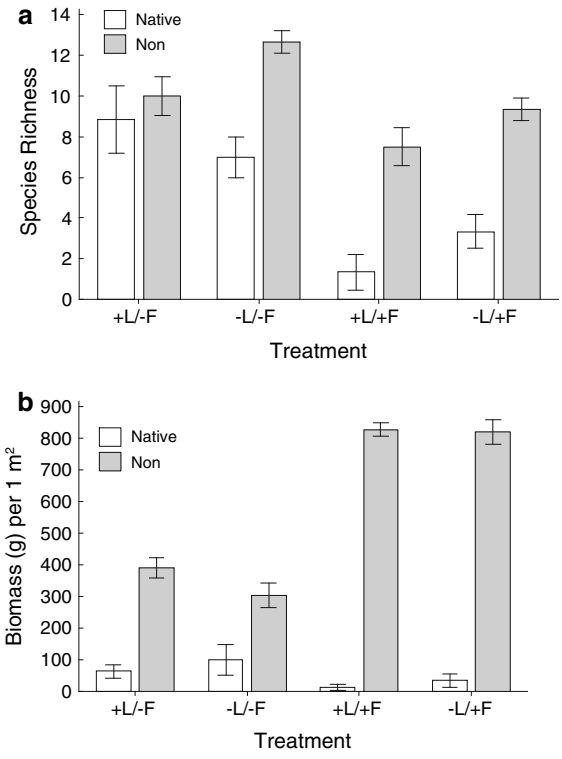

Fig. 4 (**a**) Average species richness of the native and non-native plant groups within each treatment, and (**b**) average biomass of the native and non-native plant groups within each treatment. Full results of the factorial ANOVAs are in Table 3. See Fig. 1 for key to treatment symbols

litter treatments within the entire plant community as well as ecosystem-level responses to fertilization and litter treatments.

Supporting our first hypothesis and consistent with other published studies (e.g., Dyer et al. 1991; Tilman et al. 2002a; Long et al. 2003), fertilization strongly increased plant biomass while generally decreasing plant species and plant group diversity. Fertilization effects on plant biomass were strongest in those plots where litter was left in situ, reflecting not only higher standing crop production, but also plant litter production and accumulation. However, some plant groups responded to fertilization in unpredicted ways.

Forb species were the most speciose plant group overall, including a number of species rare to the site (e.g., *Hypericum punctatum* Lam., *Trifolium pratense* L., and *Calystegia sepium* (L.)). The loss of forb species due to fertilization was consistent with the abundance-based mechanism of diversity loss due to fertilization (Suding et al. 2005). However, it is striking that fertilization significantly affected only forb species richness, not forb species biomass, contradicting our first hypothesis. Also contradicting conventional theory (e.g., Tilman et al. 2002a; Suding et al. 2005), grass species richness showed no response to fertilization, but grass species biomass was strongly affected by fertilization. This effect was likely due to our experiment manipulating an established plant community with a unique mix of native and non-native grasses and forbs, as opposed to the intentionally seeded/planted plant communities more commonly used in fertilization manipulation experiments (e.g., Foster and Gross 1998; Levine et al. 1998). Our results suggest that established grassland systems may not respond to fertilization and litter manipulation in ways generally predicted by theories derived from small-scale, short-term experiments.

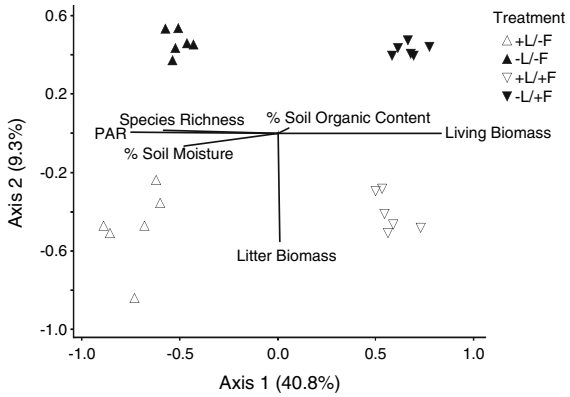

Fig. 5 Two-dimensional ordination of ecosystem-level properties from 2005 in 24 experimental plots from NMS using living biomass, species richness, litter biomass, PAR, percent soil moisture, and percent soil organic content. Vectors indicate the direction and strength of correlations between axis scores and emergent properties (R^2 cutoff for joint Biplot = 0.000 to show percent soil organic content, R^2 of all other vectors is >0.200) and ordinations are rotated to the dominant axis of living biomass. The percent of variance explained by each axis is noted next to the axis title. See Fig. 1 for key to treatment symbols

Table 4 Results of MRPP on emergent properties for 2005

Groups	*T*	*A*	*P*
All	–9.303	0.557	<0.0001
+L/–F vs. –L/–F	–3.531	0.241	0.0067
+L/–F vs. +L/+F	–5.952	0.405	0.0004
+L/–F vs. –L/+F	–6.419	0.452	0.0004
–L/–F vs. +L/+F	–6.169	0.425	0.0004
–L/–F vs. –L/+F	–5.574	0.383	0.0005
+L/+F vs. –L/+F	–3.563	0.236	0.0060

T describes the separation between groups (dissimilarity) and *A* is the chance-corrected within-group agreement. "All" indicates all four treatments included in the MRPP, and the remainders are MRPP pairwise comparisons of treatments to assess dissimilarity (lower *T* and higher *A*). +L indicates litter left in situ, –L indicates litter removed after annual mowing in early autumn, +F indicates fertilization (20 g N m^{-2}) in early spring, –F indicates no fertilization

Consistent with our second hypothesis, removing plant litter augmented Shannon's H, though as predicted the effect was dampened in fertilized plots. However, plant litter did not predictably affect the species richness and/or biomass of some plant groups. Grasses showed no responses to plant litter for either grass species richness or grass species biomass, whereas non-native plant species richness significantly increased in litter-removed plots with only a nominal, non-significant biomass response. Thus, while litter removal generally increased species richness overall, this manipulation facilitated non-native species recruitment and retention even though the effects on biomass were marginal at best.

The total biomass measurement includes the litter biomass, therefore it is intuitive that total biomass would be lower in litter-removed plots, explaining the significance of litter in the repeated PROC MIXED. The marginally significant *P*-value for litter in the repeated PROC MIXED for Shannon's H underscores the notion that litter itself affects plant diversity, though litter did not significantly affect any of the abiotic factors. Contrary to our results, Weltzin et al. (2005) found that the removal of litter in a northern fen affected abiotic factors, including increased availability of light and soil temperature. Perhaps, litter did not affect abiotic factors in our grassland because of the higher productivity of the field when compared to the fen community, and the dense mat-forming properties of the dominant grasses. We conclude that fertilization is the strongest determining factor for total plant biomass production and abiotic conditions, though litter should be considered when assessing plant species diversity, especially in consideration of the response of forbs and non-native plant species.

In support of our third hypothesis, plant litter and fertilization interacted to induce a shift in community structure as shown by fine-scale analyses of species responses to treatments, with the most abundant grasses, particularly *B. inermis* in fertilized plots and *F. arundinacea* in unfertilized plots, separating along the first CCA axis (Fig. 2). The large majority of plant species lie along the unfertilized portion of the first axis, bolstering the assertion that fertilization decreases plant diversity (Grime 1979; Tilman et al. 2002b). The distinct separation along the fertilization axis of *F. arundinacea* and *B. inermis*, the two most abundant grasses (see Table 2), indicates a stronger response to fertilization elicited by *B. inermis*, a species known to more efficiently utilize N when compared with *F. arundinacea* (Eck et al. 1981). In our study, *Poa pratensis*, our third most abundant grass, was intermediate to *B. inermis* and *F. arundinacea* along the fertilization axis, showing a weak affiliation with fertilization. The relationship between *P. pratensis* and fertilization appears to be somewhat

equivocal, as Pennings et al. (2005) report that the abundance of *P. pratensis* decreased in five of nine fertilization experiments. Indeed, *P. pratensis* was ubiquitous in our system and, therefore, its placement near the centroid of the CCA was not surprising.

The experimental plots cluster very tightly into distinct aggregates of ecosystem and plant community properties, separated along the first axis by fertilization and along the second axis by plant litter (Fig. 5). Living biomass again associates with fertilized plots, while species richness and light attenuation (PAR) were higher in unfertilized plots, consistent with other published studies that included abiotic factors (e.g., Foster and Gross 1998). Percent soil moisture increased in the direction of unfertilized plots, a surprising result given that higher litter production and lower light attenuation at the soil level would intuitively seem more conducive to increased soil moisture (Hamrick and Lee 1987); however, this may be explained by greater plant biomass in fertilized plots having a greater water demand, thus leading to soil drying. The greatest scatter within a treatment is in the control group (+L/–F), reflecting the spatial heterogeneity expected in an old field. Interestingly, fertilized plots exhibited extremely tight clustering relative to unfertilized plots, indicating strong within-group similarity and reduced heterogeneity.

The measurement and analysis of the plant community and associated abiotic properties demonstrates strong treatment effects. These highly differentiated treatments may affect ecosystem function (e.g., sequestering carbon), an effect likely to increase in magnitude through time as the communities' responses to treatments mature. While each of these biotic properties has been shown to respond individually to fertilization (e.g., Carson and Peterson 1990; Tilman et al. 2002a, b; Long et al. 2003), this is the first time that these three biotic ecosystem properties have been used in a multivariate ordination to explicitly determine whether they can define discrete and distinct plant communities and their associated abiotic properties. Most of the previous work on the effects of fertilization and plant litter has focused on a single, dominant response species or a small collection of species within the entire community over a single growing season (e.g., Foster and Gross 1997, 1998; Violle et al. 2006). We know of no other long-term, temperate system studies that have manipulated both fertilization and plant litter in an established plant community at the same (or greater) scale. However, we realize that our study has some distinct differences when compared to previous work.

Our use of an NPK fertilizer, as opposed to N-only fertilizer, is likely to have induced a stronger response to fertilization due to the added P and K. Nevertheless, our results were generally consistent with other studies that used NPK fertilizers (e.g., Carson and Barrett 1988, Turkington et al. 2002), N-only fertilizers (e.g., Tilman et al. 2002b, Long et al. 2003) as well as other studies that simulated N-only atmospheric deposition (e.g., Throop 2005). Further, our running definition of litter (see Methods) includes the vegetation mown in the previous year and not removed in our litter removal treatment, potentially altering the nutritional quality of the litter relative to naturally senesced vegetation, and the physical structure of the litter as it lay after mowing (e.g., Semmartin et al. 2004). Because the timing of the mowing was determined by the local township, litter from the annual mowing accumulated earlier than might normally be expected for our region of the USA. Were the mowing to stop, the site would very quickly yield to encroaching woody vegetation typical of early secondary succession (Cook et al. 2005).

This study expands our understanding of the long-term effects of fertilization and plant litter on plant species and specific plant groups, confirms some aspects of previous work (e.g., Tilman et al. 2002b), and extends the scope of scientific knowledge regarding the effects of terrestrial ecosystem eutrophication on ecosystem-level processes. Some plant groups did not respond to our treatments as predicted by theory, which underscores the need for larger-scale and longer-term experiments in natural systems. When viewed through multivariate ordination, the realized differences in biotic and abiotic ecosystem properties emerged, and these properties indicate that each treatment could potentially alter the trajectory of grassland ecosystem dynamics.

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