

# Bottom-up and top-down effects on plant communities: nutrients limit productivity, but insects determine diversity and composition

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Top-down effects of herbivores and bottom-up effects of nutrients shape productivity and diversity across ecosystems, yet their single and combined effects on spatial and temporal beta diversity is unknown. We established a field experiment in which the abundance of insect herbivores (top-down) and soil nitrogen (bottom-up) were manipulated over six years in an existing old-field community. We tracked plant  $\alpha$  and  $\beta$  diversity – within plot richness and among plot biodiversity- and aboveground net primary productivity (ANPP) over the course of the experiment. We found that bottom-up factors affected ANPP while top-down factors influenced plant community structure. Across years, while N reduction lowered ANPP by 10%, N reduction did not alter ANPP relative to control plots. Further, N reduction lowered ANPP by 20% relative to N addition plots. On the other hand, the reduction of insect herbivores did not alter plant richness ( $\alpha$  diversity) yet consistently promoted Shannon's evenness, relative to plots where insect herbivores were present. Further, insect herbivores promoted spatial-temporal  $\beta$  diversity. Overall, we found that the relative importance of top-down and bottom-up controls of plant ANPP, plant  $\alpha$  diversity, and composition ( $\beta$  diversity) can vary significantly in magnitude and direction. In addition, their effects varied through time, with bottom-up effects influencing ANPP quickly while the effects of top-down factors emerging only late in the experiment to influence plant community composition via shifts in plant dominance.

Arguments about the relative roles of bottom-up and top-down factors in shaping plant community structure have populated the literature for decades (Hairston et al. 1960). Nutrient amendments have clearly demonstrated that bottom-up effects can influence diversity, evenness, and aboveground net primary productivity (ANPP; Elser et al. 2007, Stevens et al. 2004), though increases in ANPP come at a cost of lower diversity levels (Suding et al. 2005). Similarly, herbivore exclusion and suppression experiments have shown that, at least in some cases, top-down factors can also affect plant community structure and ANPP, though the effects are decidedly mixed and vary among systems (Hillebrand et al. 2007, Gruner et al. 2008, Borer et al. 2014), with generally stronger impacts on aquatic than terrestrial systems (Shurin et al. 2002, Shurin and Seabloom 2005). In fact, vertebrate herbivores can remove anywhere from 15 to 70% (Cyr and Pace 1993, Peters et al. 2007) of ANPP whereas invertebrate herbivores might reduce ANPP by only 15%, but not as consistently (Carson and Root 2000, Del-Val and Crawley 2005, Gao et al. 2008).

Top-down and bottom-up factors need not act independently to influence plant diversity and productivity (Hillebrand et al. 2007, Gruner et al. 2008). In fact, in

some cases, the effects of herbivores on plant community structure depend on soil nutrient availability and appear to be consistently positive in highly productive (e.g. high nutrient) environments, but mostly negative in unproductive (e.g. nutrient poor) environments (Hillebrand et al. 2007). In highly productive environments, herbivores can attenuate competitive effects (e.g. increase resource availability) and promote plant coexistence. In low productivity environments, herbivory can negatively impact diversity due to loss of nitrogen-limited species. In other cases, the effects of top-down and bottom-up factors can counteract one another, such that herbivores decrease ANPP and increase diversity while soil nutrient addition promotes ANPP and lower diversity (Silvertown 1980, Suding et al. 2005). Ultimately, the ability of herbivores to mediate the effects of nutrient on ecosystems will depend on whether they are generalists or specialists, with generalist herbivores promoting diversity to a greater extent relative to specialist herbivores (Gruner et al. 2008).

It is also possible that top-down and bottom-up factors do not alter diversity in plant communities, but induce a shifting in community composition than the presence or abundance of species. The evidence that bottom-up factors,

especially nitrogen (N) addition, affect compositional change (i.e.  $\beta$  diversity) is strong (Chalcraft et al. 2008). Generally speaking, N addition reduces  $\beta$  diversity among plant communities in space (i.e. communities receiving the same treatments become more similar to one another in their species composition if the same over-yielding species is present across all communities). The evidence for top-down effects of herbivores on  $\beta$  diversity in plant communities is more mixed and can depend on soil-nutrient availability as well as vary among taxa and systems (Hillebrand et al. 2007); yet herbivores should promote  $\beta$  diversity by lowering producer species homogenization (Newman et al. 2014) under ambient soil N conditions. On the other hand, herbivores should promote and hinder diversity in soil N addition and soil N reduction respectively. To our knowledge, no studies have yet examined the relative and combined effects of top-down and bottom-up factors on both spatial  $\beta$  diversity (i.e. community similarity in space) and temporal  $\beta$  diversity (i.e. community similarity across time), at least in part because few studies have examined the combined and independent effects of top-down and bottom-up factors on plant communities across many years (but see Borer et al. 2014).

In this study, we examine the combined and interactive effects of insect herbivory (top-down) and soil nitrogen (N) availability (bottom-up) on metrics of plant community structure ( $\alpha$  diversity, Shannon's evenness and diversity, spatial-temporal  $\beta$  diversity) and aboveground net primary production (ANPP) in an old-field ecosystem across six years. Based on previous work in this system (Sanders et al. 2007, Blue et al. 2011), we tested three inter-related questions: 1) do herbivore reduction and nutrient addition treatments interact to shape plant community structure, and do those effects vary across time? 2) What is the effect of N addition on ANPP and plant community structure? 3) What is the effect of herbivore reduction on ANPP and community structure?

## Material and methods

### Study site

We established this field experiment in the spring 2004 within a ~10-ha old-field community at Oak Ridge National Environmental Research Park near Oak Ridge, Tennessee, USA (35°58'N, 84°17'W). The field site was used for agriculture until 1943 and left unmanaged until 2003, when the site was then annually mown in the winter (senesced vegetation) to prevent it from transitioning into a forest (Kuebbing et al. 2014). Given that mowing takes place in the winter when most vegetation is senesced, it has no direct impact on herbivore populations. The soil, classified as a Typic Hapludult, has a silty clay loam texture and is moderately well drained (Phillips et al. 2001). Precipitation is uniform throughout the year, with a mean annual rainfall of 1322 mm, a mean January minimum temperature of 2.7°C, and a mean July maximum temperature of 31.2°C. Commonly occurring plant species at the study site and surrounding old fields include *Solidago altissima*, *Rubus argutus*, *Verbesina occidentalis* and *Verbesina virginica*. Approximately 60 other sub-dominant plant species, both herbaceous and

woody, are present at the site (Sanders et al. 2007, Souza et al. 2011, Wright et al. 2014).

### Experimental design

In February 2004, we selected 36 plots that were part of a larger study encompassing 72 plots described in Sanders et al. (2007) in an area approximately 60 × 50 m. Each experimental plot was 3 × 3 m, including a 0.5-m buffer perimeter within each plot, with 2-m spacing between plots. The 2-m spacing between experimental plots was managed with continuous mowing during the growing season. A 3-m tall fence was installed around the experimental site to exclude deer. In a fully crossed, completely randomized plot design, we manipulated soil N and the abundance of insects (n = 6 replicates, n = 36 total plots) and randomly assigned plots to each treatment until the termination of the experiment: 1) insect present and ambient nutrients, 2) insect present and reduced nutrients, 3) insect present and nutrient addition, 4) insect removal and ambient nutrients, 5) insect removal and reduced nutrients, 6) insect removal and nutrient addition.

### Experimental manipulations

We manipulated soil N availability by adding N (applied as urea fertilizer, at a rate of 10 g m<sup>2</sup> year<sup>-1</sup>) or adding carbon (C) to reduce N (applied as sucrose at a rate of 167 g m<sup>2</sup> year<sup>-1</sup>) three times per year (February, May, July); the remainder of the plots were not manipulated (e.g. control) (n = 12). The addition of C in the form of sucrose provides microbial communities with a surplus of labile C, ultimately leading to N immobilization and lowering N availability (Wang et al. 2004, Craine et al. 2007, Sanders et al. 2007). Nitrogen manipulations in our experiment were similar to other studies addressing the role of N enrichment, mimicking nitrogen deposition from agriculture and industrial sources, in grasslands and old fields (McLendon and Redente 1992, Larson and Siemann 1998, Suding et al. 2005, Borer et al. 2014). In 2005, one year after the first application of the nutrient treatments, soil N availability (NO<sub>3</sub>-N + NH<sub>4</sub>-N) in the soil was 1.7 × greater in the N addition plots (11.69 ± 1.00 ppm), and 5 × lower in the N reduction plots (1.17 ± 1.00 ppm) than in the control plots (6.80 ± 0.84 ppm) (p < 0.0001). Urea additions increased both NO<sub>3</sub>-N and NH<sub>4</sub>-N (p < 0.0001), and sucrose additions decreased NO<sub>3</sub>-N (p < 0.0001) but had no effect on NH<sub>4</sub>-N (p = 0.50) (Sanders et al. 2007). Similar effects of urea and sucrose on soil nutrients have been observed in other field studies (Wilson and Gerry 1995, Morghan and Seastedt 1999). Our soil N manipulation had temporal and idiosyncratic effects on the insect populations (Lane 2006).

We had two levels of insect herbivory: 1) unmanipulated controls (in which insects were present at natural densities) and 2) reduced insect abundance (n = 18). We applied permethrin insecticide with a backpack sprayer at a rate of 0.23 l m<sup>-2</sup> every 2–3 weeks during the growing season to reduce insect abundance. The use of pyrethroid-based insecticides effectively reduced insect abundance as in other studies (Root 1996, Lynch et al. 2006, Schmitz 2006, Agrawal et al. 2012) with no effects on plant shoot

or root growth. When we sampled the plots using a combination of sweep-netting, vacuum sampling, and visual scanning, we found that insect abundance was on average  $4 \times$  lower in the insect-reduced plots ( $6.6$  individuals  $m^{-2}$ ) relative to the control plots ( $28.4$  individuals  $m^{-2}$ ; Lane 2006, Sanders et al. 2007). Based on many years of observations and detailed studies of plant–insect interactions at the site (Sanders et al. 2007, Crawford et al. 2007), we are quite certain that insect herbivores rather than insect predators were by far the most abundant trophic group. Finally, only one herbivore group, an aphid, was difficult to reduce or remove using the insecticide treatment and vacuum sampler.

### Bottom–up and top–down effects on plant community structure: ANPP and $\alpha$ diversity

To examine the main and interactive effects of herbivore suppression and nutrient amendment on plant community structure, we measured foliar cover, species  $\alpha$  diversity (the number of species per plot, *sensu* Gotelli and Colwell 2001), Shannon's diversity and evenness at the entire plot scale ( $3 \times 3$  m) each year during peak ANPP (August). To estimate foliar cover (e.g. vegetative cover including stems and leaves), we used a modified Braun–Blanquet scale that included six categories:  $< 1\%$ ,  $1\text{--}5\%$ ,  $5\text{--}25\%$ ,  $25\text{--}50\%$ ,  $50\text{--}75\%$ ,  $75\text{--}100\%$ . Similarly, we quantified  $\alpha$  diversity by determining the number of plant species (e.g. species richness) per plot. We calculated the Shannon diversity index ( $H'$ ) from foliar cover data by using the median of each cover class category as our measure of abundance.  $H'$  was calculated as:  $H' = -\sum (p_i \times \ln p_i)$  and evenness was calculated as  $J' = H' / S$ . We determined the total community aboveground biomass (ANPP) at the end of each growing season (September) by randomly placing a  $0.5 \times 1$  m quadrat within each experimental plot. We clipped aboveground biomass within each  $0.5\text{-m}^2$  quadrat to ground level and oven dried the clipped plant mass at  $60^\circ\text{C}$  for approximately 48 h and then weighed the samples to the nearest 0.1 gram.

For each of the response variables (ANPP, richness, evenness, diversity), we performed a repeated measures analyses of variance (RM ANOVA), with main and interactive effects of bottom–up (N-reduction, N-addition, N-ambient), and top–down factors (herbivores suppressed, herbivores present) as fixed effects. RM ANOVA was performed to determine whether treatment effects differed across time, controlling for non-independence among the repeated observations by estimating the correlation structure of repeated observations overtime. Plant community structure ( $\alpha$  diversity, Shannon's evenness and diversity) and ANPP were the response variables assessed individually in our RM ANOVA model. We used Wilks' Lambda test as our test statistics to determine how the effects of time (e.g. year), time  $\times$  top–down, time  $\times$  bottom–up, time  $\times$  top–down  $\times$  bottom–up on our focal response variables. We followed the RM ANOVA with subsequent univariate two-way ANOVAs if there was a significant effect of year. We used Tukey's HSD means separation test ( $\alpha = 0.05$ ) to identify which treatment means differed from one another. We conducted all univariate analyses in SAS 9.2.

### Bottom–up and top–down effects on spatial and temporal $\beta$ diversity

To determine the main and interactive effects of insect herbivores, soil nutrients, and time on spatial  $\beta$  diversity (i.e. how similar, in plant species composition, plots within the same treatment were to one another within a given year but also across years), we conducted a non-parametric, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) on a Bray–Curtis similarity matrix generated from the log transformed ( $\log x + 1$ ) plant composition (i.e. species-specific percent foliar cover) data. We chose Bray–Curtis similarity matrix because it is well-suited to indicate changes in abundance and species composition between plant assemblages using abundance data (e.g. foliar cover data). The PERMANOVA approach allowed us to compare the variability in species composition both 'within' treatments and 'among' treatments using a pseudo F-ratio to determine whether observed variability in species composition differed from the variability in species composition using a null distribution (Anderson et al. 2006). We followed up PERMANOVA analyses with PERMDISP (permutational multivariate analysis of dispersion) to determine whether differences in 'location' of treatment communities in multivariate space influenced overall dissimilarities in plant community composition or difference in 'dispersion' of communities in multivariate space (Anderson et al. 2006). We used PRIMER ver. 1.0.3 for these analyses. To illustrate species composition in multivariate space, we performed a series of principal coordinate analyses (PCO) based on the Bray–Curtis similarity matrix. We used the first PCO axes, which accounted for a significant proportion of total variation in compositional similarities, to illustrate treatment differences in  $\beta$  diversity over time. We also performed a similarity percentage analysis (SIMPER) to determine the contribution of particular plant species to the overall differences in community composition dissimilarities between bottom–up factors and top–down treatments.

To determine the main and interactive effects of insect herbivory and soil nutrients on within-plot temporal  $\beta$  diversity (i.e. how much the composition of each given plot changed from year to year in response to the treatments), we first calculated community similarity of each plot against itself for each temporal interval using a Bray–Curtis similarity matrix (e.g. compositional similarity of plot 1 in 2004 versus plot 1 in 2005) and the calculated species turnover (i.e. percent dissimilarity) within pots across time. We used JMP ver. 9.0 (SAS Inst.) to determine the main and interactive effects of insect herbivores and soil nutrients shaping within plot compositional turn over across annual (2004–2005, 2005–2007, 2007–2008, 2008–2009) and multi-annual (2004–2009) time intervals.

## Results

### Temporal dynamics in the top–down versus bottom–up effects on ANPP, plant $\alpha$ and $\beta$ diversity

ANPP differed 2.6-fold among years, with highest ANPP in 2004 ( $772 \pm 31.3$  g  $m^{-2}$ ) and lowest in 2009 ( $335 \pm 40.4$

g m<sup>-2</sup>; Table 1). Plant richness ( $\alpha$  diversity) differed by up to 15% among years and was highest in 2007 (15 plant species m<sup>-2</sup> ± 0.55) and lowest in 2005 (13 plant species m<sup>-2</sup> ± 0.44) and 2009 (13 plant species m<sup>-2</sup> ± 0.32). The effects of both bottom-up and top-down factors on ANPP and richness were consistent among years (i.e. there were no significant interactions with time; Table 1). Shannon's evenness differed by up to 15% among years, highest in 2009 (0.69 ± 0.02) and lowest in 2004 (0.58 ± 0.02). Shannon's diversity also differed among years by 15%, highest in 2008 (1.76 ± 0.04) and 2009 (1.76 ± 0.05) and lowest in 2004 (1.52 ± 0.06). The effects of both top-down and bottom-up factors on Shannon's evenness and diversity varied among years (i.e. there were significant time × bottom-up and time × top-down effects; Table 1).

### Bottom-up effects on ANPP and $\alpha$ diversity

Bottom-up factors influenced plant ANPP each year (Table 1, Fig. 1), but the magnitude of the effect varied among years. Across years, N reduction lowered ANPP between 10% and 20% (Tukey's HSD  $p < 0.05$ ) respectively, when compared to control and N addition plots (Fig. 1), and this effect emerged at the onset of the N reduction and then again in the last two years of the experiment. In contrast, N addition did not increase ANPP at any point in the study (Fig. 1).

Plant species richness responded to N manipulation in only 2007 (Table 1), when the number of species was 25% lower in N-addition plots (13.1 ± 0.9 plant species m<sup>-2</sup>) and 13% lower in N-reduction plots (14.9 ± 0.9 plant species m<sup>-2</sup>) than in control plots (16.8 ± 0.8 plant species m<sup>-2</sup>; Fig. 2) (Tukey's HSD  $p < 0.05$ ). The effects of N manipulation on Shannon's evenness and  $\alpha$  diversity, as well as community similarity patterns, varied from year to year (Table 1). Soil N influenced Shannon's evenness and richness only in 2004 (Table 1), where control plots had on average 18% and 25% greater Shannon's evenness and richness than

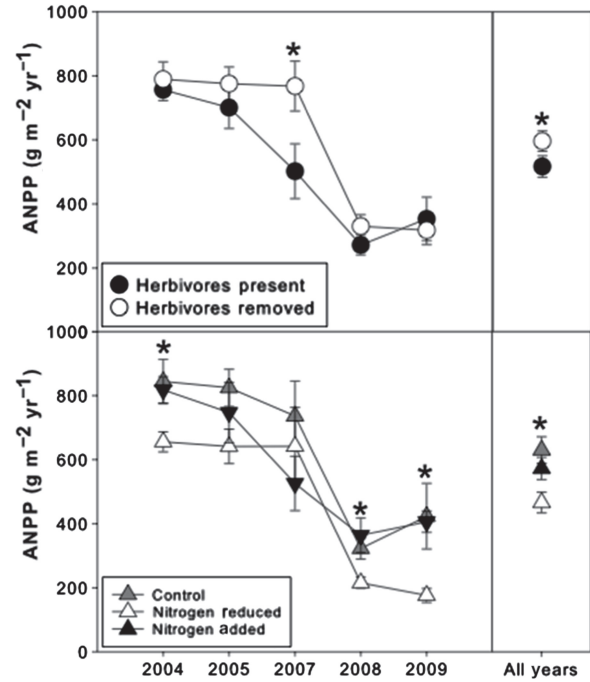


Figure 1. N-reduction plots have consistently lower ANPP when compared to N-addition and control plots, whereas the reduction of herbivores promoted ANPP only in 2007. Effects of insect herbivores (top panel) and bottom-up factors (bottom panel) on mean ( $\pm$  standard error) plant community ANPP within (2004, 2005, 2007, 2008, 2009) and across time (all years' = average across time) from two-way ANOVA. Asterisks represent statistical difference at  $\alpha = 0.05$  using Tukey's HSD means separation test.

N-reduction and N-addition plots (Tukey's HSD  $p < 0.05$ ), respectively (Fig. 2). There were no other significant bottom-up effects on plant ANPP or diversity ( $\alpha$ , evenness, Shannon's diversity) in any other year of the experiment (Table 1).

Table 1. Bottom-up and top-down factors independently influenced plant community ANPP and plant community structure in an old-field ecosystem. While bottom-up factors effects had consistent effects on plant community ANPP, top-down effects consistently affected species evenness. Results from two-way analysis of variance (ANOVA) including F-ratio (F) and p-values (p) across years (2004, 2005, 2007, 2008 and 2009).

Source	ANPP									
	2004		2005		2007		2008		2009	
	F	p	F	p	F	p	F	p	F	p
Top-down	0.15	0.7	0.87	0.36	5.07	<b>0.03</b>	1.77	0.19	0.02	0.89
Bottom-up	4.84	<b>0.02</b>	1.75	0.19	1.07	0.36	4.05	<b>0.03</b>	6.9	<0.01
Top-down × Bottom-up	1.1	0.35	1.92	0.16	0.23	0.79	0.56	0.58	0.2	0.82
<u><math>\alpha</math> diversity</u>										
Top-down	0.46	0.5	0.03	0.86	2.4	0.13	2.11	0.16	0.17	0.68
Bottom-up	2.16	0.13	0.36	0.7	<b>4.66</b>	<b>0.02</b>	1.22	0.31	0.92	0.41
Top-down × Bottom-up	2.22	0.13	0.39	0.68	0.56	0.58	0.1	0.91	0.13	0.88
<u>Shannon's evenness</u>										
Top-down	0.36	0.55	3.75	<b>0.06</b>	0.43	0.52	5.7	<b>0.02</b>	3.84	<b>0.06</b>
Bottom-up	5.34	<b>0.01</b>	0.52	0.6	1.64	0.21	0.88	0.43	1.79	0.18
Top-down × Bottom-up	0.04	0.96	0.31	0.73	0.05	0.95	2.04	0.15	0.5	0.61
<u>Shannon's diversity</u>										
Top-down	0.04	0.84	3.41	<b>0.07</b>	1.29	0.26	1.63	0.21	2.64	0.11
Bottom-up	6.06	<b>0.01</b>	0.59	0.56	1.21	0.31	2.19	0.13	0.97	0.39
Top-down × Bottom-up	0.17	0.85	0.27	0.77	0.13	0.88	1.27	0.29	0.19	0.82



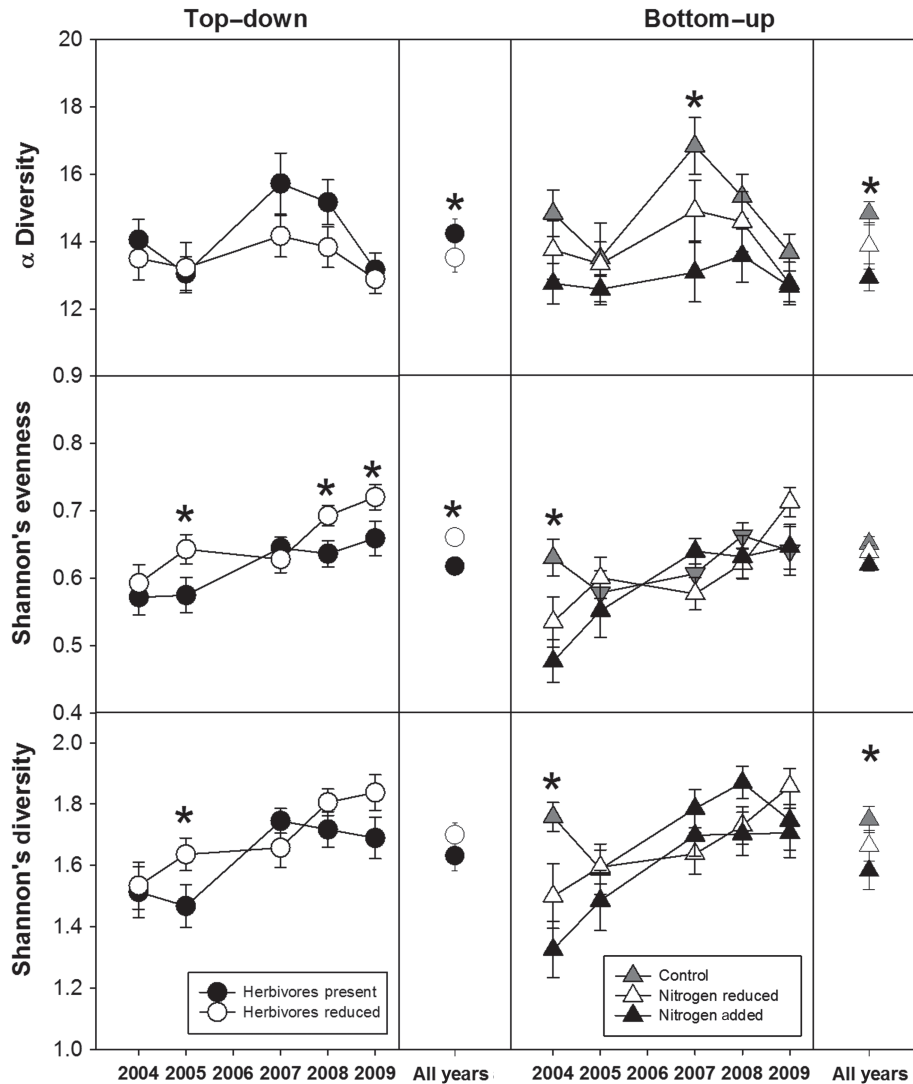


Figure 2. Both top-down and bottom-up effects influenced plant species evenness and diversity early on. Effects of insect herbivores (left panels) and bottom-up factors (right panels) on mean ( $\pm$  standard error)  $\alpha$  diversity (top panels), Shannon's evenness (middle panels) and Shannon's diversity (lower panels) within (2004, 2005, 2007, 2008, 2009) and across time ('all years' = average across time) from two-way ANOVAs. Asterisks represent statistical difference at  $\alpha = 0.05$  using Tukey's HSD means separation test.

### Bottom-up effects on spatial and temporal $\beta$ diversity

The effects of N manipulation on spatial  $\beta$  diversity were not significant until 2007, three years after the start of the experiment (Table 2, Fig. 4). In 2009, N reduction plots differed in spatial  $\beta$  diversity from both control and N-addition plots (Supplementary material Appendix 1), and together, the two most common N-fixing plant species (*Lespedeza cuneata*, *Desmodium nudiflorum*), and two common forb species (*Verbesina virginica*, *Aster pilosum*) were 60% and 40% more abundant in N-reduction than N-addition and control plots, respectively. Furthermore, woody forbs dominated N addition plots and herbaceous forbs dominated control plots, increasing spatial  $\beta$  diversity between those treatments (Supplementary material Appendix 1). Overall, changes in spatial  $\beta$  diversity were largely the result of N manipulation (Table 2, Fig. 4) as evidenced by shifts in their

location in multivariate space and marginally as the result of changes in dispersion patterns (Table 3, Fig. 4) within treatments. Finally, N manipulation did not influence temporal  $\beta$  diversity (e.g. degree of compositional dissimilarity within plots) from year to year or across years (Fig. 3).

### Top-down effects on ANPP and $\alpha$ diversity

In most years, there was no effect of herbivore reduction on ANPP. However, in 2007, ANPP was 38% greater in the plots where herbivores were reduced relative to where they were present. Herbivore reduction consistently lowered plant species dominance by increasing Shannon's evenness by 10%, relative to plots where herbivores were present (Table 1, Fig. 2). In 2004, at the beginning of the experiment, herbivore reduction promoted Shannon's diversity by 36% but reducing herbivores did not affect local diversity metrics ( $\alpha$  diversity/richness) in any other year (Table 1, Fig. 2).

Table 2. Bottom-up and top-down factors interacted to influence plant community composition in an old-field ecosystem. Results from permutation multivariate analysis of variance (PERMANOVA) including the main and interactive effects of top-down, bottom-up factors and year on pseudo F-ratio (F) and permuted p-values (p) for both across and for each year (2004, 2005, 2007, 2008 and 2009).

Source	Across years		2004		2005		2007		2008		2009	
	F	p	F	p	F	p	F	p	F	p	F	p
Top-down	4.12	<b>0.02</b>	0.9	0.53	0.87	0.57	1.89	<b>0.04</b>	1.98	<b>0.03</b>	1.78	<b>0.06</b>
Bottom-up	7.04	<b>&lt;0.01</b>	1.22	0.24	0.65	0.86	1.53	<b>0.05</b>	0.65	0.88	1.79	<b>0.02</b>
Year	8.88	<b>&lt;0.01</b>										
Top-down × Bottom-up	6.09	<b>&lt;0.01</b>	0.64	0.88	0.87	0.62	0.925	0.56	1.81	<b>0.01</b>	0.99	0.48
Top-down × Year	0.90	0.66										
Bottom-up × Year	0.53	1.00										

### Top-down effects on spatial and temporal $\beta$ diversity

Herbivore reduction consistently altered spatial  $\beta$  diversity (e.g. 2007, 2008, and 2009; Table 2, Fig. 4), leading to lower spatial  $\beta$  diversity among plots where insects were reduced from those where they were present (Supplementary material Appendix 1). In other words, when herbivores were reduced, they lowered the variability (e.g. over-dispersion) among plant communities relative to plant communities where herbivores were present; this indicates that plots where herbivores were present exhibited greater compositional dissimilarity to each other than plots where herbivory was reduced (Table 3). Further, the reduction of insect herbivores lowered plant species turnover within plots (e.g. greater within-plot dissimilarity), leading to reduced temporal  $\beta$  diversity within plots ( $F_{5,30} = 5.27$ ,  $p = 0.029$ ) and 2007–2008 ( $F_{5,30} = 3.45$ ,  $p = 0.073$ ), but not across the entire duration of the experiment (2004–2009 ( $F_{5,30} = 0.76$ ,  $p = 0.583$ ) (Fig. 3).

### Interactive effects of top-down and bottom-up factors on ANPP, plant $\alpha$ and $\beta$ diversity

Herbivore reduction and soil nutrients interacted to affect spatial  $\beta$  diversity (Table 2). For example, species composition in control plots (e.g. no N manipulation) differed significantly when herbivores were reduced than when herbivores were present, while herbivore manipulations did not alter species composition in N-addition or N-reduction plots (Supplementary material Appendix 2). In other words, reduction of herbivory altered spatial beta diversity in plots without N-manipulation, suggesting that herbivory influences beta diversity, while herbivore manipulation did not alter species composition in N-addition or N-reduction plots. On the other hand, we found no significant interactions between herbivore and soil N manipulations on ANPP,  $\alpha$  diversity, Shannon's evenness or diversity in any year (Table 1).

### Discussion

Overall, we found top-down and bottom-up factors acted independently to affect plant ANPP, plant  $\alpha$  diversity (plant richness), and composition ( $\beta$  diversity) and to vary significantly in timing, magnitude and direction. Understanding the drivers of biodiversity change rather than biodiversity loss is critical (Anderson et al. 2011, Chase et al. 2011, Dornelas et al. 2014). The drivers of plant diversity and ANPP have dominated much of the ecological literature, both in the form of ecological theory (Loreau 2000, 2010) and a large body of empirical work (Hooper et al. 2005, Dornelas et al. 2014). We found that controls on ANPP, plant community diversity and composition differed from year to year and N reduction and herbivory acted independently rather than synergistically or antagonistically. In our study system, soil N addition reduced Shannon's evenness and diversity. However, N addition did not increase ANPP (Gruner et al. 2008) or homogenize community composition, as would be expected based on other studies (Britton et al. 2009, Reinecke et al. 2014). Herbivory reduction promoted ANPP at the start of the experiment, although the effects were transient and did not persist through the 6 years of herbivore reductions. Somewhat surprisingly, the reduction of herbivores led to an increase, rather than decline, in Shannon's evenness and  $\alpha$  diversity while lowering spatial-temporal  $\beta$  diversity among and within plant communities. Yet, even generalist herbivores can selectively forage subdominant species that may exhibit higher forage quality than dominants reducing evenness.

### Bottom-up effects on plant ANPP, $\alpha$ and $\beta$ diversity

Nutrient availability had a strong and consistent influence on ANPP, but not on plant diversity metrics ( $\alpha$  and  $\beta$  diversity). We found that N reduction lowered plant ANPP early on and consistently throughout the experiment, while ANPP

Table 3. Insect herbivores and bottom-up factors interacted to affect variability (e.g. overdispersion) in plant species composition in multivariate space both in 2009 as in across years. Results from PERMDISP analysis of main and interactive effects of insect herbivores and bottom-up factors and estimated pseudo-F value (F) and permuted p-value (p). Values shown in bold are statistically significant.

Source	Across time		2004		2005		2007		2008		2009	
	F	P	F	P	F	P	F	P	F	P	F	P
Top-down	8.16	<b>0.01</b>	<0.01	0.89	1.06	0.33	0.44	0.55	0.97	0.36	7.28	<b>0.01</b>
Bottom-Up	2.43	0.12	0.33	0.76	1.63	0.25	0.65	0.58	0.36	0.74	3.64	<b>0.07</b>
Top-down × Bottom-up	2.62	<b>0.05</b>	0.75	0.72	1.11	0.52	0.7	0.79	0.45	0.91	4.09	<b>0.03</b>

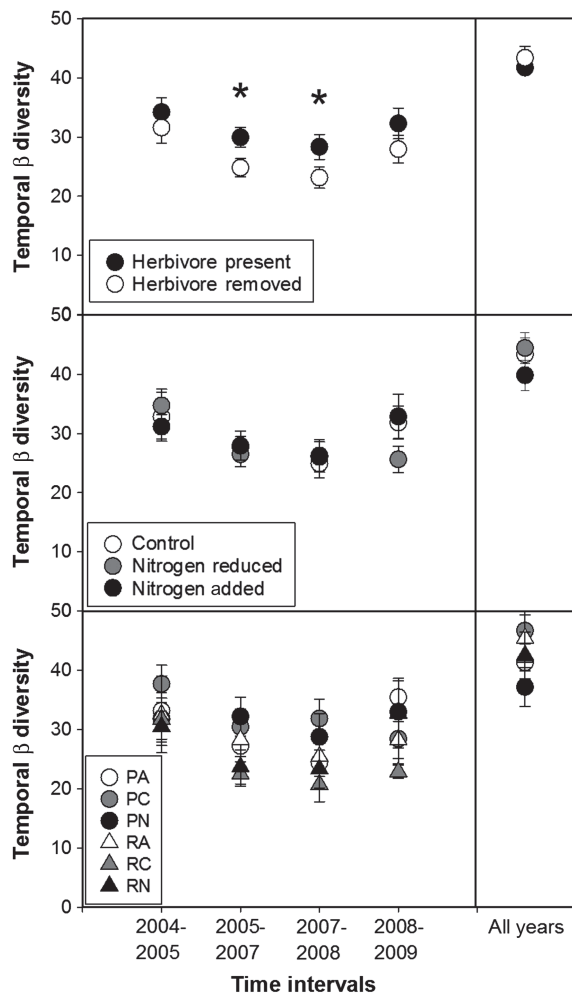


Figure 3. Herbivore reduction lowered temporal  $\beta$  diversity (e.g. species turn over) within old-field communities between short (2004–2005, 2005–2007, 2007–2008, 2008–2009) and longer (2004–2009) time intervals. In other words, plant communities experiencing less insect herbivory change less overtime than plant communities experiencing insect herbivory. Values are mean ( $\pm$  SE)  $\beta$  diversity across herbivore (Herbivore present = insect herbivore present, Herbivore removed = insect herbivore reduced); nitrogen (control, nitrogen addition, nitrogen reduction); nitrogen  $\times$  herbivores (PA = herbivores present, control; PC = herbivores present, N-reduction; PN = herbivores present, N-added; RA = herbivores reduced, control; RC = herbivores reduced, N-reduction; RN = herbivores reduced, N-addition). Asterisks denote mean differences between treatments using a post hoc t-test.

in N addition and control plots remained unchanged. This is surprising given findings from other studies that show N fertilization enhances ANPP, especially in grasslands which are often N-limited (Suding et al. 2005, Gruner et al 2008). One mechanism by which N-fertilization can increase ANPP is that a single or a few N-limited plant species show strong responses to increased N availability and over-yield in biomass production, leading to a significant increase in community-level ANPP (Tilman et al. 1997). Similarly, a reduction in N would be expected to reduce production by the same species, lowering overall community-level ANPP. The lack of ANPP response to N-addition in our

study suggests an overall lack of N limitation in this old-field plant community (Blue et al. 2011, Wright et al. 2014).

N-addition lowered both Shannon's evenness and  $\alpha$  diversity relative to control plots, potentially as a result of increasing the biomass of competitively superior, highly productive species or functional groups as shown in recent studies (Eskelinen et al. 2012, Dickson and Gross 2013). Yet, the lack of an ANPP response to N-addition likely resulted from concurrent increases in over productive species counteracted by decline in other species (e.g. generating a neutral effect). On the other hand, when N became limiting (e.g. N reduction plots), ANPP declined as a function of simultaneous reduction in biomass production of both dominant and subdominant species. Surprisingly, N-reduction and N-addition had similarly negative effects on  $\alpha$  diversity, contrary to another grassland study that found that only N-reduction promoted  $\alpha$  diversity (Baer et al. 2004). Because ANPP in N-reduction plots was significantly lower than in control and N-addition plots, it is unlikely that particular highly productive species increased in dominance and lowered Shannon's evenness and diversity. Thus, the decrease in Shannon's evenness and diversity in N-reduction plots was likely the result of increase in dominance of particular competitively subordinate species and a concurrent decline in dominance of a competitively dominant species. These changes counteract each other, generating no differences in ANPP.

Unlike the rapid but transient influence of N-availability on  $\alpha$  diversity and ANPP, the effects on spatial  $\beta$  diversity were marginal and emerged later only in the experiment with N manipulation promoting compositional dissimilarity. Bottom-up factors, both N-addition and N-reduction, promoted  $\beta$  diversity by lowering compositional similarity among high and low productivity relative to control plots. After six years of N manipulation, N reduction promoted the dominance of the native herbaceous N-fixing species *Desmodium nudiflorum* and non-native woody N-fixing species *Lespedeza cuneata* while the addition of N promoted the dominance of native woody *Rubus argutus* and herbaceous *Verbesina virginica* C<sub>3</sub> forbs, both of which are non-N-fixing species. These changes in plant dominance contributed to greater spatial  $\beta$  diversity among N addition and N reduction treatments over time. Similar to our findings, Chalcraft et al. (2008) documented that bottom-up effects on  $\beta$  diversity can be independent of effects on  $\alpha$  diversity.

### Herbivore effects on plant ANPP, $\alpha$ and spatial $\beta$ diversity

Top-down effects of herbivory on ANPP were variable. In fact, a recent meta-analysis by Coupe and Cahill (2003) documented that, on average, insect herbivores reduce NPP by 13% in temperate herbaceous plant communities. But there was considerable variation among studies – some showed that ANPP increased, or did not change (Carson and Root 2000, Coupe and Cahill 2003). In our study, the removal of insect herbivores increased ANPP, but this effect was fleeting and occurred only midway through the experiment. Chronic effects of herbivores (including insect herbivores) on ANPP in terrestrial herbaceous systems, like old fields in our study, appear to be variable and largely inconsequential, when compared to the acute effects of

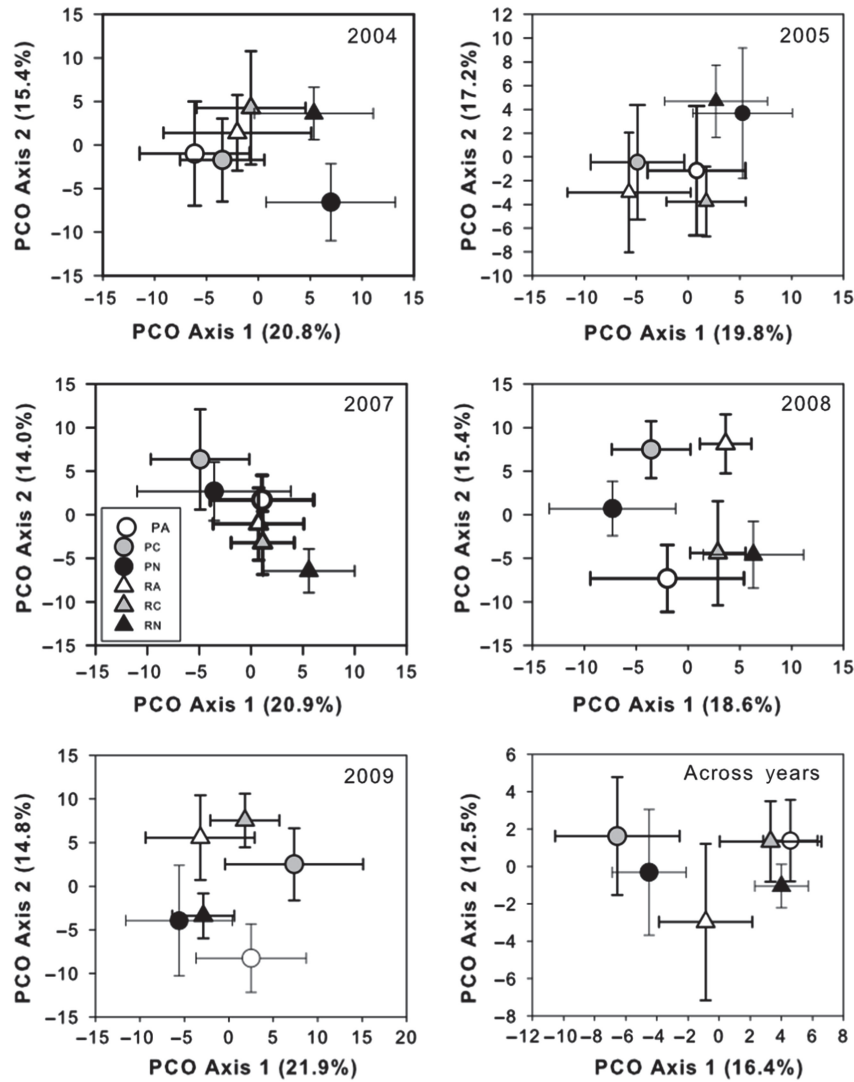


Figure 4. Top-down factors, bottom-up factors and time interact to influence spatial  $\beta$  diversity. Mean values ( $\pm$  SE) for principal coordinate ordinate axis one for all six-treatment combinations (PA = herbivores present, control; PC = herbivores present, N-reduction; PN = herbivores present, N-addition; RA = herbivores reduced, control; RC = herbivores reduced, N-reduction; RN = herbivores reduced, N-addition) overtime (2004, 2005, 2007, 2008, 2009) and across years (time not a factor, only addressing the overall effects of top-down versus bottom-up effects).

Table 4. Results from a repeated measures analysis of variance (RMANOVA) testing for the effects of year, bottom-up  $\times$  year, top-down  $\times$  year on ANPP,  $\alpha$  diversity, Shannon's evenness and diversity.

Response	Source	Wilks' $\lambda$	Wilks' $\lambda$ DF	p-value
ANPP	Year	0.11	4,27	<b>&lt;0.01</b>
	Top-down $\times$ Year	0.77	4,27	0.13
	Bottom-up $\times$ Year	0.75	8,54	0.39
$\alpha$ diversity	Year	0.53	4,27	<b>&lt;0.01</b>
	Top-down $\times$ Year	0.86	4,27	0.36
	Bottom-up $\times$ Year	0.77	8,54	0.5
Shannon's evenness	Year	0.44	4,27	<b>&lt;0.01</b>
	Top-down $\times$ Year	0.73	4,27	0.06
	Bottom-up $\times$ Year	0.47	8,54	<b>&lt;0.01</b>
Shannon's diversity	Year	0.44	4,27	<b>&lt;0.01</b>
	Top-down $\times$ Year	0.73	4,27	0.05
	Bottom-up $\times$ Year	0.58	8,54	<b>0.03</b>

herbivory in forested ecosystems (Gruner et al. 2008). Thus, the adaptation mechanisms likely encountered in grasslands target chronic, rather than episodic, herbivory. Additionally, it could be that plants are relatively tolerant of or resistant to the effects of insect herbivory and may also be capable of compensatory responses (Shurin et al. 2002, Hall et al. 2007).

In contrast to their weak effects on ANPP, herbivores consistently influenced Shannon's evenness and community composition. The reduction of herbivores promoted Shannon's evenness (i.e. lowered plant dominance) throughout our study, leading to an overall increase in diversity. This increase in  $\alpha$  diversity initially (2005) resulted from the reduction of selective consumption of subdominant species that had lower biomass in the presence of herbivores. In contrast, biomass of dominant species remained unchanged. Dominant species may defend themselves chemically and mechanically against generalist herbivores, tolerate herbivory,



or compensate by growing more plant tissue (Olf and Ritchie 1998), which may explain why dominant species biomass remained unchanged. However, by the end of our experiment (2009), changes in diversity were not a function of reduction of herbivore selective suppression of the sub-dominant species, which by 2009 had higher biomass in the presence of herbivores. Herbivory can lower diversity under low nutrient conditions (e.g. N-reduction plots) and enhance  $\alpha$  diversity under high nutrient conditions and when ANPP is high (e.g. N-addition and control plots) (Holt et al. 1994, Harpole and Tilman 2007, Hillebrand et al. 2007, Borer et al. 2014). Similar to our findings, Hillebrand and colleagues documented that herbivore reduction promoted plant diversity, but unlike our findings, herbivore reduction promoted plant  $\alpha$  diversity only in less productive environments where ANPP was low. We did not find herbivore reduction effects to be contingent on nutrient status and ANPP in an old-field community. In our study, ANPP was relatively resistant to herbivory while plant diversity was not. However, differences in the direction of species-specific responses to herbivores counteracted each other not altering ANPP. Likewise, Souza et al. (2011) also found that subordinate plant species compensatory responses to dominant species removal to alter diversity while leaving ANPP unaffected.

The effects of herbivory on community composition became apparent after four years of our experiment contrasting to the impacts of soil nutrient that took place relatively rapidly to affect productivity. For example, the reduction of herbivores promoted the dominance of a suite of N-fixing species (e.g. *Desmodium nudiflorum*, *Lespedeza cuneata*) at the expense of herbaceous forb species (e.g. *Aster pilosum*, *Verbesina occidentalis*) ultimately leading to differences in community composition. Given that legumes have higher foliage quality (e.g. nitrogen-rich) than herbaceous forbs, they may experience greater rates of herbivory and decline more in the presence of herbivores (Knops et al. 2000, Peltzer et al. 2009), unless they allocate additional N towards defense (Thamer et al. 2011, Ballhorn et al. 2013). Surprisingly, when soil N availability was manipulated (increased or decreased), the reduction of insect herbivores did not influence plant community composition. Instead, it was only in the absence of soil N manipulations that insect herbivore reduction altered plant community composition. The reduction of herbivores in control plots (no soil N manipulation), promoted the dominance of N-fixing species (e.g. *Desmodium nudiflorum*) at the demise of forb species (e.g. *Solidago altissima*), ultimately leading to increases in spatial  $\beta$  diversity.

## Conclusions

Though the question of which factors are important for regulating plant ANPP and measures of diversity is in itself important, as we gather evidence of singular controls, the next sensible step may be to address what factors mediate the relative strengths of resource limitation (bottom-up) and herbivory (top-down). In our study system, we found that the relative contributions of top-down and bottom-up controls of plant ANPP, plant diversity and composition can differ significantly. Taken together, the influence of top-down versus bottom-up effects on plant  $\alpha$  and spatial-temporal  $\beta$  diversity and ANPP changed through time, with bottom-up

effects influencing ANPP quickly and consistently while and top-down effects lagging to influence composition via shifts in plant dominance rather than identity. Explaining why the suite of factors that influence plant communities varies from year to year, even in the same system, will require new, and longer-term approaches potentially across sites with varying N limitation that may show different responses.

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Supplementary material (available online as Appendix oik-02579 at <[www.oikosjournal.org/appendix/oik-02579](http://www.oikosjournal.org/appendix/oik-02579)>). Appendix 1–3.