Changes in plant community composition, not diversity, during a decade of nitrogen and phosphorus additions drive above-ground productivity in a tallgrass prairie

Meghan L. Avolio[1]*, Sally E. Koerner[2], Kimberly J. La Pierre[3], Kevin R. Wilcox[2], Gail W. T. Wilson[4], Melinda D. Smith[2] and Scott L. Collins[5]

[1]*Department of Biology, University of Utah, Salt Lake City, UT 84112, USA;* [2]*Department of Biology and Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80523, USA;* [3]*Department of Integrative Biology, University of California, Berkeley, CA 94704, USA;* [4]*Department of Natural Resource Ecology & Management, Oklahoma State University, Stillwater, OK 74078, USA;* and [5]*Department of Biology MSC03-2020, University of New Mexico, Albuquerque, NM 87131, USA*

Summary

1. Nutrient additions typically increase terrestrial ecosystem productivity, reduce plant diversity and alter plant community composition; however, the effects of P additions and interactions between N and P are understudied.

2. We added both N (10 g m^{-2}) and three levels of P (2.5, 5 and 10 g m^{-2}) to a native, ungrazed tallgrass prairie burned biennially in northeastern Kansas, USA, to determine the independent and interactive effects of N and P on plant community composition and above-ground net primary productivity (ANPP).

3. After a decade of nutrient additions, we found few effects of P alone on plant community composition, N alone had stronger effects, and N and P additions combined resulted in much larger effects than either alone. The changes in the plant community were driven by decreased abundance of C_4 grasses, perhaps in response to altered interactions with mycorrhizal fungi, concurrent with increased abundance of non-N-fixing perennial and annual forbs. Surprisingly, this large shift in plant community composition had little effect on plant community richness, evenness and diversity.

4. The shift in plant composition with N and P combined had large but variable effects on ANPP over time. Initially, N and N and P combined increased above-ground productivity of C_4 grasses, but after 4 years, productivity returned to ambient levels as grasses declined in abundance and the community shifted to dominance by non-N-fixing and annual forbs. Once these forbs increased in abundance and became dominant, ANPP was more variable, with pulses in forb production only in years when the site was burned.

5. *Synthesis.* We found that a decade of N and P additions interacted to drive changes in plant community composition, which had large effects on ecosystem productivity but minimal effects on plant community diversity. The large shift in species composition increased variability in ANPP over time as a consequence of the effects of burning. Thus, increased inputs of N and P to terrestrial ecosystems have the potential to alter stability of ecosystem function over time, particularly within the context of natural disturbance regimes.

Key-words: above-ground net primary productivity, dominance, functional traits, grasslands, nutrients, plant community composition, plant diversity, species richness

Introduction

Above-ground net primary productivity (ANPP) of terrestrial ecosystems is limited by both nitrogen (N) and phosphorus (P), and when these nutrients are added to an ecosystem,

*Correspondence author. E-mail: meghan.avolio@utah.edu

ANPP generally increases (Elser *et al.* 2007; Vitousek *et al.* 2010). In contrast, studies have shown that N additions (Stevens *et al.* 2004; Cleland & Harpole 2010), P additions (Kirkham, Mountford & Wilkins 1996) and combined N and P additions (Willems, Peet & Bik 1993; Hejcman, Klaudisova & Schellberg 2007) often decrease plant community richness and diversity. A primary mechanism for the decreased plant diversity is enhanced above-ground competition for light as a result of increased ANPP (Suding *et al.* 2005; Hautier, Niklaus & Hector 2009; Borer *et al.* 2014). While ANPP responses to nutrients have been shown to affect measures of plant diversity, few studies have examined how nutrient-driven changes in plant community composition over time may affect ANPP.

The hierarchical response framework (HRF; Smith, Knapp & Collins 2009) predicts that chronic resource alterations will have different effects on ecosystem functioning over time depending on whether and how the plant community composition changes with increased resource availability. Initially, individual-level responses of extant species in the community are expected to drive changes in ecosystem productivity with nutrient additions. For example, nutrient additions may stimulate growth of all or a subset of species within a community leading to greater overall ANPP. The HRF predicts, however, that with chronic resource additions, eventually some species will be favoured at the expense of others or that novel species will immigrate into the community, resulting in a shift in community composition and a reordering of dominant species. Indeed, many studies have observed a change in the composition of the plant community in response to N additions (Bai, Wu & Cabido 2010; Cleland & Harpole 2010), especially long-term additions (Hejcman, Klaudisova & Schellberg 2007; Isbell *et al.* 2013), because species are differentially affected by increased nitrogen availability (Isbell *et al.* 2013; Payne *et al.* 2013). Following species reordering or species immigration, the HRF predicts large effects – either negative or positive – on ecosystem functioning depending on how community composition is impacted (Smith, Knapp & Collins 2009). Thus, short-term responses to nutrient additions might not reflect or be predictive of long-term effects, because nutrient additions may alter community composition over time.

Observed changes in the plant community in response to nutrient additions have been attributed to functional traits of individual species, where different functional classes increase or decrease in abundance in response to nutrient additions (Pennings *et al.* 2005; Suding *et al.* 2005). Plant functional trait categories, such as C_3 vs. C_4 graminoids, can clarify patterns of plant community responses to nutrient additions (Diaz & Cabido 1997; Lavorel & Garnier 2002; Suding & Goldstein 2008). For example, N-fixing forbs have a greater probability of being lost from a community with N additions than species that do not fix nitrogen (Suding *et al.* 2005). In contrast, P additions may favour N-fixing forbs because they will not be N or P limited, as has been found for N-fixing lichens (Benner & Vitousek 2007). Also, annuals have been found to increase in abundance in response to N additions (Bai, Wu &

Cabido 2010) and are less likely to be lost from the community compared with perennials (Suding *et al.* 2005). An additional key trait is the dependence of some plants, particularly C_4 grasses, on mycorrhizal fungi (Wilson & Hartnett 1997), which aid in N and especially P acquisition (Smith & Read 1997). However, mycorrhizal dependence has been understudied in explaining plant responses to nutrient additions. Placing individual species responses within a functional group response framework could substantially improve our ability to predict how plant communities will change under chronic resource additions.

To date, most grassland studies have manipulated N alone or N with P, while the effects of P alone have been understudied, in part due to the assumption that many grasslands are limited more by N than P. Here, we investigated the effects of 10 years of N and P additions on intact tallgrass vegetation at Konza Prairie in northeastern Kansas, USA. Previous studies in tallgrass prairie have found above-ground productivity responses to 10 g m^{-2} of N (Gibson, Seastedt & Briggs 1993; Tilman 1993), but a lack of response to 1 g m^{-2} of P (Gibson, Seastedt & Briggs 1993). Thus, we added N and P at levels presumed to be sufficient to overcome plant nutrient limitations: two levels of N (0 or 10 g m^{-2}) and four levels of P (0, 2.5, 5 and 10 g m^{-2}) in a fully factorial design. Since past P additions at lower levels (1 g m^{-2}) had no significant effects (Gibson, Seastedt & Briggs 1993) on community structure and ecosystem function, we included a range of P levels to better assess the interactive effects of N and P availability.

The objective of our study was to examine the independent and interactive effects of N and P additions on plant community composition and structure and ANPP, as an integrative measure of ecosystem function. We hypothesized that (i) N addition but not P addition would have large effects on ANPP and cause a reduction in plant diversity, (ii) nutrient additions would change the abundance of different functional groups, and (iii) as predicted by the HRF, these shifts in plant community composition would drive large changes in aboveground productivity and that these effects would be greatest with both N and P additions.

Materials and methods

STUDY SITE

This study was conducted at Konza Prairie Biological Station (KPBS) located in northeastern KS (39 05′N, 96 35′W), USA. KPBS is an intact, native tallgrass prairie dedicated to long-term ecological research. At KPBS, C_4 perennial grasses are dominant and drive above- and below-ground productivity (Knapp *et al.* 1998). The C_4 grasses are obligately mycorrhizal (Wilson & Hartnett 1998) potentially favouring their persistence under low N conditions (Hartnett & Wilson 1999). Plant community diversity is high and primarily a function of a diverse array of forb species (Collins & Glenn 1991). This study took place in an upland site with shallow soils (i.e., lower productivity), located within watershed 2C that has been burned in the spring biennially since 1973. The location of our experiment differs from many previous studies at Konza that were conducted in

highly productive annually burned areas on relatively deep soils (Collins *et al.* 1998). Fire removes light limitations in burn years, which can allow for increased growth of small forbs (Collins & Steinauer 1998); however, long-term annual burning favours grass dominance (Knapp *et al.* 1998; Collins & Calabrese 2012). Frequently burned sites are also more N-depleted and respond to N additions (Seastedt, Briggs & Gibson 1991; Knapp *et al.* 1998). KPBS has a weather station that records daily rainfall, which we used to calculate growing season precipitation (April–October).

EXPERIMENTAL DESIGN

In 2002, a 30×40 m area was divided into 5×5 m plots arrayed in a contiguous 6×8 plot grid. Starting in 2003, two nitrogen (0 and 10 g m^{-2}) and four phosphorus (0, 2.5, 5, and 10 g m^{-2}) treatments were applied to the plots in a fully factorial design (eight treatment combinations; hereafter referred to as N0-P0, N0-P2.5, N0-P5, N0-P10, N10-P0, N10-P2.5, N10-P5, N10-P10). There were six replicates of each treatment combination resulting a total of 48 plots. Nutrients were added by hand in an even distribution in early June. Nitrogen was added as ammonium nitrate and phosphorus as superphosphate.

SOIL NUTRIENT AVAILABILITY

We measured soil nutrient concentrations twice. In 2009, availability of soil ammonium, nitrate and phosphorus was measured during the growing season. For each plot, two resin bags were installed at a depth of 10 cm adjacent to the species composition plots in mid-June and removed in late August. All resin bags were constructed using 4.0 g of Dowex Marathon MR-3 Mixed Bed (cation and anion) ion-exchange resin in a nylon stocking. Resins were stored at 4 °C until nutrients were extracted. For each resin bag, nitrogen and phosphorus were extracted in 100 mL of 2 M NaCl in 0.1 M HCl. All extracts were analysed colorimetrically on an Astoria®2 flow analyzer (Astoria-Pacific, Clackamas, OR, USA). Values from each resin bag were averaged per plot. In June 2010, before nutrients were added, plant-available N and P were determined from three soil cores collected from each plot spaced at least 1 m apart. The soil cores were aggregated by plot and sieved through a 2-mm sieve to remove roots and rocks. KCl extractions were performed on wet-sieved soil to obtain available nitrate and ammonium). Melich III extractions were performed on dried sieved soil to obtain available P.

PLANT COMMUNITY DIVERSITY, COMPOSITION AND SPECIES TURNOVER

Within each plot, permanent species composition plots were designated. Species composition plots were 0.5×2 m long and were divided into 4, 0.5×0.5 m subplots. In each subplot, percentage aerial cover was estimated to the nearest 1% for each species that was rooted in the plot in early June and late August. Maximum cover estimates for each species were averaged across the four quadrats for each plot and used to calculate plant community richness (S), Shannon's diversity [H'; Magurran 2004) and evenness ($J = H'/\log(S)$].

To examine changes in functional groups over time, each species encountered was classified as a C_4 perennial grass, a C_3 perennial grass, an annual grass, a non-N-fixing perennial forb or shrub, an N-fixing perennial forb or shrub, or an annual forb. We then summed the total cover of each functional type in each replicate for each year of study.

We investigated species turnover by determining which species were lost from plots and which species immigrated into the plots. For species lost from a plot, we first assessed whether a species that was present in a given plot in 2002 was present in that same plot in each subsequent year. To calculate the species loss for each plot, we subtracted the summed number of species lost from a plot in each year from the number of species found in 2002 and divided this by the number of species found in 2002, thus measuring the proportion each year of species remaining from the original species pool in 2002. We calculated immigration differently. First, we included all species that were present in all plots in 2002 as the original species pool. For each given plot, we calculated the proportion of all species sampled in each year that were not in the original pool and divided this by the number of species found that year, thus reflecting the proportion of species per plot that were new to the experiment each year.

MYCORRHIZAL FUNGI

Measures of root colonization by mycorrhizal fungi were assessed twice over the course of the experiment and extra-radical mycorrhizal hyphal biomass once. In 2003 and 2008, soil cores were taken near the base of three *Andropogon gerardii* individuals in each plot in August and thus contain mostly *A. gerardii* roots. The soil cores were pooled, roots were cleaned and stained with trypan blue (Koske & Gemma 1989), and the percentage mycorrhizal colonized roots were determined microscopically using the gridline intersect method (McGonigle *et al.* 1990). To measure the amount of extra-radical fungal hyphae in the soil, hypha was isolated from the soil and standing crop was quantified using mesh in-growth cores (Wallander *et al.* 2001; van Diepen *et al.* 2010). Hyphal in-growth bags were constructed from nylon mesh (50-μm mesh, $10 \times 5 \times 2$ cm; Sefar American Inc., Depew, NY, USA) that allows mycelia to grow into the bag but excludes roots. The bags were filled with 80 cm^3 of muffled furnaced (6 h at 500 °C) quartz sand (>300 μm) and sealed, creating a carbon-free matrix through which mycorrhizal fungi will grow but parasitic fungi will avoid. In 2009, three in-growth cores were placed in each plot at a depth of 0–10 cm. In-growth bags were installed in June in the proximity of *A. gerardii* individuals and removed in September. Once removed from the soil, cores were stored at −20 °C until fungal hyphae were isolated following the methods of van Diepen *et al.* (2010). After isolation, hyphae were freeze-dried for 3 days and then weighed.

ABOVE-GROUND PRODUCTIVITY

Each September, above-ground biomass was clipped to ground level within 2 20×50 cm quadrats randomly located in each plot and sorted into graminoids (grasses and sedges), non-graminoid forbs, woody plants and previous year's dead (in years when there was no burn; Buis *et al.* 2009). Care was taken to not resample areas that were previously clipped, and the permanent species composition plots were never clipped. After clipping, biomass was dried at 60 °C for ca. 48 h and then weighed. Previous year's dead biomass (unburned years) was not included in estimates of annual productivity.

STATISTICAL ANALYSES

All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA), and statistical significance was set at $\alpha = 0.05$. For the biomass, plant community and abundance of functional type data,

we ran three-way mixed-model repeated-measures analysis of variance using the MIXED procedure with year as a repeated effect, nitrogen and phosphorus treatments as the main effects. We tested different covariance structures and selected the best model using AIC. We did not include data from 2002 in these analyses because they were pre-treatment data, and in 2002, there were no community or productivity differences between any of the treatments (data not shown). For the mycorrhizal data, we performed separate analysis of variance tests for each year using the GLM procedure. H' was anti-log–transformed, and mycorrhizal fungal root colonization and soil hyphae data were log-transformed for normality. We used least-squared means for all post-hypothesis-testing comparisons. Regression was used to test the effect of time on loss and immigration of species from/to each treatment.

Results

The experiment spanned a range of growing season precipitation amounts over the 10-year study period (Fig. 1), with a high of 875 mm in 2008 and a low of 412 mm in 2012. In the control plots, ANPP was correlated with annual precipitation ($r = 0.793$; $P = 0.004$) and species richness was generally higher in years that burning did not occur except in 2012 when precipitation was well below average (Fig. 1).

Based on both integrative measures of soil nutrients via resin bags (2009) and measures of extractable soil nutrient availability (2010), we found that adding N and P increased the amounts of N and P in the soil during the growing season and that there was a carry-over of P over time, where higher levels of P were found in the P addition plots before P was added for the 2010 growing season (See Appendix S1 in Supporting Information). N did not carry over across years.

PLANT COMMUNITY DIVERSITY

Overall, we saw small effects of nutrient additions on measures of plant community diversity (S, J and H'). The effects of N addition varied significantly over time for all measures of community diversity, whereas the effects of P varied significantly over time for only J (Table 1). Over time, N alone reduced S and increased J from 2006 to 2009 and as a consequence H' increased from 2006 to 2008 (Appendix S2). The effects of P on J over time depended on the amount of P added, where in 2007 and 2008, there was higher J in plots that received 5 g m^{-2} of P (Appendix S2). The N and P additions had main effects as well, with 10 g m^{-2} of N or P alone reducing richness by only ~8% (Fig. 2). Lastly, there were significant N × P interactions for H' only, where the greatest reduction in H' was observed in the N10-P10 treatment, but only ~10% lower than the control (Fig. 2).

PLANT FUNCTIONAL GROUPS

We grouped all plant species into six functional groups and assessed how these functional groups responded to the nutrient addition treatments over time (Table 2; Fig. 3). The addition of N and the combination of N and P changed the relative abundance of the functional groups starting in 2006; C$_4$ grasses declined substantially from ~80% to ~40% cover, whereas non-N-fixing forbs increased from ~10% to ~40% cover (Fig. 3a). Also, abundance of annual forbs increased in 2008 and was maintained thereafter, and annual grasses started to consistently appear in the treatment plots where N and P were added together after 2007 (Fig. 3a). These changes in abundance resulted in reordering of functional groups over time. After 10 years, the shifts in abundance resulted in non-N-fixing forbs replacing C$_4$ grasses as the dominant functional group in the N10-P10 treatment (Fig. 3b). When averaged across years, we found significant effects of N on all functional groups and of P and an N × P interaction on C$_4$ grasses, perennial
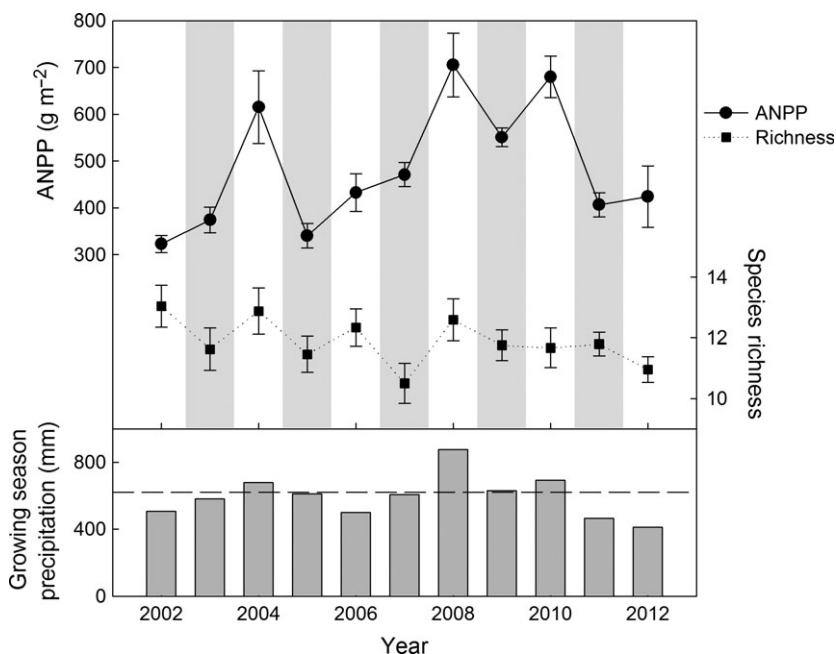

Fig. 1. Average above-ground net primary productivity (ANPP) and plant species richness for the control plots from 2002 to 2012 (top panel). Burn years are shaded in grey. Growing season precipitation (April 1–October 31) amounts from 2002 to 2012 (bottom panel). Long-term average (~620 mm over a 20-year period) is shown as a dashed line.

Table 1. Effects of nitrogen (0 and 10 g m^{-2}) and phosphorus (0, 2.5, 5 and 10 g m^{-2}) additions over time (year) on plant community diversity [richness (S), evenness (J) and Shannon's diversity (H′)], and ecosystem productivity based on mixed-model repeated-measures anova. Significant differences ($P \le 0.05$) are bolded. Shown are the *F*-values and with *P*-values in parentheses

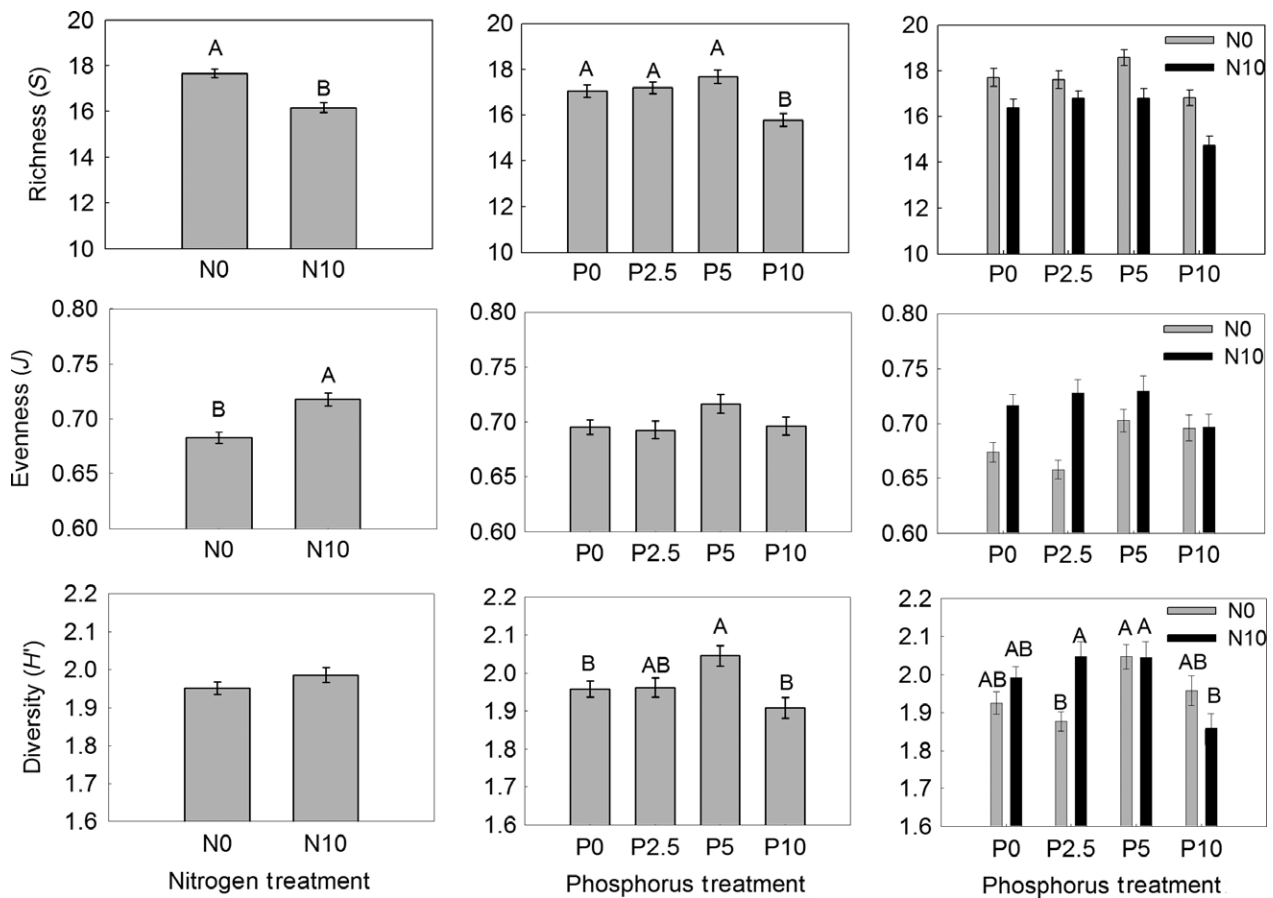| Effect	Plant community diversity				Ecosystem productivity measures			
	d.f.	S	J	H′	d.f.	ANPP	Grass	Forb
Year	9, 360	**39.89 (< 0.001)**	**12.29 (< 0.001)**	**23.32 (< 0.001)**	9, 358	**41.75 (< 0.001)**	**31.91 (< 0.001)**	**9.65 (< 0.001)**
Nitrogen	1, 40	**14.94 (< 0.001)**	**12.10 (< 0.001)**	1.79 (0.188)	1, 40	**60.92 (< 0.001)**	**13.85 (0.001)**	**41.19 (< 0.001)**
Phosphorus	3, 40	**4.30 (0.010)**	1.18 (0.331)	**2.91 (0.046)**	3, 40	1.07 (0.373)	0.70 (0.555)	**3.20 (0.034)**
Yr × N	9, 360	**2.52 (0.008)**	**3.38 (0.001)**	**2.76 (0.004)**	9, 358	**6.26 (< 0.001)**	**5.47 (< 0.001)**	**3.59 (< 0.001)**
Yr × P	27, 360	0.96 (0.815)	**1.24 (0.196)**	1.18 (0.248)	27, 358	1.32 (0.138)	0.55 (0.969)	**2.35 (0.001)**
N × P	3, 40	0.48 (0.695)	2.06 (0.120)	**3.00 (0.042)**	3, 40	0.28 (0.836)	0.65 (0.588)	**3.71 (0.019)**
Yr × N × P	27, 360	0.71 (0.857)	0.91 (0.596)	0.81 (0.737)	27, 358	**1.55 (0.042)**	0.85 (0.680)	**2.03 (0.002)**

Fig. 2. Effects of nitrogen (left) and phosphorus (middle) additions and their interactions (right) on measures of plant community diversity [richness (S), evenness (J) and Shannon's diversity (H′)] across all years. Shown are mean (±SE). Letters denote significant differences at $P \le 0.05$.

non-N-fixing forbs and shrubs, and annual forbs (Table 2; Appendix S3).

SPECIES TURNOVER

For each plot, we investigated the number of species lost over time. We did not find a significant trend of species loss over time in plots that received only P, but when N was added, species were lost over time regardless whether P was added or not (Fig. 4a). We also investigated the proportion of new species that appeared in each plot after 2002. The greatest accumulation of new species over time was in plots with both N and P additions, with the steepest slope in the N10-P10 treatment (Fig. 4b).

MYCORRHIZAL FUNGI

In 2003, N additions resulted in significantly greater end-of-season mycorrhizal root colonization in *A. gerardii* ($F_{1,40} = 53.25$; $P < 0.001$), 5 g m^{-2} of P additions increased

Table 2. Effects of nitrogen (0 and 10 g m^{-2}) and phosphorus (0, 2.5, 5 and 10 g m^{-2}) additions over time (year) on abundance of plant functional groups-based mixed-model repeated-measures anova. G1; perennial C$_4$ grasses; G2; perennial C$_3$ grasses; G3; annual grasses; F1; non-N-fixing perennial forbs; F2; N-fixing perennial forbs; F3; annual forbs. Significant differences ($P \leq 0.05$) are bolded. Shown are the *F*-values and with *P*-values in parentheses

| Effect	d.f.	G1	G2	G3	F1	F2	F3
Year	9, 360	**63.41 (< 0.001)**	**45.85 (< 0.001)**	**3.62 (< 0.001)**	**14.35 (< 0.001)**	**9.98 (< 0.001)**	**10.68 (< 0.001)**
Nitrogen	1, 40	**107.3 (< 0.001)**	**6.29 (0.016)**	**5.46 (< 0.001)**	**70.58 (< 0.001)**	**5.78 (0.021)**	**27.87 (< 0.001)**
Phosphorus	3, 40	**6.59 (0.001)**	1.55 (0.216)	1.21 (0.320)	**10.56 (< 0.001)**	0.31 (0.820)	**3.27 (0.031)**
Yr × N	9, 360	**16.57 (< 0.001)**	**7.88 (< 0.001)**	**3.62 (< 0.001)**	**8.90 (< 0.001)**	**7.18 (< 0.001)**	**7.99 (< 0.001)**
Yr × P	27, 360	**1.60 (0.031)**	1.21 (0.216)	**1.68 (0.019)**	0.57 (0.959)	1.01 (0.450)	1.41 (0.087)
N × P	3, 40	2.27 (0.096)	1.23 (0.312)	1.21 (0.320)	**3.25 (0.032)**	0.11 (0.954)	**3.94 (0.015)**
Yr × N × P	27, 360	1.21 (0.223)	**2.46 (< 0.001)**	**1.68 (0.019)**	0.78 (0.781)	0.73 (0.838)	1.21 (0.221)
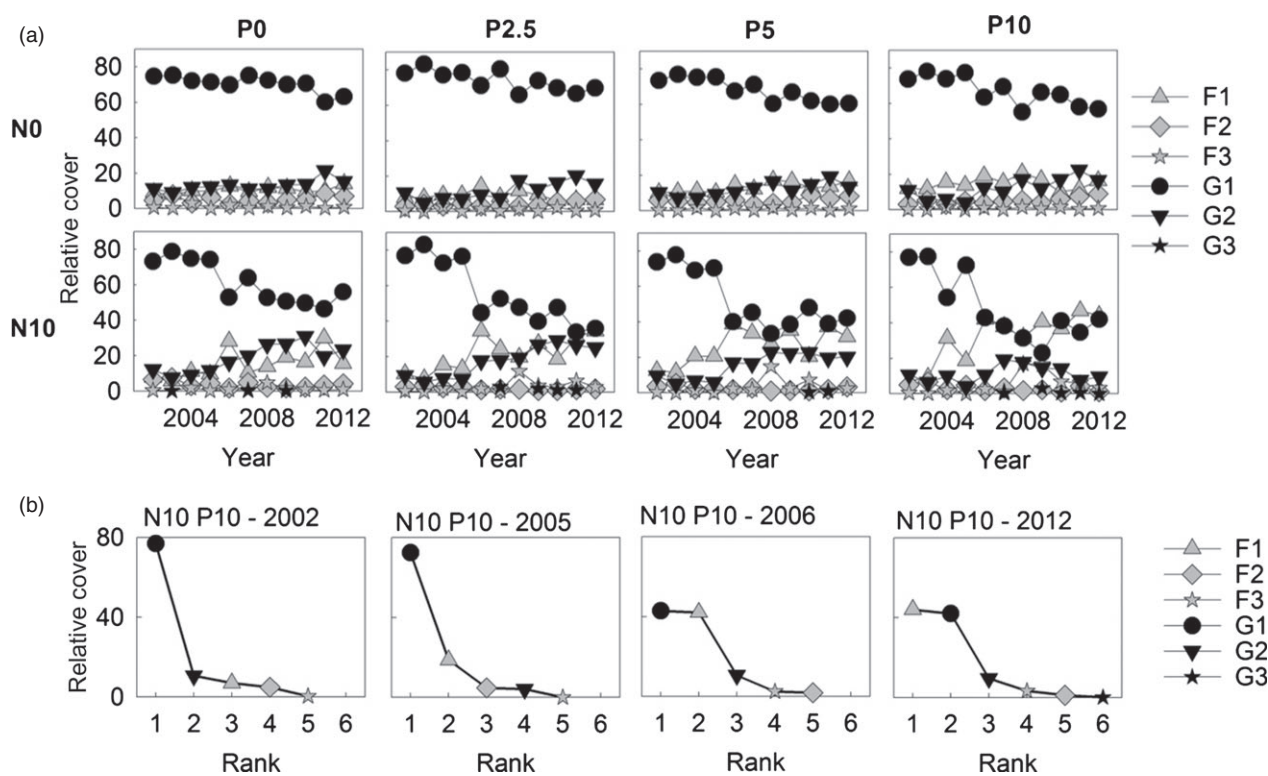
Fig. 3. Relative cover of six plant functional groups to nitrogen and phosphorus additions over time. G1: perennial C$_4$ grasses; G2: perennial C$_3$ grasses; G3: annual grasses; F1: non-N-fixing forb or shrubs; F2: N-fixing forbs or shrubs; F3: annual forbs. (a) Relative abundance of each plant functional group through time for each of the eight treatment combinations. The top left panel (N0-P0) is the control. (b) Rank-abundance curve of plant species for the high N and high P treatment in 2002 (pre-treatment), 2005 (before the community reordering), 2006 (after the community reordering) and 2012.

A. gerardii root colonization while 10 g m^{-2} of P additions reduced root colonization ($F_{3,40} = 5.31$, $P = 0.004$), and there was no interaction between N and P additions ($F_{3,40} = 0.89$, $P = 0.456$; data not shown). In 2008, there was a different effect of N and P additions ($F_{1,40} = 334.14$, $P < 0.001$; $F_{3,40} = 289.56$, $P < 0.001$, respectively) and there was an N × P interaction ($F_{3,40} = 39.10$, $P < 0.001$; Fig. 5a) on mycorrhizal root colonization of *A. gerardii*. In 2008, P additions alone reduced root colonization, and N10-P0, N10-P2.5 and N10-P5 treatments had increased root colonization, while

the N10-P10 treatments reduced mycorrhizal root colonization (Fig. 5a). In 2009, soil hyphal biomass was increased by N ($F_{1,39} = 20.42$; $P < 0.001$) and, depending on amount added, reduced by P ($F_{3,39} = 3.37$; $P = 0.028$), but there were no interactive effects of N and P ($F_{3,39} = 1.27$; $P = 0.297$). Adding 2.5 or 10 g m^{-2} of P reduced fungal biomass, and 5 g m^{-2} of P had no effect. There was the same amount of mycorrhizal fungal biomass in all the plots, regardless of treatment (Fig. 5b), with the exception of the N10-P0 plots, which had greater fungal biomass.
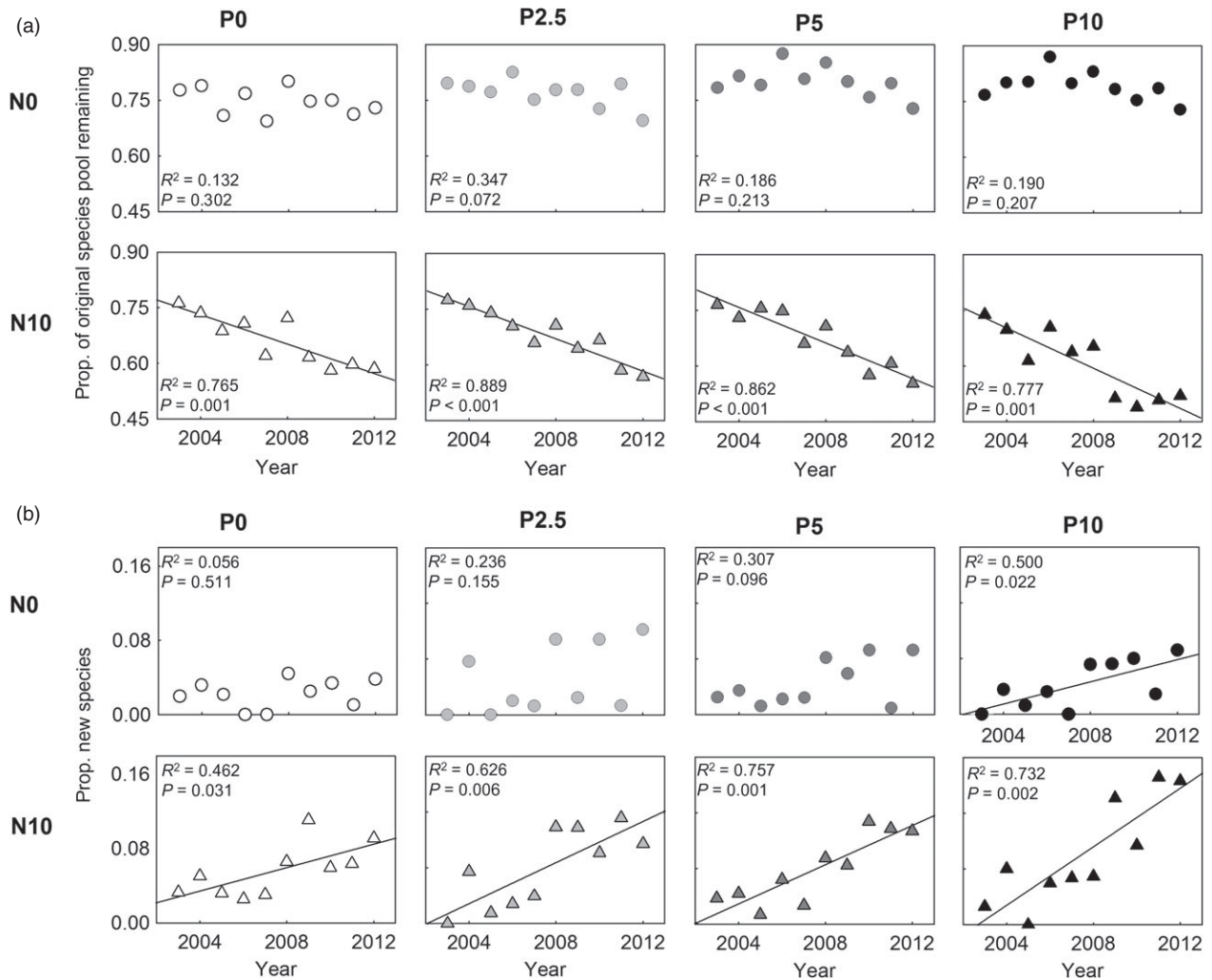
(a)

(b)

Fig. 4. Species loss (a) and immigration (b) of the entire plant community with a decade of nitrogen and phosphorus additions. In both graphs, the top left panel (N0-P0) is the control. Regression lines are only shown when there is a significant relationship. Species loss was calculated as the proportion of the original species pool of each plot in 2002 that remained each year. Species immigration was calculated as the proportion of species in each plot that were new to the experiment (not present in 2002). Equations of significant relationships can be found in Appendix S4.

ABOVE-GROUND NET PRIMARY PRODUCTIVITY

The N and P additions interacted over time to affect ANPP and forb biomass, whereas only N affected grass biomass significantly over time (Table 1). Overall, ANPP, grass biomass and forb biomass (Table 1; Fig. 6) were 20%, 18% and 169% greater, respectively, when N was added, whereas there was no effect of P alone on ANPP. In 2004, there was greater biomass in all N and P combined treatments, while in 2005, ANPP was greater only in the N10-P5 and N10-P10 treatments (Fig. 6). In 2009, there was greater biomass in the N10-P0, N10-P5 and N10-P10 treatments (Fig. 6). Grass biomass was greater with only N additions in 2003-2005; however, after 2005, the effect of N on grass biomass disappeared for all years but 2009. Lastly, N and P combined had pronounced effects on forb biomass (Fig. 6). Starting in 2007, forbs dominated the combined N and P addition treatment plots, and forb biomass was significantly higher in years when the site was burned relative to the controls (Fig. 6).

Discussion

After adding N and P to tallgrass prairie for a decade, we observed that combined N and P drastically changed plant community composition and ANPP. As hypothesized, P alone had few effects on measures of plant community diversity and no effect on ANPP, because tallgrass prairie plants are N but not P limited while N alone affected both community structure and ANPP. However, N addition in combination with even the lowest amount of P (2.5 g m^{-2}) resulted in greater alterations in community composition and ANPP than observed with N alone. After 7 years of combined N and P additions, we observed shifts in the dominant functional groups, whereby non-N-fixing forbs became most abundant and C$_4$ grasses were relegated to a subordinate position in the community. Further, we observed an increase in the abundance of annual species, especially in the combined high N and P treatment (N10-P10). The HRF proposes three key processes that underlie ecosystem responses to chronic resource
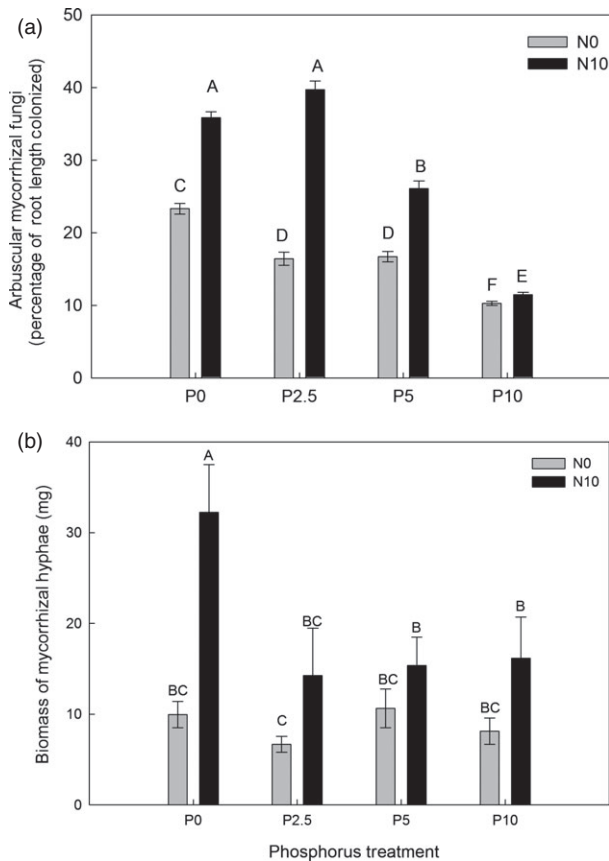
Fig. 5. Mycorrhizal fungal (a) percentage of root length colonized in 2008 and (b) biomass of hyphae in the soils in 2009. Shown are means ($\pm$SE), and letters denote significant differences at $P < 0.05$.

additions: (i) individual species responses, (ii) species reordering and (iii) species immigration (Smith, Knapp & Collins 2009). Consistent with the HRF, individual species responses, species reordering and immigration occurred in our experiment. Moreover, changes in productivity in plots that received N and P were also consistent with predictions from the HRF. Initially, ANPP increased with the N and combined N and P additions because of the individual response of grasses to N. However, after extensive shifts in species composition driven by both species reordering and gain of novel species, ANPP responses were larger and more variable due to increased abundance of forbs and their high responsiveness to biennial burning. Our results demonstrate that community responses to nutrient manipulation change over time. Moreover, these changes may not result in large shifts in plant diversity but can interact with natural disturbance regimes including burning to affect productivity.

Temperate grasslands have historically been considered to be N, but not P limited (Vitousek *et al.* 2010), including North American tallgrass prairie (Knapp *et al.* 1998), and thus the effects of P on productivity and plant community composition and structure are less often studied. In a meta-analysis, Elser *et al.* (2007) found N and P additions had similar effects on productivity in grasslands and interacted synergistically to have the greatest effects on

productivity. Here, we found no evidence of an effect of P alone on productivity. Instead, our results are similar to previous experiments in tallgrass prairie where ANPP responded to N but not P additions (Moser & Anderson 1964; Gibson, Seastedt & Briggs 1993). We did, however, find an effect of P alone on the plant community, where the highest P treatment (N0-P10) had lower plant species richness than controls and the N0-P5 treatment had increased diversity, which suggests that N and P availability may affect community diversity through different mechanisms. N additions result in increased above-ground biomass, and the subsequent light reduction changes the community through above-ground competition for light typically resulting in reduced species richness (Suding *et al.* 2005; Hautier, Niklaus & Hector 2009). We observed that P additions did not increase productivity and thus did alter above-ground competition. However, the P additions could have changed the nature of below-ground competition for nutrients (Johnson 2010), which may have caused our observed changes in plant diversity. Although Gibson, Seastedt & Briggs (1993) found that adding 1 g m^{-2} of P for 8 years had no effect on richness or diversity, our results demonstrate that higher levels of P addition over the long term can directly affect plant community structure in tallgrass prairie, and possibly grasslands in general.

N additions generally increase productivity over the short term (Gibson, Seastedt & Briggs 1993; Elser *et al.* 2007; Isbell *et al.* 2013). In our experiment, regardless of whether P was added or not, the initial productivity response to N was driven by an increase in biomass of the C$_4$ grasses (Fig. 6), which were the most abundant species (Fig. 3a). However, after 3 years, N and P additions began to have an interactive effect on plant community composition (Fig. 3a), resulting in no difference in ANPP between N and P addition plots and controls from 2006 to 2008 (Fig. 6) despite average and above-average growing season precipitation in 2007 and 2008, respectively. Over the course of the experiment, perennial C$_4$ grass abundance declined by 30% in the N10-P10 treatment relative to the controls and 13% in response to N alone. Thus, the initial increase in productivity disappeared after 3 years of combined N and P addition as abundance of the dominant C$_4$ grasses changed over time in response to chronic resource additions.

The mechanisms that caused the C$_4$ grasses to lose their dominance remain unclear. One possible mechanism is that the nutrient additions changed the interactions between the C$_4$ grasses and mycorrhizal fungi. Studies have shown that mycorrhizal mutualisms are essential to maintain dominance by C$_4$ grasses under ambient low-nutrient conditions (Hartnett & Wilson 1999; Smith, Hartnett & Wilson 1999), and it is theorized that mycorrhizal fungi help maintain dominance in general (Bever 1999; Umbanhowar & McCann 2005). Nutrient additions, however, change the nature of the symbiosis between plant hosts and mycorrhizal fungi, which has been attributed to changes in mycorrhizal community composition (Johnson 1993; Eom *et al.* 1999; Jumpponen *et al.* 2005), and when both N and P are abundant, it is predicted the symbiosis will switch from mutualism to parasitism (Johnson
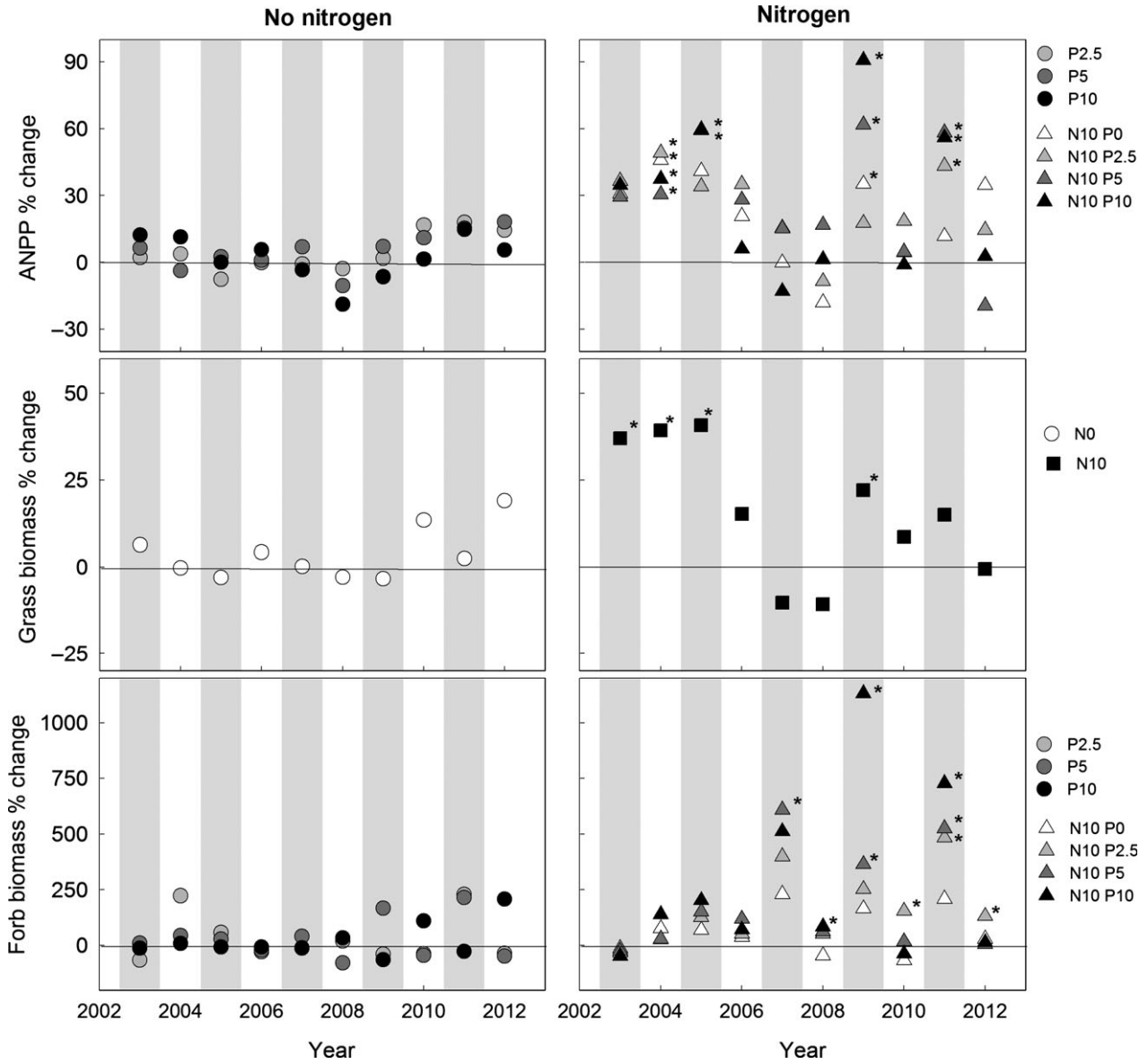
Fig. 6. Change in above-ground net primary production (ANPP) and grass and forb biomass. Since both a fire and precipitation signal were observed in the control treatment, productivity data for each treatment are presented relative to the control treatment. On the right are all treatments without N, and the left are the N and P combinations. An asterisk denotes a significant difference from the control treatment (see Table 1). Burn years are shown in grey. Note that for grass biomass, there was not a three-way interaction between N, P and year, only N and year (see Table 1). Productivity data not relative to the controls can be found in Appendix S5.

2010). We found that N additions increased mycorrhizal root colonization of *A. gerardii*, while N and P together reduced mycorrhizal root colonization. This fits the expectation of mycorrhizal responses to nutrient additions (Johnson *et al.* 2003), where plants increase allocation to mycorrhizal fungi when P is limiting (N addition plots) and reduce allocation to mycorrhizal fungi when P is no longer limiting (N and P addition plots). Although there was a reduction in root colonization with P alone and in the N10-P10 treatment, there was no change in fungal biomass relative to the controls in these treatments, suggesting that mycorrhizal fungi were still getting the same amount of C from their host plants even if they were no longer benefiting their host in the high-nutrient plots.

Our findings support the hypothesis that the benefits of mycorrhizal symbionts disappear under high soil nutrient conditions as they no longer give dominant species a competitive edge, but are still a C drain (Johnson, Grahan & Smith 1997; Johnson 2010). Regardless of what ultimately caused the C_4 grass decline, the vegetative structure of tallgrass prairie is determined by the competitive dominance of C_4 grasses (Collins & Steinauer 1998; Smith, Hartnett & Wilson 1999; Smith & Knapp 2003), and in the absence of their dominance, other functional groups were able to increase in abundance.

After individual responses to nutrient additions, the HRF predicts that species reordering should occur. In this experiment, C_4 grasses eventually declined in abundance under high

resource availability, and after 5 years of nutrient additions, non-N-fixing forbs became the dominant functional type in the N10-P10 plots. With just N additions alone, non-N-fixing forbs increased 44% in abundance relative to the controls, while we observed a 197% increase in abundance with N10-P10. Gough *et al.* (2012) hypothesized that tall clonal species that produce runners respond most strongly to N additions, which was also found by Dickson & Gross (2013). Of the non-N-fixing forbs in our study, the abundance of understorey forbs, such as *Oxalis stricta*, *Ambrosia psilostachya* and *Solidago missouriensis,* responded most strongly to the N10-P10 treatment, demonstrating that while clonal species can respond to increased resource availability, a species need not be tall and clonal to have a strong response (as shown here by *O. stricta*).

Nutrient additions that increase ANPP, besides increasing N and P availability, create other novel environmental conditions such as reduced light (Hautier, Niklaus & Hector 2009; Borer *et al.* 2014). The HRF predicts that new species will appear when these novel environmental conditions create invasion opportunities (Shea & Chesson 2002). As predicted, we found that nutrient additions led to an increase of immigration of new species into the community over time. In contrast, we only observed species loss in plots that received N. Furthermore, we found that N and P together (N10-P5 and N10-P10) resulted in the fastest rate of appearance of new species and we expect the plant communities in these high nutrient plots will continue to change over time. This does not appear to be a sampling effect given that species did not increase over time in the control plots. A large proportion of the new species in the high resource addition plots were annual grasses and forbs. Cover of annual forbs increased fivefold in the N10-P10 treatment but only 32% in the N10-P0 treatment, demonstrating that, like the perennial non-N-fixing forbs, annual forbs were also responding strongly to the two nutrients together. In our experiment, the most abundant annual forbs were *Ambrosia artemisifolia* and *Conyza canadensis* neither of which occurred in the experiment (2002 species pool) prior to the start of nutrient addition treatments. Many other studies have reported increases of annual species with N addition (i.e. Suding *et al.* 2005; Bai, Wu & Cabido 2010; Isbell *et al.* 2013). Annual species are less conservative with their resource-use compared with perennials (Suding *et al.* 2005) and would best take advantage of the pulses of resources occurring after burns in plots where N and P were added.

The change in community composition – that is the reduction of C_4 grasses, increase of non-N-fixing forbs and the appearance of annual forbs – resulted in ANPP shifting from being driven by increased growth of the C_4 grasses to being driven by forbs responding to fire in burn years. Although the cover of C_4 grasses was reduced, generally they were not lost from plots receiving N. In response to N additions (which alone drove the loss of species), of the three co-dominant C_4 grasses at this site, *A. gerardii* was never lost from a nutrient addition plot, *Sorghastrum nutans* was lost from 8% of the N addition plots, and *Schizachyrium scoparium* was lost from 20% of the N addition plots. Thus, the alterations in ANPP were not from species loss, as was observed by Isbell *et al.* (2013), but from a large reduction of the abundance of the dominant C_4 grasses and the increase in abundance and immigration of new forbs. We found after community reordering and species immigration occurred, forbs responded strongly to nutrient additions in burn years, and thus, ANPP began to have a strong fire signal starting in 2009, where the biomass in combined N and P addition plots was higher than the controls in burn years. It is not clear what drove the response of forbs to fire, but it may be due to increased light availability or alterations in soil microclimate and moisture (Knapp & Seastedt 1986). Isbell *et al.* (2013) also found that lagged community changes directly affected above-ground productivity responses, although they attributed the change in productivity to species loss, while we found alterations in community composition and species immigration were more important. Overall, the productivity responses observed in plots that received N and P are most likely driven by changes in plant community composition and not variations in interannual rainfall. Recent work across a precipitation gradient in the Great Plains has shown that productivity responses to nutrient additions were not affected by intra- or interannual variation in precipitation (La Pierre 2013).

We found that while the long-term addition of N or P alone can affect productivity and diversity, it was their interactive effects that drastically altered this grassland plant community. Although we observed large changes in plant community composition, measures of plant community diversity were not strongly affected by the nutrient additions. There were, on average, 17.7 species in the control treatment, 16.4 species in the N only treatment and 14.7 in the high N and P treatment. Thus, loss of species does not explain changes in ANPP over time. Instead, we observed that shifts in the identity of the dominant functional groups and species – grasses to forbs – in response to chronic resource additions drove changes in ANPP. Our findings demonstrate a need for greater focus on plant community responses, beyond richness alone, to explain productivity responses to nutrient additions. Overall, we found that N and P together control plant community composition, and the composition of the plant community determines ecosystem productivity responses to nutrient additions. In our study, community change had strong consequences for stability of productivity, where a natural disturbance resulted in greater overall production as well as higher variability in production over time. Thus, although increased N and P availability may initially enhance ecosystem function, compositional and functional stability may be compromised over the long term, particularly within the backdrop of natural disturbance regimes.

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Data accessibility

The species composition (data set PVC03) and productivity (data set PAB06) data will be publically available one year after publication and can be accessed through the Konza LTER website: http://www.konza.ksu.edu/knz/pages/data/knzdata.aspx.

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Supporting Information

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

Appendix S1. Soil nutrient analyses.

Table S1. Soil resin bags anova table.

Table S2. Soil nutrient carry-over.

Figure S1. 2009 resin bags demonstrate that there is higher ammonium (means ± SE) in the plots with N added, amount of nitrate depends on P added, and there is more P in the P5 and P10 treatments, but not P2.5 relative to controls. Letters denote significant differences at $P \leq 0.05$.

Appendix S2. Diversity effects over time.

Figure S2. Interactions between year and nitrogen (left) and year and phosphorus (right) additions on measures of plant community diversity [richness (S), evenness (J) and Shannon's diversity (H')] Since both a fire and precipitation signal was observed in the control treatment, diversity data for each treatment is presented relative to the control treatment.

Appendix S3. Effects of nitrogen and phosphorus on the relative cover of plant functional types.

Figure S3. The interactive effects of nitrogen and phosphorus treatments or the effects of nitrogen alone on the relative cover (mean ± SE) of the six plant functional groups.

Appendix S4. Equations from Fig. 4.

Appendix S5. Above-ground net primary productivity over time.

Figure S4. Above-ground net primary productivity (ANPP) means (±SE) over the course of the experiment.