

Nutrient additions cause divergence of tallgrass prairie plant communities resulting in loss of ecosystem stability

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Summary

1. Nitrogen (N) deposition and phosphorus (P) deposition due to pollution and land-use change are dramatically altering biogeochemical cycles. These altered nutrient inputs affect plant communities by generally increasing dominance and reducing diversity, as well as altering community variability (heterogeneity). Less well studied are the effects of changes in community variability on ecosystem functions, such as productivity, or the stability of those functions.

2. Here, we use a twelve-year nutrient addition experiment in tallgrass prairie to determine the variability in community responses to N and P additions and link these responses to ecosystem productivity and stability. We added two levels of N and four levels of P in a fully factorial design to 25-m² plots in native tallgrass prairie in north-eastern Kansas, USA. Each year percentage cover of each species was measured in June and August in a 1-m² subplot of each plot, and annual net primary productivity was measured in two 0.1-m² subplots in each plot at the end of each growing season.

3. The addition of N and P together increased plant community variability across space (i.e. the replicates were significantly more different from each other in the N + P treatments than they were in the control treatment). We also found that the variability of the plant community within a single plot through time increased with the addition of N alone and N and P together. The highest level of both spatial and temporal variability occurred in plots with the highest level of nutrient addition (10 g m⁻² of both N and P).

4. While we found no linkage between spatial variability of community composition and the spatial stability of productivity, the temporal stability of productivity decreased with increasing temporal plant community variability. Additionally, the ability to predict the productivity response to growing season precipitation, a key environmental variable, also decreased under higher temporal community variability.

5. Synthesis. Using a 12-year nutrient addition experiment, we found that nutrient addition leads to both spatial and temporal community variability in mesic tallgrass prairie. The changes in community variability through time were directly related to ecosystem stability. While overall shifts in community structure in response to nutrient additions are important, the change in variability of local communities has significant implications for our ability to predict how patterns of biodiversity and ecosystem function will respond to a rapidly changing world.

Key-words: ANPP, convergence, dispersion, grassland, heterogeneity, nitrogen, phosphorus, plant population and community dynamics, productivity, stability, variability

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Introduction

Humans have more than doubled the amount of reactive nitrogen (N) in the terrestrial N cycle through the combustion of fossil fuels, the production of synthetic fertilizers and the farming of N-fixing crops (Galloway *et al.* 2004; Vitousek *et al.* 2010). Additionally, world-wide use of phosphorus (P)-containing fertilizer has resulted in a fourfold increase in available P, which accumulates in soils (Falkowski *et al.* 2000). This increase in key limiting resources can alter species interactions (LeBauer & Treseder 2008; Cleland & Harpole 2010) and change the patterns of spatial and temporal variability within terrestrial plant communities (i.e. the variance around the mean response; Avolio *et al.* (2015)). Changes in variability of community composition may in turn have significant consequences for ecosystem function, a link that few studies have examined to date.

Grasslands cover approximately one-third of the terrestrial surface and, like most terrestrial systems, they have been shown to be limited by both N and P (Vitousek *et al.* 1997; Elser *et al.* 2007; Fay *et al.* 2015). In a global meta-analysis, N or P individually stimulated grassland productivity by ~20% and N and P in combination increased productivity by 60% (Elser *et al.* 2007). Community richness and diversity in mesic grasslands often decrease with nutrient additions (LeBauer & Treseder 2008; Cleland & Harpole 2010; Simkin *et al.* 2016) due to an increased grass biomass and the accompanying reduction in light (Hautier, Niklaus & Hector 2009; Borer *et al.* 2014). This loss of diversity can decrease the ecosystem stability through time (Tilman 1996). However, even when richness does not decrease, community composition and structure can change due to differential responses to nutrient availability by individual species (Hejman *et al.* 2007; Bai *et al.* 2010; Isbell *et al.* 2013), altering the functional traits of the plant community (La Pierre & Smith 2015) and thereby ecosystem function (Avolio *et al.* 2014).

In addition to mean responses, community variability across space or time is also an important response variable (Benedetti-Cecchi 2003; Fraterrigo & Rusak 2008; Houseman *et al.* 2008; Murphy & Romanuk 2012; Avolio *et al.* 2015) with potential consequences for patterns of biodiversity and ecosystem function (Benedetti-Cecchi *et al.* 2006). In theory, the variability of a community may decrease, resulting in convergence across space or time (i.e. increased homogeneity of replicates); increase, resulting in divergence across space or time (i.e. increased heterogeneity of replicates); or stay the same in response to environmental changes (Houseman *et al.* 2008; Avolio *et al.* 2015). Higher productivity has been linked to higher spatial variability of plant communities both regionally (Chase & Leibold 2002; Chase 2010) and even within a site such as tallgrass prairie uplands (Chalcraft *et al.* 2004). However, a recent synthesis of 18 nitrogen enrichment experiments in terrestrial herbaceous communities across North America found inconsistent effects of nutrient availability on spatial variability across studies even though nitrogen addition generally increased

productivity (Chalcraft *et al.* 2008). Indeed, nitrogen enrichment has been shown to both increase (Inouye & Tilman 1988; Houseman *et al.* 2008) and decrease community variability (Inouye & Tilman 1995). Thus, further research on how nutrient enrichment affects community variability is needed.

Changes in community dispersion have important implications for ecosystem stability (Collins & Xia 2015), such as the variability of annual net primary productivity (ANPP). ANPP is a function of the plant community (Smith, Knapp & Collins 2009; Isbell *et al.* 2013; Avolio *et al.* 2014). Therefore, if community variability increases (i.e. divergence occurs) either across space or through time, variability of ANPP could also increase reflecting lower stability. Such responses by ANPP would depend considerably on community composition as well as other external drivers of dynamics. For example, many studies have shown a positive and linear relationship between annual or growing season precipitation and ANPP in natural grasslands (Huxman *et al.* 2004; Knapp *et al.* 2006; Robinson *et al.* 2013). If community dispersion increases, the tight linkage between precipitation and ANPP could be lost. While findings on the effects of richness on stability are frequently examined (Jiang & Pu 2009; van Ruijven & Berendse 2010; Grossiord *et al.* 2014; Isbell *et al.* 2015), little is known about the implications of altered community variability on the functional stability of ANPP.

We investigated the effects of 12 years of N and P additions on community variability and ecosystem stability of a mesic grassland in Kansas, USA. Starting in 2003, one of two levels of N (0 or 10 g m⁻²) and one of four levels of P (ranging from 0 to 10 g m⁻²) were added to upland, intact tallgrass prairie. Initially, these nutrient additions increased the productivity by C₄ grasses, but eventually productivity only increased in years the site was burned as grasses declined in abundance and the community shifted to dominance by forbs (Avolio *et al.* 2014). Throughout the first 10 years of this study, mean richness, evenness and diversity were minimally affected, with richness decreasing by only 1–2 species in the high N and P plots. Changes in ANPP were attributed to strong shifts in mean community composition through time. In the present study, we examine how *variability* in plant community composition responded to N and P additions over time. We tested three main hypotheses. First, we predicted that nutrient additions would increase the spatial variability of community composition among replicates (i.e. divergence across space). Secondly, we predicted that nutrient additions would increase the temporal variability of community composition among replicates (i.e. divergence over time). For both the spatial and temporal predictions above, we also predicted that the magnitude of divergence would increase with increasing levels of nutrient additions. Thirdly, we predicted that changes in spatial and temporal variability would be linked with changes in ecosystem stability. Specifically, we expected that increased community variability either across space or through time would lead to more variable ANPP.

Materials and methods

STUDY SITE AND EXPERIMENTAL DESIGN

We established our nutrient manipulation study at the Konza Prairie Biological Station (KPBS), Kansas, USA (39° 05' N, 96° 35' W). KPBS contains one of the last remaining large remnants of native unploughed tallgrass prairie in North America. Specifically, this study took place in upland ungrazed tallgrass prairie that has been burned every other year since 1973 (Watershed 2C; 23 ha). The site is dominated by a small number of C₄ perennial grasses, such as *Andropogon gerardii* and *Schizachrium scoparium*, which account for the majority of herbaceous primary productivity (Knapp *et al.* 1998), while community diversity is generally a function of forb species (Collins & Glenn 1991). The climate is temperate (July mean temperature = 27 °C), and precipitation averages 835 mm year⁻¹, with mean growing season (April–September) precipitation being 609 mm year⁻¹ (Knapp *et al.* 2006). During our study, growing season precipitation ranged from a high in 2008 of 875 mm to a low in 2012 with 412 mm, with a mean (±SD) growing season precipitation of 598 (±126) mm year⁻¹. Soils are relatively shallow, fine-textured Udic Argiustolls underlain by cherty limestone and shales (Melzer *et al.* 2010).

In 2002, a 30 × 40 m area was divided into 5 × 5 m plots arrayed into a six by eight matrix (a total of 48 plots), with no spacing between plots. 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 added to the plots in a fully randomized factorial design ($n = 6$ per treatment). Hereafter, the treatment combinations will be referred to in abbreviated form that lists first nitrogen (N) and then phosphorus (P) amounts (N0P0, N0P2.5, N0P5, N0P10, N10P0, N10P2.5, N10P5, N10P10). For example, N10P2.5 signifies the treatment that received 10 g m⁻² of nitrogen and 2.5 g m⁻² of phosphorus. Nutrients were evenly broadcast throughout each plot by hand in early June. Nitrogen was added as ammonium nitrate, and phosphorus as superphosphate.

Each year of the 13-year study (1 year of pre-treatment, 12 of treatment), we surveyed the plant community composition at the beginning and end of the growing season (June and August) to capture peak abundance of early and late-season species, respectively. We sampled vegetation in a 0.25 × 2 m permanent subplot located within each 5 × 5 m plot. Although treatment plots were contiguous, all sampling occurred at least 0.5 m from the edge of a plot in order to eliminate any edge effects that may occur, and the subplots were located 3.0–5.0 m apart. The 0.25 × 2 m subplot was divided into four 0.25 × 0.25 m quadrats, and within each quadrat, we estimated percentage aerial cover (to the nearest 1%) for each species rooted inside. Plot-level maximum (June vs. August) cover estimates for each species were calculated by averaging across the four quadrats, and these plot-level estimates were used in all calculations. Total above-ground net primary production (ANPP) was sampled each year from two 0.1-m² quadrats where all vegetation was clipped at ground level at the end of the growing season (late September), outside of the permanent species composition plots. The mean of the two quadrats in each plot was used in all calculations. See Avolio *et al.* (2014) for further details.

The effects of nitrogen and phosphorus additions on plant diversity and productivity were analysed in detail by Avolio *et al.* (2014). With two additional years of data, we found similar trends of statistically significant, yet small effects of nitrogen and phosphorus on mean plant community responses (see Appendix A for details).

COMMUNITY METRIC CALCULATIONS

We calculated two types of plant community variability – spatial and temporal. Plant community variability across space was calculated on a plot level for each year. Within each year, a Bray–Curtis dissimilarity matrix was created comparing species composition of each replicate of a treatment to all the other replicates of that treatment ($n = 6$ replicates per treatment; therefore, each plot had 5 comparisons). Spatial dissimilarity of a plot was calculated as the mean compositional difference between those five comparisons, and spatial variability of a treatment was calculated as the mean dissimilarity of the 6 plots within a treatment. This is a slight variation on Collins (1992) to eliminate issues with autocorrelation ($n = 6$ rather than $n = 15$). Plant community variability through time was also calculated. A Bray–Curtis dissimilarity matrix was created comparing species composition of each plot in a year to that same plot in every other year ($n = 13$ years, therefore 12 combinations per plot per year). Temporal variability was calculated as the mean compositional dissimilarity of a single plot in a year to all other years (i.e. mean of 12 comparisons) and then averaged for all the plots of the same treatment ($n = 6$ replicates per treatment). By using these calculations, we maintained an n of 6 for both spatial and temporal variabilities.

To examine the effects of community variability on primary productivity, we calculated the spatial and temporal stability of primary productivity. Spatial stability was calculated as μ/σ , where μ is the average productivity of a treatment across all six replicates in a year and σ is the standard deviation of productivity across the six replicates of that same year (Tilman 1999). Temporal stability was calculated as μ/σ , using all 13 years of data collected on ANPP in each plot, where μ is the average productivity of a plot across all years and σ is the temporal standard deviation in the productivity of a plot across all years. Stability of richness through time was calculated the same way as temporal stability.

PATTERNS OF SPATIAL AND TEMPORAL VARIABILITY

For spatial variability, we used three-way mixed-model repeated-measures ANOVA with year as the repeated effect and nitrogen and phosphorus treatments as the main effects. For examining the temporal variability, we used two-way mixed-model ANOVAs with nitrogen and phosphorus as 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 (Avolio *et al.* 2014). We used least-squared means for all *post hoc* comparisons. Significance was set at $\alpha = 0.05$, and statistics were generated using Proc MIXED in SAS (SAS Institute V.9.3; Cary, NC).

VARIABILITY AND THE UNDERLYING COMMUNITY COMPOSITION

To explore the mechanisms behind the changes in community variability in response to nutrient additions seen in the above ANOVAs, we used multivariate analyses to test the simultaneous responses of each species in the community to treatment factors. We used permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersions (PERMDISP) to test for differences in group centroids and dispersions (measures of central tendency and variance in multivariate space) among the treatment types (and among years for PERMANOVA). Statistical significance was calculated by generating pseudo- F -values using 999 unrestricted permutations of raw

data. All multivariate tests were conducted with the PERMANOVA+ expansion for PRIMER (Primer-E Ltd V.6; United Kingdom).

Two methods were used to visualize the changes in communities due to treatments that were detected in multivariate statistical tests. First, non-metric multi-dimensional scaling (NMDS) plots were used to visualize the differences in community composition between treatments within a year or of a single plot through time. Secondly, rank abundance curves (RACs) were calculated to plot the rank of the first five most abundant species (rank 1 being the most abundant) versus their abundance. These were used to visualize how the differences in replicates of a treatment varied across treatments. The linking of ordination plots with RACs provides detailed information about the patterns of change that are yielding the alterations in variability (Avolio *et al.* 2015).

VARIABILITY EFFECTS ON ECOSYSTEM FUNCTION

We followed similar statistical methods to those used to determine N and P effects on community variability as we did for determining N and P effects on ecosystem stability (spatial stability: three-way mixed-model repeated-measures ANOVA; temporal stability: two-way mixed-model ANOVA). Linear regressions were then used to determine the effects of community change on ecosystem function. First, we used linear regression to test the effect of community variability on stability using all years of data (2002–2014) integrating both spatial and temporal approaches. Secondly, we used linear regression to test the effect of growing season precipitation on ANPP for the control treatment and the N10P10 treatment using 2006–2014. The first 3 years of data were dropped from this last analysis as the community had not yet shifted due to N10P10 treatment (see Fig. 1 black Δ) and was therefore behaving like control plots. Significance was set at $\alpha = 0.05$, and statistics were generated using SAS (SAS Institute V.9.3; Cary, N.C.).

Results

SPATIAL VARIABILITY

Spatial variability among replicates was significantly impacted by the interaction between the N and P treatments over time

(Table 1). The addition of P only (NOP2.5, NOP5, NOP10) or N only (N10P0) had a minimal impact on spatial variability with only a few (and temporally scattered) years being significantly different from the control in any of those treatments (Fig. 1a). However, the addition of N and P (at any level) increased spatial variability from 2006 onwards, with the greatest difference from the control generally occurring in the N10P10 treatment (Fig. 1b).

We picked 2011 to highlight the relative effects of the different treatments because this was the year that had the highest variability in the N10P10 treatment. A severe drought occurred in 2012 (Knapp *et al.* 2015), which likely caused community convergence and reduced spatial variability. Community variability has since then been increasing, but has not yet reached the same magnitude as prior to the

Table 1. Effects of nitrogen (nitrogen, N), phosphorus (phosphorus, P) and time (year, Yr) on variability among plots (1 m²) from mixed-model repeated-measures ANOVAs and effects of nitrogen and phosphorus on variability of a plot (1 m²) across time from mixed-model ANOVAs using data from 2003 to 2014. Shown are the *F*-values with *P*-values in parentheses. Significant differences ($P \leq 0.05$) are bolded.

Effect	Variability			
	d.f.	Among 1-m ² plots	d.f.	Across Time
Year	11, 480	9.29 (< 0.001)		
Nitrogen	1, 480	388.10 (< 0.001)	1, 616	1307.11 (< 0.001)
Yr \times N	11, 480	11.96 (< 0.001)		
Phosphorus	3, 480	51.95 (< 0.001)	3, 616	38.67 (< 0.001)
Yr \times P	33, 480	4.67 (< 0.001)		
N \times P	3, 480	13.15 (< 0.001)	3, 616	11.87 (< 0.001)
Yr \times N \times P	33, 480	3.84 (< 0.001)		

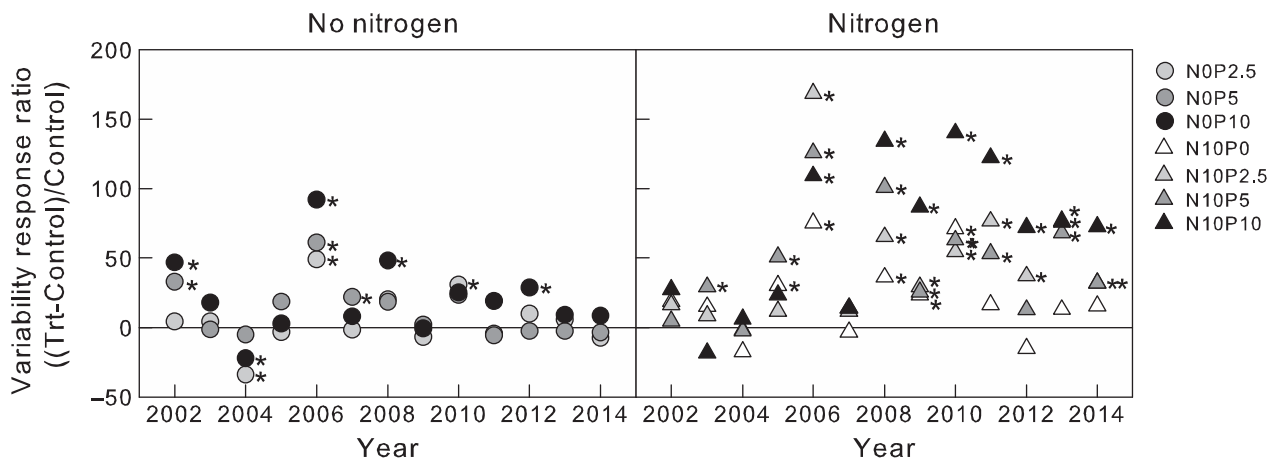


Fig. 1. Effects of nitrogen and phosphorus treatments on the spatial variability in community composition among replicates through time (2002–2014). Using a Bray–Curtis dissimilarity index, variability was calculated as the mean compositional difference among all six replicates of the same treatment ($n = 6$; average of all five possible comparisons for a plot) in a given year. Shown is the response ratio ($((\text{treatment-control})/\text{control}) \times 100$). If the point is above 0, then the treatment plots are more dissimilar from each other than the control plots are from each other. The left panel shows the no nitrogen treatments, and the right panel shows the treatments with nitrogen addition. * denotes significant differences within a year ($P \leq 0.05$) between treatment and control plots based on a three-way mixed-model repeated-measures ANOVA.

drought. In 2011, spatial variability in the single nutrient addition treatments was not different from the control, while N10P2.5 and N10P5.0 were significantly higher than control and both of the single nutrient addition treatments. N10P10 was significantly higher than all other treatments with dissimilarity values of nearly 100% (Fig. 2 left). N and P also significantly interacted to affect the community composition (PERMANOVA: $N \times P$ – pseudo- $F = 1.730$, $P(\text{perm}) = 0.005$) and dispersion (PERMDISP: $N \times P$ – pseudo- $F = 3.488$, $P(\text{perm}) = 0.040$) with the N10P10 being significantly more dispersed than the control (Fig. 2 right). Similar trends to 2011 occurred in 2014 (Appendix B: Fig. B). For reference, in 2002 neither N, P, or their combination significantly impacted the community composition (N: $F_1 = 0.746$; $P(\text{perm}) = 0.641$; P: $F_3 = 1.071$; $P(\text{perm}) = 0.373$; N \times P: $F_3 = 0.380$; $P(\text{perm}) = 0.997$) or dispersion (N \times P: $F_{7,40} = 0.192$; $P(\text{perm}) = 0.671$).

Rank abundance curves (RACs) were used to examine which species caused the shifts in community variability with N and P additions. RACs of the top five species show that in 2002 (pre-treatment) the control and N10P10 replicates, as well as the 2011 control replicates, were similarly structured (Appendix B: Fig. B2 top 3 row panels). All replicates for each of these sets of plots were dominated by tall C_4 grasses (*Andropogon gerardii* or *Schizachyrium scoparium*), shared similar subdominant species and had few, if any, forbs or woody species. In contrast, the replicates from the N10P10 treatment in 2011 were considerably more variable (Appendix B: Fig. B2 bottom panel). While three of the replicates were still dominated by *Andropogon gerardii*, the other three were dominated by different forb species, and even in the plots dominated by grasses, forb abundance was higher overall than grass abundance. RACs in 2014 show similar yet slightly weaker differences (Appendix B: Fig. B3).

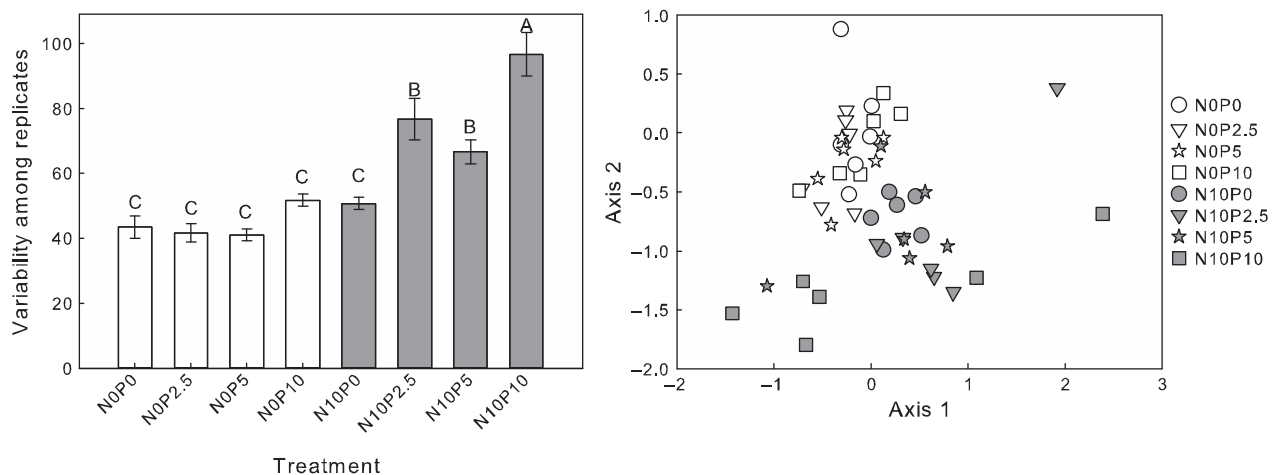


Fig. 2. Effects of nitrogen and phosphorus treatments on plant community composition in 2011. (left) Variability among replicates in 2011. Using a Bray–Curtis dissimilarity index, variability was calculated as the mean compositional difference among all plots of the same treatment. Shown are means across 6 plots ($n = 6$; average of all five possible comparisons for a single plot). Error bars represent standard error (\pm SE). Letters denote significant differences at $P \leq 0.05$ based on a three-way mixed-model repeated-measures ANOVA. (right) Non-metric multidimensional scaling ordination of the plant communities in each replicate plot in 2011 for each of the 8 treatments (stress = 0.17). Note the shift in mean with the addition of N (grey symbols) and the increase in variability with the addition of N and any level of P.

TEMPORAL VARIABILITY

N and P addition interacted to affect the temporal variability of individual plots (2002–2014; Table 1) with variability increasing under increasing nutrient levels (Fig. 3 left). N addition significantly increased the temporal variability over those treatments that did not receive N, with N10P10 being significantly more dissimilar through time than all other treatments. The control replicates were closely aggregated over years in non-metric multidimensional space (Fig. 3). In contrast, the N10P10 replicates had much higher standard error around the centroid (Fig. 3), indicating greater variation in year-to-year community composition. These patterns of temporal variability were not due to strong directional change, but instead due to seemingly stochastic annual variations in community structure. NMDS of mean community composition of the control and N10P10 treatments revealed that while the N10P10 community was changing in multidimensional space (Appendix: Fig. C1 right) more than the control (Appendix: Fig. C1 left), the change was not directional.

IMPACTS OF COMMUNITY VARIABILITY ON ECOSYSTEM FUNCTION

Variation in primary production among replicates (spatial stability) was not significantly impacted by either N or P or their interaction (N: $F_{1,88} = 1.38$, P -value = 0.243; P: $F_{3,88} = 2.15$, P -value = 0.100; N \times P: $F_{3,88} = 0.01$, P -value = 0.999), and spatial variability of the community was not significantly related to spatial stability of ANPP (Appendix: Fig. D1). In contrast, the stability of primary production of a replicate through time (temporal stability) significantly decreased with N addition (N: $F_{1,40} = 18.49$, P -value < 0.001; P: $F_{3,40} = 0.88$, P -value = 0.461; N \times P: $F_{3,40} = 1.21$, P -value = 0.320). Likewise, temporal variability of the community also negatively

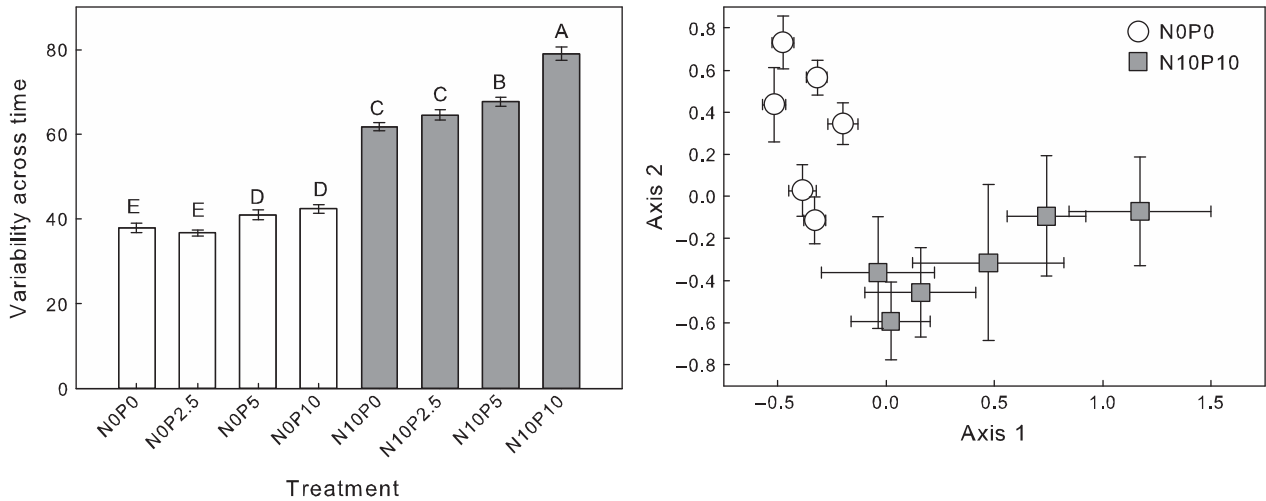


Fig. 3. Effects of nitrogen and phosphorus treatments on plant community variability through time. (left) Variability for each plot among years (2002–2014). Using a Bray–Curtis dissimilarity index, the variability was calculated as the compositional difference of a single plot in a given year to each other year (12 combinations per plot) averaged across all years (13 years) and then averaged for all the plots of the same treatment ($n = 6$ replicates per treatment). Shown are means for each treatment. Error bars represent standard error (\pm SE). Letters denote significant differences at $P \leq 0.05$ based on a two-way mixed-model ANOVA. (right) Non-metric multidimensional scaling plot of the mean plant community in each plot across all years for two treatments – control (N0P0) and N10P10. Error bars represent standard error (\pm SE). Note the much larger error bars on the N10P10 treatment compared to the control.

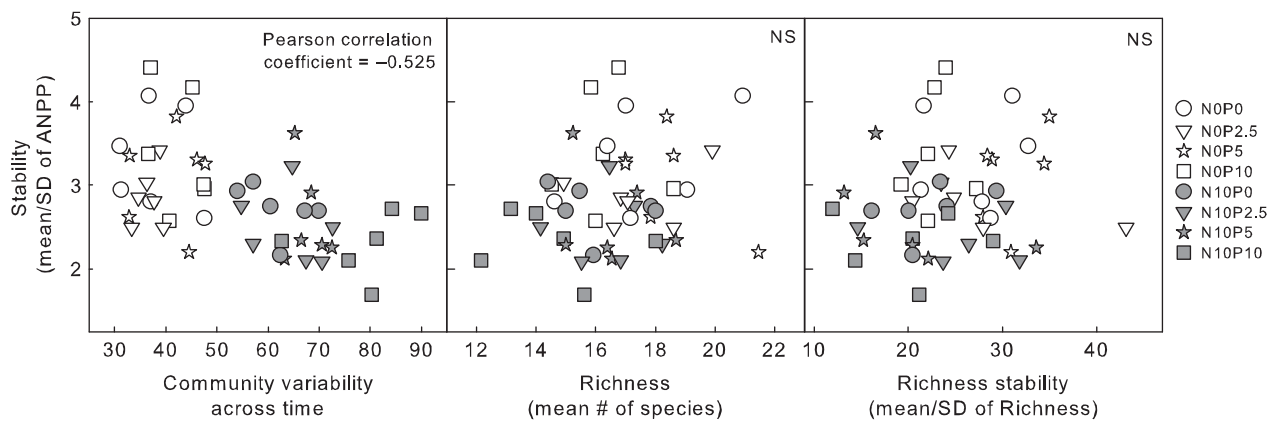


Fig. 4. Effects of plant community structure and variability on stability of ANPP through time. (left) Plant compositional variability through time effects on ANPP stability. Using a Bray–Curtis dissimilarity matrix, variability was calculated as the mean compositional difference of a single plot (mean of 12 possible comparisons) among years (2002–2014). Stability was calculated as the mean ANPP of a plot among all years of the study (2002–2014) divided by the standard deviation of that mean. (middle) Plant community richness effects on ANPP stability. Richness was calculated as the mean richness of a plot through time (2002–2014). (right) Plant community richness stability effects on ANPP stability. Richness stability was calculated as mean richness of a plot through time (2002–2014) divided by the standard deviation of that mean.

impacted the temporal stability of ANPP (Fig. 4 left). Temporal stability of ANPP had no significant relationship with either richness or stability of richness (Fig. 4 middle, right). In addition, predictability of grassland ANPP response to growing season precipitation (Fig. 5) decreased in the N10P10 treatment. While control plots showed a strong positive linear relationship between ANPP and growing season precipitation (Fig. 5), the N10P10 treatment showed no significant relationship with growing season precipitation (Fig. 5).

Discussion

Twelve years of nutrient additions to a native mesic grassland produced two main findings. First, as predicted, the addition

of N and P lead to increased spatial and temporal community variability as the dissimilarity of composition increased both among replicate plots and among years of a single plot. Secondly, and perhaps most important, increased temporal variability in community composition negatively affected the temporal stability of productivity. Neither mean richness nor temporal changes in richness were related to the stability of productivity over time; instead, stability decreased because of changes in the relative abundance of common species. Further, the ability to predict the productivity response to growing season precipitation, a key environmental variable, also decreased as temporal variability increased.

In our study, the addition of N and P in any combination increased spatial variability among replicate plots

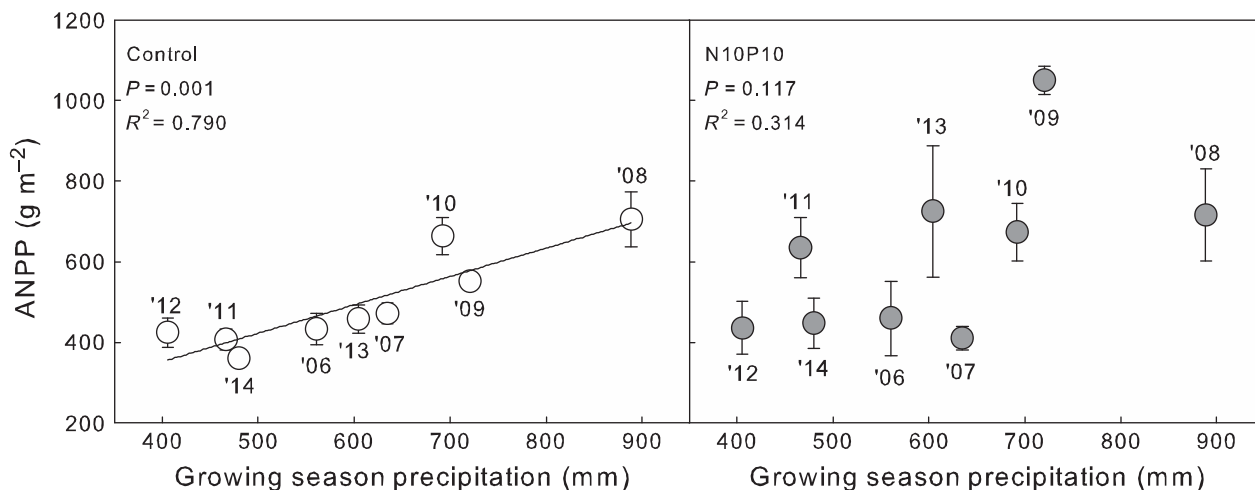


Fig. 5. Effects of growing season precipitation on ANPP from 2006 to 2014. (left) Control plots displayed typical and predictable response to precipitation ($\text{ANPP} = 0.705 \times \text{Precip} + 69.749$), while (right) N10P10 plots showed no significant relationship with precipitation ($\text{ANPP} = 0.775 \times \text{Precip} + 146.861$). Shown are means \pm SE. Years 2003–2005 were not used in linear regression analysis as the community had not yet shifted due to the N10P10 treatment (see Fig. 1 right) and were behaving like natural prairie; thus, those years were dropped from both treatment linear regressions in order to keep the number of years comparable.

relative to the control treatments after only 3 years of treatment. This spatial divergence was stronger with increasing amounts of P addition. Short-term nutrient addition studies (3–4 years) at other tallgrass prairie sites observed similar increases in spatial variability (Inouye & Tilman 1988; Houseman *et al.* 2008). Although a meta-analysis across North American grasslands suggested that N addition generally reduced the community variability in high productivity sites (Chalcraft *et al.* 2008), our results are not consistent with this prediction. Indeed, three of the four experiments from Konza, which were included in Chalcraft *et al.*'s (2008) meta-analysis, found no change in community variability in response to N additions alone, corresponding with our findings. Therefore, it appears that community variability responses to resource alterations are not universal for all resources.

Inouye & Tilman (1995) hypothesized that spatial variability in communities with similar resource levels will eventually decline over time (i.e. convergence). Indeed, Inouye & Tilman (1995) initially observed increased community variability among replicate plots with nutrient additions at the tallgrass prairie site, Cedar Creek, with the communities subsequently converging after 3–6 years. However, increased community variability in our study persisted for 9 years. The communities found in the N10P10 treatments were typically dominated by annual species after ~2006 (Avolio *et al.* 2014). Therefore, if convergence were going to occur, we expect that it should occur rapidly as annual species often respond quickly to nutrient additions (Xia & Wan 2008). The difference between the results from these two sites may be the relatively low abundance of exotic and invasive species at Konza compared to Cedar Creek where N addition led to strong dominance by a single naturalized exotic grass (Inouye & Tilman 1995). Overall, grasslands with nitrophilic exotics may be more prone to convergence as these species often increase in dominance and reduce species diversity.

The initial species composition of a plot likely determines the trajectory of that plot following nutrient addition. Although our experiment was located at a site with a relatively homogenous grassland community, small compositional differences did exist at the start of our experiment – especially in the composition of the subdominant and rare species (Koerner & Collins 2013). These seemingly trivial differences in composition likely contributed to the observed increase in spatial variability following the reduction in abundance of dominant grasses by chronic, high-level additions of both N and P (Appendix B: Fig. B2 bottom row panel, Inouye & Tilman 1988; Houseman *et al.* 2008). Spatial variability can be higher under high productivity scenarios (Steiner & Leibold 2004; Houseman *et al.* 2008), as fertilization increases both biomass and species growth rates. These shifts can lead to stronger species interactions, resulting in high levels of species turnover that could potentially increase the community variability. This could also lead to persistently high spatial variability over time (Steiner & Leibold 2004). Surprisingly, the observed spatial variability had no effect on spatial stability of ANPP; however, we suspect that the stability of other unmeasured ecosystem functions is likely impacted by the changes in spatial variability of the plant community.

In this study, temporal variability increased with both nitrogen alone and N and P together. Each plot for the control treatment occupied a very small area in community space (note the small error bars in Fig. 3b), while the N10P10 treatment exhibited much higher variability throughout the 13 years. We hypothesize that this occurred for two reasons. First, annuals began to dominate the N and P plots (Avolio *et al.* 2014), which benefitted from the decreased dominance of *Andropogon gerardii* (a tolerator of low nutrient availability environments, Wedin & Tilman 1993; Yu *et al.* 2015) and out-competed the typically dominant C_4 grasses. Secondly, higher resources tend to favour invasibility and compositional turnover (Davis, Grime & Thompson 2000), resulting in

perpetual invasion by a subset of species in the regional pool. In our case, this subset tended to be native annuals, and thus, annual turnover was high.

Unlike with the spatial variability, we found a strong negative relationship between temporal variability and temporal stability of ANPP: as temporal variability increased through time, temporal stability of ANPP declined. Although richness has been posited to be a strong driver of ecosystem stability (e.g. Tilman & Downing, 1994; Isbell, Polley & Wilsey, 2009; Jiang & Pu, 2009), we found no evidence that plant richness was related to temporal stability of ANPP. Instead, our findings suggest that spatial variability in local community composition (i.e. species identities and abundance) drives the patterns of temporal variability. At our study site, like in many ecosystems, a few species contribute to the majority of the biomass (Chapin *et al.* 1997; Smith & Knapp 2003), and only examining the effects of richness on stability ignores the important role that dominance plays in spatial and temporal variability (Hallett *et al.* 2014).

Spatial variability in plant composition *did not* impact the spatial stability of ANPP, while temporal variability in plant composition *did* impact temporal stability of ANPP. We hypothesize that this difference between spatial and temporal linkages occurred because of external environmental drivers. Within a given year, all plots experience similar temperatures, rainfall amounts and patterns, and levels of light limitation (i.e. burn years have lower light limitation than non-burn years). While spatial variability could potentially yield more variable ANPP, similar environmental conditions dampen community dissimilarity effects on ANPP. Conversely, a single plot is exposed to unique environmental conditions each year, which exacerbates ANPP differences due to the local changes in plant community composition through time. We hypothesize that any system limited by a driver that varies year-to-year would see a similar mismatch between the impact of temporal and spatial community variability on the stability of ecosystem function.

One important goal of ecological theory is to predict ecosystem responses to global environmental change. We show here that highly divergent communities are also highly unpredictable at small spatial scales. Under unmanipulated conditions, we observed a strong linear relationship between productivity and rainfall, corresponding with previous findings both within ecosystems (Turner *et al.* 1989) and across spatial gradients (Sala *et al.* 1988, 2012). Land managers use these robust relationships to predict forage availability and calculate acceptable stocking rates, and ecologists use this knowledge to derive theory on global patterns of productivity and make predictions about ecosystem carbon balance. However, under high levels of nitrogen and phosphorus availability, the linear relationship we observed between precipitation and productivity became non-significant – signifying increased variability and reduced predictability. In systems where chronic resource additions increase the temporal variability, predicting ecosystem responses to other environmental drivers may prove to be a difficult task (Hautier *et al.* 2014).

Using a 13-year nutrient addition experiment, we show for the first time that nutrient addition leads to increases in spatial

and temporal variability in tallgrass prairie, and we link these changes in temporal variability directly to the changes in ecosystem stability through time. This study is an important first step in understanding the link between community variability and ecosystem stability, and our findings highlight the need for additional experimental studies manipulating variability independent of nutrient additions. While *overall shifts* in community structure with nutrient additions are clearly important, this study shows that the *variability* of local communities has significant implications for the patterns of biodiversity, ecosystem function and stability, and our ability to predict how ecosystems will respond to a rapidly changing world.

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Data accessibility

The species composition (data set PVC03) and productivity (data set PAB06) data are publically available 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 A. Mean responses – plot diversity and ANPP statistical results and figures.

Table A1. Effects of nitrogen (nitrogen, N), phosphorus (phosphorus, P), and time (year, Yr) on plant community diversity at the plot

(1 m²) scale from mixed-model repeated-measures ANOVAS. Three measures of plant community diversity were tested – richness (S), evenness (J), and the antilog of Shannon's diversity (H') – using data from 2003 to 2014. Shown are the *F*-values with *P*-values in parentheses. Significant differences ($P \leq 0.05$) are bolded.

Table A2. Effects of nitrogen (nitrogen, N), phosphorus (phosphorus, P), and time (year, Yr) on annual net primary production (ANPP) from mixed-model repeated-measures ANOVAS. Three measures of ANPP were tested – total ANPP, grass ANPP, and forb and woody ANPP – using data from 2003 to 2014. Shown are the *F*-values with *P*-values in parentheses. Significant differences ($P \leq 0.05$) are bolded.

Figure A1. Effects of nitrogen (left) and phosphorus (right) on plant community richness at the plot scale (A&B) and total annual net primary production (C&D) across all years of the experiment (2003–2014). The interactive effects of nitrogen and phosphorus were not significant. Richness decreased due to nitrogen addition (~1.8 species·m⁻²) while total ANPP increased in 7 out of 12 years of nutrient addition (~110 g m⁻²). Phosphorus had variable effects on richness with significantly higher richness found at the P5.0 level compared to the control (~1 species·m⁻²) and significantly lower richness at P10 than the control (~1 species·m⁻²). Phosphorus had no effect on total ANPP. Diversity and evenness were significantly impacted by the interaction of nitrogen and phosphorus (see Table A1) and while treatments were different from each other few were different from the control including the N10P10 treatment (data not shown). See Appendix A: Tables A1 and A2 for statistics. Shown are means. Error bars represent standard error (\pm SE). Letters denote significant differences at $P \leq 0.05$.

Appendix B. Plant community dissimilarity between replicates (spatial variability).

Figure B1. Effects of 12 years (2014) of nitrogen and phosphorus treatments on plant community structure. (left) Variability among replicates in 2014. Using a Bray-Curtis dissimilarity index, variability was calculated as the mean compositional difference among all plots of the same treatment. Shown are means across 6 plots ($n = 6$; average of all five possible comparison for a single plot). Error bars represent standard error (\pm SE). Letters denote significant differences at $P \leq 0.05$. (right) Non-metric multidimensional scaling plot of the plant communities in each plot in 2014 for each of the eight treatments (stress = 0.21). Plots that received N were significantly different in community composition compared to plots that did not have N added (PERMANOVA: N – pseudo- $F = 11.546$, $P(\text{perm}) = 0.001$), and

all treatments that received both nutrients had higher variability than the control (PERMDISP: NxP – pseudo- $F = 4.774$, $P(\text{perm}) = 0.005$).

Figure B2. Rank abundance curves (RACs) for each plot in 2002 (pre-treatment) and 2011 for the control treatment (top 2) and high N high P treatment (bottom 2). Numbers symbolize graminoids, and letters denote forbs and woody species. Note the consistency in shape of the curve, species, and dominance by grass across plots in 2002 and the control in 2011 RACs (top 3 rows). In total, these three sets of plots (2002 control, 2002 N10P10, and 2011 control = 21 plots total) had nine graminoid, three forb, and two woody species. Conversely, the RACs of the high N high P treatment in 2011 (bottom row) are much more variable in shape of the curve, species, and dominance by grass. In the six N10P10 replicates, there were only four species of graminoids but nine different forb/woody species.

Figure B3. Rank abundance curves (RACs) for each plot in 2014 for the control treatment (top) and N10P10 treatment (bottom). Numbers symbolize graminoids, and letters denote forbs and woody species. Note the consistency in shape of the curve, species, and dominance by grass across plots in the control treatment RACs, while the RACs of the high N high P treatment are much more variable in these three traits.

Appendix C. Plant community dissimilarity through time (temporal variability).

Figure C1. Non-metric multidimensional scaling ordination of the plant community composition (mean of six replicates) in each year of the study for control (left) and N10P10 (right) treatments. The numbers inside the points identify the calendar year of that point. The grey rectangle in the upper left hand corner of the N10P10 (right) plot represents the area shown in the control (left) plot to help express the difference in scale.

Appendix D. Community dissimilarity effects on ecosystem function.

Figure D1. Effects of plant community spatial variability on spatial stability of ANPP. Using a Bray-Curtis dissimilarity matrix, variability across space was calculated as the mean compositional difference among all six replicates of the same treatment ($n = 6$; average of all five possible comparison for a single plot) for each year of data (2002–2014). Spatial Stability was calculated as the mean ANPP of a plot across all replicates of a treatment divided by the standard deviation of that mean for each year of the study (2002–2014).