

Functional trait expression of grassland species shift with short- and long-term nutrient additions

Kimberly J. La Pierre · Melinda D. Smith

Received: 1 May 2014 / Accepted: 26 November 2014 / Published online: 2 December 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Humans are altering nutrient availability worldwide, likely affecting plant trait expression, with consequences for community composition and ecosystem function. Here, we examined the responses of plant species dominant under ambient nutrient conditions (baseline species) versus those that become dominant under increased nutrient conditions (enriched species) in a tallgrass prairie ecosystem. The expression of 8 functional traits was quantified for 3 baseline and 3 enriched species within one short-term and one long-term nutrient addition experiment. We found that enriched species occupied a trait space characterized by traits that generally correspond with

faster growth rates than baseline species. Additionally, the enriched species shifted in their trait expression relative to the control more than the baseline species with nutrient additions, particularly within the long-term experiment. The trait space shifts of individual species with nutrient additions scaled up to affect community aggregate trait values within both experiments. However, traits that responded to nutrient additions at the community level were not strong predictors of aboveground net primary productivity (ANPP) within the short-term experiment. In contrast, in the long-term experiment, one response trait (community aggregate height) strongly correlated with variation in ANPP with nutrient additions. The link between plant functional traits and community and ecosystem responses to chronic nutrient additions shown here will provide important insight into key mechanisms driving grassland responses to global change.

Communicated by Sarah M. Emery.

Electronic supplementary material The online version of this article (doi:[10.1007/s11258-014-0438-4](https://doi.org/10.1007/s11258-014-0438-4)) contains supplementary material, which is available to authorized users.

K. J. La Pierre
Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA

Present Address:
K. J. La Pierre (✉)
Department of Integrative Biology, University of California, Berkeley, CA 94720, USA
e-mail: kimberly.lapierre@berkeley.edu

M. D. Smith
Department of Biology, Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80526, USA

Keywords Baseline species · Effect trait · Enriched species · Nutrient addition · Response trait · Tallgrass prairie

Introduction

Anthropogenic activities are driving chronic increases in resource availability in ecosystems worldwide (Vitousek et al. 1997b; Smith et al. 2009). One well-studied aspect of this phenomenon is the chronic

increase in nutrient availability that is resulting from atmospheric nitrogen (N) deposition and fertilizer inputs to ecosystems (Vitousek et al. 1997a, 2010). A consistent outcome of increases in nutrients, such as N and phosphorus (P), is a shift in terrestrial plant community composition and diversity (Suding et al. 2005; Clark et al. 2007; Cleland and Harpole 2010), often with effects on ecosystem functions, such as aboveground productivity (Inouye and Tilman 1995; LeBauer and Treseder 2008; Harpole et al. 2011; Isbell et al. 2013; Avolio et al. 2014). While a number of studies have documented these responses, less is known about the potential mechanisms underlying shifts in plant community composition and alterations in ecosystem function. Recently, it has been proposed that plant functional traits are key for elucidating mechanisms driving plant community and ecosystem responses to chronic resource additions (McGill et al. 2006; Suding et al. 2008).

Plant functional traits, defined as traits that indirectly impact the fitness of an individual through their effects on growth, mortality, and survival (Violle et al. 2007), are known to influence community and ecosystem processes across a wide array of ecosystems (Diaz and Cabido 2001; Suding et al. 2003, 2008). Patterns of plant functional trait expression have been studied extensively across natural environmental gradients, including precipitation and soil fertility (Chapin 1980; Cowling and Campbell 1980; Cunningham 1999; Poorter and De Jong 1999; Fonseca et al. 2000; Wright et al. 2001; McDonald et al. 2003; Westoby and Wright 2006; Ordoñez et al. 2009) and, to a lesser degree, in experimental frameworks (Suding et al. 2008). Many of these studies have focused on fixed plant traits, such as photosynthetic pathway or growth form. However, we lack an understanding of how changes in nutrient availability will affect non-fixed (quantitative) plant functional traits at a local scale, and how this change in trait expression may then scale up to influence community and ecosystem level processes.

Plant species are known to have particular suites of functional trait values (Grime 1977; Grime 1977; Chapin 1980; Reich et al. 1992; Chapin et al. 1993; Fonseca et al. 2000) that are thought to relate to the species' role in the plant community (Chapin et al. 1993; Schellberg and da Silveira Pontes 2012). Therefore, it may be expected that plant species that are known to increase in abundance with chronic

nutrient additions (hereafter referred to as 'enriched species') may occupy a different functional trait space as compared to species that dominate under ambient (unmanipulated) conditions (hereafter referred to as 'baseline species'). In addition to interspecific trait variation, traits can also vary within a species. Indeed, differential trait expression within a species under different environmental conditions can be as great as interspecific or inter-functional type variation (Wright et al. 2005; Messier et al. 2010). This differential trait expression within a species may be an important factor in determining a species' response to chronic nutrient additions, as species that exhibit a high degree of variation in their trait expression in response to environmental drivers may be able to take advantage of novel environmental conditions (Funk 2008; Albert et al. 2010, 2011; Drenovsky et al. 2012; Kichenin et al. 2013).

Ultimately, changes in plant functional traits at the species level with chronic nutrient additions may scale up to be expressed at the community level. Alterations in community aggregate trait values could be due to shifts in the expression of individual species' traits or changes in species composition (Walker et al. 1999; Knops and Reinhart 2000). By scaling plant functional traits to the community (stand) level (Suding et al. 2008), trait responses to an environmental driver (Walker et al. 1999; Diaz and Cabido 2001; Violle et al. 2007), as well as trait effects on critical ecosystem processes (Walker et al. 1999; Suding et al. 2008; Lavorel and Grigulis 2012), can be determined. Response traits likely play a role in ecological sorting and community assembly (Suding and Goldstein 2008; Webb et al. 2010; Messier et al. 2010), and are therefore important in determining how a community may respond to chronic nutrient additions. In contrast, effect traits are those that drive variation in ecosystem properties, such as productivity (Walker et al. 1999; Suding et al. 2008). While response and effect traits may be critical in determining community and ecosystem responses to environmental variation, across ecosystems different functional traits may be identified as those that respond to perturbation or affect ecosystem function. Therefore, identifying community-level response and effect traits and whether these overlap is an important step in determining the effect of chronic resource alteration on community and ecosystem processes (Suding et al. 2008).

Here, we examine how chronic additions of N and P affect the expression of plant functional traits and how the trait expression of these plant species relates to a key ecosystem function in grasslands—aboveground net primary production (ANPP). First, we examined whether the trait space occupied by baseline and enriched species differed within a natural tallgrass prairie ecosystem under natural resource conditions (i.e., within control plots). We then examined the trait responses of these species to chronic nutrient additions within two experiments—one short-term (4-year) and one long-term (9-year). Third, we examined whether the shifts in trait space observed at the species level scaled up to affect community aggregate trait responses to chronic nutrient additions and whether this effect depended upon shifts in trait expression (intraspecific trait variation) or differences in species abundances (interspecific trait variation). Finally, we determined whether community aggregate traits correlated with ANPP, an important ecosystem function, and whether the strength of this relationship varied with experiment length.

We hypothesized that the enriched plant species would occupy a different functional trait space from the baseline plant species. Specifically, we predicted that the enriched species would be characterized by trait values associated with faster growth, such as higher specific leaf area (SLA) or rates of leaf formation (number of emerging leaves). We further hypothesized that the enriched species would shift in trait space with both short- and long-term experimental nutrient additions, which would confer an advantage to these species with chronic nutrient additions, whereas baseline species would not shift in their trait space. At the community level, we hypothesized that the community aggregate trait values would not exhibit a large response to chronic nutrient additions in the short-term experiment, whereas community aggregate traits would exhibit large shifts in trait space with chronic nutrient additions in the long-term experiment due to shifts in species abundances (interspecific trait variation) being more important than variation in trait expression within species (intraspecific trait variation) in driving the shift in community aggregate trait space. Finally, we hypothesized that the traits that drive the community aggregate response to nutrient additions should also be related to variation in ANPP, as these traits are representative of the physiological responses of

individuals to nutrient additions, which underlie primary productivity within a system.

Materials and methods

Experimental design

Our study utilized two ongoing experiments at the Konza Prairie Biological Station located in northeastern KS. The site receives an average of 835 mm of precipitation per year and is dominated by C_4 grasses. The experiments were established adjacent to each other in an upland site in a watershed burned biennially in the spring (in odd years). Both nutrient addition experiments consisted of two nutrient addition treatments crossed in a fully factorial design: control, nitrogen (N), phosphorus (P), and NP. The first experiment, hereafter referred to as the ‘short-term experiment’, was established in 2008 and consists of 24 5×5 m plots ($n = 6$ replicates per treatment) fertilized in the spring of each year, with N added as ammonium nitrate in 2008 and as timed-release urea from 2009 to 2011 and P added as calcium phosphate. Half the plots in the short-term experiment also received potassium additions ($10 \text{ gm}^{-2} \text{ year}^{-1}$ of potash); however, no effects of potassium were observed and therefore all analyses are pooled across the potassium treatments for this experiment. The second experiment, hereafter referred to as the ‘long-term experiment’, was established in 2003 and consists of 24 5×5 m plots ($n = 6$ replicates per treatment) that have been fertilized in the spring of each year, with N added as ammonium nitrate and P as calcium phosphate. All nutrients were added at a rate of $10 \text{ gm}^{-2} \text{ year}^{-1}$. Nutrient addition treatments significantly elevated soil N and P levels in both the short- and long-term experiments (La Pierre unpub. data and Avolio et al. 2014, respectively).

Focal species

We measured the traits of 6 plant species populations within a natural tallgrass prairie ecosystem at Konza. Plant species at the site were identified as either ‘baseline species’—those that dominate under ambient (control) conditions—or ‘enriched species’—those that increase in abundance and dominate with chronic nutrient additions. All baseline and enriched

species chosen for this experiment are perennial species that are native to North American tallgrass prairie.

The three species with the greatest abundances within the unmanipulated plots ($n = 6$) established at the site (see “[Experimental Design](#)” section, below) were identified as the baseline species. These baseline species consistently dominate in tallgrass prairie through time in the absence of externally driven perturbations in resources. Baseline species used for this study were *Andropogon gerardii* (C_4 grass), *Sorghastrum nutans* (C_4 grass), and *Schizachyrium scoparium* (C_4 grass). Enriched species were identified from the long-term (9-year) chronic nutrient addition experiment at the site, within which large shifts in plant community composition have been observed (Avolio et al. 2014). Only the enriched species that could be found within the majority (>95 %) of the treated plots were chosen for this study, resulting in 3 enriched species used in this study. Enriched species used for this study were *Dichanthelium oligosanthes* (C_3 grass), *Ambrosia psilostachya* (forb), and *Solidago missouriensis* (forb).

Data collection

Four traits relating to leaf attributes [SLA, leaf dry matter content (LDMC), leaf thickness, and leaf toughness] and four traits relating to growth (leaf turnover, leaf formation, height, and individual biomass) were measured for individuals of each focal species at three separate sampling dates (June 2010, June 2011, and August 2011). These sampling dates corresponded to years 3–4 in the short-term experiment and years 8–9 in the long-term experiment. On each sampling date, 3 individuals of each species were selected along a 2 m transect through the center of each plot (both unmanipulated and nutrient addition plots) and destructively harvested (harvested individuals were at least 1 m away from each other). For each individual harvested, the number of senesced (>50 % dead), fully emerged, and emerging [rolled (for grasses) or curled inward (for forbs) young leaves] leaves was counted and the height was measured to the nearest 0.5 cm. Then the centermost fully emerged leaf was collected from each individual. The leaf's area was determined by scanning the leaf using a flatbed scanner (Canon, CanoScan LiDE 110) and

analyzing the image using the imaging software ImageJ. The thickness of each leaf was measured at the midpoint point of the leaf (avoiding the mid-veins) using a dial caliper to the nearest 0.1 mm. The wet weight of the leaf was also measured. Finally, the force necessary to penetrate the leaf at its widest part (avoiding the mid-veins) was measured using a simple penetrometer consisting of a 3.25 mm² puncturing surface weighted from above using sand. The leaf and remaining biomass of the individual were then dried at 60 °C for 48 h, and the dry weight of the leaf and total biomass of the individual were measured.

SLA was calculated as leaf area divided by leaf dry weight. LDMC was calculated as leaf wet weight divided by leaf dry weight. Leaf toughness was calculated as the force necessary to break the leaf (g of sand divided by the area of the puncturing surface). The number of senesced leaves was divided by the number of green leaves to relativize the measurement across species; this relativized number was used as a proxy for the rate of leaf turnover. The number of emerging leaves was divided by the number of green leaves and used as a proxy for the rate of leaf formation. All trait data have been archived in the TRY plant trait database.

In addition to functional traits, plant species composition and ANPP were measured within each plot (both control and nutrient addition plots). Species composition was measured in a permanent 1 × 1 m subunit within each of the experimental plots twice per growing season, once at the beginning (mid-May to mid-June) to determine the abundance of early season forbs and C_3 grasses and once at the end (mid-August to mid-Sept) to determine the abundance of late season forbs and C_4 grasses. Percent aerial cover was determined separately for each species to the nearest 1 %. Maximum cover values were used to calculate the relative cover of each species within each plot. ANPP was measured by clipping all biomass within two 0.1 m² quadrats to ground level in each plot at peak biomass each year (late August to early September). The locations of the clipping quadrats were moved within the plots each year to prevent a clipping effect. Biomass was separated into current year's biomass and previous year's dead and was dried at 60 °C for 48 h prior to weighing. ANPP was estimated as current year's biomass and was averaged across the two strips within each plot for all analyses.

Statistical analyses

For each sampling date, species, and trait measured, averages were taken across the 3 individuals sampled within each plot prior to analysis. Additionally, all trait data were square root transformed prior to analysis. Redundancy analysis (RDA) was used to create a trait space using data for all 8 traits for each species, plot, and experiment length, with traits constrained by experiment length, species type (i.e., baseline vs enriched), and nutrient treatment (see Appendix 1 in ESM for eigenvectors and -values and Appendix 2 in ESM for a scree plot). A permutational test of the RDA (999 perms.) was used to determine whether trait space differs between baseline and enriched species and/or by nutrient treatment or experiment length, with species type, experiment length, and N and P treatments as fixed factors and species as replicates.

Community aggregate trait values were calculated for each plot as follows (Garnier et al. 2004):

Aggregate trait = Σ (species trait value \times species relative abundance).

On average, the 6 species measured made up 55.3 % of the relative plant cover in each plot. RDA was used to create a community aggregate trait space using the community aggregate trait values for each plot within each experiment (see Appendix 3 in ESM for eigenvectors and -values and Appendix 4 in ESM for a scree plot). A permutational test of the community aggregate RDA was used to determine whether nutrient additions resulted in a shift in community aggregate trait space and whether this response differed with experiment length using data from the short- and long-term experiments, with experiment length, N, and P as fixed factors and plots as replicates. Additionally, the shift in trait space from the control (vector length) was calculated for the N, P, and NP treatments for the short- and long-term experiments within the community aggregate RDA.

We assessed what factors potentially contributed to the shift in community aggregate trait space with nutrient additions by calculating the dissimilarity of the plant community between nutrient added versus control plots, the mean of the baseline species aggregate trait values, and the mean of the enriched species aggregate traits values for each treatment within the short- and long-term experiments. The community dissimilarity of each plot from each other plot for both experiment lengths was calculated using Euclidean distance matrices based on the percent cover data of all

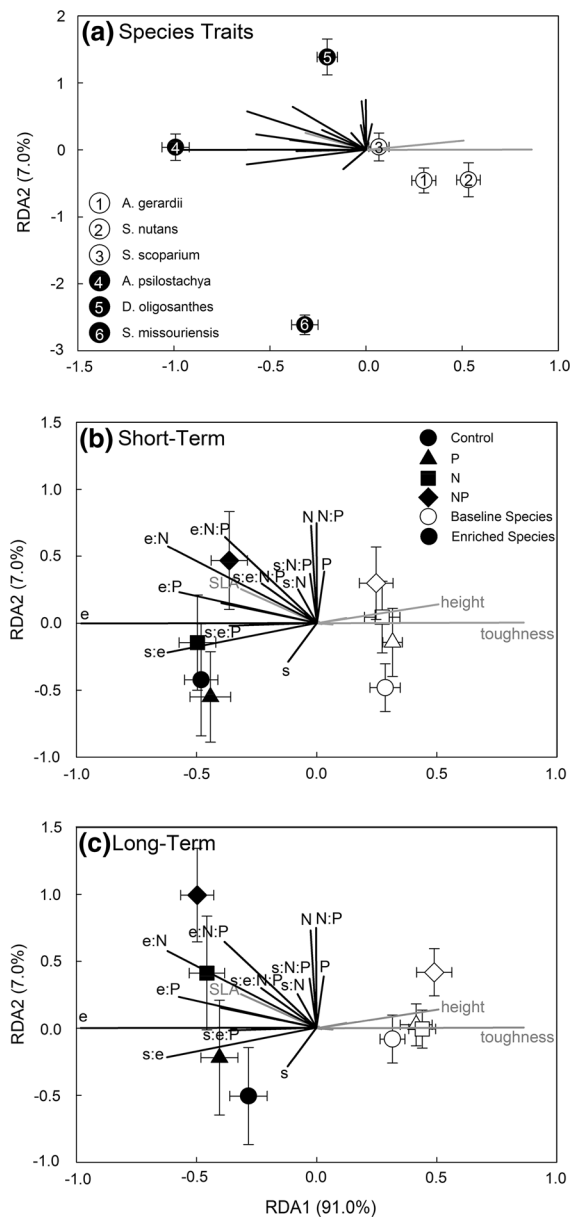
species; the mean dissimilarity from the controls was determined for each treatment within each experiment length. The means of the baseline and enriched species aggregate trait values were calculated as the average community aggregate trait values for each species type averaged across all traits measured within each experiment length and nutrient treatment. These mean aggregate trait values were used as metrics of changes in trait expression within either baseline or enriched species, as opposed to shifts in community aggregate trait expression due to interspecific trait differences. The community dissimilarity from the control, the mean baseline species aggregate trait values, and the mean enriched species aggregate trait values were compared to the shift in trait space from the control using multiple linear regression to determine what factors were related to the shift in community aggregate trait space with nutrient additions (i.e., variation in trait expression of the baseline or enriched species and/or shifts in species abundances).

We identified the individual community aggregate traits that responded to chronic nutrient additions (response traits) within the short-term experiment using multi-factor ANOVAs. These ANOVAs were conducted separately for each community aggregate trait within each experiment, with N and P as fixed factors. Finally, to determine which traits may be related to ANPP (effect traits), a stepwise multiple regression was conducted for each experiment, regressing ANPP against 7 of the 8 community aggregate traits measured, excluding individual biomass, which is essentially a measure of total aboveground biomass when aggregated at the community level. All statistical analyses were performed in R v. 3.1.1 (R Core Team 2014). The RDAs were performed using the *vegan* package (Oksanen et al. 2013), the relative importance of the factors in the multiple linear regression was determined using the *relaimpo* package (Grömping 2006), and the stepwise multiple regression was performed using the *MASS* package (Venables and Ripley 2002).

Results

Trait space occupied by baseline versus enriched species

Enriched species differed significantly from baseline species in their trait space occupied under



unmanipulated conditions (i.e., control plots; Fig. 1a). In general, the enriched species had higher SLA, but lower height, leaf toughness, leaf thickness, and rates of leaf turnover than the baseline species (Fig. 1a).

Shift in trait space occupied by baseline species versus enriched species with chronic nutrient additions

The baseline and enriched species differed in their functional trait responses to chronic N additions

Fig. 1 Functional trait space quantified using RDA, with the first two axes explaining 98.0 % of the variation in the data. Trait loadings on each axis are identified by gray lines and text, while biplot loadings for experiment length *s* short-term experiment, species type *e* enriched species, and nutrient treatments *N* nitrogen addition, *P* phosphorus addition are indicated by black lines and text. Open symbols represent baseline species (those that are dominant under ambient conditions) and filled symbols represent enriched species (those that are dominant with chronic additions of N and P). The symbol types indicate nutrient treatments. Panel **a** shows trait values for each individual species under control conditions. Symbols indicate the mean trait space occupied by each species (identified by numbers), with error bars representing standard error within each species. Panel **b** shows trait values for the short-term and **c** shows trait values for the long-term, where symbols are mean values, with standard errors across species indicated. Note that all data presented in this figure were analyzed within one RDA, but are shown in separate panels here for clarity (see text for details). Additionally, text indicating trait and biplot loadings were omitted from panel **a** for clarity; however, the loadings from panels **b** and **c** can be used as reference for panel **a**

between the short- and long-term experiments (significant length \times type \times N interaction; Table 1; Fig. 1). Both enriched and baseline species increased in SLA and height with N additions in the short-term experiment (Fig. 1b), with this increase being much stronger for enriched species than baseline species in the long-term experiment (Fig. 1c).

Community aggregate trait responses to chronic nutrient additions

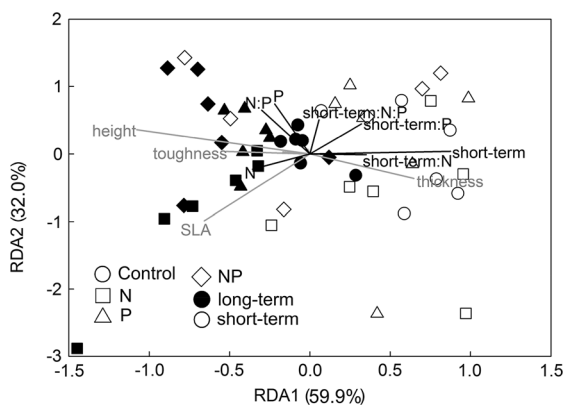
The community aggregate trait space occupied differed between the short- and long-term experiments (Fig. 2; Table 2), with plots in the long-term experiment having lower aggregate leaf thickness and higher aggregate leaf toughness, SLA, and height than plots in the short-term experiment (Fig. 2). Additionally, a significant N \times P interaction was observed, with N alone increasing leaf thickness and SLA and decreasing height, while NP added in combination increased height, leaf toughness, and SLA and decreased leaf thickness (Fig. 2).

Linear regression showed that both intraspecific trait variation and changes in community composition were related to shifts in community aggregate trait space with nutrient additions (multiple $R^2 = 0.975$, $F_{1,3} = 25.56$, $p = 0.038$); the shift in mean enriched species aggregate trait values had the strongest effect on the shift in

Table 1 Effect of experiment length, nutrient additions (N = nitrogen and P = phosphorus), and plant species type (i.e., baseline or enriched; see text for details) on RDA loadings for individual species traits based on a permutational test of the RDA (number of permutations = 999)

	Pseudo- <i>F</i>	<i>df</i>	<i>p</i>
Length	3.59	1,259	0.010
Type	145.73	1,259	<0.001
Length × type	0.98	1,259	0.424
N	6.32	1,259	<0.001
Length × N	0.08	1,259	0.950
Type × N	1.64	1,259	0.212
Length × type × N	2.28	1,259	0.030
P	2.28	1,259	0.172
Length × P	0.15	1,259	0.970
Type × P	0.19	1,259	0.899
Length × type × P	1.12	1,259	0.253
N × P	0.47	1,259	0.677
Length × N × P	0.03	1,259	0.990
Type × N × P	1.39	1,259	0.182
Length × type × N × P	0.33	1,259	0.657

Bold text indicates significant differences ($p < 0.05$)

**Fig. 2** Community aggregate trait space quantified using RDA for short-term (*open symbols*) and long-term (*filled symbols*) chronic nutrient addition experiments in a tallgrass prairie ecosystem. The nutrient addition treatments are indicated by the different symbol shapes. *Symbols* represent each community aggregate trait loadings for each plot in each experiment. Trait loadings for the four dominant trait responses are indicated by *gray lines* and *text*. Biplot loadings for experiment length and nutrient treatments are indicated by *black lines* and *text*

community aggregate trait space with nutrient additions (relative importance = 45.3 %), followed by the shift in mean baseline species aggregate trait values (relative

Table 2 Effect of experiment length and nutrient additions (N = nitrogen and P = phosphorus) on RDA loadings for community aggregate traits based on a permutational test of the RDA (number of permutations = 999)

	Pseudo- <i>F</i>	<i>df</i>	<i>p</i>
Length	10.05	1,40	<0.001
N	2.78	1,40	0.030
Length × N	0.39	1,40	0.777
P	4.40	1,40	0.007
Length × P	0.32	1,40	0.850
N × P	2.65	1,40	0.044
Length × N × P	0.77	1,40	0.525

Bold text indicates significant differences ($p < 0.05$)

importance = 31.1 %), and changes in community composition (relative importance = 23.5 %).

Identifying response and effect traits

Different community aggregate traits responded to N and P additions between the short- and long-term experiments. In the short-term experiment, a decrease in community aggregate LDMC with N additions and an increase in community aggregate rate of leaf turnover, height, and biomass with P additions was observed (Table 3). Additionally, community aggregate SLA increased with N additions, but not N and P additions combined, in the short-term experiment (Table 3). In the long-term experiment, a decrease in community aggregate LDMC and leaf thickness with N additions and an increase in community aggregate height with P additions and biomass with N and P additions were observed (Table 3).

In the short-term chronic nutrient addition experiment, community aggregate leaf toughness explained 20.9 % of the variation in ANPP ($F_{1,23} = 5.80$, $p = 0.024$; Table 3). In the long-term experiment, 39.0 % of the variation in ANPP was explained by community aggregate height ($F_{2,47} = 29.46$, $p < 0.001$; Table 3).

Discussion

With chronic additions of resources, the expectation is that some plant species may be better able cope with changing environmental conditions associated with the nutrient additions ('enriched' species) than other

Table 3 Response of seven community aggregate traits to nitrogen (N) and phosphorus (P) additions (shown are F values from ANOVAs, with degrees of freedom in parentheses; bold indicates significant effect of N or P additions at $p < 0.05$) and

their effects on ANPP (shown are partial R^2 values from stepwise multiple regressions) in both short-term and long-term chronic nutrient addition experiments in tallgrass prairie

	Short-term				Long-term			
	Response			Effect	Response			Effect
	N	P	N \times P		N	P	N \times P	
Turnover	0.44(1,30)	4.95(1,30)	1.80(1,30)	–	0.06(1,66)	1.84(1,66)	1.71(1,66)	–
Formation	0.12(1,30)	0.75(1,30)	0.72(1,30)	–	2.69(1,66)	0.03(1,66)	1.27(1,66)	–
SLA	2.42(1,30)	4.45(1,30)	4.24(1,30)	–	3.43(1,66)	2.75(1,66)	3.42(1,66)	–
LDMC	4.37(1,30)	0.00(1,30)	0.25(1,30)	–	9.82(1,66)	0.04(1,66)	0.50(1,66)	–
Thickness	0.13(1,30)	3.13(1,30)	3.11(1,30)	–	5.19(1,66)	3.02(1,66)	0.42(1,66)	–
Toughness	0.14(1,30)	0.00(1,30)	0.00(1,30)	0.209	0.12(1,66)	1.99(1,66)	1.02(1,66)	–
Height	2.30(1,30)	10.31(1,30)	0.32(1,30)	–	3.72(1,66)	6.52(1,66)	0.43(1,66)	0.390

‘–’ indicates that the variable was not included in the final multiple regression model

species in the community, thereby increasing in abundance and potentially displacing species that were abundant under ambient nutrient conditions (‘baseline’ species). A number of nutrient addition experiments have shown that plant community composition can change dramatically with chronic nutrient additions (Suding et al. 2005; Harpole and Tilman 2007; Clark and Tilman 2008). Potential mechanisms underlying changes in community composition are that enriched species may occupy a different trait space than baseline species that allows them to become abundant with chronic nutrient additions and/or that enriched species are able to respond to the nutrient additions by shifting their trait space more than baseline species. Our study was aimed at assessing whether enriched and baseline grassland plant species differ in their trait space, whether this trait space varies in response to either short- or long-term nutrient additions, and whether shifts in trait space scale up to the community level to impact aboveground productivity.

As hypothesized, the baseline and enriched species examined here occupied different trait spaces. Notably, the baseline and enriched species studied here also differ in their growth form and photosynthetic pathway, with the baseline species comprised of all C_4 grasses and the enriched species comprised of two forbs and one C_3 grass. Thus, it is not surprising that these species differ in traits associated with growth and leaf-level characteristics. However, the direction and magnitude of these differences is important for

building a more descriptive understanding of what differentiates species in these broad functional categories and why one group may perform better under altered environmental conditions than the other. Under unmanipulated (control) nutrient conditions, the enriched species occupied a trait space associated with higher SLA and lower leaf toughness, leaf thickness, height, and rates of leaf turnover than the baseline species. This is consistent with previous studies that have found that species with fast growth traits, such as high SLA, tend to perform better under conditions of high nutrient availability (Grime 1977; Chapin 1980; Laliberté et al. 2012). The traits exhibited by the enriched species likely confer an advantage under high nutrient conditions by allowing for the quick uptake and utilization of the abundant resources (Grime 1977).

We also found that the baseline and enriched species differed in their responses to nutrient additions. With shorter-term (4 years) N additions, both the baseline and enriched species shifted in their trait space to have higher SLA and height than under control conditions. Interestingly, the shift in the trait space of the enriched species was greater with longer-term (9 years) N additions than in the short-term experiment. Although not directly tested here, we speculate that the enriched species are initially exhibiting phenotypic plasticity, allowing them to adapt quickly to enriched environmental conditions and therefore rapidly shift in their trait space in the short-term experiment. In addition to phenotypic plasticity,

trait variation in the long-term experiment may be due to selection on underlying genetic variation in the enriched species. Because of the long lifespans of the species studied here, selection on genetic variation in these species may only emerge over longer time scales, such as in the long-term nutrient addition experiment studied here. Thus, as the genetic structure of the enriched species' populations shift toward genotypes with, for example, higher SLA, shifts in their phenotypic expression become more pronounced. Similar shifts in the genetic structure and phenotypic expression the dominant grass species, *A. gerardii*, in response to 10 years of altered precipitation variability have been observed in tallgrass prairie (Avolio et al. 2013).

In contrast to the enriched species, trait variation in the baseline species with N additions was consistent across the short- and long-term experiments, with the baseline species exhibiting less breadth in trait variation than the enriched species. This difference in the ability of enriched and baseline species to shift in their trait space in response to N additions may be due to lower levels of phenotypic plasticity for the latter. Additionally, while no changes in community composition have been observed in the short-term experiment (La Pierre, unpub. data), large scale shifts in community composition have been observed in the long-term experiment with N additions (Avolio et al. 2014), with reductions in the abundance of the baseline species presenting the opportunity for selection for genotypes with traits better suited to a high nutrient environment to occur. Thus, as the variation in trait space of the baseline species was lower than that of the enriched species and did not differ across the short- and long-term experiments, baseline species may have a lower capacity for trait variation than the enriched species.

It has been suggested that for a trait to be useful for the study of community ecology, it should exhibit more variation between than within species (McGill et al. 2006). However, differential trait expression of a species under different environmental conditions may in itself be considered a functional trait. Trait variation in response to altered environmental conditions may enhance the fitness of an individual by allowing it to quickly adapt to a novel environment (*sensu* Grime 1977). A similar mechanism has been proposed for the success of invasive species, suggesting that invasive species are able to succeed in their novel range by

exhibiting trait variation that allows them to take advantage of the novel environmental conditions (Funk 2008; Drenovsky et al. 2012; Bachmann et al. 2012). However, trait variation may not always lead to fitness gains in novel environments. For example, Firn et al. (2012) has shown that invasive species that increase the concentration of N in their leaf tissue in high nutrient environments tend to be eaten at a higher rate than those species that do not alter their leaf tissue chemistry. Thus, it is important to consider whether trait variation has a positive effect under novel environmental conditions, which may be system- and species-specific.

Variation in functional traits may scale up to influence community aggregate trait expression (Walker et al. 1999). In this study, we found that variation in trait expression of the enriched species was the primary driver of shifts in community aggregate trait expression with N additions, followed closely by variation in trait expression of the baseline species. Thus, our results demonstrate that intraspecific trait variation alone can impact community aggregate trait expression. In addition, we found that shifts in community composition were also predictive of changes in community aggregate trait space, although to a lesser extent than intraspecific variation in trait expression. As previous studies have shown that altered plant communities can persist long after nutrient addition treatments have stopped (e.g., Milchunas and Lauenroth 1995), trait variation both within and among species may play a large role in the stability of systems in the face of future environmental change.

Many previous studies have identified traits that can be classified as both response and effect traits (Knops and Reinhart 2000; Diaz et al. 2004; Suding et al. 2008; Yang et al. 2011; Lavorel et al. 2011; Minden and Kleyer 2011; Lanta et al. 2011; García-Palacios et al. 2013). In this study, we found that many traits responded to nutrient additions without having an effect on ANPP. However, the response traits differed between the short- and long-term experiments, suggesting that these traits may be important in structuring community changes with chronic nutrient additions (Suding and Goldstein 2008; Suding et al. 2008; Ceulemans et al. 2011). Overall, two traits were classified as effect traits in this study. Within the short-term nutrient addition experiment, community aggregate leaf toughness was correlated with ANPP;

however, no response traits were also classified as effect traits in the short-term experiment. In contrast, in the long-term nutrient addition experiment, height explained the majority of the variation in ANPP, in addition to responding to variation in nutrient availability. The amount of variation in ANPP explained by community aggregate traits in the long-term experiment was almost twice that explained in the short-term experiment. This may be due to the shift in species composition that has occurred with N additions in the long-term experiment, resulting in a shift toward species whose traits better predict ANPP (Suding et al. 2008; Pakeman 2011).

Here, we present evidence that the plant species that are most abundant under ambient nutrient conditions (baseline species) differ from those that are uncommon under ambient conditions but increase in abundance under chronic nutrient additions (enriched species) in both their trait expression and the degree to which they vary in their trait expression in response to nutrient additions. The enriched species studied here had traits that favor rapid growth, and these traits were found to respond rapidly to novel environmental conditions. The shift in trait space of the enriched species was sufficient to result in changes in trait expression at the community level, with consequent effects on ecosystem function. Thus, functional traits appear to be key predictors of species responses to chronic resource alterations and their effects on ecosystem processes.

Acknowledgments The authors are grateful for field and lab help from M. Avolio, D. Blumenthal, C. Chang, S. Koerner, A. Kuhl, B. La Pierre, J. Nippert, A. Potter, R. Ramundo, and K. Wilcox. The Post and Vasseur labs, as well as two anonymous reviewers, provided helpful feedback on earlier manuscript drafts. Funding was provided by a Yale Institute for Biospheric Studies Center for Field Ecology Pilot Grant to K. La Pierre, a grant from the Lee Pierce Fund to K. La Pierre, and the Konza LTER. This work was generated using data from a site within the Nutrient Network collaborative experiment, funded at the site-scale by individual researchers and coordinated through Research Coordination Network funding from NSF to E. Borer and E. Seabloom (NSF-DEB-1042132). Nitrogen fertilizer was donated by Crop Production Services, Loveland, CO. K. La Pierre was supported by an NSF Graduate Research Fellowship.

References

- Albert CH, Thuiller W, Yoccoz NG, Douzet R, Aubert S, Lavorel S (2010) A multi-trait approach reveals the structure and the relative importance of intra- vs. inter-specific variability in plant traits. *Funct Ecol* 24:1192–1201
- Albert CH, Grassein F, Schurr FM, Vieilledent G, Violle C (2011) When and how should intraspecific variability be considered in trait-based plant ecology? *Perspect Plant Ecol Evol Syst* 13:217–225
- Avolio ML, Beaulieu JM, Smith MD (2013) Genetic diversity of a dominant C4 grass is altered with increased precipitation variability. *Oecologia* 171:571–581
- Avolio ML, Koerner SE, La Pierre KJ, Wilcox KR, Wilson GWT, Smith MD, Collins SL (2014) Changes in plant community composition, not diversity, during a decade of nitrogen and phosphorus additions drive above-ground productivity in a tallgrass prairie. *J Ecol* 102:1649–1660
- Bachmann D, Both S, Bruehlheide H, Ding B-Y, Gao M, Härdtle W, Scherer-Lorenzen M, Erfmeier A (2012) Functional trait similarity of native and invasive herb species in subtropical China—environment-specific differences are the key. *Environ Exp Bot* 83:82–92
- Ceulemans T, Merckx R, Hens M, Honnay O (2011) A trait-based analysis of the role of phosphorus vs. nitrogen enrichment in plant species loss across North-west European grasslands. *J Appl Ecol* 48:1155–1163
- Chapin FS (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst* 11:233–260
- Chapin FS, Autumn K, Pugnaire F (1993) Evolution of suites of traits in response to environmental stress. *Am Nat* 142:S78–S92
- Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451:712–715
- Clark CM, Cleland EE, Collins SL, Fargione JE, Gough L, Gross KL, Pennings SC, Suding KN, Grace JB (2007) Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecol Lett* 10:596–607
- Cleland EE, Harpole WS (2010) Nitrogen enrichment and plant communities. *Year in ecology and conservation biology* 2010. Blackwell Publishing, Oxford, pp 46–61
- Cowling R, Campbell B (1980) Convergence in vegetation structure in the mediterranean communities of California, Chile and South Africa. *Plant Ecol* 197:191–197
- Cunningham S (1999) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecol Monogr* 69:569–588
- Diaz S, Cabido M (2001) Vive la difference: plant functional diversity matters to ecosystem processes. *Trends Ecol Evol* 16:646–655
- Diaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen JHC, Jalolo A, Montserrat-Marti G, Grime JP, Zarrinkamar F, Asri Y, Band S, Basconcelo S, Castro-Diez P, Funes G, Hamzehee B, Khoshnevi M, Perez-Harguindeguy N, Perez-Rontome M, Shirvany F, Vendramini F, Yazdani S, Abbas-Azimi R, Bogaard A, Boustani S, Charles M, Dehghan M, de Torres-Espuny L, Falczuk V, Guerrero-Campo J, Hynd A, Jones G, Kowsary E, Kazemi-Saeed F, Maestro-Martinez M, Romo-Diez A, Shaw S, Siavahs B, Villar-Salvador P, Zak M (2004) The plant traits that drive ecosystems: evidence from three continents. *J Veg Sci* 15:295–304

- Drenovsky RE, Grewell BJ, D'Antonio CM, Funk JL, James JJ, Molinari N, Parker IM, Richards CL (2012) A functional trait perspective on plant invasion. *Ann Bot* 110:141–153
- Firn J, Prober SM, Buckley YM (2012) Plastic traits of an exotic grass contribute to its abundance but are not always favourable. *PLoS One* 7:e35870
- Fonseca CR, Overton JM, Collins B, Westoby M (2000) Shifts in trait-combinations along rainfall and phosphorus gradients. *J Ecol* 88:964–977
- Funk JL (2008) Differences in plasticity between invasive and native plants from a low resource environment. *J Ecol* 96:1162–1173
- García-Palacios P, Maestre FT, Milla R (2013) Community-aggregated plant traits interact with soil nutrient heterogeneity to determine ecosystem functioning. *Plant Soil* 364:119–129
- Garnier E, Cortez J, Billès G, Navas M (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85:2630–2637
- Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* 111:1169–1194
- Grömping U (2006) Relative importance for linear regression in R: the package relaimpo. *J Stat Softw* 17:1–27
- Harpole WS, Tilman D (2007) Grassland species loss resulting from reduced niche dimension. *Nature* 446:791–793
- Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE (2011) Nutrient co-limitation of primary producer communities. *Ecol Lett* 14:852–862
- Inouye RS, Tilman D (1995) Convergence and divergence of old-field vegetation after 11 yr of nitrogen addition. *Ecology* 76:1872–1887
- Isbell F, Reich PB, Tilman D, Hobbie SE, Polasky S, Binder S (2013) Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proc Natl Acad Sci* 110:11911–11916
- Kichenin E, Wardle DA, Morse CW, Freschet GT (2013) Contrasting effects of plant inter- and intraspecific variation on community-level trait measures along an environmental gradient. *Funct Ecol* 27:1254–1261
- Knops JMH, Reinhart K (2000) Specific leaf area along a nitrogen fertilization gradient. *Am Midl Nat* 144:265–272
- Laliberté E, Shipley B, Norton DA, Scott D (2012) Which plant traits determine abundance under long-term shifts in soil resource availability and grazing intensity? *J Ecol* 100:662–677
- Lanta V, Klimešová J, Martincová K, Janeček Š, Doležal J, Rosenthal J, Lepš J, Klimeš L (2011) A test of the explanatory power of plant functional traits on the individual and population levels. *Perspect Plant Ecol Evol Syst* 13:189–199
- Lavorel S, Grigulis K (2012) How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *J Ecol* 100:128–140
- Lavorel S, Grigulis K, Lamarque P, Colace M-P, Garden D, Girel J, Pellet G, Douzet R (2011) Using plant functional traits to understand the landscape distribution of multiple ecosystem services. *J Ecol* 99:135–147
- LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89:371–379
- McDonald PG, Fonseca CR, Overton JM, Westoby M (2003) Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? *Funct Ecol* 17:50–57
- McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. *Trends Ecol Evol* 21:178–185
- Messier J, McGill BJ, Lechowicz MJ (2010) How do traits vary across ecological scales? A case for trait-based ecology. *Ecol Lett* 13:838–848
- Milchunas DG, Lauenroth W (1995) Inertia in plant community structure: state changes after cessation of nutrient-enrichment stress. *Ecol Appl* 5:452–458
- Minden V, Kleyer M (2011) Testing the effect-response framework: key response and effect traits determining above-ground biomass of salt marshes. *J Veg Sci* 22:387–401
- Oksanen JF, Blanchet G, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) Vegan: community ecology package. R package version 2.0–7. <http://cran.rproject.org/web/packages/vegan/index.html>
- Ordoñez JC, Van Bodegom PM, Witte J-PM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Glob Ecol Biogeogr* 18:137–149
- Pakeman RJ (2011) Multivariate identification of plant functional response and effect traits in an agricultural landscape. *Ecology* 92:1353–1365
- Poorter H, De Jong R (1999) A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytol* 143:163–176
- R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Reich PB, Walters M, Ellsworth D (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecol Monogr* 62:365–392
- Schellberg J, da Silveira Pontes L (2012) Plant functional traits and nutrient gradients on grassland. *Grass Forage Sci* 67:305–319
- Smith MD, Knapp AK, Collins SL (2009) A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. *Ecology* 90:3279–3289
- Suding KN, Goldstein LJ (2008) Testing the Holy Grail framework: using functional traits to predict ecosystem change. *New Phytol* 180:559–662
- Suding KN, Goldberg DE, Hartman KM (2003) Relationships among species traits: separating levels of response and identifying linkages to abundance. *Ecology* 84:1–16
- Suding KN, Collins SL, Gough L, Clark CM, Cleland EE, Gross KL, Milchunas DG, Pennings SC (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Natl Acad Sci* 102:4387–4392
- Suding KN, Lavorel S, Chapin FS, Cornelissen JHC, Diaz S, Garnier E, Goldberg DE, Hooper DU, Jackson ST, Navas ML (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biol* 14:1125–1140

- Venables WN, Ripley BD (2002) *Modern applied statistics with S*, 4th edn. Springer, New York
- Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the concept of trait be functional! *Oikos* 116:882–892
- Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson P, Schindler DW, Schlesinger WH, Tilman GD (1997a) Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl* 7:737–750
- Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997b) Human domination of Earth's ecosystems. *Science* 277:494–499
- Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol Appl* 20:5–15
- Walker B, Kinzig A, Langridge J (1999) Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. *Ecosystems* 2:95–113
- Webb CT, Hoeting Ja, Ames GM, Pyne MI, LeRoy Poff N (2010) A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecol Lett* 13:267–283
- Westoby M, Wright IJ (2006) Land-plant ecology on the basis of functional traits. *Trends Ecol Evol* 21:261–268
- Wright IJ, Reich PB, Westoby M (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Funct Ecol* 15:423–434
- Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Garnier E, Hikosaka K, Lamont BB, Lee W, Oleksyn J, Osada N, Poorter H, Villar R, Warton DI, Westoby M (2005) Assessing the generality of global leaf trait relationships. *New Phytol* 166:485–496
- Yang H, Li Y, Wu M, Zhang ZHE, Li L, Wan S (2011) Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. *Global Change Biol* 17(9):2936–2944