LETTER

Life-history constraints in grassland plant species: a growth-defence trade-off is the norm

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Abstract

Plant growth can be limited by resource acquisition and defence against consumers, leading to contrasting trade-off possibilities. The competition-defence hypothesis posits a trade-off between competitive ability and defence against enemies (e.g. herbivores and pathogens). The growth-defence hypothesis suggests that strong competitors for nutrients are also defended against enemies, at a cost to growth rate. We tested these hypotheses using observations of 706 plant populations of over 500 species before and following identical fertilisation and fencing treatments at 39 grassland sites worldwide. Strong positive covariance in species responses to both treatments provided support for a growth-defence trade-off: populations that increased with the removal of nutrient limitation (poor competitors) also increased following removal of consumers. This result held globally across 4 years within plant life-history groups and within the majority of individual sites. Thus, a growth-defence trade-off appears to be the norm, and mechanisms maintaining grassland biodiversity may operate within this constraint.

Keywords

Coexistence, competition-defence hypothesis, life history, mammalian herbivory, Nutrient Network (Nut-Net), resource limitation, tolerance, top-down bottom-up, trade-offs..

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INTRODUCTION

Plants use different strategies to maximise fitness in the face of limits to available resources and attack by consumers. Heterogeneity of both resource supply and herbivory rates in ecological communities may prevent convergence of plant strategies into a single optimal combination (Grime 1977; Tilman 1982; Herms & Mattson 1992; Wise & Abrahamson 2005). Plant strategies also reflect environmental pressures underlying the evolutionary history of existing taxa (Coley *et al.* 1985; Herms & Mattson 1992; Antonelli *et al.* 2011). Plant life history includes variance in adaptive traits, such as competitive ability for limiting resources (tolerance of low-resource levels), ability to disperse into new habitats and resistance to or tolerance of pathogens and herbivores. Predicting the constraints on, and trade-offs among, these strategies remains an active area of theoretical debate (Stamp 2003; Wright *et al.* 2004; Craine 2005; Grime 2007; Tilman 2007; Wise & Abrahamson 2007) and empirical investigation (Siemens *et al.* 2002; Ridenour *et al.* 2008; Viola *et al.* 2010; Agrawal 2011; Kempel *et al.* 2011).

The hypothesis that defence against herbivory, inclusive of resistance and tolerance, is costly has been largely borne out by experimental work, establishing one axis of a potential trade-off (reviewed in Bergelson & Purrington 1996 and Strauss *et al.* 2002; Fine *et al.* 2006; see Siemens *et al.* 2002 for an exception). Yet, while documentation of among-species trade-offs with defensive investment may be common and widespread (Kneitel & Chase 2004), there is a lack of generality in terms of which morphological traits or physiological processes are predicted to benefit from lower investment in defence. On one hand, increased investment in defence may come at the cost of competitive ability (Baldwin & Hamilton 2000; Chase *et al.* 2002; Viola *et al.* 2010). Strategies promoting competitive success vary with environment, but superior competitors are often those more thoroughly able to exploit a limiting resource, such as soil nutrients, and which can persist at the lowest levels of availability of that resource (Tilman 1982). On the other hand, increased investment in defence may result in reduced growth rate, leading to differential investment into growth or defence by species based on the environmental conditions experienced through evolutionary his-

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tory (the resource availability hypothesis; Coley *et al.* 1985; Fine *et al.* 2006; Endara & Coley 2011). Because growth rate does not necessarily correlate with success in interspecific competition (Kempel *et al.* 2011), a fundamental disparity exists between these two models. In the competition-defence trade-off (*sensu* Chase *et al.* 2002; Viola *et al.* 2010), well-defended plants invest less in mechanisms to outcompete neighbours for nutrients. In the growth-defence trade-off (*sensu* Coley *et al.* 1985; Herms & Mattson 1992), well-defended plants invest less in mechanisms to add photosynthetic tissue.

The constraints on resource allocation to various plant morphological and physiological functions, and trade-offs among these allocations, fundamentally occur at the level of individual plants, yet manifest themselves in a given environmental and community context as population trajectories (Tilman 1990). Therefore, examination of changes in species abundance, especially in the context of particular communities and environmental conditions, can reveal how these constraints and trade-offs differ across species (Tilman 1990; Meiners 2007). The covariance in limitations to plant fitness thus can be tested by examining responses of populations to the experimental removal of multiple limitations (Hawkes & Sullivan 2001; Wise & Abrahamson 2005; Viola *et al.* 2010). Although many such studies are reported from species in controlled environments (Hawkes & Sullivan 2001), responses of wild plants may differ from those of cultivated plants (Chapin 1980), and trade-offs will depend on the environment in which species are found (Kneitel & Chase 2004). Thus, replicated examinations of plant population responses to community-level manipulations of herbivory and nutrient supply *in situ* are needed to test the generality of alternative trade-offs among plant resource allocation strategies.

In an experimental removal of mammalian herbivores and nutrient resource limitations, allocation strategies are revealed by the population responses. Good resource competitors (i.e. low R^*, Tilman 1982) will decline when nutrients are added, whereas poor resource competitors (high R^*) will increase with nutrient addition. Likewise, well-defended species will decrease in abundance when mammalian herbivores are excluded, and poorly defended species will increase with mammalian herbivore removal. Combining these responses gives four potential combinations of (non-zero) responses defining the two opposing trade-offs (Fig. 1). If plant strategies are constrained by a competition-defence trade-off, species that *decline* in abundance with nutrient addition should *increase* in abundance with mammalian herbivore removal (and vice versa; dashed line Fig. 1). In contrast, where plant strategies are constrained by a growth-defence trade-off (solid line Fig. 1), species that *increase* in abundance under nutrient addition should *also increase* in abundance under mammalian herbivore removal, as these species add poorly defended tissue. At the same time, good competitor species will decrease under nutrient addition and mammalian herbivore removal alike, as investment in defence costs these plants the ability to add photosynthetic tissue at a rate matching their less well-defended neighbours. Finally, plants in some environments may respond only along one of these axes, implying that herbivory or nutrient supply is the determining selective force in plant strategy (dotted lines Fig. 1).

To empirically test the generality of competition-defence or growth-defence trade-offs in diverse naturally occurring herbaceous ecosystems, we used data from the Nutrient Network (NutNet), a coordinated distributed experiment manipulating nutrient levels and
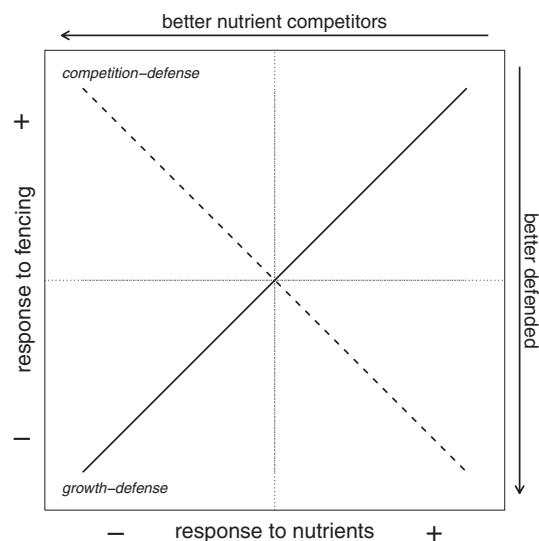

Figure 1 Hypothesised trade-offs in plant strategies revealed by environmental manipulation. By quantifying change in abundance in response to nutrient addition and fencing to exclude mammalian herbivores, the constraints on plant species allocation strategies can be inferred. If the better-defended species (benefitting least from fencing) tend to be weaker nutrient competitors (benefitting most from nutrient addition), this suggests a competition-defence trade-off (dashed line). In contrast, if the least-defended species (benefitting most from fencing) tend to be weaker nutrient competitors as well, this indicates a growth-defence trade-off (solid line). Where trade-offs do not exist, species may respond only to nutrient supply or herbivory level (dotted lines).

herbivory in grasslands. We focus on a subset of the NutNet experimental design, which includes the addition of nitrogen, phosphorus, potassium and micronutrients; fences to exclude mammalian herbivores; and no-nutrient, no-fence controls. At all NutNet sites, abundance was recorded at the species level for all vascular plants in permanent plots, prior to and annually after treatment initiation. Specifically, we investigated whether (1) the slope of species responses in aggregate indicated general competition or growth trade-offs with consumer defence, (2) trade-offs differed across plant life-history groups, (3) trade-offs differed by site and (4) variation in trade-offs by site could be explained by local environmental conditions.

MATERIALS AND METHODS

Data were compiled from 1–4 years of responses across more than three hundred and fifty 25-m^2 plots at 39 NutNet sites implementing the nutrient × fence experiment (Table 1). These plots were established in grassland sites starting in 2007, in homogenous blocks without respect to the identity of the plant species within plots. Most sites (35 of 39) implemented three blocks of 10 plots at a site, although sites varied from one to six blocks. Each block contained a full factorial combination of nutrient addition treatment (Control or All Nutrients) and consumer removal treatment (Control or Fenced) for a total of four treatments (the other six plots in a block were allocated to factorial combinations of nutrients with no fences). Fences were 120–180 cm tall, designed to exclude aboveground mammalian herbivores as appropriate at each site. To exclude digging animals, the first 90 cm of each fence was 1 cm woven wire mesh with a 30 cm outward-facing flange stapled to the

Table 1 Sites in the Nutrient Network contributing data used to estimate trade-off slopes

Site name	Latitude	Longitude	Country	Habitat	Pre-treatment year	Number years treatment	Distinct plant species*
Azi	33.67	101.87	CN	Alpine grassland	2007	4	46
Barta Brothers	42.244 586	−99.6518	USA	Mixedgrass prairie	2007	4	22
Boulder South Campus	39.972022	−105.233544	USA	Shortgrass prairie	2008	3	9
Bunchgrass (Andrews LTER)	44.27668543	−121.9680169	USA	Montane grassland	2007	4	9
Bogong	−36.874	147.254	AU	Alpine grassland	2009	2	23
Burrawan	−27.734896	151.139517	AU	Semi-arid grassland	2008	3	17
Chichaqua Bottoms	41.78506667	−93.38538333	USA	Tallgrass prairie	2009	2	15
Cedar Creek LTER	45.401	−93.201	USA	Tallgrass prairie	2007	4	15
Cedar Point Biological Station	41.2	−101.63	USA	Shortgrass prairie	2007	4	32
Cowichan	48.46	−123.38	CA	Old field	2007	4	7
Elliott Chaparral	32.875	−117.052243	USA	Annual grassland	2008	3	17
Fruebuel	47.113187	8.541821	CH	Pasture	2008	3	16
Mt Gilboa	−29.28424	30.29174	ZA	Montane grassland	2010	2	27
Hall's Prairie	36.871944	−86.70167	USA	Tallgrass prairie	2007	4	14
Hart Mountain	42.723745	−119.49767	USA	Shrub steppe	2007	4	17
Heronsbrook (Silwood Park)	51.411	−0.639	UK	Mesic grassland	2007	3	23
Hanover	43.41927	−72.13806	USA	Old field	2007	3	12
Hopland REC	39.01275 343	−123.0603134	USA	Annual grassland	2007	4	31
Kinypanial	−36.2	143.75	AU	Semi-arid grassland	2007	2	20
Konza LTER	39.070856	−96.582821	USA	Tallgrass prairie	2007	4	20
Lancaster	53.98562471	−2.628417566	UK	Mesic grassland	2008	3	9
Lookout (Andrews LTER)	44.20517707	−122.1284473	USA	Montane grassland	2007	4	6
Mclaughlin UCNRS	38.86427212	−122.4064063	USA	Annual grassland	2007	4	11
Mt. Caroline	−31.782138	117.610853	AU	Savanna	2008	3	19
Papenburg	53.086	7.4728	DE	Old field	2007	4	5
Rookery (Silwood Park)	51.406	−0.644	UK	Mesic grassland	2007	3	18
Sagehen Creek UCNRS	39.43	−120.24	USA	Montane grassland	2007	4	24
Saline Experimental Range	39.05	−99.1	USA	Mixedgrass prairie	2007	4	17
Savannah River	33.343894	−81.650936	USA	Savanna	2007	3	10
Serengeti	−2.254503	34.512613	TZ	Savanna	2008	2	19
Shortgrass Steppe LTER	40.81667	−104.76667	USA	Shortgrass prairie	2007	4	13
Sheep Experimental Station	44.242989	−112.198391	USA	Shrub steppe	2007	4	30
Sierra Foothills REC	39.23550963	−121.2836963	USA	Annual grassland	2007	4	17
Smith Prairie	48.20658068	−122.624754	USA	Mesic grassland	2007	2	31
Spindletop	38.135833	−84.500556	USA	Pasture	2007	4	10
Trelease	40.075	−88.829	USA	Tallgrass prairie	2008	3	5
Tyson	38.519092	−90.564792	USA	Old field	2007	2	10
Duke Forest	36.00828	−79.020423	USA	Old field	2007	4	20
Val Mustair	46.631345	10.372252	CH	Alpine grassland	2008	3	40

*Number of plant taxa observed at least once in both fencing and nutrient addition treatment plots within a block. CH = Switzerland; CN = China; DE = Germany; TZ = Tanzania; ZA = South Africa.

ground; fully subterranean animals may have accessed plots. Nutrients were applied at the following rates and sources annually, prior to the beginning of the growing season: 10 g nitrogen (N) m^{-2} $year^{-1}$ as time-release urea or ammonium nitrate, 10 g phosphorus (P) m^{-2} $year^{-1}$ as triple-super phosphate and 10 g potassium (K) m^{-2} $year^{-1}$ as potassium sulphate. In the first treatment year, 100 g m^{-2} of a micronutrient mix (Fe, S, Mg, Mn, Cu, Zn, B, Mo) was applied to the nutrient treatment plots, but micronutrients were not added thereafter to avoid toxicity.

Prior to treatment application, 1-m^2 quadrats were randomly selected and established in one of four sectors in each 25-m^2 plot, and the %cover of each species was recorded using a modified Daubenmire method (Daubenmire 1959). After establishing treatments, plant species %cover values were recorded annually in the same quadrat used to estimate pre-treatment cover. Data used in the analysis include 2–4 years of post-treatment data (seven sites with 2 years, 12 sites with 3 years, 20 sites with 4 years; see

Table 1). Cover was estimated independently for each species such that total summed cover could exceed 100% for multilayer canopies. Cover was estimated at peak biomass as estimated by the local site investigator, typically at the end of the growing season. At some sites with strongly seasonal communities, cover was estimated twice during the year and the maximum cover of each species in that year was used in the analyses.

Because trade-offs might not be consistent across different plant life histories, we grouped plants by life form and lifespan. We categorised species into one of four life forms: graminoid (order Poales), legume (family Fabaceae), woody (non-Fabaceae woody species) and forb (non-Fabaceae, non-Poales herbaceous species). The lifespan of each species was categorised by the investigator at each site as annual, biennial or perennial.

Additional site-level data from the NutNet experiment were tested for explanatory power of local trade-offs (see below): mean live biomass productivity (g m^{-2} $year^{-1}$) in control, fenced and

nutrient-supplemented plots (see Adler *et al.* 2011 for methods) as well as mean annual precipitation (MAP) as estimated in the WorldClim climate database (Hijmans *et al.* 2005).

Soil nutrients were also estimated prior to treatments at a majority of sites ($n = 30$). From each plot, 250 g of soil was collected, homogenised and air-dried. The Ecosystems Analysis Laboratory at University of Nebraska assayed soils for %C and %N using dry combustion GC analysis (COSTECH ESC 4010 Element Analyzer). Extractable soil P and K were extracted using the Mehlich-3 method and p.p.m concentration estimated using ICP (A&L Analytical Laboratory, Memphis, TN, USA).

We used species abundance data from plots receiving nutrients alone, or fences alone, as well as control plots, but did not consider the plots with both fences and nutrients added. While precluding analysis of any interactive effects on species or groupings, this framework allows us to focus on the theoretical question of the relationship, if any, between independent responses to nutrient addition and mammalian herbivore removal. We included only those species recorded at least once either pre- or post-treatment in both the nutrient treatment plot and the fence treatment plot within a block. Thus, we included species that disappeared from, or appeared into, focal plots over time, but we did not include species observed elsewhere in a site but never appearing in the treated plots.

We quantified the responses of plant species to the removal of limitations from nutrients and herbivores as the log response ratio of plant cover to each treatment, adjusted to the trajectory of each species in control plots. Following Viola *et al.* (2010), the global minimum observed %cover (0.01) was added to all values so that log response ratios would be informative for species present in only one of the compared years. Baseline cover was defined as the abundance within a plot for a plant species prior to treatment application (%cover$_{y0}$). The log ratio response (LRR) of the species in year t in the plot is then $\ln[(\%cover_{yt})/(\%cover_{y0})]$. The LRR in control plots was subtracted from the LRR for each treatment plot in that year ($LRR_{treatment\ yt} - LRR_{control\ yt}$), so that the final response variables represent the difference in species response from the ambient control environment. These responses were calculated at the plot level within each block, and then averaged across blocks to give the mean response by species within sites. These species-site responses were the units of observation for our analysis.

We used standardised major axis regression (SMA) to quantify the relationship between response to nutrient addition and response to fencing across plant species. In contrast to linear regression models, SMA does not assume a causal relationship between related variables and is therefore appropriate to quantify the association of variables when both might respond to similar underlying processes (Warton *et al.* 2006). We used the 'smatr' package in R version 2.14 (R Development Core Team 2011) to quantify slopes for the relationship between response of species to fencing and response to nutrient addition. We further tested these slopes for differences from zero and then used likelihood ratio tests for homogeneity within three different groupings: life form, lifespan and site (Warton *et al.* 2006).

To quantify the proportion of the species exposed to the treatments that responded to them, and how those responses were distributed, we classified each species in a site in a year by whether it responded to (1) neither treatment, (2) fence only, (3) nutrient addition only or (4) responded independently to both nutrients and fences ('trade-off'). Responses were tallied if the species %cover either increased or decreased following treatment. We further

divided the trade-off category into species with positively correlated responses ('growth-defence') and species with negatively correlated responses ('competition-defence'). To allow for natural variation and account for possible sampling error, we used a doubling/halving threshold for counting a species as responsive, meaning LRR was > 0.693 or < −0.693 (corresponding to ln[2/1] and ln[1/2] respectively). This conservative threshold allows for considerable natural variation in plant %cover before assigning a response to the treatment. We also tested the sensitivity of our conclusions to threshold values much lower and higher than the cut-off we chose, and we found them robust to thresholds below LRR of 1.3, or a change nearly quadruple the original cover value (Fig. S2). Following categorisation, we tested for the equivalence of the positive and negative trade-off categories using chi-square, under the null hypothesis that responding species would occur equally in all four quadrants of the trade-off space (as defined in Fig. 1).

Finally, following estimation of SMA slopes by site, we used regression tree analysis (De'ath & Fabricius 2000) to determine whether background ecological processes or environmental conditions explained variance in slopes across sites in each year. Regression trees split observations into homogeneous groups based on explanatory variables, revealing the predictors explaining the most variation in the response even in nonlinear data sets (De'ath & Fabricius 2000). Using the SMA slope within sites as the response variable, we tested a tree model with the following explanatory variables, all as site means: mean site productivity (g m^{-2} year^{-1}); site mean log response of biomass to fencing (unitless); site mean log response of biomass to nutrient addition (unitless); MAP (mm); soil %C; soil %N; soil P (p.p.m) and soil K (p.p.m). We used package 'rpart' in R v 2.14 for this analysis.

RESULTS

We observed a total of 706 unique site-species combinations represented in the nutrient addition and fenced plots across 4 years of

Table 2 Within life-form estimates and significance of trade-off slopes by years of treatment

Life-form group	Treatment year	N	SMA slope*	SMA P value†	R^2
Forb	1	283	1.07	<0.001	0.14
	2	272	1	<0.001	0.15
	3	167	1.01	<0.001	0.2
	4	120	0.89	<0.001	0.27
Graminoid	1	234	0.98	<0.001	0.16
	2	217	0.97	<0.001	0.09
	3	147	0.93	<0.001	0.23
	4	104	0.88	<0.001	0.22
Legume	1	47	1.17	0.0067	0.15
	2	45	1.22	<0.001	0.33
	3	30	0.96	0.0001	0.45
	4	22	1.37	<0.001	0.11
Woody	1	10	1.05	0.0069	0.62
	2	12	1.35	0.4808	0.05
	3	9	1.54	0.1765	0.24
	4	4	0.8	0.1987	0.64

*Standard major axis regression (SMA) slope of response to fencing vs. response to nutrient addition.
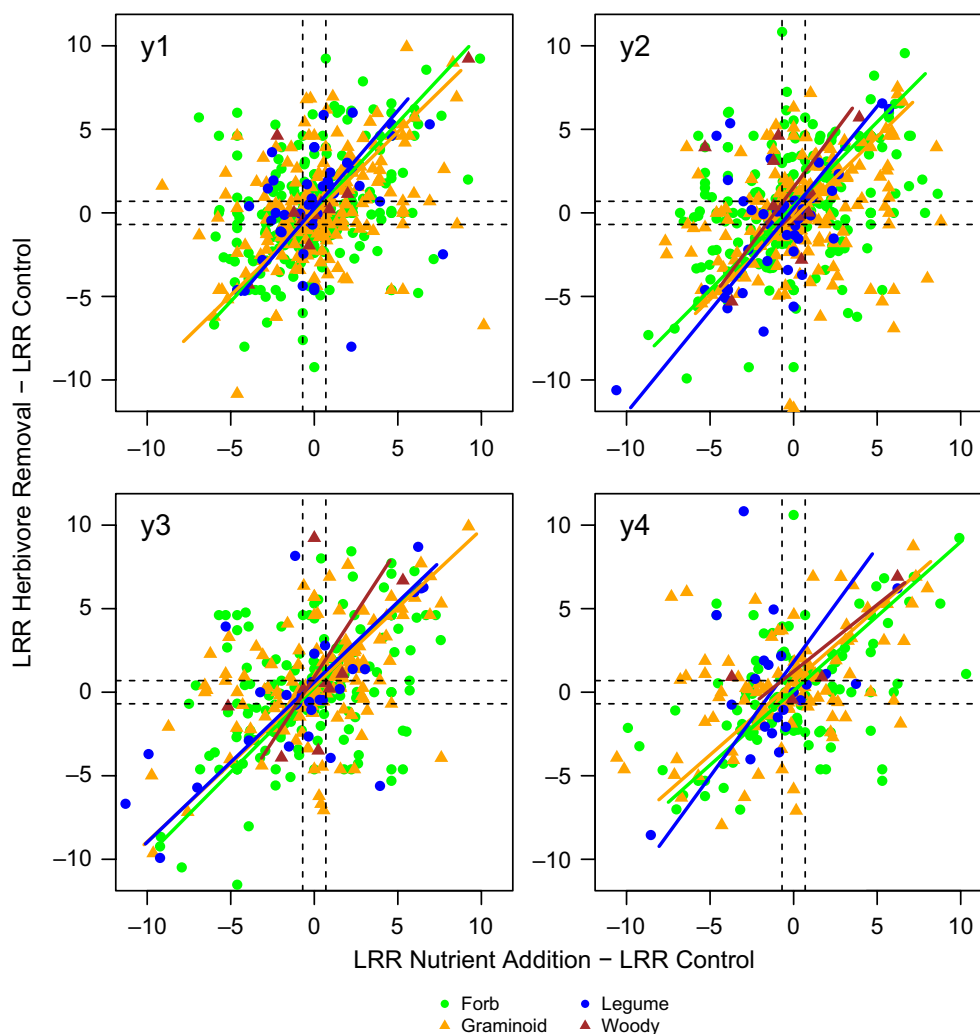†Probability of observing SMA slope given null hypothesis that slope is actually zero.

Figure 2 Plant species response by life form and years of treatment to the removal of two key limitations (nutrient availability and mammalian herbivory). Points are site-species means of the log response ratio of abundance (%cover) of plant species naturally present in 1-m^2 plots after 1–4 years of treatment compared with baseline (pre-treatment) abundance in the same plot, corrected by subtracting the ratio from control plots. Plant species included in the analysis were naturally present in one plot of each treatment within a block. Standardised major axis regression (SMA) slopes significantly different from zero ($P < 0.05$) are shown as solid lines by life form. Dashed lines indicate areas along each axis classified as zero response.

treatment, though replication differed across years based on the year each site originated the experiment ($n = 574$ in year 1, $n = 546$ in year 2, $n = 353$ in year 3, $n = 250$ in year 4). These comprised observations of 514 distinct taxa, 90% of which were only observed at one or two sites. Ten species were observed at more than four sites cumulatively across the 4 years, and the maximum number of sites for any one species was nine (Table S3). Globally, grassland plant species response to the removal of mammalian herbivory was significantly, positively associated with response to the removal of nutrient limitation in each year (Fig. 2; year 1 slope = 1.04, $P < 0.001$, $R^2 = 0.16$; year 2 slope = 1.01, $P < 0.001$, $R^2 = 0.14$; year 3 slope = 0.99, $P < 0.001$, $R^2 = 0.24$; year 4 slope = 0.91, $P < 0.001$, $R^2 = 0.23$), supporting a growth-defence trade-off. Slopes did not differ significantly by life form in any year (all $\chi^2_{\text{d.f.} = 3} < 1.5$, $P > 0.05$), and although groups differed in the slope and significance of the relationship, slopes were positive within each life form across years (Table 2; Fig. 2). With only one exception (biennial plants in year 2), species responses by lifespan (annual,

biennial, perennial) were significantly, positively correlated throughout the duration of the experiment (Table S4; Fig. S5). There was no significant difference in SMA slope among lifespan types in any year (all $\chi^2_{\text{d.f.} = 2} < 1.5$, $P > 0.05$).

In each year, most plants (47–60%) independently responded to both treatments (Fig. 3). Of those plants that responded to both treatments with changes in per cent cover greater than our response threshold ($^{\vert}\text{LRR}^{\vert} > 0.693$), those having positively correlated responses – consistent with a growth-defence trade-off – were significantly more numerous than those having negatively correlated responses (Fig. 3; all $\chi^2_{\text{d.f.} = 1} > 23$, $P < 0.001$). Between 10 and 20% of plants in each year showed no response to either treatment ($^{\vert}\text{LRR}^{\vert} < 0.693$), meaning these plants were present in similar abundance before and after 1–4 years of treatment.

Slopes within sites were largely clustered around a value near 1, and statistically homogeneous (year 1 $\chi^2_{37} = 48.58$, $P = 0.10$; year 2 $\chi^2_{38} = 28.88$, $P = 0.10$; year 3 $\chi^2_{29} = 41.18$, $P = 0.07$; year 4 $\chi^2_{19} = 21.48$, $P = 0.31$). Within each year, a few sites had negative
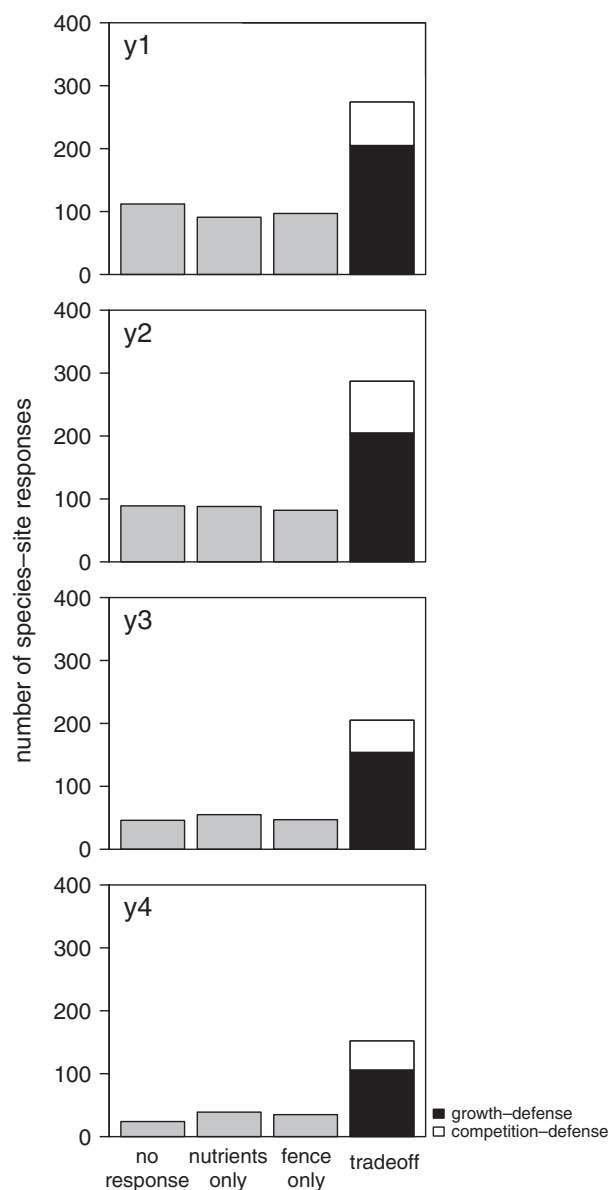
Figure 3 Frequency of species responses and non-responses to nutrient addition and fencing treatments following 1–4 years of treatment. Species were classified as having 'no response' to a treatment if the corrected log response ratio was > −0.693 (halving) or < 0.693 (a doubling). Species with responses outside this boundary along an axis (dashed lines Fig. 2) were considered responsive to that treatment. Species responsive to both treatments were classified as 'trade-off' species; if a response was correlated positively, the response was categorised as growth-defence trade-off, and if negatively correlated as a competition-defence trade-off. In each year, growth-defence trade-offs were significantly more prevalent than would be expected by chance ($\chi^2_{\text{d.f.} = 1} > 23$, $P < 0.001$).

SMA slope estimates (Fig. 4, Table S6), but in general, only positive within-site SMA slopes were significantly different from zero ($P < 0.05$). One exception occurred in year 2, when one single site had a significantly negative slope, though the slope was derived from only five species responses, and this site did not have a significant trade-off slope in any other year (Table S6).

The homogeneity of within-site trade-off slopes allowed little variance to be explained by the site-level covariates. For the 30 sites with available soil data, regression tree analysis identified no

site-level variables explaining significant variance in species response slopes (data not shown).

DISCUSSION

Evidence from our globally distributed network of grassland experiments clearly supports the growth-defence trade-off over the alternative nutrient competition-defence trade-off. Overall, within plant life-history types, and within sites, species responses to nutrient addition and to mammalian consumer removal were largely positively correlated. In each year, many more species responded to both treatments than were unaffected or responded to only one treatment, and a significant majority of those species had positively correlated responses (positive for both treatments or negative for both treatments). Because these data capture the dynamics of 514 plant species in naturally occurring plant communities, they provide robust support for the hypothesis that species profiting most from alleviation of nutrient limitation pay the price in vulnerability to consumers – a model of negative correlation between growth and defence.

Because we are utilising data from an experiment not explicitly designed to answer species-level questions, we evaluated the robustness of our results to the observed species that were included using two data reduction ('jacknife') approaches. First, we randomly sampled a third of the observed species-site responses, calculated the SMA slope and significance as above, and repeated this process 100 times. Second, we reduced the data to include only the top third of species in terms of pre-treatment %cover (roughly corresponding to a 5% cover threshold), and calculated the SMA slope and significance as above. In both cases, the slopes were significantly positive and centred around one (Fig. S7). We thus conclude that our results supporting the growth-defence trade-off are unlikely to be due to sample bias.

The growth-defence trade-off is consistent with the resource availability hypothesis (Coley *et al.* 1985), which bases predictions on the abiotic environmental context in which species have evolved. That is, species that arose in a resource-rich environment intrinsically should grow faster, with faster leaf turnover and higher tolerance to herbivory. Conversely, species should be well-defended against herbivory and grow more slowly if they evolved in low-nutrient environments. Studies in woody plants of tropical forest understory (Fine *et al.* 2006) and in a clade of temperate forbs (Mooney *et al.* 2010) provide strong support for this hypothesis, with a broader group of temperate herbaceous plants providing mixed support (Van Zandt 2007). Although it appears true that '[a successful] adaptation to a low-resource environment is to have a low demand for resources' (Endara & Coley 2011), the evidence for the universality of the resource availability hypothesis with respect to herbivory has been more equivocal. A further challenge for ecologists is that plants (and plant communities) are commonly limited by multiple resources, and the relative importance of these resources can change in space and time (Harpole *et al.* 2011). These shifts can, in turn, influence the optimal level of investment in defence, in some cases favouring no defence at all (Ito & Sakai 2009). Our results suggest that costs from both nutrient limitation and mammalian herbivory are pervasive in grassland environments.

Nutrient addition experiments are made both more important and more challenging by anthropogenic changes to 'ambient' nutrient availability, particularly nitrogen and phosphorus, in natural systems (Rockström *et al.* 2009), which may alter soil nutrient levels
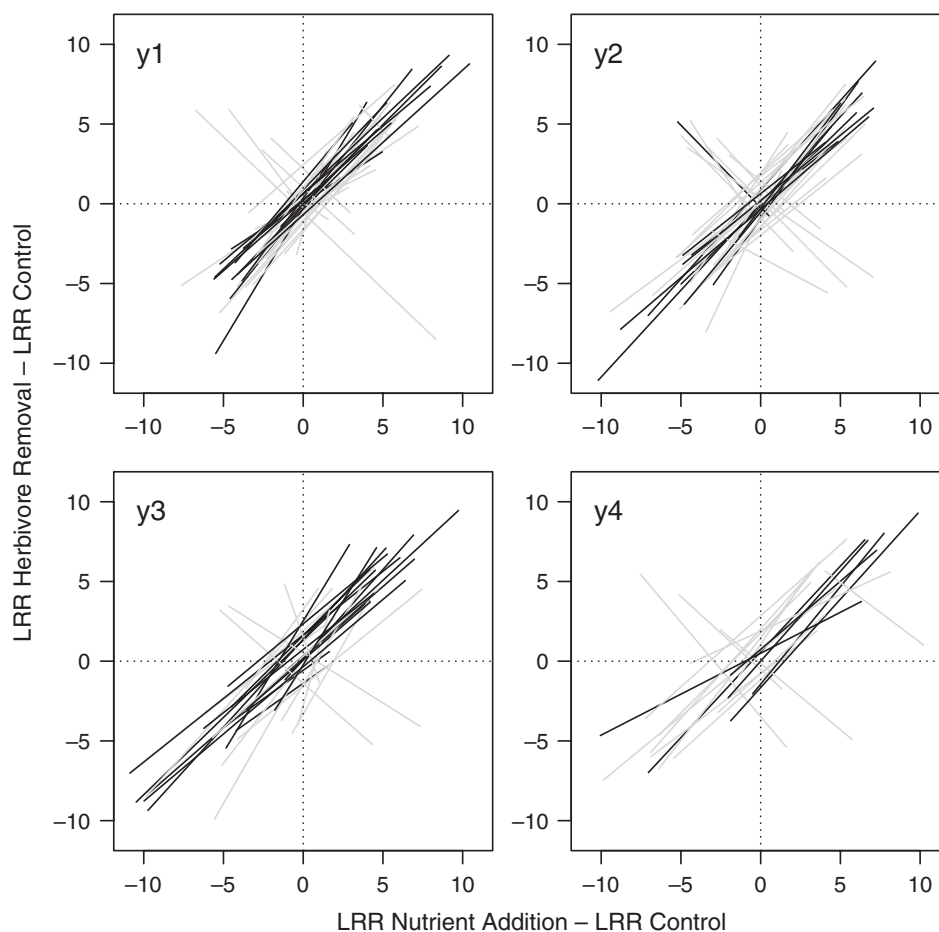
Figure 4 Plant trade-off slopes within sites following 1–4 years of treatment. Each line is the standardised major axis regression (SMA) slope of responses of plant species to fencing and nutrient addition within a site. Observation points (species responses within sites, Fig. 2) have been omitted for clarity. Dark lines represent sites with SMA slopes significantly different from zero ($P < 0.05$); grey lines represent sites with slopes not different from zero ($P \geq 0.05$). See Table S6 for slope estimates and significances by site.

from those in which plant strategies have evolved. However, experimental manipulation of nutrients, especially the high inputs compared with anthropogenically modified levels used here, should nonetheless inform the ultimate question of how species responses to changing nutrient availability are trading off with defence against herbivory.

The positive correlation in species responses we observed was consistent across 4 years of treatment. Nonetheless, the response measurement we are using here (change in population abundance through 4 years) may not reflect longer term dynamics of colonisation and local extirpation, and it may be that with the continued treatment application the trade-off axis may shift. For example, in a longitudinal study of abandoned agricultural field succession, species population growth rates in plots slowed as the community shifted to dominance by perennial and especially woody species over decades (Meiners 2007). Continued application of this high nutrient availability and removal of mammalian herbivory may drive similarly drastic changes in community composition over the long term. Such changes may also depend on how the strength of the responses relate to the abundance of species, as responses of common species may carry more ecological weight.

Although we did not observe much site-to-site variation in the constraints on plant strategies, environmental variation in resource

supply and herbivory are both thought to influence these trade-off axes. Shifts in the factors defining plant trade-offs can also occur via herbivory itself. The limiting resource model of Wise & Abrahamson (2005, 2007) predicts that the ability of plant species to tolerate herbivory depends on the identity of the limiting resources and the impact of herbivory on these resources. In grasslands, both herbivory and lack of nutrients can be limiting to plants, but herbivory removes above-ground tissue which primarily impacts an alternate resource (light and thus carbon supply). Under this model, the key question is whether the alternate resource impacted by herbivory is also limiting to plant growth (Wise & Abrahamson 2007). If available light is limiting under high nutrient supply (Hautier *et al.* 2009), the limiting resource model predicts that tolerance to herbivory should be lower in high nutrient conditions (Wise & Abrahamson 2007). Notably, this would also suggest the addition of an orthogonal major axis of plant–plant competition, of light or light : nutrient ratios.

The lack of a universal trade-off between the ability to compete for scarce nutrients and defend against mammalian herbivory (dashed line, Fig. 1) seems counter to commonly accepted mechanisms for maintaining species diversity, in which consumers preferentially increase mortality in those species that otherwise would become competitively dominant (Chase *et al.* 2002; Terborgh 2012).

In a meta-analysis across ecosystems, Hillebrand *et al.* (2007) found in terrestrial habitats that fertilisation decreased and herbivory increased plant diversity. Based on this meta-analysis, Viola *et al.* (2010) tested for a competition-defence trade-off between consumer resistance and nutrient competition in plant species. They found only a third of studies exhibited the negative relationship they predicted, whereas the overall trend was for a positive association (Viola *et al.* 2010).

In this context, it was surprising that we did not detect differential trade-off relationships among plant life-form groups. A meta-analysis of studies, where single species were factorially exposed to nutrient fertilisation and herbivory, demonstrated that graminoids responded with greater tolerance to herbivory and higher growth under enriched conditions (Hawkes & Sullivan 2001). These and other authors (e.g. Diaz *et al.* 2007) hypothesise that plants with basal meristems are better suited to tolerate and compensate for mammalian herbivory than most forbs, which actively grow from apical meristems that are more exposed to herbivory.

One limitation of this study is that we based our investigation of plant defence on responses exclusively to mammalian herbivory. However, plants face an array of other enemies from fungal, bacterial and viral pathogens to specialist and generalist invertebrate herbivores; in some ecosystems, these enemies can equal or exceed effects of vertebrate herbivory, though invertebrate effects may take more time to accumulate (Allan & Crawley 2011). This diversity of consumers likely maintains a complex suite of plant defence and tolerance strategies, and the higher specificity of these interactions may make it less likely that any one strategy will achieve broadly effective defence (Kotanen & Rosenthal 2000).

In our effort to determine the generality of trade-offs in natural herbaceous plant communities, we did not quantify the traits mediating plant defence. Specifically, we lack the data to examine trade-offs among types of defences within species (Kempel *et al.* 2011). However, plant defences may be understood best in a multivariate framework as suites of potentially redundant strategies (Agrawal & Fishbein 2006). For example, low tissue nutrient content on its own can be as effective in reducing herbivory as physical and chemical defence of more nutrient-rich tissue (Agrawal & Fishbein 2006). Leaf traits such as nutrient ratios, area, thickness and toughness are predictive of functions (including growth), ecological habit and herbivory (Wright *et al.* 2004; Diaz *et al.* 2007; Schuldt *et al.* 2012), suggesting consistent evolutionary trade-offs between resource acquisition, deployment into growth and defence of acquired resources. Additional data on the nutrient content, morphology and defensive characteristics of the plants in the NutNet experiment may provide a mechanistic understanding of this growth-defence trade-off.

The strength and ubiquity of the growth-defence trade-off we have demonstrated here suggests an important question: how can poor nutrient competitors that are susceptible to mammalian herbivory persist at all? Clearly, other factors critical to plant success must be contributing to coexistence. Environmental heterogeneity is one likely candidate, as such heterogeneity can act as a stabilising mechanism (*sensu* Chesson 2000) and reduce competitive exclusion in communities where species differ in their response to environmental gradients (i.e. their niche). In this case, species will be more strongly limited by intraspecific competition than interspecific competition in the habitats where they have the highest fitness (Chesson 2000). With patchily distributed plants and resources in soils, differences in the rate of exploitation of nutrient resources, rather than the ultimate level of depletion tolerated by a given species, can also facilitate coexistence (Bolker & Pacala 1999). Grime's (1977) 'ruderal' strategy and Tilman's (1994) competition-colonisation model also emphasise the importance of dispersal away from sites where a plant species may be a poor competitor. Thus, local plant community diversity is likely maintained by spatial heterogeneity combined with the growth-defence trade-off, which represents a fundamental fitness constraint in terrestrial herbaceous plants.

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AUTHORSHIP

See Table S1 for full matrix of author contributions.

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