



Belowground Biomass Response to Nutrient Enrichment Depends on Light Limitation Across Globally Distributed Grasslands

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ABSTRACT

Anthropogenic activities are increasing nutrient inputs to ecosystems worldwide, with consequences for global carbon and nutrient cycles. Recent meta-analyses show that aboveground primary production is often co-limited by multiple nutrients; however, little is known about how root production responds to changes in nutrient availability. At twenty-nine grassland sites on four continents, we quantified shallow root biomass responses to nitrogen (N), phosphorus (P) and potassium plus micronutrient enrichment and compared below- and aboveground responses. We hypothesized that optimal allocation theory would predict context dependence in root biomass responses to nutrient enrichment, given variation among sites in the resources limiting to plant growth (specifically light versus nutrients). Consistent with the predictions of optimal allocation theory, the *proportion* of total biomass belowground declined with N or P addition, due to increased biomass aboveground (for N and P) and decreased biomass belowground (N, particularly in sites with low canopy light penetration). *Absolute* root biomass increased with N addition where light was abundant at the soil surface, but declined in sites where the grassland canopy intercepted a large proportion of incoming light. These results demonstrate that belowground responses to changes in resource supply can differ strongly from aboveground responses, which could significantly modify predictions of future rates of nutrient cycling and carbon sequestration. Our results also highlight how optimal allocation theory developed for individual plants may help predict belowground biomass responses to nutrient enrichment at the ecosystem scale across wide climatic and environmental gradients.

Key words: belowground biomass; fertilization; nitrogen; Nutrient Network; optimal allocation; phosphorus; roots.

HIGHLIGHTS

- Both N and P addition reduced the proportion of total biomass in shallow roots

Received 2 August 2018; accepted 21 January 2019;
published online 11 March 2019

Electronic supplementary material: The online version of this article (<https://doi.org/10.1007/s10021-019-00350-4>) contains supplementary material, which is available to authorized users.

Author Contributions EEC analyzed the data and wrote the paper with input from all co-authors. All co-authors contributed to data collection.

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- N addition decreased roots most where there was low light beneath the canopy
- These results show plant allocation to roots vs shoots depends on limiting resources

INTRODUCTION

Grasslands and other herbaceous plant communities cover 20–40% of the terrestrial land surface (Leith 1978), provide critical ecosystem services such as rangeland forage, and play an important role in the global carbon (C) cycle, with grassland soils containing up to 30% of the world's soil C (Anderson 1991). Across the world's biomes, grasslands have some of the highest fractions of total biomass as roots (Poorter and others 2012). There is large variation in partitioning of biomass and productivity across sites; however, for instance, Sims and Singh (1978) estimated between 24 and 87% of net primary production was belowground across ten North American grassland sites, and Hui and Jackson (2006) found similar levels of variation across grasslands worldwide (40–86%). This variation in the proportion of growth allocated belowground is important not only for regional estimates of primary production and C sequestration (Scurlock and Hall 1998; Mokany and others 2006) but also for understanding ecosystem responses to global change (Friedlingstein and others 1999; Jackson and others 2000).

Anthropogenic activities are increasing global nutrient availability, with effects on net primary production (Elser and others 2007), plant allocation above- and belowground (Poorter and others 2012), and net ecosystem C balance (Mack and others 2004). Fossil fuel combustion and agricultural intensification have doubled annual nitrogen (N) inputs into terrestrial ecosystems and have increased phosphorous (P) inputs more than fourfold (Falkowski and others 2000). Shifts in C balance resulting from nutrient enrichment could depend on allocation above- versus belowground (Friedlingstein and others 1999; Smithwick and others 2014). High proportional allocation to root biomass increases the potential for ecosystem C sequestration because root-derived C is more likely to enter long-lasting soil organic C pools than C from aboveground tissues (Rasse and others 2005), and roots can promote physical stabilization of soil organic matter via soil aggregate formation (Jastrow 1996).

Optimal allocation theory, developed for individual plants, predicts that plant allocation below-

ground should depend on the identity of the most growth-limiting resource (Thornley 1972; Bloom and others 1985; Wilson 1988). Specifically, proportional root allocation should decline when plant growth is limited by aboveground resources (for example, light) and increase when plant growth is limited by belowground resources such as water and nutrients (Gleeson and Tilman 1992). A recent meta-analysis summarizing the results of nearly 800 experimental manipulations of resource availability found strong support for optimal allocation theory; the proportion of biomass allocated to roots was higher under water or nutrient limitation, and lower under light limitation (Poorter and others 2012). Most of these studies were focused at the species level, and if there is significant interspecific variation in allocation responses to environmental change (Craine and others 2003), then the predictions of optimal allocation theory might not explain community-level variation in root allocation. However, patterns observed across environmental gradients also support the hypothesis that community-level allocation to roots declines as belowground resources increase. For instance, proportional root allocation in grasslands is inversely correlated with mean annual precipitation and is highest in xeric regions where water is the predominant factor limiting plant growth (Hui and Jackson 2006; Mokany and others 2006).

Although many studies have evaluated how allocation responds to variation in individual environmental factors, few have evaluated how allocation responds when multiple factors change simultaneously. This is a critical knowledge gap because primary production is frequently co-limited by multiple resources as opposed to single resources (Hooper and Johnson 1999; Elser and others 2007; Harpole and others 2011; Fay and others 2015) and ecosystem responses to multiple aspects of global change often deviate from predictions based on single factor experiments (Norby and Luo 2004). The importance of community-scale biomass partitioning for understanding regional and global C budgets (Scurlock and Hall 1998; Jackson and others 2000; Smithwick and others 2014) underscores the need for a framework that effectively predicts both the absolute quantities and proportion of biomass above- versus belowground, in response to global changes such as eutrophication. Further, although regional and global estimates of total net primary production generally rely on modeled estimates of root allocation (Friedlingstein and others 1999; Woodward and Osborne 2000; Gill and others 2002; Michaletz and others 2014), these estimates are rarely vali-

dated because continental and global relationships between biomass allocation and climate and soil variables remain poorly characterized (Smithwick and others 2014).

To evaluate how community-scale root biomass production and allocation respond to local experimental nutrient enrichment across environmental gradients, we leveraged a global network of grassland sites where nutrient availability was manipulated using common protocols, the Nutrient Network (Borer and others 2014a). By using this experimental network that spans a broad range of climates and grassland soils, we characterized both global trends in allocation patterns in response to eutrophication as well as regional contingencies in this response. Prior efforts from this network have demonstrated that aboveground primary production across these sites is frequently co-limited by multiple nutrients (Fay and others 2015) and that the impact of soil nutrients on species richness depends on light limitation (Borer and others 2014b); however, belowground biomass responses to multiple nutrient enrichment have not yet been evaluated.

Here, we refer to “biomass allocation” as reflecting static pools of biomass, distinct from efforts aimed at identifying the dynamic partitioning of new photosynthates (*sensu* Poorter and others 2012, also discussed in Reich 2002, and alternatively referred to as “biomass distribution” in Reich and others 2014). We focus on root responses near the soil surface (top 10 cm), because 80–90% of root biomass in grasslands is concentrated near the surface, in the top 30 cm (Jackson and others 1996). Surface roots play a disproportionate role in nutrient acquisition because the greatest concentrations of N, P, and K are found high in soil profiles (Sposito 1989; Jobbagy and Jackson 2001), and both experimental and anthropogenic nutrient inputs occur at the soil surface. Furthermore, grasslands store the greatest proportion of soil C near the soil surface (Jobbagy and Jackson 2000), contributing to greater microbial biomass (Blume and others 2002; Eilers and others 2012) and fueling greater microbial activity in surface versus subsurface soils. Hence, C pools with potential for high turnover and release to the atmosphere are likely most sensitive to fertilization at shallow depths. Accordingly, a meta-analysis of 257 studies across a variety of ecosystems found that N addition tended to reduce carbon stocks in shallow but not deep soil layers, correlated with a decline in root allocation in shallow soil layers (Lu and others 2011).

Across the Nutrient Network sites, we hypothesized that (1) *absolute* belowground biomass would respond positively and synergistically to the addi-

tion of multiple nutrients, consistent with patterns of multiple nutrient limitation of aboveground plant biomass observed across these sites (Fay and others 2015). We expected that *relative* biomass allocation to roots (root biomass as a proportion of total biomass) would (2) decline with increasing light limitation (associated with low light availability below the grassland canopy, for example, Gleeson and Tilman 1992), (3) increase with increasing water limitation (in more arid sites, for example, Hui and Jackson 2006), and (4) decrease with nutrient enrichment particularly when multiple nutrients are added together (Yuan and Chen 2012), as predicted by optimal allocation theory. Finally, we expected that (5) there might be statistical interactions among the factors predicting belowground biomass and allocation, due to the importance of environmental context in determining community responses to resource enrichment (Cleland and Harpole 2010). Specifically, we expected that root biomass responses to nutrient enrichment would be constrained in sites where plant growth was limited by water (more arid sites) and that root biomass might even decline with nutrient addition at sites where there is strong competition for light, in favor of increased allocation to aboveground biomass.

METHODS

This research was conducted within the Nutrient Network, a globally replicated network of sites manipulating nutrients (nitrogen N, phosphorus P, and potassium plus micronutrients K_{μ}) and vertebrate herbivore exclusion (Borer and others 2014a). The micronutrients were only added in year one and included Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn. For the effort described here, we analyzed data from 29 sites where the experimental treatments had been applied for 3–5 years. At most sites, plots were arranged in three blocks, each block containing the ten focal treatments: control unfenced & unfertilized, +N, +P, + K_{μ} , +NP, + NK_{μ} , + PK_{μ} , +NPK $_{\mu}$, fenced & unfertilized, and fenced +NPK $_{\mu}$. At each site, 30 plots (each 5 x 5 m) were sampled, except where noted in Supplementary Material, resulting in 874 plots sampled in total. For this manuscript, only data from the factorial nutrient addition treatments were analyzed (that is, all fenced plots were excluded). The sites span four continents and, more importantly, wide environmental gradients in mean annual precipitation (274–2314 mm/year, summarized in Table S1). All sites are dominated by herbaceous

vegetation but vary in the relative abundance of graminoids versus other functional types (Table S1). Vegetation types included, for instance, alpine meadows, prairie, pasture, savannah, and steppe, but we refer to these sites as grasslands for brevity.

Above- and belowground biomass was collected at the time of peak biomass in either 2011 (Northern Hemisphere) or early 2012 (Southern Hemisphere). According to Nutrient Network protocols (Borer and others 2014a), aboveground biomass was destructively harvested in two 1 m x 0.1 m strips per experimental plot, sorted to separate the current year's production from litter, dried to constant mass, and weighed to the nearest 0.01 g. Immediately following the aboveground biomass harvest, five soil cores were taken to a depth of 10 cm in the harvest area. Root cores were collected using standard corers or sharpened PVC tubes with an inside diameter of 2.5 cm, for a total ground area of 24.5 cm². Exceptions to this protocol are noted in the Supplementary Material. All cores from each plot were combined in one sealed plastic bag, packed into coolers with cold packs, and sent via next day air to a central processing laboratory (USGS at Corvallis, Oregon, USA).

Total soil weights for each bulked sample were recorded, and a homogenized subsample comprising 1/5th of the total soil weight (20–150 g) was weighed and sent to the University of California, San Diego, for root extraction. Soil subsamples were kept cool with icepacks throughout transit and refrigerated while in the laboratory until processing.

Live root biomass was estimated using a modification of the standard Long Term Ecological Research method for measuring standing fine root biomass in soil cores (Bledsoe and others 1999). Soil subsamples were immersed in water; live roots were light in color and floated to the surface, whereas dead roots and organic matter were darker in color. Live roots were extracted with tweezers, rinsed to remove residual mineral soil, dried to a constant mass, and weighed to the nearest 0.001 g. Above- and belowground (to 10 cm) biomass estimates were expressed on a common scale (g/m²). Our key metric of proportional biomass allocation is the root mass fraction (RMF) following the method in Reich (2002). The RMF was calculated as the root biomass divided by the sum of root and aboveground live biomass on an equal area basis. Detailed methods are provided in Supplementary Material.

Our estimates of belowground biomass are based on one-time destructive harvests at the time of

peak biomass; while this reflects a reasonable estimate of aboveground production, this is an underestimate of belowground production (Gill and others 2002). Hence, we proceed with the caveat that this effort documents comparable patterns of shallow root biomass and allocation across plots and sites, but additional estimates of root turnover and deep root biomass would be needed to estimate total belowground production and allocation of net primary production. However, a survey of published and unpublished data on the distribution of root biomass at our sites shows that the majority of root biomass is captured by shallow root sampling efforts, such as ours (Table S2).

We assembled site-level metrics of water limitation and light availability at the soil surface, for inclusion as covariates in our analyses. We extracted measures of the Global Aridity Index (CGIAR-CSI Global-Aridity and Global-PET Database, Zomer and others 2008), based on data from the WorldClim database (Hijmans and others 2005). Hereafter referred to as GAI, this index is calculated as mean annual precipitation divided by mean annual potential evapotranspiration and hence accounts for both precipitation inputs and soil water loss due to high temperature, solar radiation, and wind. Low GAI indicates more arid sites with low soil water availability (low inputs and/or high rates of water loss).

Using a linear multi-sensor light meter, we measured the proportional decrease in photosynthetically active radiation (PAR) from above the canopy to below the canopy as a proxy of light limitation. The proportion of PAR reaching the soil surface was calculated as the average of two PAR measurements taken at the soil surface perpendicular to one another in a 1 m² undisturbed subplot, divided by PAR measured above the canopy immediately afterward, under full light conditions. We averaged the proportion of PAR reaching the soil surface across all years of measurement in the control plots from each site (unfenced, unfertilized) as a *site-level metric of the degree of light limitation*. This metric is abbreviated hereafter as “light.” Resource depletion is the key mechanism by which plants compete with neighbors (Goldberg 1990), and hence we use “light” as a proxy for community-level light depletion.

The fractions of the community comprised by graminoids and by perennial species were calculated as two additional site-level metrics of species composition, based on visual percent cover estimates collected in 1 × 1 m plots adjacent to the biomass harvests described above. These values

were calculated only from control plots at each site (unfenced, unfertilized).

Statistical Analysis

Data analysis was performed in R version 3.3.3 (R Core Team 2017). Pearson correlations were performed to evaluate associations among site-level parameters: aridity, light, live aboveground biomass (AGB), live belowground root biomass to 10 cm depth (BGB), RMF, graminoid fraction, and perennial fraction. Each data point in the correlation analysis was a site-level mean for each parameter, calculated for the control plots only.

Examination of the BGB data with quantile–quantile plots showed these data were lognormally distributed (Figure S2), as is common with ecological datasets involving measures of growth (Bolker 2008), and hence the BGB data were natural-log-transformed prior to analysis. The RMF data were continuous proportions bounded by 0 and 1, and as expected, initial inspection with quantile–quantile plots indicated the data were non-normally distributed (Figure S3). Following the recommendation of Warton and Hui (2011), the RMF data were logit-transformed. After transformation, BGB and RMF had normally distributed errors and were analyzed with a general linear mixed model using the lme call in the package nlme (Pinheiro and others 2013).

To evaluate the responses of BGB and RMF to the addition of individual nutrients and their combinations, N, P, and K_p were each included as factorial fixed factors, site was treated as a random factor, and light and aridity were included as site-level covariates. As described above, our metric of light availability was based on site-level mean light penetration of the grassland canopy only in control plots and hence was independent from aboveground biomass responses to nutrient enrichment (and resulting effects on light penetration through the canopy). Significance for each factor was evaluated with Type II Wald Chi-square tests using the Anova function in the car package (Fox and Weisberg 2011). Supplementary Information contains the R code for all tests.

RESULTS

We found wide variation across sites in root biomass (BGB, 60–1675 g/m²) and proportional allocation of biomass to roots from 0–10 cm depth (RMF, 7–90%), as summarized in Table S1 in Supplementary Material. When considering mean values in control plots (unfenced, unfertilized) at

Table 1. Correlations Among Site-level Values of Aridity (GAI, see Methods), the Proportion of Photosynthetically Active Radiation Passing Through the Grassland Canopy to Reach the Soil Surface (Light), the Proportion of Community Cover Comprised by Graminoids/Monocots (gram.frac), the Proportion of Community Cover Comprised by Perennial Species (per.frac), the Average Root Mass Fraction (RMF), Live Aboveground Biomass (AGB), and Belowground Biomass (BGB).

	Aridity	Light	Gram.frac	Per.frac	RMF	ABG
Light	– 0.16					
gram.frac	0.05	– 0.14				
per.frac	0.40*	– 0.24	0.35			
RMF	0.18	0.43*	0.01	0.01		
AGB	0.17	– 0.57**	– 0.02	0.32	– 0.61***	
BGB	0.28	– 0.14	0.19	0.15	0.70***	– 0.07

Site-level mean values were used in this analysis, for control plots only (unfenced, unfertilized). Values are Pearson correlation coefficients with significant values in bold. Asterisks indicate level of statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

each of the 29 sites across four continents, there were a number of correlations among response and predictor variables (correlation coefficients in Table 1). Aridity (GAI) was positively correlated with the proportion of perennial cover ($p = 0.03$, meaning annuals were more common in drier sites). At the site level, the proportion of PAR reaching the soil surface (light) was negatively correlated with AGB ($p = 0.001$), but was not associated with community composition (proportion of graminoid or perennial cover in control plots). AGB and BGB were not correlated; however, both variables were correlated with RMF (as expected, because AGB and BGB are used in the calculation of RMF). There was low RMF in sites with low light beneath the grass canopy ($p = 0.02$, as expected, because of the negative correlation between AGB and RMF), but RMF was not correlated with GAI or community composition.

When analyzing the full dataset (treatment plots as well as controls), both light and GAI were significant site-level covariates in the analysis (statistics in Table 2, parameter estimates for significant factors in Figure 1). GAI and light were both positive predictors of BGB, whereas only light was a significant predictor of RMF.

Previously, a synergistic increase in aboveground biomass with N and P addition was observed across the Nutrient Network sites (that is, significant N $\times$ P interaction, Fay and others 2015). In contrast, N, P, and K_{μ} each had an overall negative effect on BGB (parameter estimates for all terms shown in Table 2 and Figure S4). Only N addition had a statistically significant effect on BGB with the response characterized by a N $\times$ light interaction (Table 2); in sites with high light at the soil surface, N addition increased root biomass, but in sites where light competition likely limited growth (low light at the

soil surface), N addition reduced root biomass (Figure 2).

Mean values calculated across sites for RMF in each of the Nutrient Network treatments are shown in Figure 3. Addition of N and P each significantly reduced RMF, with no interaction. As with BGB, there was a significant N $\times$ light interaction, where the reduction in RMF with N addition was greatest in sites where a lower proportion of incoming light reached the soil surface under control conditions (statistics in Table 2, significant parameter estimates in Figure 1).

DISCUSSION

Across grasslands on four continents, N enrichment quickly (within 3–5 years) influenced community belowground biomass and allocation, and light availability at ground level was a key predictor of the response of belowground biomass allocation to N addition, despite significant variation among sites in plant community composition, climate, and soils. Interestingly, no other nutrient treatment positively affected absolute root biomass, and nutrient enrichment tended to lower proportional biomass allocation to roots. The findings of this analysis are consistent with the predictions of optimal allocation theory, demonstrating that allocation patterns predicted for individual plants scale to the community level, with total belowground allocation jointly influenced by nutrient enrichment and light limitation predictably across wide climatic and environmental gradients.

Variation in Root Biomass and Root Mass Fraction Across Sites

Similar to prior regional studies (Sims and Singh 1978; Scurlock and others 2002; Hui and Jackson

Table 2. Analysis of Deviance Table (Type II tests) Showing the Regression Parameter Estimate (Est), Chi-square Test Statistic, and *p* Value for Each Term in the Mixed Effects Models Described in the Methods.

Model term	RMF			Root biomass (g/m ²)		
	Est	χ^2	<i>p</i>	Est	χ^2	<i>p</i>
Light	2.09	13.3	< 0.001	0.28	1.43	0.23
Aridity	0.66	2.55	0.11	0.61	6.03	0.014
N	– 0.38	26.31	< 0.001	– 0.53	2.11	0.15
P	– 0.05	6.96	0.008	– 0.07	0.69	0.41
K _μ	0.11	0.84	0.36	– 0.21	0.04	0.84
Light/N	0.45	4.89	0.03	0.63	8.66	0.0032
Light/P	0.24	1.45	0.23	0.25	0.02	0.88
N/P	– 0.27	0.01	0.98	0.36	0.03	0.86
Light/K _μ	– 0.12	0.19	0.66	0.07	0.02	0.90
N/K _μ	– 0.15	0.84	0.36	0.34	0.08	0.77
P/K _μ	– 0.27	0.00	0.95	0.12	0.46	0.50
N/aridity	– 0.16	0.24	0.62	0.13	0.07	0.79
P/aridity	– 0.32	0.00	0.96	– 0.05	0.44	0.51
K _μ /aridity	– 0.17	0.46	0.50	0.15	0.21	0.65
Light/N/P	– 0.19	0.42	0.52	– 0.33	0.68	0.41
Light/N/K _μ	0.23	0.16	0.70	0.03	0.06	0.81
Light/P/K _μ	0.25	0.19	0.66	– 0.17	0.13	0.72
N/P/K _μ	0.26	2.65	0.10	– 0.33	2.45	0.12
N/P/aridity	0.59	1.81	0.18	– 0.05	0.12	0.73
N:K _μ /aridity	0.15	0.70	0.40	– 0.25	2.25	0.13
P/K _μ /aridity	0.37	0.08	0.77	0.05	0.04	0.85
Light/N/P/K _μ	– 0.14	0.03	0.86	0.09	0.00	0.95
N/P/K _μ /aridity	– 0.63	2.42	0.12	– 0.02	0.02	0.89

This analysis evaluated how factorial nitrogen (N), phosphorus (P), and potassium plus micronutrient (K_μ) enrichment influenced the proportion of biomass allocated to roots (RMF), and root biomass (g/m²). Aridity and light (mean proportion of PAR reaching the soil surface) were included as site-level covariates, including their interactions with experimental treatments. Significant terms highlighted in bold.

2006), this global study documents wide variation across sites in plant allocation to belowground biomass. Based on prior syntheses, we expected that root biomass and allocation would vary with soil water supply (Hui and Jackson 2006; Mokany and others 2006; but see Yang and others 2009; Reich and others 2014). Root biomass increased with increasing soil water availability (high GAI), but the relationship with RMF was only marginally significant. Although most root production in grasslands occurs in shallow soil layers (Jackson and others 1996), total belowground allocation was under-sampled in this study since we restricted our sampling to the top 10 cm of soil, possibly contributing to the lack of a relationship between site aridity and RMF. Under-sampling may have been relatively greater in dry sites; a global analysis of rooting depths found that arid sites were more likely to have a greater proportion of roots found at deeper depths (Schenk and Jackson 2002).

Past studies have found that variation among species could contribute to variation in the proportion of biomass allocated belowground (Craine

and others 2003). For instance, eudicots had higher fractional allocation aboveground compared with monocots in a comprehensive meta-analysis (Poorter and others 2012), and perennial species in some systems allocate more to roots than annuals (Reynolds and D'Antonio 1996). Functional composition of the grasslands in this study varied widely; however, neither the fraction of perennial species nor the fraction of graminoids present in control plots was correlated with RMF, suggesting these coarse metrics of community composition did not contribute in a predictable way to the variation in RMF observed across sites.

Across the wide range of site conditions, canopy light depletion was the aspect of environmental context most important for predicting variation in RMF; we observed higher proportional allocation aboveground (low RMF) in sites with low light availability beneath the grassland canopy. This effect was driven by AGB, which was negatively correlated with RMF. (BGB and AGB were not correlated.) This pattern is consistent with a shift from light limitation in highly productive sites to

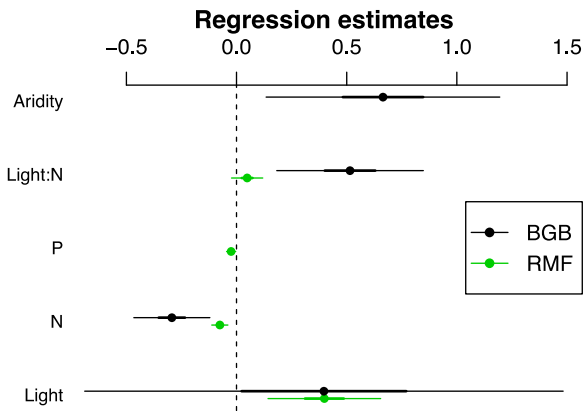


Figure 1. Mean parameter estimates and confidence intervals (thin and thick lines indicate 95% and 50% confidence intervals, respectively) for fixed effects in models evaluating the response of root mass fraction (RMF, in green) and root biomass (BGB, in black) to experimental addition of multiple nutrients, including nitrogen (N) and phosphorus (P). Average light availability at the soil surface in control plots and aridity (Global Aridity Index, see Methods) were included as site-level covariates. Only statistically significant parameter estimates from Table 2 are displayed (note the main effect of light on BGB is not significant, but is displayed because of the significant light:N interaction).

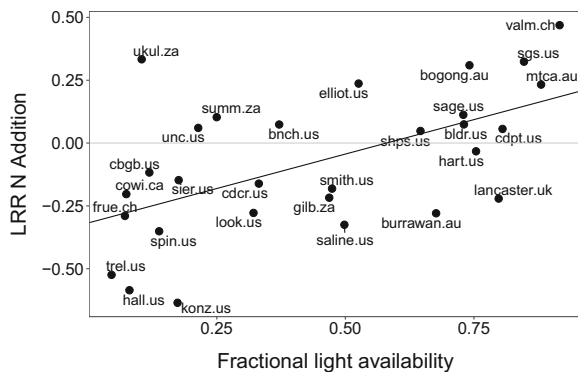


Figure 2. The root biomass response to N addition depended on site-level light limitation. Fractional light availability (light) is the proportion of photosynthetically active radiation passing through the grassland canopy. The natural-log response ratio of root biomass to N addition (LRR N addition) is equivalent to the average $\ln$ -root biomass (g/m^2) in plots without N addition subtracted from the average $\ln$ -root biomass (g/m^2) in N addition plots. The gray line indicates LRR = 0 or no difference in root biomass between ambient and N enriched plots. Negative values indicate a decline in root biomass in plots with N addition compared to plots without N addition. Black trend line shows the best linear fit, indicating that N addition increased root biomass only where abundant light passed through the canopy. Data labels indicate site names as in Table S1.

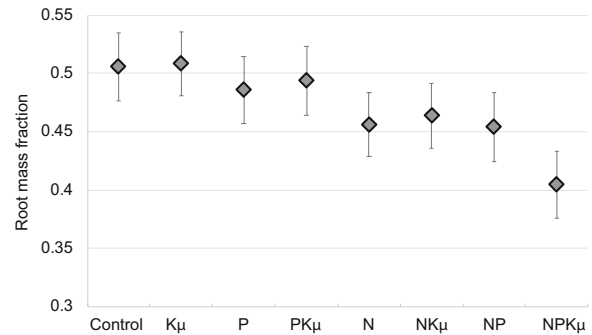


Figure 3. Mean root mass fraction (RMF) in each of experimental nutrient addition treatments, including nitrogen (N), phosphorus (P) or potassium plus micronutrients (K_{μ}), singly and in combination. Means are averages of plot level data across all sites, and error bars indicate one standard error of the mean. Addition of N and P both resulted in a significant reduction in RMF (N and P as main effects, without significant interactions).

limitation by belowground resources (nutrients, water) in low-productivity sites (Gleeson and Tilman 1992), thus providing a new empirical lens into the context dependence of root allocation.

Root Biomass Responses to Nutrient Addition

Across the Nutrient Network sites, aboveground net primary production (estimated by peak aboveground live biomass) responded positively and synergistically to the additions of N and P in approximately 75% of the sites examined (Fay and others 2015), and hence we expected that while *absolute* root biomass (BGB) would also increase in response to additions of these nutrients, root biomass as a *fraction* of total biomass (RMF) would decline with nutrient addition. Instead, our analysis shows an average decline in BGB with N addition, although the direction and magnitude of the BGB response depended on light availability (N $\times$ light interaction), with the greatest declines in BGB observed at sites with lower average light availability beneath the grassland canopy. These results are still consistent with the expectations of optimal allocation theory, whereby plants would be expected to allocate to roots when limitation by aboveground ground resources (for example, light) is small relative to limitation by belowground resources (nutrients). Importantly, increasing nutrient supply reduced the absolute biomass of shallow roots, with important implications for carbon and nutrient cycling in grasslands (Sposito 1989; Jobbagy and Jackson 2001).

Although other recent experiments in both temperate (Bardgett and others 1999) and semiarid (Zeng and others 2010) grasslands have documented declining root biomass in response to N addition, our findings are in direct contrast to recent meta-analyses finding no response (Liu and Greaver 2010), or positive responses of fine root biomass to N addition (Xia and Wan 2008). Some of this variation may reflect different expectations for effects of fertilization on standing pools of biomass versus on productivity. Nadelhoffer and others (1985) showed that forest communities with high rates of N mineralization (high N supply) had low standing pools of fine root biomass, but high rates of annual root production, due to higher rates of root turnover in the more fertile sites. A recent meta-analysis of fine root productivity based on root ingrowth cores found positive and synergistic influences of N and P additions on fine root production (Yuan and Chen 2012). Because their root production responses were smaller in magnitude than the response of aboveground productivity, their analysis found lower proportional allocation belowground with N and P addition. Therefore, although we document an average decline in standing root biomass with N addition dependent on light, we recognize this is a static measurement and that additional measures of root longevity and turnover would be required to predict the responses of ecosystem productivity across these sites.

Our results show that variation in root biomass response to N addition (but not P or K) was predictable based on light limitation at the site level. This finding is consistent with prior studies demonstrating that light becomes increasingly limiting to growth as nutrient limitation is alleviated through fertilization (Hautier and others 2009). It also demonstrates how community and ecosystem responses to nutrients are context dependent. Other studies within the Nutrient Network have also highlighted the role of context dependence; for instance, Borer and others (2014b) found greater diversity loss with nutrient enrichment at sites with low light penetration below the canopy, and Fay and others (2015) found that aboveground biomass did not respond to nutrient addition in 25% of the sites included in their analysis, which they suggested was potentially due to water limitation.

Root Mass Fraction Response to Nutrient Addition

When considering relative root biomass allocation (RMF), our results were consistent with the pre-

dictions of optimal allocation theory (Thornley 1972; Wilson 1988), with additions of both N and P reducing RMF. As with BGB, there was an interaction between N and Light, where the greatest reduction in RMF with N addition occurred in sites with low light penetration through the canopy. Because there was not a significant impact of P addition on BGB, we infer that the reduction in RMF with P addition was caused by an increase in aboveground biomass (Fay and others 2015). Together these results suggest that the predictions of optimal allocation theory with respect to N limitation are robust across wide environmental gradients, but interestingly, that allocation responses to P limitation are not as strong. Given the high—and increasing—rates of N and P fertilization of Earth's ecosystems (Falkowski and others 2000), the mechanisms underlying these differences are worthy of further investigation.

Potential Mechanisms Underlying Belowground Responses to Nutrient Enrichment

In addition to the plastic allocation responses already discussed, allocation to roots, stem, and leaves can also vary with the size of an individual according to allometric scaling theory (Weiner 2004). An analysis of a global forest biomass dataset found intraspecific variation in allocation along environmental gradients consistent with optimal allocation theory, but not intraspecific variation in allocation, and suggested that allometric changes with individual plant size could be responsible for unexplained variation in allocation (McCarthy and Enquist 2007). With respect to our analysis, allometric scaling rules associated with increasing plant size could potentially explain the proportional decline in RMF with N enrichment, but could not explain the absolute decline in root biomass.

Shifts in species diversity and composition could also alter community-level allocation of belowground biomass as a result of nutrient enrichment, particularly at the multi-year timescales considered in this study (Olf 1992; Dybzinski and McNickle 2013; Mueller and others 2013). Species with high root allocation tend to grow slowly but are often competitively dominant (Gurevitch and others 1990; Aerts and others 1991), particularly under low resource supply, due to their ability to draw down levels of soil water and nutrients (Tilman and Wedin 1991). With nutrient enrichment and a shift toward light limitation, species with lower allocation to roots but a capacity for faster aboveground growth are likely to shade and competitively sup-

press slower growing, lower-statured species (Grime and others 1991).

Nutrient enrichment often reduces species richness (Suding and others 2005; Bobbink and others 2010). Across the Nutrient Network, local loss of species diversity in response to N addition was increased by light limitation (Borer and others 2014b), and individual species responses to nutrient enrichment were predictable based on a trade-off in growth-defense strategy (Lind and others 2013). This suggests that species composition shifts contributed to the belowground biomass and allocation responses to N enrichment and light limitation documented here, but without monocultures to supplement our naturally assembled diverse communities, it is not possible to quantify the relative contribution of intraspecific (plastic) versus interspecific responses to the observed shifts in allocation at the community level. Future work should aim to evaluate the influence of shifting species composition in community-level biomass allocation and resulting feedbacks to ecosystem function.

Conclusions: Ramifications for Understanding Ecosystem Responses to Global Change

Ecosystem responses to global environmental change have the potential to either dampen or intensify the magnitude of future climate change through C-cycle feedbacks (Field and others 2007). Despite the importance of grasslands to the terrestrial C sink (Scurlock and Hall 1998; Follett and Reed 2010), belowground responses to environmental changes are often not considered in synthesis efforts (for example, Elser and others 2007; LeBauer and Treseder 2008; Lee and others 2010). Recent database efforts are aiming to address this need, for instance with the creation of the Fine Root Ecology Database (Iversen and others 2017). This study demonstrates that global changes interact with the local environment to influence allocation above- versus belowground, that shallow roots respond in predictable ways to globally pervasive changes, and that measurements of allocation, root production, and turnover will be necessary to accurately predict the ramifications for ecosystem-level processes.

ACKNOWLEDGEMENTS

This work was generated using data from the Nutrient Network (<http://www.nutnet.org>) experiment, funded at the site-scale by individual re-

searchers. Coordination and data management have been supported by funding to E. Borer and E. Seabloom from the National Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (NSF-DEB-1234162 to Cedar Creek LTER) programs, and the Institute on the Environment (DG-0001-13). We also thank the Minnesota Supercomputer Institute for hosting project data and the Institute on the Environment for hosting Network meetings. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

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