

1 **Title:** Belowground biomass response to nutrient enrichment depends on light-limitation across  
2 globally distributed grasslands

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4 **Running title:** Root response to nutrients depends on light

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67 **Author contributions:**

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

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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.

92 **Keywords:** belowground biomass, fertilization, nitrogen, Nutrient Network, optimal allocation,  
93 phosphorus, roots

94 **Manuscript highlights**

- 95 • Both N and P addition reduced the proportion of total biomass in shallow roots
- 96 • N addition decreased roots most where there was low light beneath the canopy
- 97 • These results show plant allocation to roots vs shoots depends on limiting resources

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99 **Introduction**

100 Grasslands and other herbaceous plant communities cover 20 - 40% of the terrestrial land  
101 surface (Leith, 1978), provide critical ecosystem services such as rangeland forage, and play an  
102 important role in the global carbon (C) cycle, with grassland soils containing up to 30% of the  
103 world's soil C (Anderson, 1991). Across the world's biomes, grasslands have some of the highest  
104 fractions of total biomass as roots (Poorter and others, 2012). There is large variation in  
105 partitioning of biomass and productivity across sites, however; for instance, Sims and Singh  
106 (1978) estimated between 24% and 87% of net primary production was belowground across ten  
107 North American grassland sites, and Hui and Jackson (2006) found similar levels of variation  
108 across grasslands worldwide (40-86%). This variation in the proportion of growth allocated  
109 belowground is important not only for regional estimates of primary production and C  
110 sequestration (Scurlock & Hall, 1998, Mokany and others, 2006) but also for understanding  
111 ecosystem responses to global change (Friedlingstein and others, 1999, Jackson and others,  
112 2000).

113 Anthropogenic activities are increasing global nutrient availability, with effects on net  
114 primary production (Elser and others, 2007), plant allocation above- and belowground (Poorter

115 and others, 2012), and net ecosystem C balance (Mack and others, 2004). Fossil fuel combustion  
116 and agricultural intensification have doubled annual nitrogen (N) inputs into terrestrial  
117 ecosystems and have increased phosphorous (P) inputs more than fourfold (Falkowski and  
118 others, 2000). Shifts in C balance resulting from nutrient enrichment could depend on allocation  
119 above- versus belowground (Friedlingstein and others, 1999, Smithwick and others, 2014). High  
120 proportional allocation to root biomass increases the potential for ecosystem C sequestration  
121 because root-derived C is more likely to enter long-lasting soil organic C pools than C from  
122 aboveground tissues (Rasse and others, 2005), and roots can promote physical stabilization of  
123 soil organic matter via soil aggregate formation (Jastrow, 1996).

124         Optimal allocation theory, developed for individual plants, predicts that plant allocation  
125 belowground should depend on the identity of the most growth-limiting resource (Thornley,  
126 1972, Bloom and others, 1985, Wilson, 1988). Specifically, proportional root allocation should  
127 decline when plant growth is limited by aboveground resources (e.g. light) and increase when  
128 plant growth is limited by belowground resources such as water and nutrients (Gleeson &  
129 Tilman, 1992). A recent meta-analysis summarizing the results of nearly 800 experimental  
130 manipulations of resource availability found strong support for optimal allocation theory; the  
131 proportion of biomass allocated to roots was higher under water or nutrient limitation, and lower  
132 under light limitation (Poorter and others, 2012). Most of these studies were focused at the  
133 species level, and if there is significant interspecific variation in allocation responses to  
134 environmental change (Craine and others, 2003), then the predictions of optimal allocation  
135 theory might not explain community-level variation in root allocation. However, patterns  
136 observed across environmental gradients also support the hypothesis that community-level  
137 allocation to roots declines as belowground resources increase. For instance, proportional root

138 allocation in grasslands is inversely correlated with mean annual precipitation and is highest in  
139 xeric regions where water is the predominant factor limiting plant growth (Hui & Jackson, 2006,  
140 Mokany and others, 2006).

141 While many studies have evaluated how allocation responds to variation in individual  
142 environmental factors, few have evaluated how allocation responds when multiple factors change  
143 simultaneously. This is a critical knowledge gap because primary production is frequently co-  
144 limited by multiple resources as opposed to single resources (Hooper & Johnson, 1999, Elser and  
145 others, 2007, Harpole and others, 2011, Fay and others, 2015) and ecosystem responses to  
146 multiple aspects of global change often deviate from predictions based on single factor  
147 experiments (Norby & Luo, 2004). The importance of community-scale biomass partitioning for  
148 understanding regional and global C budgets (Scurlock & Hall, 1998, Jackson and others, 2000,  
149 Smithwick and others, 2014) underscores the need for a framework that effectively predicts both  
150 the absolute quantities as well as proportion of biomass above- versus belowground, in response  
151 to global changes such as eutrophication. Further, while regional and global estimates of total net  
152 primary production generally rely on modeled estimates of root allocation (Friedlingstein and  
153 others, 1999, Woodward & Osborne, 2000, Gill and others, 2002, Michaletz and others, 2014),  
154 these estimates are rarely validated because continental and global relationships between  
155 biomass allocation and climate and soil variables remain poorly characterized (Smithwick and  
156 others, 2014).

157 To evaluate how community-scale root biomass production and allocation respond to  
158 local experimental nutrient enrichment across environmental gradients, we leveraged a global  
159 network of grassland sites where nutrient availability was manipulated using common protocols,  
160 the Nutrient Network (Borer and others, 2014a). By using this experimental network that spans a

161 broad range of climates and grassland soils, we characterized both global trends in allocation  
162 patterns in response to eutrophication as well as regional contingencies in this response. Prior  
163 efforts from this network have demonstrated that aboveground primary production across these  
164 sites is frequently co-limited by multiple nutrients (Fay and others, 2015), and that the impact of  
165 soil nutrients on species richness depends on light limitation (Borer and others, 2014b); however  
166 belowground biomass responses to multiple nutrient enrichment have not yet been evaluated.

167 Here we refer to “biomass allocation” as reflecting static pools of biomass, distinct from  
168 efforts aimed at identifying the dynamic partitioning of new photosynthates (*sensu* Poorter and  
169 others, 2012, also discussed in Reich 2002, and alternatively referred to as "biomass distribution  
170 in Reich and others, 2014). We focus on root responses near the soil surface (top 10 cm), because  
171 80-90% of root biomass in grasslands is concentrated near the surface, in the top 30 cm (Jackson  
172 and others, 1996). Surface roots play a disproportionate role in nutrient acquisition because the  
173 greatest concentrations of N, P, and K are found high in soil profiles (Sposito, 1989, Jobbagy &  
174 Jackson, 2001), and both experimental and anthropogenic nutrient inputs occur at the soil  
175 surface. Furthermore, grasslands store the greatest proportion of soil C near the soil surface  
176 (Jobbagy & Jackson, 2000), contributing to greater microbial biomass (Blume and others, 2002,  
177 Eilers and others, 2012) and fueling greater microbial activity in surface versus subsurface soils.  
178 Hence, C pools with potential for high turnover and release to the atmosphere are likely most  
179 sensitive to fertilization at shallow depths. Accordingly, a meta-analysis of 257 studies across a  
180 variety of ecosystems found that N addition tended to reduce carbon stocks in shallow but not  
181 deep soil layers, correlated with a decline in root allocation in shallow soil layers (Lu and others  
182 2011).



183           Across the Nutrient Network sites, we hypothesized that 1) *absolute* belowground  
184 biomass would respond positively and synergistically to the addition of multiple nutrients,  
185 consistent with patterns of multiple nutrient limitation of aboveground plant biomass observed  
186 across these sites (Fay and others, 2015). We expected that *relative* biomass allocation to roots  
187 (root biomass as a proportion of total biomass) would 2) decline with increasing light limitation  
188 (associated with low light availability below the grassland canopy, e.g. Gleeson & Tilman,  
189 1992), 3) increase with increasing water limitation (in more arid sites, e.g. Hui & Jackson, 2006),  
190 and 4) decrease with nutrient enrichment particularly when multiple nutrients are added together  
191 (Yuan & Chen, 2012), as predicted by optimal allocation theory. Finally, we expected that 5)  
192 there might be statistical interactions among the factors predicting belowground biomass and  
193 allocation, due to the importance of environmental context in determining community responses  
194 to resource enrichment (Cleland & Harpole, 2010). Specifically, we expected that root biomass  
195 responses to nutrient enrichment would be constrained in sites where plant growth was limited  
196 by water (more arid sites), and that root biomass might even decline with nutrient addition at  
197 sites where there is strong competition for light, in favor of increased allocation to aboveground  
198 biomass.

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## 200 **Methods**

201           This research was conducted within the Nutrient Network, a globally replicated network  
202 of sites manipulating nutrients (nitrogen – N, phosphorus – P, and potassium plus  
203 micronutrients–  $K_{\mu}$ ) and vertebrate herbivore exclusion (Borer and others, 2014a). The  
204 micronutrients were only added in year one, and included Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn.  
205 For the effort described here, we analyzed data from 29 sites where the experimental treatments

206 had been applied for 3-5 years. At most sites plots were arranged in three blocks, each block  
207 containing the ten focal treatments: control unfenced & unfertilized, +N, +P, + K<sub>μ</sub>, +NP, +NK<sub>μ</sub>,  
208 + PK<sub>μ</sub>, +NPK<sub>μ</sub>, fenced & unfertilized, and fenced +NPK<sub>μ</sub>. At each site, 30 plots (each 5 x 5 m)  
209 were sampled, except where noted in Supplementary Material, resulting in 874 plots sampled in  
210 total. For this manuscript, only data from the factorial nutrient addition treatments were analyzed  
211 (i.e. all fenced plots were excluded). The sites span four continents and, more importantly, wide  
212 environmental gradients in mean annual precipitation (274-2314 mm/year, summarized in Table  
213 S1). All sites are dominated by herbaceous vegetation but vary in the relative abundance of  
214 graminoids versus other functional types (Table S1). Vegetation types included, for instance,  
215 alpine meadows, prairie, pasture, savannah, and steppe, but we refer to these sites as grasslands  
216 for brevity.

217 Above- and belowground biomass were collected at the time of peak biomass in either  
218 2011 (Northern Hemisphere) or early 2012 (Southern Hemisphere). According to Nutrient  
219 Network protocols (Borer and others, 2014a), aboveground biomass was destructively harvested  
220 in two 1 m x 0.1 m strips per experimental plot, sorted to separate the current year's production  
221 from litter, dried to constant mass, and weighed to the nearest 0.01 g. Immediately following the  
222 aboveground biomass harvest, five soil cores were taken to a depth of 10 cm in the harvest area.  
223 Root cores were collected using standard corers or sharpened PVC tubes with an inside diameter  
224 of 2.5 cm, for a total ground area of 24.5 cm<sup>2</sup>. Exceptions to this protocol are noted in the  
225 Supplementary Material. All cores from each plot were combined in one sealed plastic bag,  
226 packed into coolers with cold packs, and sent via next day air to a central processing lab (USGS  
227 at Corvallis, Oregon, USA).

228 Total soil weights for each bulked sample were recorded, and a homogenized subsample  
229 comprising 1/5<sup>th</sup> of the total soil weight (20-150 grams) was weighed and sent to the University  
230 of California, San Diego for root extraction. Soil sub-samples were kept cool with icepacks  
231 throughout transit and refrigerated while in the lab until processing.

232 Live root biomass was estimated using a modification of the standard Long Term  
233 Ecological Research method for measuring standing fine root biomass in soil cores (Bledsoe and  
234 others, 1999). Soil subsamples were immersed in water; live roots were light in color and floated  
235 to the surface, while dead roots and organic matter were darker in color. Live roots were  
236 extracted with tweezers, rinsed to remove residual mineral soil, dried to a constant mass, and  
237 weighed to the nearest 0.001 g. Above- and belowground (to 10 cm) biomass estimates were  
238 expressed on a common scale (g/m<sup>2</sup>). Our key metric of proportional biomass allocation is the  
239 root mass fraction (RMF) following the method in Reich (2002). The RMF was calculated as the  
240 root biomass divided by the sum of root and aboveground live biomass on an equal area basis.  
241 Detailed methods are provided in Supplementary Material.

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

251           We assembled site-level metrics of water limitation and light availability at the soil  
252 surface, for inclusion as co-variates in our analyses. We extracted measures of the Global Aridity  
253 Index (CGIAR-CSI Global-Aridity and Global-PET Database, Zomer and others, 2008), based  
254 on data from the WorldClim database (Hijmans and others, 2005). Hereafter referred to as GAI,  
255 this index is calculated as mean annual precipitation divided by mean annual potential  
256 evapotranspiration, and hence accounts for both precipitation inputs and soil water loss due to  
257 high temperature, solar radiation, and wind. Low GAI indicates more arid sites with low soil  
258 water availability (low inputs and/or high rates of water loss). Using a linear multi-sensor light  
259 meter, we measured the proportional decrease in photosynthetically active radiation (PAR) from  
260 above the canopy to below the canopy as a proxy of light limitation. The proportion of PAR  
261 reaching the soil surface was calculated as the average of two PAR measurements taken at the  
262 soil surface perpendicular to one another in a 1 m<sup>2</sup> undisturbed subplot, divided by PAR  
263 measured above the canopy immediately afterwards, under full light conditions. We averaged the  
264 proportion of PAR reaching the soil surface across all years of measurement in the control plots  
265 from each site (unfenced, unfertilized) as a *site-level metric of the degree of light-limitation*. This  
266 metric is abbreviated hereafter as "light". Resource depletion is the key mechanism by which  
267 plants compete with neighbors (Goldberg 1990), and hence we use "light" is a proxy for  
268 community-level light depletion.

269           The fractions of the community comprised by graminoids and by perennial species were  
270 calculated as two additional site-level metrics of species composition, based on visual percent  
271 cover estimates collected in 1 x 1 m plots adjacent to the biomass harvests described above.  
272 These values were calculated only from control plots at each site (unfenced, unfertilized).

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### *Statistical analysis*

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Data analysis was performed in R version 3.01 (R Core Team, 2013). 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  $K_{\mu}$  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

296 function in the car package (Fox & Weisberg, 2011). Supplementary Information contains the R  
297 code for all tests.

298

## 299 **Results**

300 We found wide variation across sites in root biomass (BGB, 60-1675 g/m<sup>2</sup>) and  
301 proportional allocation of biomass to roots from 0-10 cm depth (RMF, 7-90%), as summarized in  
302 Table S1 in Supplementary Material. When considering mean values in control plots (unfenced,  
303 unfertilized) at each of the 29 sites across four continents, there were a number of correlations  
304 among response and predictor variables (correlation coefficients in Table 1). Aridity (GAI) was  
305 positively correlated with the proportion of perennial cover ( $p = 0.03$ , meaning annuals were  
306 more common in drier sites). At the site level, the proportion of PAR reaching the soil surface  
307 (light) was negatively correlated with AGB ( $p = 0.001$ ), but was not associated with community  
308 composition (proportion of graminoid or perennial cover in control plots). AGB and BGB were  
309 not correlated, however both variables were positively correlated with RMF (as expected,  
310 because AGB and BGB are used in the calculation of RMF). There was low RMF in sites with  
311 low light beneath the grass canopy ( $p = 0.02$ , as expected, because of the negative correlation  
312 between AGB and RMF), but RMF was not correlated with GAI or community composition.

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

317 Previously, a synergistic increase in aboveground biomass with N and P addition was  
318 observed across the Nutrient Network sites (i.e. significant N x P interaction, Fay and others

319 2015). In contrast, N, P, and  $K_u$  each had an overall negative effect on BGB (parameter estimates  
320 for all terms shown in Table 2 and Figure S4). Only N addition had a statistically significant  
321 effect on BGB with the response characterized by a N x light interaction (Table 2); in sites with  
322 high light at the soil surface, N addition increased root biomass, but in sites where light  
323 competition likely limited growth (low light at the soil surface), N addition reduced root biomass  
324 (Figure 2).

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

330

## 331 **Discussion**

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

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*Variation in root biomass and root mass fraction across sites*

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Similar to prior regional studies (i.e. Sims & Singh, 1978, Scurlock and others, 2002, Hui & Jackson, 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 & 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. While 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 & 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 & 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.





387 biomass of shallow roots, with important implications for carbon and nutrient cycling in  
388 grasslands (Sposito, 1989, Jobbagy & Jackson, 2001).

389         While other recent experiments in both temperate (Bardgett and others, 2009) and semi-  
390 arid (Zeng and others, 2010) grasslands have documented declining root biomass in response to  
391 N addition, our findings are in direct contrast to recent meta-analyses finding no response (Liu &  
392 Greaver, 2010), or positive responses of fine root biomass to N addition (Xia & Wang, 2008).  
393 Some of this variation may reflect different expectations for effects of fertilization on standing  
394 pools of biomass versus on productivity. Nadelhoffer and others (1985) showed that forest  
395 communities with high rates of N mineralization (high N supply) had low standing pools of fine  
396 root biomass, but high rates of annual root production, due to higher rates of root turnover in the  
397 more fertile sites. A recent meta-analysis of fine root productivity based on root ingrowth cores  
398 found positive and synergistic influences of N and P addition on fine root production (Yuan &  
399 Chen, 2012). Because their root production responses were smaller in magnitude than the  
400 response of aboveground productivity, their analysis found lower proportional allocation  
401 belowground with N and P addition. Therefore, while we document an average decline in  
402 standing root biomass with N addition dependent on light, we recognize this is a static  
403 measurement, and that additional measures of root longevity and turnover would be required to  
404 predict the responses of ecosystem productivity across these sites.

405         Our results show that variation in root biomass response to N addition (but not P or K)  
406 was predictable based on light-limitation at the site level. This finding is consistent with prior  
407 studies demonstrating that light becomes increasingly limiting to growth as nutrient limitation is  
408 alleviated through fertilization (Hautier and others, 2009). It also demonstrates how community  
409 and ecosystem responses to nutrients are context dependent. Other studies within the Nutrient

410 Network have also highlighted the role of context-dependence; for instance, Borer and others  
411 (2014b) found greater diversity loss with nutrient enrichment at sites with low light penetration  
412 below the canopy, and Fay and others (2015) found that aboveground biomass did not respond to  
413 nutrient addition in 25% of the sites included in their analysis, which they suggested was  
414 potentially due to water-limitation.

415

416 *Root mass fraction response to nutrient addition*

417 When considering relative root biomass allocation (RMF), our results were consistent  
418 with the predictions of optimal allocation theory (Thornley, 1972, Wilson, 1988), with additions  
419 of both N and P reducing RMF. As with BGB, there was an interaction between N and Light,  
420 where the greatest reduction in RMF with N addition occurred in sites with low light penetration  
421 through the canopy. Because there was not a significant impact of P addition on BGB we infer  
422 that the reduction in RMF with P addition was caused by an increase in aboveground biomass  
423 (Fay and others, 2015). Together these results suggest that the predictions of optimal allocation  
424 theory with respect to N limitation are robust across wide environmental gradients, but  
425 interestingly, that allocation responses to P limitation are not as strong. Given the high – and  
426 increasing – rates of N and P fertilization of Earth’s ecosystems (Falkowski and others, 2000),  
427 the mechanisms underlying these differences are worthy of further investigation.

428

429 *Potential mechanisms underlying belowground responses to nutrient enrichment*

430 In addition to the plastic allocation responses already discussed, allocation to roots, stem  
431 and leaves can also vary with the size of an individual according to allometric scaling theory  
432 (Weiner 2004). An analysis of a global forest biomass dataset found intraspecific variation in

433 allocation along environmental gradients consistent with optimal allocation theory, but not  
434 intraspecific variation in allocation, and suggested that allometric changes with individual plant  
435 size could be responsible for unexplained variation in allocation (McCarthy and Enquist 2007).  
436 With respect to our analysis, allometric scaling rules associated with increasing plant size could  
437 potentially explain the proportional decline in RMF with N enrichment, but could not explain the  
438 absolute decline in root biomass.

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

448         Nutrient enrichment often reduces species richness (Suding and others, 2005, Bobbink  
449 and others, 2010). Across the Nutrient Network, local loss of species diversity in response to N  
450 addition was increased by light-limitation (Borer and others, 2014b), and individual species  
451 responses to nutrient enrichment were predictable based on a trade-off in growth-defense  
452 strategy (Lind and others, 2013). This suggests that species composition shifts contributed to the  
453 belowground biomass and allocation responses to N enrichment and light-limitation documented  
454 here, but without monocultures to supplement our naturally assembled diverse communities, it is  
455 not possible to quantify the relative contribution of intra-specific (plastic) versus inter-specific

456 responses to the observed shifts in allocation at the community level. Future work should aim to  
457 evaluate the influence of shifting species composition in community-level biomass allocation  
458 and resulting feedbacks to ecosystem function.

459

460 *Conclusions: ramifications for understanding ecosystem responses to global change*

461 Ecosystem responses to global environmental change have the potential to either dampen  
462 or intensify the magnitude of future climate change through C-cycle feedbacks (Field and others,  
463 2007). Despite the importance of grasslands to the terrestrial C sink (Scurlock & Hall, 1998,  
464 Follett & Reed, 2010), belowground responses to environmental changes are often not  
465 considered in synthesis efforts (e.g. Elser and others, 2007, LeBauer & Treseder, 2007, Lee and  
466 others, 2010). Recent database efforts are aiming to address this need, for instance with the  
467 creation of the Fine Root Ecology Database (Iversen and others, 2017). This study demonstrates  
468 that global changes interact with the local environment to influence allocation above- versus  
469 belowground, that shallow roots respond in predictable ways to globally pervasive changes, and  
470 that measurements of allocation, root production, and turnover will be necessary to accurately  
471 predict the ramifications for ecosystem-level processes.

472

### 473 **Supplementary Material**

474 Supplementary material includes additional methods, tables, detailed statistical analyses and R  
475 code.

476

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485

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683 Mechanism afforestation and reforestation. *Agriculture, Ecosystems & Environment* 126:  
684 67-80.

685 Table 1. Correlations among site-level values of aridity (GAI, see Methods), the proportion of  
686 photosynthetically-active radiation passing through the grassland canopy to reach the soil surface  
687 (Light), the proportion of community cover comprised by graminoids/monocots (gram.frac), the  
688 proportion of community cover comprised by perennial species (per.frac), the average root mass  
689 fraction (RMF), live aboveground biomass (AGB) and belowground biomass (BGB). Site-level  
690 mean values were used in this analysis, for control plots only (unfenced, unfertilized). Values are  
691 Pearson correlation coefficients with significant values in bold. Asterisks indicate level of  
692 statistical significance (\*= $p < 0.05$ , \*\*= $p < 0.01$ , \*\*\*= $p < 0.001$ ).

693

	Aridity	Light	gram.frac	per.frac	RMF	ABG
Light	-0.16					
gram.frac	0.05	-0.14				
per.frac	<b>0.40 *</b>	-0.24	0.35			
RMF	0.18	<b>0.43 *</b>	0.01	0.01		
AGB	0.17	<b>-0.57 **</b>	-0.02	0.32	<b>-0.61 ***</b>	
BGB	0.28	-0.14	0.19	0.15	<b>0.70 ***</b>	-0.07

694

695

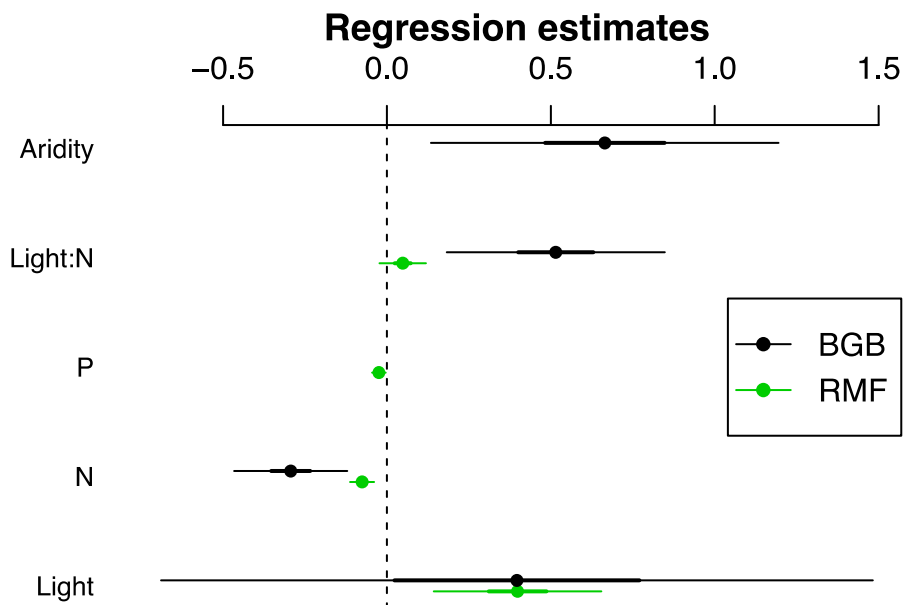


696 Table 2. Analysis of deviance table (Type II tests) showing the regression parameter estimate  
697 (Est),  $\chi^2$  test statistic and p-value for each term in the mixed effects models described in the  
698 Methods. This analysis evaluated how factorial nitrogen (N), phosphorus (P) and potassium plus  
699 micronutrient ( $K_{\mu}$ ) enrichment influenced the proportion of biomass allocated to roots (RMF),  
700 and root biomass ( $g/m^2$ ). Aridity and light (mean proportion of PAR reaching the soil surface)  
701 were included as site-level covariates, including their interactions with experimental treatments.  
702 Significant terms highlighted in bold.  
703

Model term	RMF			root biomass ( $g/m^2$ )		
	Est	$\chi^2$	p	Est	$\chi^2$	p
Light	<b>2.09</b>	<b>13.3</b>	<b>&lt;0.001</b>	0.28	1.43	0.23
Aridity	0.66	2.55	0.11	<b>0.61</b>	<b>6.03</b>	<b>0.014</b>
N	<b>-0.38</b>	<b>26.31</b>	<b>&lt;0.001</b>	-0.53	2.11	0.15
P	<b>-0.05</b>	<b>6.96</b>	<b>0.008</b>	-0.07	0.69	0.41
$K_{\mu}$	0.11	0.84	0.36	-0.21	0.04	0.84
Light:N	<b>0.45</b>	<b>4.89</b>	<b>0.03</b>	<b>0.63</b>	<b>8.66</b>	<b>0.0032</b>
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: $K_{\mu}$	-0.12	0.19	0.66	0.07	0.02	0.90
N: $K_{\mu}$	-0.15	0.84	0.36	0.34	0.08	0.77
P: $K_{\mu}$	-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
$K_{\mu}$ :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: $K_{\mu}$	0.23	0.16	0.70	0.03	0.06	0.81
Light:P: $K_{\mu}$	0.25	0.19	0.66	-0.17	0.13	0.72
N:P: $K_{\mu}$	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: $K_{\mu}$ :Aridity	0.15	0.70	0.40	-0.25	2.25	0.13
P: $K_{\mu}$ :Aridity	0.37	0.08	0.77	0.05	0.04	0.85
Light:N:P: $K_{\mu}$	-0.14	0.03	0.86	0.09	0.00	0.95
N:P: $K_{\mu}$ :Aridity	-0.63	2.42	0.12	-0.02	0.02	0.89

704

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

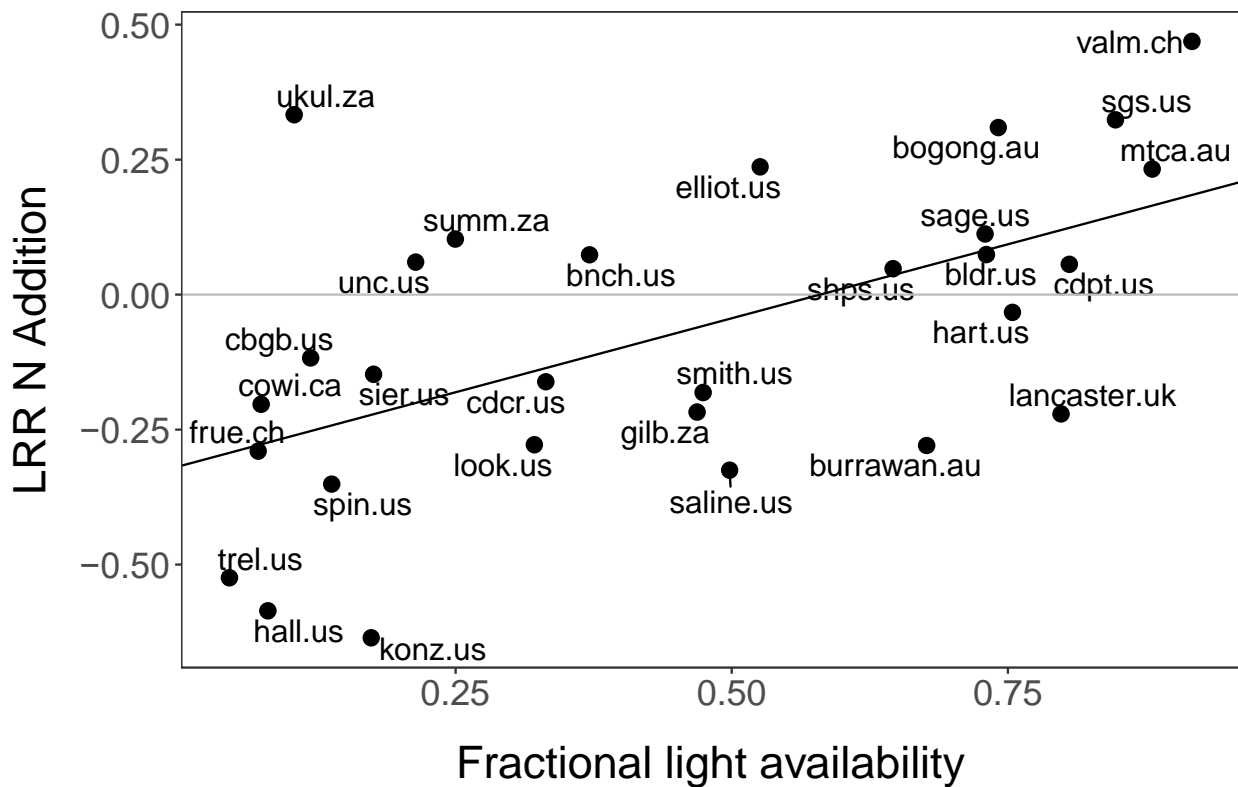


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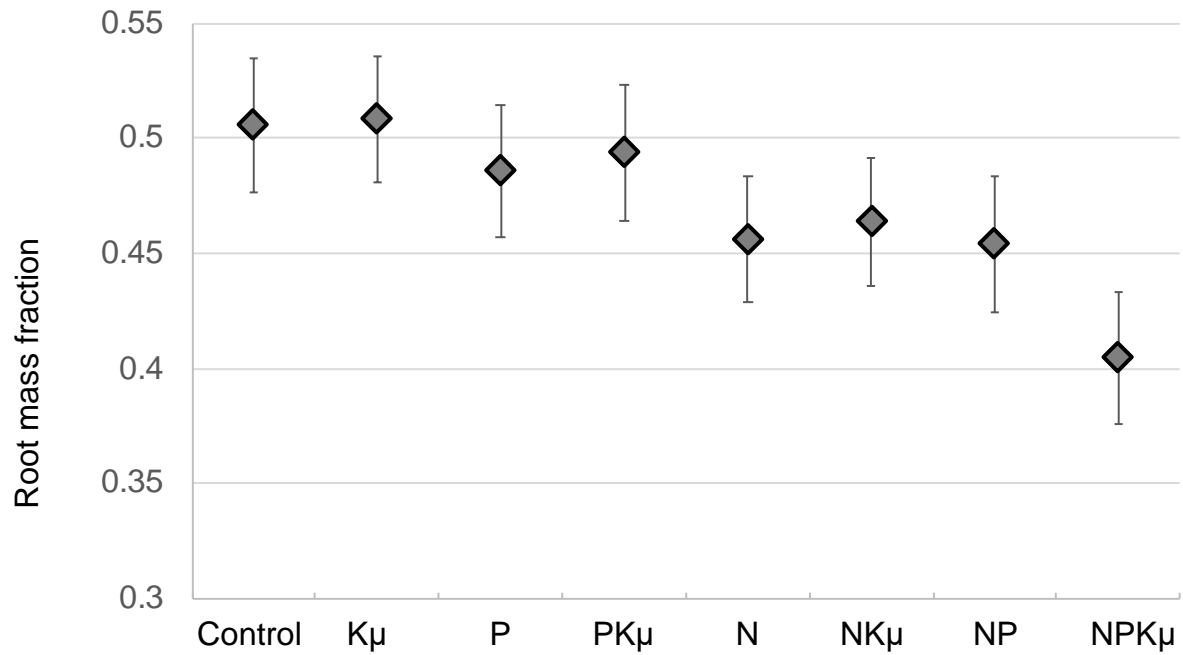
715

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



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



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