

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

3
4 **Running title:** Root response to nutrients depends on light

5
6 **Authors:** Elsa E. Cleland¹, Eric M. Lind², Nicole M. DeCrappeo³, Elizabeth DeLorenze³, Rachel
7 Abbott Wilkins⁴, Peter B. Adler⁵, Jonathan D. Bakker⁶, Cynthia S. Brown⁷, Kendi F. Davies⁸,
8 Ellen Esch⁹, Jennifer Firn¹⁰, Scott Gressard¹, Daniel S. Gruner¹¹, Nicole Hagenah¹², W. Stanley
9 Harpole^{13,14,15}, Yann Hautier¹⁶, Sarah E. Hobbie², Kirsten S. Hofmockel^{17,18}, Kevin Kirkman¹⁹,
10 Johannes Knops²⁰, Christopher W. Kopp²¹, Kimberly J. La Pierre²², Andrew MacDougall⁹,
11 McCulley, Rebecca L.²³, Brett A. Melbourne⁸, Joslin L. Moore²⁴, Suzanne M. Prober²⁵, Charlotte
12 Riggs², Anita C. Risch²⁶, Martin Schuetz²⁶, Carly Stevens²⁷, Peter D. Wragg²⁸, Justin Wright²⁹,
13 Elizabeth T. Borer², Eric W. Seabloom²

14
15 **Author Affiliations:**

16 ¹ Ecology, Behavior & Evolution Section, University of California San Diego, La Jolla CA
17 92093

18 ² Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul MN,
19 55108, USA

20 ³ U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, OR 97331

21 ⁴ Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA

22 ⁵ Department of Wildland Resources and the Ecology Center, Utah State University, Logan, UT
23 84103

24 ⁶ School of Environmental and Forest Sciences, University of Washington, Box 354115, Seattle
25 WA 98195-4115

26 ⁷ Department of Bioagricultural Sciences and Pest Management, Graduate Degree Program in
27 Ecology, 1177 Campus Delivery, Colorado State University, Fort Collins CO 80523 USA

28 ⁸ Department of Ecology and Evolutionary Biology, UCB 334 University of Colorado, Boulder,
29 CO 80309 USA

30 ⁹ University of Guelph, Department of Integrative Biology, Guelph, Ontario Canada N1G 2W1

31 ¹⁰ Queensland University of Technology, School of Earth, Environmental and Biological
32 Sciences, Brisbane, Australia

33 ¹¹ Department of Entomology, University of Maryland, 4112 Plant Sciences Bldg, College Park,
34 MD 20742 USA

35 ¹² Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria,
36 Pretoria, South Africa

37 ¹³ Department of Physiological Diversity, Helmholtz Center for Environmental Research – UFZ,
38 Permoserstrasse 15, Leipzig 04318, Germany

39 ¹⁴ German Centre for Integrative Biodiversity Research iDiv. Halle-Jena-Leipzig, Deutscher
40 Platz 5e, Leipzig 04103, Germany

41 ¹⁵ Institute of Biology, Martin Luther University Halle- Wittenberg, Am Kirchtor 1, Halle Saale.
42 06108, Germany

43 ¹⁶ Ecology and Biodiversity Group, Department of Biology, Utrecht University, Padualaan 8,
44 3584 CH Utrecht, Netherlands

45 ¹⁷ Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames
46 Iowa, USA

47 ¹⁸ Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory,
48 Richland WA, USA

49 ¹⁹ School of Life Sciences, University of KwaZulu-Natal, Scottsville, South Africa

50 ²⁰ School of Biological Sciences, University of Nebraska, Manter Hall 402, Lincoln, NE 68588
51 USA

52 ²¹ Department of Botany, University of British Columbia, Vancouver, BC V6T1Z4 Canada

53 ²² Smithsonian Environmental Research Center, 647 Contees Wharf Road
54 Edgewater, MD 21037 USA

55 ²³ Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546 USA

56 ²⁴ School of Biological Sciences, Monash University VIC 3800 Australia

57 ²⁵ CSIRO Land and Water, Underwood Avenue, Floreat WA 6014 Australia

58 ²⁶ Swiss Federal Institute for Forest, Snow and Landscape Research, Zuercherstrasse 111, 8903
59 Birmensdorf Switzerland

60 ²⁷ Lancaster Environment Center, Lancaster University, Lancaster U.K. LA14YQ

61 ²⁸ Department of Forest Resources, University of Minnesota, Saint Paul, MN 55108

62 ²⁹ Department of Biology, Duke University, Durham NC 27708 USA

63

64 **Corresponding author:** Elsa E. Cleland; University of California San Diego, 9500 Gilman Dr.
65 #0116, La Jolla CA 92093-0116 USA; 8582460509; eccleland@ucsd.edu

66

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.

70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91

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

98

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.

199

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_{μ}) 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_μ, +NP, +NK_μ,
208 + PK_μ, +NPK_μ, fenced & unfertilized, and fenced +NPK_μ. 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 cm². 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 1/5th 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/m²). 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 m² 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).

273

274

Statistical analysis

275

276

277

278

279

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.

280

281

282

283

284

285

286

287

288

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).

289

290

291

292

293

294

295

To evaluate the responses of BGB and RMF to the addition of individual nutrients and their combinations, N, P and K_{μ} 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/m²) 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.

342

343

Variation in root biomass and root mass fraction across sites

344

345

346

347

348

349

350

351

352

353

354

355

356

357

358

359

360

361

362

363

364

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.

365 Across the wide range of site conditions, canopy light depletion was the aspect of
366 environmental context most important for predicting variation in RMF; we observed higher
367 proportional allocation aboveground (low RMF) in sites with low light availability beneath the
368 grassland canopy. This effect was driven by AGB, which was negatively correlated with RMF
369 (BGB and AGB were not correlated). This pattern is consistent with a shift from light limitation
370 in highly productive sites to limitation by belowground resources (nutrients, water) in low-
371 productivity sites (Gleeson & Tilman 1992), thus providing a new empirical lens into the
372 context-dependence of root allocation.

373

374 *Root biomass responses to nutrient addition*

375 Across the Nutrient Network sites, aboveground net primary production (estimated by
376 peak aboveground live biomass) responded positively and synergistically to the additions of N
377 and P in approximately 75% of the sites examined (Fay and others 2015), and hence we expected
378 that while *absolute* root biomass (BGB) would also increase in response to additions of these
379 nutrients, root biomass as a *fraction* of total biomass (RMF) would decline with nutrient
380 addition. Instead, our analysis shows an average decline in BGB with N addition, although the
381 direction and magnitude of the BGB response depended on light availability (N x light
382 interaction), with the greatest declines in BGB observed at sites with lower average light
383 availability beneath the grassland canopy. These results are still consistent with the expectations
384 of optimal allocation theory, whereby plants would be expected to allocate to roots when
385 limitation by aboveground ground resources (e.g. light) is small relative to limitation by
386 belowground resources (nutrients). Importantly, increasing nutrient supply reduced the absolute

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

477 **Acknowledgements**

478 This work was generated using data from the Nutrient Network (<http://www.nutnet.org>)
479 experiment, funded at the site-scale by individual researchers. Coordination and data
480 management have been supported by funding to E. Borer and E. Seabloom from the National
481 Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term
482 Ecological Research (NSF-DEB-1234162 to Cedar Creek LTER) programs, and the Institute on
483 the Environment (DG-0001-13). We also thank the Minnesota Supercomputer Institute for
484 hosting project data and the Institute on the Environment for hosting Network meetings.

485

486 **References**

- 487 Aerts RR, Boog GA, Van Der Aart PJM. 1991. The relation between above- and belowground
488 biomass allocation patterns and competitive ability. *Oecologia* 87: 551-559.
- 489 Anderson JM. 1991. The effects of climate change on decomposition processes in grassland and
490 coniferous forests. *Ecological Applications* 1: 326–347.
- 491 Bardgett RD, Mawdsley JL, Edwards S, Hobbs PJ, Rodwell JS, Davies WJ. 1999. Plant species
492 and nitrogen effects on soil biological properties of temperate upland grasslands.
493 *Functional Ecology* 13: 650-60.
- 494 Bledsoe CS, Fahey TJ, Day FP, Ruess RW. 1999. Measurement of static root parameters:
495 biomass, length, and distribution in the soil profile. In: *Soils Methods for Long-Term*
496 *Ecological Research*. New York, New York, USA, Oxford University Press. pp 413-436.
- 497 Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants--an economic analogy.
498 *Annual Review of Ecology and Systematics* 16: 363-392.

499 Blume E, Bischoff M, Reichert JM, Moorman T, Konopka A, Turco RF. 2002. Surface and
500 subsurface microbial biomass, community structure and metabolic activity as a function
501 of soil depth and season. *Applied Soil Ecology* 20: 171-181.

502 Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M,
503 Cinderby S, Davidson E, Dentener F, Emmett B. 2010. Global assessment of nitrogen
504 deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20:
505 30-59.

506 Bolker B. 2008. Chapter 4: Distributions, in: *Ecological Models and Data in R*. Princeton
507 University Press, pp 103-146.

508 Borer ET, Harpole WS, Adler PB, Lind EM, Orrock JL, Seabloom EW, Smith MD. 2014a.
509 Finding generality in ecology: a model for globally distributed experiments. *Methods in*
510 *Ecology and Evolution* 5: 65–73.

511 Borer ET, Seabloom EW, Gruner DS, Harpole WS, Hillebrand H, Lind EM, Adler PB, Alberti J,
512 Anderson TM, Bakker JD, Biederman L, Blumenthal D, Brown CS, Brudvig LA,
513 Buckley YM, Cadotte M, Chu C, Cleland EE, Crawley MJ, Daleo P, Damschen EI,
514 Davies KF, DeCrappeo NM, Du G, Firn J, Hautier Y, Heckman RW, Hector A,
515 HilleRisLambers J, Iribarne O, Klein JA, Knops JMH, La Pierre KJ, Leakey ADB, Li W,
516 MacDougall AS, McCulley RL, Melbourne BA, Mitchell CE, Moore JL, Mortensen B,
517 O'Halloran LR, Orrock JL, Pascual J, Prober SM, Pyke DA, Risch AC, Schuetz M, Smith
518 MD, Stevens CJ, Sullivan LL, Williams RJ, Wragg PD, Wright JP, Yang LH. 2014b.
519 Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508:
520 517-520.

521 Cleland EE, Harpole WS. 2010. Nitrogen enrichment and plant communities. *Annals of the New*
522 *York Academy of Sciences* 1195: 46-61.

523 Craine JM, Wedin DA, Chapin FS, Reich PB. 2003. Relationship between the structure of root
524 systems and resource use for 11 North American grassland plants. *Plant Ecology* 165: 85-
525 100.

526 Dybzinski R, McNickle G. 2013. Game theory and plant ecology. *Ecology Letters*, 16, 545-555.

527 Eilers KG, Debenport S, Anderson S, Fierer N. 2012. Digging deeper to find unique microbial
528 communities: The strong effect of depth on the structure of bacterial and archaeal
529 communities in soil. *Soil Biology & Biochemistry* 50: 58-65.

530 Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom
531 EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of
532 primary producers in freshwater, marine, and terrestrial ecosystems. *Ecology Letters* 10:
533 1115-1211.

534 Falkowski P, Scholes R, Boyle E, Canadell J, Canfield D, Elser J, Gruber N, Hibbard K,
535 Högberg P, Linder S, Mackenzie FT. 2000. The global carbon cycle: a test of our
536 knowledge of earth as a system. *Science* 290: 291-296.

537 Fay PA, Prober SM, Harpole WS, Knops JMH, Bakker JD, Borer ET, Lind EM, MacDougall
538 AS, Seabloom EW, Wragg PD, Adler P, Blumenthal DM, Buckley YM, Chu C, Cleland
539 EE, Collins SL, Davies KF, Du G, Feng X, Firn J, Gruner DS, Hagenah N, Hautier Y,
540 Heckman RW, Jin VL, Kirkman KP, Klein J, Ladwig LM, Li Q, McCulley RL,
541 Melbourne BA, Mitchell CE, Moore JL, Morgan JW, Risch AC, Schütz M, Stevens CJ,
542 Wedin DA, Yang YH. 2015. Grassland productivity limited by multiple nutrients. *Nature*
543 *Plants* 1: 15080.

544 Field CB, Lobell DB, Peters HA, Chiariello NR. 2007. Feedbacks of terrestrial ecosystems to
545 climate change. *Annual Review of Environment and Resources* 32: 1-29.

546 Follett R, Reed D 2010. Soil carbon sequestration in grazing lands: societal benefits and policy
547 implications. *Rangeland Ecology Management* 63: 4–15.

548 Fox J, Weisberg S 2011. *An R Companion to Applied Regression, Second Edition*. Thousand
549 Oaks, California.

550 Friedlingstein P, Joel G, Field CB, Fung IY. 1999. Toward an allocation scheme for global
551 terrestrial carbon models. *Global Change Biology* 5: 755-770.

552 Gill RA, Kelly RH, Parton WJ, Day KA, Jackson RB, Morgan JA, Scurlock JMO, Tieszen LL,
553 Castle JV, Ojima DS, Zhang XS. 2002. Using simple environmental variables to
554 estimate belowground productivity in grasslands. *Global Ecology and Biogeography* 11:
555 79–86.

556 Gleeson SK, Tilman D. 1992. Plant allocation and the multiple limitation hypothesis. *American*
557 *Naturalist* 139: 1322-1343.

558 Goldberg, DE. 1990. Components of resource competition in plant communities. In: *Perspectives*
559 *on plant competition*, pp. 27-49.

560 Grime JP, Campbell BD, Mackey JMI, Crick JC. 1991. Root plasticity, nitrogen capture and
561 competitive ability. In: *Plant Root Growth: an Ecological Perspective*. In: Atkinson D. ed.
562 Oxford, Blackwell Scientific Publications.

563 Gurevitch J, Wilson P, Stone JL, Tees P, Stoutenburgh RJ. 1990. Competition among old-field
564 perennials at different levels of soil fertility and available space. *Journal of Ecology* 78:
565 727-744.

566 Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner
567 DS, Hillebrand H, Shurin JB, Smith JE. 2011. Nutrient co-limitation of primary producer
568 communities. *Ecology Letters* 14: 852-862.

569 Hautier Y, Niklaus PA, Hector A. 2009. Competition for light causes plant biodiversity loss after
570 eutrophication. *Science*, 324, 636-638.

571 Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated
572 climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–
573 1978.

574 Hooper DU, Johnson L. 1999. Nitrogen limitation in dryland ecosystems: responses to
575 geographical and temporal variation in precipitation. *Biogeochemistry* 46: 247 – 293.

576 Hui DF, Jackson RB. 2006. Geographical and interannual variability in biomass partitioning in
577 grassland ecosystems: a synthesis of field data. *New Phytologist* 169: 85–93.

578 Iversen CM, McCormack ML, Powell AS, Blackwood CB, Freschet GT, Kattge J, Roumet C,
579 Stover DB, Soudzilovskaia NA, Valverde - Barrantes OJ, Bodegom PM. 2017. A global
580 Fine-Root Ecology Database to address belowground challenges in plant ecology. *New*
581 *Phytologist* 215: 15-26.

582 Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. 1996. A global
583 analysis of root distributions for terrestrial biomes. *Oecologia* 108: 389-411.

584 Jackson RB, Schenk HJ, Jobbágy EG, Canadell J, Colello GD, Dickinson RE, Field CB,
585 Friedlingstein P, Heimann M, Hibbard K, Kicklighter DW. 2000. Belowground
586 consequences of vegetation change and their treatment in models. *Ecological*
587 *Applications* 10: 470-483.

588 Jastrow JD. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated
589 organic matter. *Soil Biology and Biochemistry* 28: 665-676.

590 Jobbagy EG, Jackson RB. 2000. The vertical distribution of soil organic carbon and its relation
591 to climate and vegetation. *Ecological Applications* 10: 423-436.

592 Jobbagy EG, Jackson RB. 2001. The distribution of soil nutrients with depth: Global patterns and
593 the imprint of plants. *Biogeochemistry* 53: 51-77.

594 LeBauer DS, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial
595 ecosystems is globally distributed. *Ecology* 89: 371–379.

596 Lee M, Manning P, Rist J, Power SA, Marsh C. 2010. A global comparison of grassland biomass
597 responses to CO₂ and nitrogen enrichment. *Philosophical Transactions of the Royal
598 Society of London B: Biological Sciences* 365: 2047-2056.

599 Leith HHF. 1978. Primary productivity in ecosystems: Comparative analysis of global patterns.
600 In: Leith HFH, ed. *Patterns of primary production in the biosphere*. Stroudberg, PA
601 USA., Dowden, Hutchinson and Ross. pp. 342

602 Lind EM, Borer E, Seabloom E, Adler P, Bakker JD, Blumenthal DM, Crawley M, Davies K,
603 Firm J, Gruner DS, Harpole WS. 2013. Life-history constraints in grassland plant species:
604 a growth-defence trade-off is the norm. *Ecology Letters* 16: 513-521.

605 Liu L, Greaver TL. 2010. A global perspective on belowground carbon dynamics under nitrogen
606 enrichment. *Ecology Letters* 13: 819-28.

607 Lu M, Zhou X, Luo Y, Yang Y, Fang C, Chen J, Li B. 2011. Minor stimulation of soil carbon
608 storage by nitrogen addition: a meta-analysis. *Agriculture, Ecosystems &
609 Environment* 140: 234-244.

610 Mack MC, Schuur EA, Bret-Harte MS, Shaver GR, Chapin FS. 2004. Ecosystem carbon storage
611 in arctic tundra reduced by long-term nutrient fertilization. *Nature* 431: 440-443.

612 McCarthy MC, Enquist BJ. 2007. Consistency between an allometric approach and optimal
613 partitioning theory in global patterns of plant biomass allocation. *Functional Ecology* 21:
614 713-720.

615 Mueller KE, Hobbie SE, Tilman D, Reich PB. 2013. Effects of plant diversity, N fertilization,
616 and elevated carbon dioxide on grassland soil N cycling in a long - term experiment.
617 *Global Change Biology* 19: 1249-1261.

618 Michaletz ST, Cheng D, Kerkhoff AJ, Enquist BJ. 2014. Convergence of terrestrial plant
619 production across global climate gradients. *Nature* 512: 39-43.

620 Mokany K, Raison RJ, Prokushkin AS. 2006. Critical analysis of root : shoot ratios in terrestrial
621 biomes. *Global Change Biology* 12: 84–96.

622 Nadelhoffer KJ, Aber JD, Melillo JM. 1985. Fine roots, net primary production, and soil nitrogen
623 availability: a new hypothesis. *Ecology* 66: 1377–1390

624 Norby RJ, Luo Y. 2004. Evaluating ecosystem responses to rising atmospheric CO₂ and global
625 warming in a multi-factor world. *New Phytologist* 162: 281-293.

626 Olf H 1992. Effects of light and nutrient availability on dry matter and N allocation in six
627 successional grassland species. *Oecologia* 89: 412-421.

628 Pinheiro J, Bates D, Debroy S, Sarkar D, R Core Development Team. 2013. nlme: Linear and
629 Nonlinear Mixed Effects Models. R package version 3.1

630 Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012. Biomass allocation to
631 leaves, stems and roots: meta-analyses of interspecific variation and environmental
632 control. *New Phytologist* 193: 30-50.

633 R Core Team 2018. R: A language and environment for statistical computing. Vienna, Austria, R
634 Foundation for Statistical Computing.

635 Rasse DP, Rumpel C, Dignac MF. 2005. Is soil carbon mostly root carbon? Mechanisms for a
636 specific stabilisation. *Plant and Soil* 269: 341–356.

637 Reynolds HL, D’Antonio C. 1996. The ecological significance of plasticity in root weight ratio
638 in response to nitrogen: Opinion. *Plant and Soil* 185: 75–97.

639 Reich PB. 2002. Root-shoot relations: optimality in acclimation and adaptation or the ‘Emperor’s
640 New Clothes’. In: *Plant Roots: The Hidden Half*, pp. 205-220.

641 Reich PB, Luo Y, Bradford JB, Poorter H, Perry CH, Oleksyn J. 2014. Temperature drives
642 global patterns in forest biomass distribution in leaves, stems, and roots. *Proceedings of
643 the National Academy of Sciences* 111: 13721-13726.

644 Schenk HJ, Jackson RB. 2002. The global biogeography of roots. *Ecological Monographs* 72:
645 311-328.

646 Scurlock JMO, Hall DO. 1998. The global carbon sink: a grassland perspective. *Global Change
647 Biology* 4: 229–233.

648 Scurlock JMO, Johnson K, Olson RJ. 2002. Estimating net primary productivity from grassland
649 biomass dynamics measurements. *Global Change Biology* 8: 736-753.

650 Sims PL, Singh JS. 1978. The structure and function of ten western North American grasslands:
651 III. Net primary production, turnover and efficiencies of energy capture and water use.
652 *Journal of Ecology* 66: 573-597.

653 Smithwick EA, Lucash MS, McCormack ML, Sivandran G. 2014. Improving the representation
654 of roots in terrestrial models. *Ecological Modelling* 291: 193-204.

655 Sposito G. 1989. *The chemistry of soils*, New York, New York U.S.A., Oxford University Press.

656 Suding KN, Collins SL, Gough L, Clark C, Cleland EE, Gross KL, Milchunas DG, Pennings S.
657 2005. Functional-and abundance-based mechanisms explain diversity loss due to N
658 fertilization. *Proceedings of the National Academy of Sciences of the United States of*
659 *America* 102: 4387-4392.

660 Thornley JHM. 1972. A balanced quantitative model for root: shoot ratios in vegetative plants.
661 *Annals of Botany* 36: 431-441.

662 Tilman D, Wedin D. 1991. Plant traits and resource reduction for five grasses growing on a
663 nitrogen gradient. *Ecology* 72: 685-700.

664 Warton DI, Hui FK. 2011. The arcsine is asinine: the analysis of proportions in ecology.
665 *Ecology*, 92, 3-10.

666 Weiner, J. 2004. Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology,*
667 *Evolution and Systematics* 6: 207-215.

668 Wilson JB 1988. A review of evidence on the control of shoot: root ratio, in relation to models.
669 *Annals of Botany* 61: 433-449.

670 Woodward FI, Osborne CP. 2000. The representation of root processes in models addressing the
671 responses of vegetation to global change. *New Phytologist* 147: 223–232.

672 Xia J, Wan S. 2008. Global response patterns of terrestrial plant species to nitrogen addition.
673 *New Phytologist* 179: 428-439.

674 Yang Y, Fang J, Ji C, Han W. 2009. Above- and belowground biomass allocation in Tibetan
675 grasslands. *Journal of Vegetation Science* 20: 177-184.

676 Yuan ZY, Chen HYH. 2012. A global analysis of fine root production as affected by soil
677 nitrogen and phosphorus. *Proceedings of the Royal Society of London B: Biological*
678 *Sciences* 279: 3796-3802.

679 Zeng DH, Li LJ, Fahey TJ, Yu ZY, Fan ZP, Chen FS. 2010. Effects of nitrogen addition on
680 vegetation and ecosystem carbon in a semi-arid grassland. *Biogeochemistry* 98: 185-93.

681 Zomer RJ, Trabucco A, Bossio DA, van Straaten O, Verchot LV. 2008. Climate change
682 mitigation: A spatial analysis of global land suitability for Clean Development
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	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

694

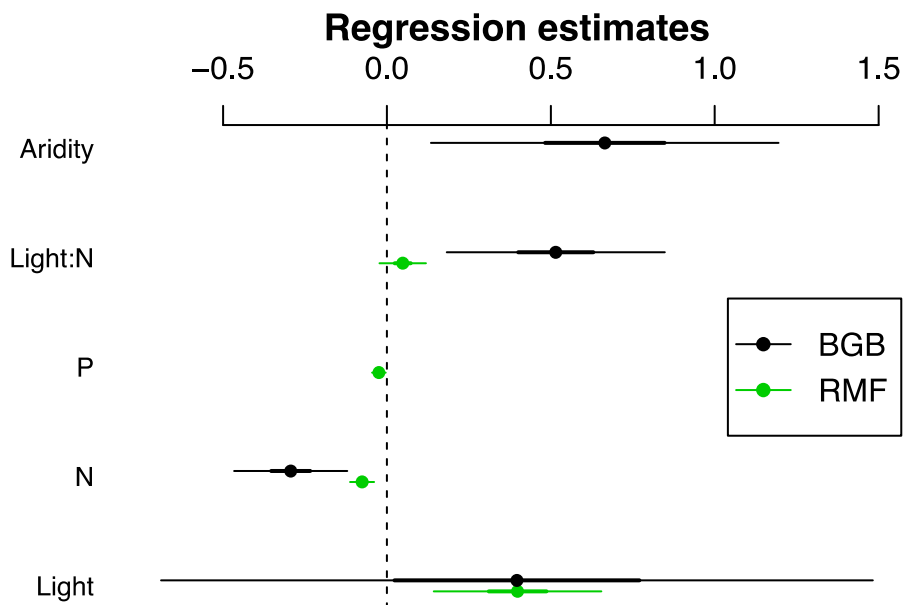
695

696 Table 2. Analysis of deviance table (Type II tests) showing the regression parameter estimate
697 (Est), χ^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_{μ}) 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	χ^2	p	Est	χ^2	p
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

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).

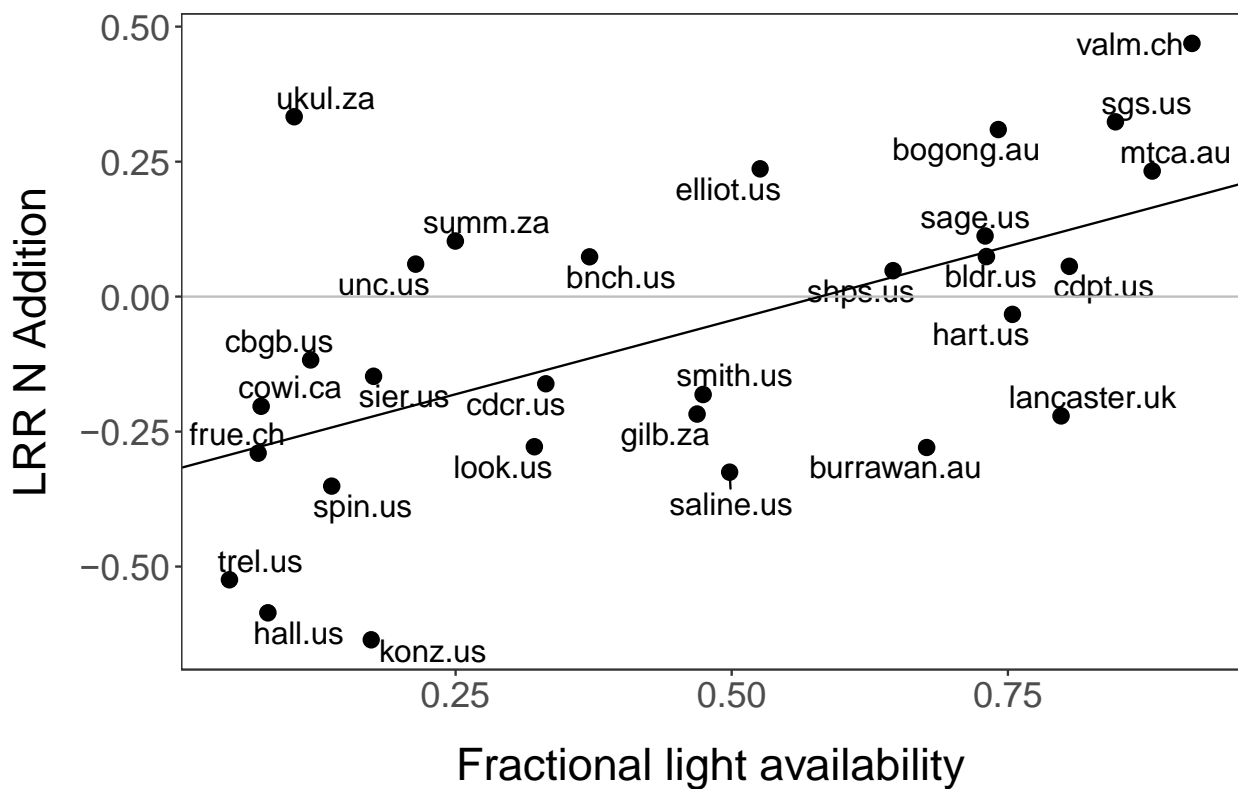


713

714

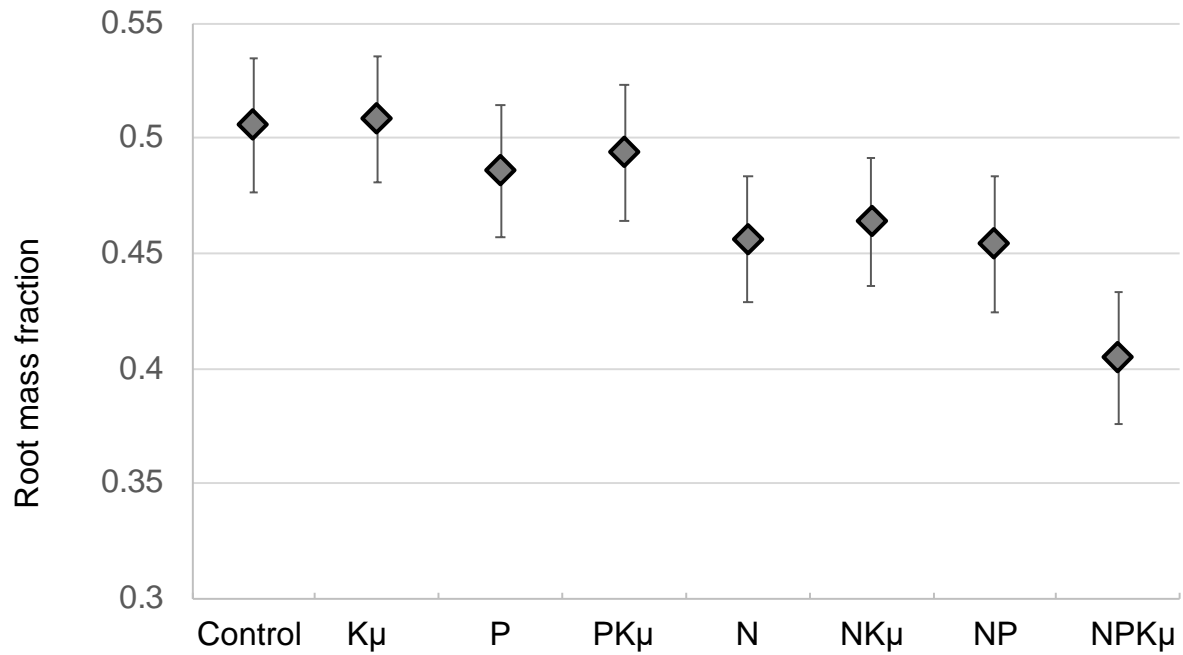
715

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 (g/m^2) in plots without N addition
 720 subtracted from the average \ln -root biomass (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.



725
 726
 727

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_{μ}), 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).



733

734