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

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Authors

Cleland, Elsa E
Lind, Eric M
DeCrappeo, Nicole M
et al.

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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

- 1
2
3 24 ⁶ School of Environmental and Forest Sciences, University of Washington, Box 354115, Seattle
4
5 25 WA 98195-4115
6
7
8 26 ⁷ Department of Bioagricultural Sciences and Pest Management, Graduate Degree Program in
9
10 27 Ecology, 1177 Campus Delivery, Colorado State University, Fort Collins CO 80523 USA
11
12 28 ⁸ Department of Ecology and Evolutionary Biology, UCB 334 University of Colorado, Boulder,
13
14 29 CO 80309 USA
15
16
17 30 ⁹ University of Guelph, Department of Integrative Biology, Guelph, Ontario Canada N1G 2W1
18
19 31 ¹⁰ Queensland University of Technology, School of Earth, Environmental and Biological
20
21 32 Sciences, Brisbane, Australia
22
23
24 33 ¹¹ Department of Entomology, University of Maryland, 4112 Plant Sciences Bldg, College Park,
25
26 34 MD 20742 USA
27
28 35 ¹² Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria,
29
30 36 Pretoria, South Africa
31
32
33 37 ¹³ Department of Physiological Diversity, Helmholtz Center for Environmental Research – UFZ,
34
35 38 Permoserstrasse 15, Leipzig 04318, Germany
36
37
38 39 ¹⁴ German Centre for Integrative Biodiversity Research iDiv. Halle-Jena-Leipzig, Deutscher
39
40 40 Platz 5e, Leipzig 04103, Germany
41
42 41 ¹⁵ Institute of Biology, Martin Luther University Halle- Wittenberg, Am Kirchtor 1, Halle Saale.
43
44 42 06108, Germany
45
46
47 43 ¹⁶ Ecology and Biodiversity Group, Department of Biology, Utrecht University, Padualaan 8,
48
49 44 3584 CH Utrecht, Netherlands
50
51 45 ¹⁷ Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames
52
53 46 Iowa, USA
54
55
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57
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59
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- 1
2
3 47 ¹⁸ Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory,
4
5 48 Richland WA, USA
6
7 49 ¹⁹ School of Life Sciences, University of KwaZulu-Natal, Scottsville, South Africa
8
9
10 50 ²⁰ School of Biological Sciences, University of Nebraska, Manter Hall 402, Lincoln, NE 68588
11
12 51 USA
13
14 52 ²¹ Department of Botany, University of British Columbia, Vancouver, BC V6T1Z4 Canada
15
16
17 53 ²² Smithsonian Environmental Research Center, 647 Contees Wharf Road
18
19 54 Edgewater, MD 21037 USA
20
21 55 ²³ Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546 USA
22
23 56 ²⁴ School of Biological Sciences, Monash University VIC 3800 Australia
24
25
26 57 ²⁵ CSIRO Land and Water, Underwood Avenue, Floreat WA 6014 Australia
27
28 58 ²⁶ Swiss Federal Institute for Forest, Snow and Landscape Research, Zuercherstrasse 111, 8903
29
30 59 Birmensdorf Switzerland
31
32
33 60 ²⁷ Lancaster Environment Center, Lancaster University, Lancaster U.K. LA14YQ
34
35 61 ²⁸ Department of Forest Resources, University of Minnesota, Saint Paul, MN 55108
36
37 62 ²⁹ Department of Biology, Duke University, Durham NC 27708 USA
38
39
40
41

42 64 **Corresponding author:** Elsa E. Cleland; University of California San Diego, 9500 Gilman Dr.
43
44 65 #0116, La Jolla CA 92093-0116 USA; 8582460509; ecleland@ucsd.edu
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49 67 **Author contributions:**

50
51 68 EEC analyzed the data and wrote the paper with input from all co-authors. All co-authors
52
53 69 contributed to data collection.
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5 71 **Abstract:**
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8 72 Anthropogenic activities are increasing nutrient inputs to ecosystems worldwide, with
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10 73 consequences for global carbon and nutrient cycles. Recent meta-analyses show that
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12 74 aboveground primary production is often co-limited by multiple nutrients, however little is
13
14 75 known about how root production responds to changes in nutrient availability. At twenty-nine
15
16 76 grassland sites on four continents, we quantified shallow root biomass responses to nitrogen (N),
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18 77 phosphorus (P) and potassium plus micronutrient enrichment and compared below- and
19
20 78 aboveground responses. We hypothesized that optimal allocation theory would predict context
21
22 79 dependence in root biomass responses to nutrient enrichment, given variation among sites in the
23
24 80 resources limiting to plant growth (specifically light versus nutrients). Consistent with the
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26 81 predictions of optimal allocation theory, the *proportion* of total biomass belowground declined
27
28 82 with N or P addition, due to increased biomass aboveground (for N and P) and decreased
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30 83 biomass belowground (N, particularly in sites with low canopy light penetration). *Absolute* root
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32 84 biomass increased with N addition where light was abundant at the soil surface, but declined in
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34 85 sites where the grassland canopy intercepted a large proportion of incoming light. These results
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36 86 demonstrate that belowground responses to changes in resource supply can differ strongly from
37
38 87 aboveground responses, which could significantly modify predictions of future rates of nutrient
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40 88 cycling and carbon sequestration. Our results also highlight how optimal allocation theory
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42 89 developed for individual plants may help predict belowground biomass responses to nutrient
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44 90 enrichment at the ecosystem scale across wide climatic and environmental gradients.
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3 92 **Keywords:** belowground biomass, fertilization, nitrogen, Nutrient Network, optimal allocation,
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5 93 phosphorus, roots
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8 94 **Manuscript highlights**
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- 10 95 • Both N and P addition reduced the proportion of total biomass in shallow roots
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12 96 • N addition decreased roots most where there was low light beneath the canopy
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14 97 • These results show plant allocation to roots vs shoots depends on limiting resources
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19 99 **Introduction**
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22 100 Grasslands and other herbaceous plant communities cover 20 - 40% of the terrestrial land
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24 101 surface (Leith, 1978), provide critical ecosystem services such as rangeland forage, and play an
25
26 102 important role in the global carbon (C) cycle, with grassland soils containing up to 30% of the
27
28 103 world's soil C (Anderson, 1991). Across the world's biomes, grasslands have some of the highest
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30 104 fractions of total biomass as roots (Poorter and others, 2012). There is large variation in
31
32 105 partitioning of biomass and productivity across sites, however; for instance, Sims and Singh
33
34 106 (1978) estimated between 24% and 87% of net primary production was belowground across ten
35
36 107 North American grassland sites, and Hui and Jackson (2006) found similar levels of variation
37
38 108 across grasslands worldwide (40-86%). This variation in the proportion of growth allocated
39
40 109 belowground is important not only for regional estimates of primary production and C
41
42 110 sequestration (Scurlock & Hall, 1998, Mokany and others, 2006) but also for understanding
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44 111 ecosystem responses to global change (Friedlingstein and others, 1999, Jackson and others,
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46 112 2000).
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52 113 Anthropogenic activities are increasing global nutrient availability, with effects on net
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54 114 primary production (Elser and others, 2007), plant allocation above- and belowground (Poorter
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3 115 and others, 2012), and net ecosystem C balance (Mack and others, 2004). Fossil fuel combustion
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5 116 and agricultural intensification have doubled annual nitrogen (N) inputs into terrestrial
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7 117 ecosystems and have increased phosphorous (P) inputs more than fourfold (Falkowski and
8
9 118 others, 2000). Shifts in C balance resulting from nutrient enrichment could depend on allocation
10
11 119 above- versus belowground (Friedlingstein and others, 1999, Smithwick and others, 2014). High
12
13 120 proportional allocation to root biomass increases the potential for ecosystem C sequestration
14
15 121 because root-derived C is more likely to enter long-lasting soil organic C pools than C from
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17 122 aboveground tissues (Rasse and others, 2005), and roots can promote physical stabilization of
18
19 123 soil organic matter via soil aggregate formation (Jastrow, 1996).

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24 124 Optimal allocation theory, developed for individual plants, predicts that plant allocation
25
26 125 belowground should depend on the identity of the most growth-limiting resource (Thornley,
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28 126 1972, Bloom and others, 1985, Wilson, 1988). Specifically, proportional root allocation should
29
30 127 decline when plant growth is limited by aboveground resources (e.g. light) and increase when
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32 128 plant growth is limited by belowground resources such as water and nutrients (Gleeson &
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34 129 Tilman, 1992). A recent meta-analysis summarizing the results of nearly 800 experimental
35
36 130 manipulations of resource availability found strong support for optimal allocation theory; the
37
38 131 proportion of biomass allocated to roots was higher under water or nutrient limitation, and lower
39
40 132 under light limitation (Poorter and others, 2012). Most of these studies were focused at the
41
42 133 species level, and if there is significant interspecific variation in allocation responses to
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44 134 environmental change (Craine and others, 2003), then the predictions of optimal allocation
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46 135 theory might not explain community-level variation in root allocation. However, patterns
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48 136 observed across environmental gradients also support the hypothesis that community-level
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50 137 allocation to roots declines as belowground resources increase. For instance, proportional root
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3 138 allocation in grasslands is inversely correlated with mean annual precipitation and is highest in
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5 139 xeric regions where water is the predominant factor limiting plant growth (Hui & Jackson, 2006,
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7 140 Mokany and others, 2006).

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10 141 While many studies have evaluated how allocation responds to variation in individual
11
12 142 environmental factors, few have evaluated how allocation responds when multiple factors change
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14 143 simultaneously. This is a critical knowledge gap because primary production is frequently co-
15
16 144 limited by multiple resources as opposed to single resources (Hooper & Johnson, 1999, Elser and
17
18 145 others, 2007, Harpole and others, 2011, Fay and others, 2015) and ecosystem responses to
19
20 146 multiple aspects of global change often deviate from predictions based on single factor
21
22 147 experiments (Norby & Luo, 2004). The importance of community-scale biomass partitioning for
23
24 148 understanding regional and global C budgets (Scurlock & Hall, 1998, Jackson and others, 2000,
25
26 149 Smithwick and others, 2014) underscores the need for a framework that effectively predicts both
27
28 150 the absolute quantities as well as proportion of biomass above- versus belowground, in response
29
30 151 to global changes such as eutrophication. Further, while regional and global estimates of total net
31
32 152 primary production generally rely on modeled estimates of root allocation (Friedlingstein and
33
34 153 others, 1999, Woodward & Osborne, 2000, Gill and others, 2002, Michaletz and others, 2014),
35
36 154 these estimates are rarely validated because continental and global relationships between
37
38 155 biomass allocation and climate and soil variables remain poorly characterized (Smithwick and
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40 156 others, 2014).

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42 157 To evaluate how community-scale root biomass production and allocation respond to
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44 158 local experimental nutrient enrichment across environmental gradients, we leveraged a global
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46 159 network of grassland sites where nutrient availability was manipulated using common protocols,
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48 160 the Nutrient Network (Borer and others, 2014a). By using this experimental network that spans a
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3 161 broad range of climates and grassland soils, we characterized both global trends in allocation
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5 162 patterns in response to eutrophication as well as regional contingencies in this response. Prior
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7 163 efforts from this network have demonstrated that aboveground primary production across these
8
9 164 sites is frequently co-limited by multiple nutrients (Fay and others, 2015), and that the impact of
10
11 165 soil nutrients on species richness depends on light limitation (Borer and others, 2014b); however
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13 166 belowground biomass responses to multiple nutrient enrichment have not yet been evaluated.
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17 167 Here we refer to “biomass allocation” as reflecting static pools of biomass, distinct from
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19 168 efforts aimed at identifying the dynamic partitioning of new photosynthates (*sensu* Poorter and
20
21 169 others, 2012, also discussed in Reich 2002, and alternatively referred to as "biomass distribution
22
23 170 in Reich and others, 2014). We focus on root responses near the soil surface (top 10 cm), because
24
25 171 80-90% of root biomass in grasslands is concentrated near the surface, in the top 30 cm (Jackson
26
27 172 and others, 1996). Surface roots play a disproportionate role in nutrient acquisition because the
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29 173 greatest concentrations of N, P, and K are found high in soil profiles (Sposito, 1989, Jobbagy &
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31 174 Jackson, 2001), and both experimental and anthropogenic nutrient inputs occur at the soil
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33 175 surface. Furthermore, grasslands store the greatest proportion of soil C near the soil surface
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35 176 (Jobbagy & Jackson, 2000), contributing to greater microbial biomass (Blume and others, 2002,
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37 177 Eilers and others, 2012) and fueling greater microbial activity in surface versus subsurface soils.
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39 178 Hence, C pools with potential for high turnover and release to the atmosphere are likely most
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41 179 sensitive to fertilization at shallow depths. Accordingly, a meta-analysis of 257 studies across a
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43 180 variety of ecosystems found that N addition tended to reduce carbon stocks in shallow but not
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45 181 deep soil layers, correlated with a decline in root allocation in shallow soil layers (Lu and others
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47 182 2011).
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3 183 Across the Nutrient Network sites, we hypothesized that 1) *absolute* belowground
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5 184 biomass would respond positively and synergistically to the addition of multiple nutrients,
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7 185 consistent with patterns of multiple nutrient limitation of aboveground plant biomass observed
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9 186 across these sites (Fay and others, 2015). We expected that *relative* biomass allocation to roots
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11 187 (root biomass as a proportion of total biomass) would 2) decline with increasing light limitation
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13 188 (associated with low light availability below the grassland canopy, e.g. Gleeson & Tilman,
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15 189 1992), 3) increase with increasing water limitation (in more arid sites, e.g. Hui & Jackson, 2006),
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17 190 and 4) decrease with nutrient enrichment particularly when multiple nutrients are added together
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19 191 (Yuan & Chen, 2012), as predicted by optimal allocation theory. Finally, we expected that 5)
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21 192 there might be statistical interactions among the factors predicting belowground biomass and
22
23 193 allocation, due to the importance of environmental context in determining community responses
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25 194 to resource enrichment (Cleland & Harpole, 2010). Specifically, we expected that root biomass
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27 195 responses to nutrient enrichment would be constrained in sites where plant growth was limited
28
29 196 by water (more arid sites), and that root biomass might even decline with nutrient addition at
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31 197 sites where there is strong competition for light, in favor of increased allocation to aboveground
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33 198 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_{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

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3 206 had been applied for 3-5 years. At most sites plots were arranged in three blocks, each block
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5 207 containing the ten focal treatments: control unfenced & unfertilized, +N, +P, +K_μ, +NP, +NK_μ,
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8 208 +PK_μ, +NPK_μ, fenced & unfertilized, and fenced +NPK_μ. At each site, 30 plots (each 5 x 5 m)
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10 209 were sampled, except where noted in Supplementary Material, resulting in 874 plots sampled in
11
12 210 total. For this manuscript, only data from the factorial nutrient addition treatments were analyzed
13
14 211 (i.e. all fenced plots were excluded). The sites span four continents and, more importantly, wide
15
16 212 environmental gradients in mean annual precipitation (274-2314 mm/year, summarized in Table
17
18 213 S1). All sites are dominated by herbaceous vegetation but vary in the relative abundance of
19
20 214 graminoids versus other functional types (Table S1). Vegetation types included, for instance,
21
22 215 alpine meadows, prairie, pasture, savannah, and steppe, but we refer to these sites as grasslands
23
24 216 for brevity.

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28 217 Above- and belowground biomass were collected at the time of peak biomass in either
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30 218 2011 (Northern Hemisphere) or early 2012 (Southern Hemisphere). According to Nutrient
31
32 219 Network protocols (Borer and others, 2014a), aboveground biomass was destructively harvested
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34 220 in two 1 m x 0.1 m strips per experimental plot, sorted to separate the current year's production
35
36 221 from litter, dried to constant mass, and weighed to the nearest 0.01 g. Immediately following the
37
38 222 aboveground biomass harvest, five soil cores were taken to a depth of 10 cm in the harvest area.
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40 223 Root cores were collected using standard corers or sharpened PVC tubes with an inside diameter
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42 224 of 2.5 cm, for a total ground area of 24.5 cm². Exceptions to this protocol are noted in the
43
44 225 Supplementary Material. All cores from each plot were combined in one sealed plastic bag,
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46 226 packed into coolers with cold packs, and sent via next day air to a central processing lab (USGS
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48 227 at Corvallis, Oregon, USA).
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3 228 Total soil weights for each bulked sample were recorded, and a homogenized subsample
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5 229 comprising 1/5th of the total soil weight (20-150 grams) was weighed and sent to the University
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8 230 of California, San Diego for root extraction. Soil sub-samples were kept cool with icepacks
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10 231 throughout transit and refrigerated while in the lab until processing.

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12 232 Live root biomass was estimated using a modification of the standard Long Term
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14 233 Ecological Research method for measuring standing fine root biomass in soil cores (Bledsoe and
15
16 234 others, 1999). Soil subsamples were immersed in water; live roots were light in color and floated
17
18 235 to the surface, while dead roots and organic matter were darker in color. Live roots were
19
20 236 extracted with tweezers, rinsed to remove residual mineral soil, dried to a constant mass, and
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22 237 weighed to the nearest 0.001 g. Above- and belowground (to 10 cm) biomass estimates were
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24 238 expressed on a common scale (g/m²). Our key metric of proportional biomass allocation is the
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26 239 root mass fraction (RMF) following the method in Reich (2002). The RMF was calculated as the
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28 240 root biomass divided by the sum of root and aboveground live biomass on an equal area basis.
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31 241 Detailed methods are provided in Supplementary Material.

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35 242 Our estimates of belowground biomass are based on one-time destructive harvests at the
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37 243 time of peak biomass; while this reflects a reasonable estimate of aboveground production, this is
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39 244 an underestimate of belowground production (Gill and others, 2002). Hence, we proceed with the
40
41 245 caveat that this effort documents comparable patterns of shallow root biomass and allocation
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43 246 across plots and sites, but additional estimates of root turnover and deep root biomass would be
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45 247 needed to estimate total belowground production and allocation of net primary production.
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47 248 However, a survey of published and unpublished data on the distribution of root biomass at our
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49 249 sites shows that the majority of root biomass is captured by shallow root sampling efforts, such
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51 250 as ours (Table S2).
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3 251 We assembled site-level metrics of water limitation and light availability at the soil
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5 252 surface, for inclusion as co-variates in our analyses. We extracted measures of the Global Aridity
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8 253 Index (CGIAR-CSI Global-Aridity and Global-PET Database, Zomer and others, 2008), based
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10 254 on data from the WorldClim database (Hijmans and others, 2005). Hereafter referred to as GAI,
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12 255 this index is calculated as mean annual precipitation divided by mean annual potential
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14 256 evapotranspiration, and hence accounts for both precipitation inputs and soil water loss due to
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16
17 257 high temperature, solar radiation, and wind. Low GAI indicates more arid sites with low soil
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19 258 water availability (low inputs and/or high rates of water loss). Using a linear multi-sensor light
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21
22 259 meter, we measured the proportional decrease in photosynthetically active radiation (PAR) from
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24 260 above the canopy to below the canopy as a proxy of light limitation. The proportion of PAR
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26 261 reaching the soil surface was calculated as the average of two PAR measurements taken at the
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28 262 soil surface perpendicular to one another in a 1 m² undisturbed subplot, divided by PAR
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31 263 measured above the canopy immediately afterwards, under full light conditions. We averaged the
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33 264 proportion of PAR reaching the soil surface across all years of measurement in the control plots
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35 265 from each site (unfenced, unfertilized) as a *site-level metric of the degree of light-limitation*. This
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37 266 metric is abbreviated hereafter as "light". Resource depletion is the key mechanism by which
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40 267 plants compete with neighbors (Goldberg 1990), and hence we use "light" is a proxy for
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42 268 community-level light depletion.

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44 269 The fractions of the community comprised by graminoids and by perennial species were
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46 270 calculated as two additional site-level metrics of species composition, based on visual percent
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49 271 cover estimates collected in 1 x 1 m plots adjacent to the biomass harvests described above.
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51 272 These values were calculated only from control plots at each site (unfenced, unfertilized).
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3 274 *Statistical analysis*
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5 275 Data analysis was performed in R version 3.3.3 (R Core Team, 2017). Pearson
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7 276 correlations were performed to evaluate associations among site-level parameters: aridity, light,
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9 277 live aboveground biomass (AGB), live belowground root biomass to 10 cm depth (BGB), RMF,
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11 278 graminoid fraction, and perennial fraction. Each data point in the correlation analysis was a site-
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13 279 level mean for each parameter, calculated for the control plots only.
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17 280 Examination of the BGB data with Quantile-Quantile plots showed these data were
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19 281 lognormally distributed (Figure S2), as is common with ecological datasets involving measures
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21 282 of growth (Bolker, 2008), and hence the BGB data were natural-log transformed prior to
22
23 283 analysis. The RMF data were continuous proportions bounded by 0 and 1 and, as expected,
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25 284 initial inspection with Quantile-Quantile plots indicated the data were non-normally distributed
26
27 285 (Figure S3). Following the recommendation of Warton and Hui (2011) the RMF data were logit
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29 286 transformed. After transformation, BGB and RMF had normally distributed errors and were
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31 287 analyzed with a general linear mixed model using the lme call in the package nlme (Pinheiro and
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33 288 others, 2013).
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37 289 To evaluate the responses of BGB and RMF to the addition of individual nutrients and
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39 290 their combinations, N, P and K_{μ} were each included as factorial fixed factors, site was treated as
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41 291 a random factor, and light and aridity were included as site-level covariates. As described above,
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43 292 our metric of light availability was based on site-level mean light penetration of the grassland
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45 293 canopy only in control plots, and hence was independent from aboveground biomass responses
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47 294 to nutrient enrichment (and resulting effects on light penetration through the canopy).
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51 295 Significance for each factor was evaluated with Type II Wald chi-square tests using the Anova
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3 296 function in the car package (Fox & Weisberg, 2011). Supplementary Information contains the R
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5 297 code for all tests.
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9
10 299 **Results**

11
12 300 We found wide variation across sites in root biomass (BGB, 60-1675 g/m²) and
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14 301 proportional allocation of biomass to roots from 0-10 cm depth (RMF, 7-90%), as summarized in
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16 302 Table S1 in Supplementary Material. When considering mean values in control plots (unfenced,
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18 303 unfertilized) at each of the 29 sites across four continents, there were a number of correlations
19
20 304 among response and predictor variables (correlation coefficients in Table 1). Aridity (GAI) was
21
22 305 positively correlated with the proportion of perennial cover ($p = 0.03$, meaning annuals were
23
24 306 more common in drier sites). At the site level, the proportion of PAR reaching the soil surface
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26 307 (light) was negatively correlated with AGB ($p = 0.001$), but was not associated with community
27
28 308 composition (proportion of graminoid or perennial cover in control plots). AGB and BGB were
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30 309 not correlated, however both variables were positively correlated with RMF (as expected,
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32 310 because AGB and BGB are used in the calculation of RMF). There was low RMF in sites with
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34 311 low light beneath the grass canopy ($p = 0.02$, as expected, because of the negative correlation
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36 312 between AGB and RMF), but RMF was not correlated with GAI or community composition.
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42 313 When analyzing the full data set (treatment plots as well as controls), both light and GAI
43
44 314 were significant site-level covariates in the analysis (statistics in Table 2, parameter estimates for
45
46 315 significant factors in in Figure 1). GAI and light were both positive predictors of BGB, while
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48 316 only light was a significant predictor of RMF.
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51 317 Previously, a synergistic increase in aboveground biomass with N and P addition was
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53 318 observed across the Nutrient Network sites (i.e. significant N x P interaction, Fay and others
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3 319 2015). In contrast, N, P, and K_{μ} each had an overall negative effect on BGB (parameter estimates
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5 320 for all terms shown in Table 2 and Figure S4). Only N addition had a statistically significant
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7 321 effect on BGB with the response characterized by a N x light interaction (Table 2); in sites with
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9 322 high light at the soil surface, N addition increased root biomass, but in sites where light
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11 323 competition likely limited growth (low light at the soil surface), N addition reduced root biomass
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13 324 (Figure 2).
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17 325 Mean values calculated across sites for RMF in each of the Nutrient Network treatments
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19 326 are shown in Figure 3. Addition of N and P each significantly reduced RMF, with no interaction.
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21 327 As with BGB, there was a significant N x light interaction, where the reduction in RMF with N
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23 328 addition was greatest in sites where a lower proportion of incoming light reached the soil surface
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25 329 under control conditions (statistics in Table 2, significant parameter estimates in Figure 1).
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31 331 **Discussion**

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33 332 Across grasslands on four continents, N enrichment quickly (within 3-5 years) influenced
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35 333 community belowground biomass and allocation, and light availability at ground level was a key
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37 334 predictor of the response of belowground biomass allocation to N addition, despite significant
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39 335 variation among sites in plant community composition, climate, and soils. Interestingly, no other
40
41 336 nutrient treatment positively affected absolute root biomass, and nutrient enrichment tended to
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43 337 lower proportional biomass allocation to roots. The findings of this analysis are consistent with
44
45 338 the predictions of optimal allocation theory, demonstrating that allocation patterns predicted for
46
47 339 individual plants scale to the community level, with total belowground allocation jointly
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49 340 influenced by nutrient enrichment and light-limitation predictably across wide climatic and
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51 341 environmental gradients.
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5 343*Variation in root biomass and root mass fraction across sites*6
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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.

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3 365 Across the wide range of site conditions, canopy light depletion was the aspect of
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5 366 environmental context most important for predicting variation in RMF; we observed higher
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7 367 proportional allocation aboveground (low RMF) in sites with low light availability beneath the
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9 368 grassland canopy. This effect was driven by AGB, which was negatively correlated with RMF
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11 369 (BGB and AGB were not correlated). This pattern is consistent with a shift from light limitation
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13 370 in highly productive sites to limitation by belowground resources (nutrients, water) in low-
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15 371 productivity sites (Gleeson & Tilman 1992), thus providing a new empirical lens into the
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17 372 context-dependence of root allocation.
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24 374 *Root biomass responses to nutrient addition*

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26 375 Across the Nutrient Network sites, aboveground net primary production (estimated by
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28 376 peak aboveground live biomass) responded positively and synergistically to the additions of N
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30 377 and P in approximately 75% of the sites examined (Fay and others 2015), and hence we expected
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32 378 that while *absolute* root biomass (BGB) would also increase in response to additions of these
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34 379 nutrients, root biomass as a *fraction* of total biomass (RMF) would decline with nutrient
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36 380 addition. Instead, our analysis shows an average decline in BGB with N addition, although the
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38 381 direction and magnitude of the BGB response depended on light availability (N x light
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40 382 interaction), with the greatest declines in BGB observed at sites with lower average light
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42 383 availability beneath the grassland canopy. These results are still consistent with the expectations
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44 384 of optimal allocation theory, whereby plants would be expected to allocate to roots when
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46 385 limitation by aboveground ground resources (e.g. light) is small relative to limitation by
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48 386 belowground resources (nutrients). Importantly, increasing nutrient supply reduced the absolute
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3 387 biomass of shallow roots, with important implications for carbon and nutrient cycling in
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5 388 grasslands (Sposito, 1989, Jobbagy & Jackson, 2001).

7 389 While other recent experiments in both temperate (Bardgett and others, 2009) and semi-
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9 arid (Zeng and others, 2010) grasslands have documented declining root biomass in response to
10 390
11 N addition, our findings are in direct contrast to recent meta-analyses finding no response (Liu &
12 391
13 Greaver, 2010), or positive responses of fine root biomass to N addition (Xia & Wang, 2008).
14 392
15 Some of this variation may reflect different expectations for effects of fertilization on standing
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17 pools of biomass versus on productivity. Nadelhoffer and others (1985) showed that forest
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19 communities with high rates of N mineralization (high N supply) had low standing pools of fine
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21 root biomass, but high rates of annual root production, due to higher rates of root turnover in the
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23 more fertile sites. A recent meta-analysis of fine root productivity based on root ingrowth cores
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25 found positive and synergistic influences of N and P addition on fine root production (Yuan &
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27 Chen, 2012). Because their root production responses were smaller in magnitude than the
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29 response of aboveground productivity, their analysis found lower proportional allocation
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31 belowground with N and P addition. Therefore, while we document an average decline in
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33 standing root biomass with N addition dependent on light, we recognize this is a static
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35 measurement, and that additional measures of root longevity and turnover would be required to
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37 predict the responses of ecosystem productivity across these sites.
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44 405 Our results show that variation in root biomass response to N addition (but not P or K)
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46 was predictable based on light-limitation at the site level. This finding is consistent with prior
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48 studies demonstrating that light becomes increasingly limiting to growth as nutrient limitation is
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50 alleviated through fertilization (Hautier and others, 2009). It also demonstrates how community
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52 and ecosystem responses to nutrients are context dependent. Other studies within the Nutrient
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3 410 Network have also highlighted the role of context-dependence; for instance, Borer and others
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5 411 (2014b) found greater diversity loss with nutrient enrichment at sites with low light penetration
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7 412 below the canopy, and Fay and others (2015) found that aboveground biomass did not respond to
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9 413 nutrient addition in 25% of the sites included in their analysis, which they suggested was
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11 414 potentially due to water-limitation.
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17 416 *Root mass fraction response to nutrient addition*

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19 417 When considering relative root biomass allocation (RMF), our results were consistent
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21 418 with the predictions of optimal allocation theory (Thornley, 1972, Wilson, 1988), with additions
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23 419 of both N and P reducing RMF. As with BGB, there was an interaction between N and Light,
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25 420 where the greatest reduction in RMF with N addition occurred in sites with low light penetration
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27 421 through the canopy. Because there was not a significant impact of P addition on BGB we infer
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29 422 that the reduction in RMF with P addition was caused by an increase in aboveground biomass
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31 423 (Fay and others, 2015). Together these results suggest that the predictions of optimal allocation
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33 424 theory with respect to N limitation are robust across wide environmental gradients, but
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35 425 interestingly, that allocation responses to P limitation are not as strong. Given the high – and
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37 426 increasing – rates of N and P fertilization of Earth’s ecosystems (Falkowski and others, 2000),
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39 427 the mechanisms underlying these differences are worthy of further investigation.
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46 429 *Potential mechanisms underlying belowground responses to nutrient enrichment*

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49 430 In addition to the plastic allocation responses already discussed, allocation to roots, stem
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51 431 and leaves can also vary with the size of an individual according to allometric scaling theory
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53 432 (Weiner 2004). An analysis of a global forest biomass dataset found intraspecific variation in
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3 433 allocation along environmental gradients consistent with optimal allocation theory, but not
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5 434 intraspecific variation in allocation, and suggested that allometric changes with individual plant
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7 435 size could be responsible for unexplained variation in allocation (McCarthy and Enquist 2007).
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10 436 With respect to our analysis, allometric scaling rules associated with increasing plant size could
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12 437 potentially explain the proportional decline in RMF with N enrichment, but could not explain the
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14 438 absolute decline in root biomass.

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17 439 Shifts in species diversity and composition could also alter community-level allocation of
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19 440 belowground biomass as a result of nutrient enrichment, particularly at the multi-year timescales
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21 441 considered in this study (Olf, 1992, Dybzinski & McNickle, 2013, Mueller and others, 2013).
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23 442 Species with high root allocation tend to grow slowly but are often competitively dominant
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25 443 (Gurevitch, and others, 1990, Aerts and others, 1991), particularly under low resource supply,
26
27 444 due to their ability to draw down levels of soil water and nutrients (Tilman & Wedin, 1991).
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30 445 With nutrient enrichment and a shift towards light limitation, species with lower allocation to
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32 446 roots but a capacity for faster aboveground growth are likely to shade and competitively suppress
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34 447 slower growing, lower-statured species (Grime and others, 1991).

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36
37 448 Nutrient enrichment often reduces species richness (Suding and others, 2005, Bobbink
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39 449 and others, 2010). Across the Nutrient Network, local loss of species diversity in response to N
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41 450 addition was increased by light-limitation (Borer and others, 2014b), and individual species
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43 451 responses to nutrient enrichment were predictable based on a trade-off in growth-defense
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45 452 strategy (Lind and others, 2013). This suggests that species composition shifts contributed to the
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47 453 belowground biomass and allocation responses to N enrichment and light-limitation documented
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49 454 here, but without monocultures to supplement our naturally assembled diverse communities, it is
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51 455 not possible to quantify the relative contribution of intra-specific (plastic) versus inter-specific
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3 456 responses to the observed shifts in allocation at the community level. Future work should aim to
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5 457 evaluate the influence of shifting species composition in community-level biomass allocation
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8 458 and resulting feedbacks to ecosystem function.
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12 460 *Conclusions: ramifications for understanding ecosystem responses to global change*

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14 461 Ecosystem responses to global environmental change have the potential to either dampen
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16 462 or intensify the magnitude of future climate change through C-cycle feedbacks (Field and others,
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18 463 2007). Despite the importance of grasslands to the terrestrial C sink (Scurlock & Hall, 1998,
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20 464 Follett & Reed, 2010), belowground responses to environmental changes are often not
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22 465 considered in synthesis efforts (e.g. Elser and others, 2007, LeBauer & Treseder, 2007, Lee and
23
24 466 others, 2010). Recent database efforts are aiming to address this need, for instance with the
25
26 467 creation of the Fine Root Ecology Database (Iversen and others, 2017). This study demonstrates
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28 468 that global changes interact with the local environment to influence allocation above- versus
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30 469 belowground, that shallow roots respond in predictable ways to globally pervasive changes, and
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32 470 that measurements of allocation, root production, and turnover will be necessary to accurately
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34 471 predict the ramifications for ecosystem-level processes.
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41 473 **Supplementary Material**

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43 474 Supplementary material includes additional methods, tables, detailed statistical analyses and R
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45 475 code, as well as a table of author contributions.
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49 476

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For Peer Review

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

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	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

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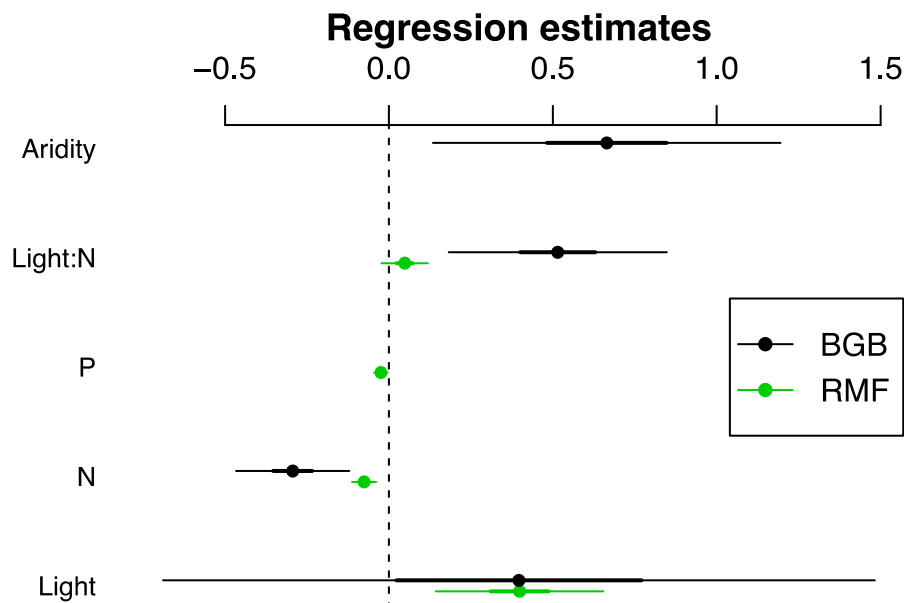
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698 Table 2. Analysis of deviance table (Type II tests) showing the regression parameter estimate
 699 (Est), χ^2 test statistic and p-value for each term in the mixed effects models described in the
 700 Methods. This analysis evaluated how factorial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

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

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3 707 Figure 1. Mean parameter estimates and confidence intervals (thin and thick lines indicate 95%
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5 708 and 50% confidence intervals, respectively) for fixed effects in models evaluating the response
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7 709 of root mass fraction (RMF, in green) and root biomass (BGB, in black) to experimental addition
8
9 710 of multiple nutrients, including nitrogen (N) and phosphorus (P). Average light availability at the
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11 711 soil surface in control plots and aridity (Global Aridity Index, see Methods) were included as
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13 712 site-level covariates. Only statistically significant parameter estimates from Table 2 are displayed
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15 713 (note the main effect of light on BGB is not significant, but is displayed because of the
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17 714 significant light:N interaction).
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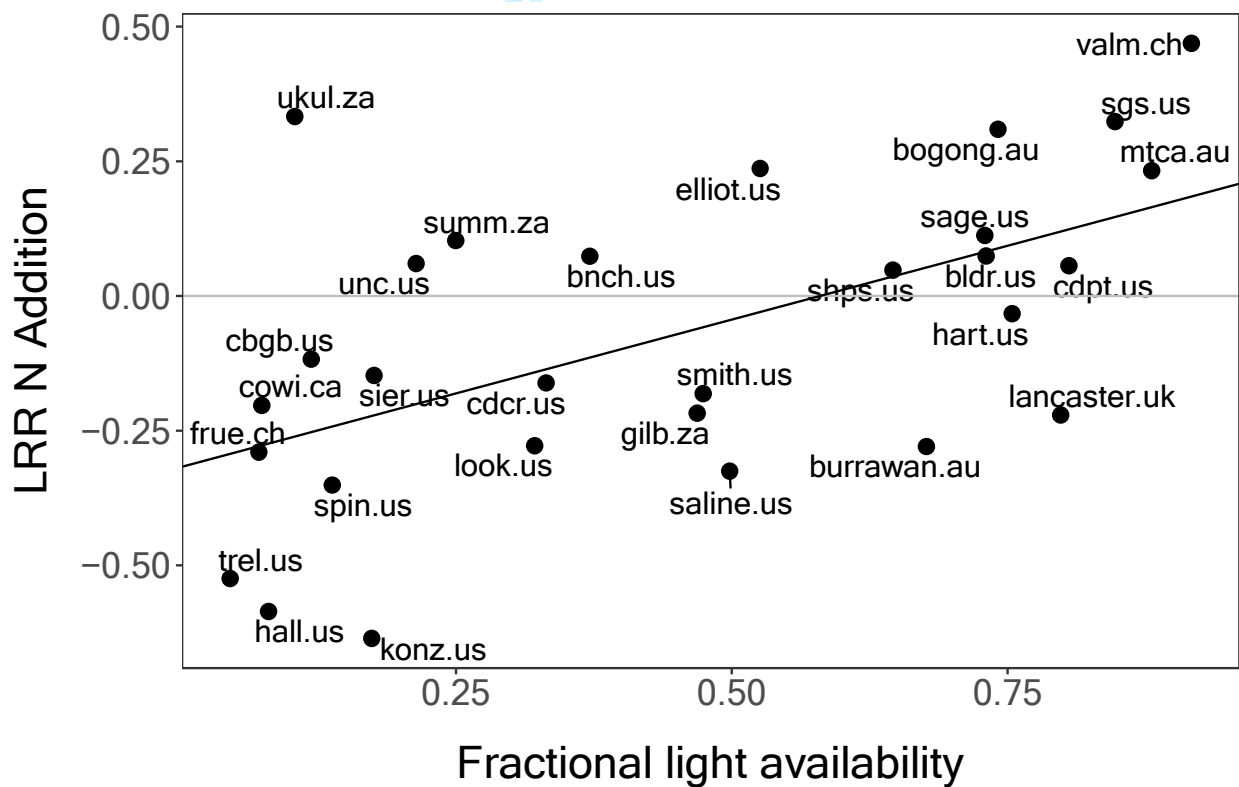


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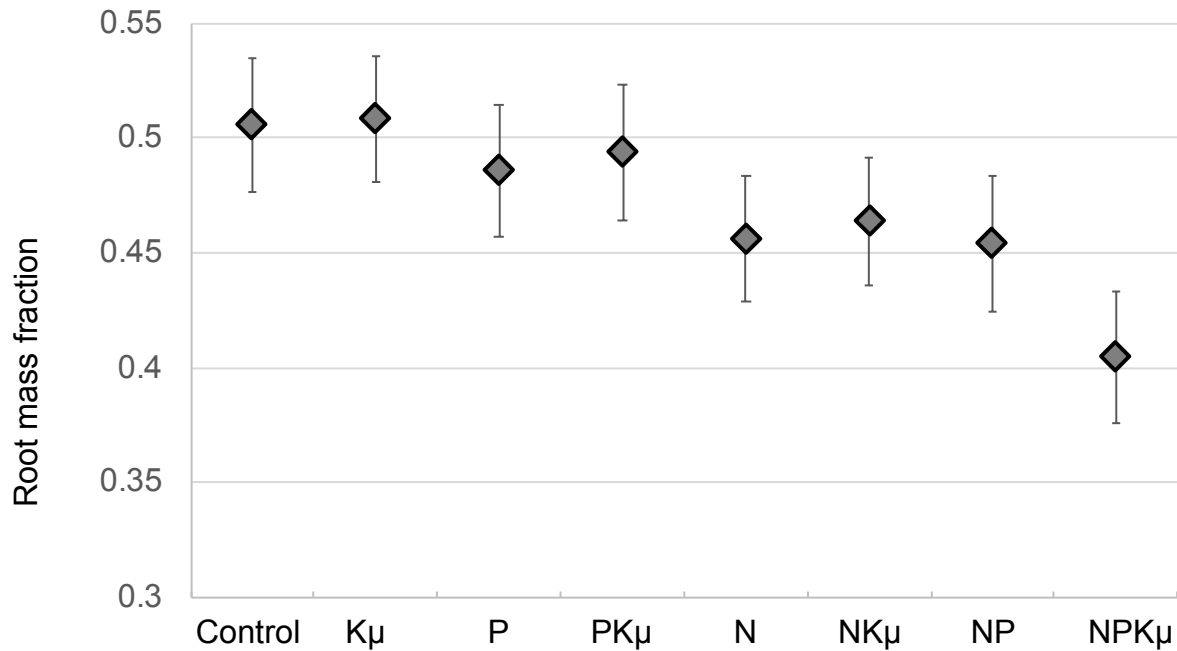
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3 718 Figure 2. The root biomass response to N addition depended on site-level light limitation.
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5 719 Fractional light availability (light) is the proportion of photosynthetically active radiation passing
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7 720 through the grassland canopy. The natural-log response ratio of root biomass to N addition (LRR
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9 721 N addition) is equivalent to the average \ln -root biomass (g/m^2) in plots without N addition
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11 722 subtracted from the average \ln -root biomass (g/m^2) in N addition plots. The grey line indicates
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13 723 LRR=0 or no difference in root biomass between ambient and N enriched plots. Negative values
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15 724 indicate a decline in root biomass in plots with N addition compared to plots without N addition.
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17 725 Black trend line shows the best linear fit, indicating that N addition increased root biomass only
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19 726 where abundant light passed through the canopy. Data labels indicate site names as in Table S1.
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730 Figure 3. Mean root mass fraction (RMF) in each of experimental nutrient addition treatments,
731 including nitrogen (N), phosphorus (P) or potassium plus micronutrients (K_{μ}), singly and in
732 combination. Means are averages of plot level data across all sites, error bars indicate one
733 standard error of the mean. Addition of N and P both resulted in a significant reduction of RMF
734 (N and P as main effects, without significant interactions).



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