

Supplementary Material – Supplemental methods, tables figures, and statistical analyses

Experimental design, number of replicates, and exceptions

The Nutrient Network experimental design and methods are described in detail in Borer and others, (2014a); this Appendix provides detailed methods not included in the main text or the Borer and others, (2014a) descriptions. For this effort we sampled all plots within the participating sites, including plots with vertebrate herbivore exclusion; however, the fenced plots were excluded from all analyses in this manuscript. The following text includes details on the fencing treatments in some sites for the sake of completeness, as these data are available as part of the complete dataset. The 29 sites included in this analysis are summarized in Table S1. Most sites have 30 plots arrayed in three blocks. Exceptions (for sites included in this study) are as follows: bldr.us has two blocks (20 plots); cbgb has six blocks, but only three contain the fencing treatments (54 plots sampled for this effort), lancaster.uk sampled only 26 of their 30 plots due to a broken corer. ukal.za and summ.za do not have the fencing treatments and hence have eight plots in each of three blocks resulting 24 plots.

While most sites were dominated by herbaceous vegetation, one site (shps.us, a shrub steppe community) had significant cover by woody species. When woody species were present in the above-ground biomass harvest, only the leaves and new twigs from the woody species were harvested (herbaceous species were harvested as usual), hence any above-ground biomass allocation to woody stems was not included in this sampling. Below-ground perennial tissues from woody species would also have been included in the root biomass sampling, but this is unlikely to have impacted the below-ground allocation estimates because the fraction of perennial vegetation at shps.us is similar to the other grassland sites included in the analysis (Table S1). To ensure that this site was not biasing the results, all of the final statistical models were run without shps.us, and in no case was the statistical significance of any model term changed by the omission of this site. Hence, this site was included in all analyses.

Soil sampling in brief:

As described in the main text, five 1” diameter cores were taken from each plot in the area harvested for above-ground biomass. These cores were bulked into one sample and the total sample weight was recorded. The total sample was homogenized and approximately 1/5th was subsampled for root biomass. The weight of this subsample was recorded and used in the calculation of root biomass on a g/m² basis (see below). The remaining soil was distributed for additional analyses (e.g. Prober and others, 2015, see www.nutnet.org for ongoing efforts).

Root extraction from soil:

The subsample was submersed in deionized water (2 mL per gram of soil). The sample was stirred to break up large aggregates, sat on the bench top for 30 minutes, and the pH of the slurry was recorded with a pH meter (Denver Instrument UP-25). This slurry was then added to 1L of tap water. The mineral soil sank while roots and organic matter floated to the surface, and

roots were separated from organic matter by color and shape with tweezers (live roots are lighter in color than dead roots or other organic matter). The live roots were subsequently rinsed on a 0.5 mm sieve to remove any remaining soil, dried to constant mass, weighed to the nearest 0.001 g, and archived for future elemental analysis. The remaining soil and water were passed through a 2mm sieve; the captured rocks were collected from the sieve, rinsed to remove residual soil, dried and weighed to calculate the rock weight in the soil sample. Rock weight was not used to calculate root mass per unit area, but these data were archived so that a calculation of grams roots per gram dry rock-free soil was available for future analysis.

Table S1. Nutrient Network sites included in this analysis. The country in which each site is located is abbreviated as the suffix of the site code (au=Australia, ca=Canada, ch=Switzerland, uk=United Kingdom, us=United States, za=South Africa). The Principle Investigator/s (PI) for each site is listed. Additional information includes site location (latitude and longitude), mean annual precipitation in mm (MAP), Global Aridity Index (GAI) and elevation (m). From the control plots at each site (unfenced, unfertilized) we calculated the mean proportion of photosynthetically active radiation reaching the soil surface (light fraction), fraction of the community made up of graminoid or perennial species, shallow root mass fraction (RMF) and average live plant biomass above- and below-ground. Two sites do not have fencing treatments (indicated with * after the site code).

site code	PI	community type	continent	latitude	longitude	elevation (m)	MAP (mm/yr)	Aridity (GAI)	Light fraction (controls)	gramminoid fraction	perennial fraction	RMF	Shoot live mass (g/m ²)	Root mass (g/m ²)
bldr.us	Brett Melbourne; Kendi Davies	shortgrass prairie	N. America	39.97	-105.23	1633	425	0.366	0.73	0.43	0.4	0.45	95	87
bnch.us	Elizabeth Borer; Eric Seabloom	montane grassland	N. America	44.28	-121.97	1318	1647	1.7054	0.37	0.29	0.55	0.9	142	1264
bogong.au	Joslin L. Moore; John Morgan	alpine grassland	Australia	-36.87	147.25	1760	1592	1.9159	0.74	0.46	0.85	0.37	573	339
burrawan.au	Jennifer Firm	semiarid grassland	Australia	-27.73	151.14	425	683	0.432	0.68	0.58	0.57	0.43	180	136
cbgb.us	Lori A. Biederman; W. Stanley Harpole	tallgrass prairie	N. America	41.79	-93.39	275	855	0.849	0.12	0.39	0.53	0.27	312	107
cdcr.us	Eric Seabloom; Elizabeth Borer	tallgrass prairie	N. America	45.4	-93.2	270	750	0.8351	0.33	0.42	0.44	0.46	192	139
cdpt.us	Johannes M. H. Knops	shortgrass prairie	N. America	41.2	-101.63	965	445	0.4028	0.81	0.73	0.12	0.79	244	960
cowi.ca	Andrew MacDougall	old field	N. America	48.46	-123.38	50	764	1.0491	0.07	0.51	0.55	0.69	758	1675
elliott.us	Elsa Cleland	annual grassland	N. America	32.88	-117.05	200	331	0.2565	0.53	0.7	0.11	0.31	312	128
frue.ch	Yann Hautier; Sabine Gusewell	pasture	Europe	47.11	8.54	995	1355	1.9924	0.07	0.4	0.8	0.16	1126	222
gilb.za	Peter D. Wragg	montane grassland	Africa	-29.28	30.29	1748	926	0.7803	0.47	0.65	0.94	0.84	221	1204
hall.us	Rebecca L. McCulley	tallgrass prairie	N. America	36.87	-86.7	194	1282	1.0541	0.08	0.5	0.78	0.22	392	104
hart.us	David Pyke; Nicole M. DeCrappeo	shrub steppe	N. America	42.72	-119.5	1508	272	0.2506	0.75	0.16	0.32	0.73	46	127
konz.us	Melinda Smith; Kimberly J. La Pierre	tallgrass prairie	N. America	39.07	-96.58	440	877	0.7617	0.17	0.78	0.99	0.34	458	232
lancaster.uk	Carly Stevens	mesic grassland	Europe	53.99	-2.63	180	1322	2.2341	0.8	0.76	0.96	0.85	123	546
look.us	Elizabeth Borer; Eric Seabloom	montane grassland	N. America	44.21	-122.13	1500	1898	2.1509	0.32	0.26	0.45	0.93	43	988
mtca.au	Suzanne M Prober	savanna	Australia	-31.78	117.61	285	330	0.2221	0.88	0.24	0.19	0.38	172	122
sage.us	Daniel S. Gruner; Louie Yang	montane grassland	N. America	39.43	-120.24	1920	882	0.9016	0.73	0.18	0.64	0.58	145	174
saline.us	Kimberly J. La Pierre; Melinda Smith	mixedgrass prairie	N. America	39.05	-99.1	440	607	0.491	0.5	0.64	0.9	0.51	270	299
sgs.us	Cynthia S. Brown; Dana M. Blumenthal	shortgrass prairie	N. America	40.82	-104.77	1650	365	0.3255	0.85	0.52	0.61	0.8	87	405
shps.us	Peter Adler	shrub steppe	N. America	44.24	-112.2	910	262	0.2626	0.65	0.16	0.52	0.66	182	349
sier.us	W. Stanley Harpole; Eric Seabloom; Elizabeth Borer	annual grassland	N. America	39.24	-121.28	197	935	0.6639	0.18	0.4	0.12	0.15	324	60
smith.us	Jonathan D. Bakker	mesic grassland	N. America	48.21	-122.62	62	597	0.7796	0.47	0.21	0.3	0.5	326	322
spin.us	Rebecca L. McCulley	pasture	N. America	38.14	-84.5	271	1140	1.0008	0.14	0.47	0.57	0.68	238	859
summ.za*	Peter D. Wragg	mesic grassland	Africa	-29.81	30.72	679	939	0.7308	0.25	0.65	0.98	0.8	333	1379
trel.us	Andrew Leakey	tallgrass prairie	N. America	40.08	-88.83	200	982	0.8927	0.05	0.2	0.82	0.07	1609	114
ukul.za*	Nicole Hagenah; Kevin P Kirkman	mesic grassland	Africa	-29.67	30.4	843	880	0.6317	0.1	0.66	0.97	0.23	774	264
unc.us	Charles Mitchell; Justin Wright	old field	N. America	36.01	-79.02	141	1163	0.9121	0.21	0.73	0.85	0.48	317	356
valm.ch	Anita C. Risch; Martin Schuetz	alpine grassland	Europe	46.63	10.37	2320	1098	1.6479	0.92	0.28	0.9	0.69	207	466

Calculation of root biomass on per area basis:

Although all sites were sampled to 10 cm depth, six of the 29 sites used a non-standard corer, and hence the total ground area sampled deviated slightly from the majority of sites: bnch.us and look.us took 3, 4.0 cm diameter cores for a total area of 37.7 cm² per plot; spin.us and summ.za took 5, 3.1 cm diameter cores for a total area of 37.7 cm² per plot; cdrc.us took 3, 3.17 cm diameter cores for a total area of 26.6 cm² per plot; valm.ch took 6, 2.3 cm diameter cores for a total area of 24.9 cm² per plot; hart.us used a 2.0 cm diameter corer for a subset of the samples, resulting in 22 cm² and 15.7 cm² total areas for plots where they collected 7 or 5 cores respectively; gilb.za had extremely thin soils over an impermeable rock layer and hence collected variable numbers of cores per plot, all less than 10 cm depth, with a 3.1 cm diameter corer for a total of 39-90 cm² per plot). As it is unlikely there were roots reaching into the rock layer, we assume the sampling captured all roots within the 10 cm depth. Regardless of sampling method, root biomass data from all sites was calculated as grams roots per m² of total area sampled. For each plot root biomass (g/m²) was calculated as follows:

$$\text{root biomass (g/m}^2\text{)} = \frac{\text{extracted root weight (g)} * \text{total weight of soil cores (g)}}{\text{area of combined soil cores (m}^2\text{)} * \text{weight of soil subsample for roots(g)}}$$

Subsequently, the root mass fraction (RMF) was calculated as the proportion of biomass below-ground, compared to total biomass:

$$\text{RMF} = \frac{\text{root biomass g/m}^2}{(\text{root biomass g/m}^2 + \text{above-ground live biomass g/m}^2)}$$

Estimate of site-level light limitation:

As described in the main text, the degree of light limitation was estimated as a site-level covariate, based on measures of the proportion of photosynthetically active radiation (PAR) reaching the soil surface in control plots (unfenced, unfertilized). Light was only measured during full-sun conditions; due to cloudy conditions six of the 29 sites did not collect light measurements during the year corresponding to above- and below-ground biomass harvests. Hence, the mean proportion of PAR reaching the soil surface in the control plots was calculated across all experiment years at each site, referred to as “Light” throughout the manuscript. For the 23 sites that measured the proportion of PAR reaching the soil surface in the focal year, these values were highly correlated with Light when evaluated with a Pearson correlation ($r=0.98$, $p < 0.0001$, see Figure S1 below, point labels are site codes listed in Table S1).

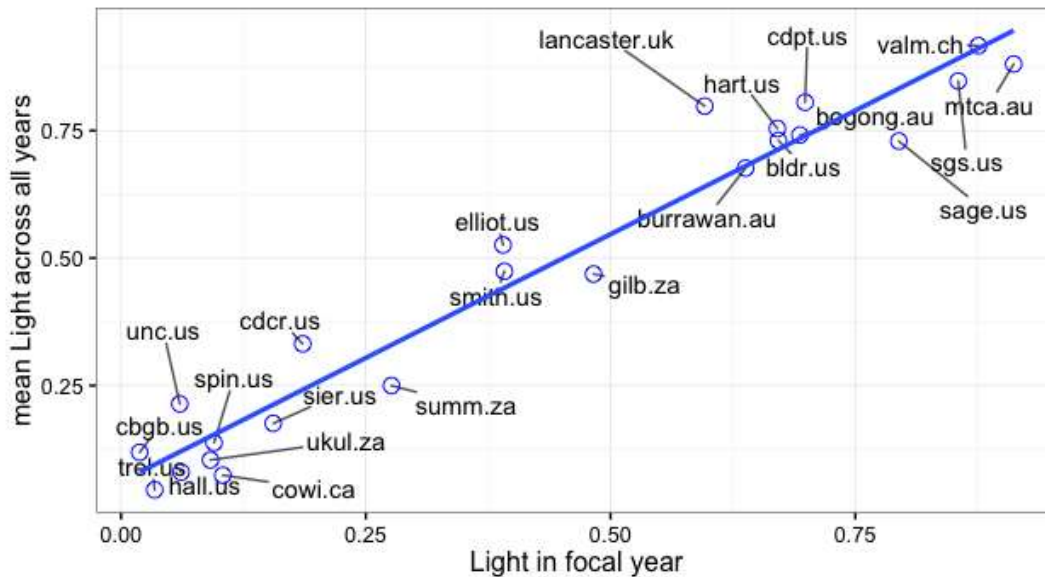
Statistical analysis:

Identification of co-variates:

The Global Aridity Index (GAI) and light-limitation (Light, discussed above) were a priori identified as two site-level co-variates with potential to influence the response of absolute

below-ground production and relative biomass distribution to nutrient enrichment. Pearson correlations were conducted to evaluate the co-variation of these and other site-level characteristics, including the root mass fraction, above-ground biomass, and the proportion of vegetative cover comprised by monocots and perennial species, all in the control plots (unfenced, unfertilized). Correlation coefficients and significance are shown in Table 1 in the main text.

Figure S1. The correlation between fractional Light availability under the canopy in control plots in the year of sampling for this manuscript, and averaged across all years at each site.



Inspection of data for normality, transformation, and analysis:

Initial visual data inspection employed quantile-quantile plots from the MASS package (Venables & Ripley, 2002). Visual inspection of the below-ground root biomass dataset on a g/m^2 basis (BGB) showed that the data were log-normally distributed (Figure S2). Following the recommendations of Bolker and others, (2009), the data were natural log-transformed. Root mass fraction (RMF) was also non-normally distributed (Figure S3), as is common for proportional data (Bolker 2008). Following the recommendation of Warton and Hui (2011) the RMF data were logit transformed. Transformed data were visually re-assessed for normality (Figures S2 and S3) and subsequently analyzed with general linear mixed models using the lme call in nlme (Pinheiro and others, 2013). The parameter estimates for all model terms are displayed in Table 2 in the main text, and are displayed along with their confidence intervals in Figure S4.

Figure S2. Quantile-quantile plots showing the distribution of errors for the root biomass (g/m^2 , left) versus natural-log transformed root biomass (right). The solid blue line shows the estimated values for data defined by normal distributions, with the dashed lines showing the 95% confidence intervals for these estimates.

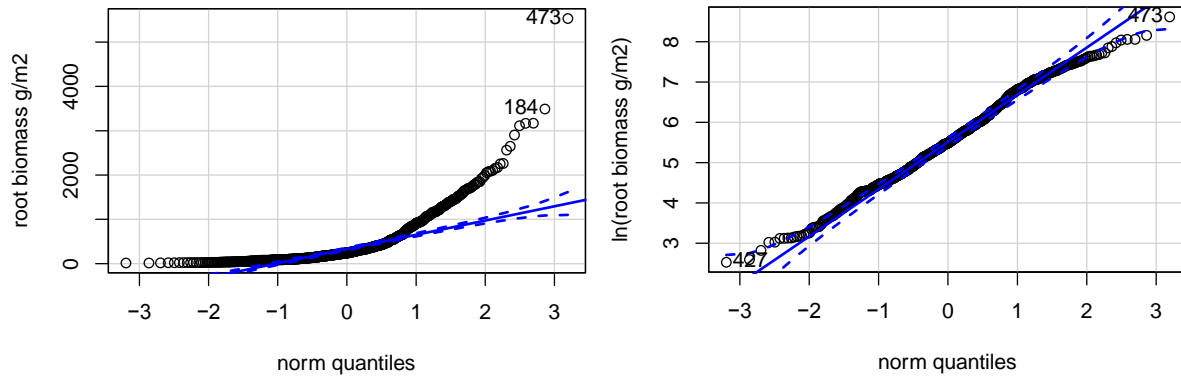


Figure S3. Quantile-quantile plots showing the distribution of errors for root mass fraction (RMF, left) versus logit transformed RMF (right). The solid blue line shows the estimated values for data defined by normal distributions, with the dashed lines showing the 95% confidence intervals for these estimates.

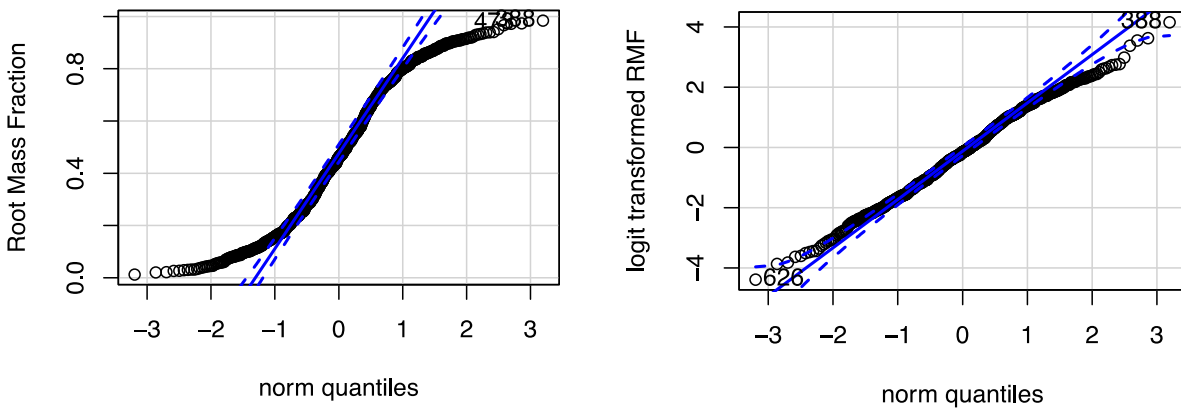
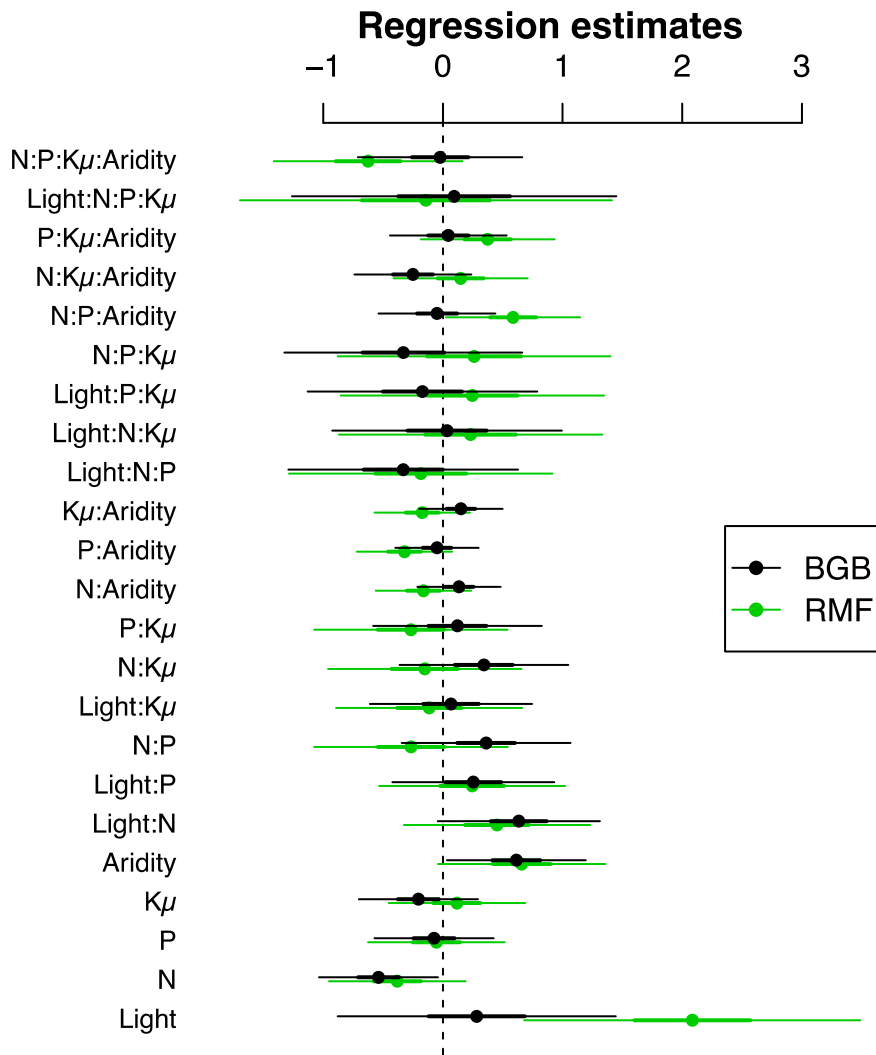


Figure S4. Parameter estimates and 95% confidence intervals (thin lines, thicker lines indicate 50% confidence intervals) for fixed effects in models evaluating the response of logit-transformed shallow root mass fraction (RMF, in green) and natural log-transformed root biomass (BGB in black) to experimental addition multiple nutrients, including nitrogen (N), phosphorus (P) and potassium plus micronutrients (K_{μ}). Average light availability at the soil surface in control plots, and aridity (Global Aridity Index, see Methods) were included as site-level covariates.



R code for the analyses presented in the main text:

Code for statistical output in Table 1 in the main text, using only data from control plots:

```
rcorr(as.matrix(dataforcorr))
```

Models associated with statistical output for Table 2 in the main text:

```
Inrootsfullmodel<-lme(Inroots~Light*N*P*Kμ + Aridity*N*P*Kμ, data=multnut1,  
random = ~1|site_code, na.action="na.omit")  
Anova(Inrootsfullmodel)
```

```
RMFfullmodel <-lme(logitRMF~Light*N*P*Kμ + Aridity*N*P*Kμ, data=multnut1,  
random = ~1|site_code)  
Anova(RMFfullmodel)
```

Code for displaying parameter estimates in Figure S4 (code modified to display only significant terms in Figure 1 in the main text):

```
colvec <- c(11,1)  
coefplot2(list(RMF= RMFfullmodel, Inroots=Inrootsfullmodel), legend=TRUE,  
legend.x="right", col=colvec)
```


Table S2. Summary of published and unpublished data on root depth distributions at the Nutrient Network sites included in this analysis (site abbreviations correspond to those in Table S1).

site code	PI	community type	Data available	citations	Communication from
bldr.us	Brett Melbourne; Kendi Davies	shortgrass prairie	none available, very rocky		Brett Melbourne
bnch.us	Elizabeth Borer; Eric Seabloom	montane grassland	none available		Eric Seabloom
bogong.au	Joslin L. Moore; John Morgan	alpine grassland	none available		Joslin Moore
burrawan.au	Jennifer Firn	semiarid grassland	none available		Jenn Firn
cbgb.us	Lori A. Biederman; W. Stanley Harpole	tallgrass prairie	none available		Lori Biederman, Stan Harpole, Lauren Sullivan
cdr.us	Eric Seabloom; Elizabeth Borer	tallgrass prairie	75% of root biomass in most diverse treatment (16 species) was in top 60 cm of 100 cm (Mueller et al 2013).	Mueller et al. 2013 Ecology	Eric Seabloom
cdpt.us	Johannes M. H. Knops	shortgrass prairie	Too rocky to sample deeper than 10 cm, we assume our effort sampled majority of root biomass		Jean Knops
cowi.ca	Andrew MacDougall	old field	37% of roots to 50 cm found in top 10 cm	Ziter and MacDougall 2013 Ecology Figure 1.	Andrew MacDougall
elliott.us	Elsa Cleland	annual grassland	none available		Elsa Cleland
frue.ch	Yann Hautier; Sabine Gusewell	pasture	none available		Yann Hautier; Sabine Gusewell
glib.za	Peter D. Wragg	montane grassland	Too rocky to sample deeper than 10 cm, we assume our effort sampled majority of root biomass		Peter Wragg
hall.us	Rebecca L. McCulley	tallgrass prairie	60% of roots in top 1 m were in the top 10 cm	unpublished data from Nutnet site	Rebecca McCulley
hart.us	David Pyke; Nicole M. DeCrappeo	shrub steppe	49% of root organic carbon is in the top 15 cm, measured to a depth of 90 cm (extracted from Figure 4, phase 2, Rau et al. 2011). Note Hart Mountain has 2 of 7 sites summarized in these data (Grey Butte and Rock Creek)	Rau et al. 2011, Transition From Sagebrush Steppe to Annual Grass (<i>Bromus tectorum</i>): Influence on Belowground Carbon and Nitrogen, Rangeland Ecology and Management, 64:139–147	David Pyke
konz.us	Melinda Smith; Kimberly J. La Pierre	tallgrass prairie	approximately 50% of root biomass to a depth of 1 m is in the top 10 cm (ungrazed sites, Nippert et al 2012), grazed sites have less biomass but follow same distributional profile	Nippert et al. 2012 Plant and Soil (Figure 1)	Kim La Pierre
lancaster.uk	Carly Stevens	mesic grassland	none available		Carly Stevens
look.us	Elizabeth Borer; Eric Seabloom	montane grassland	Too rocky to sample deeper than 10 cm, we assume our effort sampled majority of root biomass		Eric Seabloom
mtca.au	Suzanne M Prober	savanna	65-80% of roots in the top 10 cm compared to top 100 cm (for native versus exotic dominated areas respectively)	Creamer, Farrell & Prober, unpublished data	Suzanne Prober
sage.us	Daniel S. Gruner; Louie Yang	montane grassland	Too rocky to sample deeper than 10 cm, we assume our effort sampled majority of root biomass		Dan Gruner
saline.us	Kimberly J. La Pierre; Melinda Smith	mixedgrass prairie	76% of root biomass is in the top 15 cm, when sampled to a depth of 120 cm (Hopkins 1953)	Hopkins et al. 1953 Journal of Range Management	Kim La Pierre
sgs.us	Cynthia S. Brown; Dana M. Blumenthal	shortgrass prairie	About 30% of roots in the top 10 cm (to a depth of 1 m), with 60% in the top 30 cm, for areas dominated by <i>Bouteloua gracilis</i>	Lee & Lauenroth 1994, American Midland Naturalist	Cini Brown
shps.us	Peter Adler	shrub steppe	61% of root biomass in the top 10 cm, of 110 cm total depth sampled	Andrew Kulmatiski (unpublished data)	Peter Adler
sier.us	W. Stanley Harpole; Eric Seabloom; Elizabeth Borer	annual grassland	55% of roots to 30 cm are in top 10 cm (from "controlRain" plots containing both grasses and forbs)	Lauren Hallett & Katie Suding (unpublished data)	Katie Suding
smith.us	Jonathan D. Bakker	mesic grassland	Too rocky to sample deeper than 10 cm, we assume our effort sampled majority of root biomass		Jon Bakker
spin.us	Rebecca L. McCulley	pasture	69% of roots in top 1m were in top 10 cm	unpublished data from Nutnet site	Rebecca McCulley
summ.za	Peter D. Wragg	mesic grassland	none available		Peter Wragg
trel.us	Andrew Leakey	tallgrass prairie	none available		Andrew Leakey
ukul.za	Nicole Hagenah; Kevin P Kirkman	mesic grassland	Most root biomass in top 15 cm, samples taken to 40 cm (unpublished data)		Kevin Kirkman
unc.us	Charles Mitchell; Justin Wright	old field	none available		Justin Wright
valm.ch	Anita C. Risch; Martin Schuetz	alpine grassland	Too rocky to sample deeper than 10 cm, we assume our effort sampled majority of root biomass		Anita Risch

Supplementary References:

Bolker, B.M., 2008. Chapter 4: Distributions, in: Ecological models and data in R. Princeton University Press. pp 103-146.

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

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