

Root depth distribution and the diversity–productivity relationship in a long-term grassland experiment

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Abstract. The relationship between plant diversity and productivity in grasslands could depend, partly, on how diversity affects vertical distributions of root biomass in soil; yet, no prior study has evaluated the links among diversity, root depth distributions, and productivity in a long-term experiment. We used data from a 12-year experiment to ask how plant species richness and composition influenced both observed and expected root depth distributions of plant communities. Expected root depth distributions were based on the abundance of species in each community and two traits of species that were measured in monocultures: root depth distributions and root-to-shoot ratios. The observed proportion of deep-root biomass increased more than expected with species richness and was positively correlated with aboveground productivity. Indeed, the proportion of deep-root biomass explained variation in productivity even after accounting for legume presence/abundance and greater nitrogen availability in diverse plots. Diverse plots had root depth distributions that were twice as deep as expected from their species composition and corresponding monoculture traits, partly due to interactions between C₄ grasses and legumes. These results suggest that the productivity of diverse plant communities was partly dependent on belowground plant interactions that caused roots to be distributed more deeply in soil.

Key words: aboveground biomass; C₄ grass; complementarity; interspecific interactions; legume; root biomass; species richness.

INTRODUCTION

The positive diversity–productivity relationship in experimental grasslands is facilitated, partly, by greater capture of soil resources in more diverse plant communities (Tilman et al. 1996, Scherer-Lorenzen et al. 2003, Spehn et al. 2005, Fornara and Tilman 2009). Several factors can enhance resource acquisition in diverse plant communities, including: (1) the presence of nitrogen-fixing legumes (Spehn et al. 2002); (2) positive feedbacks from plant productivity and plant nutrient concentrations to soil nutrient availability (Fornara and Tilman 2009, Reich et al. 2012); (3) high root biomass and root activity (Tilman et al. 1996, de Kroon et al. 2012); and (4) niche differentiation with respect to resource requirements and extraction (Berendse 1982, McKane et al. 1990, Fargione and Tilman 2005a, von Felten et al. 2009). In this paper, we focus on one related, but understudied, factor that could greatly influence soil resource use and partitioning: the vertical distribution of roots in soil.

The vertical distribution of roots could influence the amount and complementarity of soil resource extraction in two ways. First, combinations of species with *inherently* different rooting distributions; for example, shallow- and deep-rooted species could facilitate coexistence and more exhaustive use of soil resources (Berendse 1982, Mommer et al. 2010). Second, *adjustments* in root:shoot ratios or rooting depths by one or more species in a community could facilitate coexistence and increase total resource extraction. For instance, in response to depletion of surface soil resources in diverse communities, some species might allocate more root biomass to deep soil (Fargione and Tilman 2005b, Schenk 2008, Skinner and Comas 2010). Species in diverse communities might also alter the depth distribution of roots in response to the density and identity of neighboring roots (Schenk 2006, Mommer et al. 2010, de Kroon et al. 2012).

In this study, we explored the relationships among plant diversity, root depth distributions, and productivity using data from the 12th year of a grassland plant diversity experiment (Tilman et al. 2001). First, we evaluated how root depth distributions, at the community level, are influenced by plant species richness and the presence and abundance of plants from different

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functional groups. We then assessed the implications of community-level root depth distributions for the diversity–productivity relationship. Previous studies of this experiment showed that both above- and belowground plant biomass were positively correlated with plant species richness.

METHODS

Study site

The experiment, located near Bethel, Minnesota (USA), was initiated in 1993 and planted in 1994 (Tilman et al. 2001). For the growing season, approximately May thru September, the average maximum daily temperature between 1994 and 2006 was 24.4°C, the average minimum temperature was 11.7°C, and the average precipitation was 480 mm. Soils are derived from glacial outwash and have coarse texture (>90% sand). The percentages of carbon and nitrogen (N) in soil are typically lower than 1% and 0.1%. The upper 6–8 cm of soil was removed prior to seeding. Plots (9 × 9 m) were seeded to achieve five different levels of plant species richness (1, 2, 4, 8, and 16 species). Each level of species richness was replicated >25 times. Species composition of each plot was determined by random draws from a pool of 18 plant species that included four non-legume forbs (hereafter forbs), four non-woody legumes, four C₃ grasses, four C₄ grasses, and two *Quercus* species. Thus, all 16-species plots contained at least two species from each of the herbaceous plant types. All plots were ignited in the spring of each year and weeded ~3 times per year to remove non-planted species. Following Tilman et al. (2006), we focused on 152 plots that burn well and have very little *Quercus* biomass.

Sampling

In August 2006, we sampled root biomass in three different depth increments: 0–30 cm, 30–60 cm, and 60 to 100 cm. Three soil cores, 5 cm in diameter, were removed and composited for each plot before roots were isolated from the soil by rinsing with water over a 1.5-mm mesh screen. Roots were dried at 40°C for 10 d and weighed (Fornara and Tilman 2008). Aboveground biomass was sampled in August, and it approximates aboveground productivity due to annual spring burning (Tilman et al. 2006).

Estimating net adjustments of rooting depth in multi-species communities

For each plant species and each rooting depth increment (0–30, 30–60, and 60–100 cm), we calculated the ratio of root biomass to aboveground biomass using data from the monoculture plots of each species. Then, for each species in a multispecies plot, the monoculture-derived root : shoot ratios were multiplied by the relative aboveground abundance of that species in the mixture (i.e., the proportion of total aboveground biomass attributed to that species). Finally, the calculated root

biomass values for each species in a plot were summed to produce an “expected” root depth distribution for each plot. The expected root depth distributions reflect a null hypothesis for each experimental plant community, based on the null expectation that species do not adjust their root : shoot ratios or root depth distributions in response to changes in community composition or resource availability. Consequently, deviations of observed root depth distributions from expected values reflect adjustments in rooting depth and/or root : shoot ratios of individual species that cause the root depth distribution of the whole community to become deeper, or more shallow, than expected based on community composition and monoculture traits.

We estimated the expected root depth distributions for a subset of plots dominated by species with well-characterized root depth distributions in monoculture. We defined well-characterized species as those for which >70% of aboveground biomass in monoculture plots was derived from the target species. Twelve of the 16 focal species met this criterion; two C₃ grasses, including *Poa pratensis*, and two forbs did not meet this criterion. However, we have confidence in our estimate of the root depth distribution of *Poa pratensis* monocultures because a similar value, within 1% of our estimate, was observed in a neighboring experiment (P. B. Reich, unpublished data). Expected root depth distributions were then calculated for plots where >70% of the aboveground biomass was accounted for by these 13 species (137 out of 152 plots). More and less strict cutoffs yielded similar results.

Statistics

All data were assessed for normality and transformed accordingly, frequently using a square-root transformation. We then used ANOVA models with different combinations of factors to tease out their effects on dependent variables. Type III sums of squares were used for significance tests, such that the contribution of each factor was evaluated *after* accounting for the effects of the other predictors (Hector et al. 2010). Community functional composition was evaluated using binary variables coded for the presence/absence of different plant functional groups (e.g., legumes, C₄ grasses). To assess the effects of individual species, we used separate analyses with binary variables coded for the presence/absence of each of the 13 focal species (species richness was not included as a covariate). Finally, to build on previous studies that identified plant N concentrations and soil N availability as important predictors of productivity in our experiment (Fargione et al. 2007, Fornara and Tilman 2009), we compared the effect of root depth distributions and N-related parameters on aboveground biomass and total root biomass in additional regression models. All analyses were performed using JMP (SAS Institute 2007).

RESULTS

Depth distribution of root biomass

Across all plots, the upper 30 cm of soil contained between 51% and 100% of the total root biomass (sampled to a depth of 1 m). Root biomass in the 30–60 cm and 60–100 cm depth increments showed similar patterns with species richness (Appendix: Fig. A1) and community composition (not shown), so for statistical analyses we combined these two depth increments into one: root biomass between 30 cm and 100 cm.

Planted species richness was positively correlated with root biomass in the surface soil (0–30 cm; $R^2 = 0.43$, $P < 0.0001$) and in the deeper soil (30–100 cm; $R^2 = 0.34$, $P < 0.0001$), even after accounting for variation in the presence of different functional groups (Appendix: Table A1). Species richness had a greater positive effect on deep-root biomass; the median root biomass below 30 cm was ~ 7 times higher in 16-species plots than in monocultures, while the median root biomass in the upper 30 cm of soil was ~ 3.5 times higher in 16-species plots as compared to monocultures. Consequently, species richness had a positive effect on the proportion of total root biomass present below 30 cm (hereafter, the deep-root proportion; $P < 0.001$; Fig. 1C; Appendix: Table A2).

The effects of plant functional composition on root biomass at different depths were consistent with patterns observed in monocultures. Among monocultures, legumes had the deepest rooting systems, with $>20\%$ of root biomass typically below 30 cm (Appendix: Table A3). Considering all plots, the presence of legumes was associated with higher root biomass in each depth increment, especially in the 30–100 cm increment ($P < 0.001$; Appendix: Table A1), such that plots with legumes had higher deep-root proportions ($P < 0.0001$; Appendix: Table A2). For example, the deep-root proportion in mixtures with legumes was three times larger than in mixtures without legumes (Fig. 1G). Contrastingly, C_3 grasses had the shallowest root systems among monocultures, with typically $<1\%$ of total root biomass occurring below 30 cm (Appendix: Table A3). Accordingly, across all plots, the presence of C_3 grasses had negative effects on both deep-root biomass ($P = 0.01$; Appendix: Table A1) and the deep-root proportion ($P < 0.01$; Appendix: Table A2). In monocultures, C_4 grasses and forbs had intermediate and more species-specific depth distributions of root biomass (Appendix: Table A3), while across all plots, the presence of C_4 grasses and forbs had no main effect on the deep-root proportion (Table A2). For models of root biomass and deep-root proportion, there were significant interaction terms related to plant functional composition; but, species richness and the main functional group effects typically explained more variation, i.e., had higher Type III sums of squares, and had smaller P values (Appendix: Tables A1 and A2).

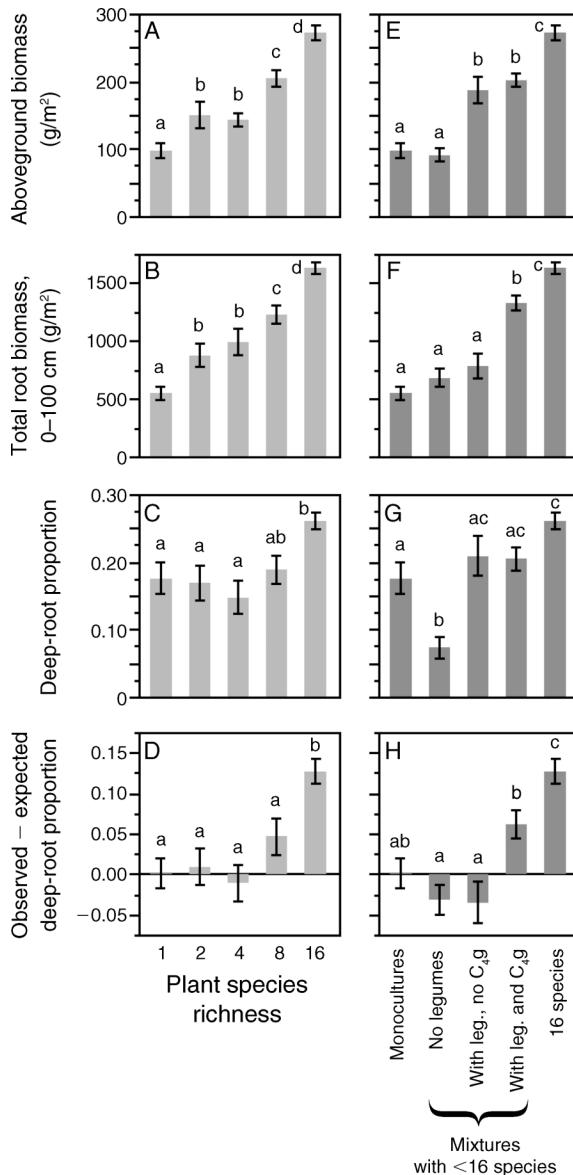


FIG. 1. Effects of species richness and community composition on plant biomass, the deep-root proportion, and the difference between observed and expected deep-root proportions. The categories describing community composition (leg., legumes; and C_{4g} , C_4 grasses) were chosen based on results of statistical models of the deep-root proportion and the difference between observed and expected deep-root proportions (Appendix: Tables A2 and A4). Error bars indicate standard error. Within each panel, bars labeled with different letters are significantly different according to Tukey tests ($P < 0.05$).

Expected vs. observed root depth distributions

The differences between observed and expected root depth distributions, which we expressed as differences between observed and expected deep-root proportions, were also related to species richness and functional composition. The observed deep-root proportion in the most diverse plots was two times higher than the

TABLE 1. Results of four models of aboveground biomass with different sets of predictor variables.

Factor	Model with N parameters only ($R^2 = 0.44$)			Model with N parameters and percentage of deep roots ($R^2 = 0.55$)			Model with N parameters, legume presence, and no. species ($R^2 = 0.68$)			Model with all predictors ($R^2 = 0.70$)		
	SS (%)	<i>t</i>	Effect size	SS (%)	<i>t</i>	Effect size	SS (%)	<i>t</i>	Effect size	SS (%)	<i>t</i>	Effect size
Initial soil N (%)	1	1.9	22	1	2.0	21	2	3.2	28	2	3.1	27
Δ soil N (%) [†]	6	4.1	45	5	3.8	39	2	3.3	29	2	3.3	28
Δ root N (%) [‡]	5	3.6	44	3	3.2	36	ns	ns	ns	ns	ns	ns
Net N mineralization	11	5.3	73	4	3.8	49	2	3.0	34	1	2.5	28
Soil NO ₃ [§]	13	-5.8	-78	9	-5.5	-67	2	-3.2	-38	2	-3.1	-35
Deep roots (%)	na	na	na	11	6.0	69	na	na	na	2	3.3	35
No. species	na	na	na	na	na	na	6	5.3	65	6	5.2	62
Legume presence [¶]	na	na	na	na	na	na	9	6.2	75	5	4.8	59

Notes: All models include five predictor variables related to nitrogen (N) availability. For comparison, deep root proportion, legume presence, and species richness were included as predictors in only two of the four models shown. SS is the percentage of total sums of squares for aboveground biomass that can be uniquely attributed to each predictor variable, using Type III sums of squares. Effect size was estimated by multiplying the model coefficient by two standard deviations of the predictor (similar to Bring [1994]). The effect size is the amount of aboveground biomass (g/m²) predicted to be gained or lost when each predictor shifts from one SD below the mean to one SD above the mean. Abbreviations are: na, not included in the model; and ns, not significant ($P > 0.1$). For each model category, $n = 152$.

[†] The increase in the percentage of soil N between 1994 and 2006.

[‡] The increase in the percentage of root N between 1995 and 2006.

[§] Soil nitrate concentrations. For details on N-related parameters, see Fornara and Tilman (2009).

[¶] Similar results were observed when using legume abundance.

expected value (26% vs. 13%; $P < 0.0001$). The deep-root proportion for plots planted with eight species was 33% higher than expected (19% vs. 14%; $P < 0.05$), whereas observed and expected deep-root proportions were not significantly different for lower levels of species richness (Fig. 1D; significance was evaluated using paired *t* tests). The co-occurrence of legumes and C₄ grasses was strongly associated with higher deep-root proportions than expected ($P \leq 0.001$; Appendix: Table A4). Most diverse plots contained both of these plant types, but species richness had a significant effect on the deviations from expected deep-root proportions even when legume and C₄ grass presence were included as covariates ($P < 0.001$; Appendix: Table A4). Also, when comparing among plots with at least one legume and C₄ grass present, the deviations from expected deep-root proportions were larger for 16-species plots than for plots with eight or less species (Fig. 1H). The presence of forbs and C₃ grasses was associated with deep-root proportions that were lower than expected, but only when species richness was included as a covariate ($P < 0.05$; Appendix: Table A4).

Effects of individual plant species

Lupinus perennis, *Lespedeza capitata*, and *Amorpha canescens* each had significantly positive effects on the deep-root proportion ($P < 0.01$), but the presence of *Petalostemum purpureum* was not a significant factor. According to calculations based on model coefficients, the deep-root proportion increased by 17% when *Lupinus perennis* was present, compared to 5% and 6% when *Lespedeza capitata* and *Amorpha canescens*, respectively, were present. The presence of other species did not have apparent effects on the deep-root

proportion. The presence of *Lespedeza capitata*, *Lupinus perennis*, and *Schizachyrium scoparium* (a C₄ grass) were associated with higher deep-root proportions than expected ($P < 0.05$), with the predicted effect sizes (using model coefficients) of *Lupinus perennis* and *Schizachyrium scoparium* more than double that of *Lespedeza capitata*. None of the species were linked with lower than expected deep-root proportions based on their presence/absence.

Covariance of root depth distributions and plant biomass

Across all levels of species richness, both aboveground and belowground biomass were positively correlated with deep-root proportion ($R^2 = 0.31$ and 0.24 , respectively, $P < 0.0001$, $n = 152$) and with the difference between observed and expected deep-root proportions ($R^2 = 0.19$ and 0.37 , respectively, $P < 0.0001$, $n = 137$). Since deep-root proportions were strongly positively correlated with the differences between observed and expected deep-root proportions ($R^2 = 0.57$, $P < 0.0001$, $n = 137$), we focused on deep-root proportions in multiple regression analyses of aboveground biomass. These analyses show that deep-root proportion explains variance in aboveground biomass that is not accounted for by planted species richness, legume presence, legume abundance, or various parameters related to N availability, including root N content, total soil N, extractable soil nitrate, and the rate of net N mineralization (Table 1). This apparent effect of deep-root proportion on aboveground biomass is not simply a result of the correlation between deep-root proportion and total root biomass, since both deep-root proportion and total root biomass were significant predictors of aboveground biomass when included in multiple regression models ($P < 0.05$, regardless of

whether other predictors discussed above were included or excluded). When compared to the N-related parameters, deep-root proportion explained as much or more variance in aboveground biomass, according to sums of squares, and had an effect size that was as large or larger, according to *t* values and standardized model coefficients (Table 1; Bring 1994). Results were similar for regression models of total root biomass that used the difference between observed and expected deep-root proportions as a predictor instead of the observed deep-root proportion; deviations from expected deep-root proportions explained variation in root biomass that was not accounted for by species richness, legume presence or abundance, or N-related parameters (not shown).

DISCUSSION

In this experiment, the most diverse and productive plant communities also had the deepest distributions of root biomass (Fig. 1A–C, E–G). The relationship between diversity and deep-root proportion arose, not because diverse plant communities contained a higher proportion of deep-rooted species, but because of *plasticity* in root biomass allocation in diverse communities. This conclusion is supported by trends in the difference between observed and expected root depth distributions (Fig. 1D, H). In plant communities with less than eight species, observed deep-root proportions were similar to expected values based on the relative abundance of species and the rooting characteristics of those species in monoculture (i.e., root depth distributions and root:shoot ratios). However, communities with eight or more plant species had higher deep-root proportions than expected, reflecting the *net* effect of adjustments to rooting depth and/or root:shoot ratios by one or more plant species. Furthermore, the covariance of root depth distributions and plant biomass, both above and belowground, depended not only on plant species richness, but also on the presence of different plant functional groups (Fig. 1E–H).

Collectively, our results suggest that diversity-dependent shifts in rooting depth, which were enhanced by plasticity in root allocation, contribute to the positive diversity–productivity relationship in this experimental grassland. We hypothesize that deeper root distributions (at the community level) enhanced plant productivity by enabling three related attributes of plant communities to increase, including: (1) spatial complementarity among species, (2) biomass of absorptive roots, and (3) uptake of limiting resources in soils. To test this hypothesis requires data on the distribution of root biomass *for each species* in a community (e.g., Mommer et al. 2010) and uptake of resources from different soil depths (e.g., Kulmatiski and Beard 2012).

Notably, even after we accounted for root depth distributions and other factors underlying the diversity–productivity relationship at our site, such as legume presence and N availability (Fornara and Tilman 2009), species richness explained additional variance in aboveground productivity. Therefore, other, unidentified

factors likely contributed to the higher productivity of diverse plots, such as the amelioration of pathogen effects (Maron et al. 2011, Schnitzer et al. 2011, de Kroon et al. 2012) or phenological complementarity (Fargione and Tilman 2005a).

Why do more diverse communities have deeper root distributions?

The presence of legumes was strongly associated with deep-root depth distributions, but several lines of evidence suggest the positive effects of species richness on the deep-root proportion were not simply due to the presence or dominance of deep-rooting legumes in diverse plots. First, species richness still explained variation in root depth distributions after accounting for legume presence or abundance (Appendix: Table A2). Second, root depth distributions of the most diverse plots were deeper than expected according to species' abundance and monoculture traits (Fig. 1D, H). Finally, for plots planted with 16 species, the plots with the lowest abundance of legumes (aboveground) had the deepest root depth distributions and the most apparent plasticity in root allocation (Appendix: Fig. A2). Below, we discuss how interspecific interactions and the presence of particular species might explain the residual effects of species richness on root depth distributions (i.e., the effects of species richness that cannot be explained by the presence or abundance of legumes).

What caused root allocation to be more plastic in diverse plots?

Only communities with both legumes and C₄ grasses consistently had deeper root depth distributions than expected according to monoculture traits (Fig. 1H); these communities also had the most root biomass (Fig. 1F). One possible explanation for this apparent plasticity in root allocation is that C₄ grasses might grow and maintain more deep roots if legumes increased N availability in deep soils, through both N fixation and mineralization of N in dead, N-rich legume roots. Earlier studies documented that plant productivity in this experiment increased when both legumes and C₄ grasses were present, but explanations of this interaction focused on the complementarity of relatively fixed, inherent traits. For example, compared to C₃ grasses and forbs, the extensive root systems and low N tissues of C₄ grasses probably allow greater uptake of legume-derived N and more efficient conversion of this N into biomass (HilleRisLambers et al. 2004, Fargione and Tilman 2005b, 2006, Fargione et al. 2007, Fornara and Tilman 2008). In this study, we show that plasticity of root allocation could, through unknown mechanisms, also contribute to the effect of legumes and C₄ grasses on plant biomass, particularly belowground.

Effects of individual species

When using the presence or abundance of plant functional groups as predictors of plant biomass, some

of the variance in biomass that is attributed to species richness could be accounted for by strong impacts of individual species within functional groups. In our study, *Lupinus perennis* had the highest relative abundance of the legumes, the largest effect of any species on the deep-root proportion, and a large effect on plasticity in root allocation. This is likely attributable not only to the abundance of *Lupinus perennis*, but also to its possession of one or more unique traits relative to other legumes. For example, *Lupinus perennis* is the only legume species that actively grows in early spring. Since *Lupinus perennis* was planted in nearly all of the most diverse plots (33 of 35 plots), its presence likely contributed to the effects of planted species richness on the deep-root proportion.

The presence of the C₄ grass *Schizachyrium scoparium* in multispecies communities was also associated with deeper than expected root biomass distributions. *Schizachyrium scoparium* is a shallow-rooting species in monoculture (Appendix: Table A3) with a strong ability to reduce shallow soil nutrient concentrations (Fargione and Tilman 2005a), two characteristics that might induce co-occurring species to shift allocation of root biomass to deeper soil horizons.

How do our results relate to theory and results from other studies?

Investment in deep roots is expected to be more advantageous when shallow soil horizons reach low levels of nutrient or water availability as compared to deep soil horizons (Schenk 2008, Mommer et al. 2010, Skinner and Comas 2010). Alternatively, some species might root more deeply in response to changes in the presence or density of roots from conspecifics or other plant species (Schenk 2006, Mommer et al. 2010), regardless of nutrient gradients (de Kroon et al. 2012). More data on species-level rooting patterns, nutrient gradients, and nutrient uptake from different depths is required to distinguish among these different possibilities. The limited data we have suggests a potential role for nutrient gradients; nitrate concentrations in upper soil horizons were negatively correlated with species richness ($P < 0.0001$, $R^2 = 0.17$, using nitrate concentrations sampled in mid-August 2006; see also Fargione and Tilman 2005b) and soil moisture in the upper 20 cm of soil was depleted by the presence of legumes (not shown; see also Fornara and Tilman 2009).

Earlier studies of pasture forage species, including legumes, also found that root depth distributions were deeper and plant productivity was higher for more diverse plant mixtures (Skinner et al. 2004, 2006, Skinner and Comas 2010). Yet, because the most diverse plots contained species that were not present in any replicate of lower diversity plots, the apparent richness effect is difficult to evaluate (Sanderson et al. 2004). Other field and laboratory experiments have observed that the depth distribution of root biomass did *not* increase with plant species richness (Bessler et al. 2009,

Wacker et al. 2009, Mommer et al. 2010). There are several reasons that could explain the contrasting results of these studies: (1) the absence of legumes (Mommer et al. 2010) or the low levels of species richness (≤ 6 species; Wacker et al. 2009, Mommer et al. 2010) in some studies; (2) use of soils that are more nutrient rich than our study site (Bessler et al. 2009), fertilized soils (Wacker et al. 2009), or soils that do not have realistic vertical resource gradients (Mommer et al. 2010); and (3) differences among studies with respect to how species richness and functional composition influence soil resource gradients. For example, at an experiment in Jena, Germany, that has a comparable design to our experiment, N availability in soil is generally higher and diverse plots did not reduce nitrate concentrations in soil after the first year (Oelmann et al. 2011); thus, increases in aboveground biomass with species richness might be supported without additional investment in root biomass, evident in reduced root-to-shoot biomass ratios (Bessler et al. 2009).

Conclusions

In this 12-year experiment, the most productive and diverse plant communities had the deepest distributions of root biomass, partly as a consequence of plasticity in root allocation that arose when both legumes and C₄ grasses were present. Future studies should address the role of root depth distribution and belowground plasticity in other grassland diversity experiments. Additional research is also needed to evaluate whether spatial complementarity and uptake of soil resources were enhanced in diverse plots by root plasticity.

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SUPPLEMENTAL MATERIAL

Appendix

Tables reporting the deep-root proportion of monocultures and results of statistical models of root biomass, deep-root proportions, and differences between observed and expected deep-root proportions; and figures showing root biomass for each depth increment and species richness, and correlations of the deep-root proportion and the difference between observed and expected deep-root proportion with the abundance of legumes and C₄ grasses in diverse plots (*Ecological Archives* E094-067-A1).