

Root Responses To Neighbors Depend On Neighbor Identity And Resource Distribution

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Abstract

Purpose: In a complex soil environment, competitive and environmental factors will interact with individual traits to influence a plant's root growth patterns and ability to compete for resources. Here, we examine how root growth of a focal plant, *Plantago lanceolata* L., responds to resource heterogeneity and to presence of two neighbor species, *Centaurea jacea* L. and *Poa pratensis* L.

Methods: A full factorial experiment tested the effects of nutrient heterogeneity, neighbors, and their interaction on root responses of *Plantago*. Roots in shared quadrants of a pot were harvested and quantified by qPCR for plants grown alone or with a neighbor, in patchy or even soil. The effects of experimental treatments on *Plantago* root mass distribution were tested with two-way ANOVA.

Results: When soil resources were evenly distributed, *Plantago* individuals increased root allocation to soil shared with a *Centaurea* neighbor but not a *Poa* neighbor. When soil resources were patchy, *Plantago* responded more strongly to *Poa* than to *Centaurea*, and placed more roots in the high-resource patch.

Conclusions: These results demonstrate that plants can respond differently to neighbors depending on species and that integrating multiple cues results in non-additive effects on root behavior.

Introduction

Plants are able to detect various cues from their environment both above and below ground (de Kroon et al. 2009; Cahill and McNickle 2011), and their responses to those cues can determine competitive outcomes and ultimately community structure (Fransen et al. 2001; Mommer et al. 2012; Padilla et al. 2013; Oram et al. 2018). Belowground, roots are known to respond to water, nutrients, chemicals, microbes and the roots of neighbors (reviewed by Cahill and McNickle 2011). In a complex soil environment, the combination of cues present, as well as individual traits and plasticity, will influence a plant's overall root growth patterns and its ability to compete for resources (Craine and Dybzinski 2013; Pierik et al. 2013).

Root responses to spatial variation in soil nutrients are well documented. Plants are capable, to varying degrees, of responding to spatial heterogeneity in nutrient availability (Johnson and Biondini 2001; Kembel and Cahill 2005). Plants tend to proliferate more roots in higher nutrient locations, which then can lead to increased nitrogen uptake and increased biomass (Hodge 2004; Mommer et al. 2012). Species vary in their foraging precision, the degree to which they grow roots selectively in nutrient-rich patches (Johnson and Biondini 2001; Grime and Mackey 2002; Ravenek et al. 2016). Potential patterns in this variation and its adaptive significance are debated (Kembel and Cahill 2005; Grime 2007; Kembel et al. 2008).

Plants also respond to the presence of neighbors, independent of their response to nutrients (Cahill et al. 2010; Belter and Cahill 2015). Three categories of responses have been observed: over-production, neighbor avoidance, or neutral (Cahill and McNickle 2011). Multiple studies have shown root over-

production in response to neighbors (e.g. Gersani et al. 2001; O'Brien et al. 2005), avoidance of root overlap with neighbors (reviewed by Schenk et al. 1999; Pierik et al. 2013), or found both responses (e.g. Mommer et al. 2012). McNickle and Brown (2014) showed that the response of *Brassica rapa* plants to neighbors could be explained by their effects on soil resources.

Variation in response to neighbors is also not well understood. A great deal of debate has focused on elements of experimental design (reviewed by Chen et al. 2012), rather than traits of species or aspects of the environment. Comparative studies have shown both that species differ in root growth responses to competitors, and that those responses are dependent on the identity of the neighbor (Semchenko et al. 2007; Belter and Cahill 2015; Paya et al. 2015; Sattler and Bartelheimer 2018). Furthermore, neighbor identity effects are not consistent among focal species (Semchenko et al. 2007; Belter and Cahill 2015; Sattler and Bartelheimer 2018).

A few studies have investigated plant responses to the two simultaneous cues of resource heterogeneity and neighbors, but no consistent patterns have emerged. Addition of soil nutrients has been observed to shift the response to neighbors from neutral to over-production (Sattler and Bartelheimer 2018), from over-production to neutral (McNickle et al. 2016), from avoidance to neutral (Cahill et al. 2010), or from avoidance to greater avoidance (Nan et al. 2013). The interactive effects of resources and neighbors become even more complex when additional cues are introduced (e.g. earthworms, Wang et al. 2020)

In order to understand how plants integrate information about multiple cues and why responses vary, we need to better understand how plant traits and environmental variables drive those responses. A game theoretic model of root competition (McNickle and Brown 2012) offers some testable predictions. Previous models used game theory to predict that plants will over-produce roots in soil shared with neighbors (Gersani et al. 2001; O'Brien and Brown 2008). McNickle and Brown (2012) modified the game theory model to keep total soil volume constant for plants with or without neighbors, as in most competition experiments, and to allow the competing plants to differ in traits. Their model predicts that plants will generally over-produce roots when a neighbor is present, and that overproduction will be greater when resources are high. When neighbor traits differ, a plant with low cost of producing roots and high uptake rate should overproduce roots to preempt resources, whereas a plant with costly roots and low uptake rate should conserve resources and produce fewer roots.

Here, we test some of those predictions by examining root responses of *Plantago lanceolata* to nutrient heterogeneity, the presence of two different neighbors, and the combination of the two cues. Based on the results of the McNickle and Brown model, we predict that

(1) *Plantago* will respond to a neighbor by increasing root growth in shared soil.

(2) The response to a neighbor will be greater when the neighbor has traits associated with a greater cost of root production.

(3) In heterogeneous soil, the response to a neighbor will be greater in a nutrient-rich patch than in a nutrient-poor patch, resulting in greater foraging precision when a neighbor is present.

Measuring root production in the presence of neighbors requires a method to determine each species' abundance in mixed samples of roots. Recently, DNA-based methods (Mommer et al. 2008) to quantify roots in mixed-species samples have been successfully applied in plant competition experiments (Mommer et al. 2012; Hendriks et al. 2015; Ravenek et al. 2016; Oram et al. 2018). Here, we modify that approach to develop species-specific DNA markers for each of our study species and apply those markers to test the predictions above.

Materials And Methods

Study species

Our study species are grassland perennials that co-occur in old fields on the UMass Dartmouth campus (41.6309° N, 71.0067° W). *Plantago lanceolata* L., our focal plant, is a perennial forb. In previous studies, this species has exhibited foraging precision (Wijesinghe et al. 2001; Maestre and Reynolds 2007; Mommer et al. 2010) and over-production with neighbors (Mommer et al. 2010; Padilla et al. 2013). Two species with contrasting traits were chosen as neighbors. *Centaurea jacea* L., a forb, has a lower specific root length than *Plantago* and a greater concentration of N and P in its roots, while *Poa pratensis* L. a grass, has greater specific root length than *Plantago* and a lower root concentration of N and P (data from Schroeder-Georgi et al. 2016; Ravenek et al. 2016; Herz et al. 2017; as published in the Global Root Traits database, Guerrero-Ramirez et al. 2020). Both neighbors have higher root N uptake rates than *Plantago* (Schroeder-Georgi et al. 2016).

Marker development and validation

We developed species-specific DNA markers for the three species in the current study using a strategy similar to that of Mommer et al. (2008). However, instead of using the ISSR approach to randomly sample the genomes of target species for sequences flanked by microsatellites, we adapted AFLP methods (Vos et al. 1995) to sample sequences flanked by specific restriction enzyme sites. This approach allows the option of sampling different sets of sequences by changing the restriction enzymes. We found this to be an effective alternative but did not empirically evaluate it in comparison to the ISSR approach.

Briefly, 200 ng of genomic DNA from each species was digested with *HpaII* and *EcoRI* restriction enzymes (New England Bio Labs, Ipswich MA), and AFLP libraries were prepared following Blouin et al. (2010). After the PCR amplification step, the AFLP libraries were cloned using the TOPO TA Cloning Kit for Sequencing (Thermo-Fisher Scientific, Grand Island NY), following the manufacturer's recommended procedure. For each species, 15-30 colonies, each containing one fragment insert, were randomly selected and sequenced using standard M13 primers at the MGH CCIB DNA Core facility (Massachusetts General Hospital, Cambridge, MA). The resulting sequences were screened for data quality, and chloroplast

sequences were identified and excluded by comparing the sequences to the NCBI nonredundant nucleotide database using BLAST (Altschul et al. 1990). Primer-probe assays were designed using the Universal Probe Library (UPL) Assay Design Center (Roche Diagnostics, Pleasanton, CA). Each DNA marker contained a primer set that amplified a small PCR product (80-120 bp) and a UPL MGB probe containing a 5' FAM fluorescent dye and a 3' dark quencher dye (Table 1).

All potential DNA markers were evaluated for species-specificity and reliability for estimating species ratios in root samples. First, each marker was tested on DNA from each of the three species in the current study as well as three others that are routinely used in our experiments (*Dianthus armeria*, *Bromus inermis*, and *Schizachyrium scoparium*), and was considered species-specific if it produced signal with DNA from the target species and no signal with DNA from non-target species. Additionally, we tested the markers on samples containing known mixtures of DNA (50:50 and 10:90 target to non-target species) to ensure that amplification was not affected by the presence of non-target DNA. Finally, we extracted DNA from artificially mixed root samples (total 25 mg, \pm 0.5 mg) with known fresh weights of target and non-target species to determine the accuracy and precision of the relationship between fresh weight percentage and estimated percentage by qPCR. Samples containing 1:9, 1:3, 1:1, 3:1, and 9:1 fresh weights of target species root tissue were made for all pairs of species.

All qPCR was performed using a Lightcycler 480 (Roche Diagnostics). Each sample was run in duplicate, including negative controls. To calculate amplification efficiency, a standard curve was run in triplicate consisting of a four-point, 1:4 serial dilution of a pure sample of root DNA from the target species. The qPCR profile was as follows: 95°C for 3 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Each 20 μ l reaction contained 1 μ M of each primer, 0.1 μ M UPL MGB probe (Roche), and PrimeTime Gene Expression Master Mix (Integrated DNA Technologies, Skokie, IL). For samples of mixed DNA (50:50 and 10:90), the total DNA added to each reaction varied proportionally so that the target amount of DNA stayed constant at 10ng. For samples of artificially mixed roots and all unknown samples, 10ng of total DNA was added to each reaction.

Experimental design

The greenhouse experiment used square pots (12.7 cm x 16.5 cm deep; Fig. 1) in which one quadrant was home to a focal *Plantago* seedling and the opposite quadrant was home to a *Centaurea* neighbor, a *Poa* neighbor, or no neighbor. The two remaining quadrants were designated "away" quadrants. Soil resources in each pot were evenly distributed, with resource availability equal in all quadrants, or heterogeneously distributed, with resource availability higher in one of the two away quadrants and lower in the remaining three quadrants. Neighbor treatments and soil treatments were fully crossed, with ten replicates per treatment, for a total of 60 pots (3 neighbors x 2 soils x 10 replicates).

Resource distribution was manipulated by creating different mixtures of commercial play sand and compost (Just Natural Mushroom Compost, Oldcastle Lawn and Garden, Poland Spring, ME) in varying ratios. Pots with even resources were filled with a mixture of 80% sand and 20% compost by volume. To create patchy soil resources, pots were divided into quadrants with plastic barriers. One quadrant was

filled with 50% sand/50% compost, and the remaining three quadrants were filled with 90% sand/10% compost. Barriers between quadrants were removed after filling. Total resource availability was the same in both soil treatments. We did not attempt to manipulate or test for presence of soil microbes, including mycorrhizal fungi.

Seeds collected on campus of all three study species were sown in trays filled with 80% sand/20% compost and transplanted into pots three weeks after germination. A single *Plantago* seedling was planted in the center of the focal home quadrant of each pot. A single seedling of either *Centaurea* or *Poa* was planted in the center of the neighbor home quadrant, or the neighbor quadrant was left empty, to create the three neighbor treatments.

Harvest and data collection

Seedlings were planted on 29 July 2015 and grew for approximately 50 days. The pots were harvested over six days so that harvested roots could be promptly preserved for DNA extraction. Ten pots had one or more plants die during the experiment and were not used for analysis, leaving three replicates with *Poa* neighbors in even soil, and 8-10 replicates of each remaining treatment. At the time of harvest, aboveground biomass was cut with scissors at the base of the plant and dried at 80 °C for 48 hours before weighing. Soil from away quadrants was removed and left to air dry for 1-2 hours in order to easily sift out roots. Roots were patted dry and weighed fresh before being put in 1.5 mL microcentrifuge tubes and stored at -80°C until DNA was extracted. Because isolating roots from soil was time consuming, and harvested roots needed to be preserved while fresh, roots were not collected from home quadrants.

For pots with neighbors, DNA was extracted from the entire root sample from each away quadrant. Quantitative PCR was conducted with a Lightcycler 480 Real-Time PCR System (Roche Diagnostics, Pleasanton, CA), using DNA markers designed for each species, as described above. All samples were run in duplicate for each species in each sample (4 qPCR reactions per root sample). A 1:4 serial dilution standard curve done in triplicate was included for each species, and reference samples with known ratios of each species DNA were included for quality control.

Unknown samples were standardized relative to 1:1 reference samples, following the approach used by Mommer et al. (2008). Twenty reference samples were made for each species pair by combining 25 mg (\pm 0.5 mg) of fresh root tissue from each species. DNA was extracted as described above. Relative species proportions in test samples were calculated from raw Ct values and reference values using formulas from Mommer et al. (2008) and then multiplied by the total root mass in each away quadrant to determine the root mass from each species.

Data analysis

The effects of experimental treatments on root growth were tested with ANOVA, with summed root mass in the two away quadrants as the response variable, and with soil environment (even or patchy), neighbor identity (none, *Centaurea*, or *Poa*) and their interaction included as fixed factors. Shoot mass of the focal

plant was included as a covariate, so that treatment effects on root mass reflected changes in allocation, rather than absolute biomass. To interpret a significant interaction term, we used posthoc pairwise comparisons of neighbor treatments within each soil treatment.

In pots with heterogeneous soil, root allocation between the high-resource and low-resource away quadrants was analyzed with mixed-effects ANOVA, with focal root mass within a quadrant as the response variable, focal shoot mass as a covariate, neighbor treatment as a between-subjects factor, and quadrant as a within-subjects factor. To interpret a significant interaction term, we used posthoc pairwise comparisons of neighbor treatments within each quadrant. To test for foraging precision, we used pairwise comparisons of quadrants within each neighbor treatment.

To compare potential competitive effects of the two neighbor species, two-way ANOVA was used to test for effects of soil environment, neighbor species, and their interaction on total neighbor root mass in the two away quadrants. Neighbor shoot mass was not included as a covariate, because the value of interest was neighbor root abundance, not allocation.

Analyses were completed with R statistical software, version 4.0.0 (R Core Team 2020). Root and shoot mass values were natural log transformed to improve normality and homogeneity of variance. ANOVAs with mixed effects were fitted with the 'lme4' package (Bates et al. 2015). Model significance was evaluated with Type III sums of squares with the package 'car' (Fox and Weisberg 2019). Posthoc comparisons were tested with the 'emmeans' package, using Tukey's method to adjust for multiple comparisons (Length 2020).

Results

Marker development and validation

Each DNA marker had acceptable qPCR efficiencies across a 64-fold range of DNA concentrations, indicating that amplification was consistent regardless of the amount of initial DNA. Moreover, amplification was not affected by the addition of equal (50%) or large (90%) relative amounts of DNA from non-target species. Finally, as expected, there was no amplification signal in samples lacking the target species, such as samples containing only DNA from non-target species and negative controls. The estimated percentages of target species were highly correlated with the initial fresh weight percentages for each species (Fig. 2), indicating that these markers can accurately predict the starting fresh weight for a target species using qPCR. For example, *Poa* fresh weight estimates based on qPCR ranged from 87-90% for 9:1 reference samples, 9-16% for 1:9 reference samples, and 37-65% for 1:1 reference samples. DNA markers for *Centaurea* and *Plantago* had more variation in estimated percentages, but all were well within acceptable ranges (Fig. 2). Each regression had an R^2 of 0.80 or better and relatively narrow confidence and prediction intervals (Fig. 2), comparable to that of Mommer et al. (2008). Based on these findings, we were satisfied that the DNA markers were species-specific and reliable for quantification of relative species abundances in mixed root samples.

Greenhouse experiment

Root allocation of the focal plant to shared quadrants depended on the soil environment and neighbor treatment (significant interaction term, Table 2). *Plantago* produced about twice as much root mass in away quadrants with a *Centaurea* neighbor than when grown alone in either soil type (Fig. 3). With a *Poa* neighbor, *Plantago* produced about four times as much root mass, but only when soil was patchy (Fig. 3). Shoot biomass was a significant covariate for root mass (Table 2), but when analyzed separately, *Plantago* shoot biomass was not affected by soil environment or neighbor treatment (results not shown).

In pots with patchy soil, *Plantago*'s root distribution between shared quadrants depended on the neighbor treatment (significant interaction term, Table 3). With a *Centaurea* neighbor, *Plantago* increased root mass in the high-resource quadrant but not in the low-resource quadrant. With a *Poa* neighbor, *Plantago* increased root mass in both low and high resource quadrants (Fig. 4). Without a neighbor, root mass was similar in the two quadrants, indicating no foraging precision. With a neighbor present, *Plantago* exhibited foraging precision, producing significantly more roots in the high-resource quadrant (Fig. 4).

Although *Centaurea* neighbors tended to have greater root mass in shared soil than *Poa* neighbors (Fig. 5), neighbor root mass was highly variable and not significantly affected by species or soil environment (Table 4).

Discussion

Root growth of *Plantago lanceolata* responded to both neighbors and resources. As predicted, root growth in shared soil increased in the presence of a neighbor. When resource levels varied in the shared soil, *Plantago* showed a stronger response to neighbors where resources were high, resulting in increased foraging precision. These responses differed between two neighbor species, with a stronger response to *Centaurea* when soil resources were evenly distributed and a stronger response to *Poa* when resources had a patchy distribution..

Neighbor detection and discrimination

When a neighbor was present in the pot, *Plantago* allocated more root biomass to the "away" quadrants of the pot than when alone. This increased allocation could reflect either an overall increase in root mass, or a shift in placement of roots. We were unable to distinguish these alternatives because we did not measure root production in the home quadrant. In one previous study, *Plantago* increased allocation to roots in intraspecific competition (Berendse and Möller 2009), but in other studies it decreased allocation to roots (Janeček et al. 2014) or made no change in allocation (Robinson et al. 2010) in interspecific competition.

The signal that triggers a shift in either allocation or root placement might be nutrient depletion by the neighbor or direct detection of the neighbor through root exudates (Pierik et al. 2013). Given the short duration of our experiment (seven weeks), depletion of nutrients was unlikely to be important. There was

no evidence of decreased biomass due to resource competition. Similar overproduction responses to neighbors have been observed in nutrient-rich and nutrient-poor soil (Mommer et al. 2010), demonstrating that the response to neighbors was not dependent on nutrient depletion. Root growth responses have also been observed when root exudates from potential neighbors are added to the soil, even though the neighbors themselves are absent (Biedrzycki et al. 2010; Semchenko et al. 2014).

We found that *Plantago's* root growth response to *Centaurea* neighbors was different from its response to *Poa* neighbors. Species-specific responses to neighbors appear to be common, but not universal. Among a set of 20 co-occurring species, each grown alone or with one of two neighbors, twelve species shifted root growth toward or away from a neighbor, changed rooting depth, changed allocation to roots, or had some combination of responses (Belter and Cahill 2015). These responses differed between the two neighbors. In an experiment comparing root distribution responses to four neighbors, only one of two focal species showed species-specific responses (Sattler and Bartelheimer 2018). Two recent studies that each compared only two neighbor species concluded that neighbor identity does not influence root responses (e.g. Paya et al. 2015; Jacob et al. 2017).

A model of root growth in competition experiments (McNickle and Brown 2012) proposes traits that may drive species-specific responses. The model predicts that when two competitors differ in foraging efficiency, the individual with the higher nutrient uptake rate and/or lower cost of roots will produce more root than it would alone. The optimal strategy for the less efficient individual is to produce less root than it would alone. Comparisons of specific root length and root nitrogen content (data from Schroeder-Georgi et al. 2016; Herz et al. 2017; Garlick 2016) suggest that *Poa* may have a lower cost of root production than *Plantago*, while *Centaurea* may have a higher cost. When soil resources were evenly distributed, *Plantago* behaved as predicted, increasing root growth with *Centaurea* but not *Poa*. However, *Plantago* responded strongly to *Poa* when resources were concentrated in one quadrant of the pot. With only one focal species and two neighbor species, we are unable to draw generalizable conclusions about the relationship between plant traits and root responses to neighbors.

Other studies have had limited success in identifying traits associated with responses to neighbors. Responses were not predicted by traits related to size, habitat occupancy, or root architecture in the set of 20 species tested by Belter and Cahill (2015). Sattler and Bartelheimer (2018) hypothesized that roots of non-legumes would grow towards a legume neighbor, especially when nitrogen was limiting, but found only one species combination for which that was true. In another study, a perennial grass shifted its rooting depth profile in response to the growth habit of its neighbor, growing progressively deeper with a tree, a shrub, and a perennial forb (Chen et al. 2020).

Integrating information about neighbors and resources

Plantago's responses to neighbors were also affected by resource heterogeneity. *Plantago* overproduced roots with a *Centaurea* neighbor to a similar degree in both even and patchy soil, but when soil was patchy, all of the increased root growth occurred in the resource-rich patch. *Plantago* responded to a *Poa* neighbor only when resources were patchy. This specificity suggests that the benefit of pre-empting a

volume of soil depends on the resources available there and on the identity of the competitor for the space, and that the effects of those factors may be non-additive. In a mixture of four grassland species, the effect of neighbors on root production was also dependent on resource heterogeneity (McNickle et al. 2016). Mixtures produced more roots than expected based on root growth of single plants, but only when resources were heterogeneous, and only in background soil, not in patches. In another study, the foraging strategies of two species responded to neighbors, resource heterogeneity, and a third cue, presence of earthworms (Wang et al. 2020).

We found that *Plantago* did not forage precisely in patchy soil when grown alone but did so when a neighbor was present. A larger study of eight species, including *Plantago*, *Centaurea*, and *Poa*, also observed changes in precision with different competitors (Ravenek et al. 2016). Shifts in precision may help explain why precision measured on isolated individuals is often unrelated to competitive outcomes in heterogeneous soil (e.g. Cahill and Casper 1999; Rajaniemi 2007).

Conclusion

The ability to integrate multiple cues into an appropriate response is crucial to maintaining a root strategy that is beneficial in many environmental and competitive contexts (Cahill and McNickle 2011). Our results are consistent with others (Cahill et al. 2010; Mommer et al. 2012; Nan et al. 2013; McNickle et al. 2016) in concluding that plants can change their responses depending on the combination of cues they experience. In addition, the integrated response to resources and neighbors incorporates not just the presence of a neighbor, but the identity of the neighbor. More comprehensive experiments involving a large sets of species are needed to elucidate the functional traits that underlie these differential responses.

Declarations

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Tables

Table 1

Primer sequences and probes used in this study to quantify the abundances of three species using quantitative PCR

Species	UPL probe #	Marker Name	Primers
Plantago lanceolata	46	Pla44	5'-TGAGTCTAGAACGGGGATGTTT-3'
			5'-TTGGTTGGTGCAAAAAGAATC-3'
Centaurea jacea	75	Cen54	5'-AGCGGTGGTAGAAGAGCTTG-3'
			5'-CCTAGATCATCCTTGATGACACC-3'
Poa pratensis	147	Ppr49	5'-CAACAAAATCTGGTCAGAATGC-3'
			5'-AGAAGGGGCCAGGTTGTACT-3'

Table 2

ANOVA results for root mass of *Plantago* focal plants summed over two away quadrants. Root mass and shoot mass were natural log transformed for analysis

Source of variation	F	df	P
shoot mass	46.53	1, 43	< 0.001
neighbor	6.42	2, 43	0.004
soil	0.04	1, 43	0.851
neighbor x soil	5.07	2, 43	0.011

Table 3

ANOVA results for root mass of *Plantago* focal plants in high-resource and low-resource quadrants, for the patchy soil environment only. Root mass and shoot mass were natural log transformed for analysis

Source of variation	χ^2	df	P
shoot mass	12.79	1	< 0.001
neighbor	9.16	2	0.010
quadrant	0.12	1	0.724
neighbor x quadrant	10.41	2	0.005

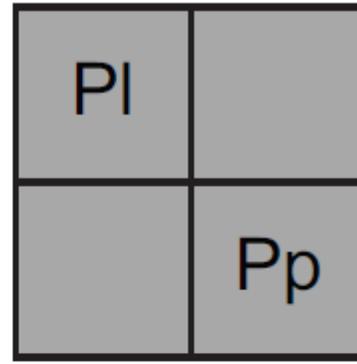
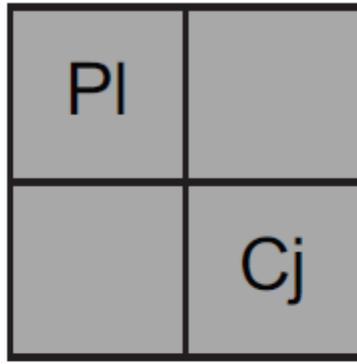
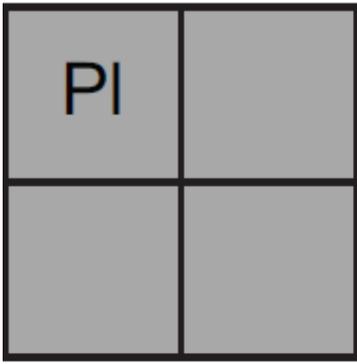
Table 4

ANOVA results for root mass of neighbor plants summed over two away quadrants. Root mass was natural log transformed for analysis

Source of variation	F	df	P
neighbor species	2.81	1, 26	0.106
soil	0.40	1, 26	0.535
neighbor species x soil	0.11	1, 26	0.742

Figures

even



patchy

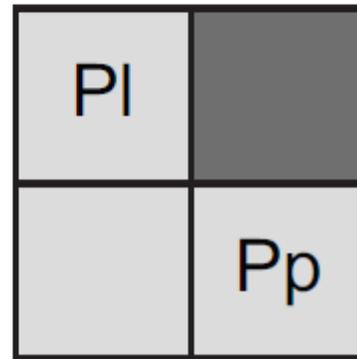
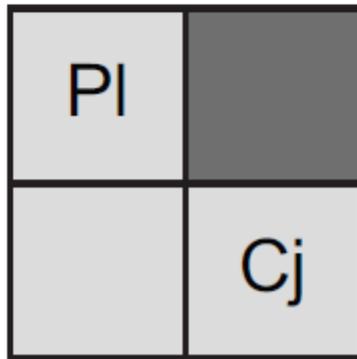
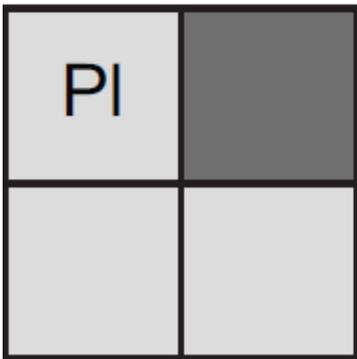


Figure 1

Experimental design. Each pot was divided into four quadrants, without barriers between quadrants. Resource levels are indicated by darkness of shading. Even pots had equal resource availability in all four quadrants; patchy pots had high resource availability in one quadrant. Each pot had a focal *Plantago* plant (PI) with no neighbor, a *Centaurea* (Cj) neighbor, or a *Poa* (Pp) neighbor

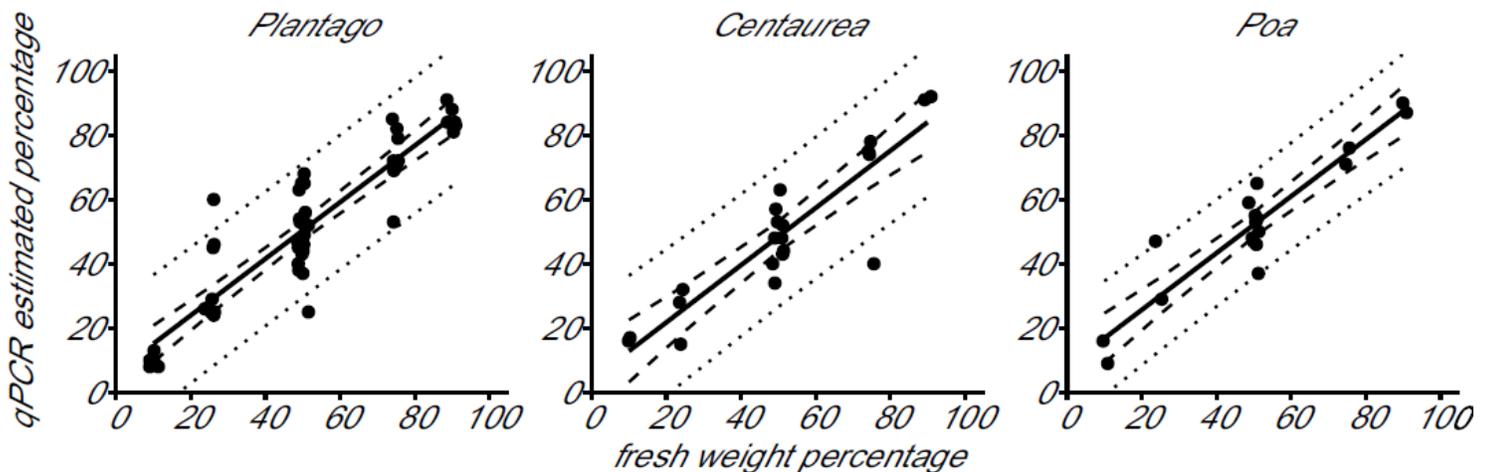


Figure 2

Regression of estimated species percentage versus fresh weight species percentage for known root mixtures. The dashed lines indicate the 95% confidence interval; dotted lines indicate the 95% prediction interval

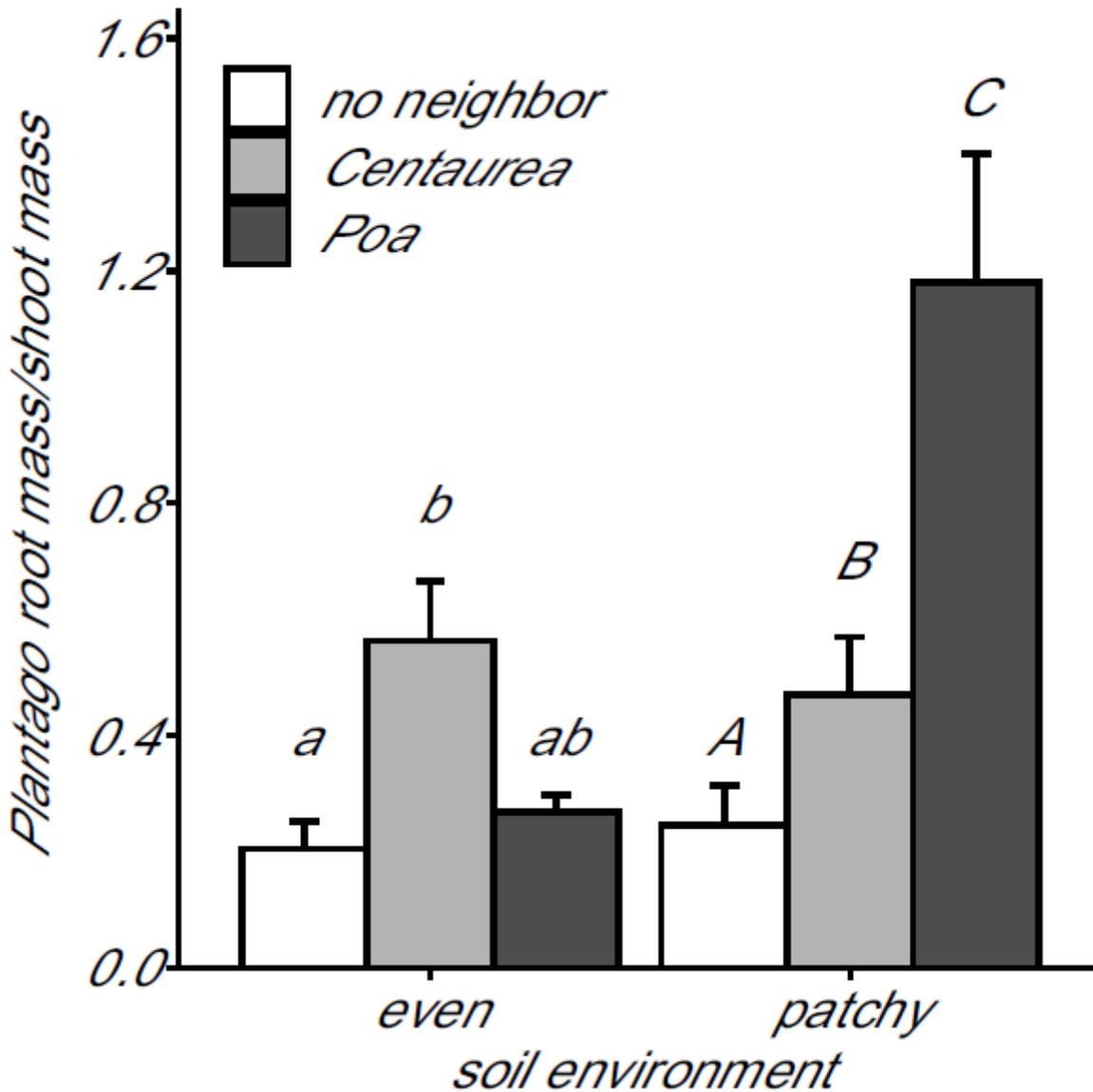


Figure 3

Root mass of *Plantago* focal plants in two away quadrants combined, divided by shoot mass, in three neighbor treatments and two soil environments. Root mass was estimated using qPCR in treatments with neighbors and measured directly in the treatment with no neighbors. Effects of treatments on root mass were analyzed with shoot mass as a covariate. Different letters indicate significant difference ($p < 0.01$) between neighbor species within a soil treatment. For pots with no neighbor, $n=10$ for both soils; *Centaurea* neighbor, $n=10$ and 8 for even and patchy soils; *Poa* neighbor, $n=3$ and 9

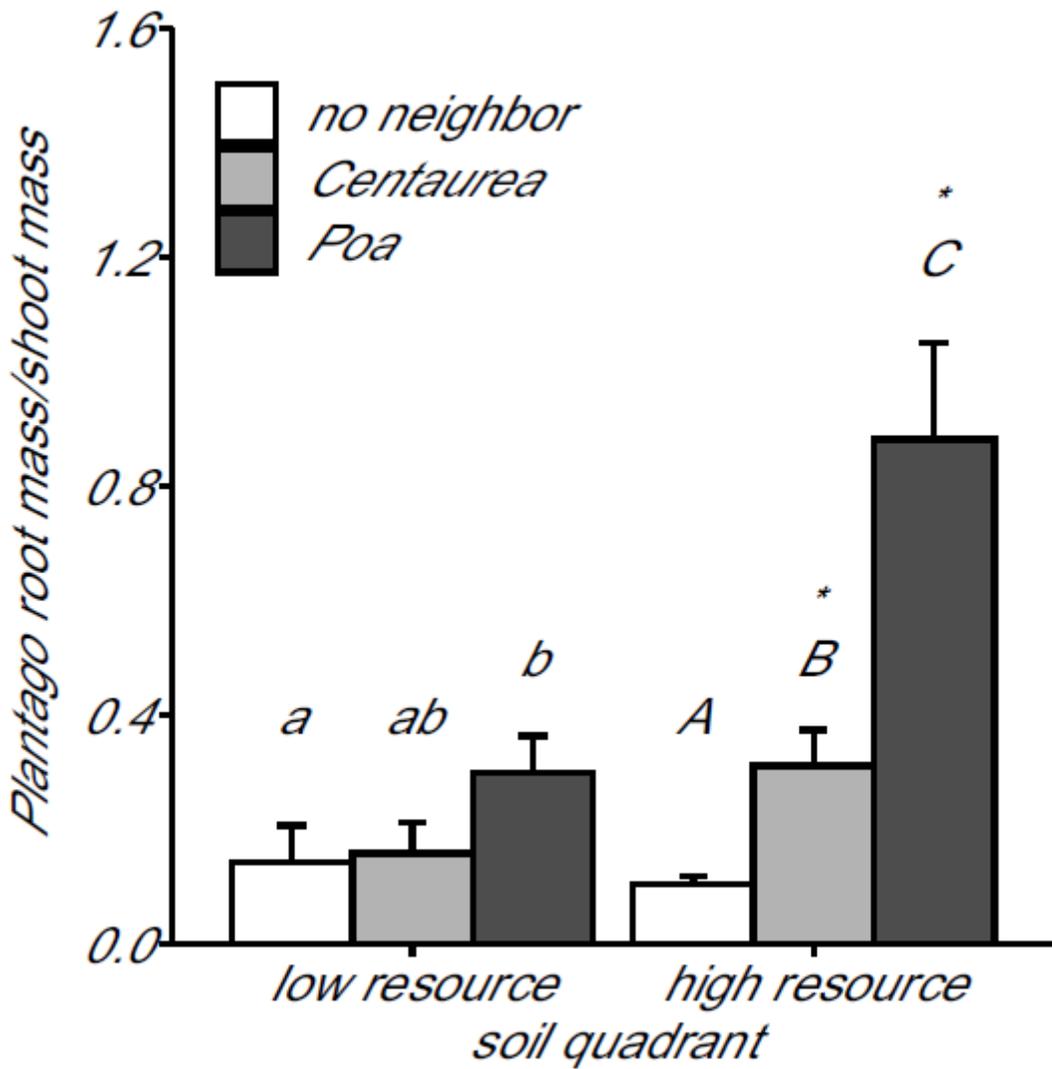


Figure 4

Root mass of *Plantago* focal plants in the low- and high-resource away quadrants, divided by shoot mass, in three neighbor treatments. Root mass was estimated using qPCR in treatments with neighbors and measured directly in the treatment with no neighbors. Data are for the patchy soil environment only. Effects of treatments on root mass were analyzed with shoot mass as a covariate. Different letters indicate significant differences ($p < 0.05$) between neighbor species within a quadrant type. Stars indicate that root mass differs significantly ($p < 0.01$) between the two quadrants within a neighbor treatment. Samples sizes as in Fig. 2

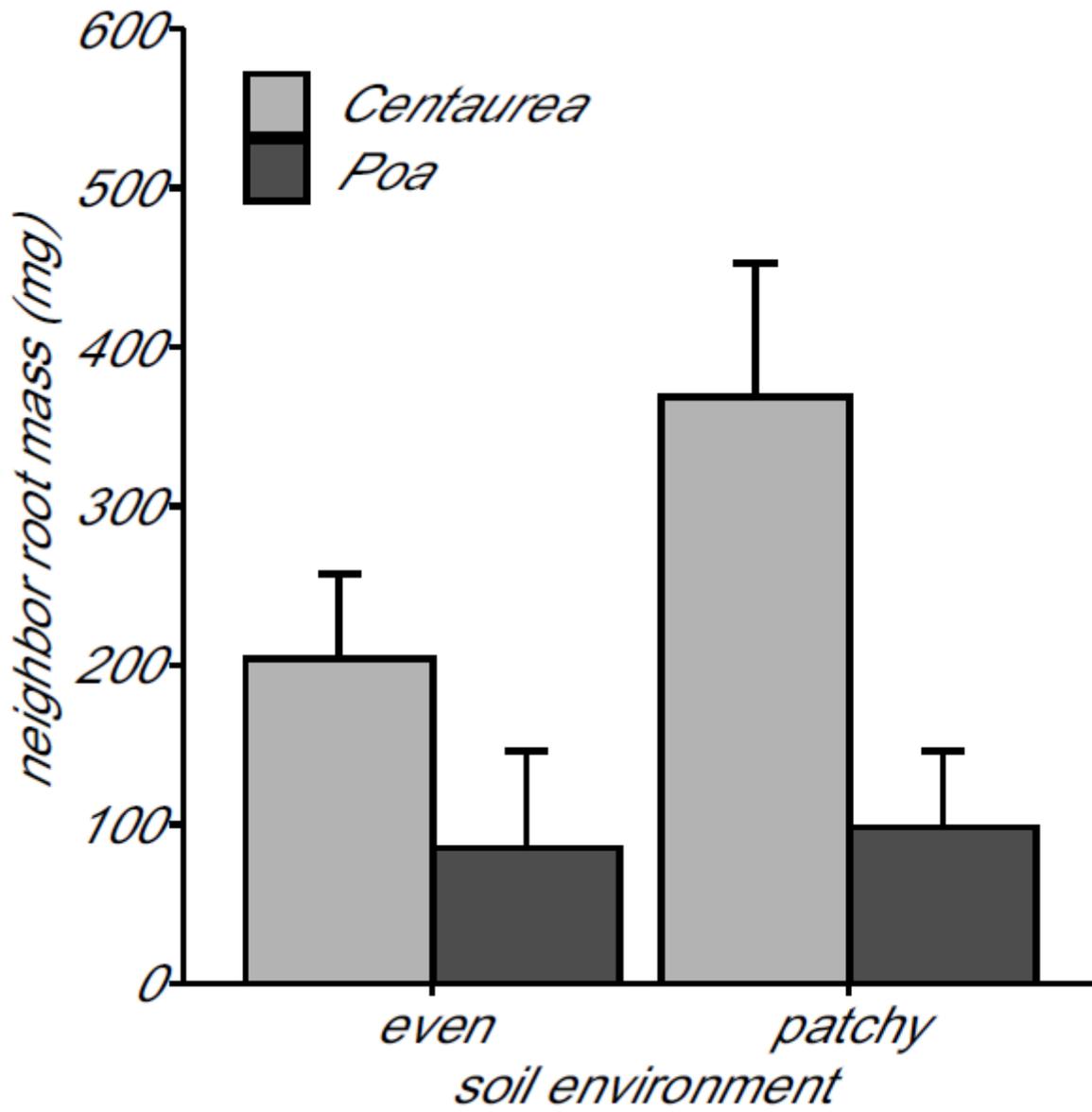


Figure 5

Root mass estimated by qPCR in two away quadrants combined of *Centaurea* and *Poa* neighbors plants, in two soil environments. Sample sizes as in Fig. 2