Plant Ecology (2006) 183:65–75
DOI 10.1007/s11258-005-9007-1

Small-scale spatial heterogeneity in the vertical distribution of soil nutrients has limited effects on the growth and development of *Prosopis glandulosa* seedlings

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Received 2 January 2005; accepted in revised form 2 June 2005

Key words: Grass-shrub transition, Nutrient patchiness, *Prosopis glandulosa*, Root proliferation, Seedling, Vertical heterogeneity

Abstract

We examined the above- and below-ground responses of seedlings of the woody shrub *Prosopis glandulosa* to the spatial heterogeneity of soil nutrients within the root zone. We performed a microcosm experiment where seedlings were grown with different combinations of nutrients [nitrogen (N), phosphorus (P), and both combined (NP)] and under different levels of nutrient heterogeneity (nutrients supplied as patches located in the bottom and/or upper portion of rooting zone versus homogeneous distribution). Seedling morphology and biomass did not show a strong response to changes in nutrient ion or spatial heterogeneity. Height, number of leaves, and specific leaf area did not vary significantly between treatments. The number of leaves, foliar biomass, stem biomass and biomass allocation to stems of seedlings showed more responsiveness to the addition of N and NP than to the addition of P. The spatial heterogeneity of nutrients affected the diameter, root biomass and leaf N content. Seedlings had higher diameter and root biomass when the nutrients were homogeneously distributed as compared to their placement as patches in the bottom of the microcosms. Their leaf N concentration increased in those treatments where the nutrient patch was located in the lower half as compared to the upper half of the microcosms. Root foraging responses to nutrient patches varied with their location. Significant root proliferation was observed when patches of N, P and NP were located in the upper portion of the rooting zone; when they were located in the lower portion such a response was observed only for P. Despite our findings that *Prosopis* seedlings have a low overall responsiveness to small-scale vertical differences in soil nutrient heterogeneity, our results suggest that these differences may modify the growth dynamics of the secondary roots of this ecologically important dryland species during the early stages of its development.

Introduction

It is well known that soils are highly heterogeneous both in space and time, and that this heterogeneity has strong consequences for both plant performance and ecosystem function (Huber-Sannwald and Jackson 2001). At a landscape level, the spatial patterning of soil resources affects the distribution (Pan et al. 1998), productivity (Mou et al. 1995) and diversity (Anderson et al. 2004) of plant communities. At smaller scales, it influences plant establishment (Maestre et al. 2003) and the outcome of plant–plant interactions (Robinson et al. 1999). The spatial heterogeneity of soil resources is especially important for the functioning of arid ecosystems, where the heterogeneous distribution of vegetation promotes the formation and maintenance of resources underneath the canopy of plants, including water and nutrients (Reynolds et al. 1999). These 'resource islands' not only act as hot spots of soil nutrients, microbial activity, and mycorrhizal inoculum (Titus et al. 2002), but also are focal points for plant interactions and seedling establishment (Aguiar and Sala 1999).

In the last years there has been an increased interest in the response of plants to the small-scale spatial heterogeneity of soil nutrients (i.e. within the rooting volume of individual plants), and on the ecological significance and consequences of this response (Huber-Sannwald and Jackson 2001; Hutchings et al. 2003; Hodge 2004). Many studies have shown that the presence of localized nutrient patches at distances within the rooting volume induces the proliferation of fine roots (Drew et al. 1973; Mou et al. 1997; Farley and Fitter 1999; Einsmann et al. 1999 *inter alia*). This proliferation is both species- and nutrient ion-specific (Robinson 1994; Hodge et al. 1998), and also depends on the size and quality of the nutrient patch (Wijesinghe and Hutchings 1999; Fitter et al. 2000). However, the mechanisms underlying root proliferation, and the benefits that plants obtain from it, are still poorly understood (Fitter et al. 2000; Hodge 2004). While root proliferation can increase biomass and nutrient capture, and thus may confer competitive advantage when plants are grown in the presence of neighbors (e.g., Robinson et al. 1998; Hodge et al. 1999; Fransen et al. 2001), this may not be always the case (e.g., Cahill and Casper 1999; Fransen and de Kroon 2001).

Evidence on the role of spatial heterogeneity of soil nutrients has been acquired mainly from studies of horizontal heterogeneity. Other components of soil heterogeneity, such as temporal variations and vertical patterns, are less commonly described (Fransen et al. 1999; Fitter et al. 2000), although they can exert strong influence on community structure (Berendse 1981; Fitter 1982). Vertical heterogeneity in soils may be especially relevant in arid and semiarid ecosystems (Sala et al. 1997; Reid et al. 1999) due to phenomena such as hydraulic lift (Caldwell and Manwaring 1994), accumulation of litter and plant material (Zaady et al. 1996), plant rooting behavior (Soriano et al. 1987) and bioturbation by animals (Whitford 1993). However, studies exploring the response of roots to small-scale, vertical heterogeneity in nutrients are lacking (Hodge 2004) and, furthermore, most studies have been conducted with herbaceous plants, which have high plasticity and quick response time to nutrient patches (Robinson 1994).

In this study we examine the above- and below-ground responses of seedlings of a woody desert shrub, *Prosopis glandulosa* Torr., to vertical heterogeneity of soil nutrients within the root zone. This species is one of the most important shrubs in the arid and semiarid ecosystems of North America, and is one of the main species involved in shrub encroachment in semiarid grasslands (Van Auken 2000). *Prosopis* typically develops a prominent tap root that can reach 5 m deep, but also a dense network of lateral shallow roots that may grow upward to within 4 cm of the soil surface (Gibbens and Lenz 2001). These rooting patterns, together with a low root turnover rate (Reynolds et al. 1999), allow this species to use both shallow and deep sources of water, and promotes a high degree of overlap and contact between the roots of this species and those of native grasses (Gibbens and Lenz 2001; Jurena and Archer 2003). The analysis of the response of *Prosopis* seedlings to soil nutrient heterogeneity can provide insights into the establishment of this species under natural conditions. Our objectives are: (i) to evaluate if the performance of *Prosopis* seedlings is affected by soil nutrient heterogeneity (i.e. an heterogeneous supply of nutrients in the form of discrete patches); (ii) to test if responses to this heterogeneity, if any, are modified by small-scale differences in the vertical placement of the nutrient patches; and (iii) to assess if these responses are modified by the nutrient ion forming the nutrient patches.

Methods

Experimental design

We conducted a microcosm experiment in the National Phytotron at Duke University between October and December 2001. In this experiment, seedlings of *Prosopis* were grown under different combinations of nutrients [nitrogen (N), phosphorus (P), and both combined (NP)] and under different levels of nutrient heterogeneity (nutrients supplied as patches located in the bottom and/or upper portion of the rooting zone versus homogeneous distribution), resulting in 13 different treatment combinations (Table 1). Microcosms consisted of PVC pipe (length 51 cm, internal diameter 15 cm) filled with, from the base, 5 cm of gravel (for drainage) and then 36 cm of inert, non-sterilized, sand. In the homogeneous treatments (indicated by the symbols N^h and P^h in Table 1), the nutrients were mixed thoroughly with the sand before setting up the microcosms. In the heterogeneous treatments, the nutrients were either placed in the upper (heterogeneous top, indicated by upper case N or P in Table 1) or lower (heterogeneous bottom, indicated by lower case n or p in Table 1) half of the microcosms. Within the relevant half, the nutrients were localized within discrete 244 cm^3 volumes of sand. To create one of these patches we mixed 244 cm^3 of sand with the nutrients and introduced the resulting mix into a 244 cm^3 plastic cylinder (length 140 mm and internal diameter 50 mm) consisting of a light mesh with square pores 7×7 mm in size. We refer to this as the patch cylinder. A second (control) cylinder, filled only with sand, was placed 2.5 cm apart and alongside the patch cylinder. Both cylinders allow the free movement of roots into them. Heterogeneous bottom and top patches were placed 5 cm and 31 cm, above the gravel layer, respectively. In the microcosms belonging to the control and homogeneous treatments, the same plastic cylinders were placed at the same depth, but were filled with inert sand (control treatment) or with the mixture of sand and nutrients used to fill the rest of the microcosm (homogeneous treatments). In those treatments containing nitrogen (Np, Pn, N, n, N^h, Table 1), 4 g of ammonium nitrate (NH_4NO_3; Nitrex, Arcadian corporation, Memphis, TN, USA) were added to the microcosms. In those treatments containing phosphorus (Np, Pn, P, p, P^h, Table 1), 6 g of Superphosphate (P_2O_5, 0-18-0, Voluntary Purchasing Groups Inc., Bonham, TX, USA) were added to the microcosms. In the treatments containing both nutrients (NP, np, NhPh, Table 1), the same amount of NH_4NO_3 (4 g) and P_2O_5 (6 g) was added in combination, except in the treatment NPnp (Table 1), where half of the concentration (2 and 3 g of NH_4NO_3 and P_2O_5, respectively) was added to both the upper and lower patch cylinders.

Prosopis seeds were collected from the Jornada Basin LTER site at the Jornada Experimental Range near Las Cruces, New Mexico. Seeds were germinated in a glasshouse, and uniformly sized seedlings (1.43 ± 0.16 mm diameter; 3.19 ± 0.40 cm height [mean ± SD, $n = 20$]) were transplanted into microcosm units (one seedling per unit) five days after germination. Once the seedlings were transplanted, the microcosms were placed in two walk-in growth chambers that were maintained at a day/night air temperature of 27/20 °C, PAR of 1000 $\mu mol\ m^{-2}\ s^{-1}$ with a 15 h photoperiod, atmospheric partial pressure of 35 Pa, and an average relative humidity of 40% during the day

Table 1. Summary of the treatments employed in the experiment.

Treatment code[a]	Nutrient used and vertical placement in the microcosm
Np	Nitrogen *top*, Phosphorus *bottom*
Pn	Phosphorus *top*, Nitrogen *bottom*
NPnp	1/2 Nitrogen and Phosphorus *top* and *bottom*
NP	Nitrogen and Phosphorus (combined) *top*
np	Nitrogen and Phosphorus (combined) *bottom*
N^hP^h	Nitrogen and Phosphorus (combined) *homogeneous*
0	Control (no nutrients added)
N	Nitrogen *top*
n	Nitrogen *bottom*
P	Phosphorus *top*
p	Phosphorus *bottom*
N^h	Nitrogen *homogeneous*
P^h	Phosphorus *homogeneous*

The vertical placement of nutrient patches is indicated by *top*, upper half of microcosm; *bottom*, lower half of microcosm; and *homogeneous*, distributed evenly throughout the microcosm. See the text for details on the amounts of fertilizer added in each case.[a]N, P (*top*); n,p (*bottom*); N^h·P^h (*homogeneous*).

(from 06:00 until 21:00) and 70% during the night. In each chamber, half of the microcosm units per treatment (3 replicates of each treatment, 39 microcosm units in total) were randomly grouped in eight trolleys containing five microcosm units each (one empty unit was added in each chamber to maintain the same spatial configuration of units in all trolleys). Trolleys were randomly rotated within each chamber once per week, and were changed between chambers every two weeks. Each microcosm unit was irrigated to free drainage twice a day, with a modified Hoagland's solution (1/2 strength for all nutrients except for P and N, which was 1/6 strength) in the morning (8:00–9:00 h) and distilled water in the midday (14:30–15:30 h).

Despite *Prosopis* has the potential to symbiotically fix atmospheric N_2, this poses no complications to interpret the results of our experiment. Available evidence indicates that N fixation takes place in the deep roots of mature individuals (Virginia et al. 1986). An extensive survey of the depth distribution of *Prosopis* root-nodulating bacteria at the Jornada Experimental Range found that surface soils had very low populations of effective rhizobia, and that root nodules were absent from *Prosopis* seedlings (Jenkins et al. 1988). Thus, it is likely that fixation of N_2 is not prevalent during the early stages of *Prosopis* development due to factors such as low symbiotic populations, fluctuating soil moisture conditions, and high availability of nitrogen in the surface as compared to the subsurface soil (Jenkins et al. 1987). Furthermore, the time required for the development of an effective symbiosis may preclude the fixation of atmospheric N_2 during this time period. For these reasons, we believe that the use of inorganic fertilizer –an approach previously used with *Prosopis* seedlings (BassiriRad et al. 1997; Causin et al. 2004) – is a reasonable approximation of field conditions. We did not find any evidence of nodulation during the course of our experiment (F.T. Maestre, personal observations).

Harvest

Plants were grown in the chambers for 53 days. At this time, the above-ground part of each plant was cut at the soil surface, and plant height, basal diameters and number of branches and leaves were measured. Leaves and stems were dried at 60 °C until constant weight. Before drying, leaf area for each plant was measured with a LI-3100 leaf area meter (Li-Cor Inc., Lincoln, NE, USA). Once the above-ground parts of the plants were harvested, the soil was carefully removed from the microcosm unit. The roots within each of the plastic cylinders were extracted by cutting those outside the volume of the cage. The rest of the root system was also collected, and all the roots were dried as described above. Before drying, we estimated the length, area and mean diameter of the roots within the plastic cylinders by scanning and analyzing digitized images with the program Winrhizo V. 2002c Pro (Regent Instruments Inc., Quebec, Canada). Some root samples coming from these cylinders were accidentally destroyed during the drying of this material. To account for this, total root biomass in those seedlings with missing samples from the plastic cylinders was estimated using the relationship between the total root biomass (TRB) and the biomass of the root system outside the cylinders (TRO), since the latter variable was available for all seedlings. The regression used was obtained using those seedlings from which we had all the root biomass samples available, and was highly significant (TRB = 0.19 + 1.04*TRO; $R^2 = 0.977$, $p < 0.001$; $n = 34$ in total). To evaluate the effect of treatments in nutrient uptake, all the harvested leaves were triturated, and a representative sample was analyzed for tissue N (Kjeldahl method; Bremner and Mulvaney 1982) and P (inductively coupled plasma-atomic emission spectrometry after acid digestion; Dean and Ando 1997) concentrations.

Root foraging precision was estimated using the RII index (Armas et al. 2004). It is calculated as $(RLD_p - RLD_c)/(RLD_p + RLD_c)$, where RLD_p and RLD_c are the root length density (cm cm^{-3}) in the patch and control cylinders, respectively. RII ranges from -1 to $+1$; a value of zero indicates equal root growth in patches and background sand and hence no precision to foraging. Increasing positive values indicate increasing precision and negative values the opposite (i.e. avoidance of nutrient patches by roots). In the homogeneous treatment, one of the cylinders was selected as a patch cylinder (the one located in the same place as the patch cylinder in the heterogeneous treatments), and the other as a control. Calculations of the RII index were performed separately for the cylinders located in the upper and lower half of the microcosms.

Statistical analyses

Seedling performance data (height, diameter, leaf area, specific leaf area, number of leaves, biomass, biomass allocation and N and P concentration in leaves) were initially analyzed to test for differences between treatments with one-way ANOVA. The specific effects of Nutrient ion (N, P, and NP) and Heterogeneity (heterogeneous top, heterogeneous bottom, and homogeneous) on seedling performance data were evaluated with a two-way ANOVA. Separate analyses were conducted for each of the variables evaluated. In order to keep a balanced design for these analyses, only data from treatments NP, np, N^hP^h, N, n, P, p, N^h and P^h (Table 1) were used. Differences in root foraging precision between treatments were analyzed separately at each depth with one-way ANOVA. To evaluate if the magnitude in root foraging responses differed with differences in the vertical placement of nutrient patches, a two-way ANOVA with Nutrient ion (N, P, and NP) and Location (upper or lower half of the microcosm) as main factors was conducted. For this analysis, only data from the treatments NP, np, N, n, P and p (Table 1) were used.

In all ANOVA analyses, we used the Tukey *B*-test for *post-hoc* comparisons when appropriate. Data were log-transformed when necessary to achieve the homogeneity of variances and to improve the normality of the data. All the statistical analyses were performed using the SPSS 9.0 package (SPSS Inc., Chicago, IL, USA). As suggested by Gotelli and Ellison (2004), we did not adjust the experiment-wide error rate, and rather we based our interpretations of the effects of the different treatments by evaluating the raw *p*-values.

Results

Morphology and biomass of *Prosopis* seedlings did not show a strong response to changes in nutrient ion or in the vertical spatial pattern of nutrient availability (Figure 1). Height, number of leaves, and specific leaf area did not vary significantly between treatments. When significant differences were found, higher values as compared to the control treatment were generally found in those treatments containing N, alone or in combination with P (treatments Np, NP, np, N and n). Patterns of biomass allocation between leaf, stem and root did not vary between treatments (Figure 2).

We found a significant effect of nutrient ion on the number of leaves, allocation of biomass to stems, leaf biomass, and stem biomass of *Prosopis* seedlings (Table 2). *Post-hoc* comparisons (not shown) revealed that seedlings had more leaves when N and P were added to the growing medium as compared to the addition of P alone; differences between any of these treatments and the addition of N alone were not significant. *Prosopis* seedlings allocated more biomass to stems when N was added to the growing medium as compared to the addition of P alone; seedlings that experienced the addition of N and P did not show significant differences with those growing only with any of the two nutrients. For leaf and stem biomass, the effect of nutrient ion, despite significant, was not strong enough to reveal *post-hoc* differences.

We found a significant effect of heterogeneity on the diameter and mass of roots of seedlings (Table 2). In both cases, higher values were found when the nutrients were homogeneously distributed as compared to their placement as patches in the bottom of the microcosms. Differences between any of these treatments and the placement of nutrients as patches located in the upper half of the microcosms were not significant. We did not find any significant Nutrient×Heterogeneity interaction, suggesting that observed responses to the heterogeneity of nutrients were not modified by the identity of the nutrient ion added.

Leaf P concentration varied significantly among treatments, but the N concentration did not (Figure 3). Higher leaf P concentration (as compared to the control) was found in those treatments containing P supplied homogeneously or as a patch in the upper half of the microcosms (treatments P^h and P, respectively). The effect of Nutrient ion was marginally significant for leaf P concentration ($p = 0.093$, Table 2). The effect of Heterogeneity was significant for leaf N concentration, which increased in those treatments where the nutrient patch was located in the lower half as compared to the upper half of the microcosms. Differences between any of these treatments and the homogeneous treatment were not significant.

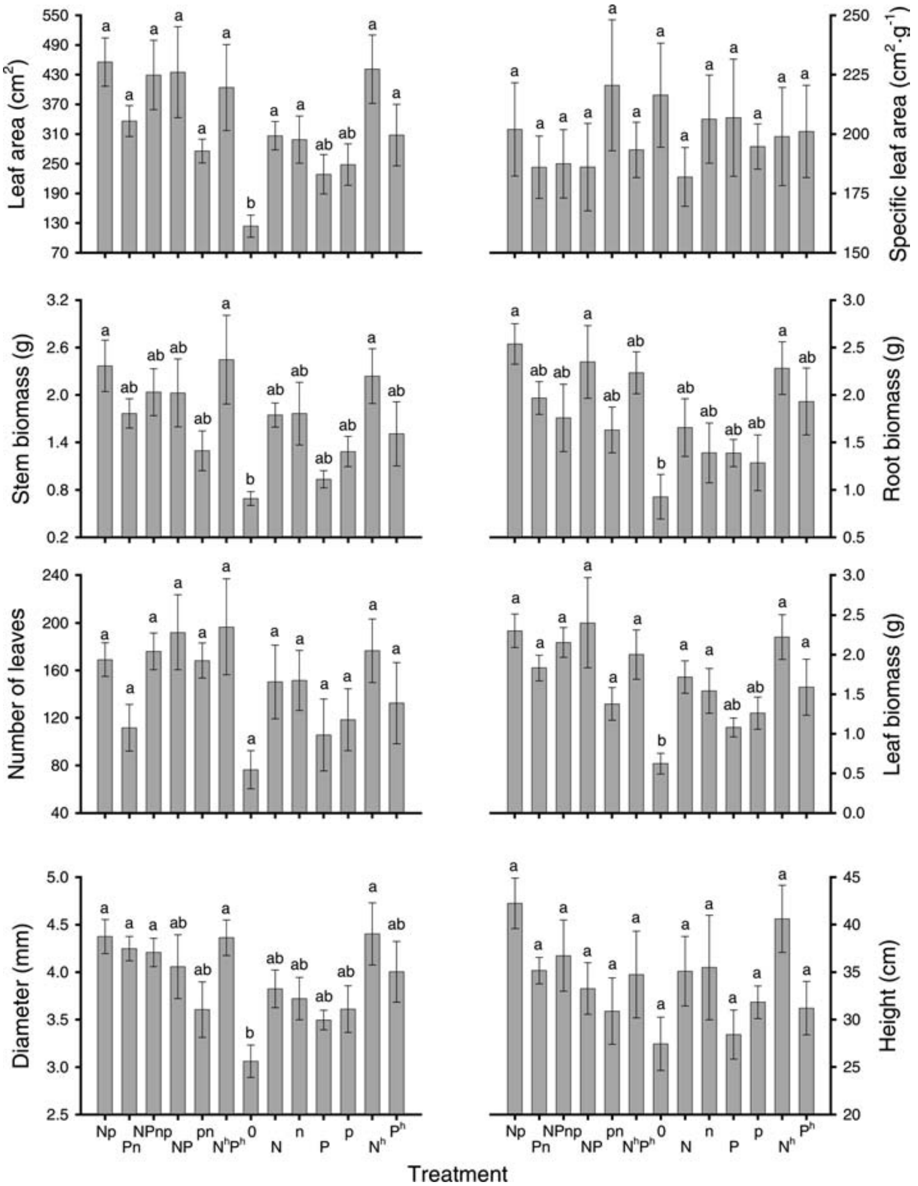

Figure 1. Morphological responses of *Prosopis glandulosa* seedlings to the different treatments. Data represent means ± SE ($n = 6$). Different lowercase letters indicate significant differences between treatments ($p < 0.05$, *post-hoc* Tukey's *B*-test after one-way ANOVA). For explanation of *x*-axis labels see Table 1.

Root foraging responses were characterized by substantial within-treatment variability (Figure 4). Despite this, *Prosopis* seedlings showed precise root foraging patterns in response to heterogeneity. Significant root foraging responses in the upper cylinders was observed when N or P were added, alone or in combination, as a patch located in the upper half of the microcosms (treatments NPnp, NP, N and P, Figure 4a). However, such proliferation was higher compared to the control only when N and P were added together (treatments NPnp and NP). In the lower cylinders, significant root foraging was observed only when P was added as a patch in the lower half of the microcosm (treatment p, Figure 4b), but such response did not differ between treatments. The magnitude of the root foraging responses did not differ with differences in the vertical placement of

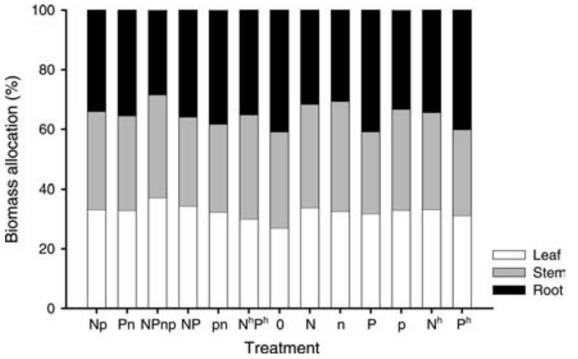

Figure 2. Percent biomass allocated to leaves, stems and roots by *Prosopis glandulosa* seedlings in the different treatments (average values). Biomass allocation patterns did not differ between treatments (one-way ANOVA, $p_{\text{treatment}} > 0.1$ for leaves, stems and roots). For explanation of *x*-axis labels see Table 1.

nutrient patches (two-way ANOVA; $F_{\text{Nutrient ion}} = 1.42$, df = 2,30, $p = 0.257$; $F_{\text{Location}} = 1.21$, df = 1,30, $p = 0.281$; $F_{\text{Nutrition} \times \text{Location}} = 1.09$, df = 2,30, $p = 0.350$).

Discussion

Robinson's (1994) survey of the literature demonstrated that individual plant responses to the heterogeneous supply of nutrients show a great degree of variation among plant species and functional types. However, many empirical studies – working largely with herbaceous species – have identified three basic responses to a heterogeneous supply of nutrients: the proliferation of fine roots in nutrient patches (i.e. a significant root foraging precision), an increase in biomass (especially root biomass), and a higher ratio of below- to above-ground biomass (Birch and Hutchings 1994; Einsmann et al. 1999; Farley and Fitter 1999; Wijesinghe and Hutchings 1999). Our results, obtained for a dryland woody species, do not match some of these responses. They suggest that *Prosopis* has a low overall responsiveness to changes in the spatial heterogeneity of nutrient supply, at least during its seedling stage. We found that *Prosopis* seedlings modified stem diameter, root biomass and leaf N content in response to changes in the spatial heterogeneity of nutrient supply, but in any case the addition of nutrients in a spatially homogeneous configuration led to a decrease in the values obtained for these variables.

Root proliferation responses, significant in some cases, were relatively small, especially when the nutrient patches were located in the lower half of the microcosms. These results may be explained by several factors. First, the lack of precise foraging may be a response to an insufficient contrast of the nutrient patches with the background soil concentration. Although lateral roots often strongly respond to localized nutrient supply, this may be

Table 2. Results of a two-way ANOVA to assess the significance of the Nutrient ion (nitrogen, phosphorous, or both combined) and Heterogeneity (heterogeneous top, heterogeneous bottom or homogeneous) effects on seedling performance variables.

	ANOVA results					
	Nutrient ion (N)		Heterogeneity (H)		N×H	
	$F_{2,45}$	p	$F_{2,45}$	p	$F_{4,45}$	p
Diameter	1.29	0.286	4.57	**0.016**	0.35	0.845
Height	2.61	0.085	0.73	0.487	0.29	0.886
Number of leaves	4.32	**0.019**	0.35	0.708	0.13	0.972
Leaf biomass	3.37	**0.043**	2.34	0.108	0.67	0.615
Stem biomass	3.84	**0.029**	2.68	0.079	0.76	0.558
Root biomass	2.04	0.143	3.24	**0.049**	0.67	0.619
Leaf area	2.90	0.065	1.68	0.199	0.38	0.824
SLA (specific leaf area)	0.06	0.938	0.51	0.604	0.47	0.756
Biomass allocation to leaves	0.30	0.746	0.62	0.545	0.41	0.804
Biomass allocation to stems	3.64	**0.034**	0.98	0.382	2.00	0.111
Biomass allocation to roots	2.33	0.109	0.45	0.642	0.78	0.542
Leaf nitrogen concentration	0.59	0.559	3.62	**0.035**	0.84	0.508
Leaf phosphorus concentration	2.50	0.093	0.24	0.788	1.91	0.125

p-values below 0.05 are shown in boldface.

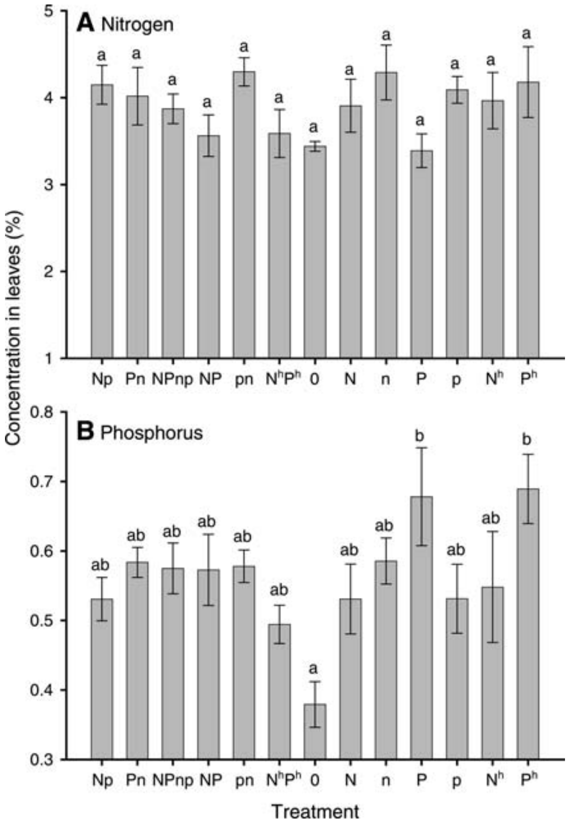

Figure 3. Concentration of nitrogen (a) and phosphorus (b) in leaves of *Prosopis glandulosa* seedlings. Data represent means ± SE ($n = 6$). Different lowercase letters indicate significant differences between microsites ($p < 0.05$, *post-hoc* Tukey's *B*-test). For explanation of x-axis labels see Table 1.

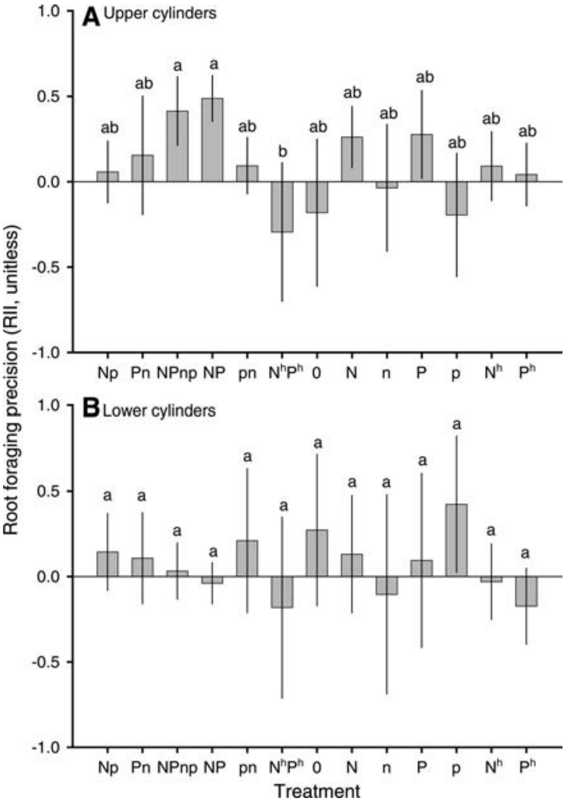

Figure 4. Foraging precision of *Prosopis glandulosa* seedlings in the cylinders located in the upper (a) and lower (b) half of the microcosms. Positive values indicate precise root foraging responses. See Methods for calculation of the RII index. Data are means and 95% confidence intervals ($n = 6$). Significant root proliferation is shown by confidence intervals that do not overlap zero. Different lowercase letters indicate significant differences between treatments ($p < 0.05$, *post-hoc* Tukey's *B*-test after one-way ANOVA). For explanation of x-axis labels see Table 1.

affected by the nutrient supply to the rest of the root system (Forde and Lorenzo 2001). Root foraging in response to localized N and P patches has been observed in instances where the remainder of the root system was subjected to low nutrient concentrations (Drew et al. 1973; Snapp et al. 1995). Second, it is also possible that the low responsiveness we observed is characteristic of *Prosopis*' root system. Morphological plasticity may be of great importance for the exploitation of localized soil resources (de Kroon and Hutchings 1995), and it has been suggested that there is a trade-off between the size of the root system and its capacity to proliferate roots when exposed to nutrient-rich patches (Campbell et al. 1991). If applicable to woody species, this suggests that species with dense and coarse root systems, such as *Prosopis*, might have limited root proliferation in response to nutrient-rich patches in the soil. Our results may have also been influenced by our use of root length as the sole measure of response, which may have underestimated it because of the large errors associated with this variable (Robinson, 1994), and by the irrigation schedule used, which could have enhanced the leaching of nutrients from the microcosms. Thus, they should be interpreted with certain caution.

Despite low overall responsiveness, we found that root proliferation responses were more sensitive to nutrient patches when they were located in the upper half of the microcosms. This was observed despite the fact that *Prosopis* very quickly forms a major taproot (Gibbens and Lenz 2001), and suggests that small-scale vertical differences in the spatial distribution of soil nutrients could modify the growth dynamics of secondary roots of this species during the first stages of seedling development. This proliferation could have important implications for the establishment of *Prosopis* under field conditions, which is highly dependent upon below-ground competition with grasses (Van Auken and Bush 1988; Bush and Van Auken 1989; Jurena and Archer 2003). Pot experiments have shown that root proliferation may confer competitive advantages when plants are in competition (Hodge et al. 1999; Fransen et al. 2001) thus, it may be possible that, if the pattern observed is reproduced in the field, the presence of nutrient patches in the upper soil layers could increase competitive performance of *Prosopis* seedlings when growing in competition with native grasses. However, it is unknown if these grasses respond to such patchiness. If so, this response could even increase their competitive ability against *Prosopis* due to a higher uptake capacity for N and P (BassiriRad et al. 1997) and a very plastic root system, which would allow them to reach faster to the nutrient patch. Further studies are needed to evaluate the effects of small-scale soil nutrient patchiness on the outcome of the interaction between *Prosopis* and native grasses, with the aim to see if this reduces or increases grassland susceptibility to *Prosopis* competition.

Despite precise root foraging responses of *Prosopis* seedlings where found in some heterogeneous treatments, these had negligible consequences for above-ground seedling development, as the performance variables evaluated did not differ among heterogeneous and homogeneous treatments. A similar link between root proliferation and lack of aboveground response has been reported for *Prosopis* in the field (BassiriRad et al. 1999) and in other woody (Mou et al. 1997) and herbaceous (Hodge et al. 1998) species in greenhouse experiments. Whereas adult *Prosopis* individuals typically have substantial amounts of viable roots in the upper soil horizons (Freckman and Virginia 1989; Gibbens and Lenz 2001), BassiriRad et al. (1999) found that this does not necessarily translate into a rapid response to water and nutrient pulses applied to the shallow portions of the soil profile. These authors suggested that this apparent lack of function of the shallow roots may be a response to heat inactivation (Lin et al. 1996). It is possible that the high temperatures and radiation levels used in the experimental growth chambers could have precipitated a similar response in the seedlings, although we have no direct evidence for this.

Seedlings had larger stem diameter and root biomass when the nutrients were homogeneously distributed in the microcosm as compared to when the nutrients were supplied as a patch in the lower half of the microcosms. Mou et al. (1997) found similar results with temperate forest species, and speculated that this could be in response to the need in heterogeneous conditions to translocate nutrients internally to those parts of the root system located in nutrient-poor soil areas. On the other hand, the content of N in leaves was higher when the nutrient patches were located in the lower half of the microcosm than when they were located in the upper half, a pattern that did not match that of root proliferation. Lack of coincidence between root proliferation and N acquisition has been reported by Hodge et al. (1998) for five grass species, and they concluded that the capture of N from a patch of decomposing organic matter was not related strongly to root proliferation in the patch. Hence, our observed uncoupled responses suggest that mechanisms other than root proliferation may be more important for nutrient acquisition in *Prosopis*. High root turnover rates typically associated to root proliferation are disadvantageous in nutrient-poor habitats such as deserts (Grime et al. 1986), and low turnover rates have been measured in adult *Prosopis* individuals in the field (Reynolds et al. 1999).

In addition to the responses observed to changes in the spatial patterning of nutrient supply, we found a low overall responsiveness of *Prosopis* seedlings to fertilization. The lack of significant differences between the control and the rest of treatments for many response variables was initially unexpected, and may have been promoted by an adequate nutrient availability in all treatments. One of the most commonly observed effects of nutrient deficiency on plant development is an increase in the below- to above-ground biomass ratio (Chapin 1980). Since we did not find significant differences in the allocation of biomass between treatments, this suggests that we may have failed to create a nutrient deficiency, even in the control treatment.

Overall, our results suggest that *Prosopis* seedlings have a low responsiveness to small-scale vertical differences in soil nutrient heterogeneity. Despite this, the ecological consequences of some of the responses observed (precise root foraging patterns in response to shallow N, P and NP patches), merit further study. Increasing our understanding of the effects of shallow nutrient heterogeneity on the outcome of the competition between *Prosopis* and native grasses will provide valuable insights to elucidate those mechanisms underlying invasion of grasslands by *Prosopis*, which remains a major ecological challenge.

Acknowledgements

We thank Guy Telesnicki and Rocío Paramá for their help during the harvesting, scanning and weighing of plant material, David Tremmel for statistical advice and logistic support, and Scott Wilson and two anonymous referees for useful comments on a previous version of the manuscript. This research was supported by the National Phytotron via the Operations Grant (NSF-IBN-99-85877 and Duke University). Further support was provided by USDA Specific Cooperative Agreement #58-1270-3-070 and NSF-DEB-02-12123. F. T. Maestre was supported by FPU and Fulbright (FU2003-0398) fellowships from the Spanish Ministerio de Educación, Cultura y Deporte. The latter was funded by Secretaría de Estado de Universidades and Fondo Social Europeo.

References

Aguiar M.R. and Sala O.E. 1999. Patch structure, dynamics and implications for the functioning of arid ecosystems. Trends Ecol. Evol. 14: 273–277.

Anderson T.M., McNaughton S.J. and Ritchie M.E. 2004. Scale-dependent relationships between the spatial distribution of a limiting resource and plant species diversity in an African grassland ecosystem. Oecologia 139: 277–287.

Armas C., Ordiales R. and Pugnaire F.I. 2004. Measuring plant interactions: a new comparative index. Ecology 85: 2682–2686.

BassiriRad H., Reynolds J.F., Virginia R.A. and Brunelle M.H. 1997. Growth and root NO_3^- an PO_4^{-3} uptake capacity of three desert species in response to atmospheric CO_2 enrichment. Aust. J. Plant Physiol. 24: 353–358.

BassiriRad H., Tremmel D.C., Virginia R.A., Reynolds J.F., De Soyza A.G. and Brunell M.H. 1999. Short-term patterns in water and nitrogen acquisition by two desert shrubs following a simulated summer rain. Plant Ecol. 145: 27–36.

Berendse F. 1981. Competition between plants with different rooting depths. II. Pot experiments. Oecologia 48: 334–341.

Birch C.P.D. and Hutchings M.J. 1994. Exploitation of patchily distributed soil resources by the clonal herb *Glechoma hederacea*. J. Ecol. 82: 653–664.

Bremner J.M. and Mulvaney C.S. 1982. Nitrogen-total. In: Page A.L., Miller R.H. and Keeney D.R. (eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, American Society of Agronomy and Soil Science Society of America, Madison, pp. 595–624.

Bush J.K. and Van Auken O.W. 1989. Soil resource levels and competition between a woody and herbaceous species. Bull. Torrey Bot. Club. 116: 22–30.

Cahill J.F.J. and Casper B.B. 1999. Growth consequences of soil nutrient heterogeneity for two old-field herbs, *Ambrosia artemisiifolia* and *Phytolacca americana*, grown individually and in combination. Ann. Bot. 83: 471–478.

Caldwell M.M. and Manwaring J.J. 1994. Hydraulic lift and soil nutrient heterogeneity. Isr. J. Plant Sci. 42: 321–330.

Campbell B.D., Grime J.P. and Mackey J.M.L. 1991. A trade-off between scale and precision in resource foraging. Oecologia 87: 532–538.

Causin H.F., Tremmel D.C., Rufty T.W. and Reynolds J.F. 2004. Growth, nitrogen uptake, and metabolism in two semiarid shrubs grown at ambient and elevated atmospheric CO_2 concentrations: effects of nitrogen supply and source. Am. J. Bot. 91: 565–572.

Chapin F.S. III. 1980. The mineral nutrition of wild plants. Annu. Rev. Ecol. Syst. 11: 233–260.

Dean J.R. and Ando D.J. 1997. Absorption and Plasma Spectroscopy 2nd ed. John Wiley & Sons, Chichester.

de Kroon H. and Hutchings M.J. 1995. Morphological plasticity in clonal plants: the foraging concept reconsidered. J. Ecol. 83: 143–152.

Drew M.C., Saker L.R. and Ashley T.W. 1973. Nutrient supply and the growth of the seminal root system in barley. I. The effect of nitrate concentration on the growth of axes and laterals. J. Exp. Bot. 24: 1189–1202.

Einsmann J.C., Jones R.H., Mou P. and Mitchell R.J. 1999. Nutrient foraging traits in 10 co-occurring plant species of contrasting life forms. J. Ecol. 87: 609–619.

Farley R.A. and Fitter A.H. 1999. The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches. J. Ecol. 87: 849–859.

Fitter A.H. 1982. Influence of soil heterogeneity on the coexistence of grassland species. J. Ecol. 70: 139–148.

Fitter A., Hodge A. and Robinson D. 2000. Plant response to patchy soils. In: Hutchings M.J., John E.A. and Stewart A.J.A (eds.), The Ecological Consequences of Environmental Heterogeneity, Blackwell Science, Cambridge, pp. 71–90.

Forde B. and Lorenzo H. 2001. The nutritional control of root development. Plant Soil 232: 51–68.

Fransen B., Blijjenberg J. and de Kroon H. 1999. Root morphological and physiological plasticity of perennial grass species and the exploitation of spatial and temporal heterogeneous nutrient patches. Plant Soil 211: 179–189.

Fransen B. and de Kroon H. 2001. Long-term disadvantages of selective root placement: root proliferation and shoot biomass of two perennial grass species in a 2-year experiment. J. Ecol. 89: 711–722.

Fransen B., de Kroon H. and Berendse F. 2001. Soil nutrient heterogeneity alters competition between two perennial grass species. Ecology 82: 2534–2546.

Freckman D.W. and Virginia R.A. 1989. Plant-feeding nematodes in deep-rooting desert ecosystems. Ecology 70: 1665–1678.

Gibbens R.P. and Lenz J.M. 2001. Root systems of some Chihuahuan Desert plants. J. Arid Environ. 49: 221–263.

Gotelli N.J. and Ellison A.M. 2004. A Primer of Ecological Statistics. Sinauer Associates, Sunderland, USA.

Grime J.P., Crick J.C. and Rincon J.E. 1986. The ecological significance of plasticity. In: Jennings D.H. and Trewaras A.J. (eds.), Plasticity in Plants, Cambridge University Press, Cambridge, pp. 5–29.

Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 162: 9–24.

Hodge A., Robinson D., Griffiths B.S. and Fitter A.H. 1999. Nitrogen capture by plants grown in N-rich organic patches of contrasting size and strength. J. Exp. Bot. 50: 1243–1252.

Hodge A., Stewart J., Robinson D., Griffiths B.S. and Fitter A.H. 1998. Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. New Phytol. 139: 479–494.

Huber-Sannwald E. and Jackson R.B. 2001. Heterogeneous soil-resource distribution and plant responses-from individual-plant growth to ecosystem functioning. Prog. Bot. 61: 451–476.

Hutchings M.J., John E.A. and Wijesinghe D.K. 2003. Toward understanding the consequences of soil heterogeneity for plant populations and communities. Ecology 84: 2322–2334.

Jenkins M.B., Virginia R.A. and Jarrell W.M. 1987. Rhizobial ecology of the woody legume mesquite (*Prosopis glandulosa*) in the Sonoran Desert. Appl. Environ. Microbiol. 53: 36–40.

Jenkins M.B., Virginia R.A. and Jarrell W.M. 1988. Depth distribution and seasonal populations of mesquite-nodulating rhizobia in warm desert ecosystems. Soil Sci. Soc. Am. J. 52: 1644–1650.

Jurena P.N. and Archer S. 2003. Woody plant establishment and spatial heterogeneity in grasslands. Ecology 84: 907–919.

Lin G., Phillips S.L. and Ehleringer J.R. 1996. Monsoonal precipitation responses of shrubs in a cold desert community on the Colorado Plateau. Oecologia 106: 8–17.

Maestre F.T., Cortina J., Bautista S., Bellot J. and Vallejo R. 2003. Small-scale environmental heterogeneity and spatiotemporal dynamics of seedling survival in a degraded semiarid ecosystem. Ecosystems 6: 630–643.

Mou P., Jones R.H., Mitchell R.J. and Zutter B. 1995. Spatial distribution of roots in Swetgum and Lobolly Pine monocultures and relations with above-ground biomass and soil nutrients. Funct. Ecol. 9: 689–699.

Mou P., Mitchell R.J. and Jones R.H. 1997. Root distribution of two tree species under a heterogeneus nutrient environment. J. Appl. Ecol. 34: 645–656.

Pan D., Bouchard A., Legendre P. and Domon G. 1998. Influence of edaphic factors on the spatial structure of inland halophytic communities: a case study in China. J. Veget. Sci. 9: 797–804.

Reid K.D., Wilcox B.P., Breshears D.D. and MacDonald L. 1999. Runoff and erosion in a pinyon-juniper woodland: influence of vegetation patches. Soil Sci. Soc. Am. J. 63: 1869–1879.

Reynolds J.F., Virginia R.A., Kemp P.R., De Soyza A.G. and Tremmel D.C. 1999. Impact of drought on desert shrubs: effects of seasonality and degree of resource island development. Ecol. Monogr. 69: 69–106.

Robinson D. 1994. The responses of plants to non-uniform supplies of nutrients. New Phytol. 127: 635–674.

Robinson D., Hodge A., Griffiths B.S. and Fitter A.H. 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proc. R. Soc. London B 266: 431–435.

Sala O.E., Lauenroth W.K. and Golluscio R.A. 1997. Plant functional types in temperate semi-arid regions. In: Smith T.M., Shugart H.H. and Woodward FI (eds.), Plant Functional Types: Their Relevance to Ecosystem Properties and Global Change, Cambridge University Press, New York, pp. 217–233.

Snapp S., Koide R. and Lynch J. 1995. Exploitation of localized phosphorus-patches by common bean roots. Plant Soil 177: 211–218.

Soriano A., Golluscio R.A. and Satorre E.H. 1987. Spatial heterogeneity of the root system of grasses in the Patagonian arid steppe. Bull. Torrey Bot. Club. 114: 103–108.

Titus J.H., Nowak R.S. and Smith S.D. 2002. Soil resource heterogeneity in the Mojave Desert. J. Arid Environ. 52: 269–292.

Van Auken O.W. 2000. Shrub invasion of North American semiarid grasslands. Annu. Rev. Ecol. Syst. 31: 197–215.

Van Auken O.W. and Bush J.K. 1988. Competition between *Schizachyrium scoparium* and *Prosopis glandulosa*. Am. J. Bot. 75: 782–789.

Virginia R.A., Jenkins M.B. and Jarrell W.M. 1986. Depth of root symbiont occurrence in soil. Biol. Fert. Soils 2: 127–130.

Whitford W.G. 1993. Animal feedbacks in desertification: an overview. Rev. Chil. Hist. Nat. 66: 243–251.

Wijesinghe D.K. and Hutchings M.J. 1999. The effects of environmental heterogeneity on the performance of *Glechoma hederacea*: the interactions between patch contrast and patch scale. J. Ecol. 87: 860–872.

Zaady E., Groffman P.M. and Shachak M. 1996. Litter as a regulator of N and C dynamics in macrophytic patches in Negev desert soils. Soil Biol. Biochem. 28: 39–46.