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Soil heterogeneity and community composition jointly influence grassland biomass

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Abstract

Question: Does the spatial pattern of nutrient supply modify community biomass responses to changes in both species composition and richness?

Location: Duke University Phytotron (Durham, North Carolina, USA).

Methods: We conducted a microcosm experiment to evaluate individual plant and whole community responses to species richness, species composition and soil nutrient heterogeneity. The experiment consisted of seven levels of species composition (all possible combinations of *Lolium perenne*, *Poa pratensis* and *Plantago lanceolata*) crossed with three levels of soil nutrient distribution (homogeneous, heterogeneous-up, and heterogeneous-down, where up and down indicates the location of a nutrient patch in either the upper or the lower half of the soil column, respectively).

Results: Communities containing *Plantago* and *Lolium* responded to nutrient heterogeneity by increasing above- and below-ground biomass. Nutrient heterogeneity also increased size inequalities among individuals of these species. Significant species composition × nutrient heterogeneity interactions on community biomass and individual size inequality were observed when nutrient patches were located in the upper 10 cm of the soil columns. However, root proliferation in nutrient patches was equivalent regardless of the vertical placement of the patch.

Conclusions: Our results suggest that nutrient heterogeneity may interact with plant species composition to determine community biomass, and that small-scale vertical differences in the location of nutrient patches affect individual and community responses to this heterogeneity.

Keywords: *Lolium perenne*; Microcosm; Nutrient heterogeneity; *Plantago lanceolata*; *Poa pratensis*; Species richness; Species composition.

Nomenclature: Tutin et al. (1964-1980).

Introduction

In terrestrial ecosystems, soil resources are spatially heterogeneous at various scales (Kelly & Canham 1992; Jackson & Caldwell 1993; Maestre & Cortina 2002). This heterogeneity has profound consequences for plants, as it affects their establishment (Maestre et al. 2003), neighbourhood interactions (Robinson et al. 1999), distribution (Pan et al. 1998), productivity (Stein et al. 1997) and diversity (Désilets & Houle 2005). In the last decade there has been a renewed interest in the effects of small-scale nutrient heterogeneity on plant performance (for recent reviews see Robinson 1994; Huber-Sannwald & Jackson 2001; Hodge 2004). At the spatial scale of root systems, soil nutrient heterogeneity promotes a variety of responses, including changes in biomass allocation, root morphology, nutrient uptake kinetics and root production. These responses determine the competitive ability and survival of individual plants within communities (Hutchings et al. 2003), and are dependent upon species traits and on nutrient patch characteristics (Robinson 1994; Robinson & van Vuuren 1998).

In spite of the fact that plants occur in complex communities, most of what is known about plant responses to soil nutrient heterogeneity has originated from studies using plants grown singly or in pairs (Robinson 1994; Hodge 2004). Hence, extrapolation to natural situations is hindered by the simplicity of the employed experimental systems (Hodge 2004). To address these concerns, studies conducted at the population and community level are growing in number (Casper & Cahill 1996, 1998; Šmilauerová & Šmilauer 2002; Day et al. 2003a, b; Wijesinghe et al. 2005; Maestre et al. 2005). These studies have shown that soil nutrient heterogeneity can modify net primary productivity, which is one of the most important ecosystem attributes

(McNaughton et al. 1989). Productivity is also strongly influenced by biotic attributes of plant communities such as composition, richness and evenness (Hooper et al. 2005). However, it is unknown whether observed productivity responses to changes in these attributes are modified by soil nutrient heterogeneity. The potential for them to be so is certainly great: nutrient heterogeneity is both ubiquitous and has been shown to have multiple effects on individual plant performance and plant-plant interactions (Hutchings et al. 2003; Hodge 2004). The effects of the biotic attributes of plant communities on productivity largely depend on the traits of the species forming them (Hooper et al. 2005). As some of these traits, like root foraging scale and precision, may be relevant only under heterogeneous soil conditions (Fransen et al. 2001), soil heterogeneity may potentially modify the effects of biotic attributes on net primary productivity.

Bliss et al. (2002) evaluated the effects of soil nutrient heterogeneity on root foraging patterns and competitive interactions among species within artificial communities of varying composition and richness levels. However, we are not aware of previous experimental studies that have explicitly evaluated the role of simultaneous changes in soil nutrient heterogeneity, species composition and richness on the productivity of plant communities. Such studies are required to advance our understanding of the ecological consequences of soil nutrient heterogeneity, and to test if it interacts with biotic attributes to determine productivity. Here we report results from a factorial experiment designed to evaluate individual plant and whole community responses in model grassland communities – containing monocultures and all possible mixtures of *Lolium perenne*, *Plantago lanceolata* and *Poa pratensis* – to species richness, species composition (defined as the list of species present in a particular community) and soil nutrient heterogeneity. The plant species used commonly co-occur in semi-natural temperate grasslands (Joshi et al. 2000) but differ in their ability to proliferate roots into nutrient patches: *Lolium* and *Plantago* are usually more responsive to the presence of nutrient patchiness than *Poa* (Wurst et al. 2003; Hodge 2004). We tested the hypotheses that soil nutrient heterogeneity will interact with plant species composition to determine the performance of individual plants and with both species composition and richness to determine the productivity and biomass allocation patterns of communities.

Material and Methods

Experimental design

We conducted a microcosm experiment in the Duke University Phytotron between January and April 2004. The experiment consisted of 21 different treatment combinations: 7 levels of species composition (*Lolium* monocultures, *Plantago* monocultures, *Poa* monocultures, *Lolium* + *Plantago* mixtures, *Lolium* + *Poa* mixtures, *Plantago* + *Poa* mixtures and *Lolium* + *Plantago* + *Poa* mixtures) crossed with 3 levels of soil nutrient distribution (homogeneous, heterogeneous-up, and heterogeneous-down, where up and down indicates the location of a nutrient patch in either the upper or the lower half of the soil column, respectively).

Microcosms consisted of PVC pipe (length 43 cm, internal diameter 10 cm) filled with 5 cm of gravel at the base (for drainage) and then 35 cm of soil (App. 1). Two 31-cm^3 plastic cylinders (length 75 mm and internal diameter 23 mm) consisting of a light mesh with square pores 5 × 10 mm in size were placed in all the microcosms at both 10 cm and 30 cm above the bottom of the microcosm (four cylinders per microcosm). These cylinders were used to house the organic material in the heterogeneous treatments, and to measure root foraging precision (see below). The soil, a sandy loam of the White Store series, was collected from the top 30 cm of mineral soil at a site in the Duke Forest (35º55' N, 78º52' W) near Durham, NC. We steam-treated (two 2-h treatments at 75 ºC) the soil to kill soil macrofauna, which Wurst et al. (2003) showed can modify the responses to nutrient heterogeneity of some of the plant species used here. After this steam-treatment, we leached the soils for one week with distilled water to minimize the associated nutrient pulse. We then mixed the steamed soil with sand and fresh soil to produce a 90:5:5 steamed soil/sand/fresh soil mixture. The sand was included to ensure efficient soil drainage and the fresh soil to provide a microbial inoculum to re-introduce any microbial species killed during steaming. The resulting mix (hereafter 'background soil') had 7.24 mg N-NH_4^+.g^{-1} dry soil and 0.37 mg N-NO_3^-.g^{-1} dry soil.

To each microcosm we added 0.8 g of air-dried and ground (< 2 mm) *Trifolium repens* shoots (3.9% N, 10.8 C:N), which constituted the organic material and is equivalent to an addition of 31 mg of nitrogen per microcosm (3.95 g.m^{-2} of nitrogen). In the homogeneous treatment, we thoroughly mixed the organic material with the background soil before introducing it into the PVC pipe. In this treatment, the whole microcosm (including all the cylinders) was filled with this mixture. In the heterogeneous treatments, the organic material

was either placed in the upper (heterogeneous-up) or lower (heterogeneous-down) half of the soil columns. To create nutrient patches in these treatments, we mixed 31 cm^3 of background soil with the organic material and introduced the resulting mix into a plastic cylinder (patch cylinder). A second (control) cylinder, filled only with background soil, was placed 2 cm apart and alongside the patch cylinder. The remaining two cylinders located in the upper (heterogeneous-down) or lower (heterogeneous-up) half of the microcosm, as well as the rest of the microcosm, were also filled with background soil.

Seeds from the three species were obtained from commercial suppliers (*Lolium* seeds were provided by Granite Seed Company, Lehi, UT; *Plantago* and *Poa* seeds by V & J Seed Service, Woodstock, IL). Seeds were germinated in Petri dishes and placed in a growth chamber at 20 ºC, with a PAR of 500 $\mu mol.m^{-2}.s^{-1}$ and a 14 h photoperiod. Due to differences in germination times and relative growth rate between species, we staggered the germination start dates to ensure all seedlings were of similar size (one-leaf stage) at the start of the experiment: height in cm (means ± SD, $n = 20$) of
Poa = 1.21 ± 0.46; *Plantago* = 1.10 ± 0.38; *Lolium* = 1.32 ± 0.47.

On 5 January 2004, uniformly sized seedlings of each species were randomly transplanted into each microcosm unit. Monocultures contained six seedlings of a single species, two-species mixtures three seedlings of each of two species, and three-species mixtures two seedlings of each species. Thus, total plant density across microcosms was constant (764 seedlings·m^{-2}). Seedlings that died during the first 10 days of the experiment were replaced. No further mortality was observed after that date.

We established six replicate microcosms for each treatment combination, providing 126 microcosms total. All microcosms were maintained in a walk-in growth chamber having a day/night air temperature of 25/15 ºC and a 16 h photoperiod. PAR was maintained at 500 $\mu mol.m^{-2}.s^{-1}$ during the first week of the experiment, 750 $\mu mol.m^{-2}.s^{-1}$ during the second week of the experiment, and at 1000 $\mu mol.m^{-2}.s^{-1}$ thereafter. This gradual ramping-up of light intensity was used to preclude the plants from high-light shock. Each microcosm was irrigated twice a day with 50 ml of distilled water during the first six weeks of the experiment, and once a day with the same amount thereafter. The positions of the microcosms within the growth chamber were randomized every 14 days.

Measurements and harvest

The microcosms were maintained in the growth chamber for 85 days. At this time, the above-ground biomass of each individual plant was clipped at the soil surface and then dried at 60 ºC to constant weight. Next, roots (diameter > 0.4 mm) were harvested. There was a high degree of root entanglement, so we were unable to separate roots by species and instead restricted our root biomass estimates to standing totals in the background soil, in the control cylinders, and in the patch cylinders. Roots were washed and then dried at 60 ºC to constant weight. At harvest there were no visible remnants of the added organic material in either the homogeneous or heterogeneous treatments. The percentage of biomass accounted for by each species in the mixtures was evaluated using above-ground biomass data.

Root foraging precision in the heterogeneous treatments was estimated using the RII index (Armas et al. 2004). It is calculated as (RBp – RBc)/(RBp + RBc), where RBp and RBc are the root biomass 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 soil and hence no precision to foraging. Increasing positive values indicate increasing precision and negative values the opposite.

Statistical analyses

We evaluated the effects of species richness (SR, three levels), species composition (SC, seven levels) and nutrient heterogeneity (NH, three levels) on plant community biomass (total, above-ground and below-ground) and the below: above-ground ratio with a three-way nested ANOVA, with SC nested within SR. In these analyses, richness terms were tested against the appropriate composition terms; all other terms were tested against the error term. This approach tests whether there is a significant effect of increasing species richness over and above possible effects of species composition (Schmid et al. 2002). To control for differences in plant size when evaluating the patterns of biomass allocation (Reich 2002), we analysed not the below: above-ground ratio but instead the residuals from a regression between the log-transformed below: above-ground ratio (dependent variable) and the log-transformed total biomass data (independent variable). To evaluate differences in foraging precision between heterogeneous-up and -down treatments, we used a similar three-way nested ANOVA model with SR, SC (nested within SR) and Depth as the main factors. The effect of NH on the percentage of above-ground biomass accounted for by each species was evaluated separately for each mixture with MANOVA. These data did not meet the homoge-

neity of variance-covariance matrices assumption (Box's *M*-test, $P < 0.05$ in all cases). Despite this, we conducted the MANOVA using the Pillai's trace statistic, which is robust to deviations from this assumption, especially when the sample sizes are equal (Quinn & Keough 2002).

To test predictions at the individual plant level, for each species we evaluated the average above-ground biomass per individual and the individual size inequality (as measured by the coefficient of variation of individual above-ground biomass per microcosm). These data were analysed with a two-way ANOVA with SC and NH as the main fixed factors. Differences in plant size variation were further examined by calculating the combined biomass of the two largest individuals in each microcosm. These data were analysed with the three-way nested ANOVA described for the community-level data. Where appropriate, Tukey's HSD test was used for pair-wise *post-hoc* comparisons. Data were log-transformed (biomass) or arcsine-transformed (species composition) prior to statistical analyses, which were performed using SPSS 9.0 (SPSS Inc., Chicago, IL, USA). Although we conducted a large number of statistical tests, *P* values were not adjusted for multiple testing as this approach is considered overly conservative (Gotelli & Ellison 2004).

Table 1. Results of the nested ANOVAs of log-transformed community biomass data. Significant effects ($P < 0.05$) are shown in bold.

Variable	Source of variation	ANOVA results *df*	*F*	*P*
Total biomass	Species richness (SR)	2, 4	< 0.1	0.969
	Species composition (SC)	4, 105	36.0	**< 0.001**
	Nutrient heterogeneity (NH)	2, 105	23.4	**< 0.001**
	SR × NH	4, 8	0.4	0.795
	SC × NH	8, 105	2.5	**0.015**
Above-ground biomass	SR	2, 4	< 0.1	0.952
	SC	4, 105	33.6	**< 0.001**
	NH	2, 105	26.7	**< 0.001**
	SR × NH	4, 8	0.2	0.954
	SC × NH	8, 105	2.2	**0.035**
Below-ground biomass	SR	2, 4	<0.1	0.971
	SC	4, 105	35.7	**< 0.001**
	NH	2, 105	19.3	**< 0.001**
	SR × NH	4, 8	0.7	0.587
	SC × NH	8, 105	2.4	**0.023**
Below: Above-ground biomass*	SR	2, 4	0.2	**0.002**
	SC	4, 105	4.5	**0.002**
	NH	2, 105	6.4	0.050
	SR × NH	4, 8	3.8	0.565
	SC × NH	8, 105	0.9	0.825

* Analyses were conducted with the residuals from a regression between the log-transformed below: above-ground ratio and total biomass.

Results

Species composition and nutrient heterogeneity, but not species richness, significantly affected community biomass (Table 1; Fig. 1). From a composition perspective, communities that included *Plantago* and/or *Lolium* had substantially higher biomass than *Poa* monocultures.

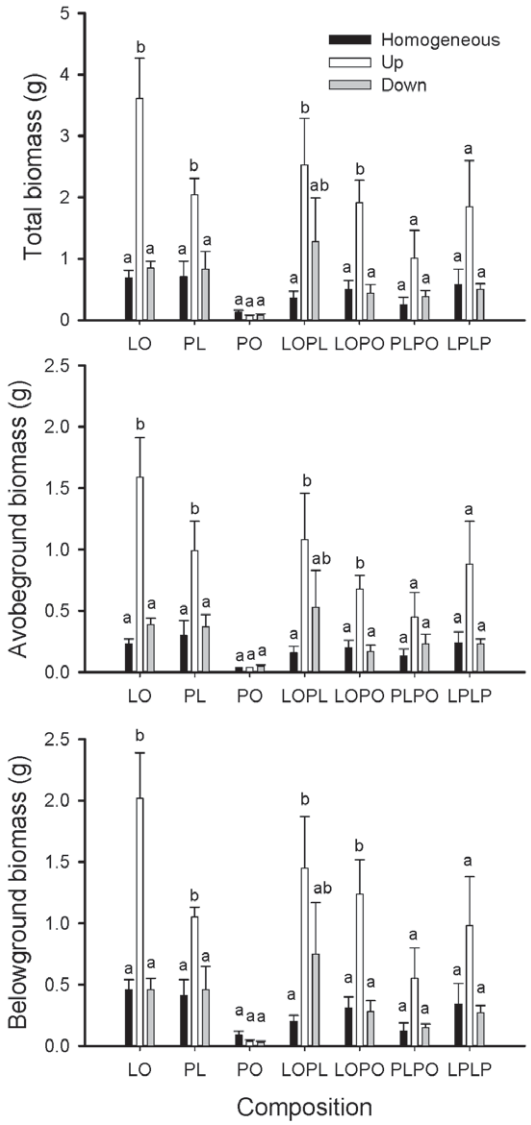

Fig. 1. Total community, above-ground and below-ground biomass compared among composition and nutrient heterogeneity levels. Data are means + 1 SE ($N = 6$). Up and down refers to heterogeneous-up and -down treatments, respectively. Where significant species composition × nutrient heterogeneity interactions were found (shown in Table 1), lowercase letters are used to denote significant differences among nutrient heterogeneity levels ($P < 0.05$, Tukey's HSD test). LO, PL, PO = monocultures of *Lolium perenne*, *Plantago lanceolata*, *Poa pratensis*; LOPL = *Lolium* + *Plantago* mixture; LOPO = *Lolium* + *Poa*, PLPO = *Plantago* + *Poa*; LPLP = *Lolium* + *Plantago* + *Poa* mixture. Note different scales on the *y*-axes.

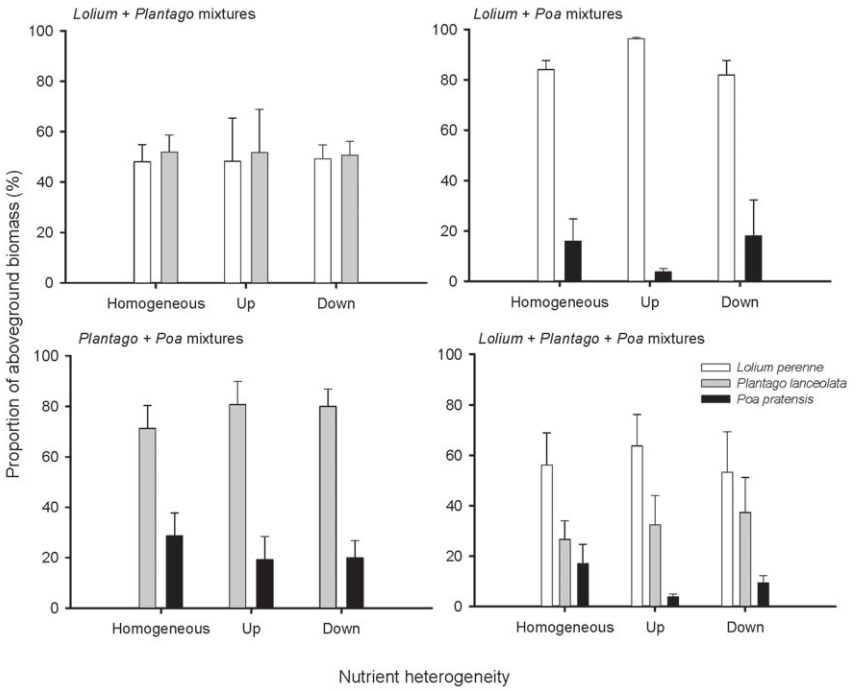

Fig. 2. Proportion of the total above-ground biomass accounted for by each of the different species in the mixtures. Data are means + 1 SE ($N = 6$). Up and down refers to heterogeneous-up and -down treatments, respectively.

From a nutrient heterogeneity perspective, communities with *Plantago* and/or *Lolium* had higher biomass when nutrients were distributed heterogeneously as opposed to homogeneously, but this was only true when the nutrient patch was located in the upper half of the soil column (Fig. 1). *Poa* monocultures did not respond significantly to the heterogeneity treatment (Fig. 1) and this most likely explains the significant species composition × nutrient heterogeneity interaction found for total, above- and below-ground biomass (Table 1). *Post-hoc* analyses conducted for each species composition level revealed that, in most cases, biomass in the homogeneous treatment did not differ significantly from the heterogeneous-down treatment (Fig. 1). Patterns of biomass allocation revealed a different picture: the marginally significant species richness × nutrient heterogeneity interaction ($P = 0.05$) found for the below-ground: above-ground ratio (Table 1) occurred because this variable was higher in the homogeneous than heterogeneous treatments in the monocultures, but equivalent in the two- and three-species mixtures (App. 2).

Nutrient heterogeneity had negligible effects on the proportion of above-ground biomass accounted for by each species in the *Lolium* + *Plantago*, *Plantago* + *Poa* and *Lolium* + *Plantago* + *Poa* mixtures (Fig. 2, MANOVA, $P > 0.05$ in all cases). In the *Lolium* + *Poa* mixtures, the contribution of *Lolium* to the total above-ground biomass increased significantly in the heterogeneous-up treatment relative to that in the heterogeneous-down and homogeneous treatments (MANOVA, $F_{2,15} = 4.77$, $P = 0.025$).

Most communities showed precise root foraging patterns (Fig. 3). Despite the observed differences in biomass characteristics between heterogeneous-up and -down treatments (Fig. 1), we did not find significant differences in foraging precision with depth ($F_{1,70} < 0.1$, $P = 0.841$). Indeed, in our analyses of root foraging data, only the main effect of species composition was significant ($F_{4,70} = 6.5$, $P < 0.001$). The *Poa* monoculture was the only community where root foraging precision was not significantly different from zero in both the 'up' and

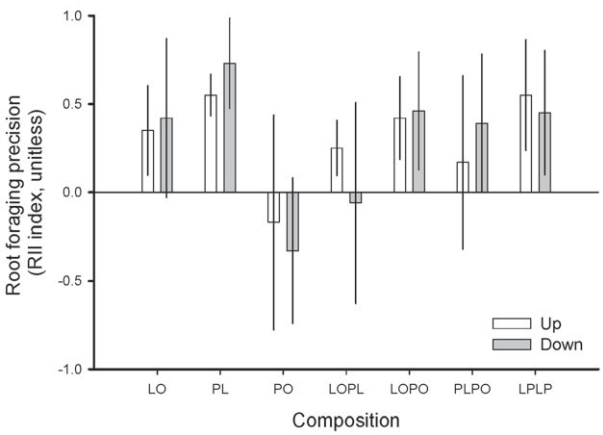

Fig. 3. Root foraging precision into nutrient patches in the heterogeneous treatments. Positive values indicate precise root foraging (proliferation) responses into nutrient patches. See Methods for calculation of the RII index. Data are means and 95% confidence intervals ($N = 6$). Significant root proliferation is shown by confidence intervals that do not overlap zero. For explanation of x-axis labels see the legend for Fig. 1.

the 'down' patch; in all other communities significant positive foraging responses were observed in at least one of the two patches (Fig. 3).

The average above-ground biomass per individual was not significantly affected by composition for any of the species (Table 2). For both *Plantago* and *Lolium*, above-ground biomass per individual was significantly greater in the heterogeneous-up than in the homogeneous or heterogeneous-down treatments (Fig. 4, Table 2). Individual size inequalities of *Lolium* and *Plantago*, but not of *Poa*, were affected by composition and nutrient heterogeneity (Table 2). Both *Lolium* and *Plantago* had higher CVs in the heterogeneous-up as compared to the homogeneous treatment, and in the monocultures as compared to the majority of the mixtures (Fig. 4).

A significant species composition × nutrient heterogeneity interaction was found when analysing the combined biomass of the two largest plants ($F_{8,105} = 2.4$, $P = 0.020$). An analysis of this interaction revealed that this variable was significantly larger in the heterogeneous-up than in the homogeneous treatment for all of the composition levels excepting the *Poa* monocultures and the *Poa* + *Plantago* mixtures (Fig. 5). No significant differences between the heterogeneous-down and the homogeneous treatments were observed for any composition level.

Table 2. Results of the two-way ANOVAs to evaluate the effects of species composition and nutrient heterogeneity on the average above-ground biomass per individual and on its coefficient of variation. Significant effects ($P < 0.05$) are shown in bold.

Species	Source of variation	*F*	*df*	*P*
Average above-ground biomass per individual				
Lolium perenne	Species composition (SC)	0.4	3, 60	0.761
	Nutrient heterogeneity (NH)	11.2	2, 60	**< 0.001**
	SC × NH	0.2	6, 60	0.967
Plantago lanceolata	SC	1.1	3, 60	0.375
	NH	8.4	2, 60	**0.001**
	SC × NH	0.4	6, 60	0.893
Poa pratensis	SC	1.4	3, 60	0.241
	NH	0.2	2, 60	0.856
	SC × NH	0.5	6, 60	0.812
Coefficient of variation of the average above-ground biomass per individual				
Lolium perenne	SC	7.3	3, 60	**< 0.001**
	NH	6.4	2, 60	**0.003**
	SC × NH	1.3	6, 60	0.274
Plantago lanceolata	SC	6.7	3, 60	**0.001**
	NH	9.1	2, 60	**< 0.001**
	SC × NH	0.6	6, 60	0.725
Poa pratensis	SC	0.7	3, 60	0.553
	NH	0.3	2, 60	0.716
	SC × NH	1.1	6, 60	0.359

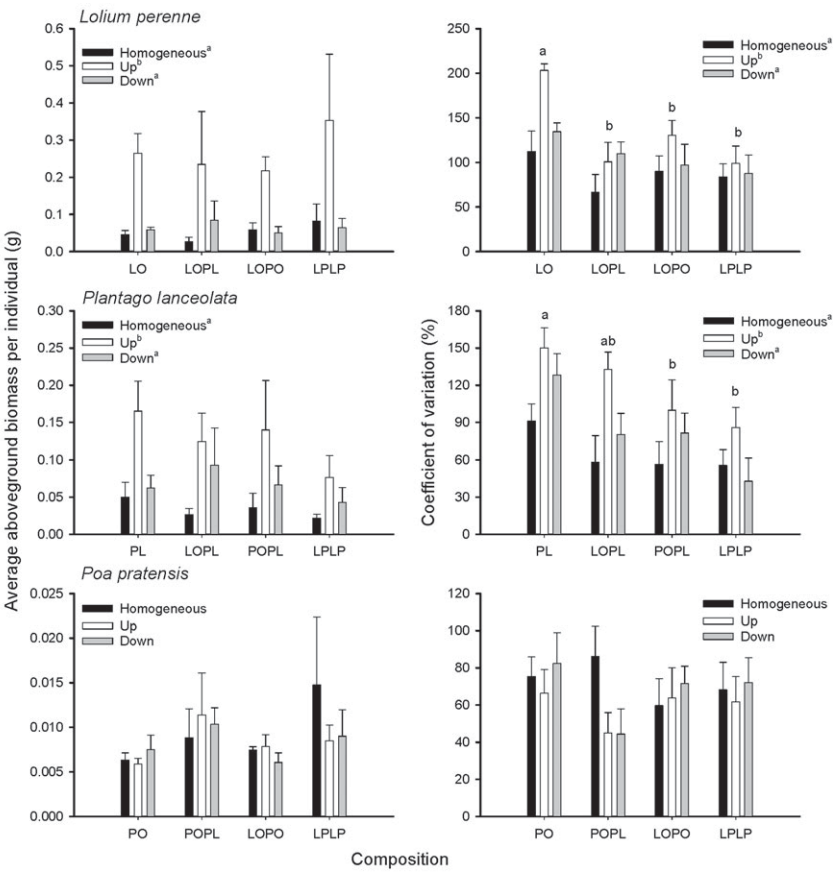

Fig. 4. Average above-ground biomass per individual and coefficient of variation of this measure among composition and nutrient heterogeneity levels. Data are means + 1 SE ($N = 6$). Where significant main effects were detected (shown in Table 2), superscript and lowercase letters are used to denote significant differences between nutrient heterogeneity and species composition levels, respectively ($P < 0.05$, Tukey's HSD test). For explanation of x-axis labels see the legend for Fig. 1. Note different scales on the *y*-axes.

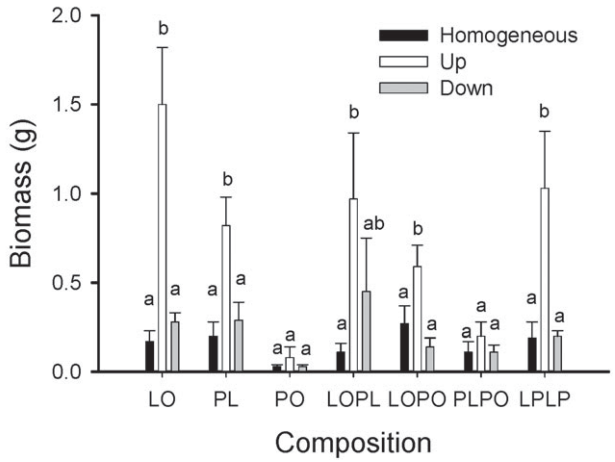

Fig. 5. Combined biomass of the two largest plants in each microcosm compared among composition and nutrient heterogeneity levels. Data are means + 1 SE ($N = 6$). Lower-case letters are used to denote significant differences among nutrient heterogeneity levels ($P < 0.05$, Tukey's HSD test). For explanation of *x*-axis labels see the legend for Fig. 1.

Discussion

Our working hypotheses, i.e. that there would be significant interactions between soil nutrient heterogeneity and species composition to determine the performance of individual plants and with both species composition and richness to determine the productivity and biomass allocation patterns of communities, were partially supported by our data. We found significant composition × nutrient heterogeneity interactions for above, below-ground and total biomass, and a marginally significant species richness × heterogeneity interaction for the below-ground : above-ground ratio. The composition × nutrient heterogeneity interactions were mainly driven by the response of *Lolium* to the presence of nutrient patches in the upper parts of the microcosm, and by the lack of response to such patches by *Poa*. While we did not expect *Poa* to respond strongly to nutrient heterogeneity (Hodge et al. 1998, 2000), it also grew poorly. Why it did so is not clear but may be related to the relatively low fertility levels of the microcosm soils. Despite the fact that this species can thrive under different nutrient availability levels, it requires large amounts of nutrients during active growth stages (Jiang & Sullivan 2004), and the organic material added may have not provided this, especially at early stages of the experiment.

We tested whether the poor growth of *Poa* alone could explain the biomass responses observed at the community level by conducting analyses of community biomass data that explored, in a likelihood-based fashion (Burnham & Anderson 2002), how well competing ANOVA models performed (App. 3). These analyses indicated that models including more than a single species are required in most cases to explain observed biomass responses. Further, the poor growth of *Poa* did not, by itself, explain the marginally significant species richness × nutrient heterogeneity interaction found for the below: above-ground biomass ratio (because communities without *Poa* exhibited the same responses as those with *Poa*). Our experimental design precludes us from making strong inferences on the mechanisms underlying this interaction, as well as on its relevance under natural conditions. However, given the ubiquitous nature of soil nutrient heterogeneity and the important role that biomass allocation patterns play in growth dynamics of individual plants and communities (Belcher et al. 1995), it deserves further attention.

Higher biomass at the community level was observed under heterogeneous conditions of nutrient supply, but only when the nutrient patch was located in the upper part of the soil columns. Other studies have shown that plant community biomass is greater when nutrients are supplied heterogeneously (Wijesinghe et al. 2005; Maestre et al. 2005). The suggested explanation for this is that when a fixed amount of nutrients are made available to a plant, their acquisition will be more efficient if the nutrients are spatially concentrated because of preferential root allocation in these areas (Jackson & Caldwell 1996). This more efficient nutrient acquisition is reflected in a greater rate of root biomass development, at least until the supply of nutrients is exhausted (Day et al. 2003a). The increase in biomass we observed was not achieved through an increase in root biomass at the expense of above-ground biomass. As found by Maestre et al. (2005), the responses to soil nutrient heterogeneity were similar for both above- and below-ground biomass. These results contrast with those of Wijesinghe et al. (2005), who reported a significant increase of the below-ground : above-ground ratio of grassland communities growing under heterogeneous conditions of nutrient supply. However, these authors did not control for existing differences in the size of plants among homogeneous and heterogeneous treatments, and thus their results should be interpreted with caution. The mechanisms underlying the equal above- and below-ground responses we observed cannot be elucidated from our study and require further investigation.

Soil nutrient heterogeneity had limited effects on the structure of the mixtures evaluated, as only in the *Lolium* + *Poa* mixtures it affected the relative contribution of each species to the total above-ground biomass. Using model grassland communities, Maestre et al. (2005) found no significant effect of heterogeneity on the proportion of above-ground biomass accounted for by each

species, but Wijesinghe et al. (2005) found significant effects of heterogeneity on the cover, relative abundance and population sizes of some of the species forming their community. These contrasting results, and the low number of studies conducted so far, warrant further research into the effects of nutrient heterogeneity on the structure of plant communities.

Contrary to our expectations, soil nutrient heterogeneity and species composition did not interact to determine the average biomass and relative growth rate per individual of any of the species evaluated. Both factors, however, affected individual size inequality of *Lolium* and *Plantago*. When the nutrients were located in the upper part of the soil columns, individuals of these species were larger. This response promoted changes in the size inequality of these species, with greater CVs of the average above-ground biomass per individual being observed in the heterogeneous-up treatment when compared to the homogeneous treatment. These results agree with previous studies conducted with populations (Day et al. 2003b, but see Casper & Cahill 1998; Day et al. 2003a). We speculate that soil nutrient heterogeneity shifted the symmetry of competitive interactions. It has been suggested that below-ground size asymmetric competition may occur under conditions of heterogeneous nutrient supply because, if larger plants can exploit nutrient patches more quickly than smaller plants, they would have an advantage disproportionate to their size (Schwinning & Weiner 1998). Our results provide evidence to support this hypothesis, given that maximum plant size increased in the heterogeneous-up microcosms for most composition levels, an effect observed in previous studies conducted with other grass species (Fransen et al. 2001; Rajaniemi 2003, but see Casper & Cahill 1998).

It has been established that below- and above-ground competitive interactions interact (for a review see Casper & Jackson 1997), and that the direction of these interactions may change with species identity and nutrient availability (Cahill 1999, 2002). The consequences of nutrient heterogeneity on such interactions are, however, largely unknown. If our observed responses to nutrient heterogeneity for *Plantago* and *Lolium* hold under field conditions, they may play a key role in determining the interaction between above- and below-ground competition in semi-natural and managed grasslands. In these habitats shoot competition for light, which is usually size asymmetric (Schwinning & Weiner 1998), is intense (Teyssonneyre et al. 2002). Under these conditions soil nutrient heterogeneity could magnify or diminish the effects of light on the size symmetry of competitive interactions.

The responses to soil nutrient heterogeneity at both the individual and the community levels were strictly dependent upon the vertical placement of the nutrient patch. In most instances, when the patch was located in the lower (as opposed to upper) part of the soil columns, community and individual responses did not differ from those obtained in the homogeneous treatment. Strikingly, we did not find significant differences in foraging precision patterns between the heterogeneous-up and -down treatments (Fig. 3). The most logical explanation for our results is that the 'up' nutrient patch would have been encountered earlier in the experiment and thus there would have been more time for foraging responses to feed into community and above-ground biomass responses. Differences in nutrient leaching or in decomposition rates between depths may also have led to the significant differences in the response to the heterogeneous-up and -down treatments. However, our irrigation scheme did not promote a substantial leaching from the microcosms, and the lack of differences in root foraging patterns between depths clearly suggests that the availability of nutrients derived from the patches was similar at the two depths evaluated.

The use of experimental microcosms in this study is not intended to mimic the full complexity of nature but, instead, permits us to elucidate interactions that may occur in the field (Lawton 1995). In the field, heterogeneity in resource distribution arises as a result of organic inputs and their subsequent microbial decomposition. Such decomposition will release nutrients for plant capture in a spatio-temporal fashion more complex than that resulting from placing a patch of inorganic nutrients directly in the soil, the most utilized approach in experimental work on soil nutrient heterogeneity (Hutchings et al. 2000). The use of natural soil and organic material in our experiment allows us to interpret plant responses to soil nutrient heterogeneity in a more realistic context (Hodge 2004). Our results show that soil nutrient heterogeneity profoundly affects the performance of individual plants and the productivity of low-diversity communities, at least within experimental microcosms, and also that this heterogeneity may interact with both plant species richness and composition to determine biomass responses at the community level. Further, they suggest that small-scale vertical differences in the location of nutrient patches may affect the way in which plant communities and individuals respond to soil nutrient heterogeneity. Differences in vertical placement have been largely ignored in the literature on plant responses to soil nutrient heterogeneity (but see Berendse 1981; Fitter 1982; Maestre & Reynolds in press) but can influence plant responses to nutrient heterogeneity (this paper), and thus our perceptions of the importance of the spatial pattern of nutrient supply for governing interactions in plant communities.

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References

Armas, C., Pugnaire F.I. & Ordiales, R. 2004. Measuring plant interactions: A new comparative index. *Ecology* 85: 2682-2686.

Belcher, J.W., Keddy, P.A. & Twolan-Strutt, L. 1995. Root and shoot competition intensity along a soil depth gradient. *J. Ecol.* 83: 673–682.

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

Bliss, K.M., Jones, R.H., Mitchell, R. & Mou, P. 2002. Are competitive interactions influenced by spatial nutrient heterogeneity and root foraging behavior? *New Phytol.* 154: 409-417.

Burnham, K.P. & Anderson, D.R. 2002. *Model selection and multimodel inference. A practical information-theoretic approach*. 2nd. ed. Springer, New York, NY, US.

Cahill, J.F. Jr. 1999. Fertilization effects on interactions between above- and belowground competition in an old field. *Ecology* 80: 466-480.

Cahill, J.F. Jr. 2002. Interactions between root and shoot competition vary among species. *Oikos* 99: 101-112.

Casper, B.B. & Cahill, J.F. Jr. 1996. Limited effects of soil nutrient heterogeneity on populations of *Abutilon theophrasti* (*Malvaceae*). *Am. J. Bot.* 83: 333-341.

Casper, B.B. & Cahill, J.F. Jr. 1998. Population-level responses to nutrient heterogeneity and density by *Abutilon theophrasti* (*Malvaceae*): an experimental neighborhood approach. *Am. J. Bot.* 85: 1680-1687.

Casper, B.B. & Jackson, R.B. 1997. Plant competition underground. *Annu. Rev. Ecol. Syst.* 28: 545-570.

Day, K.J., Hutchings, M.J. & John, E.A. 2003a. The effects of spatial pattern of nutrient supply on yield, structure and mortality in plant populations. *J. Ecol.* 91: 541-553.

Day, K.J., Hutchings, M.J. & John, E.A. 2003b. The effects of spatial pattern of nutrient supply on the early stages of growth in plant populations. *J. Ecol.* 91: 305-315.

Désilets, P. & Houle, G. 2005. Effects of resource availability and heterogeneity on the slope of the species-area curve along a floodplain-upland gradient. *J. Veg. Sci.* 16: 487-496.

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

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

Gotelli, N.J. & Ellison, A.M. 2004. *A Primer of ecological statistics*. Sinauer Associates, Sunderland, MA, US.

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

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

Hodge, A., Stewart, J., Robinson, D., Griffiths, B.S. & Fitter. A.H. 2000. Spatial and physical heterogeneity of N supply from soil does not influence N capture by two grass species. *Funct. Ecol.* 14: 645-653.

Hooper, D.U., Chapin, F.S. III, Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setäla, H., Symstad, A.J., Vandermeer, J. & Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecol. Monogr.* 75: 3-35.

Huber-Sannwald, E. & Jackson, R.B. 2001. Heterogeneous soil-resource distribution and plant responses-from individual-plant growth to ecosystem functioning. *Progr. Bot.* 62: 451-476.

Hutchings, M.J., Wijesinghe, D.K. & John, E.A. 2000. The effects of heterogeneous nutrient supply on plant performance: a survey of responses, with special reference to clonal herbs. In: Hutchings, M.J., John, E.A. & Stewart, A.J.A. (eds.) *The ecological consequences of environmental heterogeneity*, pp. 91-110. Blackwell Science, Oxford, UK.

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

Jackson, R.B. & Caldwell, M.M. 1993. Geostatistical patterns of soil heterogeneity around individual perennial plants. *J. Ecol.* 81: 683-692.

Jackson, R.B. & Caldwell, M.M. 1996. Integrating resource heterogeneity and plant plasticity: Modelling nitrate and phosphate uptake in a patchy soil environment. *J. Ecol.* 84: 891-903.

Jiang, Z. & Sullivan, W.M. 2004. Nitrate uptake of seedling and mature Kentucky bluegrass plants. *Crop Sci.* 44: 567-574.

Joshi J., Matthies, D. & Schmid, B. 2000. Root hemiparasites and plant diversity in experimental grassland communities. *J. Ecol.* 88: 634-644.

Kelly, V.R. & Canham, C.D. 1992. Resource heterogeneity in old fields. *J. Veg. Sci.* 3: 545-552.

Lawton, J.H. 1995. Ecological experiments with model systems. *Science* 269: 328-331.

Maestre, F.T. & Cortina, J. 2002. Spatial patterns of surface soil properties and vegetation in a Mediterranean semiarid steppe. *Plant Soil* 241: 279-291.

Maestre, F.T. & Reynolds, J.F. In press. Small-scale spatial heterogeneity in the vertical distribution of soil nutrients

has limited effects on the growth and development of *Prosopis glandulosa* seedlings. *Plant Ecol*.

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

Maestre, F.T., Bradford, M.A. & Reynolds, J.F. 2005. Soil nutrient heterogeneity interacts with elevated CO_2 and nutrient availability to determine species and assemblage responses in a model grassland community. *New Phytol*. 168: 637-650.

McNaughton, S.J., Oesterheld, M., Frank, D.A. & Williams, K.J. 1989. Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. *Nature* 341: 142-144.

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

Quinn, G.P. & Keough, M.J. 2002. *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, UK.

Rajaniemi, T.K. 2003. Evidence for size asymmetry of belowground competition. *Basic Appl. Ecol*. 4: 239-247.

Reich, P.B. 2002. Root-shoot relations: Optimality in acclimation and adaptation or the "Emperor's new clothes"? In: Waisel, Y., Eshel, A. & Kafkafi, U. (eds.) *Plant roots: The hidden half*, pp. 205-220. Marcel Dekker, New York, NY, US.

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. & Fitter, A.H. 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proc. R. Soc. Lond. B* 266: 431-435.

Robinson, D. & van Vuuren. M.M.I. 1998. Responses of wild plants to nutrient patches in relation to growth rate and life-form. In: Lambers, H., Poorter, H. & van Vuuren, M.M.I. (eds.) *Variation in plant growth*, pp. 237-257. Backhuys. Leiden, NL.

Schmid, B., Hector, A., Huston, M.A., Inchausti, P., Nijs, I., Leadley, P.W. & Tilman, D. 2002. The design and analysis of biodiversity experiments. In: Loreau, M., Naeem, S. & Inchausti, P. (eds.) *Biodiversity and ecosystem functioning*, pp. 61-75. Oxford University Press, New York, NY, US.

Schwinning, S. & Weiner. J. 1998. Mechanisms determining the degree of size asymmetry in competition among plants. *Oecologia* 113: 447-455.

Šmilauerová, M. & Šmilauer, P. 2002. Morphological responses of plant roots to heterogeneity of soil resources. *New Phytol*. 154: 703-715.

Stein, A., Brouwer, J. & Bouma, J. 1997. Methods for comparing spatial variability patterns of millet yield and soil data. *Soil Sci. Soc. Am. J*. 61: 861-870.

Teyssonneyre, F., Picon-Cochard, C. & Soussana, J.F. 2002. How can we predict the effects of elevated CO_2 on the balance between perennial C_3 grass species competing for light? *New Phytol*. 154: 53-64.

Tutin, G.T., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H, Walters, S.M. & Webb, DA. (eds.) 1964-1983. *Flora Europaea*. Cambridge University Press, Cambridge, UK.

Wijesinghe, D.K., John, E.A. & Hutchings, M.J. 2005. Does pattern of soil resource heterogeneity determine plant community structure? An experimental investigation. *J. Ecol*. 93: 99-112.

Wurst, S., Langel, R., Reineking, A., Bonkowski, M. & Scheu, S. 2003. Effects of earthworms and organic litter distribution on plant performance and aphid reproduction. *Oecologia* 137: 90-96.

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