

Nutrient Availability and Atmospheric CO₂ Partial Pressure Modulate the Effects of Nutrient Heterogeneity on the Size Structure of Populations in Grassland Species

FERNANDO T. MAESTRE^{1,*} and JAMES F. REYNOLDS^{1,2}

¹Department of Biology, Duke University, Phytotron Building, Box 90340, Durham, NC 27708, USA and

²Nicholas School of the Environment, Division of Environmental Science and Policy, Duke University, Durham, NC 27708, USA

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• **Background and Aims** Size-asymmetric competition occurs when larger plants have a disproportionate advantage in competition with smaller plants. It has been hypothesized that nutrient heterogeneity may promote it. Experiments testing this hypothesis are inconclusive, and in most cases have evaluated the effects of nutrient heterogeneity separately from other environmental factors. The aim of this study was to test, using populations of *Lolium perenne*, *Plantago lanceolata* and *Holcus lanatus*, two hypotheses: (a) nutrient heterogeneity promotes size-asymmetric competition; and (b) nutrient heterogeneity interacts with both atmospheric CO₂ partial pressure (P_{CO_2}) and nutrient availability to determine the magnitude of this response.

• **Methods** Microcosms consisting of monocultures of the three species were grown for 90 d in a factorial experiment with the following treatments: P_{CO_2} (37.5 and 70 Pa) and nutrient availability (NA; 40 and 120 mg of N added as organic material) combined with different spatial distribution of the organic material (NH; homogeneous and heterogeneous). Differences in the size of individual plants within populations (size inequality) were quantified using the coefficient of variation of individual above-ground biomass and the combined biomass of the two largest individuals in each microcosm. Increases in size inequality were associated with size-asymmetric competition.

• **Key Results** Size inequality increased when the nutrients were heterogeneously supplied in the three species. The effects of NH on this response were more pronounced under high nutrient supply in both *Plantago* and *Holcus* (significant NA × NH interactions) and under elevated P_{CO_2} in *Plantago* (significant P_{CO_2} × NA × NH interaction). No significant two- and three-way interactions were found for *Lolium*.

• **Conclusions** Our first hypothesis was supported by our results, as nutrient heterogeneity promoted size-asymmetric competition in the three species evaluated. Nutrient supply and P_{CO_2} modified the magnitude of this effect in *Plantago* and *Holcus*, but not in *Lolium*. Thus, our second hypothesis was partially supported.

Key words: *Lolium perenne*, *Plantago lanceolata*, *Holcus lanatus*, elevated CO₂, nutrient heterogeneity, nutrient availability, below-ground competition, size inequality.

INTRODUCTION

In most ecosystems, the distribution of soil nutrients is spatially heterogeneous at scales ranging from individual root systems to the landscape (e.g. Jackson and Caldwell, 1993; Cain *et al.*, 1999). Heterogeneity of nutrient supply (hereafter called nutrient heterogeneity), occurring at the scale of the root system, induces many physiological and morphological plant responses, proliferation of roots in the nutrient patches and changes in nutrient uptake rates being two of the most important (Hodge, 2004). These responses are ecologically relevant, as they may determine the competitive ability of competing individual plants (Hodge *et al.*, 1999), the structure of populations (Day *et al.*, 2003a) and the productivity of communities (Maestre *et al.*, 2005, 2006; Wijesinghe *et al.*, 2005).

An aspect that has received increased attention in recent years is the potential influence of nutrient heterogeneity on plant co-existence via its effects on the relationship between a plant's size and its competitive effect (Weiner, 1990; Schwinning and Weiner, 1998). Size-symmetric competition occurs when individuals compete in proportion to their size, with large plants causing a large decrease in the growth of smaller plants, and small plants causing a small (but proportionate to their size) decrease in the growth of the larger plants (Schwinning and Weiner, 1998). In contrast, size-asymmetric competition occurs when larger plants have a disproportionate advantage (for their relative size) in competition with smaller plants (Schwinning and Weiner, 1998). Increases in the differences in size of individual plants within populations (hereafter referred to as size inequality) are associated with size-asymmetric competition (Casper and Cahill, 1998; Schwinning and Weiner, 1998; Nagashima *et al.*, 2003). It is often assumed that competition for nutrients is size-symmetric (Weiner, 1990; Cahill and Casper, 2000). However, it has been hypothesized that, with heterogeneous nutrient supply, large plants can have

* For correspondence. Present address: Unidad de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, 28933 Móstoles, Spain. E-mail fernando.maestre@urjc.es

a disproportionately large competitive effect if they can reach and exploit nutrient patches faster than smaller plants, a process that would promote size-asymmetric competition (Schwinning and Weiner, 1998). Experiments both support (Fransen *et al.*, 2001; Day *et al.*, 2003a; Rajaniemi, 2003) and reject (Casper and Cahill, 1998; Blair, 2001; Day *et al.*, 2003b) this prediction. Most have, however, used artificial substrates and inorganic fertilizer to simulate nutrient heterogeneity. In the field, this heterogeneity arises from deposition of organic matter (e.g. leaf litter) and its subsequent microbial decomposition. Such decomposition releases inorganic nutrients, which plants capture, in a manner which is more complex than that resulting from placing a patch of inorganic nutrients directly into the growing medium (Hodge, 2004). In addition, most of our knowledge on how plants respond to small-scale nutrient heterogeneity has been derived from studies evaluating responses to this heterogeneity isolated from other environmental factors (Robinson, 1994; Hutchings and John, 2004).

Evaluating how joint changes in nutrient heterogeneity and global change drivers, such as atmospheric CO₂ partial pressure (P_{CO_2}) and nutrient availability, affect the relationship between a plant's size and its competitive ability is relevant for several reasons. First, this relationship strongly influences plant community structure and dynamics (Newman, 1973). Secondly, there is a pressing need to increase understanding of the consequences of global change for plant individuals, populations and communities (Houghton *et al.*, 2001). Thirdly, nutrient heterogeneity may potentially interact with nutrient availability and P_{CO_2} to determine plant population responses. Maestre *et al.* (2005) evaluated the effects of P_{CO_2} and nutrient availability and heterogeneity on the responses of model communities of *Lolium perenne*, *Plantago lanceolata*, *Anthoxanthum odoratum*, *Trifolium repens* and *Holcus lanatus*. They showed that nutrient heterogeneity interacted with P_{CO_2} and nutrient availability to determine different aspects of the productivity and the nutrient uptake patterns of communities. Nagashima *et al.* (2003) investigated the effects of P_{CO_2} and nutrient availability on size inequality within populations of *Chenopodium album*. They found that size inequality increased at elevated P_{CO_2} , and that the magnitude of this response was modulated by nutrient availability.

We conducted a microcosm experiment to evaluate, for what we believe to be the first time, the effects of P_{CO_2} , nutrient availability and nutrient heterogeneity on individual size differences within populations of *Lolium*, *Plantago* and *Holcus*. These species commonly occur together in semi-natural temperate grasslands (Joshi *et al.*, 2000), but differ in the magnitude of their responses to P_{CO_2} , nutrient heterogeneity and availability (Poorter, 1993; Kirkham *et al.*, 1996; Grime and Mackey, 2002). We tested the following hypotheses: (a) nutrient heterogeneity promotes size-asymmetric competition (Schwinning and Weiner, 1998); and (b) nutrient heterogeneity interacts with both P_{CO_2} and nutrient availability to determine the magnitude of such a response (Nagashima *et al.*, 2003; Maestre *et al.*, 2005).

MATERIALS AND METHODS

Experimental design

The experiment, conducted in the Duke University Phytotron between January 3 and April 4, 2005, had four treatments: two atmospheric P_{CO_2} s (37.5 Pa and 70 Pa), two fertilization applications (40 and 120 mg of N added as organic material), two spatial distributions of the organic material (homogeneous and heterogeneous) and three species (*Lolium*, *Holcus* and *Plantago*). Microcosms consisted of PVC pipe (length 38 cm, internal diameter 10 cm) filled with, from the base, 5 cm of gravel (for drainage), and then 28 cm of a 50 : 50 mixture of sandy-loam soil and sand, as described in Maestre *et al.* (2005). On top of this was a 2 cm layer of a 50 : 50 mixture of organic soil : peat to resemble the typical accumulation of organic matter in the topsoil of temperate grasslands. To recreate realistic microbial communities, all the microcosms were irrigated with 200 mL of a fresh soil solution and with 100 mL of a solution derived from root macerations (Maestre *et al.*, 2005).

The two nutrient availabilities were 1.036 and 3.108 g of finely cut (2 mm) *Trifolium* shoots (3.9% N, 10.8 C : N) in the low and high nutrient availability treatments, respectively. These are equivalent to adding 40 and 120 mg of N per microcosm, respectively. Within each of these nutrient applications, the *Trifolium* shoots were added homogeneously (homogeneous treatment) or as a patch (heterogeneous treatment). In the homogeneous treatment, we thoroughly mixed the organic material with the soil before filling the PVC pipe. In the heterogeneous treatment, the organic material was placed within discrete 31 cm³ volumes of soil. To create one of these patches, 25 cm³ of soil was mixed with the organic material and placed into a 31 cm³ plastic cylinder (length 75 mm; internal diameter 23 mm) consisting of a light mesh with square pores 5 × 10 mm in size. We refer to this as the patch cylinder. A second (control) cylinder, filled only with background soil, was placed 2 cm away from and parallel to the patch cylinder. Cylinders were located 12 cm below the surface of the organic soil. In the homogeneous treatments, both cylinders were filled with the mixture of background soil and organic material.

Seeds from the three species were obtained from commercial suppliers (*Lolium* seeds were provided by Granite Seed Company, Lehi, UT; *Plantago* and *Holcus* seeds by V & J Seed Service, Woodstock, IL, USA). These were germinated in a growth chamber (20 °C temperature and PAR of 350 μmol m⁻² s⁻¹ with a 14 h photoperiod) on different days to ensure that all the species had a similar size (two-leaf stage) at the start of the experiment. Each microcosm contained six seedlings of a single species. The planting positions of the six seedlings were allocated at random, but were the same in all microcosms. Seedlings that died during the first week of the experiment were replaced. After that period, no further mortality was observed. We established four replicated microcosms for each of the 24 treatment combinations, resulting in 96 microcosms in total. The microcosms were placed in four walk-in growth chambers (two for each P_{CO_2}), within which

atmospheric temperature and P_{CO_2} were independently controlled. For each P_{CO_2} , half of the microcosms per combination of treatments were randomly assigned to one of the chambers (24 microcosms per chamber), and then were randomly grouped on two trolleys. To minimize possible chamber effects, the P_{CO_2} levels and trolleys were rotated between chambers every week, and the position of the microcosms within the chamber was randomized after every rotation. Strictly speaking, this does not eliminate the problem of pseudoreplication, but we are confident that any chamber effect was negligible because the P_{CO_2} and trolleys were switched 12 times during the experiment. At harvest, all the microcosms had spent the same amount of time in each chamber. The chambers were maintained at a day/night air temperature of 27/20 °C, a 15 h photoperiod and an average relative humidity of 70 % during the day and 85 % during the night. To prevent seedlings being exposed to high-light shock, PAR was maintained at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ during the first week of the experiment, $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ during the second week and at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ thereafter. These conditions included a simulated dawn and dusk period, each of 2 h duration, where temperature, radiation and humidity were gradually increased or decreased. Each microcosm was irrigated daily with 30 ml of distilled water during the first 2 weeks of the experiment, and with 50 ml thereafter. This irrigation scheme resulted in appropriate conditions for plant growth, as indicated by the lack of mortality during the course of the experiment. Because of low overall soil fertility, all the microcosms were watered with 50 mL of a nutrient solution containing 35 mg of Ca (added as $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) and 29 mg of Mg (added as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) twice during the course of the experiment (1 February and 1 March).

Measurements and harvest

After 90 d of growth, the plants were harvested. Each individual plant was clipped at the soil surface, and dried at 60 °C to constant mass. Once the above-ground biomass was removed, volumetric soil moisture (0–12 cm depth) was measured in all the microcosms using a HydroSense probe (Campbell Scientific, Logan, UT, USA). After these measurements, the soil was carefully removed from the microcosm unit and the roots were harvested and dried as above. Roots were so entangled that it was not possible to separate those belonging to each individual. We harvested the roots within control and patch cylinders separately from the bulk soil. To standardize measurement of root proliferation (defined here as the biomass of roots per unit of soil volume) into nutrient patches, the index RII was used (Armas *et al.*, 2004). RII was calculated as $(RB_p - RB_c) / (RB_p + RB_c)$, where RB_p and RB_c are the root biomass in the patch and control cylinders, respectively. In the homogeneous treatment, the cylinder placed in the same location as the patch cylinder in the heterogeneous treatment was treated as the patch cylinder for the purposes of calculating the RII index. RII ranges from -1 to $+1$: a value of zero indicates equal root growth in nutrient patches and background soil.

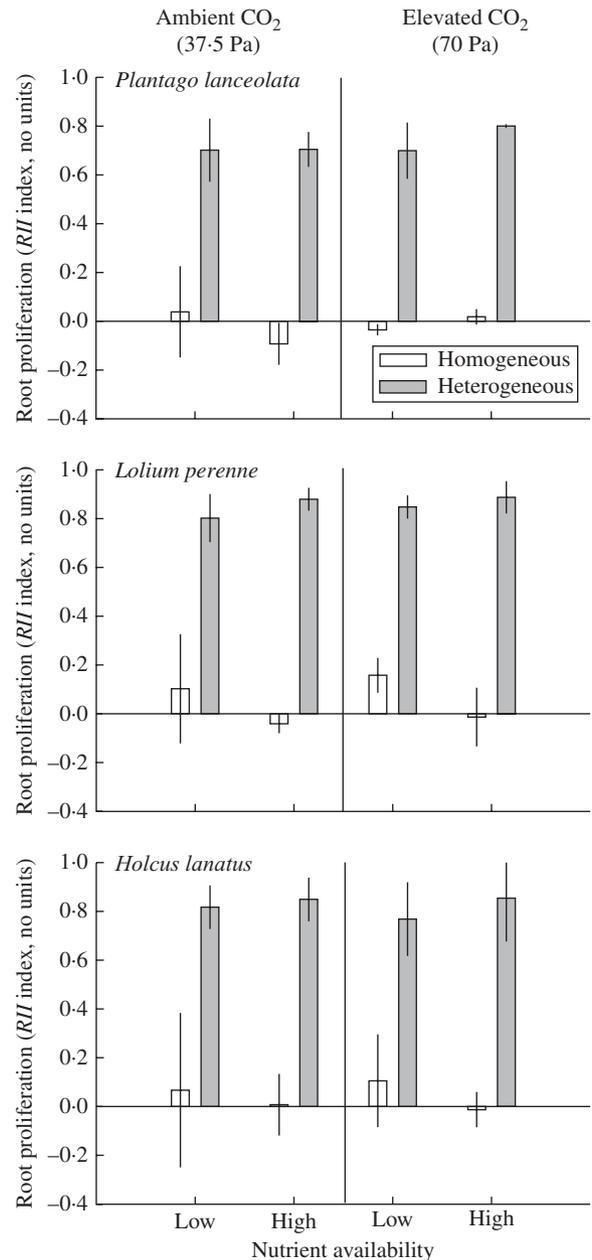


FIG. 1. Proliferation of roots into the nutrient patches, as measured with the RII index, in soil microcosms differing in nutrient availability, heterogeneity and CO_2 partial pressure. Values are means \pm 95 % confidence intervals ($n = 4$). Significant root proliferation is indicated by confidence intervals that do not overlap 0.

Size inequality within each population was estimated using two variables: the coefficient of variation of above-ground biomass per microcosm (CV_{ABO} ; Weiner, 1990) and the combined biomass of the two largest individuals per microcosm (Casper and Cahill, 1998). The combined biomass of the two smallest individuals per microcosm was also calculated. Under size-asymmetric competition, one would expect that the largest plants would get larger at the expense of the smallest plants, which would, in absolute terms, therefore get smaller. Thus, increases in

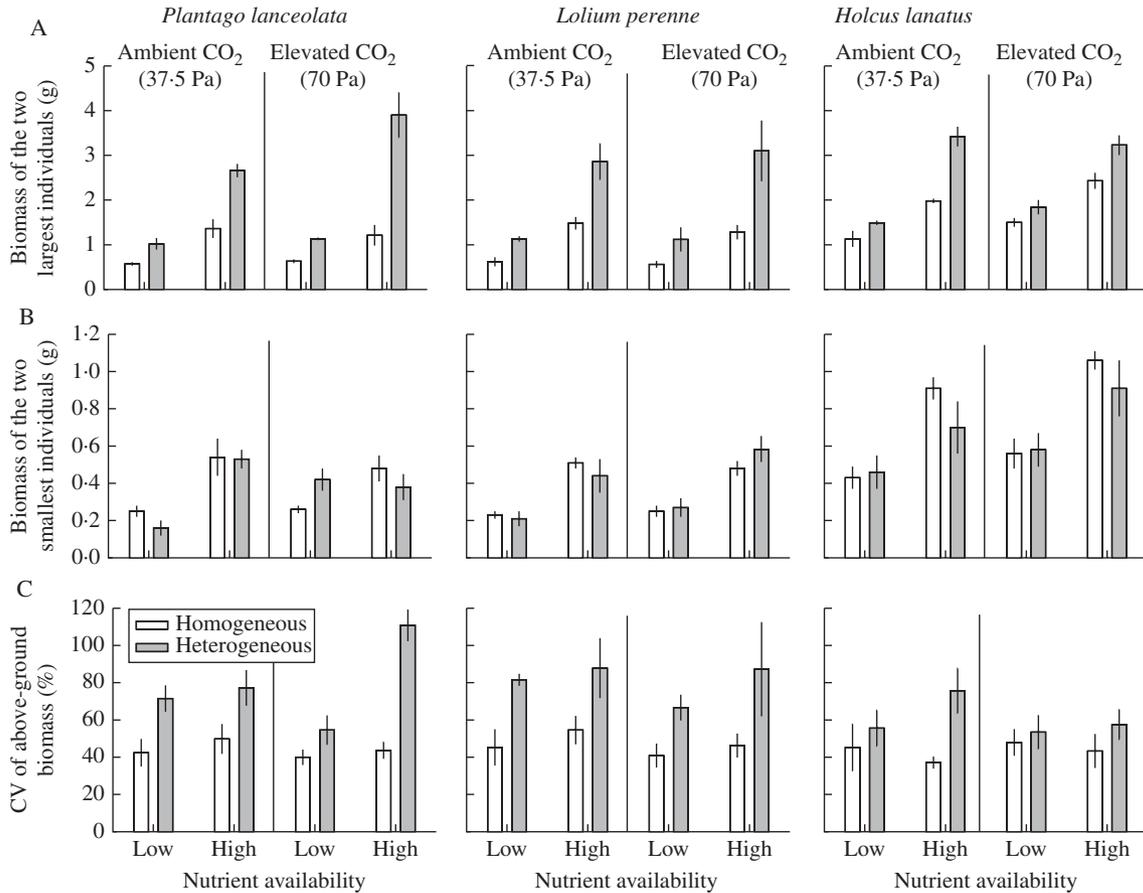


FIG. 2. Biomass of the two largest (A) and smallest (B) individuals and coefficient of variation of above-ground biomass (C) in soil microcosms differing in nutrient availability, heterogeneity and CO₂ partial pressure. Data are means \pm 1 s.e. ($n = 4$).

CV_{ABO} and/or in the biomass of the largest individuals, accompanied by a decrease in the biomass of the smallest individuals in the latter case, would indicate an increase in size inequality within the population (Casper and Cahill, 1998; Nagashima *et al.*, 2003). If such an increase occurs in response to nutrient heterogeneity, it would indicate that this factor promotes size-asymmetric competition, according to our first hypothesis.

Statistical analyses

The effects of P_{CO_2} , nutrient availability (NA) and nutrient heterogeneity (NH) on CV_{ABO}, the combined biomass of the two largest and smallest individuals and soil moisture were evaluated separately for each species with a three-way analysis of variance (ANOVA) model. In this model, all the factors were considered fixed, and all main effects and interactions were tested against the residual error. To evaluate if the initial distance to the nutrient patch (on a horizontal scale) affected plant growth in heterogeneous treatments, above-ground biomass data were also analysed separately for each species using a four-way nested ANOVA model. The model included P_{CO_2} and NA as fixed between-plots factors, planting position as a fixed within-plot factor and microcosm

as a random factor nested within P_{CO_2} and NA. In this model, the effects of P_{CO_2} and NA, and their interaction, were tested against the random effect of the microcosm. The main effect of planting position and the two- and three-term interactions involving this factor were tested against the interaction microcosm \times planting position (Quinn and Keough, 2002). Relationships between root proliferation and the estimators of size inequality were evaluated in the heterogeneous treatment using linear and non-linear (quadratic, logarithmic, power and exponential) functions. Among those models that significantly fitted the data, we selected the model that minimized the second-order Akaike's information criterion (Sugiura, 1978), after examining the residuals for normality and homoscedasticity. Prior to statistical analyses, the CV_{ABO} and the biomass of the two largest and smallest individuals of *Lolium* were log-transformed to meet the homogeneity of variances assumption of ANOVA. Where appropriate, Tukey's HSD test was used for pairwise *post hoc* comparisons. 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 and Ellison, 2004). All the statistical analyses were performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

TABLE 1. Results of analysis of variance showing the effects of CO_2 partial pressure (P_{CO_2}), nutrient heterogeneity (NH), nutrient availability (NA) and their interactions on the biomass of the two largest (B_{MAX}) and smallest (B_{MIN}) individuals, and on the coefficient of variation of above-ground biomass (CV_{ABO})

Species	Source of variation	B_{MAX}		B_{MIN}		CV_{ABO}	
		$F_{1,24}$	P	$F_{1,24}$	P	$F_{1,24}$	P
<i>Plantago lanceolata</i>	P_{CO_2}	4.10	0.054	0.15	0.701	0.24	0.628
	NH	63.25	<0.001	0.05	0.819	42.65	<0.001
	NA	86.11	<0.001	22.55	<0.001	11.53	0.002
	$P_{CO_2} \times NH$	5.45	0.028	0.81	0.376	1.75	0.199
	$P_{CO_2} \times NA$	2.23	0.148	8.09	0.009	5.54	0.027
	$NH \times NA$	24.07	<0.001	1.31	0.264	5.46	0.028
	$P_{CO_2} \times NA \times NH$	4.61	0.042	4.18	0.052	7.39	0.012
<i>Lolium perenne</i>	P_{CO_2}	0.44	0.514	1.30	0.265	1.13	0.297
	NH	43.45	<0.001	0.12	0.734	16.90	<0.001
	NA	74.85	<0.001	45.88	<0.001	1.04	0.318
	$P_{CO_2} \times NH$	0.21	0.655	1.22	0.280	0.03	0.867
	$P_{CO_2} \times NA$	0.01	0.935	0.08	0.781	<0.01	0.970
	$NH \times NA$	0.24	0.628	<0.01	0.979	0.14	0.713
	$P_{CO_2} \times NA \times NH$	0.20	0.656	0.10	0.754	0.14	0.709
<i>Holcus lanatus</i>	P_{CO_2}	4.30	0.049	4.93	0.036	0.26	0.616
	NH	38.28	<0.001	1.39	0.250	7.07	0.014
	NA	114.50	<0.001	31.77	<0.001	0.12	0.734
	$P_{CO_2} \times NH$	1.94	0.177	0.02	0.889	1.40	0.249
	$P_{CO_2} \times NA$	0.99	0.331	0.21	0.654	0.15	0.702
	$NH \times NA$	10.50	0.003	2.28	0.144	1.69	0.206
	$P_{CO_2} \times NA \times NH$	1.71	0.204	0.07	0.793	0.42	0.524

Results for *Lolium* are shown for \log_{10} -transformed data. P -values below 0.05 are in bold.

RESULTS

When the organic material was supplied heterogeneously, roots of all the species proliferated in the nutrient patches (Fig. 1). The magnitude of this response was not modified by P_{CO_2} or NA for either *Plantago* or *Holcus* (ANOVA, $P > 0.215$ in all cases). A marginally significant effect of NA on this variable was found for *Lolium* (ANOVA, $F_{1,12} = 4.09$, $P = 0.066$; no interactions), indicating a trend towards greater root proliferation in the high NA treatment (Fig. 1).

Both the CV_{ABO} and the biomass of the largest individuals increased in the three species when nutrients were heterogeneously supplied (Fig. 2). Significant $P_{CO_2} \times NA \times NH$ interactions, as well as $P_{CO_2} \times NH$ and $NA \times NH$ interactions modulated these responses in *Plantago* (Table 1), showing that the effect of NH on size inequality within the population was more pronounced under high nutrient supply, especially at elevated P_{CO_2} (Fig. 2). No significant two- or three-way interactions were found when analysing the data from *Lolium*, but the biomass of the largest individuals increased under high NA conditions. The $NH \times NA$ interaction for this variable was significant in *Holcus* (Table 1); as with *Plantago*, the effect of NH on the biomass of the largest individuals was more pronounced under high nutrient supply. Biomass was also increased under elevated P_{CO_2} for *Plantago* (Table 1, Fig. 2). The biomass of the smallest individuals increased under high nutrient supply in the three species (Fig. 2), but was not affected by NH (Table 1). Elevated P_{CO_2} also increased it for *Holcus*.

In the heterogeneous treatments, seedlings of *Plantago* and *Holcus* planted nearest to the nutrient patch had more biomass than those furthest away (Fig. 3). No significant interactions between planting position and the remaining factors were found in any species (ANOVA, $P > 0.105$ in all cases). Root proliferation in the heterogeneous treatments was positively and exponentially related to the biomass of the two largest individuals and to the CV_{ABO} of *Lolium* and *Holcus*, respectively (Fig. 4). Soil moisture increased under elevated P_{CO_2} in *Plantago* and *Holcus* microcosms (Fig. 5; *Plantago*, $F_{1,24} = 6.33$, $P = 0.019$; *Holcus*, $F_{1,24} = 11.27$, $P = 0.003$), and decreased under high NA conditions in the latter ($F_{1,24} = 30.68$, $P < 0.001$). A significant $NA \times NH$ interaction was found for this variable in *Plantago* microcosms ($F_{1,24} = 12.07$, $P = 0.002$). Soil moisture in *Lolium* microcosms was not affected by any of the factors evaluated.

DISCUSSION

Our first hypothesis, that nutrient heterogeneity promotes size-asymmetric competition, was supported by the results. This heterogeneity increased both the CV_{ABO} and the biomass of the largest individuals in the three species evaluated. Greater nutrient availability also increased the biomass of both the largest and smallest individuals within each microcosm, but the biomass of the smallest individuals remained unaffected by nutrient heterogeneity. The latter result was unexpected. In our experiment, the total biomass of the microcosms increased under heterogeneous

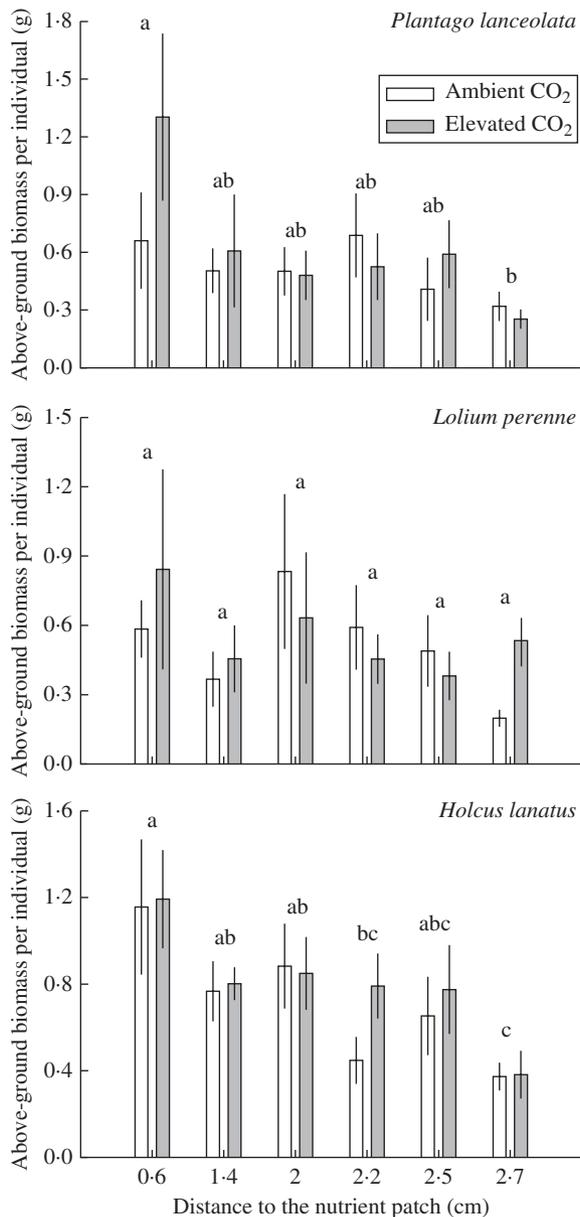


FIG. 3. The dependence of above-ground biomass of individual plants in the heterogeneous nutrient treatment on the distance each seedling was planted from the nutrient patch. For clarity, the data from the low and high nutrient availability treatments were pooled. Data are means \pm 1 s.e. ($n = 8$). Lower case letters denote significant differences among planting positions ($P < 0.05$, Tukey's HSD test after four-way nested ANOVA). *Post hoc* results for *Plantago lanceolata* are shown for \log_{10} -transformed data. Note the different scales on the y-axes.

nutrient supply (Supplementary Information), a response commonly observed with plant populations and communities (Day *et al.*, 2003a; Maestre *et al.*, 2005, 2006). Thus, it is likely that the reduction in size of the smallest plants is outweighed by increases in total microcosm biomass because of the more efficient extraction of nutrients from patches in heterogeneous environments (Jackson and Caldwell, 1996; Maestre *et al.*, 2005).

It has been suggested that small-scale nutrient heterogeneity is a requisite for below-ground size-asymmetric competition to occur (Rajaniemi, 2003). However, this has not been fully supported by experiments, which have produced conflicting results (e.g. Casper and Cahill, 1998; Blair, 2001; Fransen *et al.*, 2001; Facelli and Facelli, 2002). This is not surprising given the importance of both the experimental context and the nature of interactions (intra- or interspecific) when evaluating plant responses to small-scale nutrient heterogeneity (Wijesinghe *et al.*, 2001; Maestre *et al.*, 2006). In this regard, it is interesting that experiments to evaluate the effect of nutrient heterogeneity on the relationships between a plant's size and its competitive effect have used nutrient solutions, commercial potting mixtures or fertilizer pellets to simulate nutrient heterogeneity. Thus, previous results are not directly comparable with those presented here, where such heterogeneity was simulated using organic material.

We were unable to elucidate the relative importance of plasticity in morphological vs. physiological factors responsible for the increase in size inequality promoted by nutrient heterogeneity. However, the positive relationships found between root proliferation into the nutrient patches and the biomass of the largest individuals and the CV_{ABO} observed under heterogeneous conditions for both *Lolium* and *Holcus* suggest that this proliferation was an important mechanism driving the responses. Our results suggest that feedbacks between plant size and root proliferation are crucial in determining the effects of nutrient heterogeneity on the size symmetry of competitive interactions. Studies comparing groups of species have often found a positive relationship between plant size/growth rate and root proliferation into nutrient patches (Einsmann *et al.*, 1999; Rajaniemi and Reynolds, 2004; Kembel and Cahill, 2005). Similar comparative studies on intraspecific variability in growth and size, and in root proliferation are lacking, but it is reasonable to argue that the largest individuals were those with the greatest proliferation of roots in nutrient patches. Despite having a similar initial size, some seedlings may have encountered the nutrient patch sooner than others. Individual plants quickly proliferate roots when they encounter a nutrient patch (Hodge, 2004), which increases nutrient uptake and overall plant growth (Hodge *et al.*, 1999; Maestre *et al.*, 2005). This, in turn, further stimulates root proliferation via the relationship between this process and growth rate (Fransen *et al.*, 1999), in a positive feedback that increases growth until the supply of nutrients is exhausted (Fransen *et al.*, 2001). The fact that individuals of *Plantago* and *Holcus* close to the nutrient patch were larger than those further away supports this view.

Nutrient supply and P_{CO_2} modified the magnitude of the effects of nutrient heterogeneity in *Plantago* and *Holcus*, but not in *Lolium*. Thus, our second hypothesis, that nutrient heterogeneity interacts with both nutrient availability and P_{CO_2} to determine the magnitude of size-asymmetric competitive responses, was partially supported by the results. In terms of biomass production, the three species evaluated have been shown to respond positively to elevated P_{CO_2} (Poorter, 1993), nutrient

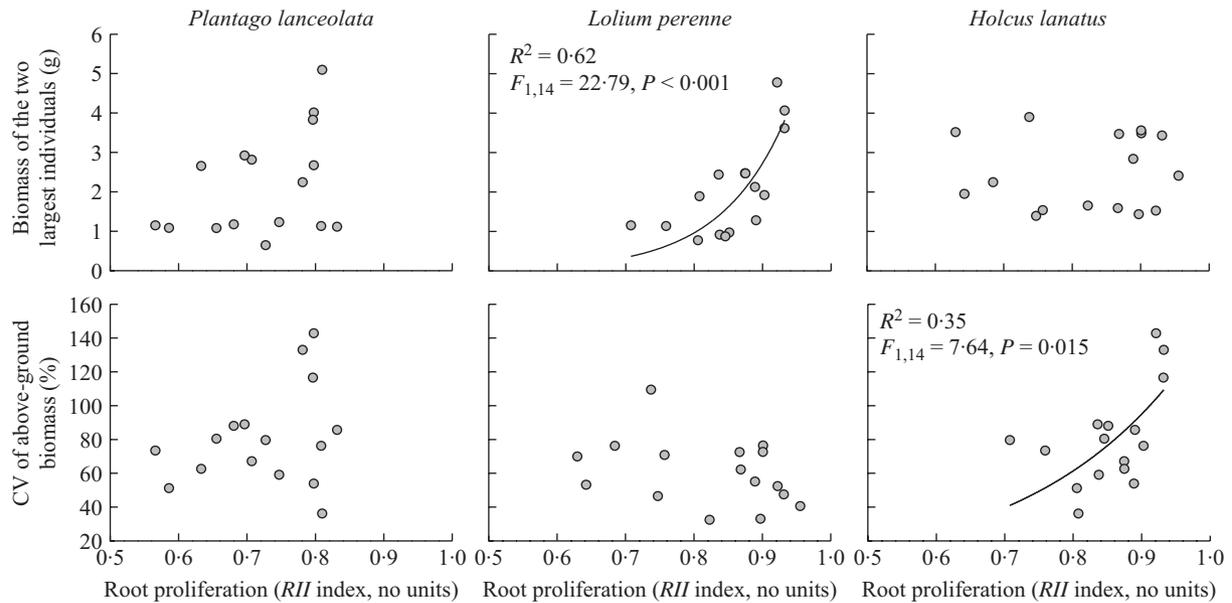


FIG. 4. The relationship between root proliferation in the heterogeneous nutrient treatment, as measured with the *RII* index, and the measures of differences in individual plant size within the population employed: biomass of the two largest individuals (upper panels) and coefficient of variation of individual above-ground biomass (lower panels). Each point represents the value obtained in a single microcosm. Root proliferation is measured in the microcosm, and reflects the summed response of all the individual plants within the microcosm. Only significant relationships ($P < 0.05$) are drawn.

heterogeneity (Grime and Mackey, 2002) and increases in nutrient supply (Kirkham *et al.*, 1996). However, they differ in the magnitude of these responses, as well as in key attributes such as relative growth rate (RGR; *Lolium* has a lower RGR than *Holcus* and *Plantago*; Grime and Hunt, 1975). These differences may help to explain why interactions among the evaluated factors were not observed in all of the species. For both *Plantago* and *Holcus*, increases in size inequality promoted by nutrient heterogeneity were more pronounced where more nutrient was available, possibly caused by the increase in plant size with fertilization. Under nutrient-limiting conditions, such as experienced by the plants growing with little nutrient, addition of nutrients typically increases plant growth, and such an increase would foster the positive feedback between growth rate and root proliferation. However, other mechanisms not accounted for in this study, such as changes in nutrient uptake rates associated with changes in nutrient availability (Robinson, 1994), could also explain the results. Elevated P_{CO_2} accentuated the combined effects of nutrient availability and heterogeneity on the differences in individual plant size within *Plantago* populations. This result is in line with observations from Nagashima *et al.* (2003), who found that elevated P_{CO_2} increased size inequality in populations of *Chenopodium album*, and that this effect was larger under high nutrient availability conditions. Our results cannot provide a fully mechanistic explanation for this synergistic effect, but it could have been mediated by P_{CO_2} -induced changes in nutrient availability and use. Despite having higher biomass, soil moisture at harvest in *Plantago* microcosms was significantly higher under elevated than under ambient P_{CO_2} conditions, a result probably caused by the increase in the water use efficiency of this species commonly observed under elevated P_{CO_2}

(Klus *et al.*, 2001). In our experiment, as in the field, the release of nutrients from added organic material depends on mineralization by the soil microbial and faunal populations, which typically increases with increasing water availability (Ebersberger *et al.*, 2003). Thus, the increased mineralization could have increased nutrient availability for plants under elevated P_{CO_2} , which would have increased the feedback between growth rate and foraging precision described above. However, this is speculative as mineralization rates were not measured. Regardless of the underlying mechanisms, our results suggest that the combined effects of nutrient heterogeneity, P_{CO_2} and increased nutrient availability on the differences in the size of individual plants within populations may be non-additive.

Our results must be interpreted with some caution because the experiment did not separate above- and below-ground competitive interactions. Both operate and interact in the field, and the direction and magnitude of such interaction may depend on factors such as species traits and nutrient availability (Cahill, 1999, 2002). Within microcosms, plants probably competed for light, and below- and above-ground competition occurred, influencing each other, especially at later stages of growth and with ample nutrients. However, such interaction did not obscure the effects of nutrient heterogeneity on the differences in individual plant size within the population. Competition for above-ground resources such as light is usually size-asymmetric (Weiner, 1990; Schwinning and Weiner, 1998). If the differences in size were the consequence of such competition, and not of changes in below-ground competition induced by nutrient heterogeneity, we should have not observed differences between homogeneous and heterogeneous treatments, as both have the

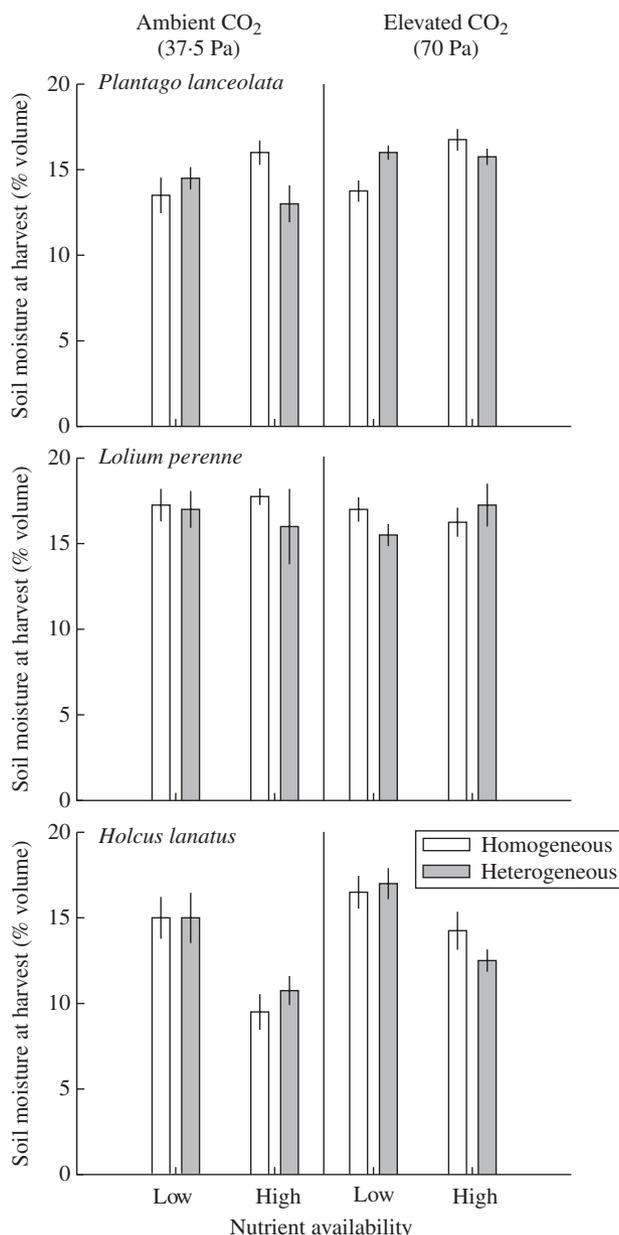


FIG. 5. Soil moisture (0–12 cm depth) at harvest for *Plantago lanceolata*, *Lolium perenne* and *Holcus lanatus* grown in microcosms with different nutrient availability, heterogeneity and CO₂ partial pressure. Data represent means \pm s.e. ($n = 4$).

same soil resources. However, competition for light could have magnified the effect of nutrient heterogeneity, as differences in size among neighbours resulting from nutrient heterogeneity may have been accentuated by larger plants reducing the light available to smaller neighbours. Detailed analyses of the effects of nutrient heterogeneity on the interactions between above- and below-ground competition are lacking, and should be addressed.

Our first hypothesis was supported by our results, as nutrient heterogeneity promoted size-asymmetric competition in the three species evaluated. They also indicate

that the proliferation of roots into nutrient patches was an important driver of this response. As predicted by our second hypothesis, nutrient supply and P_{CO_2} modified the magnitude of the effects of nutrient heterogeneity in *Plantago* and *Holcus*, but not in *Lolium*. We suggest that these differences are caused by species-specific differences in attributes such as relative growth rate. Our results add to studies evaluating the joint effects of nutrient heterogeneity and global change drivers on grassland communities (Maestre *et al.*, 2005, 2006), and suggest that this heterogeneity may be a key environmental factor when interpreting and assessing plant population responses to global change.

SUPPLEMENTARY INFORMATION

The following Supplementary Information is available online at <http://www.aob.oxfordjournals.org/>. Figure A1: total biomass in soil microcosms differing in nutrient availability, heterogeneity and CO₂ partial pressure. Table A1: results of a three-way analysis of variance showing the effects of CO₂ partial pressure, nutrient heterogeneity and availability on total microcosm biomass.

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