

Spatial heterogeneity in soil nutrient supply modulates nutrient and biomass responses to multiple global change drivers in model grassland communities

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Abstract

Changes in the atmospheric concentration of carbon dioxide ([CO₂]), nutrient availability and biotic diversity are three major drivers of the ongoing global change impacting terrestrial ecosystems worldwide. While it is well established that soil nutrient heterogeneity exerts a strong influence on the development of plant individuals and communities, it is virtually unknown how nutrient heterogeneity and global change drivers interact to affect plant performance and ecosystem functioning. We conducted a microcosm experiment to evaluate the effect of simultaneous changes in [CO₂], nutrient heterogeneity (NH), nutrient availability (NA) and species evenness on the biomass and nutrient uptake patterns of assemblages formed by *Lolium perenne*, *Plantago lanceolata* and *Holcus lanatus*. When the nutrients were heterogeneously supplied, assemblages exhibited precise root foraging patterns, and had higher above- and below-ground biomass (average increases of 32% and 29% for above- and belowground biomass, respectively). Nutrient heterogeneity also modulated the effects of NA on biomass production, complementarity in nitrogen uptake and below: aboveground ratio, as well as those of [CO₂] on the nutrient use efficiency at the assemblage level. Our results show that nutrient heterogeneity has the potential to influence the response of plant assemblages to simultaneous changes in [CO₂], nutrient availability and biotic diversity, and suggest that it is an important environmental factor to interpret and assess plant assemblage responses to global change.

Keywords: climate change, diversity, evenness, *Holcus lanatus*, *Lolium perenne*, microcosm, nutrient availability, nutrient heterogeneity, *Plantago lanceolata*, productivity

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Introduction

Changes in the atmospheric concentration of carbon dioxide ([CO₂]), nutrient availability and biotic diversity are three major drivers of global change being faced by terrestrial ecosystems worldwide (Vitousek *et al.*, 1997; Sala *et al.*, 2000). Most of the research conducted so far to understand and predict the biological consequences of these drivers has evaluated the response of ecosystem components and processes to them in isola-

tion from each other (Körner, 2001; Loureau *et al.*, 2002). However, they act simultaneously, and often interactively, to determine ecosystem responses (Körner, 2001; Shaw *et al.*, 2002). Nevertheless, few studies so far have simultaneously evaluated the joint effects of changes in [CO₂], nutrient availability and biodiversity on ecosystem attributes such as productivity and carbon fluxes (Stocker *et al.*, 1999; Reich *et al.*, 2001; He *et al.*, 2002) and, furthermore, these studies have manipulated species richness and/or composition when evaluating diversity effects, but not species evenness. Species evenness, a component of diversity that measures how equitability abundances are distributed among the species in a given community, has significant effects on productivity and related ecosystem processes (Wilsey & Potvin, 2000; Mulder *et al.*, 2004). Despite its recognized importance,

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the ecological consequences of simultaneous changes in species evenness, [CO₂] and nutrient availability are virtually unknown.

In most terrestrial ecosystems, the spatial pattern of nutrient supply is highly heterogeneous across a wide range of scales, and the elucidation of how organisms, communities and ecosystems respond to it has been a central research topic in community ecology (Hutchings *et al.*, 2000). At small spatial scales (i.e. those detected by the root system of individual plants), this heterogeneity (hereafter termed nutrient heterogeneity) determines plant competitive ability and survival (Day *et al.*, 2003; Hodge, 2004), as well as community composition and productivity (Maestre *et al.*, 2005; Wijesinghe *et al.*, 2005). However, and despite its relevance, few studies have explicitly evaluated how plant species and assemblages respond to joint changes in nutrient heterogeneity and global change drivers. Maestre *et al.* (2005) found that nutrient heterogeneity interacts with both [CO₂] and nutrient availability to determine the productivity and nutrient uptake patterns of grassland species and assemblages. However, Arnone (1997) did not find interactions between [CO₂] and nutrient heterogeneity when evaluating fine root responses in a model tropical community. Studies like these are essential to advance our understanding of the interactions between global change drivers and intrinsic ecosystem features, and to predict how such features may modify ecosystem responses to global change.

To our knowledge, no previous study has evaluated how plant communities respond to simultaneous changes in atmospheric [CO₂], nutrient heterogeneity, nutrient availability and species evenness. To address this need, we conducted a microcosm experiment to evaluate the joint effects of these factors on the biomass and nutrient uptake patterns of assemblages formed by *Lolium perenne* L., *Plantago lanceolata* L. and *Holcus lanatus* L., which commonly co-occur in temperate grasslands of the Northern hemisphere (Joshi *et al.*, 2000). We varied species evenness (1:1:1 or 4:1:1 ratio among species) and the identity of the dominant species in the 4:1:1 mixtures for each combination of [CO₂], nutrient heterogeneity and availability. Using this model system, we tested the following hypotheses:

- (i) Assemblage responses to [CO₂], nutrient availability, nutrient heterogeneity and species evenness are not predictable from the observed responses to any one of them in isolation. Interactions are predicted because these factors modify productivity and biomass allocation patterns in grassland communities (Wilsey & Potvin, 2000; Niklaus & Körner, 2004; Wijesinghe *et al.*, 2005), and because interactive responses may arise when two or more factors act

in combination (Reich *et al.*, 2004; Maestre *et al.*, 2005).

- (ii) The interactions among the factors evaluated will vary depending upon which species is dominant in the 4:1:1 mixtures. Such an effect is predicted because the species used differ in their ability to respond to the different factors evaluated (Poorter, 1993; Grime & Mackey, 2002; Maestre *et al.*, 2005).

Materials and methods

Experimental design

A factorial microcosm experiment was conducted in the Duke University Phytotron between January and April 2005. The experiment had four treatments: [CO₂] (two levels: 37.5 and 70 Pa), nutrient availability (two levels: 40 and 120 mg of N added as organic material), nutrient heterogeneity (two levels: homogeneous and heterogeneous), and species composition (seven levels: monocultures and three-species mixtures). In the mixtures, species abundances were completely equitable (maximum evenness; 1:1:1 ratio) or distributed in a 4:1:1 ratio with each of the three species used as the dominant in a single 4:1:1 mixture. 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 (hereafter referred as background soil), as described in Maestre *et al.* (2005). On top of the background soil we placed 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 150 mL of a fresh soil solution and with 100 mL of a solution derived from root macerations as described in Maestre *et al.* (2005).

To generate the two nutrient availabilities, different amounts of finely cut (2 mm) *Trifolium repens* L. shoots (3.9% N, 10.8 C:N) were added: 1.036 and 3.108 g in the low and high nutrient availability treatments, respectively, which is equivalent to adding 40 and 120 mg of N per microcosm, respectively. Within each level, the organic material was added as a patch (heterogeneous treatment) or homogeneously (homogeneous treatment). In the heterogeneous microcosms, we mixed 25 cm³ of background soil with the organic material and introduced this mix into a 31 cm³ plastic cylinder (length 75 mm and internal diameter 23 mm) consisting of a light mesh with square pores 5 mm × 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 apart and alongside the patch cylinder. Cylinders

were located 12 cm below the surface of the organic soil. In the homogeneous microcosms, we thoroughly mixed the organic material with the background soil before introducing it into the PVC pipe. In these microcosms, two plastic cylinders filled with this mixture were introduced as described above.

Seeds from the three species, obtained from commercial suppliers (*Lolium* seeds were provided by Granite Seed Company, Lehi, UT, USA; *Plantago* and *Holcus* seeds by V & J Seed Service, Woodstock, IL, USA), were placed in trays with potting soil and germinated in a growth chamber [20 °C temperature and photosynthetic active radiation (PAR) of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a 14 hours photoperiod]. Species were germinated on different days to ensure that all seedlings were of similar size at the start of the experiment (aboveground biomass; *Plantago* = 0.0030 \pm 0.0010 g; *Lolium* = 0.0027 \pm 0.0011 g; *Holcus* = 0.0033 \pm 0.0015 g; means \pm SD, n = 30). Within each microcosm, the planting positions of the six seedlings were allocated at random, but the same planting grid was maintained in all the microcosms by using a wire grid pattern secured to the top of the containers. 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 56 treatment combinations, resulting in 224 microcosms in total. The microcosms were introduced in four walk-in growth chambers (two for each CO₂ level), within which atmospheric temperature and [CO₂] were independently controlled. For each [CO₂] level, half of the microcosms per treatment were randomly assigned to one of the chambers (56 microcosms per chamber), and then were randomly grouped in five wheeled trolleys. To minimize possible chamber effects, the [CO₂] levels and trolleys were rotated between chambers every week, and the position of the microcosms within each chamber was randomized with every rotation. This process was repeated 12 times during the experiment, and at harvest all the microcosms spent the same amount of time in each chamber.

Temperatures in the growth chambers ranged from 12 °C at night to 21 °C during the day, and this regime included a simulated dawn and dusk period, each of 2 hours duration, where temperature was gradually ramped up or down. Relative humidity followed a similar pattern ranging from 85% to 70%, as did the lights. PAR was maintained at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the first week of the experiment, at 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the second week of the experiment, and at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, thereafter. Microcosms were irrigated daily with 30 mL of distilled water during the first 2 weeks of the experiment, and with 50 mL, hereafter. To reduce limitations to plant growth because of

low overall fertility, all the microcosms were watered with 50 mL of a nutrient solution containing 35 mg of Ca (added as CaCl₂·2H₂O) and 29 mg of Mg (added as MgSO₄·7H₂O) twice during the course of the experiment (1 February and 1 March).

Measurements and harvest

After 90 days of growth, the aboveground biomass of all the microcosms was harvested and sorted by species. Leaves and stems were dried at 60 °C to constant mass. Once dried, all aboveground plant samples were ground to a fine powder, transferred into tin capsules and injected into an elemental analyzer (Costech CHN Analyser, Milan, Italy) to calculate their carbon and nitrogen concentrations. The aboveground nitrogen content (ANC) of each species was estimated by multiplying its aboveground nitrogen concentration by its aboveground biomass. Summed ANC values for all species in a mixture are that mixture's ANC. This variable was used to calculate complementarity in nitrogen uptake by the mixtures (*sensu* Hooper, 1998), which was estimated with the relative yield total (de Wit, 1960). The relative yield (RY) for a given species in a mixture was calculated as its ANC divided by the average ANC of its monocultures for the same combination of growth chamber, [CO₂], nutrient availability and heterogeneity treatments. Summed RYs for all species in a mixture are that mixture's relative yield total (hereafter RYT-N). The nitrogen use efficiency (NUE) of the mixtures was estimated as the grams of aboveground biomass produced per gram of nitrogen (van Ruijven & Berendse, 2005).

After aboveground harvesting, the soil was carefully removed from the microcosm unit and the roots were harvested. They were so large and entangled that it was impossible to separate them by species. To measure root foraging precision, we extracted the roots within each cylinder by cutting those outside it. The rest of the root system was also collected, and all the roots were dried as described above. Root foraging precision was estimated with the index RII (Armas *et al.*, 2004), as described in Maestre *et al.* (2005). RII ranges from -1 to +1: a value of zero indicates equal root growth in nutrient patches and background soil (no precision of foraging). Increasing positive values indicate increasing precision (i.e. root proliferation into the nutrient patch).

Statistical analyses

The effects of species evenness (SE), [CO₂], nutrient availability (NA) and nutrient heterogeneity (NH) on above- and belowground assemblage biomass, below: aboveground biomass ratio (BAR), aboveground N

concentration, RYT-N, and NUE were analyzed with a four-way analysis of variance (ANOVA). In order to control for differences in plant size when analyzing the BAR data (Reich, 2002), total biomass was used as a covariate in this analysis. The effects of SE, $[\text{CO}_2]$ and NA on root foraging precision in the heterogeneous treatment were evaluated with a three-way ANOVA. To assess if species identity modified assemblage responses to the factors evaluated, separate analyses were conducted for each of the three 4:1:1 mixtures evaluated. To investigate higher-order interactions, data were divided into subsets based on one of the factors of the interaction and then were subjected to ANOVA. When appropriate, biomass data were square-root transformed to achieve the homogeneity of variances assumption of ANOVA. Relationships between root foraging precision and both total biomass and ANC of the mixtures were evaluated using linear and nonlinear (quadratic, logarithmic, power and exponential) functions. When significant relationships were found, the function that minimized the second-order Akaike information (Sugiura, 1978) was chosen. P values were not adjusted for multiple testing because this approach is considered overly conservative (Gotelli & Ellison, 2004). All the statistical analyses were performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Regardless the species dominating the 4:1:1 mixtures, significant $\text{NA} \times [\text{CO}_2]$ and $\text{NA} \times \text{NH}$ interactions modulated their aboveground biomass responses (Fig. 1a, $F_{1,48} > 10.6$, $P < 0.003$ in all cases). ANOVAS conducted at each NA level showed that aboveground biomass increased under heterogeneous nutrient supply at both NA levels ($F_{1,24} > 10.4$, $P < 0.005$), albeit this response was larger under high NA (Fig. 1a), and that it increased in response to elevated $[\text{CO}_2]$ only under high NA in both *Lolium* and *Holcus* ($F_{1,24} > 10.4$, $P < 0.005$ in both cases). When *Lolium* dominated the 4:1:1 mixtures, assemblages had higher aboveground biomass under maximum SE conditions (Fig. 1a, $F_{1,48} = 18.0$, $P < 0.001$). The magnitude of this response was larger under high NA, as indicated by a significant $\text{NA} \times [\text{CO}_2] \times \text{SE}$ interaction ($F_{1,48} = 3.9$, $P = 0.055$).

Belowground biomass increased under heterogeneous nutrient supply in all cases (Fig. 1b, $F_{1,48} > 20.3$, $P < 0.001$). When *Lolium* dominated the 4:1:1 mixtures, this response was especially evident under high NA ($F_{\text{NA} \times \text{NH}} = 5.5$, $\text{df} = 1,48$, $P = 0.024$). Significant $\text{NA} \times [\text{CO}_2]$ interactions were found for this variable ($F_{1,48} > 6.1$, $P < 0.018$ in all cases). ANOVAS conducted at each NA level revealed that belowground biomass increased in response to elevated $[\text{CO}_2]$ only under high

NA ($F_{1,24} > 7.5$, $P < 0.012$ in all cases). When *Holcus* dominated the 4:1:1 mixtures, belowground biomass decreased under maximum SE (Fig. 1b, $F_{1,48} = 4.3$, $P = 0.044$).

When *Lolium* dominated the 4:1:1 mixtures, assemblages had a lower below:aboveground biomass ratio (BAR) under elevated $[\text{CO}_2]$, high NA, maximum SE, and heterogeneous nutrient supply (Fig. 1c, $F_{1,47} > 5.7$, $P < 0.022$; no interactions). The same response to NH ($F_{1,47} = 22.8$, $P < 0.001$) and $[\text{CO}_2]$ ($F_{1,47} = 30.5$, $P < 0.001$) was observed when *Plantago* was the dominant species, albeit in the later case the decrease was larger under high NA ($F_{\text{NA} \times [\text{CO}_2]} = 4.6$, $\text{df} = 1,47$, $P = 0.037$). When *Holcus* was the dominant species in the 4:1:1 mixtures, a significant $\text{NA} \times \text{NH}$ ($F_{1,47} = 4.1$, $P = 0.050$) interaction was found. ANOVAS conducted at each NH level showed a reduction in BAR values under high NA at both NH levels ($F_{1,23} > 5.8$, $P < 0.025$), a reduction also found in response to elevated $[\text{CO}_2]$ under heterogeneous nutrient supply ($F_{1,23} = 13.9$, $P = 0.001$). ANOVAS conducted at each NA level revealed that nutrient heterogeneity decreased the BAR under high NA ($F_{1,23} = 5.7$, $P = 0.025$).

Precise root foraging patterns were evident in the assemblages when the organic material was supplied as a patch (Fig. 1d). In all cases, root proliferation into nutrient patches increased with increasing nutrient availability ($F_{1,24} > 12.2$, $P < 0.003$; no interactions), and was not affected by either SE or $[\text{CO}_2]$.

Significant $\text{NA} \times [\text{CO}_2]$ (*Plantago* and *Holcus* as dominant species in the 4:1:1 mixtures) and $\text{NA} \times \text{NH}$ (*Holcus* as dominant) interactions were found when evaluating the RYT-N (Fig. 2a, $F_{1,48} > 5.1$, $P < 0.029$). ANOVAS conducted at each NA level showed that elevated $[\text{CO}_2]$ promoted a decrease in the RYT-N of the assemblages under low NA (*Plantago*; $F_{1,24} = 5.7$, $P = 0.026$; *Holcus*; $F_{1,24} = 11.0$, $P = 0.003$; no interactions). These analyses revealed that, when *Holcus* was the dominant species in the 4:1:1 mixtures, the RYT-N of the assemblages was slightly lower under heterogeneous conditions of nutrient supply at low NA levels (low NA: $F_{\text{NH}} = 3.2$, $\text{df} = 1,24$, $P = 0.085$; high NA: $F_{\text{NH}} = 2.5$, $\text{df} = 1,24$, $P = 0.130$).

Regardless the species dominating the 4:1:1 mixtures, the concentration of N in aboveground tissues decreased under elevated $[\text{CO}_2]$ (Fig. 2b, $F_{1,48} > 34.8$, $P < 0.001$; no interactions). When *Lolium* and *Holcus* were dominant, it increased under high nutrient supply ($F_{1,48} = 5.6$, $P = 0.022$) and maximum species evenness ($F_{1,48} = 7.9$, $P = 0.007$), respectively.

Significant and marginally significant $[\text{CO}_2] \times \text{NA} \times \text{NH}$ interactions were found when evaluating the NUE of the assemblages (Fig. 2c, *Plantago*: $F_{1,48} = 3.7$, $P = 0.060$; *Lolium*: $F_{1,48} = 6.9$, $P = 0.011$; *Holcus*:

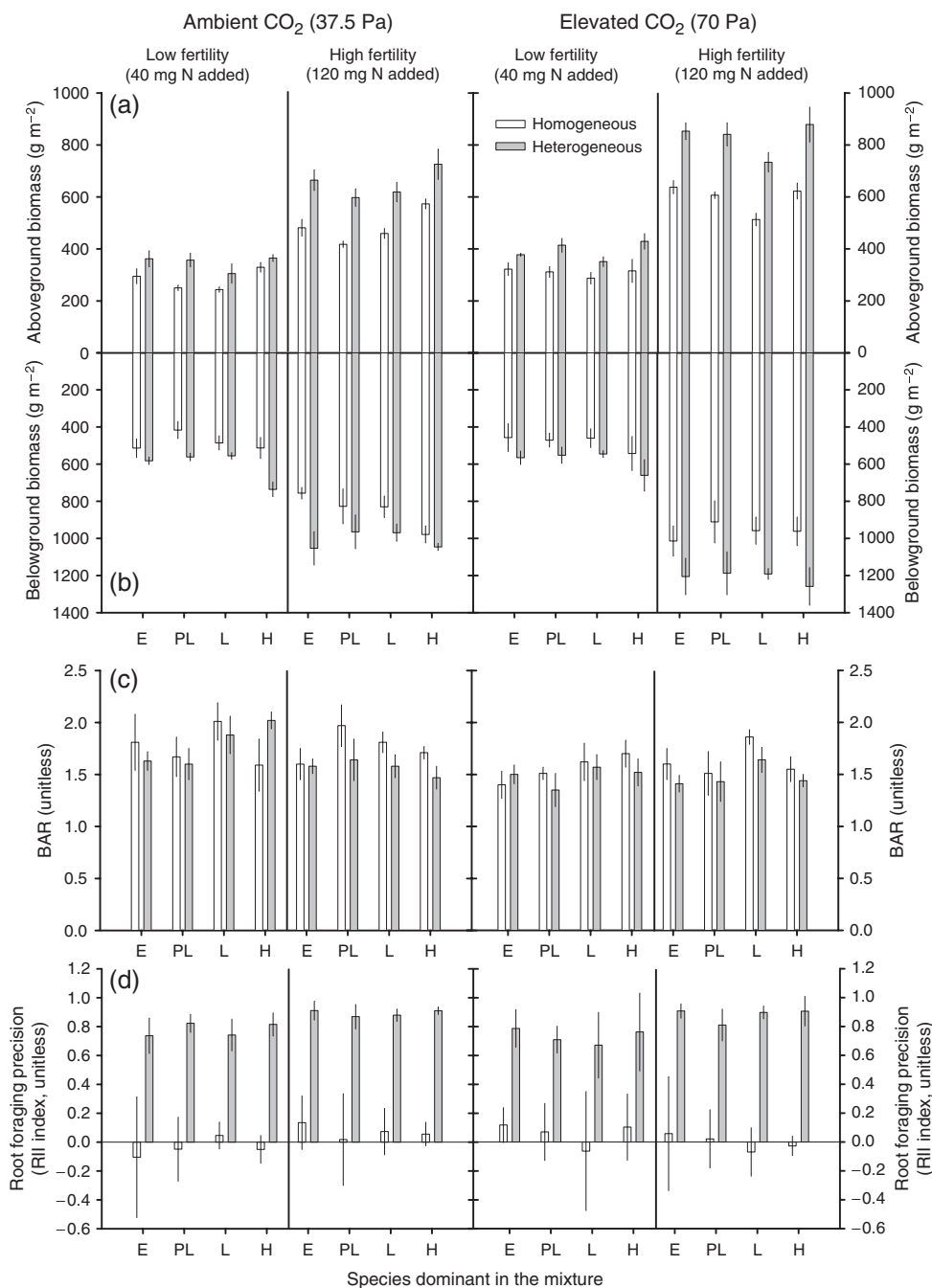


Fig. 1 Aboveground biomass (a), belowground biomass (b), below: aboveground biomass ratio (BAR, c), and root foraging precision into nutrient patches (RII index, d) of the mixtures compared across CO₂, nutrient availability, and nutrient heterogeneity levels. Data are means \pm 95% confidence intervals (RII index), and means \pm 1 SE (rest of variables); $n = 4$ in all cases. Positive values of the RII index indicate precise root foraging (proliferation) responses. Significant root proliferation is shown by confidence intervals that do not overlap zero. E = equal evenness mixture (1 *Plantago*:1 *Lolium*:1 *Holcus*), P = mixture dominated by *Plantago lanceolata* (4 *Plantago*:1 *Lolium*:1 *Holcus*), L = mixture dominated by *Lolium perenne* (1 *Plantago*:4 *Lolium*:1 *Holcus*), and H = mixture dominated by *Holcus lanatus* (1 *Plantago*:1 *Lolium*:4 *Holcus*).

$F_{1,48} = 6.5$, $P = 0.014$). Separate ANOVAS conducted at each NH and NA levels revealed that, under homogeneous nutrient supply, increases in [CO₂] and NA

increased and decreased, respectively, the NUE of the assemblages ($F_{1,24} > 11.8$, $P < 0.003$ in all cases; no interactions). Under heterogeneous nutrient supply,

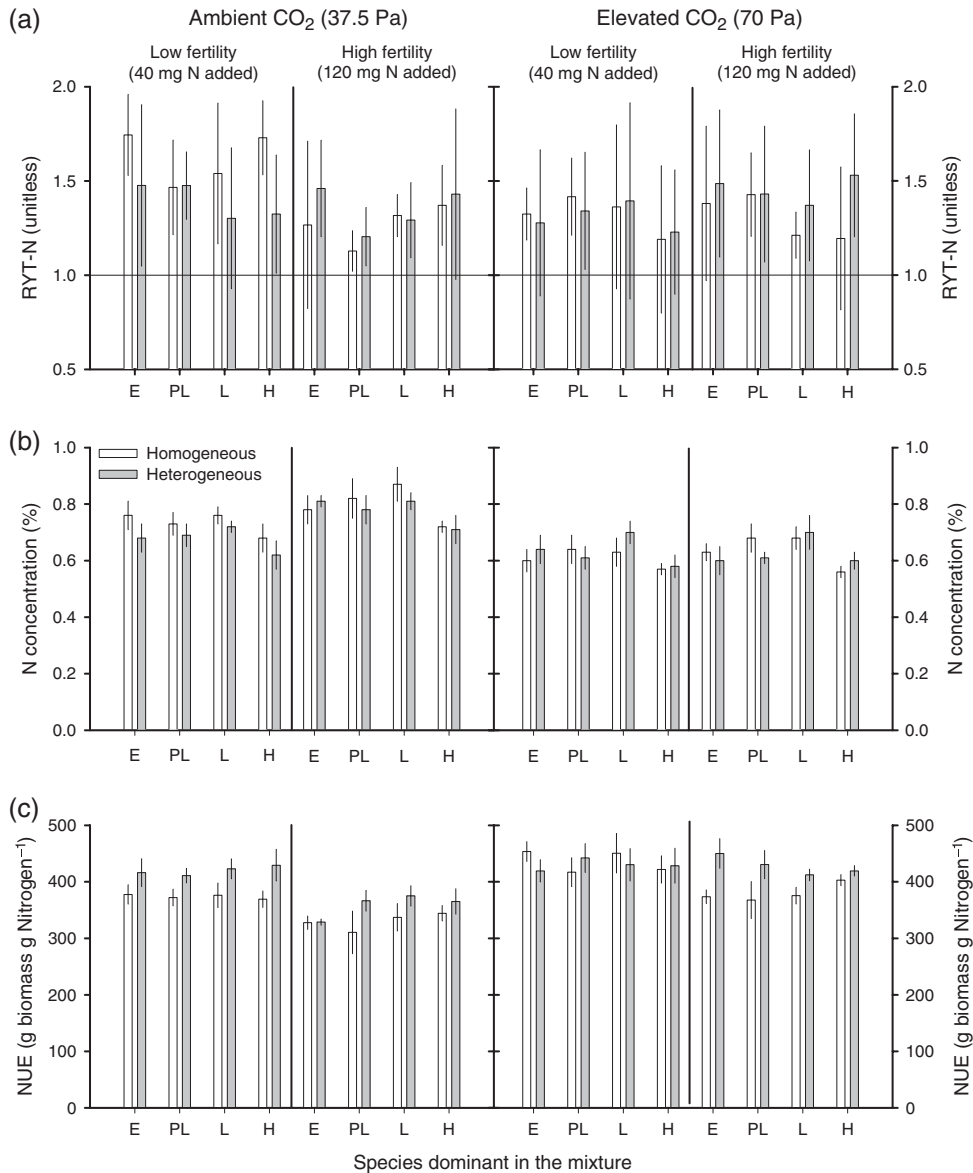


Fig. 2 Relative yield total (RYT-N, a), aboveground nitrogen concentration (b) and grams of aboveground biomass per gram of nitrogen in aboveground tissues (NUE, c) compared across CO_2 , nutrient availability, and nutrient heterogeneity levels. Data are means \pm SE ($n = 4$). For explanation of x -axis labels see Fig. 1 caption.

the decrease in NUE observed when increasing NA was more pronounced at ambient than at elevated $[\text{CO}_2]$ ($F_{\text{NA} \times [\text{CO}_2]} > 6.5$, $df = 1,24$, $P < 0.018$ in all cases). Nutrient heterogeneity increased the NUE of assemblages under high NA in all cases ($F_{1,24} > 6.9$, $P < 0.015$).

In the heterogeneous treatments, both the total biomass and the ANC of the assemblages increased exponentially with increases in root foraging precision in all the mixtures except those dominated by *Plantago* (Fig. 3). In the homogeneous treatments, no significant relationships were found between root foraging precision and either total biomass or ANC.

Discussion

Our first hypothesis (i.e. that assemblage responses to $[\text{CO}_2]$, nutrient availability, nutrient heterogeneity and species evenness are not predictable from the isolated responses of any of them), was partially supported by our results. Although we did not observe significant four-term interactions in any of the response variables measured, we found a series of two- and three-term interactions determining key biomass and nutrient responses in most of the assemblages (Table 1). Nutrient heterogeneity significantly affected most of the perfor-

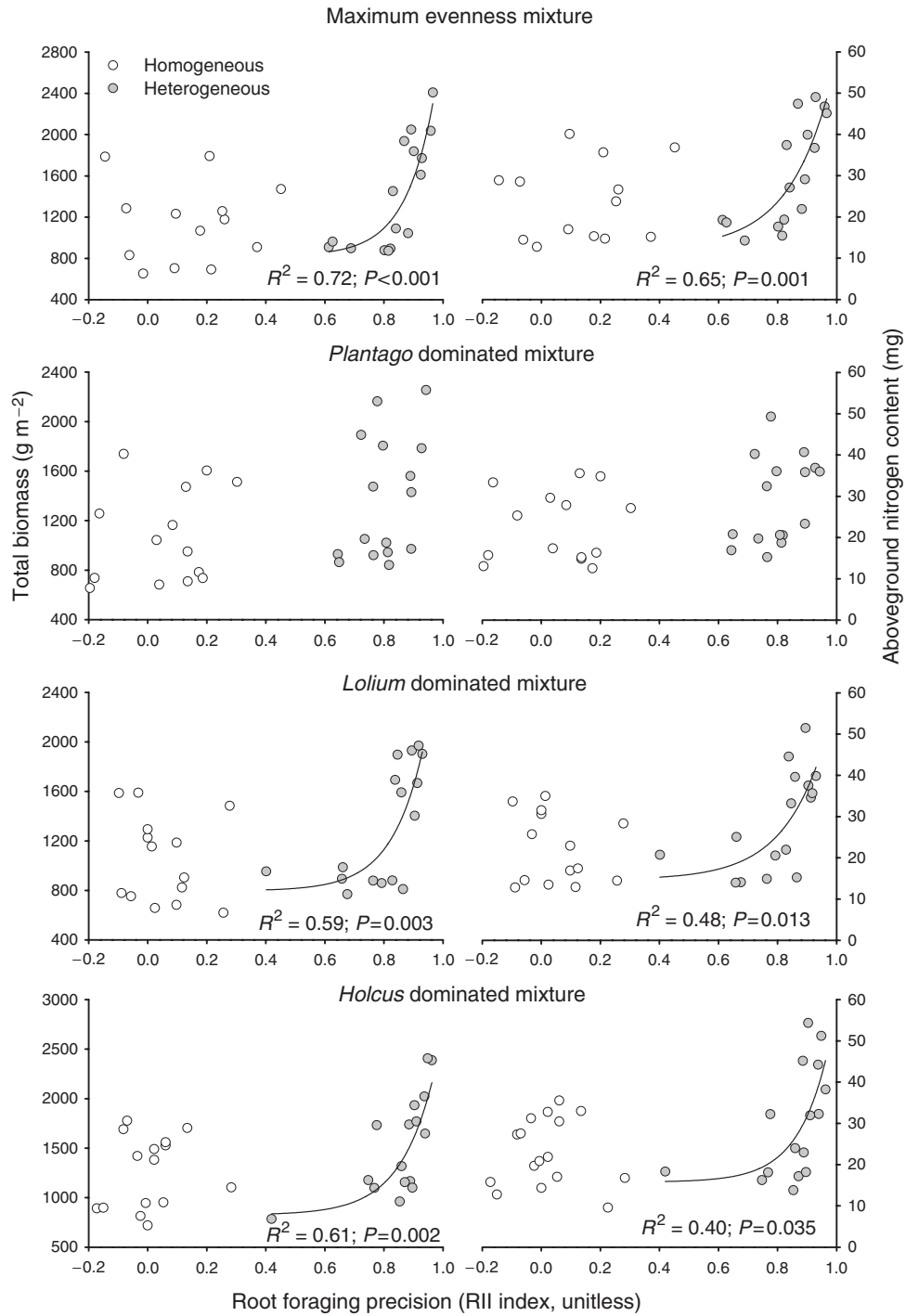


Fig. 3 Relationships between root foraging precision (RII index) and total biomass (left panels) and aboveground nitrogen content (right panels) of the different mixtures evaluated. Each point represents the value of a microcosm for a given combination of nutrient heterogeneity, nutrient availability and CO₂ concentration (*n* = 16 in each case). Only significant relationships (*P* < 0.05) are shown. The model fitted is in all cases an exponential function of the form $y = y_0 + ae^{bx}$.

mance variables evaluated, and modulated the effects of (i) nutrient availability on biomass production, complementarity in nitrogen uptake and the below : aboveground biomass ratio, and (ii) [CO₂] on nutrient use

efficiency (Table 1). These results suggest that it may be an extremely important environmental factor when assessing and interpreting the effects of global change drivers on plant assemblages.

Table 1 Overview of the effects of the factors on the observed assemblage responses, as evaluated with ANOVA analyses

Species dominating the 4:1:1 mixture	Response variable	Species evenness (se)	CO ₂	Nutrient heterogeneity (nh)	Nutrient availability (na)
<i>Plantago lanceolata</i>	AB	•	X _{na}	X _{na}	X _{CO₂,nh}
	BB	•	X _{na}	+	X _{CO₂}
	R:S	•	X _{na}	–	X _{CO₂}
	RII	•	•	+	+
	RYT-N	•	x _{na}	•	X _{CO₂}
	[N]	•	–	•	•
	NUE	•	+	+	–
<i>Lolium perenne</i>	AB	+	X _{na}	X _{na}	X _{CO₂} , X _{nh}
	BB	•	X _{na}	X _{na}	X _{CO₂} , X _{nh}
	R:S	–	–	–	–
	RII	•	•	+	+
	RYT-N	•	–	•	•
	[N]	•	–	•	+
	NUE	•	X _{na, nh}	X _{na,CO₂}	X _{CO₂,nh}
<i>Holcus lanatus</i>	AB	•	X _{na}	X _{na}	X _{CO₂} , X _{nh}
	BB	–	X _{na}	+	X _{CO₂}
	R:S	•	–	X _{na}	X _{nh}
	RII	•	•	+	+
	RYT-N	•	X _{na}	x _{na}	X _{CO₂,nh}
	[N]	+	–	•	•
	NUE	•	X _{na, nh}	X _{na,CO₂}	X _{CO₂,nh}

CO₂, [CO₂]; AB, aboveground biomass; BB, belowground biomass; R:S = below : aboveground biomass ratio; RII, root foraging precision (responses to factors other than heterogeneity are shown for the heterogeneous treatment only); RYT-N, complementarity in nitrogen use; [N], aboveground nitrogen concentration; NUE, nitrogen use efficiency; •, no significant effect ($P > 0.05$); +, increased values of this factor led to increased response ($P < 0.05$); –, increased values of this factor led to reduced response ($P < 0.05$); X, significant main effect ($P < 0.05$) and interaction ($P < 0.05$) with other factor; x, no significant effect ($P > 0.05$) but interaction ($P < 0.05$) with other factor. In the latter two cases, the factor/s involved in the interaction are denoted as subscripts. Two “X” in the same cell indicate that the factor is involved in two independent interactions.

In a recent study, Maestre *et al.* (2005) evaluated, using the same microcosm approach as employed here, the joint effects of nutrient heterogeneity, nutrient availability and [CO₂] on assemblages formed by *Lolium*, *Plantago*, *Holcus*, *Trifolium* and *Anthoxanthum odoratum* L. As found in this study, root proliferation into nutrient patches increased with increases in nutrient availability, and above- and belowground biomass increased in response to nutrient heterogeneity. They also found that elevated [CO₂] had a positive effect on aboveground biomass production under homogeneous nutrient supply only. This response was not observed in our study, as indicated by the lack of a significant [CO₂] × nutrient heterogeneity interaction when analyzing either above- or belowground biomass data. Our study differed from that of Maestre *et al.* (2005) in important aspects, including total plant density (764 vs. 1273 individuals m⁻²), number of species forming the assemblages (three vs. five species per microcosm), nutrient availability levels (40–120 vs. 40–80 mg N added per microcosm), and growing conditions (characteristics of the

organic material used as fertilizer, light intensity and watering regime). Given the strong influence of both environmental context and plant–plant interactions on plant responses to nutrient heterogeneity (Wijesinghe *et al.*, 2001) and global change drivers (Poorter & Navas, 2003), these discrepancies are not fully surprising. Further research is, thus, needed to assess the generality, direction and magnitude of the effect induced by nutrient heterogeneity on plant responses to elevated [CO₂].

Whereas, we cannot ascertain the mechanisms underlying observed responses to nutrient heterogeneity, our results suggest that they are driven by root proliferation into nutrient patches and, up to a less degree, by changes in the nutrient use efficiency of assemblages. Individual plants begin to proliferate roots quickly after they encounter a nutrient patch, and this response increases nutrient uptake and overall plant growth (Hodge, 2004). This, in turn, further stimulates root proliferation because of the positive relationship between plant size and/or relative growth rate and root

foraging precision (Einsmann *et al.*, 1999; Fransen *et al.*, 1999; Rajaniemi & Reynolds, 2004; Kembel & Cahill, 2005), in a positive feedback that increases growth until the supply of nutrients is exhausted (Fransen *et al.*, 2001). This positive feedback should be amplified under high nutrient availability conditions because of increases in plant size in response to the addition of nutrients. The increase in root foraging precision observed in response to this addition, and the positive relationships found between root foraging precision and both total biomass and aboveground nitrogen content (in most cases, see Fig. 3) support this view. It is interesting to mention that the nutrient use efficiency of the assemblages increased under heterogeneous nutrient supply, albeit only at the high nutrient availability. This response could have enhanced the positive effects of the feedback process described above on biomass production. It has been shown that when a fixed amount of nutrients are made available to a plant, their acquisition will be more efficient if these are spatially concentrated because of preferential root allocation in these areas (Fransen *et al.*, 1999). Our results agree with this view, and indicate that this effect may be dependent on overall nutrient availability.

As commonly observed with grassland species (Cotrufo *et al.*, 1998), elevated [CO₂] promoted a decrease in the aboveground N concentration ([N]) of the mixtures. Similar [N] values to those found in our study have been reported by previous experiments that have grown the same species using field soil as growing medium and plant material as fertilizer (e.g. Hodge *et al.*, 2000; Wurst *et al.*, 2003; Maestre *et al.*, 2005). The decrease in the BAR of the assemblages observed under elevated [CO₂] deserves some attention. It does not match the results of a recent synthesis of published data that did not account for plant size, which showed that [CO₂], *per se*, had negligible effects on the BAR of individual plants (Poorter & Nagel, 2000). Our results may be partially explained by the effects of [CO₂] on soil moisture. The decrease in BAR observed under elevated [CO₂] was concomitant in most cases with a significant increase in soil moisture at harvest (data not shown). Decreases in water availability not only promote a decrease in water uptake per unit of root mass, but also a reduced nutrient uptake because of the delivery of nutrients by mass flow is impeded in dry soils (Marschner, 1995). These factors are expected to increase BAR values, a view supported by Poorter & Nagel (2000).

Studies evaluating the effects of species evenness on the productivity of grassland assemblages have found that these range from negative to positive, and that they are largely dependent upon species-specific traits (Wilsey & Potvin, 2000; Polley *et al.*, 2003; Mulder *et al.*,

2004). Our results agree with these findings, as the magnitude and direction of the observed effects of species evenness on productivity (either negative, neutral or positive) were dependent on the identity of the dominant species in the mixtures. They also indicate that these effects may be modulated by nutrient availability, a response not reported previously. It has been suggested that positive effects of species evenness are mediated by greater complementarity in resource use (Mulder *et al.*, 2004). Albeit we found higher [N] under maximum evenness in the *Holcus*-dominated mixtures, our results link the observed responses with patterns of biomass allocation. When *Lolium* was the dominant species in mixtures – the only situation where increasing evenness led to an increase in biomass –, the assemblages allocated less biomass to roots with increasing species evenness. Such a response should have reduced belowground competition (Aerts *et al.*, 1991), and this amelioration could have promoted the increase in aboveground biomass observed in the 1:1:1 mixtures.

As observed with species evenness, other main effects and interactions were dependent on the species dominating the 4:1:1 mixtures. Thus, our second hypothesis, (i.e. the interactions among the factors evaluated will depend on the identity of the species whose density is varied in the 4:1:1 mixtures), was also supported by our results. In terms of biomass production, the species evaluated here have been shown to respond positively to elevated CO₂ (Poorter, 1993), nutrient heterogeneity (Grime & Mackey, 2002) and increases in nutrient availability (Kirkham *et al.*, 1996). However, they differ in the magnitude of their responses, as well as in key competitive attributes such as relative growth rate (RGR, Grime & Hunt, 1975). These differences may help to explain some of the responses we observed. For instance, the positive effects of species evenness observed when *Lolium* was dominant in the 4:1:1 mixtures may be partially explained by the differences in RGR between species (*Lolium* has a lower RGR than *Holcus* and *Plantago*, Grime & Hunt, 1975). It has been shown that productivity responses of assemblages to elevated CO₂ and nutrient availability may depend on species richness and composition (Reich *et al.*, 2001; He *et al.*, 2002). Available evidence indicates that individual species are key determinants of assemblage responses to global change factors, especially if they have the ability to fix atmospheric nitrogen (Craine *et al.*, 2003). Our results also point in this direction, suggesting that species composition is a relevant factor to fully disentangle the multiplicity of potential responses of plant assemblages to global change.

The importance of interactive responses and the need to utilize multifactorial experimental approaches to

understand the biological consequences of global change has been stressed in recent years (Körner, 2001; Norby & Luo, 2004). However, the interactions among global change drivers and intrinsic ecosystem features (such as nutrient heterogeneity) have only begun to be explored (Arnone, 1997; Maestre *et al.*, 2005). Our microcosm results – using model grassland communities – have shown that nutrient heterogeneity interacts with several global change drivers ([CO₂], species diversity, nutrient availability) to determine key biomass and nutrient responses. The extrapolation of these results to the natural world must be, however, done with caution because of the following limitations of our approach: (i) growing assemblages in microcosms may alter root foraging responses due to physical restriction of lateral root growth; (ii) patterns of nutrient heterogeneity and degree of contrast to the background soil may not reflect those found in the field; and (iii) standardized climatic conditions may amplify plant responses to soil nutrient availability and heterogeneity over those that may be observed in the field. Nevertheless, we are confident that our findings demonstrate potential plant responses to simultaneous changes in nutrient heterogeneity and global change drivers (Jones *et al.*, 2000). We suggest that future studies should explicitly consider nutrient heterogeneity when evaluating plant responses to single and multiple global change drivers, especially if the species under study are known to respond to this heterogeneity.

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