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Earthworms modify plant biomass and nitrogen capture under conditions of soil nutrient heterogeneity and elevated atmospheric CO_2 concentrations

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ABSTRACT

Earthworms modify the way roots respond to soil nutrient patchiness. However, few studies have evaluated the joint effects of earthworms and soil heterogeneity on plant community biomass and species dominance, and none of them have assessed the influence of different patch features and environmental conditions on such effects. We evaluated how soil nutrient heterogeneity, earthworms (*Eisenia fetida*), organic material quality (^{15}N-labelled leaves and roots of contrasting C: N ratios) and elevated atmospheric CO_2 concentrations (phytotron chambers) affected the resource-use strategy, biomass and species dominance of mixtures formed by *Lolium perenne* L. and *Plantago lanceolata* L. Soil heterogeneity decreased N capture from the organic material, especially in the presence of earthworms. Mixtures experienced a 26 and 36% decrease in shoot and root biomass when earthworms were added to the heterogeneous microcosms, but only with high quality organic material. The dominance of *L. perenne* was lower under conditions of elevated CO_2, nutrient heterogeneity and earthworms. Our data suggest that earthworms can neutralize positive plant growth responses to soil heterogeneity by exacerbating decreases in the supply of N to the plant. Specifically, earthworms foraging for high quality patches may stimulate microbial N immobilization, translating into lower N capture by plants. Increases in casting activity under elevated CO_2, and hence in microbial N immobilization, may also explain why earthworms modulated the effects of soil heterogeneity and CO_2 concentrations on plant community structure. We show that earthworms, absent from most soil nutrient heterogeneity studies, mediate plant biomass responses to nutrient patchiness by affecting N capture. Future plant-foraging behaviour studies should consider the roles played by soil engineers such as earthworms, so that results can be better extrapolated to natural communities.

1. Introduction

The distribution of nutrients in soils is naturally heterogeneous at multiple spatial scales (Jackson and Caldwell, 1993; Farley and Fitter, 1999). Soil nutrient heterogeneity promotes a variety of plant responses, including morphological (e.g., root proliferation into nutrient patches; Hutchings and de Kroon, 1994) and physiological adjustments (e.g., changes in nutrient uptake kinetics; Jackson et al., 1990). These individual responses have performance implications, which are well addressed in the root foraging literature, both at the population (Day et al., 2003; Maestre and Reynolds, 2006a) and community (Wijesinghe et al., 2005; García-Palacios et al., 2011) levels. However, most previous research on soil heterogeneity has concentrated on the addition of inorganic patches of phosphate or nitrate (Hodge, 2004), whose patterns of nutrient release differ markedly to those of organic patches. Indeed, organic patches generally require degradation by soil biota before the nutrients become plant available (Fransen and

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de Kroon, 2001). Soil ecosystem engineers, such as earthworms, may then be important drivers of plant growth responses to organic nutrient patchiness because their actions directly redistribute patch material through the soil and indirectly affect patch decay because they change the environment and hence activities of decomposer microbes (Kreuzer et al., 2004; Lavelle et al., 2006; Bradford et al., 2007).

Earthworms are one of the most conspicuous groups of detritivores in terrestrial ecosystems. Their high consumption rates and burrowing activity profoundly modify the physical structure of soils and the rate at which soil microbes carry out important ecosystem processes such as N mineralization (Willems et al., 1996; Lavelle et al., 1997; Eisenhauer, 2010). Earthworms also influence root architecture and growth (Setälä and Huhta, 1991) – metrics that strongly influence N capture by plants from nutrient patches – potentially through root feeding and their effects on the distribution of patches. Together these effects of earthworms may play a key role modulating nutrient availability from organic patches, and therefore ultimately plant growth. Yet earthworms have been excluded from most soil heterogeneity studies, probably because the majority has used artificial growing media or potting soil (Hodge, 2004). To our knowledge, only a few studies have evaluated the joint effects of earthworms and nutrient heterogeneity on plant biomass (Bonkowski et al., 2000; Wurst et al., 2003; Kreuzer et al., 2004; Bradford et al., 2007), and none of them have assessed how different patch features and environmental conditions modify these effects.

The quality of the organic patch material (e.g., lignin: N and C: N ratios) may be particularly relevant in determining plant responses to soil heterogeneity, as it largely determines the rate of decomposition (Cornwell et al., 2008), and strongly regulates the effects of soil fauna on litter decay (García-Palacios et al., 2013). For example, the activity of earthworms may change in response to patches of contrasting qualities, as they selectively forage for high quality organic matter (Bradford et al., 2007). Thus, earthworms may particularly influence plant responses to patches of high quality because they redistribute them more rapidly, reducing the opportunity for roots to capture nutrients from the patch. The patch quality may also interact with abiotic environmental factors affecting the traits that determine the plant resource use strategy (García-Palacios et al., 2012). The rise in the atmospheric concentration of CO_2 is a major driver of ongoing global environmental change (IPCC, 2013). Elevated CO_2 concentrations have been found to alter plant nutrient uptake responses to soil nutrient heterogeneity, which in turn changes plant dominance (Maestre et al., 2005; Maestre and Reynolds, 2006b). Earthworms could also modify such effects because these soil engineers have been shown to influence plant biomass responses to nutrient heterogeneity and elevated CO_2 separately (Wurst et al., 2003; Arnone et al., 2013). Thus, our broader understanding of the ecological consequences of soil heterogeneity is hindered by the limited evaluation of the combined effects of earthworms, patch quality and projected global change scenarios.

Using a microcosm approach, natural soil and ^{15}N-labelled organic material, we examined the simultaneous effects of soil nutrient heterogeneity, earthworm presence, organic material quality and elevated atmospheric CO_2 on plant resource-use strategy, biomass and species dominance in mixtures formed by *Lolium perenne* L. and *Plantago lanceolata* L. We tested the following two hypotheses: i) Earthworms reduce plant biomass under heterogeneous nutrient distribution through reduction in a set of morphological (root foraging precision) and physiological (N capture) plant responses caused by earthworm preemptive use of nutrient patches. The decrease in plant biomass will be highest with high (versus low) quality nutrient patches because earthworms selectively forage for these (Bradford et al., 2007); and ii) elevated CO_2-induced increases in earthworm activity (Arnone et al., 2013) alter plant dominance in the mixtures when nutrient are heterogeneously distributed. The dominance of the more competitive species *L. perenne* – which is usually favoured by nutrient heterogeneity under elevated CO_2 (Maestre et al., 2005) – will therefore be reduced when earthworms are present under these conditions.

2. Materials and methods

The experiment was conducted at the Duke University Phytotron. The design consisted of a fully-crossed combination of two levels of spatial distribution of the organic material (NH: homogeneous and heterogeneous), earthworms (E: with and without), organic material quality (NQ: low and high) and atmospheric CO_2 concentration (ambient and elevated). *L. perenne* and *P. lanceolata* seeds were used to form two-species plant mixtures. Both species commonly co-occur in semi-natural temperate grasslands (Fowler and Antonovics, 1981), and have been previously employed in soil heterogeneity studies evaluating the interaction with earthworms (Wurst et al., 2003) and elevated CO_2 (Maestre et al., 2005).

2.1. ^{15}N-labelled organic material

We obtained ^{15}N-labelled organic material by growing *L. perenne* from seed in microcosms made out of a section of PVC pipe (length 40 cm, internal diameter 10 cm) and filled with 3: 1 sand: vermiculite mix. Seeds were germinated in a dark room on agar plates, and three days after germination were planted into the microcosms (7 seedlings per tube). These were then placed in a walk-in growth chamber that was maintained at day/night air temperature of 21/16 °C, PAR of 1000 μmol m^{-2} s^{-1} with a 16 h photoperiod, atmospheric CO_2 partial pressure of 35 Pa, and an average relative humidity of 80%. Each microcosm unit was irrigated to free drainage with distilled water twice a day, and received a modified ½ strength Hoagland's solution containing 800 mg L^{-1} of $^{15}NH_4^{15}NO_3$ (5 atom% ^{15}N, Isotec, Miamisburg, USA) twice a week for eight weeks. After eight weeks, the plants were harvested and the roots and shoots were dried at 60 °C. For the experiment, we used finely cut (2 mm length) green shoots (5.30 atom% ^{15}N, 2.88% N, 14.6C:N) and roots (4.30 atom% ^{15}N, 0.88% N, 45.2C:N) to provide nutrient patches of high and low quality for decomposition, respectively.

2.2. Microcosm setup

Plants were grown in microcosms made out of sections of PVC pipe (length 38 cm, internal diameter 10 cm) closed at the bottom by caps with a 5 mm diameter hole to allow drainage (see Maestre and Reynolds, 2007 for details). At 15 cm from the top of the pots we placed two 31-cm^3 open plastic cylinders (hair rollers with length and internal diameter of 75 and 23 mm, respectively, and a light mesh of 5 × 10 mm) separated from each other by 2 cm. The cylinders were introduced to measure root proliferation, and to enable the placement of the organic material in the heterogeneous treatments (see below). All the microcosms were filled as follows (from the bottom to the top): a 5-cm layer of gravel, a 28-cm layer (corresponding to 2.38 L) of a 50: 50 mixture of mineral soil: sand (hereafter background soil), and a 2-cm layer of a 50:50 mixture of organic soil: peat. Both the mineral (a nutrient-poor sandy loam soil) and organic soils were obtained from the Duke Forest two months prior to the beginning of the experiment. The mineral soil was a low-fertility, sandy loam classified as an ultisol-type soil. Total percentage of soil C, N and P were 1.20 ± 0.049, 0.05 ± 0.002 and 0.01 ± 0.002, respectively (mean ± 1 SE; $n = 8$; Bradford et al.,

2008). The soil texture was classified as loamy sand, with 15% silt and 5% clay; base saturation was 16% and CEC (meq 100 g dry soil^{-1}). Before mixing the mineral and organic soils with sand and peat, respectively, we steam-treated the soil (two 2 h treatments at 75 °C) to kill all macrofauna. After sterilization, we leached the soils for three weeks with distilled water to minimize any nutrient pulse associated with the steaming. To re-introduce any microbial species, and some microfauna (e.g. Protozoa, nematodes), killed during steaming, all the microcosms were irrigated with 100 mL of a soil solution. To obtain this solution, 3 kg of fresh soil from the turf communities surrounding the Phytotron (dominated by *Trifolium repens* L., *P. lanceolata* and *Anthoxanthum odoratum* L.) were introduced in 30 L of water. The resulting solution was agitated every 8 h for 2 days, filtered with a 106 μm sieve and added to the microcosms before the placement of the organic soil.

Each microcosm received either 1.59 g of *L. perenne* shoots (high quality of the organic material) or 5.22 g of *L. perenne* roots (low quality of the organic material). In both cases, the amount of N added per microcosm was the same (*ca.* 46 mg), and thus we maintained the same overall N availability but contrasted organic material quality (C: N ratio). Within each quality treatment, the organic material was distributed either as a patch (heterogeneous level) or homogeneously in the microcosm. In the patch treatment, one of the cylinders was filled with a mix of background soil and organic material. The other cylinder (control) was filled with the background soil. In the homogeneous treatment, the organic material was mixed with the background soil throughout the entire microcosm volume. Two cylinders were also placed into the homogenous treatment but, in contrast to the heterogeneous treatment, the organic material-soil mix was equivalent between both cylinders and also to the surrounding soil.

Earthworms (*Eisenia fetida* Savigny) were purchased from a commercial supplier (Happy D Ranch, Visalia, CA, USA) and were maintained in background soil for one month prior to the start of the experiment. Three adult individuals of *E. fetida* were added to half of the microcosms (E treatment level). Mean live weight (including gut content) of the earthworms added at the start of the experiment was 0.97 ± 0.16 g (mean ± SD, $n = 128$), and did not differ among CO_2, heterogeneity and quality treatments (ANOVA, $p > 0.166$ in all cases).

Seeds from *P. lanceolata* and *L. perenne* were obtained from commercial suppliers (V and J Seed Service, Woodstock, IL and Granite Seed Company, Lehi, UT, respectively). Seeds were placed in trays with plant growing medium (Metro-Mix® 200, Scotts Company, Ohio) and germinated in a growth chamber (20 °C temperature and PAR of 600 μmol m^{-2} s^{-1} with a 14 h photoperiod). Due to different germination times, the different species were germinated on different days to ensure that both species had a similar size at the start of the experiment. Three uniformly-sized seedlings of each species were transplanted into the microcosms. The planting positions for 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.

We established 4 microcosms for each of the 16 treatment combinations, resulting in 64 microcosms (2 nutrient heterogeneity levels × 2 earthworm levels × 2 nutrient quality levels × 2 CO_2 levels × 4 replicates). These were introduced in four walk-in growth chambers (two for each CO_2 level), within which air temperature and atmospheric CO_2 were independently controlled. Within each CO_2 level (ambient: 37.5 Pa and elevated: 70 Pa), half of the microcosms (16) were randomly assigned to one of the chambers, and then were randomly grouped in two trolleys. The position of the trolleys within each chamber was interchanged once a week. The chambers were maintained at a daily temperature cycle ranging from 12 (dark) to 21 °C (full light), PAR of 900 μmol m^{-2} s^{-1} with a 16 h photoperiod, and an average relative humidity varying from 70 to 85% during the day. The lights turned on gradually during the morning and shut off gradually during the evening to simulate daily changes in radiation. Similarly, temperature and relative humidity were increased and decreased gradually during each day to simulate normal daily variation in these variables. Each microcosm was irrigated daily with 30 mL of distilled water. To promote the formation of mycorrhizae in the introduced seedlings, all the microcosms were watered with 30 mL of a 106-μm sieved solution containing root washes from the turf communities surrounding the Phytotron (described above) 7 and 15 days after planting.

2.3. Harvest

From the planting to the final harvest, the experiment lasted 101 days. Shoot biomass was cut at the soil surface and sorted by species. Leaves and stems were dried at 60 °C. After aboveground harvesting, the soil was carefully removed from the microcosm unit and the roots were harvested and dried at 60 °C. They were so large and entangled that it was impossible to separate them by species. To measure root proliferation, we extracted the roots within each cylinder by cutting those outside it. Earthworms were collected by hand, counted and freeze-dried. No earthworms were recovered from four of the microcosms to which we had added them, either because they escaped from the PVC pipes (F.T. Maestre, *personal observation*) or died during the experiment. Data from these four microcosms were not used in the statistical analysis of earthworm growth, reducing the replication number from four to three for some treatments.

Precision of root foraging of the plant mixtures was measured with the RII index (Armas et al., 2004). In the heterogeneous treatments, 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. RII ranges from −1 to +1, a value of zero indicates equal root growth in patches and background soil, and no precision of foraging. Positive values indicate increasing precision. In the homogeneous treatment, the cylinders selected as the patch cylinder were located in the same position as the patch cylinders in the heterogeneous treatments.

2.4. Measurement of N capture

Plant N capture from the organic material added was determined in all the microcosms at the mixture level. After drying, leaves from the two species were randomly selected for isotopic ($\delta^{15}N$) and elemental (total N content) analyses. Leaves were ground to a fine powder and transferred into 9 × 5 mm tin capsules and injected into an elemental analyzer (Costech CHN Analyzer, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan Delta Plus Mass Spectrometer, Bremen, Germany) via a Conflo Interface (Finnigan, Bremen). Foliar samples were analysed for each species separately. The atom% ^{15}N excess was calculated by subtracting 0.366 as the atmospheric background (Maestre et al., 2005). N capture was estimated as the percentage of the N originally added which was incorporated by each species according to the equation: [(mg enriched ^{15}N in the foliar tissue)/(mg enriched ^{15}N in the organic material added)]*100 (Hodge et al., 2000a). We summed the species-level data to determine the N capture at the plant mixture level (NCA hereafter).

2.5. Statistical analyses

The effects of treatments on shoot and root biomass, root foraging precision (RII Index), N capture (NCA) and leaf N content at

the mixture level were analysed using a five-way nested ANOVA model. The model included CO_2 concentration (CO_2), nutrient heterogeneity (NH), earthworms (E) and organic material quality (NQ) as main fixed effects, chamber number as a random factor nested within CO_2, and all the interactions between the fixed factors. In this model, the effect of CO_2 (1 df) was tested against the effect of chamber nested within CO_2 (2 df); the other main effects and interactions (1 df) were tested against the residual error (46 df). The same ANOVA model was used to analyse the effects of treatments on the proportion of the shoot biomass accounted for by the dominant species. Separate ANOVAs were conducted at each treatment level to facilitate the interpretation of three- and four-way significant interactions. The effects of NH, NQ and CO_2 on earthworm growth, evaluated as the difference in weight of the earthworms (freeze-dried weight at harvest − estimated initial freeze-dried weight) were evaluated with a four-way nested ANOVA (with CO_2, NH and NQ as main fixed effects and chamber number as a factor nested within CO_2). Data on N capture were log-transformed to meet ANOVA assumptions. Analyses were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). The data from this experiment are available from figshare (Maestre et al., 2014).

3. Results

3.1. Earthworm population and individual biomass

About 60% of the earthworms added were recovered at the end of the experiment, with an average biomass decrease per microcosm of 46% (Fig. 1). This reduction in biomass, however, did not differ significantly across treatments (main and interaction effect significance values: $p > 0.190$).

3.2. Root foraging precision and N capture

Precise root foraging patterns ($p < 0.001$) were demonstrated when the organic material was supplied heterogeneously (Fig. 2). However, the intensity of this NH effect was significantly modulated by the other three treatments (Table 1). A further ANOVA conducted at each NH level to investigate the 4-way interaction found revealed a significant CO_2 × NQ interaction ($F_{1,22} = 5.91$; $p = 0.024$) in heterogeneous conditions. In ambient CO_2 settings, high *vs.* low quality patches promoted precise root foraging (Fig. 2). The opposite effect of organic material quality was found under elevated CO_2. The RII Index was not obviously affected by earthworms, and under homogeneous nutrient supply it was not modified by the other treatments.

The decrease in N uptake found under heterogeneous conditions (Fig. 3) was larger when the patches were of low quality (63% decrease in NCA compared to the homogeneous level), which presumably caused the significant NH × NQ interaction (Table 1). The NH × E interaction appeared driven by the fact earthworms emphasized the decrease in N capture by the plant mixture from a patch. Compared to homogenous nutrient conditions, N capture in the heterogeneous treatment was 35% lower from patches in the presence of earthworms, but only 15% lower when earthworms were absent (Fig. 3).

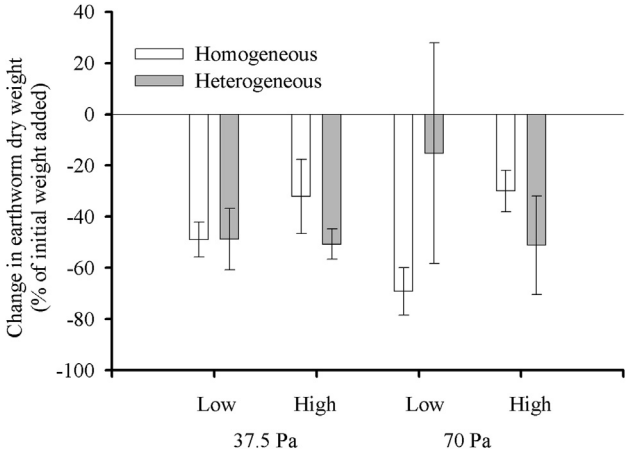

Fig. 1. Change in freeze-dry weight (as % of the initial weight added) of earthworms compared across atmospheric CO_2 concentration (CO_2: 37.5 *vs.* 70 Pa), nutrient quality (NQ: low *vs.* high) and nutrient heterogeneity (NH: homogeneous *vs.* heterogeneous). Values are means ± SE ($n = 4, 4, 4, 3, 3, 3, 4$ and 3 for the bars from left to right). The decrease in earthworm biomass was not significantly affected by any treatment (CO_2: $F_{1,2} = 4.00$; $p = 0.184$, NH: $F_{1,22} = 0.76$; $p = 0.391$, NQ: $F_{1,22} = 0.43$; $p = 0.519$; no interactions).

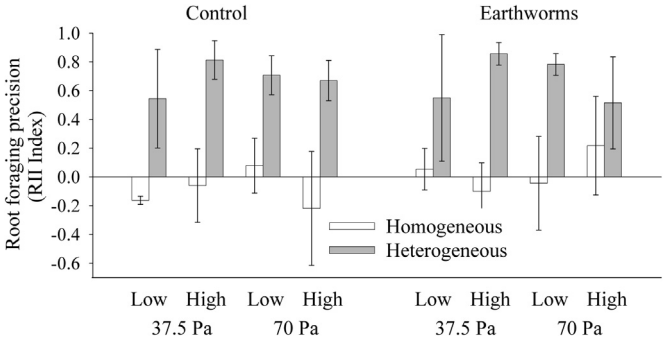

Fig. 2. Root foraging precision of mixtures into nutrient patches (RII index) compared across atmospheric CO_2 concentration (CO_2: 37.5 *vs.* 70 Pa), earthworms (E: control *vs.* earthworms), nutrient quality (NQ: low *vs.* high) and nutrient heterogeneity (NH: homogeneous *vs.* heterogeneous) levels. Positive values of the RII index indicate precise root biomass proliferation into the nutrient patch (see Materials and Methods for details on its calculation). Values are means ± 95% confidence intervals ($n = 4$). Significant root foraging patterns are indicated by confidence intervals that do not overlap 0.

Table 1
Summary results of the full ANOVA model showing the effects of CO_2 concentration (CO_2), nutrient heterogeneity (NH), earthworms (E) and organic material quality (NQ) on the root foraging precision (RII), percentage of N added as organic material captured (NCA), shoot and root biomass, and leaf N concentration (leaf N) at the mixture level. Values represent the *F* statistic. $^{*}p < 0.05$,$^{**}p < 0.01$,$^{***}p < 0.001$. Chamber number (random factor) was not included in the table as it only has interest for *F* calculations of the CO_2 effect; the other main effects and interactions were tested against the residual error.

Source of variation (d.f.)	RII	NCA	Shoot biomass	Root biomass	Leaf N
CO_2 (1,2)	0.28	0.00	1.41	0.02	2.15
NH (1, 46)	111.79***	28.90***	0.76	0.90	3.44
E (1, 46)	0.73	0.59	9.99**	5.59*	0.92
NQ (1, 46)	0.12	122.24***	8.12**	3.77	16.16***
CO_2 × NH (1, 46)	0.53	0.05	0.14	3.22	2.24
CO_2 × E (1, 46)	0.00	1.88	0.00	0.04	8.62**
CO_2 × NQ (1, 46)	2.60	2.91	3.15	1.70	0.49
NH × E (1, 46)	0.94	5.66*	7.46**	3.49	2.06
NH × NQ (1, 46)	0.45	11.28**	1.89	0.04	6.72*
E × NQ (1, 46)	0.04	0.10	1.19	6.29*	0.28
CO_2 × NH × E (1, 46)	0.25	0.34	1.23	0.04	0.34
CO_2 × NH × NQ (1, 46)	2.78	1.32	0.15	1.51	2.67
CO_2 × E × NQ (1, 46)	1.04	0.04	0.46	0.84	0.00
NH × E × NQ (1, 46)	0.84	1.91	5.25**	6.71*	0.18
CO_2 × NH × E × NQ (1, 46)	4.07*	2.19	1.91	0.17	0.00

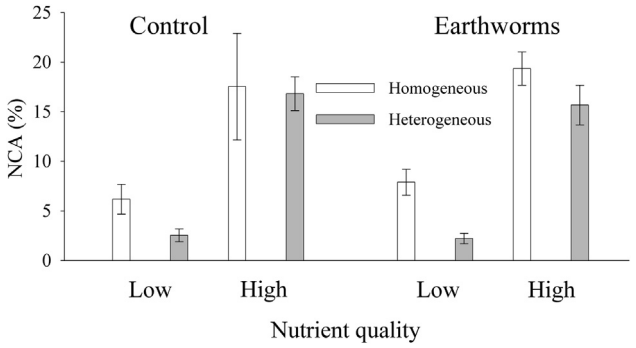

Fig. 3. Percentage of N added as organic material captured by the plant mixtures (NCA) compared across earthworms (E: control *vs.* earthworms), nutrient quality (NQ: low *vs.* high) and nutrient heterogeneity (NH: homogeneous *vs.* heterogeneous) levels. Data from the two CO_2 concentrations were pooled to highlight the significant NH × E and NH × NQ interactions. Values are means ± 1 SE ($n = 8$).

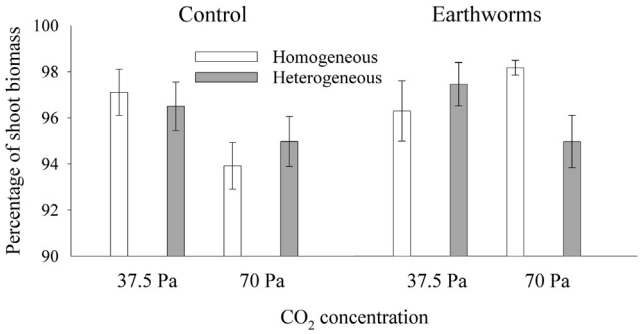

Fig. 5. Percentage of mixture shoot biomass accounted by *Lolium perenne* compared across atmospheric CO_2 concentration (CO_2: 37.5 *vs.* 70 Pa), earthworms (E: control *vs.* earthworms) and nutrient heterogeneity (NH: homogeneous *vs.* heterogeneous) levels. Data from the two nutrient quality levels were pooled to highlight the significant CO_2 × E × NH interaction. Values are means ± 1 SE ($n = 8$).

3.3. Shoot and root biomass at the plant mixture and species level

The overall effects of earthworms on mixture shoot and root biomass (15 and 20% increase, respectively, Fig. 4) were dependent on nutrient heterogeneity and quality, as significant NH × E × NQ interactions were found for both variables (Table 1). These interactions were investigated by conducting separate ANOVAs for each NQ level. The E × NH interaction was significant only with high quality organic material for shoot and root biomass ($F_{1,22} = 5.17$; $p = 0.033$, $F_{1,22} = 12.73$; $p = 0.002$, respectively). Reductions of 26 and 36% in shoot and root biomass were observed, respectively, when earthworms were present with high nutrient quality patches (Fig. 4).

L. perenne was the dominant species in the mixture, accounting for an average of 96% of the shoot biomass (Fig. 5). A significant CO_2 × E × NH interaction was found with regard to the percentage of the shoot biomass accounted for by this species ($F_{1,46} = 8.78$; $p = 0.005$). When the former three-way interaction was investigated only in the presence of earthworms, we found that CO_2 conditions modulated the effects of soil heterogeneity, with a slight but statistically significant ($F_{1,22} = 6.61$; $p = 0.005$) decrease (3%) in the dominance of *L. perenne* under elevated CO_2.

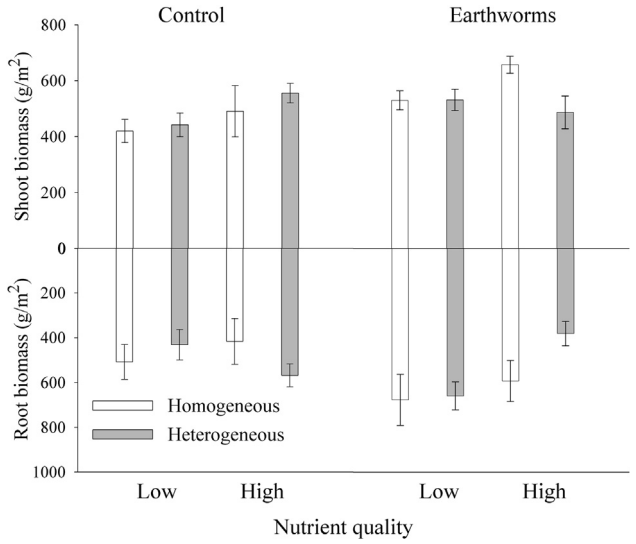

Fig. 4. Shoot and root biomass of the mixtures compared across earthworms (E: control *vs.* earthworms), nutrient quality (NQ: low *vs.* high) and nutrient heterogeneity (NH: homogeneous *vs.* heterogeneous) levels. Data from the two CO_2 concentrations were pooled to highlight the significant NH × E × NQ interaction. Values are means ± 1 SE ($n = 8$).

3.4. Plant nutritional responses

The effects of NH on leaf N concentration at the mixture level were dependent on nutrient quality, as indicated by the significant NH × NQ interaction (Table 1). Leaf N decreased (by 13%) only when heterogeneous nutrient supply co-occurred with high quality organic material (Fig. 6). We also found a significant CO_2 × E interaction ($p < 0.01$), indicating that higher leaf N was found in the presence of earthworms and ambient CO_2 conditions.

4. Discussion

4.1. Earthworms counteract the positive performance effects of nutrient heterogeneity under high nutrient quality levels

Positive effects of soil heterogeneity on plant biomass have been shown at the species (Bilbrough and Caldwell, 1997), population (Day et al., 2003; Maestre and Reynolds, 2006a) and community (Wijesinghe et al., 2005; García-Palacios et al., 2011) levels. In contrast to these observations, under conditions of high quality organic material, we found lower shoot and root biomass in heterogeneous versus homogenous treatments when earthworms were present. Specifically, heterogeneity increased shoot and root

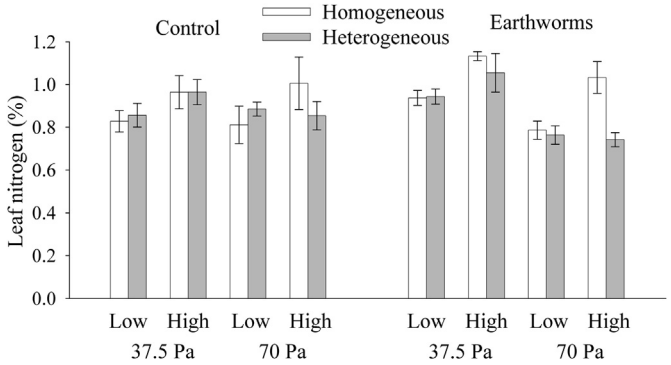

Fig. 6. Leaf N concentration of plant mixtures compared across atmospheric CO_2 concentration (CO_2: 37.5 *vs.* 70 Pa), earthworms (E: control *vs.* earthworms), nutrient quality (NQ: low *vs.* high) and nutrient heterogeneity (NH: homogeneous *vs.* heterogeneous) levels. Values are means ± 1 SE ($n = 4$).

biomass (13 and 36%, respectively) in the absence of earthworms, but the opposite response was found when they were present (26 and 36% reduction, respectively). Our observations therefore indicate that current expectations of plant responses to soil nutrient heterogeneity may only hold in the absence of earthworms. Furthermore, in line with our first hypothesis, patch nutrient quality seems an important determinant of how earthworms influence plant responses to nutrient heterogeneity, presumably because earthworms selectively feed on higher quality materials (Smith and Bradford, 2003; Bradford et al., 2007) and so caused marked reductions in plant shoot and root biomass when the patches were of high quality. Our results argue for a reconsideration of individual and community productivity responses to soil heterogeneity that explicitly incorporates soil engineers, such as earthworms, and patch quality.

The shoot and root biomass patterns matched the N capture responses to soil heterogeneity and earthworms, at least with high quality patches. This match is supported by the correlation between NCA and shoot biomass ($r = 0.575$; $p < 0.001$; $n = 64$), suggesting that the reduction in N capture in the earthworm and soil heterogeneity microcosms could explain at least some of the response of NCA and shoot biomass. This result is important because it indicates that root proliferation into nutrient patches can be disadvantageous for plant performance in the presence of earthworms. We suggest the following mechanism to explain this finding: earthworms distribute material away from the patch and also enhance microbial activity and biomass through their burrowing and the secretion and excretion of highly enriched products (Lavelle, 1997). The resulting reduction in patch material and stimulation of microbial activity by earthworms may then enhance microbial N immobilization, intensifying N competition between plants and soil microorganisms (reviewed by Hodge et al., 2000b) and leading to reductions in plant N capture from the nutrient patch. Thus, the ability of earthworms to selectively forage for high quality organic material may enhance the influence of microbial N immobilization in patches of high, but not low, quality organic material. The larger decrease in leaf N concentration under heterogeneous conditions with high quality patches (low C: N ratio) supports this interpretation. The potential for earthworms to mediate effects of soil heterogeneity on plant biomass has been demonstrated previously (Wurst et al., 2003; Kreuzer et al., 2004) but, to our knowledge, ours is the first study to experimentally show how this role may depend on nutrient patch quality.

4.2. Earthworms mediate the joint effect of soil nutrient heterogeneity and elevated CO_2 on plant species dominance

The dominance of *L. perenne* over *P. lanceolata* in model grassland communities such as those evaluated here has been previously documented (Maestre and Reynolds, 2007). In fact, in a very similar experimental design addressing the effects of soil heterogeneity, Maestre et al. (2005) found that soil heterogeneity decreased and increased the dominance of *L. perenne* in ambient and elevated CO_2 conditions, respectively. Our findings replicated these results, but only when earthworms were absent from the microcosms. Earthworms therefore have the potential to mediate plant responses to soil heterogeneity and elevated CO_2, because when they were included in the microcosms, soil heterogeneity increased and decreased the dominance of *L. perenne* in ambient and elevated CO_2 conditions, respectively. The shifts in dominance were, admittedly, minor in magnitude (2–4% absolute change). Yet they demonstrate the potential for earthworms to modify effects of elevated CO_2 and nutrient patchiness on plant community composition, even when average species dominance is higher than 90%. We hypothesized that *L. perenne* would show the observed decreases in dominance because of greater N limitation through higher casting. Earthworm cast production increases in grasslands exposed to elevated atmospheric CO_2 (Arnone et al., 2013), enhancing microbial N immobilization and therefore decreasing the dominance of fast-growing species such as *L. perenne*. Our findings indicate that earthworms can mediate the effects of soil heterogeneity on species competitive ability and plant community structure (Wurst et al., 2003; Kreuzer et al., 2004), especially when addressing interactions with global change drivers such as elevated CO_2. The potential for earthworms to modify outcomes might have been underestimated in previous studies evaluating soil heterogeneity and CO_2 conditions (Arnone, 1997; Maestre et al., 2005). Our results suggest that soil fauna should be considered when assessing the interaction between nutrient patchiness and global change drivers (García-Palacios et al., 2012).

4.3. Strengths and caveats of the approach followed

The experimental approach followed allowed us to test how earthworms modulate plant responses to nutrient heterogeneity depending on the patch quality and environmental conditions (atmospheric CO_2 concentration) considered. This complex assessment was performed without the influence of other factors that likely also determine the effects of soil resource heterogeneity in the field (e.g. soil moisture and nutrient availability; Hodge, 2004; García-Palacios et al., 2012); suggesting that further work is required to understand how increasingly complex factor combinations affect the influence of nutrient heterogeneity. However, the use of organic patches and natural soil makes our work ecologically relevant, as nutrient patches in field soils are typically organic and so decomposition processes are a fundamental component of N competition between plants and soil microorganisms. Thus, we can suggest potential mechanisms that, together or alone, could explain the decreases in plant biomass and N capture found under heterogeneous conditions. Measuring the $\delta^{15}N$ in patches, earthworms and microbial biomass would have allowed us to track the fate of N in the soil and the food web to evaluate such hypothesized mechanisms. Future work should test these proposed mechanisms to help inform projections of how plant communities and N dynamics will respond to rising atmospheric CO_2.

Our approach is not without limitations, and extrapolations to the natural world should be made with caution. Pots physically restrict lateral root growth (Fransen et al., 1999) and burrowing by earthworms (Lowe and Butt, 2005). The biomass of the earthworms declined over the course of the experiment, which may have reduced the efficacy of this treatment. As this decrease was homogeneous across the other treatments evaluated, we consider the effect of earthworms on plant biomass and nutritional status, albeit maybe diluted, to have been equal across the microcosms containing earthworms. We cannot explain the reasons for the biomass decrease, but suggest that earthworms may have run out of food during the course of the experiment (101 days), as found in previous soil nutrient heterogeneity studies (Wurst et al., 2003). In addition, earthworm species other than the one used in this study (*E. fetida*) may interact with the organic patches differently. *E. fetida* is commonly found in compost and organic soils (Sims and Gerard, 1985), and different species from contrasting habitats and functional groups may promote different patterns of plant N availability (Butenschoen et al., 2009). Hence, similar experiments need to be conducted with earthworm species that exhibit endogeic and anecic behaviours.

4.4. Conclusions

Our results show that earthworms modify plant responses to nutrient heterogeneity, but not necessarily for all variables (e.g.

they affected N capture but not root proliferation), and not necessarily under all environmental contexts (e.g. they affected plant biomass only with high quality patches and species dominance only under elevated CO_2 conditions). To be predictive, experimental heterogeneity studies should take into account the effects of earthworms (Bradford et al., 2007) and also recognize that apparently contradictory effects of soil fauna may simply be a product of environmental context. Our work demonstrates the role of soil engineers in shaping root foraging behaviour and hence the ecological consequences of nutrient heterogeneity and how it might interact with global change drivers.

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