Journal of Ecology

British Ecological Society

Journal of Ecology 2011, **99**, 551–562 doi: 10.1111/j.1365-2745.2010.01765.x

Soil nutrient heterogeneity modulates ecosystem responses to changes in the identity and richness of plant functional groups

Pablo García-Palacios[1,2]*, Fernando T. Maestre[1] and Antonio Gallardo[3]

[1]*Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, 28933 Móstoles, Spain;* [2]*Instituto de Recursos Naturales, Centro de Ciencias Medioambientales, CSIC, C/Serrano 115-bis, 28006 Madrid, Spain; and* [3]*Departmento de Física, Química y Sistemas Naturales, Universidad Pablo de Olavide, 41013 Sevilla, Spain*

Summary

1. Recent research has shown that biodiversity may have its greatest impact on ecosystem functioning in heterogeneous environments. However, the role of soil heterogeneity as a modulator of ecosystem responses to changes in biodiversity remains poorly understood, as few biodiversity studies have explicitly considered this important ecosystem feature.

2. We conducted a microcosm experiment over two growing seasons to evaluate the joint effects of changes in plant functional groups (grasses, legumes, non-legume forbs and a combination of them), spatial distribution of soil nutrients (homogeneous and heterogeneous) and nutrient availability (50 and 100 mg of nitrogen (N) added as organic material) on plant productivity and surrogates of carbon, phosphorous and N cycling (β-glucosidase and acid phosphatase enzymes and *in situ* N availability, respectively).

3. Soil nutrient heterogeneity interacted with nutrient availability and plant functional diversity to determine productivity and nutrient cycling responses. All the functional groups exhibited precise root foraging patterns. Above- and below-ground productivity increased under heterogeneous nutrient supply. Surrogates of nutrient cycling were not directly affected by soil nutrient heterogeneity. Regardless of their above- and below-ground biomass, legumes increased the availability of soil inorganic N and the activity of the acid phosphatase and β-glucosidase enzymes.

4. Our study emphasizes the role of soil nutrient heterogeneity as a modulator of ecosystem responses to changes in functional diversity beyond the species level. Functional group identity, rather than richness, can play a key role in determining the effects of biodiversity on ecosystem functioning.

5. *Synthesis*. Our results highlight the importance of explicitly considering soil heterogeneity in diversity–ecosystem functioning experiments, where the identity of the plant functional group is of major importance. Such consideration will improve our ability to fully understand the role of plant diversity on ecosystem functioning in ubiquitous heterogeneous environments.

Key-words: ecosystem functioning, legumes, plant functional groups, plant resource use strategy, plant–soil (below-ground) interactions, productivity, soil nutrient cycling, soil nutrient heterogeneity

Introduction

The past decade has seen a surge in research on the effects of biodiversity on ecosystem functioning, which has nowadays become a major ecological topic (see Loreau *et al.* 2001; Srivastava & Vellend 2005; Hooper *et al.* 2005 for reviews). The so called 'diversity–ecosystem functioning' debate (Loreau *et al.* 2001; Thompson *et al.* 2005) has elucidated the key role that plant species composition, species richness and functional group richness play in generating diversity effects on ecosystem functioning (Tilman *et al.* 1997), and their potential to independently influence processes such as biomass accumulation (Reich *et al.* 2004), soil nitrogen dynamics (Hooper & Vitousek 1997), or plant–soil feedbacks (Bezemer *et al.* 2006). While plant biodiversity may have its greatest impact on ecosystem functioning in environments where limiting resources

*Correspondence author. E-mail: pablo.palacios@urjc.es

such as nutrients are heterogeneously distributed in space (Cardinale, Nelson & Palmer 2000; Tylianakis *et al*. 2008), few diversity–ecosystem functioning studies have explicitly considered the spatial heterogeneity in the availability of soil resources (hereafter soil heterogeneity; Maestre & Reynolds 2006a, 2007a; Wacker *et al*. 2008). Therefore, little is known on the potential effects of soil heterogeneity as a modulator of ecosystem responses to changes in biodiversity, particularly when these relate to modifications in the diversity of functional groups.

In the natural world, soil heterogeneity is ubiquitous in most ecosystems (Jackson & Caldwell 1993; Gross, Pregitzer & Burton 1995; Ryel, Caldwell & Manwaring 1996; Farley & Fitter 1999). Plants have developed different foraging mechanisms to deal with such heterogeneity, including root proliferation into nutrient patches (Hutchings & de Kroon 1994), changes in nutrient uptake capacity (Jackson, Manwaring & Caldwell 1990) and modifications in biomass allocation (Hutchings, John & Wijesinghe 2003). Through these responses, soil heterogeneity can alter population structure (Day, Hutchings & John 2003) and community dynamics (Wijesinghe, John & Hutchings 2005), and has even the potential to modulate the effects of global change drivers on plant communities (Maestre, Bradford & Reynolds 2005; Maestre & Reynolds 2006a; b). Soil heterogeneity has also been found to increase the slope of the diversity–ecosystem function relationship, suggesting that biodiversity may have its greatest impact on the functioning of diverse, naturally heterogeneous ecosystems (Tylianakis *et al*. 2008). When a diverse array of niches is available through different levels of heterogeneity, complementarity resource use may lead to a positive relationship between diversity and function (Loreau, Mouquet & González 2003; Gross *et al*. 2007). Indeed, the consideration of soil heterogeneity may help to understand and reconcile the discrepancies observed in experiments and observational studies devoted to assess the effects of biodiversity on ecosystem functioning (Jiang, Wan & Li 2009), and such consideration has been advocated to fully understand the importance of biodiversity for ecosystem functioning in real ecosystems (Tylianakis *et al*. 2008).

The number of plant functional groups is considered to be especially important in generating biodiversity effects on ecosystem functioning (Reich *et al*. 2004). Each functional group encompasses a variety of ecophysiological traits and represents a fraction of total functional diversity (McLaren & Turkington 2010). It is well documented that the identity of certain functional groups, such as legumes (Mulder, Uliassi & Doak 2001; Spehn *et al*. 2002), may have higher functional significance than the number (richness) of functional groups (Hooper & Vitousek 1997; Stephan, Meyer & Schmid 2000). In addition, plant functional group diversity and identity are also crucial to improve our understanding of the ecological consequences of soil heterogeneity. The foraging scale–precision hypothesis (Campbell, Grime & Mackey 1991) proposed a trade-off between the spatial scale over which plant species forage and the precision to proliferate roots in nutrient-rich patches as a potential explanation for species coexistence. This influential hypothesis has been tested for a small number of species (Einsmann *et al*. 1999; Wijesinghe, John & Hutchings 2001; Rajaniemi & Reynolds 2004). Recent tests of this hypothesis (Kembel & Cahill 2005; de Kroon & Mommer 2006) have pointed out the necessity of placing foraging ability in the broader context of plant traits and resource economy strategies (Kembel *et al*. 2008). Differences in nutrient uptake strategies between plant functional groups influence the degree and rate of depletion of nutrient-rich patches in heterogeneous environments (Gross *et al*. 2007). This depletion affects nutrient availability for soil microbes and potentially modulates the competition between plants and microbes for them (Wardle 2002). In addition, the depletion of nutrient patches limits the potential long-term benefits of selective root proliferation (Hodge *et al*. 1998; Fransen & de Kroon 2001). Therefore, the study of resource use strategies within the context of plant functional groups can contribute to understanding how root foraging ability affects plant community structure beyond the species level.

Most experiments on the ecological consequences of soil heterogeneity carried out to date have focused on plant responses such as primary productivity (e.g. Wijesinghe, John & Hutchings 2005; Maestre & Reynolds 2007b; Wacker *et al*. 2008) and have barely evaluated its effects on nutrient cycling, even though soil heterogeneity may drive processes like N mineralization (Manzoni, Porporato & Schimel 2008). Recent investigations have shown that the study of individual ecosystem processes in isolation will underestimate the levels of biodiversity required to maintain multifunctional ecosystems (Hector & Bagchi 2007; Zavaleta *et al*. 2010), and thus the analysis of multiple ecosystem functions is being recommended in biodiversity research (Reiss *et al*. 2009).

To our knowledge, no previous study has evaluated how multiple ecosystem processes respond to simultaneous changes in plant functional group diversity, soil heterogeneity and nutrient availability. To address this need, we conducted a common-garden experiment over two growing seasons to evaluate the joint effects of these factors on the resource use strategies of a model plant community, and on ecosystem processes such as primary productivity and nutrient cycling. For the experiment, we used fast-growing plant species occurring in prairie communities of semi-arid Mediterranean regions planted in their natural soil, as this approach permits the interpretation of plant and soil responses to soil heterogeneity in a more realistic context (Hodge 2004). Using this model system, we tested the following hypotheses: (i) soil heterogeneity modulates the influence of plant functional group diversity on productivity and nutrient cycling; we expect strong positive effects of plant functional groups on these ecosystem processes to be particularly evident under heterogeneous conditions of nutrient supply (Tylianakis *et al*. 2008), (ii) soil heterogeneity improves the performance of plant communities and hence the rate of nutrient cycling through an increase in nutrient uptake and root foraging precision (Maestre, Bradford & Reynolds 2005; Wijesinghe, John & Hutchings 2005), and (iii) the identity of plant functional groups will have strong effects on ecosystem functioning; in particular, we expect legumes to enhance nitrogen dynamics (Stephan, Meyer & Schmid 2000; McLaren & Turkington 2010).

Materials and methods

EXPERIMENTAL DESIGN AND GROWING CONDITIONS

We conducted a microcosm common-garden experiment in the plant growth facilities of the Rey Juan Carlos University between February 2008 and June 2009. These facilities are located in Móstoles, in central Spain (40°18′48″N, 38°52′57″W, 632 m a.s.l.). The experiment consisted of three treatments: four levels of plant functional group (PFG) richness (three PFG monocultures and one 3-PFG mixture), two levels of nutrient availability (NA: 50 and 100 mg N added as ^{15}N-labelled *Lolium multiflorum* shoots) and two levels of nutrient heterogeneity (NH: homogeneous and heterogeneous). We established nine replicates for each of the 16 treatment combinations, totalling 144 assemblages.

For the experiment we selected 27 herbaceous perennial plant species naturally occurring in semi-arid Mediterranean roadside slopes and abandoned fields undergoing secondary succession. We assembled three different PFG pools (grasses, legumes and non-legume forbs; hereafter referred to as 'NLFs'), each one containing nine species (Table 1). This functional group classification has been widely used when working with grassland species because it is based on traits that are potentially relevant to the response variables (e.g. biomass production, resource use strategies, N-fixation ability; Reich *et al.* 2001; Gross *et al.* 2007; McLaren & Turkington 2010). The three PFG monocultures were obtained by randomly selecting six different species from each pool. The 3-PFG mixture was created with two randomly selected species of each pool, forming a six-species community. In each treatment combination, the composition of the nine replicates was modified when needed to guarantee that they differed by at least two species. This design allows us to evaluate the effects of PFGs on the response variables independently from the effect of species richness. Seeds of the 27 species were obtained from commercial suppliers (Intersemillas Ltd, Valencia, Spain). Their germination time was tested in a pilot experiment and used to correct the date of sowing. Species were pooled and sowed into three groups according to germination times. The seeds of each species were sown by hand and allocated randomly at a density of 400 seeds m^{-2}. Six weeks after sowing, some individuals were removed to correct species density to a final density of 60 individuals m^{-2} per species. Weeds were regularly removed during the experiment.

Microcosms consisted of PVC pots (depth 33 cm, diameter 24 cm) filled first with 3 cm of sand (for drainage), and then with a 60 : 40 mixture of soil and sand (7600 and 5000 cm^3, respectively). We collected the soil from a roadside slope located in the surroundings of the Rey Juan Carlos University. To minimize interactions with the soil seed bank, the first 5-cm layer of this soil was removed, which in these areas concentrates 95% of all seeds present in the seedbank (Garcia-Fayos *et al.* 1995). The resulting mix of soil (calcareous type, pH = 8.2) and sand (hereafter referred to as 'background soil') had very low fertility (0.143 ± 0.01 mg total N g^{-1} soil and 0.389 ± 0.02 mg total P g^{-1} soil; mean ± SE, $n = 10$). To recreate realistic microbial communities, all the pots were initially irrigated with 500 mL of a soil microbial inoculum (Maestre, Bradford & Reynolds 2005). To obtain this inoculum, 10 kg of fresh soil from the roadside slope (containing species from the three PFG pools) were mixed with 75 L of water. Roots from legume species were collected in the roadside slope and added to the soil microbial inoculum in order to promote the formation of bacteria nodules.

We added ^{15}N-labelled *Lolium multiflorum* shoots (3.84 atom% ^{15}N, 2.23% N, 0.44% P) to each microcosm. This material was synthesized by growing *L. multiflorum* in sterilized sand under greenhouse conditions (16 °C air temperature, 50% average relative humidity, 148 μmol m^{-2} s^{-2} PAR). We used *L. multiflorum* shoots as organic material because of its dominance in the prairie roadside slope where the soil was collected (García-Palacios, personal observation). The growing medium was irrigated twice weekly with a nutritive solution containing 800 mg L^{-1} $^{15}NH4$ $^{15}NO_3$ (5 atom% ^{15}N, Isotec, Miamisburg, OH, USA). After 8 weeks of growth, *L. multiflorum* plants were harvested and their shoots air-dried at 60 °C to constant weight.

The two NA levels (50 mg and 100 mg ^{15}N per microcosm) were obtained by adding 2.24 g and 4.48 g of ^{15}N-labelled *L. multiflorum* shoots, respectively. Within each NA level, the organic material was either added as a patch (heterogeneous treatment) or homogeneously mixed with the background soil along the entire pot volume (homogeneous treatment). In the heterogeneous microcosms, we mixed 100 cm^3 of background soil with the organic material and introduced this mix into a 137 cm^3 plastic cylinder (length 7 cm, internal diameter 5 cm) consisting of a light mesh with square pores 5 × 10 mm in size (see Appendix S1 in Supporting Information). We refer to this as the patch cylinder. A second (control) cylinder, filled only with background soil, was placed 6 cm away from the patch cylinder (Maestre, Bradford & Reynolds 2005). In the homogeneous microcosms, two plastic cylinders were placed within the pot as in the heterogeneous treatments. These patches were filled up with the same mixture of organic material and background soil present in the rest of the homogeneous pot. Cylinders in both homogeneous and heterogeneous treatments were located 10 cm below the soil surface (Appendix S1).

Table 1. Species composition of the three plant functional groups (PFGs) pools used to obtain the four PFG levels (three PFG monocultures and one 3-PFG mixture)

Grasses	Legumes	Non-legume forbs
Dactylis glomerata	*Anthyllis vulneraria*	*Achillea millefolium*
Festuca glauca	*Dorycnium pentaphyllum*	*Dianthus barbatus*
Koeleria glauca	*Lotus corniculatus*	*Gypsophila paniculata*
Lolium perenne	*Medicago lupulina*	*Hypericum perforatum*
Lygeum spartum	*Medicago sativa*	*Inula viscosa*
Pennisetum clandestinum	*Melilotus officinalis*	*Nepeta mussini*
Phleum pratense	*Onobrychis viciifolia*	*Plantago lanceolata*
Poa pratensis	*Trifolium pratense*	*Saponaria ocymoides*
Poa trivialis	*Trifolium repens*	*Viola odorata*

To simulate field conditions similar to those experienced by semi-arid grasslands in central Spain, all the microcosms were kept under ambient light, temperature and rainfall (mean monthly temperature = 14.16 ± 1.48 °C, mean monthly rainfall = 34.71 mm ± 6.68; Appendix S2). However, to facilitate seedling establishment, all microcosms were watered three times per week with 1 L per irrigation during the first 6 weeks of the experiment. Because of the black colour of the PVC pots, and the low rainfall and high temperature experienced during summer (Appendix S2), all microcosms were additionally watered once a week with 1 L per irrigation in July and August 2008 to reduce potential extreme drought conditions.

SAMPLING AND HARVESTING

Uptake of N from added organic material was determined for the foliar material at the community level. To do this, we harvested in both June 2008 and May 2009 two leaves from the upper part of the canopy of all the plants growing in each microcosm. This harvesting allowed us to compare short- vs. long-term changes in N uptake. Leaves were dried at 60 °C until constant weight, ground to a fine powder and mixed to create a composite sample for each microcosm. A subsample was injected into an elemental analyser (PDZ Europa ANCA-GSL, Sercon Ltd, Cheshire, UK) interfaced to an isotope ratio mass spectrometer (IRMS, Sercon Ltd, Cheshire, UK). Nitrogen was separated on a Carbosieve GC column (65 °C, 65 mL min^{-1}) before entering the IRMS. The atom% ^{15}N excess was calculated by subtracting 0.366 (atmospheric background). The amount of N captured was estimated as the percentage of N added in the organic material that was captured by assemblages (NCA) as [(mg ^{15}N in foliar tissue)/(mg ^{15}N in added organic material)] × 100 (Maestre, Bradford & Reynolds 2005). To compare short- vs. long-term changes in NCA, non-destructive harvests were carried out in June 2008 and in May 2009, respectively. We acknowledge that our NCA measurements do not discriminate between the root foraging behaviour of individual plant species, which commonly differ in their ability to proliferate roots into nutrient patches (Einsmann *et al.* 1999), and thus on their ability to capture soil N. However, they are a good estimator of the ability of plant assemblages to acquire N during the decomposition of the organic material (Maestre, Bradford & Reynolds 2005), and thus a suitable measure to test our second hypothesis.

Cover (estimated with the point quadrat method) and height (measured with a ruler) of each individual plant was measured at the end of the two growing seasons (June 2008 and June 2009). In June 2009, above-ground biomass of all the microcosms was cut at the soil surface and sorted to species. Plant shoots were dried at 60 °C to constant weight. The good allometric relationship found between above-ground biomass and plant cover in 2009 ($R^2 = 0.31$, $P = 0.0001$, $n = 144$) was used to calculate above-ground biomass in 2008 from cover data without the need of harvesting. Plant height was not related to biomass ($R^2 = 0.003$, $P = 0.452$, $n = 144$) and thus not further considered. We estimated above-ground net primary productivity (ANPP) in both 2008 and 2009 from above-ground biomass because the latter is an appropriate surrogate of ANPP in communities such as the one we studied (Scurlock, Johnson & Olson 2002).

After above-ground harvesting, soil was carefully removed from each microcosm and the roots were harvested. The soil was bulked, sieved (2 mm mesh) and air-dried for 15 days for further analyses. At harvesting, three randomly selected soil cores (5 × 20 cm) were removed from the same locations in all the pots to measure root density (g cm^{-3}). We washed the roots using a 500-μm mesh size sieve to retrieve fine roots. We did not attempt to distinguish between live and dead roots, or to separate them by species. All roots were dried at 60 °C to constant weight. The total root biomass from 16 microcosms containing all possible treatment combinations was measured. The good allometric relation found between total root biomass in the whole microcosm and root density in the three soil cores ($R^2 = 0.67$, $P < 0.0001$, $n = 16$) was used to calculate below-ground biomass in all the microcosms. Grasslands typically have shallow root systems (Jackson *et al.* 1996). Thus, changes to root biomass in 0–20 cm depth should serve as a reliable index of below-ground net primary productivity (BNPP) in 2009 (Douglas 2007). To measure root foraging precision, we harvested the roots within control and patch cylinders separately from the bulk soil. All roots were dried at 60 °C and weighted. Root foraging precision was estimated with the relative interaction index (RII) proposed by Armas, Pugnaire & Ordiales (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. This index 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 (root biomass proliferation into the nutrient patch). In the homogeneous treatment, the cylinder located 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. Root foraging precision and N uptake were employed to assess differences in resource use strategies by the three PFG evaluated. Root foraging precision is an important parameter in studies of root proliferation (de Kroon & Mommer 2006) and is related to a range of ecophysiological traits involved in plant resource use economy (Kembel *et al.* 2008).

SOIL ANALYSES

We measured the activity of β-glucosidase and acid phosphatase enzymes, and *in situ* N availability rate as surrogates of nutrient cycling. These two enzymes are strongly related to carbon (β-glucosidase) and phosphorous (acid phosphatase) cycling (Tabatabai 1982), which are critical determinants of the functioning of arid and semi-arid ecosystems (Whitford 2002). *In situ* soil N availability is related to N mineralization (Subler, Blair & Edwards 1995). The activity of acid phosphatase was measured by determining the amount of *p*-nitrophenol (PNF) released from 0.5 g soil after incubation at 37 °C for 1 h with the substrate *p*-nitrophenyl phosphate in MUB buffer (pH 6.5; Tabatabai & Bremner 1969). The activity of β-glucosidase was measured according to Tabatabai (1982), following the procedure for phosphatase, but using *p*-nitrophenyl-b-D-glucopyranoside as substrate and trishydroxymethyl aminomethane instead of NaOH. To estimate the *in situ* soil N availability rate, we used anionic and cationic exchange membranes (Castillo-Monroy *et al.* 2010; types I-100 and I-200, Electropure Excellion, Laguna Hills, CA, USA). Ionic exchange membranes (IEMs) were previously conditioned in the lab by immersing them in demineralized water at 82–90 °C for 48 h. One 2.5 × 2.5 cm IEM was buried between the plastic cylinders and incubated during 25 days. Surveys were carried out in May 2008 and May 2009 in 96 microcosms (six replicates per combination of treatments). Upon retrieval, we extracted the nutrients from the IEMs with a 2-M KCl solution and calculated the availability of NH_4^+ and NO_3^- by colorimetry (indophenol blue method) using a microplate reader (Sims, Ellsworth & Mulvaney 1995). Mineral N was taken as the sum of NH_4^+-N and NO_3^--N.

STATISTICAL ANALYSES

We evaluated the effects of PFG, NA and NH on root foraging precision, nitrogen uptake (NCA), above-ground net primary productivity (ANPP), below-ground net primary productivity (BNPP) and the three soil functioning variables using a three-way ANOVA. All factors were fixed. Above- and below-ground net primary productivity were introduced in the analyses of the soil variables as covariates. Relationships between plant cover and ANPP, and between root density and root biomass were evaluated using linear regression. Data were log-transformed when necessary to meet the assumptions of ANOVA and ANCOVA analyses. Where appropriate, Tukey's HSD test was used for *post hoc* comparisons. Analyses were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Although we conducted a large number of statistical tests, *P* values were not adjusted for multiple testing as this approach is considered overly conservative (Gotelli & Ellison 2004).

Results

ECOSYSTEM LEVEL RESPONSES TO SOIL HETEROGENEITY AND PLANT FUNCTIONAL GROUP DIVERSITY

Above-ground net primary productivity estimated in June 2008 and 2009 was significantly affected by soil nutrient heterogeneity ($P = 0.020$ and 0.017, respectively; Appendix S3). Higher values of this variable were found under heterogeneous nutrient supply (Fig. 1a,b). In June 2008, the legumes and 3-PFG mixtures were about two times more productive than the grasses and non-legume forbs, respectively ($P < 0.001$, *post hoc* results shown in Fig. 1a). In June 2009, we only found a marginally significant NH × PFG interaction when analysing

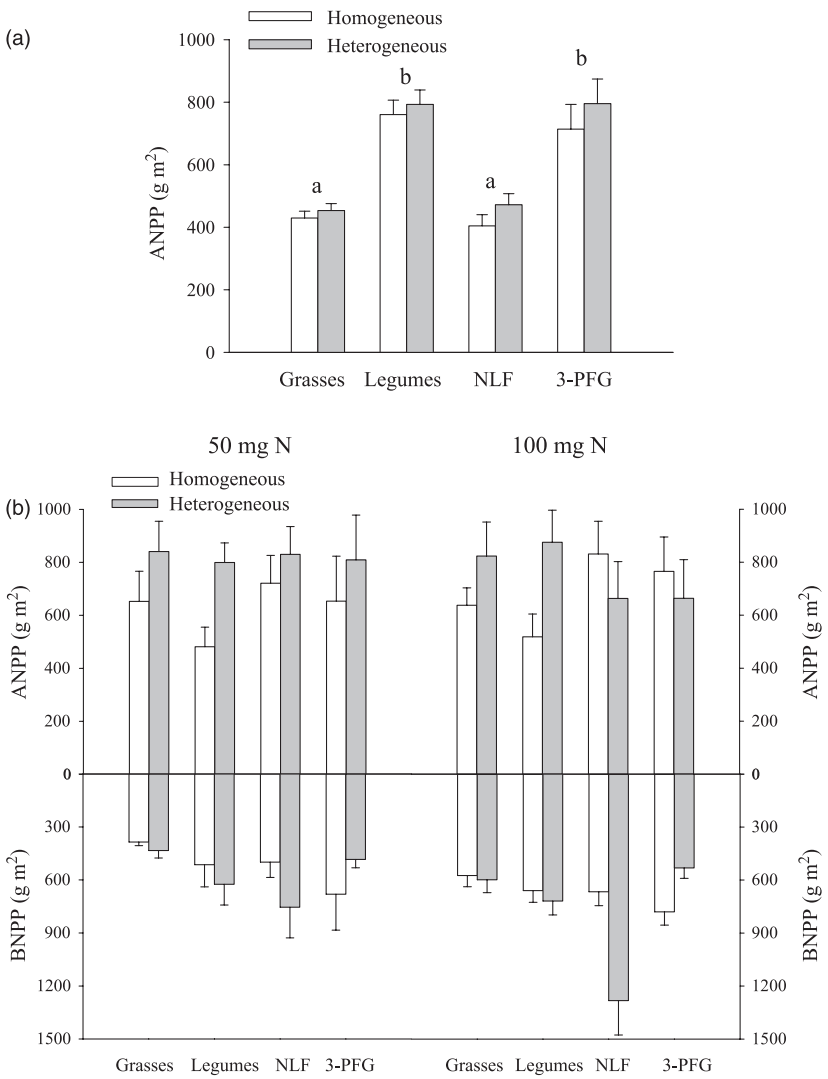

Fig. 1. Above-ground net primary productivity (ANPP) in June 2008 (a) and 2009 (b). Below-ground net primary productivity (BNPP) in June 2009 is also shown in (b). Nutrient availability factor was collapsed in (a) to highlight the main significant effects of soil nutrient heterogeneity and plant functional group. Different lowercase letters in (a) indicate significant differences among plant functional group levels ($P < 0.05$, Tukey's HSD test after a one-way ANOVA). Values are means ± 1 SE ($n = 18$ and 9 in (a) and (b), respectively). 50 and 100 mg N represent the two levels of nutrient availability provided. NLF = non-legume forbs, and 3-PFG = three plant functional group mixture. See Appendix S4 for *post hoc* analyses of the nutrient availability × PFG significant interaction found when analysing BNPP.

ANPP ($P = 0.060$; Appendix S3), suggesting that legumes and grasses were the only PFG more productive under heterogeneous nutrient supply at both low and high nutrient availabilities (Fig. 1b). Soil heterogeneity had positive effects on the estimated BNPP in 2009 (Fig. 1b, $P < 0.001$). The only significant interaction found when analysing BNPP at this time was NA × PFG ($P = 0.006$; Appendix S3); this interaction revealed a positive effect of the non-legume forbs, which was only found under conditions of high NA (see Appendix S4 for *post hoc* analyses).

Significant NH × NA and NH × PFG interactions were found when analysing the activity of β-glucosidase (Fig. 2a, $P = 0.048$ and 0.002, respectively; Appendix S3). Nonetheless, when we included ANPP as a covariate ($P = 0.002$; Appendix S5), the NH × NA interaction became non-significant ($P = 0.113$), but the NH × PFG interaction remained highly significant ($P = 0.009$; Appendix S5). Under homogeneous nutrient supply, the highest values of this variable were found in the NLF microcosms, but in the heterogeneous treatment they were found in the legume microcosms (*post hoc* results in Fig. 2a). The activity of the acid phosphatase experienced an increase in the legume microcosms (*post hoc* results in Fig. 2b, $P = 0.008$; Appendix S3), a positive effect that was still significant when we included ANPP as a covariate ($P = 0.001$; Appendix S5). Below-ground net primary productivity was not included in the analysis of β-glucosidase and acid phosphatase as a covariate because it was not significant ($F_{1,127} = 0.05$; $P = 0.816$ and $F_{1,127} = 0.28$; $P = 0.592$, respectively).

The two *in situ* N availability measurements showed a different pattern. In May 2008, the availability rate of NO_3^- was about two times higher in the 3-PFG microcosms than in any of the other PFG combinations when the nutrients were heterogeneously supplied at the highest availability level (Fig. 3a). However, this NH × NA × PFG interaction was only marginally significant ($P = 0.086$; Appendix S3). In May 2009, the NO_3^- availability rate was significantly higher in the legume and 3-PFG mixtures microcosms ($P = 0.001$; Appendix S3, *post hoc* results shown in Fig. 3b). Above-ground net primary productivity in 2008 ($F_{1,79} = 0.82$; $P = 0.369$) and both ANPP and BNPP in 2009 ($F_{1,63} = 0.32$; $P = 0.572$ and $F_{1,63} = 0.156$; $P = 0.694$, respectively) were not included in the analysis as covariates because they were not significant. The NH_4^+ availability rate was not affected by any treatment or interaction in both years.

SOIL HETEROGENEITY AND PLANT RESOURCE USE STRATEGIES

In May 2008, the only interaction found significant when analysing NCA was soil heterogeneity × PFG ($P = 0.002$; Appendix S3). *Post hoc* analyses revealed that the non-legume forbs captured the highest and lowest percentage of N when the nutrients were homogeneously and heterogeneously supplied,

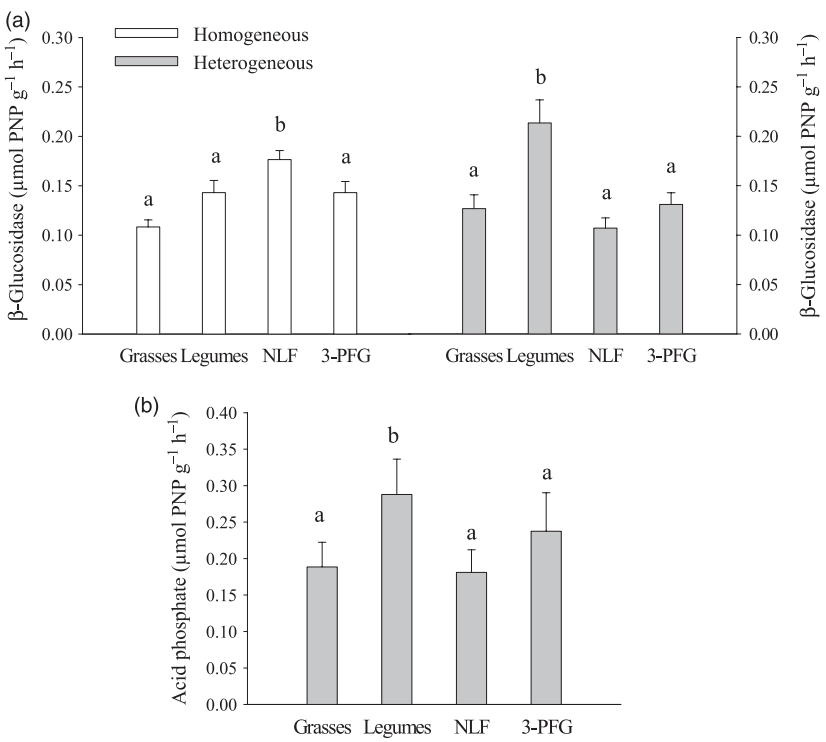

Fig. 2. Activity of the β-glucosidase (a) and acid phosphatase (b) enzymes in June 2009. Nutrient availability factor was collapsed in (a) to highlight the significant soil nutrient heterogeneity × plant functional group (PFG) interaction found (Appendix S3). Nutrient availability and heterogeneity were collapsed in (b) to highlight the main significant effect of PFG. Different lowercase letters in (a) and (b) indicate significant differences among PFGs ($P < 0.05$, Tukey's HSD test after a one-way ANOVA). Values are means ± 1 SE ($n = 18$ and 36 in (a) and (b), respectively). NLF = non-legume forbs, and 3-PFG = three plant functional group mixture.

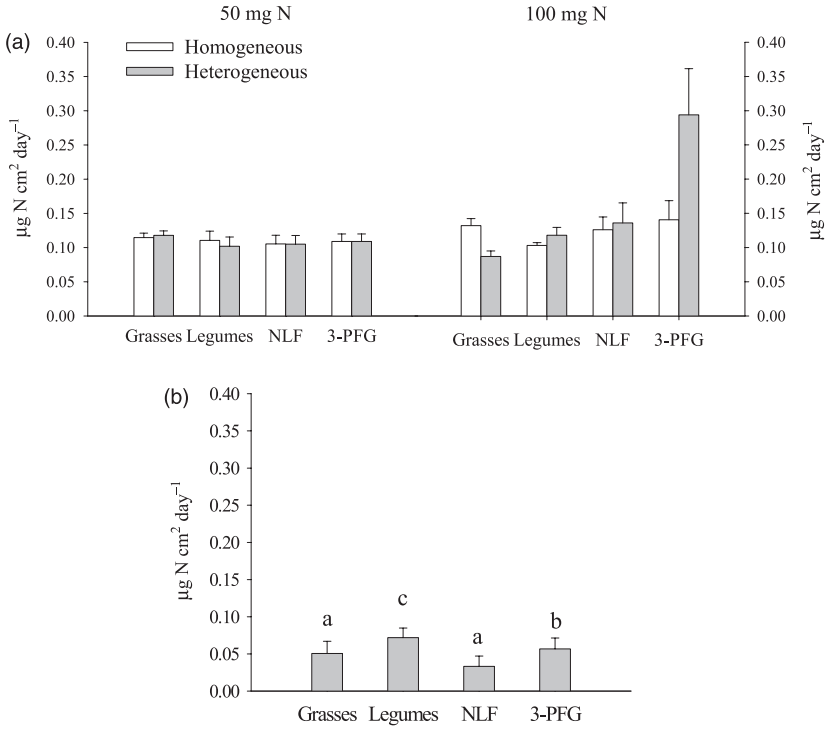

Fig. 3. NO_3^- availability rate in June 2008 (a) and May 2009 (b). Nutrient availability and heterogeneity factors were collapsed in (b) to highlight the main significant effect of plant functional group (PFG). Different lowercase letters in (b) indicate significant differences among PFG levels ($P < 0.05$, Tukey's HSD test after a one-way ANOVA). Values are means ± 1 SE ($n = 6$ and 20 in (a) and (b), respectively). About 50 and 100 mg N represent the two levels of nutrient availability provided. NLF = non-legume forbs, and 3-PFG = three plant functional group mixture. One replicate from each combination of treatments was ruled out in May 2009 due to extremely low N availability values found (below the method detection limit).

respectively (Fig. 4a). In May 2009, the pattern changed considerably (Fig. 4b), and a NH × NA × PFG significant interaction was found ($P = 0.020$; Appendix S3). The non-legume forbs were the most efficient PFG taking up N under heterogeneous nutrient supply and at the highest nutrient availability level (see Appendix S6 for *post hoc* analyses). Total N uptake and variability (as indicated by the standard errors of the mean) in all the PFGs evaluated declined from 2008 to 2009 (Fig. 4).

All the PFG assessed demonstrated precise root foraging patterns when the organic material was supplied heterogeneously ($P < 0.001$; Appendix S3; Fig. 5). We only found a marginally significant NH × NA interaction ($P = 0.059$), suggesting that root foraging precision increased in the high nutrient availability level. No significant differences in this variable were found between PFGs. The RII index observed under homogeneous nutrient supply was not statistically significant from zero in most of the situations (Fig. 5).

Discussion

SOIL HETEROGENEITY MODULATES THE DIVERSITY–FUNCTION RELATIONSHIP

Recent research has suggested that the same levels of plant diversity can exert a stronger positive effect on ecosystem function in heterogeneous than in homogeneous environments (Tylianakis *et al.* 2008; Wacker *et al.* 2008). Our results support this idea and confirm our first hypothesis. Soil heterogeneity modulated the effects of nutrient availability and PFG diversity on ecosystem processes such as productivity and soil variables related to nutrient cycling. In agreement with other studies that have shown that species level diversity can interact with nutrient heterogeneity and supply to determine nutrient uptake and plant productivity (Maestre, Bradford & Reynolds 2005; Maestre & Reynolds 2006a, 2007a), our study provided clear evidence that the same is true when diversity is defined by plant functional groups.

The effects of soil heterogeneity as a modifier of plant diversity effects on productivity varied with the PFG evaluated. Grasses and legumes increased their ANPP in the second growing season under heterogeneous nutrient supply, but the non-legume forbs and 3-PFG mixtures did not experience such a response. Specific relations between ANPP of each functional group and root foraging precision in homogenous and heterogeneous conditions did not explain this soil heterogeneity × PFG interaction (Appendix S7). Even though our experimental design and measurements cannot provide a mechanistic explanation for this interaction, we suggest that soil heterogeneity enhanced intra-functional group complementarity in resource use, which may indeed promote an increase in ANPP. Wacker *et al.* (2008) found that soil heterogeneity can promote complementarity-based biodiversity effects in experimental grassland communities comprised by

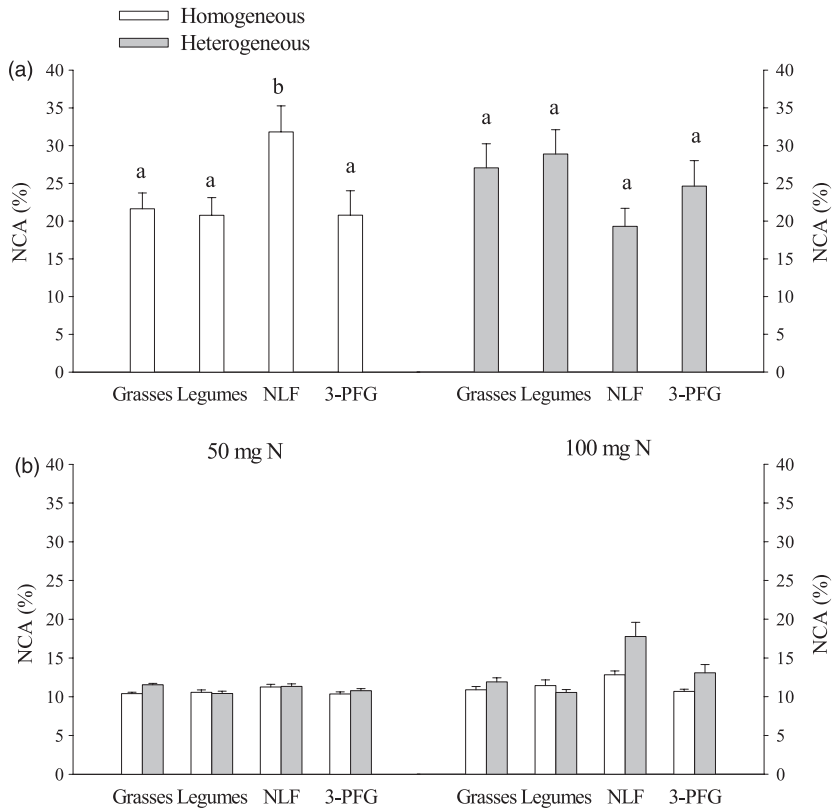

Fig. 4. Percentage of N added as organic material captured by assemblages (NCA) in June 2008 (a) and May 2009 (b). Nutrient availability levels were collapsed in (a) to highlight the significant nutrient heterogeneity × plant functional group (PFG) interaction found (Appendix S3). Different lowercase letters in (a) indicate significant differences among PFGs ($P < 0.05$, Tukey's HSD test after a one-way ANOVA). Values are means ± 1 SE ($n = 18$ and 9 in (a) and (b), respectively). About 50 and 100 mg N represent the two levels of nutrient availability provided. NLF = non-legume forbs, and 3-PFG = three plant functional group mixture. See Appendix S6 for *post hoc* analyses of the three-way significant interaction found when analysing June 2009 data (b).

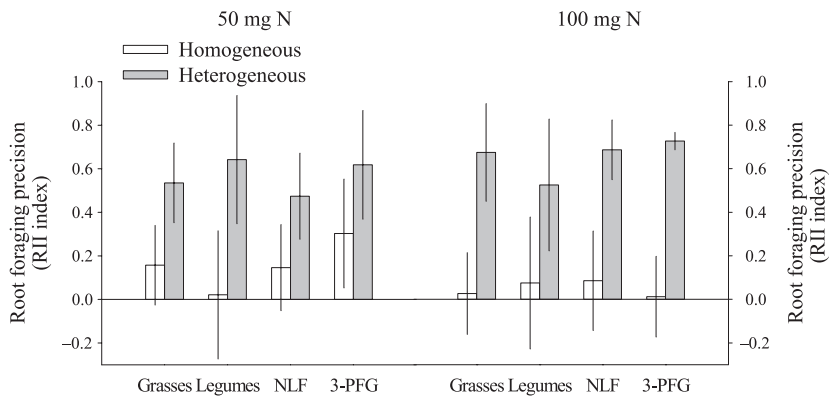

Fig. 5. Root foraging precision into nutrient patches in May 2009. Positive values of the relative interaction (RII) index indicate precise root biomass proliferation into the nutrient patch (see 'Materials and methods' section for details on its calculation). Values are means ± 95% confidence intervals ($n = 9$). Significant root foraging patterns are indicated by confidence intervals that do not overlap 0. 50 and 100 mg N represent the two levels of nutrient availability provided. NLF = non-legume forbs, and 3-PFG = three plant functional group mixture.

different functional groups. Plant species within functional groups may have also different resource use strategies that allow them to use the new potential niches created by nutrient heterogeneity (Loreau, Mouquet & González 2003; Gross *et al.* 2007), but our experimental approach does not allow us to explore these relationships. Although soil heterogeneity did not directly affect the surrogates of C, P and N cycling evaluated, it conditioned the PFG effects on the activity of β-glucosidase and the availability rate of NO_3^- in 2008. A weak positive relation between productivity and nutrient cycling was found for most of the soil variables, even though soil heterogeneity did not influence it (Appendix S8). There-

fore, the potential effect of a productivity increase modulated by soil nutrient heterogeneity on nutrient cycling cannot be explained in terms of resource inputs into the soil (Wardle *et al.* 1999). This result suggests that differences in plant morphological and physiological traits, such as the production of high decomposability litter by legumes (Wardle 2002), or species-specific associations with soil microbes enhancing nutrient cycling (Hodge, Campbell & Fitter 2001), could be potential determinants of soil heterogeneity influence on the plant diversity–nutrient cycling relationships observed.

PLANT RESOURCE USE STRATEGIES AND ECOSYSTEM PROCESSES IN HETEROGENEOUS ENVIRONMENTS

As found in many studies conducted with individuals, populations and communities (Hutchings & de Kroon 1994; Fransen, de Kroon & Berendse 2001; Hodge 2004; Maestre, Bradford & Reynolds 2005; Maestre & Reynolds 2007a; b), plants in our model communities selectively placed their roots in the nutrient patches. This root foraging pattern was particularly marked at the highest nutrient availability, a response observed by previous studies (Fransen & de Kroon 2001; Maestre, Bradford & Reynolds 2005; Maestre & Reynolds 2007b). Root proliferation under heterogeneous conditions increases with increasing contrast between the patch and the background soil, especially in nutrient-limited plants (Lamb, Haag & Cahill 2004). The high nutrient availability patch represented 10% of all N contained in our low-fertility soil, although only 1% in terms of volume. Therefore, the poor soil conditions, typical of semi-arid roadside prairie slopes (Bochet, Garcia-Fayos & Tormo 2007), may further enhance the benefits of root foraging into nutrient patches.

The positive effect of soil heterogeneity on primary productivity has been previously assessed at the species (Bilbrough & Caldwell 1997), population (Day, Hutchings & John 2003; Maestre & Reynolds 2006b) and community (Maestre, Bradford & Reynolds 2005; Wijesinghe, John & Hutchings 2005) levels. According to results from these studies, we found a general positive effect of soil nutrient heterogeneity on ANPP during the first growing season and on ANPP and BNPP after two growing seasons. However, the rate of soil nutrient cycling was not directly affected by the spatial pattern of nutrient supply. Different root responses to soil heterogeneity explained its positive effect on productivity, supporting our second hypothesis. The legumes and 3-PFG mixtures were the most productive functional groups in the first growing season, when they captured more N under heterogeneous nutrient supply, suggesting an increase in nutrient use efficiency as a rapid response to soil heterogeneity (Hodge 2004). This initial gain in nitrogen uptake experienced by legumes disappeared in the second growing season, when the low NO_3^- availability rate may suggest patch and overall soil nutrient depletion. Other soil heterogeneity studies have reported reductions in shoot biomass in the long term because of patch depletion and high nutrient losses promoted by a limited root life span (Fransen & de Kroon 2001), especially in legumes (Tjoelker *et al.* 2005). However, in the second growing season, legumes were still the most productive functional group, albeit only under heterogeneous conditions of nutrient supply. Legumes may compensate for this reduction in soil nutrient availability by increasing bacterial N fixation and/or mycorrhizal fungi activity (van der Heijden, Bardgett & van Straalen 2008). On the other hand, at the end of the experiment, the non-legume forbs turned to have the highest rates of N capture in heterogeneous treatments under high levels of nutrient supply. Although this PFG presented the lowest morphological plasticity (as measured by the root foraging precision), it showed enough physiological plasticity to acquire more N from the organic patches. The lower root foraging precision of non-legume forbs corresponded with their higher specific root length (P. García-Palacios, unpublished data), as predicted by Fitter (1994). These results do not agree with previous findings predicting an important role for root physiological plasticity and morphological responses in the short- and long-term exploitation of patches, respectively (Burns 1991; Hodge 2004), but support the findings of Fransen, de Kroon & Berendse (2001). The non-legume forbs compensated for the nutrient-poor soil conditions found in the second growing season (Fig. 3b) by investing more resources in root than in shoot production in the heterogeneous treatments. Overall these results indicate the existence of different plant resource use strategies among plant functional groups (Gross *et al.* 2007).

ECOSYSTEM RESPONSES TO CHANGES IN PLANT FUNCTIONAL GROUP IDENTITY AND DIVERSITY

Unsurprisingly, legumes increased inorganic N availability (Harrison & Bardgett 2010) in the second growing season. The same effect was found in the 3-PFG mixtures, albeit its magnitude was smaller than that produced by legumes. These results may be caused by the multispecies sampling effect for legumes proposed by Huston & McBride (2002), where more diverse pots (three PFG vs. one PFG in our study) have a higher chance of simultaneously containing legume species. However, the legumes also enhanced the activity of the acid phosphatase and β-glucosidase enzymes, but the latter only under heterogeneous nutrient supply. These positive effects of legumes on soil nutrient cycling variables were independent of their above- and below-ground biomass (Stephan, Meyer & Schmid 2000). Collectively, these findings suggest that legume effects on soil functioning are mediated by microbial communities, probably by promoting a shift towards a bacterial-based energy channel that is typically associated with rapid rates of nutrient cycling in infertile soils (van der Heijden, Bardgett & van Straalen 2008; Harrison & Bardgett 2010). Therefore, PFG identity was more important than PFG richness to recover several ecosystem processes in the studied assemblages, providing support for our third hypothesis.

CONCLUDING REMARKS

The use of natural soil and organic patches (e.g. leaves) in our experimental design, and the natural climatic conditions experienced by the microcosms, allow us to interpret our results in a

more appropriate ecological context than in studies that use artificial soils and environmental conditions (Hodge 2004). However, their extrapolation to the natural world must be done with caution because of: (i) the physical restriction of pots for lateral root growth (Fransen *et al*. 1999) and deep root systems (particularly for non-legume forb species; Weaver 1958), (ii) the patterns of patch heterogeneity and degree of contrast to the background soil, which may not reflect those found in the field, and (iii) the single nutrient application conducted, which contrasts with the highly dynamic creation of nutrient patches in nature. In addition, N uptake patterns evaluated at the community level may mix different root–shoot partitioning and temporal abilities to capture soil nitrogen of the species involved (Einsmann *et al*. 1999). Despite these methodological problems, the plant assemblage signal for N uptake was strong enough to show several significant effects. The functional classification used in this study also has limitations. Some species belonging to the same PFG can have different life forms (hemicryptophytes or geophytes) and functional attributes. However, as significant effects have been found on several resource use strategy and ecosystem variables, the species evaluated can be grouped to predict their effects on ecosystem functioning (Reich *et al*. 2004; McLaren & Turkington 2010). The PFG classification employed can be improved by measuring those plant functional traits specifically related to the ecosystem variables of interest (Wright *et al*. 2006; Gross *et al*. 2007).

As a community-level study using multiple ecosystem response variables, our research helps to determine whether soil heterogeneity is important for plant community dynamics and ecosystem functioning. In general terms, soil heterogeneity improved the ANPP and BNPP of the assemblages, but had no direct effects on the surrogates of nutrient cycling that were evaluated. However, this ecosystem feature modulated the effects of nutrient availability and plant functional groups on some soil variables and on the resource use strategy of the assemblages. Our results highlight the importance of explicitly considering soil heterogeneity in diversity–ecosystem functioning experiments (Maestre & Reynolds 2006a, 2007b; Tylianakis *et al*. 2008; Jiang, Wan & Li 2009). Such consideration will substantially improve our ability to fully understand the role of plant communities on ecosystem functioning in ubiquitous heterogeneous environments (Hector & Bagchi 2007).

Acknowledgements

We thank Adrián Escudero for his help with the experimental design, Patricia Valiente, Jorge Papadopoulos, Becky Mou, Carlos Díaz, Rafael Sendra and Santiago Soliveres for their help during the development of the experiment, José Margalet for his help with the logistic at the plant growth facilities of Rey Juan Carlos University (CULTIVE), Matt Bowker for revising the English, and Robert Jones and two anonymous referees for their constructive comments on an earlier version of this manuscript. F.T.M. is supported by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC Grant agreement no. 242658. P.G.P. is supported by a fellowship from Fundación Biodiversidad-CINTRA (EXPERTAL grant). This research was supported by the EXPERTAL and EFITAL (B007/2007/3-10.2) grants, funded by Fundación Biodiversidad-CINTRA and the Spanish Ministerio de Medio Ambiente, respectively.

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Received 28 April 2010; accepted 2 November 2010
Handling Editor: Robert Jones

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Overall view (upper photo) of the location of the two 137 cm^3 plastic cylinders during the preparation of the soil nutrient patch in a heterogeneous microcosm (a). In the homogeneous treatments, the two plastic cylinders filled with the mixture of background soil + organic matter were homogeneously distributed along the entire pot volume (b). In the heterogeneous treatments, a plastic cylinder was filled with organic material, and the other (control) was filled only with background soil (c).

Appendix S2. Climatic data (mean monthly temperature, black circles; and monthly rainfall, grey bars) obtained from a meteorological station (Onset, Pocasset, MA, USA) located in the facilities of the Rey Juan Carlos University (Móstoles, Spain).

Appendix S3. Summary of three-way ANOVA for main treatment effects and interactions on above- (ANPP) and below-ground (BNPP) net primary productivity, the activity of the β-glucosidase and acid phosphatase enzymes, the percentage of N added as organic material captured by assemblages (NCA), root foraging precision (RII) and the NO_3^- and NH_4^+ availability rates.

Appendix S4. *Post hoc* analyses of the significant nutrient availability × plant functional group (PFG) interaction found when analyzing below-ground net primary productivity (BNPP) data in June 2009.

Appendix S5. Summary of three-way ANCOVA for main treatment effects and interactions on the activity of the β-glucosidase and acid phosphatase enzymes.

Appendix S6. *Post-hoc* analyses of the significant nutrient heterogeneity × nutrient availability × plant functional group (PFG) interaction found when analyzing the percentage of N added with organic material captured by assemblages (NCA) in May 2009.

Appendix S7. Relationships between root foraging precision and above-ground net primary productivity (ANPP) of the four plant functional groups in June 2009.

Appendix S8. Relationships between above-ground net primary productivity (ANPP), below-ground net primary productivity (BNPP) and the activity of the enzymes β-glucosidase (a, d), acid phosphatase (b, e) and NO_3^- availability rate (c, f) in June 2009.

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