

## AMOUNT OR PATTERN? GRASSLAND RESPONSES TO THE HETEROGENEITY AND AVAILABILITY OF TWO KEY RESOURCES

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**Abstract.** Patterns of resource availability and heterogeneity shape the composition, productivity, and dynamics of plant assemblages in a wide variety of terrestrial ecosystems. Despite this, the responses of plant assemblages to simultaneous changes in the availability and heterogeneity of more than a single resource are virtually unknown. To fill this gap, microcosms consisting of assemblages formed by *Lolium perenne*, *Plantago lanceolata*, *Anthoxanthum odoratum*, *Holcus lanatus*, and *Trifolium repens* were grown in a factorial experiment with the following treatments: nutrient availability (NA), water availability (WA), spatial nutrient heterogeneity (NH), and temporal water heterogeneity (WH). Assemblages exhibited precise root foraging patterns in response to nutrient heterogeneity, which were modified by NA and WA. A series of two- and three-way interactions involving the four factors evaluated determined biomass production, the belowground:aboveground biomass ratio, the patterns of root biomass allocation with depth, and the relative contribution to aboveground biomass of *Lolium* and *Anthoxanthum*. In all cases, these interactions explained significant amounts of the variation found in the data. Our study demonstrates that considering the interactions between resource availability and heterogeneity allows for a refinement of predictions that can detectably reduce the error associated with extrapolating from single factor analyses.

**Key words:** *Anthoxanthum odoratum*; *competition*; *global change*; *Holcus lanatus*; *Lolium perenne*; *microcosm*; *plant assemblages*; *Plantago lanceolata*; *resource availability*; *resource heterogeneity*; *Trifolium repens*.

### INTRODUCTION

Over the past three decades, an important aspect of ecological research has focused on understanding and predicting the manner in which plant species and assemblages respond to changes in the availability and spatiotemporal heterogeneity of resources such as water, light, and nutrients (Grime 1979, Tilman 1988, Wilson and Tilman 1991, Hutchings et al. 2000, Anderson et al. 2004). This research has shown that patterns of resource availability and heterogeneity play a crucial role in shaping the structure, productivity, composition, and diversity of plant assemblages across a wide range of terrestrial ecosystems. However, little is known of the relative importance of resource heterogeneity vs. availability, as most studies conducted have evaluated their roles in isolation of one another (see reviews by DiTommaso and Aarsen [1989], Huber-Sannwald and Jackson [2001], Lawrence [2003], and Hodge [2004]). Some notable exceptions, however, are recent studies evaluating the joint effects of light availability and its

spatial pattern on the richness of grassland assemblages (Stevens and Carson 2002, Bakker et al. 2003) and the joint effects of nutrient availability and its spatial pattern (hereafter “nutrient heterogeneity”) on the structure and productivity of grassland populations and assemblages (Collins and Wein 1998, Baer et al. 2004, Maestre et al. 2005, Maestre and Reynolds 2006).

The increase in the availability of nutrients and the changes in total and within-season precipitation patterns are two important components of global change facing terrestrial ecosystems (Vitousek 1994, Houghton et al. 2001). It is thus imperative to understand how both processes simultaneously impact plant species and assemblages. Changes in the within-season distribution of precipitation may impact the survival, growth, and productivity of plant individuals and communities (Novoplansky and Goldberg 2001, Fay et al. 2003, Sher et al. 2004), yet their effects under simultaneous conditions of increased nutrient availability are largely unknown. It is also unknown whether observed responses to shifts in rainfall patterns are modified by nutrient heterogeneity, although we know it influences the competitive ability and survival of individual plants (Hodge 2004), as well as the composition, nutrient uptake, and productivity of plant assemblages (Maestre et al. 2005, 2006, Wijesinghe et al. 2005).

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Considering nutrient heterogeneity when studying the response of plant assemblages to changes in rainfall patterns and nutrient availability is relevant for several reasons. First, nutrient heterogeneity is ubiquitous in many ecosystems (Hutchings et al. 2000) with demonstrated effects at the individual, population, and community organization levels (Huber-Sannwald and Jackson 2001). Second, nutrient heterogeneity may potentially interact with nutrient availability and shifts in rainfall patterns to determine the responses of plant assemblages. Such interactions are expected because coexisting species differ in the magnitude of their response to these factors, because nutrient availability is linked to water availability, and because nutrient heterogeneity elicits plant responses that are also affected by both nutrient availability and rainfall patterns (Warren and Johnston 1964, Einsmann et al. 1999, Fay et al. 2003, Austin et al. 2004, Wijesinghe et al. 2005).

To our knowledge, no previous study has experimentally evaluated the effects of simultaneous changes in the spatial and/or temporal heterogeneity and availability of water and nutrients on the productivity and structure of plant assemblages. We aimed to do this for an assemblage formed by the grasses *Lolium perenne* L., *Anthoxanthum odoratum* L., and *Holcus lanatus* L., the forb *Plantago lanceolata* L., and the legume *Trifolium repens* L., which commonly co-occur in seminatural temperate grasslands (Joshi et al. 2000). Using a microcosm approach, we independently manipulated nutrient and water availability, as well as the spatial and temporal patterns of nutrient and water supply, respectively. We tested two hypotheses: (1) resource heterogeneity is as important as resource availability as a driver of the productivity and composition of the assemblages and (2) the effects of resource availability and heterogeneity on assemblage responses are not additive.

## METHODS

### *Experimental design*

The experiment was conducted in a greenhouse in the Duke University Phytotron (Durham, North Carolina, USA) between 21 March and 28 June 2005. The experiment had four treatments: nutrient availability (three levels: 40, 80, and 120 mg of N added as organic material), nutrient heterogeneity (two levels: organic material added homogeneously or as a patch), water availability (three levels: 125, 250, and 375 mL of water added per week) and water heterogeneity (two levels: watering five times per week or watering once per week). We used natural soil and organic material rather than growing medium and fertilizer because it allows the interpretation of plant responses to varying soil nutrient availability and heterogeneity in a realistic context (Hodge 2004). Microcosms consisted of PVC pipe (length 38 cm, internal diameter 10 cm) filled with, from the base, 5 cm of gravel (for drainage) and 28 cm of a

50:50 mixture of sandy-loam soil and sand (hereafter named "background soil"; Appendix A). On top of this mixture 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 three levels of overall nutrient availability we added different amounts of finely cut (2 mm) and dried *Trifolium* shoots (3.9% N, 10.8 C:N): 1.036, 2.072, and 3.108 g in the low, medium, and high nutrient availability levels, respectively (equivalent to adding 40, 80, and 120 mg of N per microcosm, respectively). Within each of these levels, the shoots were added homogeneously or as a patch. In both cases the same amount of nutrient was added, and thus we maintained the same overall nutrient availability. In the homogeneous microcosms, we thoroughly mixed the organic material with the background soil before introducing it into the PVC pipe. In the heterogeneous microcosms, we mixed 25 cm<sup>3</sup> of background soil with the organic material and introduced the resulting mix into a 31-cm<sup>3</sup> plastic cylinder (length 75 mm and internal diameter 23 mm) consisting of a light mesh with square pores 5 × 10 mm in size. We refer to this as the patch cylinder. A second (control) cylinder, filled only with background soil, was placed 2 cm apart and alongside the patch cylinder. Both cylinders were placed 23 cm above the bottom of the microcosm. In the homogeneous microcosms, two plastic cylinders were introduced in the same way, but in this case both were filled with the background soil previously mixed with the organic material.

To generate three levels of water availability we irrigated the microcosms with different amounts of distilled water: 125, 250, and 375 mL per week in the low, medium, and high water availability levels, respectively (equivalent to 15.92, 31.84, and 47.76 L/m<sup>2</sup> of water per week). For each of these levels, the same amount of water was supplied as a single pulse applied once per week (pulse regime) or as smaller pulses applied five times per week (regular regime). In this way, the amount of water added per event in the pulse and regular watering regimes were 125, 250, and 375 mL and 25, 50, and 75 mL, for the low, medium, and high water availability levels, respectively.

Seeds from the five species were obtained from commercial suppliers. They were germinated in a greenhouse on different days to ensure that all the species had a similar size and the same development stage (two-leaf seedling) at the start of the experiment. On 21 March 2005, two uniformly sized seedlings per species were transplanted into each microcosm. The planting positions for the 10 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; no further mortality was observed thereafter. We established four replicated microcosms for each of the 36 treatment combinations, resulting in 144 microcosms in total. The microcosms were introduced in a heated greenhouse exposed to natural sunlight (maximum photosynthetic active radiation =  $1314.2 \pm 389.6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; mean  $\pm$  SD,  $n = 100$ ) but within which the temperature was controlled ( $23^\circ\text{C}$  during the day and  $14^\circ\text{C}$  during the night). Average relative humidity ranged from 96% at night to 76% during the day. The microcosms were arranged in four blocks in which each block contained a replicate per combination of treatments formed by three wheeled trolleys containing 12 microcosms each (see Plate 1). The positions of the blocks within the greenhouse were changed twice per week and that of the microcosms within each block were randomized once per week to minimize position effects.

### Harvest

Plants were harvested after 100 d of growth. At that time, some individuals of *Anthoxanthum* had already set fruits, but no other species had begun to flower. Therefore, reproductive output was not measured in any species. None of the species had clear symptoms of water stress at harvest. The aboveground biomass of all the microcosms was cut at the soil surface and sorted to species. Once aboveground biomass was harvested, the soil in each microcosm was sliced into two depth layers: 0–15 cm and 15–30 cm. These layers capture most of root biomass under natural conditions and are of paramount importance for plant–plant interactions (Casper and Jackson 1997). In each depth layer all roots were carefully separated from the soil; 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. All the harvested material was dried at  $60^\circ\text{C}$  until constant mass.

Root foraging precision (i.e., the proliferation of roots into nutrient patches) was measured with the relative fine-root mass difference (RFRMD) index (Einsmann et al. 1999). The index is calculated as  $(\text{RB}_p - \text{RB}_c) / \text{belowground biomass}$ , where  $\text{RB}_p$  and  $\text{RB}_c$  are the root biomass in the patch and control cylinders, respectively. In the homogeneous treatment, the control 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 RFRMD index. To evaluate whether the treatments modified root allocation patterns with depth, the relative interaction intensity (RII) index (Armas et al. 2004) was estimated as  $(\text{RB}_{0-15} - \text{RB}_{16-30}) / (\text{RB}_{0-15} + \text{RB}_{16-30})$ , where  $\text{RB}_{0-15}$  and  $\text{RB}_{16-30}$  are the root biomass in the 0–15 and 16–30 cm depth, respectively. The RII ranges from  $-1$  to  $+1$ ; a value of zero would indicate equal root growth at both depths. Both the RFRMD and the RII indices allow

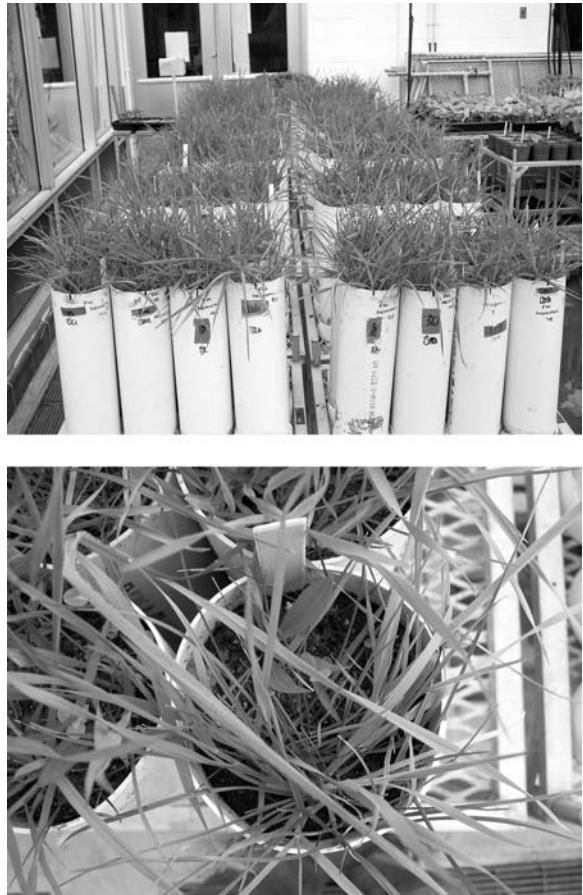


PLATE 1. (Top) Overall view and (bottom) details of the microcosms in the greenhouse after two months of growth. Photo credits: F. T. Maestre.

comparison of patterns of root proliferation and of root allocation with depth among treatments while accounting for differences in plant size.

### Statistical analyses

To look at the effects of nutrient availability (NA), nutrient heterogeneity (NH), water availability (WA), and water heterogeneity (WH) on plant assemblage biomass we used a four-way multivariate analysis of variance (MANOVA) in which the independent variables were NA, NH, WA, and WH and the dependent variables were total, above-, and belowground biomass. As the dependent variables were highly correlated among them ( $r > 0.914$ ,  $P < 0.001$  in all cases), a MANOVA analysis was preferred over individual ANOVAs on each variable (Quinn and Keough 2002). Belowground : aboveground ratio (BAR) and RII index data were analyzed with a four-way analysis of variance (ANOVA) in which the independent variables were NA, NH, WA, and WH and the dependent variables were either BAR or RII. To control for differences in plant size when analyzing BAR data (Reich 2002), the  $\log_{10}$ -transformed total biomass of the assemblages was used

as a covariate in this analysis. Root foraging precision in the heterogeneous treatments was evaluated with a three-way ANOVA in which the independent variables were NA, WA, and WH and the dependent variable was the RFRMD index.

The percentage of aboveground biomass accounted for by each species was analyzed separately for each species using the four-way ANOVA model described above. Despite the limitations of this approach (the data gathered from the different species within each microcosm are not fully independent of one another [see Anderson 2001]), we used it because it facilitates the interpretation of the effects of different factors on the performance of individual species within assemblages (Berntson et al. 1998, Grünzweig and Körner 2003, Maestre et al. 2005). In MANOVA and ANOVA analyses, all the factors were considered fixed. To investigate interactions, data were divided into subsets based on one of the factors of the interaction and then were subjected to MANOVA or ANOVA as appropriate.

To gain insights on the relative importance of each factor, we calculated in all ANOVA and MANOVA analyses the partial eta-squared ( $\eta_p^2$ ) statistic as  $SS_{\text{effect}} / (SS_{\text{effect}} + SS_{\text{error}})$ , where  $SS$  is the sum of squares and effect is either a main effect or an interaction. This statistic measures the degree of association between a main effect or an interaction and the dependent variable (Tabachnick and Fidell 1989). We also evaluated to what degree the interactions found explained significant amounts of variation in the data over that already explained by the main effects using general linear models (GLM; McCullagh and Nelder 1989). A model formed by the main effects of NH, NA, WH, and WA (M1) was used as a template against which we compared two alternative models: (2) a model containing all main effects and interactions (the full model, M2) and (2) a model containing all main effects and those interactions that were identified as significant by the MANOVA and ANOVAs (M3). The change in deviance between each pair of models (M1 vs. M2 and M1 vs. M3) is tested against an  $F$  distribution; significant changes in deviance indicate that the addition of interactions significantly increases the amount of variation in the data explained by the M1 model (Roland et al. 2000). All models assumed a Gaussian distribution of errors. As this analysis cannot handle multivariate data, total biomass was employed when evaluating biomass responses. This variable was highly correlated with both aboveground ( $r = 0.979$ ,  $P < 0.001$ ,  $n = 144$ ) and belowground ( $r = 0.976$ ,  $P < 0.001$ ,  $n = 144$ ) biomass.

Biomass and percentage data were  $\log_{10}$ - and arcsine-transformed, respectively, as needed to meet MANOVA and ANOVA assumptions. However, even with these transformations the biomass data did not satisfy the assumption of homogeneity of variance-covariance matrices (Box's  $M$  test,  $F = 1.89$ ,  $df = 204, 3911$ ,  $P <$

0.001). Despite this, we conducted the MANOVA using the Pillai's trace statistic, which is robust to deviations from this assumption, especially under equal sample sizes (Quinn and Keough 2002). Where appropriate, the Tukey's hsd test was used for post-hoc comparisons. MANOVA/ANOVA and GLM analyses were performed using SPSS 13.0 (SPSS, Chicago, Illinois, USA) and S-Plus 6.0 (Insightful Company, Seattle, Washington, USA), respectively. The experiment-wide error rate was not adjusted for multiple testing, as this approach is considered overly conservative (Gotelli and Ellison 2004).

## RESULTS

Significant nutrient heterogeneity (NH)  $\times$  water availability (WA), nutrient availability (NA)  $\times$  WA, and water heterogeneity (WH)  $\times$  WA interactions were found when analyzing assemblage biomass (Appendix B). Separate MANOVAs conducted at each WA level revealed significant effects of NH in all cases ( $F_{3,34} > 15.5$ ,  $P < 0.001$ ), with assemblages having more biomass under heterogeneous nutrient supply (Fig. 1A–C). Under low WA conditions, this effect was more pronounced as NA increased (NH  $\times$  NA interaction,  $F_{6,70} = 2.5$ ,  $P = 0.030$ ). At every WA level, increases in NA led to increases in assemblage biomass ( $F_{3,70} > 7.9$ ,  $P < 0.001$ ; Fig. 1). At the high WA level, assemblages had higher biomass under regular watering ( $F_{3,34} = 9.8$ ,  $P < 0.001$ ). However, at the low WA level assemblages had higher biomass under pulse watering ( $F_{3,34} = 24.2$ ,  $P < 0.001$ ), this response being more pronounced when the nutrients were homogeneously supplied (NH  $\times$  WH interaction,  $F_{3,34} = 3.1$ ,  $P = 0.040$ ).

Significant NH  $\times$  NA  $\times$  WA, WH  $\times$  WA, and NA  $\times$  WH interactions drove belowground: aboveground biomass ratio (BAR) responses (Fig. 1D; Appendix B). We investigated them by conducting separate ANOVAs for each WA and NA level. At the low and medium WA levels, assemblages had lower BAR when the nutrients were heterogeneously supplied ( $F_{1,35} > 1.8$ ,  $P < 0.041$ ) and when NA increased ( $F_{2,35} > 3.7$ ,  $P < 0.023$ ). At the low WA level, however, the effect of NA on the BAR was dependent on WH (WH  $\times$  NA interaction,  $F_{2,35} = 3.26$ ,  $P = 0.050$ ). At the medium and high NA levels, the BAR of assemblages decreased under heterogeneous nutrient supply ( $F_{1,35} > 5.1$ ,  $P < 0.032$  in both cases), albeit in the former case such an effect was dependent on WA (NH  $\times$  WA interaction,  $F_{2,35} = 5.73$ ,  $P = 0.007$ ). At the high NA level, the BAR of assemblages decreased under pulse watering ( $F_{1,35} = 14.9$ ,  $P < 0.001$ ), this effect being more pronounced at the low WA level (WH  $\times$  WA interaction,  $F_{2,35} = 6.9$ ,  $P = 0.003$ ).

When the organic material was supplied heterogeneously, the assemblages demonstrated highly precise root foraging patterns (Fig. 2A). The magnitude of this response in heterogeneous treatments was independently affected by NA ( $F_{2,54} = 39.7$ ,  $P < 0.001$ ; no interactions) and WA ( $F_{2,54} = 8.8$ ,  $P = 0.001$ ; no interactions). Post-

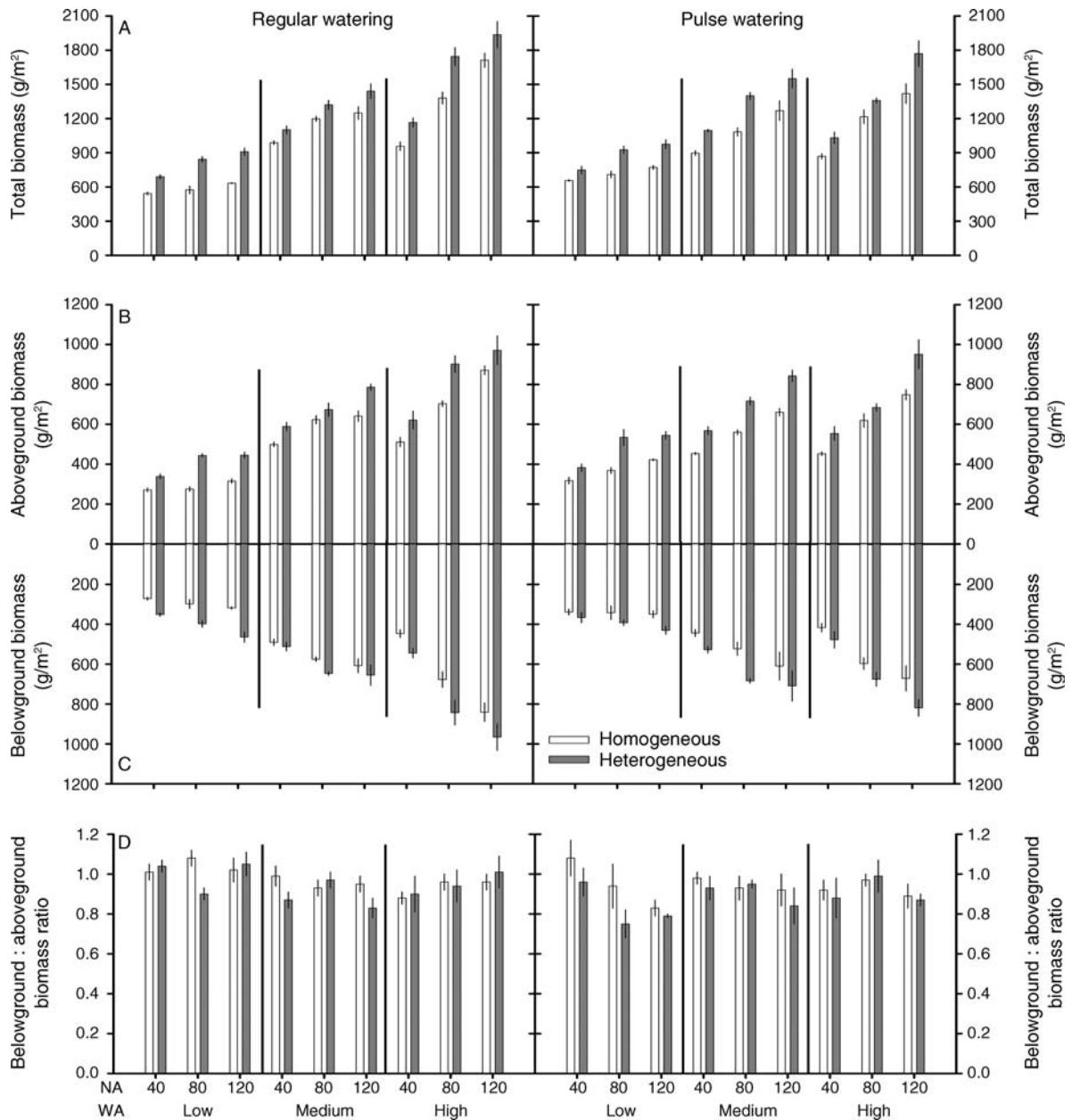


FIG. 1. (A) Total, (B) aboveground, and (C) belowground biomass, and (D) belowground : aboveground biomass ratio of the assemblages (all the species combined) in the different treatments. Data are means  $\pm$  SE ( $n=4$ ). The horizontal axes indicate both nutrient availability (NA) and water availability (WA). Units for nutrient availability are milligrams of N added as organic material. In the homogeneous treatment, the organic material was homogeneously distributed over the microcosm profile; in the heterogeneous treatment, the organic material was concentrated in a patch (see Appendix A for further details).

hoc analyses (Appendix C) revealed that root proliferation into nutrient patches increased concomitantly with increases in NA. Responses to increases in WA did not follow the same pattern, as higher root proliferation was found in the lower as compared to the medium and high WA levels.

Patterns of root allocation with depth were significantly affected by NH and NA (Appendix B). Assemblages allocated more roots to the first 15 cm of soil

under heterogeneous conditions of nutrient supply, as well as at the high compared to the low and medium NA levels (Fig. 2B; Appendix D). The effect of WA on this variable was only evident under regular watering (WA  $\times$  WH interaction; Appendix B). In this case, assemblages allocated fewer roots to the first 15 cm of soil under the high WA level as compared to the low and medium levels (Appendix D).

Main effects of NH, NA, and WA had, for most response variables measured at the assemblage level, the highest values of the  $\eta_p^2$  statistic (Appendix B), suggesting that they were the most influential drivers of observed responses. However, GLM analyses of these variables showed that the interactions found in MANOVA/ANOVA analyses explained significant amounts of the variation over that already explained by the main effects (Appendix E).

*Holcus lanatus* was the dominant species in the assemblages, accounting for up to 52% of the total aboveground biomass in some treatment combinations (Fig. 3). *Anthoxanthum* and *Lolium* were, in order, the next most dominant, with proportions ranging from 26% to 44% and from 7% to 31% of the total aboveground biomass, respectively. Biomass of *Trifolium* and *Plantago* were always below 18% and 3% of the aboveground total, respectively. Nutrient heterogeneity significantly decreased the proportion of the total aboveground biomass accounted for by *Plantago*, *Trifolium*, and *Anthoxanthum*, but increased that of *Lolium* (Fig. 3, Appendix F). The effect of NH on the proportion of total aboveground biomass accounted for by *Lolium* was especially evident at the low NA level (NH  $\times$  NA interaction; Appendix F). The decrease in the proportion accounted for by *Anthoxanthum* under heterogeneous nutrient supply was only observed at the regular watering regime (NH  $\times$  WH interaction; Appendix F). The proportion of total aboveground biomass accounted for by *Trifolium* and *Plantago* was higher at the low compared to the medium and high NA levels (Appendix G) and at the pulse compared to the regular watering regime (Fig. 3). The proportion of total aboveground biomass accounted for by *Lolium* was higher at the low compared to the medium and high WA levels, but that of *Holcus* and *Plantago* was higher at the medium and high WA levels (Appendix G). Nutrient heterogeneity was the most influential driver of the proportion of aboveground biomass accounted for by all species excepting *Holcus* (Appendix F). When significant interactions were found, they added significant amounts of variation over that explained by the main effects alone (Appendix H).

#### DISCUSSION

Our first hypothesis that resource heterogeneity is as important as resource availability as a driver of assemblage responses was supported by our results. All factors evaluated, either independently or through interactions, influenced the productivity, biomass allocation patterns, and composition of the assemblages. In fact, nutrient heterogeneity was the most influential factor driving assemblage responses. We did not find four-term interactions in any of the response variables measured. However, a series of two- and three-term interactions between the factors evaluated determined key biomass responses at both the species and assemblage levels. These interactions, which explained signif-

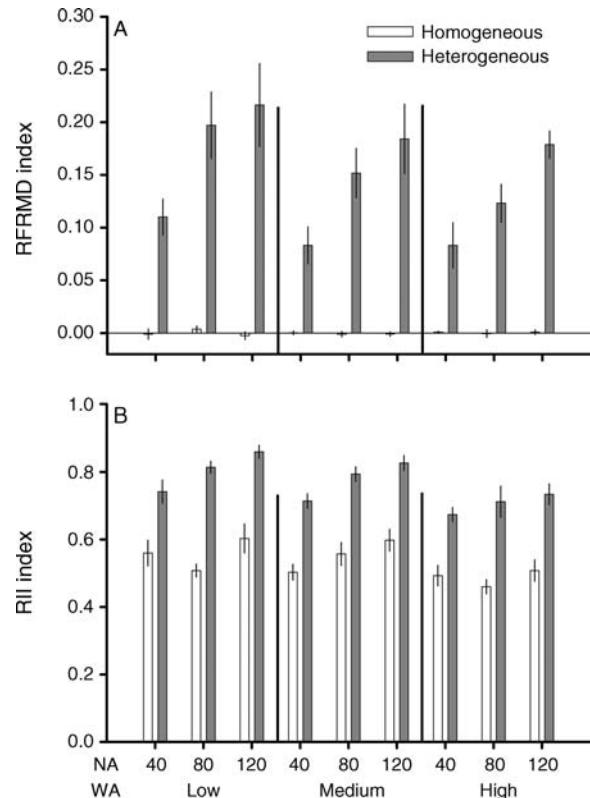


FIG. 2. (A) Root foraging precision (relative fine-root mass difference [RFRMD] index) and (B) ratio of root biomass in the 15–30-cm soil depth layer to that in the 0–15-cm depth layer in the different treatments (relative interaction intensity [RII] index) compared across nutrient heterogeneity, nutrient availability (NA), and water availability (WA) levels. Data are means  $\pm$  95% confidence intervals (RFRMD index) and means  $\pm$  SE (RII index),  $n = 8$  in all cases. Significant root proliferation into nutrient patches is shown by confidence intervals that do not overlap zero. For further explanation of treatments, see Fig. 1.

icant amounts of the variation found in the data, indicate that the effects of resource availability and heterogeneity on assemblage responses are not additive, as predicted by our second hypothesis.

Assemblage productivity increased with increases in both nutrient and water availability, albeit in a nonadditive way. The increase in biomass observed in response to increased nutrient availability was especially evident at the medium and high water availability levels. Similar responses have been observed in studies conducted with grassland communities (e.g., Kirchner 1977, Goldberg and Miller 1990, Stevens et al. 2006). It has been suggested that increases in nutrient availability may augment the sensitivity of plant individuals to water stress (Gleeson and Tilman 1992). This may be especially true if, as found in this study, increasing nutrient availability increases biomass and thus maintenance requirements (Goldberg and Novoplansky 1997).

As in previous studies conducted with grassland assemblages, productivity increased when the nutrients

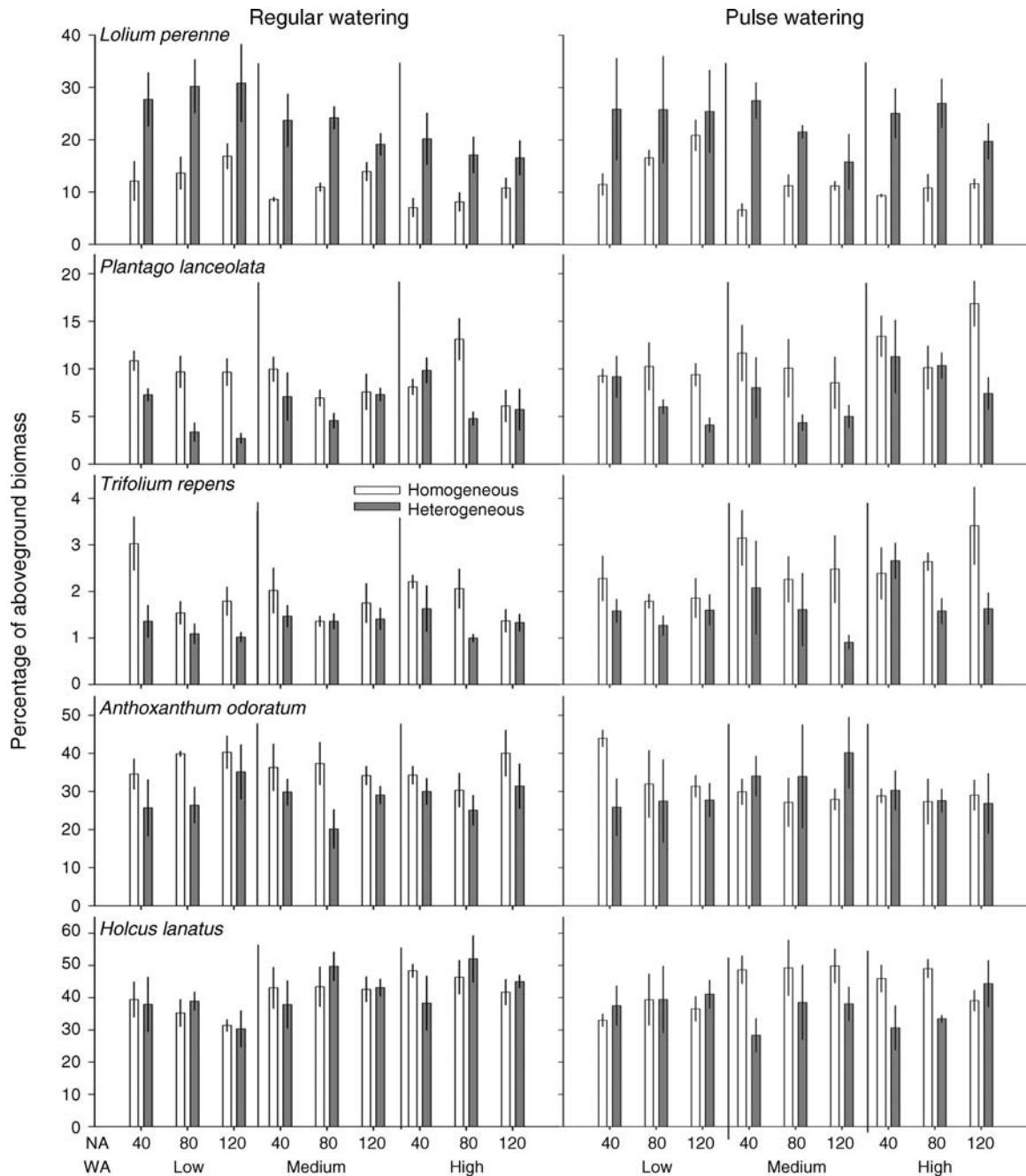


FIG. 3. Percentage of the aboveground biomass accounted for by each species. Values are means  $\pm$  SE ( $n = 4$ ). The horizontal axes indicate both nutrient availability (NA) and water availability (WA).

were supplied in a heterogeneous manner (Maestre et al. 2005, 2006, Wijesinghe et al. 2005). While our measurements cannot provide a fully mechanistic interpretation of the results obtained, they suggest that root foraging patterns were an important driver of this response. Assemblages showed precise root foraging patterns, regardless of nutrient availability, water availability, and heterogeneity. Herbaceous plants proliferate roots quickly after they encounter a nutrient patch, which

increases nutrient uptake and overall growth (Hodge 2004). This increase in growth, in turn, further stimulates root proliferation via the relationship between growth rate and root foraging precision (Fransen et al. 1999) in a positive feedback that increases plant growth until the supply of nutrients is exhausted (Fransen et al. 2001). It has been suggested that this synergistic interaction may be enhanced under high nutrient availability conditions because of increased

root proliferation (Maestre and Reynolds 2006). The positive effect of nutrient heterogeneity on biomass became more evident as nutrient availability increased, which provides partial support for this hypothesis. However, it must be noted that root foraging responses to water availability did not fully match biomass responses, as biomass increased with increases in water availability but root foraging precision did not. These results suggest that the relative importance of root foraging vs. other root responses elicited by nutrient patchiness, such as changes in nutrient uptake rates and root longevity (Hodge 2004), may be dependent on water availability.

The effects of water heterogeneity on productivity we found (from positive to negative as water availability increased) parallel those of Lundholm and Larson (2004), who found that effects of water heterogeneity on the productivity of herbaceous assemblages varied from neutral to negative as water availability increased. These results contrast with studies carried out in mesic grasslands of North America, where Fay et al. (2003), in a rainfall manipulation experiment, found that increasing variability in rainfall negatively affected productivity at either ambient or reduced (70% of ambient) rainfall levels. A recent analysis by Nippert et al. (2006) of long-term data gathered in the same system showed a positive and a negative effect of rainfall variability early and later in the growing season, respectively. These authors suggest that these seasonal patterns may be driven by differences in overall water availability, with increases in variability having a negative effect under low water availability levels. Discrepancies may be due to differences in the duration, experimental approach, and species involved. Despite these discrepancies, our results highlight the importance of temporal rainfall patterns on grassland productivity, which is consistent with previous studies (Fay et al. 2003, Nippert et al. 2006). Further, we note that the magnitude of the response to water heterogeneity under low water availability levels was modulated by nutrient heterogeneity. To our knowledge, this is the first time that such an interaction has been reported. Recent studies have shown that nutrient heterogeneity interacts with global change drivers such as nutrient availability, CO<sub>2</sub> concentration, and biotic diversity to determine the productivity of grassland populations and assemblages (Maestre et al. 2005, 2006, Maestre and Reynolds 2006). Our results complement these findings and indicate that nutrient heterogeneity may also interact with altered rainfall patterns to determine such responses.

As commonly observed with herbaceous plants (Poorter and Nagel 2000), assemblages allocated less biomass to roots as nutrient availability increased, but only at the low and medium water availability levels. Water availability also controlled the effects of nutrient heterogeneity on this variable. When significant, assemblages showed reduced belowground : aboveground biomass (BAR) values under conditions of heterogeneous

nutrient supply. Previous studies evaluating the effects of nutrient heterogeneity on the BAR of multispecies grassland assemblages have found an increase (Wijesinghe et al. 2005) or no change (Maestre et al. 2005, 2006) in this variable. It is possible that the decrease in BAR observed is the result of increasing the nutrient use efficiency under heterogeneous nutrient supply (as reported by Jackson and Caldwell 1996, Hodge 2004, Maestre et al. 2005).

Differences in the rooting patterns of co-occurring plant species have often been studied in the context of plant–plant interactions (Berendse 1982, Wardle and Peltzer 2003). However, the issues of the manner in which resource availability and heterogeneity influence root allocation patterns of coexisting species and whether differences in assemblage productivity may be attributable to divergences in the rooting depths of the species forming them remain largely unexplored. Our experimental design and measurements do not allow us to elucidate whether changes in root allocation responses were coordinated among species or were driven by one or a few species nor to fully disentangle the relative importance of root allocation patterns against potentially confounding factors such as root foraging precision as drivers of plant productivity. However, we note that values of the RII index were positively related to total assemblage biomass at the low (Spearman correlation coefficient = 0.651,  $n = 48$ ,  $P < 0.001$ ) and medium (Spearman correlation coefficient = 0.623,  $n = 48$ ,  $P < 0.001$ ) water availability levels, suggesting that patterns of root allocation with depth could be important determinants of assemblage productivity.

At the species level, the magnitude of the responses to each of the factors evaluated was species-specific, suggesting that they affected the interspecific competitive relationships between the species evaluated (Fransen et al. 2001, Novoplansky and Goldberg 2001). Nutrient heterogeneity significantly affected the contribution to aboveground biomass in four of the five species forming the assemblages and was the most influential factor affecting this variable, as it had the highest value of the  $\eta_p^2$  statistic (in most cases, see Appendix F). Interestingly, *Lolium* was the only species that positively responded to nutrient heterogeneity. Therefore, it is likely that this species largely accounted for observed patterns in root foraging at the assemblage level. The lack of positive responses to nutrient heterogeneity in *Plantago*, *Holcus*, and *Anthoxanthum* contrasts with previous studies reporting strong root proliferation and higher aboveground biomass for these species when growing in isolation or in monocultures under heterogeneous nutrient supply (Fransen et al. 1998, Maestre and Reynolds 2006). Responses of these species to nutrient heterogeneity were thus likely promoted by changes in the magnitude of their interactions with co-occurring species rather than by their lack of responsiveness to soil heterogeneity. Our results emphasize the importance of considering interspecific interactions

when evaluating responses to nutrient heterogeneity (as highlighted by Hodge [2004]) and suggest that patterns of assemblage response to this factor are likely to be strongly driven by species composition. It is interesting to note that nutrient heterogeneity interacted with both nutrient availability and water heterogeneity to determine responses in *Lolium* and *Anthoxanthum*, respectively. In a previous experiment, Maestre et al. (2005) found that nutrient heterogeneity interacted with CO<sub>2</sub> concentration to determine the proportion of above-ground biomass accounted for by *Plantago* and *Holcus*. These results contrast with those of Baer et al. (2004) and Wijesinghe et al. (2005), who found weak direct effects of soil heterogeneity on the aboveground structure of plant assemblages. These discrepancies are not surprising given the importance that both the environmental context and intra- and interspecific interactions have on the magnitude of the response of grass and forb species to nutrient heterogeneity (Wijesinghe et al. 2001).

The experimental approach used has some caveats that need to be taken into account when interpreting our results. For example, we used organic material to create different nutrient availability levels. As it needs to be mineralized before plants can take up the nutrients it contains, the "real" nutrient availability encountered by the plants could have differed between levels of the rest of factors evaluated because of differences in mineralization between them. We did not measure mineralization throughout the experiment because it would have disturbed plant growth in the microcosms. However, it must be highlighted that previous experiments using this approach have found good correlations between the amount of organic material added and the amount of nitrogen that plants capture from it (Maestre et al. 2005). We did not measure soil moisture throughout the experiment because it would have disturbed normal root growth in the microcosms. Therefore, we were unable to evaluate whether mean soil moisture content throughout the experiment differed among water heterogeneity treatments. However, the fact that assemblage biomass increased in response to increases in water availability under both regular and pulse watering suggests that the effects of water heterogeneity and availability were not confounded in our experiment.

Our experiment is the first in evaluating experimentally the effects of simultaneous changes in the spatial and temporal heterogeneity and availability of nutrients and water, respectively, on plant assemblages. It provides novel insights into how plant responses to variations in resource availability may be moderated, and even altered, by changing patterns of spatiotemporal supply. Our results demonstrate that considering the interactions between resource availability and heterogeneity allows for a refinement of predictions that can detectably reduce the error associated with extrapolating from single-factor analyses. By taking into account these interactions, considerable progress can be made toward

understanding the mechanisms that maintain and drive the dynamics of plant assemblages and toward improving our ability to predict how they will respond to the ongoing global change.

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#### APPENDIX A

Schematic representation of the microcosms used, details of the plastic cylinders and nutrient patches created in the microcosms, and view of the microcosms in the greenhouse after two months of growth (*Ecological Archives* E088-030-A1).

**APPENDIX B**

Summary results of the four-way MANOVA and ANOVA analyses for assemblage measurements (*Ecological Archives* E088-030-A2).

**APPENDIX C**

Results of post hoc analyses of root foraging precision data in the heterogeneous treatments (*Ecological Archives* E088-030-A3).

**APPENDIX D**

Post hoc analyses of the effects of nutrient availability and water availability  $\times$  water heterogeneity interaction on the ratio of root biomass in the 15–30-cm soil depth layer to that in the 0–15-cm depth layer in the heterogeneous treatments (*Ecological Archives* E088-030-A4).

**APPENDIX E**

Comparisons between alternative statistical models for assemblage measurements (*Ecological Archives* E088-030-A5).

**APPENDIX F**

Summary results of the four-way ANOVA for the effects of the different treatments on the proportion of the aboveground biomass contributed by each species (*Ecological Archives* E088-030-A6).

**APPENDIX G**

Post hoc analyses of the effects of nutrient and water availability on the proportion of the aboveground biomass contributed by *Trifolium repens*, *Lolium perenne*, *Plantago lanceolata*, and *Holcus lanatus* (*Ecological Archives* E088-030-A7).

**APPENDIX H**

Comparisons between alternative statistical models for the proportion of the aboveground biomass contributed by each species (*Ecological Archives* E088-030-A8).

**DATA REGISTRY**

Data associated with this paper are registered (ESA Data Registry: 32.1).