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# A simple method to vary soil heterogeneity in three dimensions in experimental mesocosms

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**Abstract**

Soil heterogeneity affects terrestrial plant communities both directly and indirectly. In nature, the exploration of the role of heterogeneity is made difficult because any co-varying factors (nutrients, soil depth, etc.) render it problematic to clearly link cause and effect. Attributing changes specifically to heterogeneity is facilitated if heterogeneity is varied in a controlled manner and other possible confounding factors are kept constant. The experiments conducted in such a way have up till now only considered heterogeneity in two dimensions, horizontally or vertically. In this methodological study, we present a novel technique that enables researchers to vary both qualitative and configurational heterogeneity in three dimensions by building up the soil cell by cell in experimental mesocosms. We illustrate the technique with an experiment where we test the effect of cell size (i.e. configurational heterogeneity) on the performance of grassland species that vary in nutrient preference (high N and low N species). Cell size did not affect aboveground biomass but modified species richness, both at the mesocosm and the patch scale, with most species being found when cells were small yet distinct (cell size 12 cm). High N species had significantly greater aboveground biomass and species richness than low N species, both on nutrient rich and nutrient poor cells. Remarkably, those differences disappeared when plants grew on the mesocosms with cell size close to zero. By allowing greater complexity in the design of experimental mesocosms, the 3-D approach can improve understanding of the interplay between soil heterogeneity and plant and ecosystem functioning.

**Key-words**: Configurational heterogeneity · Methodology · Qualitative heterogeneity · Patch · Pattern

## Introduction

Terrestrial plants interact with the heterogeneity in the soil in different ways. On the one hand, there are the direct influences of soil heterogeneity on the performance of individual plants and the properties of plant communities, for example, on seedling establishment, plant productivity, species composition and species diversity (Hutchings et al. 2003; Wijesinghe et al. 2005). These influences are often ascribed to soil heterogeneity promoting niche differences (Williams and Houseman 2014). On the other hand, plants respond actively to soil heterogeneity through root foraging and adapting their nutrient uptake capacity per unit root length or mass (Wijesinghe et al. 2001; Mommer et al. 2012). A third aspect of the interaction is the ability of plants to change the heterogeneity of their soil environment via plant-soil feedback (Casper and Jackson 1997; Hinsinger et al. 2005; Maestre et al. 2006; Mommer et al. 2012; Hendriks et al. 2015). The experimental investigation of these functional aspects of heterogeneity is challenging for different reasons. One is that soil heterogeneity has a qualitative and a configurational component (Kelly and Canham 1992; Maestre and Cortina 2002). Qualitative heterogeneity refers to differences in texture, nutrients, moisture, pH, etc. between locations in the soil, which can be large or small. Configurational heterogeneity, on the other hand, refers to the patch size of these locations (Dufour et al. 2006). Spatial patterns with smaller patches, for example, nutrient-poor and nutrient-rich patches alternating at short distances, are considered more heterogeneous because an "observer" such as a growing plant root or a burrowing soil animal will encounter more frequent changes when penetrating the soil. Moreover, the soil can be heterogeneous in the horizontal dimensions (Williams and Houseman 2014) and in the vertical dimension (Maestre et al. 2006;

Maestre and Reynolds 2006). Many studies have explored effects of either horizontal or vertical heterogeneity (García-Palacios et al. 2011; Brandt et al. 2013), but most soils are heterogeneous in all dimensions at the same time. No technique currently exists in experimental ecology to create fully controlled three-dimensional heterogeneity in both qualitative and configurational factors. Here we introduce such a technique in synthesized mesocosms, illustrate it with a feasibility test where plants are sown on mesocosms differing in 3-D heterogeneity, and discuss potential applications.

**Experimentally simulating 3-D heterogeneity**

The technique basically creates a mesocosm consisting of cells filled with substrates of different quality (Fig. 1). Such a "three-dimensional chessboard" is constructed layer by layer. Each layer is encased in a wooden box of which the height equals the cell size of the matrix. The wooden boxes are closed only at the sides, except for the lowest box which is also closed at the bottom. The construction starts by placing a frame consisting of vertical plastic plates with slits into the lowest wooden box, dividing the box into cells (Figs. 2a and b). Next, the cells of this lowest layer are filled with different substrates (in our case two, "black" and "white"), in an alternating fashion (Fig. 2c). The plates are subsequently removed by pulling them upwards (as a consequence the substrate will slightly subside). This completes the assembly of the bottom layer. The entire process is then repeated by placing a second wooden box with plastic plates on top of the first one, likewise filling its cells with the two substrates (black above white and vice versa), and pulling out the plates (Fig. 2d). More layers can be assembled using the same procedure, depending on the desired depth of the mesocosm. Since, apart from the lowest box, the wooden boxes are open at

89 the top and the bottom, the substrates in adjacent layers are physically connected. Given that each
90 cell has different neighbors both horizontally and vertically, heterogeneity is created in three
91 dimensions.

92 Qualitative heterogeneity can be modulated in such mesocosms by varying the difference
93 between the substrate types. For example, if nutrient-rich soil (A) and nutrient-poor soil (B) are
94 used, a series of decreasing qualitative heterogeneity could be: (i) pure A and pure B substrate in
95 alternating cells, (ii) (90% A mixed with 10% B) and (90% B mixed with 10% A) in alternating
96 cells, (iii) (80% A mixed with 20% B) and (80% B mixed with 20% A) in alternating cells, etc.
97 Configurational heterogeneity can be modulated by varying the cell size (Fig. 1), i.e. by dividing
98 the mesocosm into few large cells (low configurational heterogeneity) or many small cells (high
99 configurational heterogeneity). The two components of heterogeneity can thus be controlled
100 independently. Although we believe that a cell size equal to the box size can be used as part of a
101 heterogeneity gradient, researchers may also opt to keep the cell size smaller than the mesocosm
102 size.

103 To obtain “isotropic” mesocosms with the same density throughout, equal substrate amounts
104 should be put in each cell and similar compression applied such that soil in all cells is compacted
105 to the same degree. We recommend filling each cell in two stages while compressing already after
106 the first half. Substrates composed of a mixture of A and B soil can be homogenized with a cement
107 mixer. If the substrate is sticky, for example when using clay soil, placing two flat heavy objects
108 on top of the layer, one directly to the left and one directly to the right of the plate which is to be
109 removed, can prevent substrate from being dragged along when pulling up a plate. Drainage holes
110 may be drilled in the bottom plate of the mesocosms, and root mesh placed to avert loss of

111 substrate and roots growing through the holes.

112

113 **Demonstration of the technique**

114 We illustrate the 3-D method with an example of its use (Figs. 2a-e). Four levels of
115 configurational heterogeneity were created in cubic mesocosms (dimensions 48 cm × 48 cm × 48
116 cm) with cell size 48, 24, 12 or 0 cm. In other words, all mesocosms had identical dimensions; the
117 only difference was the cell size. Qualitative heterogeneity was the same in all, created by mixing
118 80% potting soil with 20% sand to fill the black cells (nutrient rich substrate), and 20% potting
119 soil with 80% sand to fill the white cells (nutrient poor substrate). The mesocosms with cell size
120 zero were filled with mixed nutrient rich and poor substrate so that the 'cell size' was close to zero.
121 There are numerous definitions of heterogeneity (Kolasa and Rollo, 1991; Li and Reynolds 1995;
122 Stein and Kreft, 2015), and according to some of those, our mesocosms with cell size zero could
123 be perceived as homogeneous. However, these mesocosms could also be seen as exhibiting the
124 highest soil heterogeneity because: (i) plant roots extract nutrients at the millimeter scale, and
125 would thus encounter a series of micro-patches of nutrient rich and nutrient poor substrate along a
126 very short distance (Hutchings et al. 2003), and (ii) considering the mixture as homogeneous
127 (based on macroscopic visual impression) would imply that the gradient at one point would
128 suddenly change from absolute maximum heterogeneity (very small but still distinct cells) to
129 absolute minimum heterogeneity (homogeneity), even though the cell size was systematically
130 further decreased (Eilts et al. 2011). The authors of the current study support the latter perspective,
131 but since there is no consensus in literature on this (cf. Kolasa and Rollo, 1991; Li and Reynolds
132 1995; Chen and Dong 2003) we treat and describe the cell size zero level neutrally.

Substrates were tested by a soil laboratory ("Bodemkundige Dienst van België", Heverlee, Belgium) (Table 1). Heterogeneity levels with cell sizes 24, 12 and 0 cm were represented by five replicate mesocosms each, while the heterogeneity level with cell size 48 cm was represented by five replicate mesocosms with nutrient rich substrate and five replicate mesocosms with nutrient poor substrate in order to able to determine the influences of both these substrate types. At the surface, the 48-cm-cell mesocosms can actually be considered to have an "infinite" patch size, since there is no surrounding patch with different soil that plants can access. In the analyses, nutrient rich and nutrient poor mesocosms with cell size 48 cm were therefore lumped when comparing them with other heterogeneity levels. This ensures that the average amount of nutrients (or average nutrient concentration) is identical for each cell size level, i.e. nutrient rich and nutrient poor substrate are always equally represented, precluding nutrient bias across heterogeneity levels. With respect to depth, the mesocosms with cell size 48, 24 and 12 cm were composed of one, two and four layers, respectively. All boxes were randomly distributed across the experimental site (see below) to prevent position bias.

Most previous studies on soil heterogeneity focused on monocultures or two plant species growing together, while we aimed for more complex communities with many species interacting both with each other and with the soil heterogeneity. To this end, seeds of 24 perennial plant species occurring in grasslands in Belgium were obtained from commercial suppliers (Herbiseed in England and Cruydt-Hoeck in The Netherlands). The species were arbitrarily classified into two groups: high (6-8) or low (1-4) preference for nitrogen (N) availability according to the Ellenberg ecological indicator value for N (12 species per group, Table 2). These values, which were put forward by Ellenberg according to the position of plant species' realized ecological niche along an

155 environmental gradient, indicate a species' association with particular growing conditions
156 (Ellenberg et al. 1991), and have been used in many other studies to differentiate between species
157 groups (e.g. Pärtel and Zobel 2007; Gazol et al. 2013). The rationale of this design was that
158 nutrient-rich cells might favor the biomass production of species with high N indicator values,
159 which tend to be competitive (Franzaring et al. 2007) and could outcompete species with low N
160 indicator values on this substrate (i.e. lower their species richness). High N species would also
161 compete intensely among themselves on such nutrient rich cells, likewise reducing their own
162 species richness (Hautier et al. 2009). The low N species, on the other hand, which are more
163 stress-tolerant given that they are most often found in low N environments, would be expected to
164 perform relatively better on the nutrient-poor cells. High N species might still occur on such cells
165 as any competitive exclusion will be slow in unproductive environments, leading to higher species
166 richness compared with nutrient rich cells. We expect the differences in species richness between
167 nutrient poor and nutrient rich substrates to become smaller with decreasing cell size. The
168 presence of adjacent nutrient poor cells should decrease the dominance of those species that
169 outcompete many other species on larger nutrient rich cells, while species being able to persist on
170 bigger nutrient poor cells will likely face increasing competitive pressure as the proximity to
171 nutrient rich cells increases. Depending on the balance of these changes expected on nutrient rich
172 and nutrient poor cells, the species richness at mesocosm scale may change in different directions.

173 The experiment ran from 19 May 2015 (sowing date) to 2 September 2015 (harvest) at the
174 Drie Eiken Campus of the University of Antwerp (Belgium, 51°09´N, 04°24´E). The local climate
175 in the region is characterized by mild winters and cool summers, with an average annual air
176 temperature of 9.6°C and 776 mm of rainfall, equally distributed throughout the year. Three weeks

prior to the start of the experiment we tested the germination rates and emergence times of the species in order to correct for interspecific differences in these traits in the actual experiment, i.e. to equalize the relative abundances and synchronize emergence. Only the relative abundances had to be adjusted as emergence times were similar. In the actual experiment, the adjusted seed mixture containing all 24 species was sown uniformly across the surface of the 25 3-D mesocosms, at 423 seeds per mesocosm. After sowing, the seeds were covered by a few mm of the appropriate substrate (i.e., seeds sown on nutrient rich/poor cells were covered with nutrient rich/poor substrate, respectively) and the mesocosms were kept moist to promote germination and establishment. During the experiment, water was added when needed to account for any shortage in natural rainfall, at the prevailing frequency of rainfall events in the region (every two days). Fungicide was applied once at the end of June and then one week later to avoid fungal diseases.

At the end of the experiment, we recorded the species in four subsamples of 12 cm × 12 cm for mesocosms with cell sizes 0 and 48 cm, and in eight subsamples of 12 cm × 12 cm for the mesocosms with cell sizes 12 and 24 cm (with half of the subsamples in nutrient poor and half in nutrient rich cells). These species lists were used to calculate the average species richness at patch scale (i.e., per 12 cm × 12 cm subsample, for each substrate type), and the average species richness at mesocosm scale. Next, we harvested the aboveground biomass of the plants in each mesocosm. In the mesocosms with cell sizes 24 and 12 cm, one subsample (12 cm × 12 cm) was taken from a randomly chosen nutrient poor cell and one subsample (12 cm × 12 cm) from a randomly chosen nutrient rich cell. Mesocosms with cell sizes 0 and 48 cm were sampled with only one subsample each (12 cm × 12 cm, likewise randomly selected), as these did not have different (or distinguishable) substrate types. In each of these subsamples, the plants were

harvested at the surface of the soil, grouped by species type, oven dried at 70°C for 4 days, and weighed. The remaining aboveground biomass in each mesocosm was also harvested, separated per substrate (plants growing on the same substrate were combined), and likewise dried and weighed. Overall, biomass was thus estimated both on subsamples (smaller sample area, but allowing separation of the species groups) and on entire mesocosms (larger area, but without separating the species groups).

We analyzed the data both at mesocosm scale and at cell (patch) scale. At mesocosm scale, we used one-way ANOVA to test the effect of cell size on plant biomass, and two-way ANOVA to test the effect of cell size and species type on species richness. Post-hoc analysis (pairwise comparisons with Fisher's LSD) was used to test differences between mesocosms with different cell sizes. At cell (patch) scale, we used generalized linear mixed models (GLMMs) to analyze the effect of the treatments on plant biomass and species richness, box identity was treated as the random factor. A first analysis was performed on the mesocosms with cell sizes 12, 24 and 48 cm, excluding the mesocosms with cell size 0 cm because these contained no distinguishable poor and rich cells to which plant responses could be attributed. In this analysis, species type (high or low N species) and (growing on) nutrient rich or poor substrate were explanatory fixed factors, as was cell size. A stepwise exclusion of the least significant explanatory variables was performed. Post-hoc analysis (pairwise comparisons with Fisher's LSD) was applied to explicitly test differences between mesocosms differing in configurational heterogeneity (i.e. cell size). In a second analysis at cell (patch) scale, we used Student's t-tests to investigate the effect of species type on aboveground biomass and species richness in 0-cm cell size mesocosms. These mesocosms were analyzed separately because nutrient rich and nutrient poor patches were

indistinguishable, implying that substrate type could not be used as a factor in the analysis (in contrast to the first analysis at cell scale). All statistics were conducted with SPSS 23.0 (IBM Corp., 2015).

**Results**

At the mesocosm scale, cell size did not significantly affect aboveground biomass. However, cell size and species type significantly affected species richness (Table 3, Fig. 3). More high N than low N species were found at the mesocosm scale ($p$ = 0.001), and most species tended to grow in mesocosms with cell size 12 cm ($p$ = 0.016, 0.068 and <0.001 for the post-hoc pairwise comparison with 0-, 24- and 48-cm cell mesocosms, respectively). The least species tended to be found in mesocosms with cell size 48 cm ($p$ = 0.078, <0.001 and 0.015 for the post-hoc pairwise comparison with 0-, 12- and 24-cm cell mesocosms, respectively) (Fig. 3).

At the cell (patch) scale, the first analysis of the mesocosms with cell sizes 12, 24 and 48 cm revealed no interactive effects of substrate, cell size and species type on aboveground biomass, while cell size and species type interacted significantly on species richness. Significant differences in aboveground biomass and species richness between high and low N species were found (Table 4). We found more biomass and higher species richness for high N species than for low N species on both nutrient rich and poor cells (Fig. 4). While cell size did not significantly affect aboveground biomass, it did modulate species richness (Table 4), in agreement with the aforementioned analyses at mesocosm scale. Most species were generally found in mesocosms with cell size 12 cm ($p$ = 0.02 and <0.01 for the post-hoc pairwise comparison with 24- and 48-cm cells, respectively), while no significant difference of species richness (at cell scale) between

mesocosms with cell size 24 cm and 48 cm was found ($p$ = 0.440). Note, however, that low N species responded more weakly to cell size than high N species (cf. the aforementioned significant cell size × species type interaction). The second analysis at patch (cell) scale demonstrated that low and high N species performed very similarly in the mesocosms with cell size zero, with no significant effects of species type discernible, both regarding aboveground biomass ($p$ = 0.77) and species richness ($p$ = 0.78) (Fig. 4).

## Discussion

We demonstrated that configurational and qualitative soil heterogeneity can be created in three dimensions at controlled levels and independently from each other in synthesized mesocosms. The results of a first, short experiment highlight that complex and surprising patterns may emerge from manipulating 3-D heterogeneity. Aboveground biomass at the mesocosm scale was not significantly affected by cell size, which is inconsistent with the study by Gazol et al. (2013). However, species richness was modified by cell size, with more species growing in mesocosms with small yet distinct cells (12 cm) than in mesocosms with either a more coarse distribution of substrates (cell size 24 and 48 cm) or with fully mixed substrate (cell size 0). The former is in line with earlier assertions of soil heterogeneity promoting community diversity by offering more niches (Pickett and Bazzaz 1978; Ackerly and Cornwell 2007; Williams and Houseman 2014).

At the cell (patch) scale, in line with expectations, our results indicate clear differences in productivity and realized species richness between species with varying nutrient preferences. Although we expected that especially high N species would profit from growing on nutrient rich cells, i.e. relatively increase their biomass at the expense of low N species by superior foraging

265 ability either locally or in deeper soils (Tamme et al. 2010; Gazol et al. 2013), the absence of a
266 significant species type × substrate type interaction suggests otherwise. Likewise, no clear
267 evidence of lower species richness in nutrient rich patches was found. In fact, at cell size 48 cm,
268 more species were present in nutrient rich than in nutrient poor mesocosms. In the analyses at the
269 patch scale we did observe several effects of configurational heterogeneity (cell size). First,
270 species richness increased when the cell size decreased from 48 cm to 12 cm (Fig. 4 and Table 4),
271 in line with our findings at the mesocosm scale. Because the number of species found on nutrient
272 poor and nutrient rich substrates was similar, our hypothesis that species richness differences
273 between both substrates would become smaller with decreasing cell size was not confirmed. Of
274 note is the observation that differences in both species richness and aboveground biomass between
275 species thought to be more and less competitive (high and low N species) were no longer
276 significant in mesocosms where the two substrates were fully mixed (i.e. cell size 0 cm). This
277 implies that high N species were negatively affected at this extreme end of the heterogeneity
278 gradient, while low N species were stimulated. Our experiment demonstrates that explicitly
279 varying soil heterogeneity in three dimensions can generate complex patterns. Full elucidation of
280 such patterns will probably require detailed studies of root foraging and plasticity, as plants
281 growing on nutrient poor cells likely grew into nutrient rich cells adjacent or below.

282 Similar to other methods of artificial assembly of model ecosystems, the technique is likely
283 prone to reduced ecological realism owing to the initial soil disturbance during construction, edge
284 effects, isolation or island effects, time scale limitations, etc. (De Boeck et al. 2015). Results from
285 its application should thus be interpreted and extrapolated with caution, and should preferably be
286 combined with findings from other approaches such as using the natural variation in heterogeneity

(Williams and Houseman 2014) or injecting nutrients in existing soils (McKane et al. 2002). Yet the 3-D technique can offer insights into heterogeneity – ecosystem functioning relationships which are hard to acquire from these other approaches, which are less flexible and suffer from covariation of heterogeneity with other factors (Brandt et al. 2013). Note that direct comparisons of our results with those from previous studies on 2-D soil heterogeneity are not straightforward because 2-D studies have used a wide variety of different techniques, which do not always have an equivalent of cell size (e.g. injecting nutrients, mixing different layers of existing soils, etc.), and because we sowed a mixture of two particular groups of species, which to our knowledge has not been done before in heterogeneity – ecosystem functioning research.

A promising avenue for future research opened by the 3-D technique is disentangling the combined influences of horizontal heterogeneity (patchiness) and vertical heterogeneity (stratification) in the same system. Here the method even allows one to simulate increasing uniformity away from the soil surface as found in many real soils (Kardanpour et al. 2015), by gradually augmenting the thickness of the layers with depth. Likewise, horizontal anisotropy in the soil patchiness can be simulated by locally varying the cell size within the same layer, for example, by subdividing some of the cells into smaller ones whilst keeping others larger. Moreover, more than two substrate types could be used, for example, to simulate gradients or generate a variegated mesocosm in one or more dimensions.

The 3-D method also opens perspectives to better understand the interplay between soil heterogeneity and plant heterogeneity (i.e. spatial aggregation of plant species). Growth in mono-specific patches significantly alters the competitive balance relative to a random mixture (De Boeck et al. 2006), but the interaction with the soil structure is hardly understood. This

309 interaction can be studied by planting species in specific positions (Burns and Brandt 2014),
310 manipulating both the species-specific interaction of the plants and the soil heterogeneity.

311 Finally, the 3-D technique could improve our understanding of how soil heterogeneity drives
312 plant diversity at small scales. Several theories have been proposed to explain this relationship
313 (positive, neutral or negative, Wijesinghe et al. 2005; Brandt et al. 2013), depending on the
314 relative sizes of patches and plants. When the plant size exceeds the patch size of soil
315 heterogeneity, species with good foraging ability may monopolize the resource-rich patches, thus
316 competitively excluding weaker foragers (Tamme et al. 2010; Gazol et al. 2013). This theory in
317 fact considers heterogeneity like a resource and a heterogeneity gradient like a niche axis. In the
318 microfragmentation theory (Tamme et al. 2010; Gazol et al. 2013), the plant size is similar or
319 smaller than the patch size. Here, specialists of poor soil patches would be exposed to greater risk
320 of mortality than generalist species which can use both poor and rich soil, which overall may
321 reduce plant species diversity. In the current experiment, there are two factors precluding us from
322 testing this theory. First, the relative size of species vs the patch is unclear, which could be
323 resolved by injecting stable isotopes in specific patches and analyzing aboveground biomass so
324 that the root distribution/proliferation of each species can be traced (Oburger and Schmidt 2016);
325 second, two extreme species types were used rather than a generalist and a specialist type. A third
326 theory states that soil heterogeneity may promote diversity simply because more niches are
327 available, which we think caused the pattern observed in the current experiment as mentioned
328 earlier. Future studies using the 3-D technique could test these theories by independently varying
329 plant and cell size. Since the goal in this type of research would be to understand how soil
330 heterogeneity drives plant diversity, a random seed rain of a given species mixture might be

applied as in the current experiment, allowing the different substrates to “select” the species that establish locally, producing a community structure that is “unsupervised” by the experimenter. In conclusion, the 3-D technique provides a flexible test environment for investigating these heterogeneity-diversity relationships, which should yield more insight in small-scale coexistence and diversity patterns in plant communities.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.

**Ethical approval** The authors declare that all appropriate ethics and approvals were obtained for the study.

**References**

Ackerly DD, Cornwell WK (2007) A trait-based approach to community assembly: partitioning of species trait values into within- and among-community composents. Ecol Lett 10:135-145

Brandt AJ, de Kroon H, Reynolds HL, Burns JH (2013) Soil heterogeneity generated by plant–soil feedbacks has implications for species recruitment and coexistence. J Ecol 101:277-286

Burns JH, Brandt AJ (2014) Heterogeneity in plant–soil feedbacks and resident population dynamics affect mutural invasibility. J Ecol 102:1048-1057

Casper BB, Jackson RB (1997) Plant competition underground. Annu Rev Ecol Syst 28:545-570

Chen Y, Dong M (2003) Spatical heterogeneity in ecological systems. Acta Ecol Sin 23:346-352

De Boeck HJ, Nijs I, Lemmens CM, Ceulemans R (2006) Underlying effects of spatial aggregation (clumping) in relationships between plant diversity and resource uptake. Oikos 113:269-278

De Boeck HJ, Vicca S, Roy J, Nijs I, Milcu A, Kreyling J, Jentsch A, Chabbi A, Campioli M, Callaghan T, Beierkuhnlein C, Beier C (2015) Global change experiments: challenges and opportunities. BioScience 65:922-931

Dufour A, Gadallah F, Wagner HH, Guisan A, Buttler A (2006) Plant species richness and environmental heterogeneity in a mountain landscape: effects of variability and spatial configuration. Ecography 29:573-584

Eilts JA, Mittelbach GG, Reynolds HL, Gross KL (2011) Resource heterogeneity, soil fertility, and species diversity: effects of clonal species on plant communities. Am Nat 177:574-588

Ellenberg H, Webber HE, Düll R, Wirth V, Werner W, Paulissen D (1991) Zeigerwerte von Pflanzen in Mitteleuropa. Scr Geobot 18:1-248

Franzaring J, Fangmeier A, Hunt R (2007) On the consistencies between CSR plant strategies and Ellenberg ecological indicator values. J Appl Bot Food Qual 81:86-94

García-Palacios P, Maestre FT, Gallardo A (2011) Soil nutrient heterogeneity modulates ecosystem responses to changes in the identity and richness of plant functional groups. J Ecol 99:551-562

Gazol A, Tamme R, Price JN, Hiiesalu I, Laanisto L, Pärtel M (2013) A negative heterogeneity-diversity relationship found in experimental grassland communities. Oecologia 173:545-555

Hautier Y, Niklaus PA, Hector A (2009) Competition for light cause plant biodiversity loss after eutrophication. Science 324:636-638

Hendriks M, Visser EJ, Visschers IG, Aarts BH, Caluwe H, Smit-Tiekstra AE, Putten WH, De Kroon H, Mommer L (2015) Root responses of grassland species to spatial heterogeneity of plant–soil feedback. Funct Ecol 29:177-186

Hinsinger P, Gobran G, Gregory P, Wenzel W (2005) Rhizosphere geometry and heterogeneity

arising from root-mediated physical and chemical processes. New Phytol 168:293-303

Hutchings MJ, John EA, Wijesinghe DK (2003) Toward understanding the consequences of soil heterogeneity for plant populations and communities. Ecology 84:2322-2334

Kardanpour Z, Jacobsen OS, Esbensen KH (2015) Local versus field scale soil heterogeneity characterization - a challege for representative sampling in pollution studies. Soil 1:695-705

Kelly VR, Canham CD (1992) Resource heterogeneity in oldfields. J Veg Sci 3:545-552

Kolasa J, Rollo CD (1991) Introduction: the heterogeneity of heterogeneity : a glossary. In Ecological Heterogeneity (eds. Kolasa J and Pickett STA). Springer, New York, pp 1-23

Li H, Reynolds JF (1995) On definition and quantification of heterogeneity. Oikos 73:280-284

Maestre FT, Bradford MA, Reynolds JF (2006) Soil heterogeneity and community composition jointly influence grassland biomass. J Veg Sci 17:261-270

Maestre FT, Cortina J (2002) Spatial patterns of surface soil properties and vegetation in a Mediterranean semi-arid steppe. Plant Soil 241:279-291

Maestre FT, Reynolds JF (2006) Small-scale spatial heterogeneity in the vertical distribution of

soil nutrients has limited effects on the growth and development of *Prosopis glandulosa* seedlings. Plant Ecol 183:65-75

McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA, Murray G (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415:68-71

Mommer L, van Ruijven J, Jansen C, van de Steeg HM, de Kroon H (2012) Interactive effects of nutrient heterogeneity and competition: implications for root foraging theory? Funct Ecol 26:66-73

Oburger E, Schmidt H (2016) New methods to unravel rhizosphere process. Trends Plant Sci 21:243-255

Pärtel M, Zobel M (2007) Dispersal limitation may result in the unimodal preductivity-diversity relationship: a new explanation for a general pattern. J Plant Ecol 95:90-94

Pickett STA, Bazzaz FA (1978) Organization of an assemblage of early successional species on a soil moisture gradient. Ecology 59:1248-1255

Stein A, Kreft H (2015) Terminology and quantification of environmental heterogeneity in species-richness research. Bio Rev 90:815-836

441

442 Tamme R, Hiiesalu I, Laanisto L, Szava-Kovats R, Pärtel M (2010) Environmental heterogeneity,

443 species diversity and co-existence at different spatial scales. J Veg Sci 21:796-801

444

445 Williams BM, Houseman GR (2014) Experimental evidence that soil heterogeneity enhances plant

446 diversity during community assembly. J Plant Ecol 7:461-469

447

448 Wijesinghe DK, John EA, Beurskens S, Hutchings MJ (2001) Root system size and precision in

449 nutrient foraging: responses to spatial pattern of nutrient supply in six herbaceous species. J Ecol

450 89:972-983

451

452 Wijesinghe DK, John EA, Hutchings MJ (2005) Does pattern of soil resource heterogeneity

453 determine plant community structure? An experimental investigation. J Ecol 93:99-112

454 **Table 1** Characteristics of the two substrate types used in the heterogeneity experiment.

| Substrate type | pH | C (%) | NaCl (mg $L^{-1}$) | $NO_3^-$-N (kg $ha^{-1}$) | $NH_4^+$-N (kg $ha^{-1}$) | $P_2O_5$ (mg $L^{-1}$) | $K_2O$ (mg $L^{-1}$) | MgO (mg $L^{-1}$) | CaO (mg $L^{-1}$) | $Na_2O$ (mg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Nutrient poor | 5.5 | 1.1 | 555 | 142 | 11 | 32 | 118 | 253 | 467 | 18 |
| Nutrient rich | 5.3 | 8.7 | 1264 | 420 | 12 | 188 | 228 | 1252 | 1700 | 81 |

455 **Table 2** Plant species used in the experiment and Ellenberg nitrogen (N) values of the two groups.

| Group 1 | N value | Group 2 | N value |
|---|---|---|---|
| *Brachypodium sylvaticum* (Huds.) Beauv. | 6 | *Achillea ptarmica* L. | 2 |
| *Dactylis glomerata* L. | 6 | *Agrostis capillaris* L. | 4 |
| *Epilobium hirsutum* L. | 8 | *Berteroa incana* (L.) DC. | 4 |
| *Festuca gigantea* (L.) Vill. | 6 | *Briza media* L. | 2 |
| *Festuca pratensis* Huds. | 6 | *Festuca ovina* L. | 1 |
| *Geranium robertianum* L. | 7 | *Hypericum perforatum* L. | 4 |
| *Lolium perenne* L. | 7 | *Koeleria macrantha* (Ledeb.) Schult. | 2 |
| *Nepeta cataria* L. | 7 | *Leucanthemum vulgare* Lam. | 3 |
| *Poa pratensis* L. | 6 | *Nardus stricta* L. | 2 |
| *Poa trivialis* L. | 7 | *Poa compressa* L. | 3 |
| *Silene dioica* (L.) Clairv. | 8 | *Rumex acetosella* L. | 2 |
| *Taraxacum officinale* F.H.Wigg | 8 | *Vulpia myuros* (L.) C.C.Gmel | 1 |

**Table 3** Results of the measurements at the mesocosm scale analyzed with one-way ANOVA for aboveground biomass and with two-way ANOVA for species richness. F-values, P-values and degrees of freedom (df1, df2) are given, with df1 = between-groups degrees of freedom, and df2 = within-groups degrees of freedom. Significant results ($P < 0.05$) indicated in bold. Nonsignificant variables were removed stepwise from the final model.

| Source | Aboveground biomass | | | Species richness | | |
|---|---|---|---|---|---|---|
| | df | *F* | *P* | df | *F* | *P* |
| Cell size | 3, 21 | 0.041 | 0.989 | 3, 46 | 7.669 | **< 0.001** |
| Species type | | | | 1, 48 | 18.025 | **< 0.001** |
| Cell size × Species type | | | | 3, 42 | 1.800 | 0.162 |

**Table 4** Results of the generalized linear mixed models (GLMMs): comparison of mesocosms with cell sizes 12, 24 and 48 cm (substrate types: nutrient rich and nutrient poor). F-values, P-values and degrees of freedom (df1, df2) are given, with df1 = between-groups degrees of freedom, and df2 = within-groups degrees of freedom, for aboveground biomass and species richness. Species type refers to the species' nitrogen preference (low or high) according to Ellenberg indicator values. Significant results ($P < 0.05$) indicated in bold. Nonsignificant variables were removed stepwise from the final model.

| Source | Aboveground biomass | | | Species richness | | |
|---|---|---|---|---|---|---|
| | df | *F* | *P* | df | *F* | *P* |
| Cell size | 2, 57 | 0.824 | 0.444 | 2, 237 | 5.501 | **0.005** |
| Soil type | 1, 58 | 2.405 | 0.126 | 1, 238 | 0.126 | 0.723 |
| Species type | 1, 58 | 60.002 | < **0.001** | 1, 238 | 78.314 | < **0.001** |
| Cell size × Soil type | 2, 54 | 0.501 | 0.609 | 2, 234 | 0.299 | 0.742 |
| Cell size × Species type | 2, 54 | 0.562 | 0.574 | 2, 234 | 3.453 | **0.033** |
| Soil type × Species type | 1, 56 | 1.170 | 0.284 | 1, 236 | 1.022 | 0.361 |
| Cell size × Soil type× Species type | 2, 48 | 0.161 | 0.852 | 2, 228 | 0.971 | 0.424 |

**Figure 1.** Mesocosms consisting of substrates of different quality, for example, nutrient rich (black) and nutrient poor (white) cells. Configurational heterogeneity decreases from left to right, from fine (small cells) to coarse (large cells) distribution of resources. The cell size of the full mixture of the two substrates on the left can be considered as approximately zero.

**Figure 2.** Experimental simulation of 3-D heterogeneity in mesocosms: (a) frame of vertical plastic plates with slits, to separate the mesocosm cells; (b) wooden box with the frame of vertical plastic plates placed inside to hold one mesocosm layer; (c) filled nutrient rich (black) and nutrient poor (white) soils into one mesocosms layer; (d) completed mesocosm consisting of four filled layers, with alternating nutrient rich (black) and nutrient poor (white) substrate; (e) emerging plants two weeks after sowing.

**Figure 3.** Means ± SE of (a) aboveground biomass, (b) species richness and (c) species richness separated by species differing in nitrogen preference (high N/low N), all analyzed at mesocosm scale. For mesocosms with cell size 48 cm, the average (grey dot) of mesocosms of nutrient rich (black dot) and nutrient poor (white dot) are used to connect the line. These markers are added for visual clarity and were not used in statistical analyses. Significant ($p < 0.05$) differences between treatments are indicated by different letters (post hoc analysis with Fisher's LSD).

**Figure 4.** Means ± SE of species differing in nitrogen preference (high N/low N species) in nutrient rich and poor cells of varying size: (a) aboveground biomass in nutrient poor cells, (b) aboveground biomass in nutrient rich cells, (c) species richness in nutrient poor cells and (d)

490 species richness in nutrient rich cells. Significant ($p < 0.05$) differences between mesocosms of
491 different cell size (12, 24 and 48; across substrate type) are indicated by different letters (post hoc
492 analysis with Fisher's LSD). The means ± SE are also indicated for the full mixture of rich and
493 poor substrate (cell size 0 cm).