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Abstract

Aims Soil heterogeneity is a primary mechanism explaining plant species diversity. Yet, controlled experiments yield inconsistent soil heterogeneity-diversity (SHD) relationships, ranging from positive, neutral to negative.

Methods Here we investigated the SHD relationship by experimentally alternating nutrient-rich and nutrient-poor substrate in three dimensions, creating four levels of soil configurational heterogeneity (cell sizes 0, 12, 24 and 48 cm). Across each mesocosm, a mixture of species with high and low nitrogen requirements was evenly sown.

Results Contrary to earlier experimental findings, this approach yielded a unimodal SHD relationship, peaking at cell size 12 cm. This pattern originated mainly from increased plant diversity of species with high nitrogen requirement. Diversity increases with configurational heterogeneity were not due to greater variation in light niches, and diversity decreases were not due to success of fast growing species. Strikingly, plant density increased monotonically with increasing configurational heterogeneity, indicating that not only more species but also more individuals could coexist.

Conclusions This study provides experimental evidence for unimodal SHD curves in plant communities, which has hitherto only been predicted by models. Our results carry a striking similarity with other unimodal response patterns of plant species diversity, notably in diversity–disturbance and diversity–productivity relationships.

Keywords: Abundance; Diversity index; Similarity; Soil heterogeneity; Three dimensions

Introduction

Spatial heterogeneity is likely to be an ultimate driver of plant species diversity (Tilman 1982, 1988; Tilman and Pacala 1993). However, the nature of the soil heterogeneity-diversity (SHD) relationship is not consistent across studies, and several theories have been put forward to explain the underlying mechanisms. The classical one is niche theory, which assumes that heterogeneous environments offer more niches than homogeneous environments, thus allowing more species to coexist (Tilman and Pacala 1993; Rosenzweig 1995; Williams and Houseman 2014). Positive SHD's in line with this theory were indeed found in several experimental studies (e.g. Richardson et al. 2012; Williams and Houseman 2014). In contrast, other experiments have found negative SHD relationships (e.g. Gazol et al. 2013). The decreasing pattern was attributed to rapid depletion of resource-rich patches in heterogeneous soils by species with good foraging abilities, thus suppressing other species through asymmetric root competition (Hutchings et al. 2003; Wijesinghe et al. 2005). The easy access to patchily distributed soil resources would in turn also enhance shoot biomass, further suppressing competitors through asymmetric competition for light (Hautier et al. 2009; Lamb et al. 2009; DeMalach et al. 2017). Sometimes, neutral SHD relationships are found. In the experiment of Reynolds et al. (2007), this was attributed to clonal species obscuring the effect of soil heterogeneity on plant diversity (De Kroon and Bobbink 1997; Eilts et al. 2011; Baer et al. 2015). However, to our knowledge, no experimental study simultaneously tested many possible mechanisms, which hampers assessing their relative importance.

Investigating the SHD relationship in nature is complex because soil heterogeneity has a qualitative component (texture, nutrients, moisture, pH, etc.) and a configurational component (the

size and distribution of patches) (Kelly and Canham 1992; Maestre and Cortina 2002; Dufour et al. 2006), and both these components vary in space and time (Tilman and Pacala 1993; Maestre et al. 2006; Maestre and Reynolds 2006). Experimental manipulation of soil heterogeneity, on the other hand, may bring more control and repeatability, but suffers from the lack of a standard method to vary soil heterogeneity. Some experimental studies have injected nutrients or spread fertilizer in a clumped pattern (Richardson et al. 2012), but doing so may not lead to stable patch sizes. Others have spatially redistributed soil from different layers at the same location, or soil from different locations (García-Palacios et al. 2011; Wubs and Bezemer 2016; 2017). While this may bring more realism, legacies from previous plant-soil feedback can confound current plant responses to soil heterogeneity (Brandt et al. 2013). Moreover, the studies that experimentally explored effects on plant species diversity have varied soil heterogeneity in two dimensions, yet soils are heterogeneous in three dimensions (Stewart et al. 2000). Finally, differences in species composition may explain some of the contrasting SHD findings, as in the aforementioned case of clonal species, or when N-fixing species change the original soil heterogeneity through local N-fixation.

Here we explore the SHD relationship with a mesocosm experiment where soil configurational heterogeneity is systematically varied in three dimensions using a recently developed technique (Fig.1a, Liu et al. 2017a), whilst excluding species that may significantly alter soil heterogeneity or blur response patterns such as N-fixing and clonal species (García-Palacios et al. 2012; Tamme et al. 2016). To allow different species to thrive and coexist on different substrates, as would be the case in nature, we apply the same seed rain to all mesocosms (Gazol et al. 2013). Compared with the existing literature, novel potential mechanisms

81 are put forward as well as mechanisms proposed earlier. Our hypotheses are: (1) At patch scale,
82 high availability of soil resource promotes biomass production, which in turn reduces light
83 availability (and vice versa). The fine-scale alternation of small resource-rich and resource-poor
84 patches at high levels of soil heterogeneity therefore creates greater spatial variation in light
85 intensity at mesocosm scale, and thus more light niches, than the course-scale alternation of large
86 resource-rich and resource-poor patches at low levels of soil heterogeneity. As a result, soil
87 heterogeneity would indirectly increase diversity by weakening competition for light (Fig. 1b). (2)
88 Alternatively, at mesocosm scale, soil heterogeneity decreases diversity, because, when cell size is
89 small, slow growing species are eliminated by species with better foraging abilities (often fast
90 growers, Fransen et al. 1999; Kembel and Cahill 2005) that can better exploit the more dispersed
91 soil resources (Fig. 1c). Soil heterogeneity would thus lower diversity by accelerating species
92 exclusion. (3) High soil heterogeneity (small patches) facilitates root access to resources from
93 adjacent patches because of the shorter distance. At patch scale, communities growing on
94 nutrient-poor patches will thus more easily gain resources from neighbouring nutrient-rich patches
95 when the patch size is small, and this will enhance their productivity and light competition and
96 reduce their species diversity (Fig. 1d-f). In contrast, communities growing on nutrient-rich
97 patches will more easily lose resources to ingrowing neighbours from adjacent nutrient-poor
98 patches when the patch size is small, thus reducing their productivity and light competition, and
99 allowing more species to coexist. The balance of these changes on the two substrates will
100 determine the species diversity response to heterogeneity at mesocosm scale (note that this is the
101 case for all hypotheses).
102

Materials and Methods

EXPERIMENTAL DESIGN

As details of the experimental site and design are described in Liu et al. (2017b), who studied root responses of plant communities to soil heterogeneity in the same model ecosystems, we give a succinct description here. The experiment was conducted at University of Antwerp in Wilrijk, Belgium (51°09′41″N, 04°24′29″E), which is characterized by mild winters and cool summers, with average annual air temperature 10.6 °C and rainfall 832 mm, equally distributed throughout the year (Royal Meteorological Institute of Belgium). In spring 2015 we established four levels of three-dimensional soil heterogeneity in cubic mesocosms of the same size (48 cm × 48 cm × 48 cm), by varying the cell size within these mesocosms from 0 to 12, 24 and 48 cm (Fig. 1a). The cells were filled with nutrient-rich and nutrient-poor substrate, created by thoroughly mixing potting soil and sand in a 4:1 and a 1:4 ratio, respectively, in a cement mixer. Nutrients were the main difference between these two substrates, since soil water in the experiment was kept optimal. Each level of soil heterogeneity was constructed with the same amounts of the two substrates, so that only configurational heterogeneity was varied (via cell size) and qualitative heterogeneity was kept constant (see method in Liu et al. 2017a). Mesocosms with cell size 48 cm were filled with either nutrient-rich or nutrient-poor substrate; mesocosms with cell size 24 and 12 cm were filled with nutrient-rich and nutrient-poor substrate alternating in all directions; mesocosms with the smallest cell size were filled with a mixture of the two substrates, i.e. with both of them alternating at very short distance. The exact size could no longer be accurately measured as small aggregates of both substrates remained, but for convenience we named this cell size “0” cm.

We replicated the mesocosms with cell sizes 0, 12 and 24 cm five times, and the mesocosms

125 with cell size 48 cm ten times, five with nutrient-rich and five with nutrient-poor substrate because
126 they jointly constitute the mesocosm-scale response at 48 cm (they were lumped in
127 mesocosm-scale analyses), but also to know the separate effects of both substrates. The
128 mesocosms were contained in wooden boxes with drainage holes in the bottom. Liu et al. (2017a)
129 provide further details on the technique to create soil heterogeneity in three dimensions.

130 The same seed rain was applied to all mesocosms, consisting of 24 species that naturally
131 occur in grasslands in Belgium. This seed mixture covered a broad range of Ellenberg's Indicator
132 Nitrogen Values (Ellenberg et al. 1991), in order to allow potentially different communities to
133 develop on nutrient-poor and nutrient-rich patches, as could be expected in nature. The species
134 were classified in two groups, i.e. low N (Ellenberg 1-4) and high N (Ellenberg 6-8), with each
135 group being represented by 12 species in order to avoid bias from uneven composition in the seed
136 rain (Table 1). Low N and high N species tend to be slow growing and fast growing, respectively
137 (Grime 1977; Chapin 1980; Franzaring et al. 2007). Seeds were obtained from commercial
138 suppliers (Herbiseed, Reading, UK and Cruydt-Hoeck, Nijeberkoop, The Netherlands). We tested
139 the germination rates and emergence times of these seeds three weeks before the start of
140 experiment, and took them into account when composing the seed rain to have equal
141 representation (aiming at six individuals per species) and germination timing (within a two-week
142 window) of all species. Only germination rate needed to be corrected. On 19 May 2015, each
143 mesocosm received a uniform seed rain of 423 mixed seeds, sown randomly on the surface and
144 covered with a few mm of the relevant substrate (i.e. nutrient-poor substrate on nutrient-poor cells
145 and vice versa). This seed rain aimed for a distance of 4 cm between germinating individuals in
146 each mesocosm. Mesocosms were kept moist to ensure optimal germination and establishment;

147 later on, water was added at the prevailing frequency of rainfall events in the region (every two
148 days) where natural rainfall fell short. Fungicide was added twice, one at the end of June and once
149 one week later. Weeds were regularly removed.
150
151 MEASUREMENTS AND CALCULATIONS
152 To assess the light environment of the plants, the horizontal distribution of photosynthetically
153 active radiation (PAR) was measured with a custom-made miniature sensor in each mesocosm 5
154 cm above the soil surface, at every 2 cm along two parallel lines placed at respectively 18 and 30
155 cm from the edge of the wooden box (S1). These $PAR_{below\ canopy}$ measurements were made on a
156 cloudy day (1 September 2015) to avoid disturbance by sunflecks and to obtain an average across
157 a range of solar angles. Incident PAR ($PAR_{above\ canopy}$) was measured at the same time, yielding
158 PAR transmission (T_{PAR}):

159 $$T_{PAR}\ (\%) = PAR_{under\ canopy} / PAR_{above\ canopy} \times 100.$$

160 The horizontal variation in PAR in each mesocosm, required to test Hypothesis 1, was assessed
161 with the coefficient of variation of PAR transmission:

162 $$CV(T_{PAR}) = \text{standard deviation of } T_{PAR} / \text{mean of } T_{PAR}.$$

163 Abundance (density) was recorded by species, in four samples in mesocosms with cell size 0,
164 and eight samples in mesocosms with cell sizes 12, 24 and 48 cm (four on nutrient-rich and four
165 on nutrient-poor patches), during the last week of August 2015. Sample size was 12 cm × 12 cm
166 and the squares were randomly placed within the substrate type. Values converted to m^2 at
167 mesocosm and substrate scale are shown in S2. At mesocosm scale, we also calculated
168 whole-community abundance and abundance by group of species (high N or low N), likewise

converted to m^2.

The same data were used to assess species diversity, its components species richness and species evenness, and similarity in species composition between the two substrate types in a mesocosm (Table 2). Species richness at mesocosm scale refers to the total number of different species in the four 12 cm × 12 cm samples in a mesocosm, while species richness at substrate scale reflects the same for a given substrate in a mesocosm. Species richness at mesocosm scale was also separated into high N and low N species, required to test Hypothesis 2. Simpson's diversity, Simpson's evenness, Shannon-Wiener's diversity and Shannon-Wiener's evenness were calculated from the relative abundances of the species, likewise at mesocosm scale or by substrate type. Similarity indices (Sorensen and Bray-Curtis) assess the similarity of the species composition between the two substrate types in a mesocosm. Reflecting β-diversity, these indices connect α-diversity (substrate scale) with γ-diversity (mesocosm scale).

At the end of the experiment, on 2 September 2015, plant shoots in each mesocosm were cut 2-3 cm above the soil surface, separated by substrate type, oven dried at 70 °C for 4 days and weighed. Shoot biomass was calculated at mesocosm and at substrate scale by converting to m^2. Average shoot biomass of individual plants in a mesocosm (not separated by species) was calculated as shoot biomass / plant density.

STATISTICAL ANALYSIS

We first examined the nature of the SHD relationship. At mesocosm scale, one-way MANOVA was used to explore the effect of cell size (0, 12, 24 and 48 cm) on community shoot biomass, abundance, diversity indices and CV(T_{PAR}). At substrate scale, we investigated the effects of cell

size, substrate type and their interaction with two-way MANOVA on shoot biomass, abundance, diversity indices and T_{PAR} of the local community on that substrate. Moreover, GLMM was conducted to test the performance of high N and low N species on different substrates. Cell size, species type, substrate type and their interactions were the fixed factors. Box identity was a random factor, and cell size 0 was excluded as substrates could not be distinguished in this treatment. In all these analyses, non-significant explanatory variables were excluded stepwise, and significant differences among treatments were explored further with post-hoc analysis (pairwise comparisons with Fisher's LSD). All statistics were conducted with SPSS 23.0 (IBM Corp., 2015).

Next, to test the assumptions involved in hypothesis 1 and 3, structural equation modeling (SEM) was conducted (Gozal et al. 2013). Corresponding with hypothesis 1 and 3, we assumed that soil heterogeneity (cell size) influences diversity indirectly via changes in plant shoot biomass which themselves alter light availability (hypothesis 3), or its spatial variation (hypothesis 1). Yet, we also allowed for possible other relationships between SEM variables, for example a direct effect of soil heterogeneity on diversity, in order to test for possible alternative response pathways not included in the hypotheses. Because the relationship between cell size and the diversity indices was unimodal (see Results), with a positive response from cell size 0-12 cm and a negative response from cell size 12-48 cm, the underlying mechanisms were tested at mesocosm scale in separate SEMs for these ranges. However, SEMs at mesocosm scale only lead to an acceptable model when both the cell size 0 and the biomass variation between the two substrates within the mesocosm were removed. We therefore present results for SEMs on cell size 12-24-48 cm with effects of soil heterogeneity on PAR variation and subsequently on plant diversity, whilst keeping

213 also the direct path from soil heterogeneity to plant diversity referred to above. Apart from these
214 SEMs at mesocosm scale, we also conducted SEMs at substrate scale, but here only the response
215 from 12 to 48 cm could be tested as responses to nutrient-rich and nutrient-poor substrate cannot
216 be distinguished at cell size 0 cm. The overall fit of each SEM model was assessed by the $\chi 2$
217 statistic and the root square mean error of approximation (RMSEA), with non-significant $\chi 2$ and
218 significant RMSEA indicating an acceptable fit of the model. In these final SEM diagrams, values
219 along the path arrow refer to the standardized path coefficients and values above the variable refer
220 to the proportion of variance that can be explained by relationships with other variables. SEM
221 analyses were conducted with IBM SPSS Amos 23.0.

222 To test hypothesis 2, generalized linear mixed models (GLMMs) were applied to test effects
223 of cell size, species type (high N or low N) and their interaction on community abundance and
224 species richness, with box identity as a random factor. Finally, one-way ANOVA was performed to
225 test the effect of cell size on community abundance, on the calculated average biomass of
226 individual plants and on the similarity indices between the two substrate types in a mesocosm. In
227 all these analyses, non-significant explanatory variables were excluded stepwise, and significant
228 differences among treatments were explored further with post-hoc analysis (pairwise comparisons
229 with Fisher's LSD). Statistics in this section were conducted with SPSS 23.0 (IBM Corp., 2015).
230

231 **Results**

232 The MANOVA analyses revealed that cell size significantly affected community performance
233 ($F_{3,24} = 3.02$, $P < 0.005$), whilst marginally significantly interacting with substrate type ($F_{16,34} =$
234 1.87, $P = 0.062$). The relationship between species richness and cell size at mesocosm scale was

235 unimodal, with a peak at cell size 12 cm (Fig. 2b; Table 3). A similar pattern was observed for
236 species diversity (Fig. 2c,d; Table 3), consistent with species evenness not being affected by cell
237 size (Table 3; mean Simpson's and Shannon-Wiener's evenness were 0.79 ± SE 0.02 and 0.94 ±
238 SE 0.01, respectively). The unimodal species richness response originated from nutrient-rich
239 patches, as cell size did not affect richness on nutrient-poor patches (Fig. 3b; Table 4). Beta
240 diversity between nutrient-rich and nutrient-poor patches did not contribute to the richness peak at
241 12 cm either, as Sorensen similarity was insensitive to cell size (Fig. 3g, Table 3; as mentioned
242 above, community richness at mesocosm scale can be seen as gamma diversity, produced by the
243 alpha diversities on both nutrient-rich and nutrient-poor patches, and the beta diversity between
244 them). The unimodal response trend of species diversity to cell size likewise originated from the
245 nutrient-rich patches (Fig. 3c,d, Table 4; again, cell size had no effect on nutrient-poor patches).
246 However, in this case, beta diversity between nutrient-rich and nutrient-poor patches dampened
247 the peak at mesocosm scale by reaching a minimum, i.e. Bray-Curtis similarity reaching a
248 maximum (Fig. 3h), at 12 cm (we use Bray-Curtis similarity here instead of Sorensen because
249 species diversity takes into account relative abundances). The GLMMs suggest that the higher
250 richness and diversity going from cell size 48 cm to 12 cm mainly originated from the increase of
251 high N species (Fig. 2g, Table 3), an increase that was observed on both nutrient-rich and
252 nutrient-poor patches (not shown). These patterns being established, we can now move to the
253 underlying hypotheses. In itself, a unimodal relationship excludes none of them, as it encompasses
254 both an increasing and a decreasing response.

255 Under Hypothesis 1 we expected greater diversity at higher soil heterogeneity (smaller cell
256 size) because more edges between productive vegetation on nutrient-rich patches and

257 unproductive vegetation on nutrient-poor patches would create more light niches. Cell size did not
258 affect CV(T_{PAR}) from 12-24-48 cm (Fig. 2e and SEM in Fig. 4). This result was similar when we
259 excluded the edges of the mesocosms and only used the inner 24 × 24 cm area (not shown).
260 Probably the shoot biomass on the two substrates was not different enough to generate much
261 spatial variation of light, (Fig. 3a, see also corresponding effects on PAR transmission in Fig. 3b).
262 Cell size 48 cm was the exception, with much less productive plants on nutrient-poor than on
263 nutrient-rich patches, but these productivity differences cannot cause light variation within
264 mesocosms either because there are no edges with adjacent patches (in fact 48 cm represents an
265 'infinite' cell size). Surprisingly, CV(T_{PAR}) did not influence richness at cell size 12-24-48 cm
266 (SEM in Fig. 4). Probably, CV(T_{PAR}) – diversity relationships are hard to pinpoint across the very
267 small ranges of CV(T_{PAR}) observed in this experiment (cf. Fig. 2e). The SEMs also detected a
268 direct negative effect of cell size on species richness from 12-24-48 cm (Fig. 4), corroborating the
269 declining phase of the SHD relationship in Fig. 2b. Altogether, the support for Hypothesis 1 was
270 thus limited. Paths observed in SEMs for species diversity (Simpson and Shannon-Wiener index,
271 Fig. S3-4) were highly similar compared with those for species richness.

272 Under Hypothesis 2 we postulated lower diversity at greater soil heterogeneity, owing to fast
273 growing species with good foraging abilities depleting the resource-rich cells more easily, at the
274 expense of slower growers. Although species richness did decline from cell size 12 to zero (Fig.
275 2b), this hypothesis was not supported because the richness of high N species decreased at
276 mesocosm scale over this range of cell sizes (Fig. 2g, Table 3), opposite to expectation.

277 Under Hypothesis 3 we assumed that, with increasing soil heterogeneity, a low-productive
278 and thus species-richer community on nutrient-poor substrate will become more productive

because root access to neighbouring nutrient-rich substrate is improved by the shorter distance. This would enhance light competition on the poor patches and diminish plant diversity there. This was not confirmed: although shoot biomass did increase (Fig. 3a and SEM in Fig. 5a) and PAR transmission did decrease (Fig. 3f and SEM in Fig. 5a) towards smaller cell size on nutrient-poor patches, as expected, a connection in the SEM between these two changes was not observed, nor did T_{PAR} influence species richness. On nutrient-rich substrate, a high-productive and thus species-poorer community was expected to become less productive towards smaller cell size, because resources are then more easily lost to (more nearby) neighbouring species on nutrient-poor substrate. This would decrease light competition on the nutrient-rich patches and thus promote plant diversity there. This was not confirmed either: none of these paths were retained in the SEM for nutrient-rich substrate (Fig. 5b). Still, cell size negatively influenced species richness directly, corroborating the unimodal pattern in Fig. 3b. Combined for nutrient-rich and nutrient-poor patches, the trend in richness and diversity predicted by Hypothesis 3 is confirmed, at least from cell size 48 towards 12 cm (Fig. 2b), but not the underlying mechanism. Paths observed in SEMs for species diversity (Simpson and Shannon-Wiener index, Fig. S5-6) were highly similar compared with those for species richness.

Interestingly, community abundance at mesocosm scale increased with smaller cell size (greater soil heterogeneity) (Fig. 2f, Table 3). This originated mainly from nutrient-rich patches (Fig. 3e, Table 4), and high N species also contributed more to this increase than low N species (Fig. 2h). At the same time, cell size did not affect the average biomass of individual plants in a mesocosm (P = 0.600). Likewisely, cell size did not affect shoot biomass per unit area at mesocosm scale (Fig. 2a), in agreement with the contrasting responses on the two substrates (Fig.

3a).

Discussion

The idea that soil heterogeneity drives plant species diversity has attracted much attention in recent decades (Tilman 1982, 1988; Hutchings et al. 2000; Williams and Houseman 2014). However, lack of a standard method to create soil heterogeneity experimentally and the inclusion of species that can blur fundamental trends in empirical tests (e.g. clonal or N-fixing species) may have prevented consistent SHD relationships from emerging. In the current experiment we systematically varied the patch size of each of the two used substrates (one nutrient rich, one nutrient poor), from small to large in three dimensions, whilst avoiding confounding by specific species. Contrary to previous findings in controlled experiments, we identified a novel pattern in the form of a unimodal SHD relationship, with diversity first increasing and then decreasing across the 3-D cell size range. Surprisingly, our hypothesized mechanisms to explain these responses, several of which were based on earlier assumptions in the heterogeneity-diversity literature, were not confirmed. However, our results do point to other potential mechanisms.

To test Hypothesis 1 that soil heterogeneity promotes species diversity by generating light niches, induced by the productivity differences between nutrient-rich and nutrient-poor patches, the spatial variation of PAR transmission in mesocosms was measured. This was not explicitly considered in previous studies on soil heterogeneity (Borer et al. 2014). Soil heterogeneity generating light niches is analogous to species diversity (i.e. heterogeneity in plant traits) generating light niches (Spehn et al. 2000), which in turn allows species to coexist. Yet, in our experiment, cell size did not affect the variation of light transmission [$CV(T_{PAR})$] through

323 modifying biomass (including biomass variation did not lead to an acceptable SEM) at mesocosm
324 scale: shoot biomass was too similar on nutrient-rich and nutrient-poor patches (Fig. 2a), as well
325 as high enough to produce low T_{PAR} values, across the 0-12-24 cm cell size range (Fig. 3f), thus
326 offering little potential for light niche differentiation. Liu et al. (2017b) attributed this shoot
327 biomass similarity to easier root access of plants growing on nutrient-poor patches to soil
328 resources in neighbouring nutrient-rich patches when cell size is smaller. However, we cannot
329 exclude that the observed positive SHD response across part of the cell size range was caused by
330 the presence of more light niches before full light interception was reached (Sapijanskas et al.
331 2014; Vojtech et al. 2008), as biomass would be expected to increase faster on nutrient-rich than
332 on nutrient-poor patches. Interestingly, in the SEMs, cell size directly reduced plant diversity from
333 12-48 cm. This points to other mechanisms than those hypothesized here, and thus requires further
334 research.

335 Contrary to Hypothesis 1, Hypothesis 2 postulated impoverished communities at high soil
336 heterogeneity, owing to fast growing species outcompeting slow growers through rapid depletion
337 of resource-rich cells. While species richness did drop across one part of the SHD range (from 12
338 to 0 cm), the underlying cause was opposite: high N species were lost instead of low N species.
339 Tamme et al. (2010) and Laanisto et al. (2013) proposed that negative SHD relationships might
340 also ensue from increased isolation and lack of connectivity among patches at high levels of
341 heterogeneity, but the question remains whether these principles from landscape fragmentation
342 apply across the 12 to 0 cm cell size range. Possibly, very small pockets of nutrient-rich substrate
343 (i.e., smaller than the plant size) offer insufficient resources to maintain a large diversity of fast
344 growing species because they co-occur with nutrient-poor cells, thus locally reducing the mean

345 resource availability relative to larger nutrient-rich cells where plant individuals only ‘sense’ the
346 most favourable substrate. This would also explain why these fast growers could not outcompete
347 the slow growing species from cell size 12 to 0 cm. The explanation of insufficient resources at
348 very small cell size would not be incompatible with the observed increase of the diversity of the
349 high N species on another part of the cell size range, i.e. from 48 to 12 cm. The latter could arise
350 from relaxation of intense competition among these fast growing species, and thus low diversity,
351 from cell size 48 towards 12 cm, especially in nutrient-rich patches. Note that this mechanism is
352 opposite to Hypothesis 2. Moreover, the 3D structure of soil heterogeneity in this experiment may
353 also explain this, as the roots in a nutrient-rich patch quickly encounter poor soil when they grow
354 deeper, resulting in reduced nutrient availability which would likewise relax competition.
355 Different underlying reasons for species impoverishment at both very small and large cell sizes
356 may thus explain the unimodal SHD relationship, similar to other unimodal plant diversity
357 patterns such as diversity-productivity (Fraser et al. 2015) and diversity-disturbance (Kondoh
358 2001).

359 Hypothesis 3 was based on the aforementioned greater resource loss from nutrient-rich
360 patches through extraction by species on neighbouring nutrient-poor patches as cell size gets
361 smaller (Liu et al. 2017b, Fig. 3g), thus reducing the productivity and light competition and
362 increasing the species diversity on nutrient-rich patches. However, such easier root access should
363 increase the productivity and light competition on nutrient-poor patches, reducing species
364 diversity there. Depending on the balance of these processes, increasing as well as decreasing
365 SHD relationships at mesocosm scale might thus arise, in principle also giving rise to unimodal
366 curves. Yet, though we observed higher biomass and reduced light availability on nutrient-poor

patches as cell size decreased, diversity on these patches was not reduced. On nutrient-rich patches, on the other hand, we found increases in diversity even though the expected lower biomass and decreased light availability was not observed. Nevertheless, we think that the mechanisms in Hypothesis 3, which basically consider only shading within a patch, might still hold, but could be blurred by the associated, simultaneous effects of shading by the neighbouring patches (DeMalach et al. 2016, 2017).

Previous studies on heterogeneity have to our knowledge not measured plant density along a range of controlled soil heterogeneity. In our mesocosms, plant density increased monotonically towards small cell size, so not only more species were able to coexist on the same area (up to cell size 12 cm), but also more individuals (up to cell size 0 cm). As cell size did not affect community shoot biomass, the more numerous plants growing on small cells would be expected to be less productive, which was not the case. We also considered whether small-cell mesocosms contained more species of small stature. This was not confirmed either: the average species height (derived from www.try-db.org on 19 March 2018), weighted by their relative abundance as observed in our mesocosms, was highly similar (49 and 51 cm at cell size 48 and 12 cm, respectively), indicating that the community composition probably did not shift to intrinsically smaller species and that the enhanced coexistence of more individuals may be caused by other factors. In any case, starting from the seed rain, less competitive exclusion occurred at cell size 12 than at 48 cm since species richness was higher there, pointing at the same conclusion of improved coexistence (assuming equal germination across cell sizes because mesocosms contained equal amounts of nutrient-rich and nutrient-poor surface soil). Note that our findings of greater density and similar community biomass in mesocosms with smaller cell size also point to the law of constant final yield (Kira et al.

389 1953; Weiner 2004).

390 We analysed effects of soil heterogeneity in line with the method in our earlier publications
391 (Liu et al. 2017a, b), along a gradient from very large to very small substrate patches. This
392 variation in cell size thus considers heterogeneity in the spatial, physical sense. It could be argued
393 that the way these patches of varying size are perceived, depends very much on the organism. As
394 such, heterogeneity would be different for trees, forbs, mosses, and soil bacteria on a soil with the
395 same absolute, physical spatial heterogeneity ('cell size'). What is very heterogeneous to one
396 organism, could be sensed as homogeneous by another, for example a large plant on our 12 cm
397 cells versus a bacteria in the middle of that cell. Moreover, this perception can also change during
398 the organism's life cycle, for instance, from seedling to large plant. This makes it very difficult to
399 quantify 'perceived' heterogeneity in a multi-species community consisting of very
400 differently-sized organisms, and makes the physical (cell size) approach more practical in analyses.
401 Nevertheless, much like in studies on other environmental drivers, where for example the same air
402 temperature may be perceived very differently by various coexisting plant species depending on
403 their thermal traits (e.g. Michaletz et al. 2015), the interpretation of the analyses should take into
404 account potential varying perception of the driver (here: spatial heterogeneity). It is unclear
405 whether the perception of heterogeneity changes towards the smallest cell sizes in general, i.e.
406 whether plants perceive their environment as increasingly homogeneous when substrate patches
407 get very small, which would mean spatial (physical) and perceived heterogeneity start diverging at
408 some point. To shed more light on this, new studies would need to include additional levels of
409 spatial heterogeneity on side of the gradient (between cell sizes 0 and 12 cm).

410 We conclude that species diversity responses to small-scale spatial soil heterogeneity can be

411 unimodal, which to our knowledge was not experimentally observed before for plant communities,
412 although it has been studied in simulation modelling at large spatial scale where it was attributed
413 to greater extinction risk of small plant populations from inbreeding depression and stochastic
414 events (Kadmon and Allouche 2007; Allouche et al. 2012). These mechanisms clearly do not
415 operate at the scale of our experiment. The location of the SHD peak at 12 cm suggests different
416 underlying mechanisms, which seem to switch around this point, as proposed earlier (Fitter 1994).
417 Studies at the very small scale may therefore hold the key to progress in this domain. However,
418 future studies should also include longer-term community dynamics mediated by further
419 competitive exclusion (beyond the level observed here) or by possible influences of soil
420 heterogeneity on seed production and dispersal. For example, the probability of dispersal of
421 species adapted to nutrient-rich patches into surrounding less suitable nutrient-poor habitats may
422 increase with decreasing cell size, because these less suitable habitats are then more nearby. This
423 might alter the competitive balance between species, and thus species diversity. Longer-term
424 experiments would also create their own seed rain, possibly altering species recruitment.
425
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427 Marc Wellens, Niels Van Putte, Sigi Berwaers and Toon Ramsdonck for field assistance, and
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608 **Table 1** Plant species used in this experiment and their Ellenberg nitrogen (N) and light values

Species	Family	Group	N value	Light value
Achillea ptarmica L.	Asteraceae	Low N	2	9
Agrostis capillaris L.	Poaceae	Low N	4	7
Berteroa incana (L.) DC.	Brassicaceae	Low N	4	9
Briza media L.	Poaceae	Low N	2	8
Festuca ovina L.	Poaceae	Low N	1	7
Hypericum perforatum L.	Hypericaceae	Low N	4	7
Koeleria macrantha (Ledeb.) Schult.	Poaceae	Low N	2	7
Leucanthemum vulgare Lam.	Asteraceae	Low N	3	7
Nardus stricta L.	Poaceae	Low N	2	8
Poa compressa L.	Poaceae	Low N	3	9
Rumex acetosella L.	Polygonaceae	Low N	2	8
Vulpia myuros (L.) C.C.Gmel	Poaceae	Low N	1	8
Species	Family	Group	N value	Light value
Brachypodium sylvaticum (Huds.) Beauv.	Poaceae	High N	6	3
Dactylis glomerata L.	Poaceae	High N	6	7
Epilobium hirsutum L.	Onagraceae	High N	8	7
Festuca gigantea (L.) Vill.	Poaceae	High N	6	4
Festuca pratensis Huds.	Poaceae	High N	6	8
Geranium robertianum L.	Geraniaceae	High N	7	5
Lolium perenne L.	Poaceae	High N	7	8
Nepeta cataria L.	Lamiaceae	High N	7	8
Poa pratensis L.	Poaceae	High N	6	6
Poa trivialis L.	Poaceae	High N	7	6
Silene dioica (L.) Clairv.	Caryophyllaceae	High N	8	-
Taraxacum officinale F.H.Wigg	Asteraceae	High N	8	7

609 **Table 2** Calculation formulae and ranges of diversity and similarity indices, where p_i and S refer
610 to the relative abundance of species i and the total number of species in the community,
611 respectively; H'_{max} to the maximum value of H'; a_i and c_i to the relative abundances of species i at
612 site a and c, respectively; and C, S_1 and S_2 to the number of species occurring at both site 1 and
613 site 2, at site 1, and at site 2, respectively

Index	Formula	Range (min, max)
Simpson's diversity	$D = 1 - \sum {p_i}^2$	(0, 1)
Simpson's evenness	$E = (1/\sum {p_i}^2)/S$	(1/S, 1)
Shannon-Wiener's diversity	$H' = -\sum_i^s p_i \ln p_i$	(1.5, 3.5)*
Shannon-Wiener's evenness	$E' = H'/H'_{max} = H'/\ln S$	(0, 1)
Bray-Curtis similarity	$BC = 2\left(\sum_{i=1}^{S} min(a_i, c_i) \Big/ \sum_{i=1}^{S} (a_i + c_i)\right)$	(0, 1)
Sorensen similarity	$QS = \ 2C/(S_1 + S_2)$	(0, 1)

614 *Range rarely exceeds 4.0 for ecological data

Table 3 At mesocosm scale, effects of cell size (0, 12, 24 and 48 cm) in one-way MANOVA on shoot biomass, species richness, Simpson's diversity, Simpson's evenness, Shannon-Wiener's diversity, Shannon-Wiener's evenness, plant abundance and coefficient of variation of PAR transmission [CV(T_{PAR})] of the community (Top), and effects of cell size, species type and their interaction in generalized linear mixed models (GLMMs) on species richness and plant abundance of high N and low N species separately (Bottom). F-values, *P*-values and degrees of freedom ($df_{between\text{-}groups}$, $df_{within\text{-}groups}$), with significant results ($P < 0.05$) in bold

	Shoot biomass			Species richness			Simpson's diversity		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Cell size	3, 21	0.041	0.989	3, 21	7.060	**0.002**	3, 21	3.201	**0.044**

	Simpson's evenness			Shannon-Wiener's diversity			Shannon-Wiener's evenness		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Cell size	3, 21	3.017	0.053	3, 21	4.038	**0.021**	3, 21	2.039	0.139

	Community abundance			CV(T_{PAR})		
	df	*F*	*P*	df	*F*	*P*
Cell size	3, 21	4.512	**0.014**	3, 21	0.369	0.776

	Species richness			Plant abundance		
	df	*F*	*P*	df	*F*	*P*
Cell size	3,45	7.060	**0.001**	3,45	21.126	**<0.001**
Species type	1,45	19.497	**<0.001**	1,45	38.248	**<0.001**
Cell size × Species type	3,42	2.104	0.114	3,42	2.135	0.110

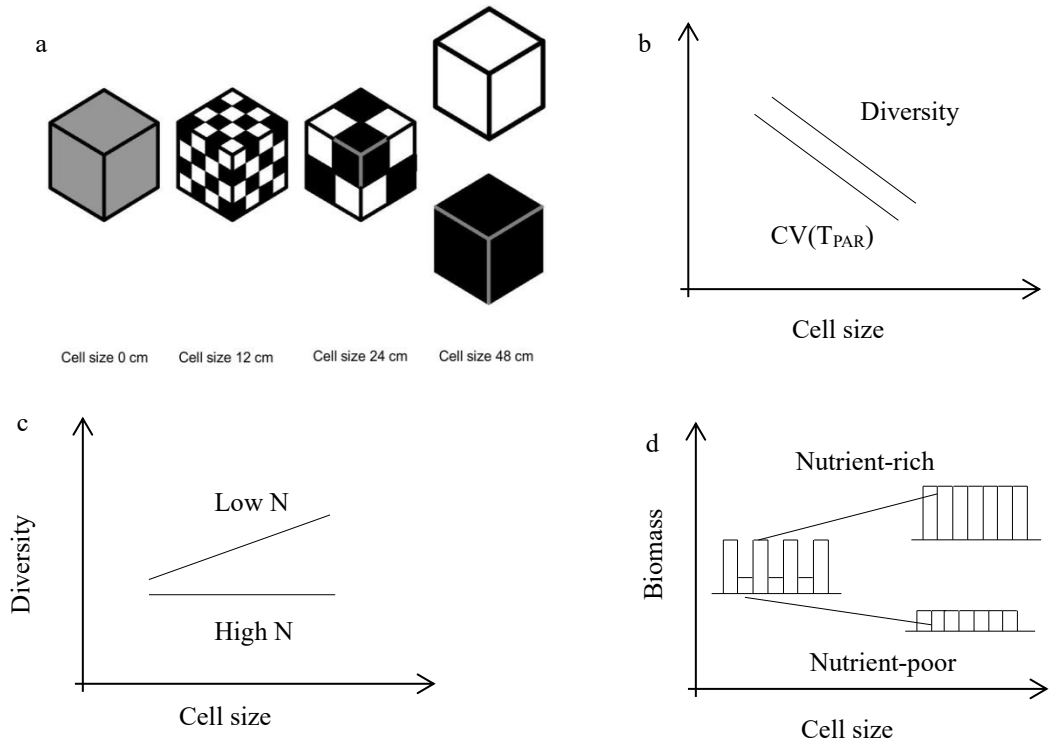
623 **Table 4** At substrate scale, effects of cell size, substrate type (nutrient-rich or nutrient-poor) and their interaction in two-way MANOVA on shoot biomass, species
624 richness, Simpson's diversity, Simpson's evenness, Shannon-Wiener's diversity, Shannon-Wiener's evenness, local community abundance and PAR transmission
625 (T_{PAR}) of mesocosms with cell size 12, 24 and 48 cm. F-values, *P*-values and degrees of freedom ($df_{between\text{-}groups}$, $df_{within\text{-}groups}$), with significant results ($P < 0.05$) in
626 bold

	Shoot biomass			Species richness			Simpson's diversity		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Cell size	2, 24	1.035	0.371	2, 27	3.466	**0.046**	2, 27	1.617	0.217
Substrate type	1, 24	17.194	**< 0.001**	1, 26	0.078	0.782	1, 26	0.181	0.674
Cell size × Substrate type	2, 24	4.202	**0.027**	2, 24	1.664	0.211	2, 24	2.756	0.084

	Simpson's evenness			Shannon-Wiener's diversity			Shannon-Wiener's evenness		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Cell size	2, 27	1.428	0.257	2, 27	2.423	0.108	2, 27	1.576	0.225
Substrate type	1, 26	1.097	0.304	1, 26	0.009	0.924	1, 26	0.746	0.396
Cell size × Substrate type	2, 24	1.634	0.216	2, 24	2.177	0.135	2, 24	2.509	0.102

	Local community abundance			Light transmission (T_{PAR})		
	df	*F*	*P*	df	*F*	*P*
Cell size	2, 27	8.584	**0.001**	2, 24	6.278	**0.006**
Substrate type	1, 26	1.071	0.310	1, 24	3.707	0.066
Cell size × Substrate type	2, 24	0.953	0.400	2, 24	9.308	**0.001**

Figure 1 (a) Three-dimensional view of the mesocosms with the two substrates used in the experiment, where black and white colour indicates nutrient-rich and nutrient-poor substrate, respectively. Configurational heterogeneity decreases from left to right, from fine (small cells) to coarse (large cells) distribution of resources. The cell size of the mixture of the two substrates on the left can be considered as approximately zero. (b) At mesocosm scale, predicted pattern of the coefficient of variation of PAR transmission [$CV(T_{PAR})$] and plant species diversity along a gradient of increasing soil heterogeneity (decreasing cell size), under Hypothesis 1. (c) At mesocosms scale, predicted pattern of the species diversity of high N and low N species along a gradient of increasing soil heterogeneity (decreasing cell size), under Hypothesis 2. (d,e,f) At substrate scale, predicted pattern of biomass, PAR transmission (T_{PAR}) and species diversity within nutrient-rich and nutrient-poor patches along a gradient of increasing soil heterogeneity (decreasing cell size), under Hypothesis 3



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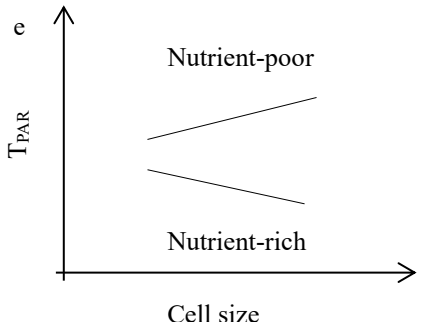


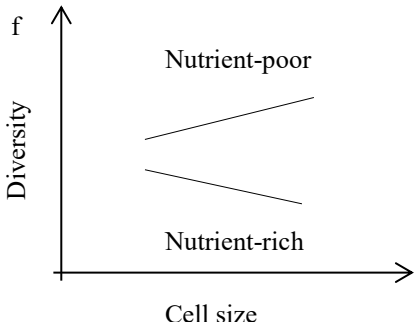
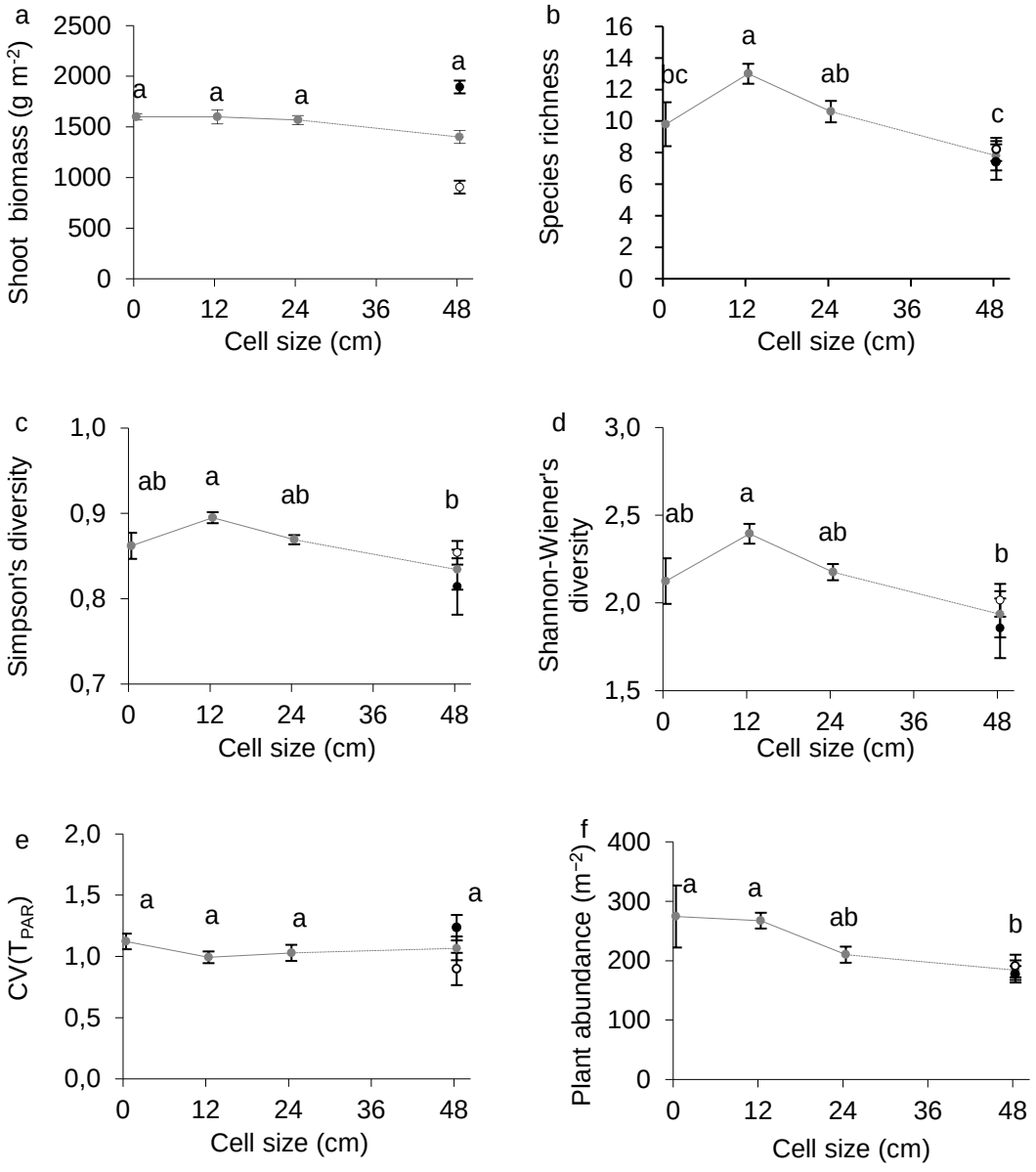
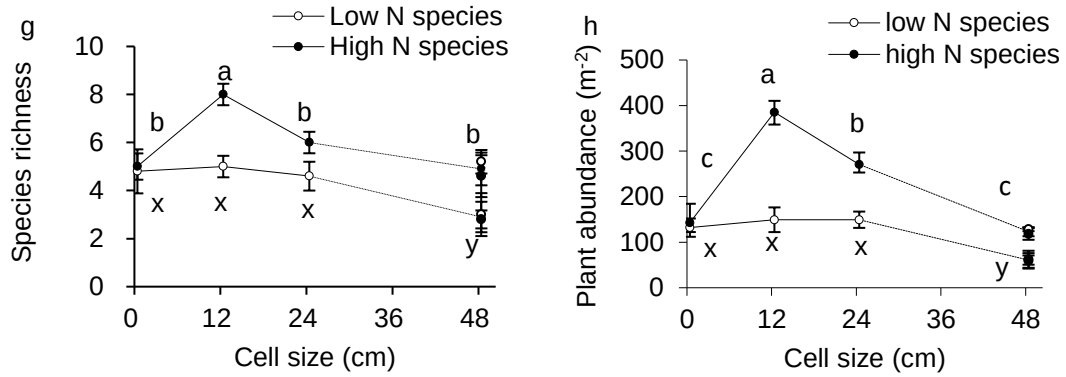


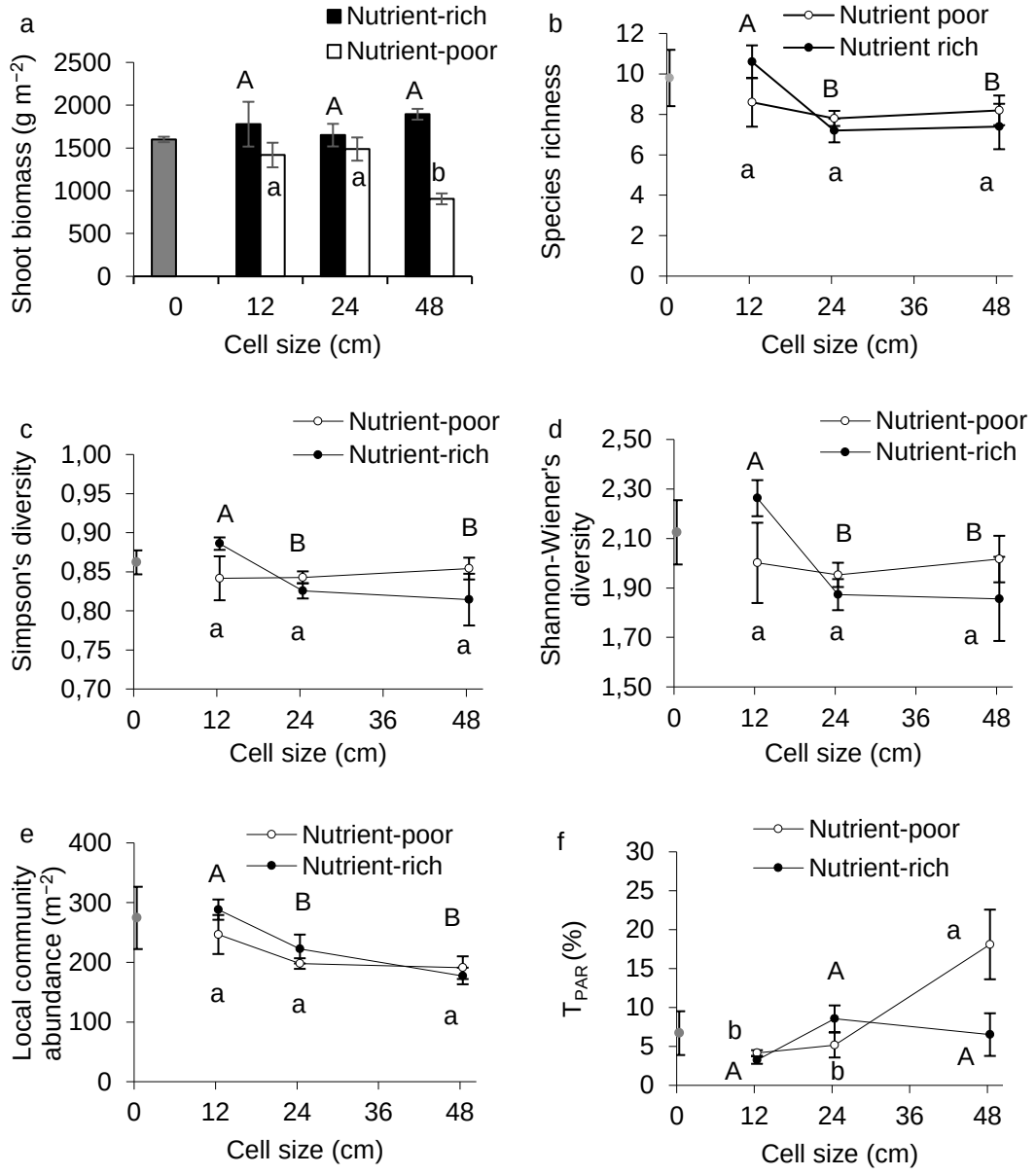
Figure 2 At mesocosm scale, mean ± SE of shoot biomass (a), species richness (b), Simpson's diversity (c), Shannon-Wiener's diversity (d), coefficient of variation of PAR transmission [$CV(T_{PAR})$] (e) and plant abundance (f) of the community, and mean ± SE of species richness (g) and plant abundance (h) of high N and low N species separately, all as a function of cell size. In (a-h), the grey symbol at 48 cm represents the average of the measurements on nutrient-rich (black symbol) and nutrient-poor (white symbol) mesocosms. Significant differences between treatments are indicated by different letters (post hoc analysis with Fisher's LSD)

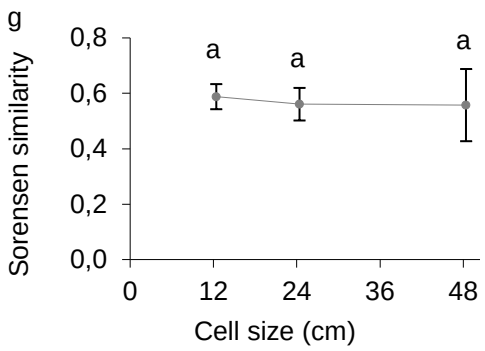


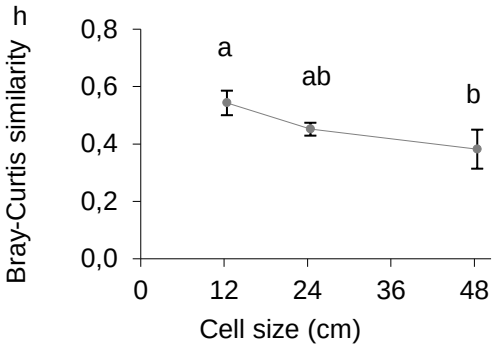

g
Species richness
Low N species
High N species
10
8
6
4
2
0
a
b
b
b
x
x
x
y
0
12
24
36
48
Cell size (cm)
h
Plant abundance (m-2)
low N species
high N species
500
400
300
200
100
0
a
b
c
c
x
x
x
y
0
12
24
36
48
Cell size (cm)

658

Figure 3 At substrate scale, mean ± SE of shoot biomass (a), species richness (b), Simpson's diversity (c), Shannon-Wiener's diversity (d), local community abundance (e), PAR transmission (T_{PAR}) (f), Sorensen similarity (g) and Bray-Curtis similarity (h) as a function of cell size, on nutrient-rich (black) and nutrient-poor (white) patches. Response variables at cell size 0 are grey. Significant differences between treatments in (a-f) are indicated by different letters (post hoc analysis with Fisher's LSD), with small and capital letters for nutrient-poor and nutrient-rich patches, respectively




g
Sorensen similarity
0,8
0,6
0,4
0,2
0,0
a
a
a
0
12
24
36
48
Cell size (cm)


h
Bray-Curtis similarity
0,8
0,6
0,4
0,2
0,0
a
ab
b
0
12
24
36
48
Cell size (cm)

669

Figure 4 At mesocosm scale, structural equation model (SEM) relating cell size (12-24-48 cm) to coefficient of variation of PAR transmission [CV(T_{PAR})] and species richness. The statistics of SEM fitting are: $\chi^2 = 0.355$, $P = 0.551$, GFI = 0.988, RMSEA < 0.001. Values above the variables refer to the proportion of variance that can be explained by relationships with other variables; values along the path arrows reflect the standardized path coefficients. Negative effects are indicated with '-'. Significant ($P < 0.05$) and marginally significant ($P < 0.10$) pathways are indicated with solid thick and dashed line, respectively

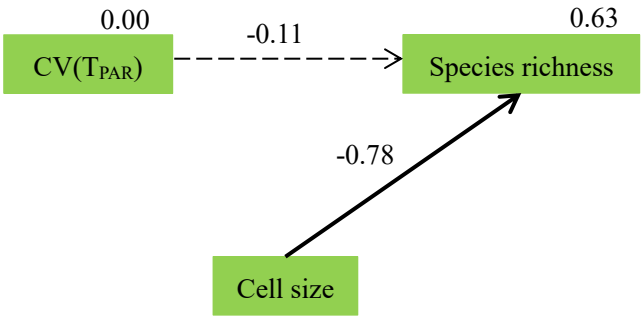
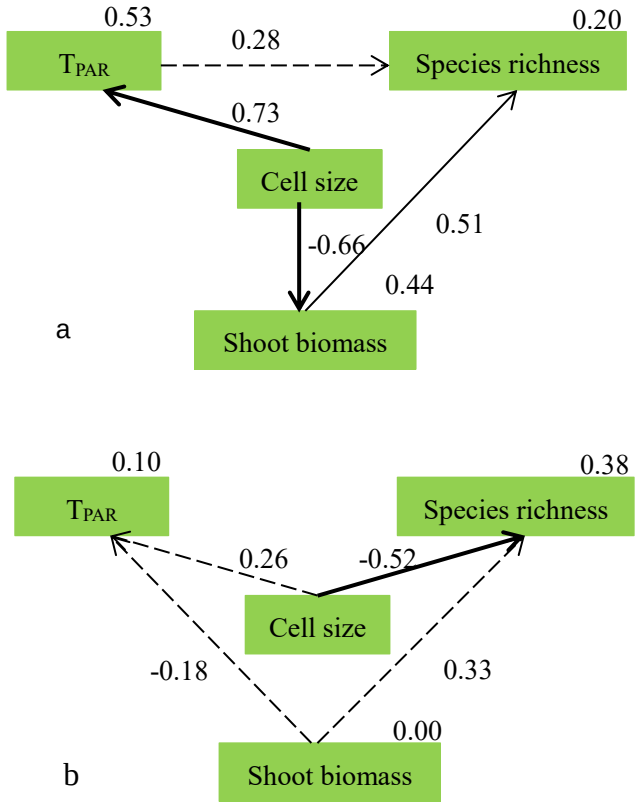
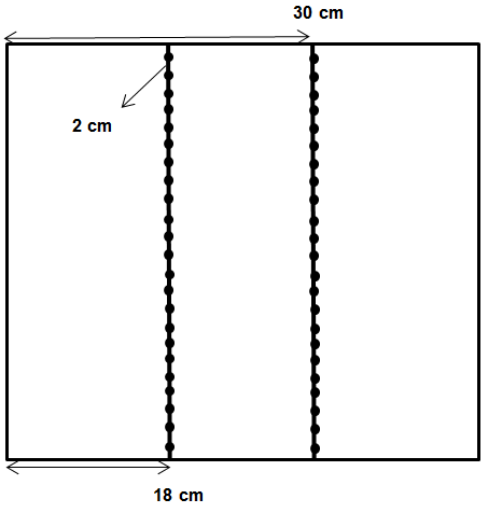


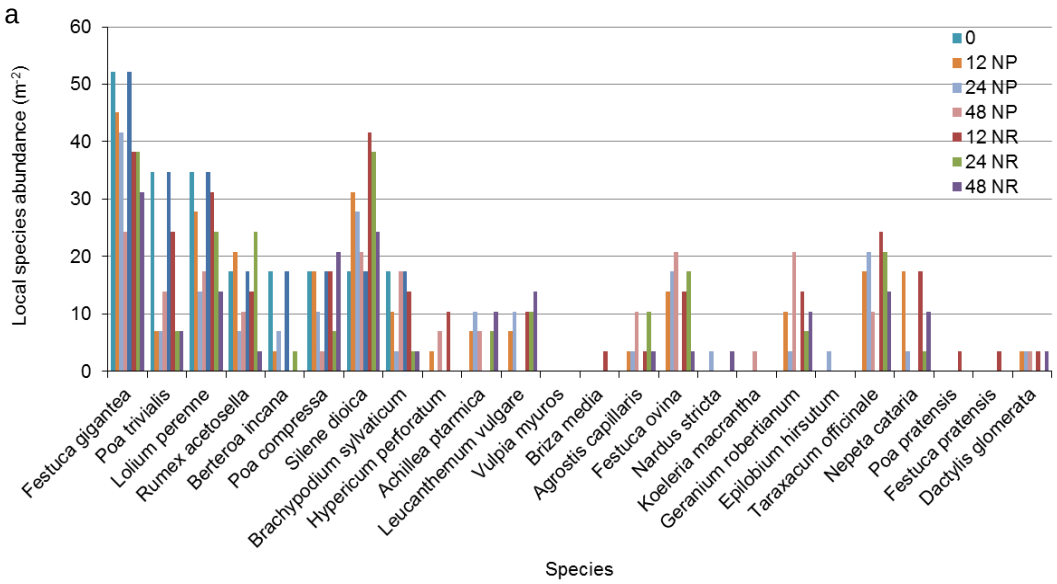
Figure 5 At substrate scale, structural equation model (SEM) relating cell size (12, 24 and 48 cm) to root biomass, shoot biomass, PAR transmission and species richness, separately for nutrient-poor (a) and nutrient-rich substrate (b). The statistics of SEM fitting are: (a) $\chi^2 = 1.020$, $P = 0.601$, GFI = 0.964, RMSEA < 0.001; (b) $\chi^2 = 0.487$, $P = 0.784$, GFI = 0.983, RMSEA < 0.001. Values above the variables refer to the proportion of variance that can be explained by relationships with other variables; values along the path arrows reflect the standardized path coefficients. Negative effects are indicated with '-'. Significant ($P < 0.05$), marginally significant ($P < 0.10$), and nonsignificant pathways are indicated with solid thick, solid thin and dashed line, respectively

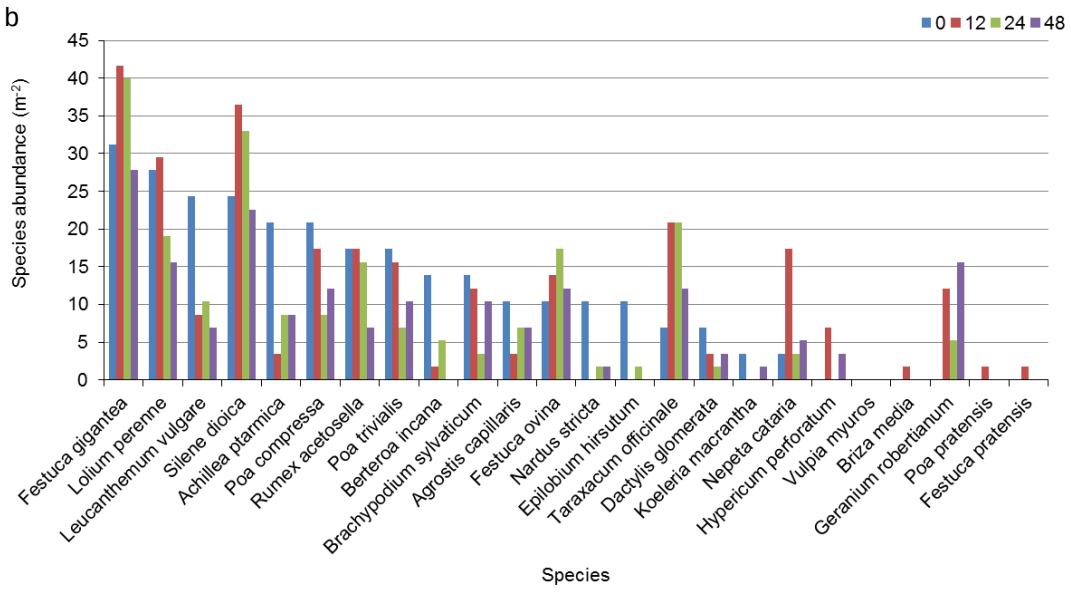


S1 Setup of the photosynthetically active radiation (PAR) measurements in a mesocosm (top view). PAR was recorded 5 cm above the soil surface at every 2 cm (dots) along each of two parallel lines, one 18 and one 30 cm from the left side of the mesocosm, respectively

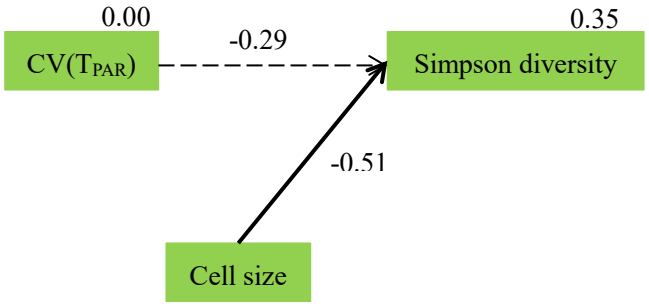


S2 Average species abundance (density) at substrate scale (a) and mesocosm scale (b) in mesocosms with different cell sizes. NP and NR refer to nutrient-poor and nutrient-rich substrate, respectively. High-N species had higher average densities than low-N species, both at mesocosm scale and on each substrate type

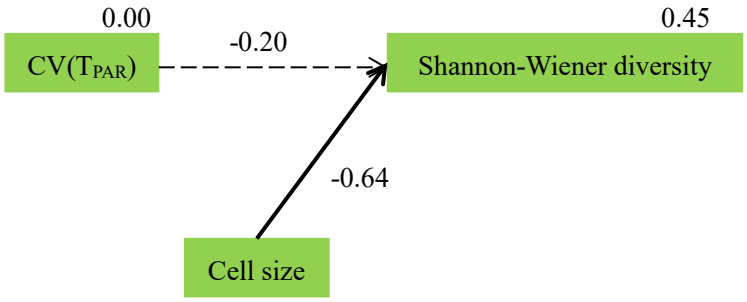




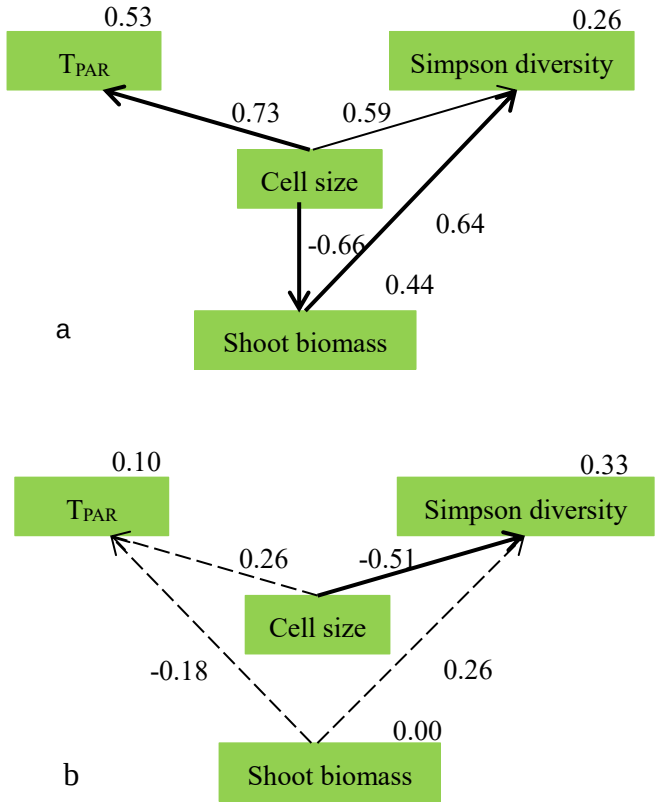
S3 At mesocosm scale, structural equation model (SEM) relating cell size (12-24-48 cm) to coefficient of variation of PAR transmission [$CV(T_{PAR})$] and Simpson diversity. The statistics of SEM fitting are: $\chi^2 = 0.355$, $P = 0.551$, GFI = 0.988, RMSEA < 0.001. Values above the variables refer to the proportion of variance that can be explained by relationships with other variables; values along the path arrows reflect the standardized path coefficients. Negative effects are indicated with '-'. Significant ($P < 0.05$) and marginally significant ($P < 0.10$) pathways are indicated with solid thick and dashed line, respectively



S4 At mesocosm scale, structural equation model (SEM) relating cell size (12-24-48 cm) to coefficient of variation of PAR transmission [$CV(T_{PAR})$] and Shannon-Wiener diversity. The statistics of SEM fitting are: $\chi^2 = 0.355$, $P = 0.551$, GFI = 0.988, RMSEA < 0.001. Values above the variables refer to the proportion of variance that can be explained by relationships with other variables; values along the path arrows reflect the standardized path coefficients. Negative effects are indicated with '-'. Significant ($P < 0.05$) and marginally significant ($P < 0.10$) pathways are indicated with solid thick and dashed line, respectively



S5 At substrate scale, structural equation model (SEM) relating cell size (12, 24 and 48 cm) to root biomass, shoot biomass, PAR transmission and Simpson diversity, separately for nutrient-poor (a) and nutrient-rich substrate (b). The statistics of SEM fitting are: (a) $\chi^2 = 0.856$, $P = 0.652$, GFI = 0.971, RMSEA < 0.001; (b) $\chi^2 = 0.901$, $P = 0.637$, GFI = 0.969, RMSEA < 0.001. Values above the variables refer to the proportion of variance that can be explained by relationships with other variables; values along the path arrows reflect the standardized path coefficients. Negative effects are indicated with '-'. Significant ($P < 0.05$), marginally significant ($P < 0.10$), and nonsignificant pathways are indicated with solid thick, solid thin and dashed line, respectively



S6 At substrate scale, structural equation model (SEM) relating cell size (12, 24 and 48 cm) to root biomass, shoot biomass, PAR transmission and Shannon-Wiener diversity, separately for nutrient-poor (a) and nutrient-rich substrate (b). The statistics of SEM fitting are: (a) $\chi^2 = 0.988$, $P = 0.610$, GFI = 0.967, RMSEA < 0.001; (b) $\chi^2 = 0.491$, $P = 0.782$, GFI = 0.983, RMSEA < 0.001. Values above the variables refer to the proportion of variance that can be explained by relationships with other variables; values along the path arrows reflect the standardized path coefficients. Negative effects are indicated with '-'. Significant ($P < 0.05$), marginally significant ($P < 0.10$), and nonsignificant pathways are indicated with solid thick, solid thin and dashed line, respectively