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1 Plant community assembly at small scales: spatial versus environmental
2 factors in a central European grassland

3

4 Sebastian Horn^{*1,4}, Stefan Hempel^{1,4}, Michael Ristow^{2,4}, Matthias C. Rillig^{1,4}, Ingo Kowarik^{3,4},
5 Tancredi Caruso^{1,5}

6

7 **1** Institut für Biologie - Ökologie der Pflanzen, Freie Universität Berlin, Altensteinstr. 6, 14195
8 Berlin, Germany

9 **2** Institut für Biochemie und Biologie - Vegetationsökologie und Naturschutz, Universität
10 Potsdam, Maulbeerallee 2, 14469 Potsdam, Germany

11 **3** Institut für Ökologie, FG Ökosystemkunde, Technische Universität Berlin, Rothenburgstr.
12 12, 12165 Berlin, Germany

13 **4** Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), 14195 Berlin,
14 Germany

15 **5** School of Biological Sciences, Queen's University Belfast. Medical Biology Centre, 97
16 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

17

18 * Corresponding author, Tel.: +49 30 838 53145. *E-mail address*: sebastian.horn@fu-berlin.de

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21 model; Plant community ecology; Variance partitioning

22

23 **Abstract**

24

25 Dispersal limitation and environmental conditions are crucial drivers of plant species
26 distribution and establishment. As these factors operate at different spatial scales, we asked: Do
27 the environmental factors known to determine community assembly at broad scales operate at
28 fine scales (few meters)? How much do these factors account for community variation at fine
29 scales? In which way do biotic and abiotic interactions drive changes in species composition?

30 We surveyed the plant community within a dry grassland along a very steep gradient of soil
31 characteristics like pH and nutrients. We used a spatially explicit sampling design, based on
32 three replicated macroplots of 15x15, 12x12 and 12x12 meters in extent. Soil samples were
33 taken to quantify several soil properties (carbon, nitrogen, plant available phosphorus, pH,
34 water content and dehydrogenase activity as a proxy for overall microbial activity). We
35 performed variance partitioning to assess the effect of these variables on plant composition and
36 statistically controlled for spatial autocorrelation via eigenvector mapping. We also applied null
37 model analysis to test for non-random patterns in species co-occurrence using randomization
38 schemes that account for patterns expected under species interactions.

39 At a fine spatial scale, environmental factors explained 18% of variation when controlling for
40 spatial autocorrelation in the distribution of plant species, whereas purely spatial processes
41 accounted for 14% variation. Null model analysis showed that species spatially segregated in a
42 non-random way and these spatial patterns could be due to a combination of environmental
43 filtering and biotic interactions. Our grassland study suggests that environmental factors found
44 to be directly relevant in broad scale studies are present also at small scales, but are
45 supplemented by spatial processes and more direct interactions like competition.

46

1. Introduction

Plant community assembly is significantly driven by filtering processes on several scales, like competition (Aarssen, 1989), dispersal limitation (Ai et al., 2012) and environmental conditions (Latimer and Jacobs, 2012). Understanding the processes involved in the assembly of communities is considered one of the most important challenges in ecology today (HilleRisLambers et al., 2012; O'Neill, 1989; Turner and O'Neill, 1995). While the understanding of community assembly has advanced significantly within the last 50 years, ecologists still lack precise insight on how the interplay of organisms and their environment determines the structure of natural communities (Götzenberger et al., 2012; Naaf and Wulf, 2012).

One common idea in ecology about the assembly of a diverse community involves filtering by the environment and interactions of organisms that establish local populations. This led to the niche-partitioning concept (Leibold, 1995; Silva and Batalha, 2011), where assemblages of species are viewed as having different tolerances to the abiotic environment and differing abilities to exploit resources. With the rise of neutral theory (Hubbell, 2001; Rosindell et al., 2012), the debate on the processes influencing biodiversity was reinvigorated and the search for a unified theory has dominated the field (Adler et al., 2007). It has been suggested that the combination of investigating both local and short-term mechanisms as well as regional processes occurring over longer timescales may be crucial for the complete understanding of ecosystem assembly and function (HilleRisLambers et al., 2012).

Grasslands cover one fourth of the Earth's land surface and harbour the majority of annual plant diversity (Shantz, 1954). A significant amount of studies on grassland ecosystems are focused on the influence of soil characteristics on plant community composition (Wellstein et al., 2007), which, together with water, wind and sunlight, represents the bulk of abiotic influences on a plant community (Callaway, 1997; Parfitt et al., 2010). Soil characteristics can be strong predictors of plant community composition (Gough et al., 2000; Tilman and Olf, 1991), although the scale of the studies influences the predictive power of soil parameters like pH, carbon, nitrogen or phosphorus content (Sebastiá, 2004). But not only abiotic factors are influenced by the scale of a study; positive and negative species-species associations can occur at small scales and disappear with increasing scale (Wiegand et al., 2012).

In this study we aimed at increasing the understanding of scale-dependence in community patterns by using a metacommunity approach to analyse the plant community composition of a semi-natural grassland (Leibold et al., 2004). While a lot of studies on grasslands are trying to

81 approach community composition mechanisms by inferring local interactions via the
82 observation of larger-scale composition (Eckhardt et al., 1996; Thomas and Palmer, 2007;
83 Toogood et al., 2008), we were aiming at understanding these processes by looking for patterns
84 of species composition that could be either deterministically or stochastically structured while
85 choosing the smallest local community possible: a single focal plant and its direct rhizosphere
86 interaction partners, making the community unit as small as possible. Other small-scale studies
87 have dealt with similar grain sizes like ours (Chu et al., 2007; Reitalu et al., 2009; Turtureanu
88 et al., 2013), however, they do not approach single plants with their rhizosphere environment
89 or combine small grain and extent. We consider everything beyond the single plant rhizosphere
90 environment a metacommunity, implicitly embodying the idea of interactions of plants with the
91 environment and each other.

92 Our study area offers unique possibilities of studying steep environmental gradients within only
93 a few meters in a very species-rich grassland which also harbours one highly abundant plant
94 species, enabling us to observe potential environmental filtering as well as spatial processes and
95 biotic interactions in a spatially well-defined small-scale area. We selected this plant species,
96 namely *Festuca brevipila* R. TRACEY (Aiken and Darbyshire, 1990; Klotz et al., 2002), as our
97 focal plant to be able to target the whole gradient of environmental conditions which our study
98 area offers, and still be able to standardize the metacommunity perspective on one species. We
99 used patterns of co-variation among plant species, environmental and spatial variables derived
100 from a neighbour matrix to answer the following questions: i) Do the environmental factors,
101 like soil characteristics, that are known to determine community assembly at broad scales also
102 operate at fine scales (1-15 meters) and how much do these factors account for community
103 variation at fine scales? ii) In which way do biotic and abiotic factors drive changes in species
104 composition? Our questions involve the disentanglement of patterns at various small scales,
105 which calls for tools able to quantify the contributions of environmental, spatial patterns and
106 their shared effect. Therefore state-of-the-art multivariate analysis will be applied (Borcard et
107 al., 1992; Dray et al., 2006). Given the small scale at which we conduct our study, we are able
108 to compare the importance of the environment found on larger scales with the processes shaping
109 our analyzed community that are strongly spatially structured. Large-scale environmental
110 effects that determine plant community structure in a range from a few to several hundred
111 kilometres, include climatic gradients (Ludewig et al., 2014), altitudinal changes (Krömer et
112 al., 2013) or differences in soil biogeochemistry (Khan et al., 2013). It might be that due to the
113 small scale of our study area the environment will only have a minor influence despite the

114 strong gradient since biotic interactions could be more influential and random effects or neutral
115 dynamics might overlay species sorting effects.
116

117 **2. Materials and Methods**

118

119 *2.1. Data collection*

120 The grassland studied is situated in a natural reserve (Mallnow Lebus, Brandenburg, Germany,
121 52°27.778' N, 14°29.349' E). The region is influenced by sub-continental climate with a mean
122 annual precipitation of below 500 mm (Ristow et al., 2011) and the area is managed by sheep
123 grazing twice a year. The sampling strategy was based on a hierarchical nesting of macroplots
124 and plots, and was done at the end of June 2011 to minimize influences by spring ephemerals.
125 Three macroplots of 15 x 15, 12 x 12 and 12 x 12 meters, respectively (Fig. 1), were located on
126 the slopes of mostly undisturbed hills in an area of about five hectares. Grazing was very limited
127 on our macroplots due to the strong slope and only minor traces of sheep trails were found. We
128 ensured that all macroplots were part of two closely related grassland communities found in
129 Mallnow, namely *Sileno otitae-Festucetum-brevipilae* Libbert 1933 corr. Kartzert & Dengler
130 1999 and *Festuco psammophilae-Koelerietum glaucae* Klika 1931. Our macroplots were
131 comparable concerning vegetation and soil related factors like distance from trees, stone content
132 or depth of A-horizon, as well as slope and sun exposure, and therefore can be considered a
133 replicated design. The uphill-downhill axes of the macroplots are characterized by a steep
134 textural gradient from highly sandy (downhill macroplot) to sandy-loamy (uphill macroplot)
135 soils. Preliminary analyses revealed that this gradient causes gradients in many other soil
136 parameters, namely pH, carbon, nitrogen and plant available phosphorus. Each macroplot was
137 divided into 3 x 3 m plots (Fig. 1). From each macroplot the vegetation of the four corner plots
138 (top left, top right, bottom left, bottom right) was sampled: For the measurement of soil
139 properties one soil core per plant was taken atop of five randomly chosen *F. brevipila* plants
140 per plot, creating 60 samples in total. In a radius of 15 cm around the chosen *F. brevipila* plant,
141 the local plant community was assessed visually as presence or absence of plant species. This
142 sampling unit represents our main community unit and below we refer to it as “sample”. With
143 regard to the smallest sampling unit (“sample”), the 15 cm radius ensures that interactions
144 within the rhizosphere of *F. brevipila* plants were captured. We preferred this method to a
145 totally random location of the sampling units (i.e. not having a focal species) for the following
146 mutually reinforcing reasons: a random location would have been strongly biased towards *F.*
147 *brevipila* in a non-controlled way because *F. brevipila* is by far the most abundant species in
148 the area (in some case the species can cover up to 70 % of one plot); by controlling for this
149 critical source of certain bias, we could minimise possible very small scale environmental
150 heterogeneity that could confound the interpretation of co-occurrence analysis based on null

151 models (see methods below) and the comparison between null models and multivariate analyses
152 based on RDA; the plant assemblage can be objectively defined at a biologically meaningful
153 small scale (i.e. rhizosphere) as the neighbourhood community of the dominant species. This
154 makes the community unit highly replicable: the average composition of this particular but
155 representative assemblage can be assessed throughout plots and macroplots as a function of
156 changes in the environment and the effects of the environments on how species interact within
157 this assemblage. By having a focal species and defining the assemblage as a function of it, we
158 lost some degree of generality but it is also true that our focal species and the genus to which it
159 belongs (*Festuca*) is one of the most abundant if not the most abundant and widespread species
160 in dry grasslands overall the world. Thus, we could compare total plant species richness of each
161 plot with the species richness found in the proximity of each of the five randomly sampled *F.*
162 *brevipila* plant per plot. The corner plots were chosen to use the maximum of the environmental
163 gradient along one direction (the downhill-uphill axis) and a minimum of it in the direction
164 orthogonal to the environmental gradient while keeping the spatial distances between plots
165 equal. Thus, the three macroplots represent three spatial replicates while the environmental
166 gradient is replicated twice within each macroplot.

167 Each soil core was thoroughly homogenized and representatively subsampled for the different
168 analyses. Soil water content was measured as the weight difference between fresh and oven-
169 dried soil cores. Soil carbon and nitrogen analysis was performed on a EuroEA 3000 Elemental
170 Analyser (EuroVector, Milano, Spain) with a TDC detector using 25 mg of pulverized soil per
171 core. Soil pH was measured in 10 mM CaCl₂ solution (van Lierop and Mackenzie, 1977) using
172 3 grams of soil per core. Plant available phosphorus was characterized following the CAL-
173 method (Sparks, 1996) using 1 gram of soil per core. Dehydrogenase assays were conducted
174 according to Rossel (1997), using 1 gram of soil per core. The pH, carbon, nitrogen and
175 phosphorus content data were used to create maps based on ordinary kriging to visualize
176 environmental gradients within the macroplots (Fig. S1). This was used to elucidate the actual
177 presence and orientation of a gradient.

178

179 2.2. Statistical analysis

180 Normality was checked with the Kolmogorov–Smirnov test and variables were transformed to
181 meet the requirements of parametric analysis when necessary. The subsequent analysis of
182 patterns in community structure was conducted in R 2.15.2 (2013), with functions from the
183 *vegan* (Oksanen et al., 2012) and the *SPACEMAKER* (Dray, 2011) package. Source code from the
184 analysis in R is provided in the supporting information. We created a presence/absence matrix

185 for the plant species recorded in each sample, containing 60 samples and 68 identified plant
186 species. Environmental factors for each sample were summarized in a matrix containing seven
187 columns for the factors and 59 rows for the samples. Eventually, one row had to be omitted
188 from all matrices since one soil core was lost prior to analysis. All the species matrices including
189 the subsets used were stripped from zero-occurrences spots and species, respectively, prior to
190 the subsequent analysis. For completeness reasons, the whole species matrix is included in the
191 supplementary information. Multivariate analysis was done on a per-sample basis, while the
192 null model analysis was conducted on various subsets of the whole data matrix (see below).

193 The species presence / absence matrix was Hellinger transformed and subjected to a
194 multivariate analysis to disentangle spatial and environmental factors influencing community
195 variation (Legendre and Legendre, 1998). At first a canonical correspondence analysis (CCA)
196 was conducted with the coordinates of the samples as constraints in order to remove linear
197 spatial patterns. The remaining spatial patterns of the detrended community data were
198 summarized, together with the geographical distance matrix of the samples, in the Moran
199 eigenvector mapping matrix (MEM) that best accounted for autocorrelation (Dray et al., 2006).

200 The final spatial matrix used for analysis then contained both the MEMs and the linear trends.

201 Spatial autocorrelation represents the predictability of a locally observed response value by
202 response variables observed in the surrounding area (Legendre, 1993). The MEM is calculated
203 by multiplying a connectivity binary matrix with a weighting matrix. The connectivity matrix
204 represents a graph in which samples are connected as networks while the weighting matrix is
205 used to quantify the sample dissimilarity by weighting each link of the network (Caruso et al.,
206 2012). In order to test multiple spatial patterns, the connectivity and/or weighting algorithms
207 were modified and the best model was selected following the Akaike Information Criterion AIC
208 (Akaike, 1973). Thus, the best linear combination of eigenvectors was chosen so the correlation
209 with the data would be maximal and the AIC values would be minimal (Dray et al., 2006). An
210 extracted eigenvector summarizes spatial patterns at a given scale; therefore the cumulative
211 matrix of eigenvectors can describe several spatial scales. This matrix then can be used in
212 multivariate regression approaches to predict spatial patterns (Dray et al., 2006). The
213 eigenvector method we utilised is able to detect patterns down to a scale of 1m, which equals
214 roughly twice the average distance between our samples. We used redundancy analysis (RDA)
215 and variance partitioning to resolve the contribution of environmental and spatial factors to the
216 total variance (Legendre and Legendre, 1998). The community matrix was used as response
217 matrix and measured environmental factors like carbon, nitrogen or pH, plus the MEM vectors
218 representing spatial autocorrelation were used as explanatory factors for the response matrix.

219 Since plants tend to respond more strongly to a change in nutrient availability when the nutrient
220 is scarce than when it is abundant, we followed suggestions by Jones et al. (2008) and tried to
221 transform the environmental data by taking their natural logarithm and generate a polynomial
222 environmental dataset prior to variance partitioning. However, this did not change the results
223 compared to untransformed data, we therefore only report results from the latter dataset.
224 Variance partitioning is a tool to quantify the unique contribution of these two components plus
225 the spatial patterning shared by the environmental data (Borcard et al., 1992). Multivariate
226 variances were visualized using principal coordinate analysis (Anderson and Willis, 2003).
227 Each of the variance partitions was subjected to a constrained redundancy analysis and
228 subsequent statistical test at $P < 0.05$, based on permutation (Oksanen et al., 2012). We applied
229 automatic stepwise model building for constrained ordination methods using the `ordistep`
230 function (Blanchet et al., 2008) with forward and backwards selection to include important
231 environmental variables only and calculate their respective P-values.

232 Since mosses and lichens can affect seedling establishment of higher plants (Soudzilovskaia et
233 al., 2011), their cover was considered as an additional environmental factor; however, this did
234 not increase the variance explained by the environment (data not shown). Lichens and mosses
235 were thus excluded from further analysis albeit their inclusion slightly increased the explained
236 variation of the spatial component.

237 Since variance partitioning quantifies variation in our community data but does not indicate a
238 positive or negative trend of the species coexistence necessary to judge the role of biotic
239 interactions, we applied a null model analysis done in PAIRS (Ulrich, 2008). In our null model
240 analysis the C-score index was used to compute values of co-occurrence for the given set of
241 presence/absence data. Since the C-score does not require perfect checkerboard distributions
242 and has a low susceptibility to type II errors, it seemed best suited for our purpose (Gotelli,
243 2000). The input matrix was randomized following the suggestions of Gotelli (2000) to
244 minimize type I errors and test for patterns of co-occurrence expected under non-random
245 assembly processes and interacting species. The algorithm used fixed sums of rows and sums
246 of columns and applied the Random Knight's Tour approach for shuffling the matrix. Retaining
247 the row and column totals preserves differences in species richness among sites and differences
248 in occurrence frequencies among species, therefore representing a conservative approach when
249 testing for patterns in species composition. We applied a nestedness analysis using the matrix
250 temperature method (Atmar and Patterson, 1993). Since the results indicated a strongly nested
251 community, the data set was split up according to geographic orientation, and in addition to the
252 whole community matrix, the subsets of the top, bottom, left and right plots were each subjected

253 to a null model analysis. The top and bottom subsets represent the spatial distance since the
254 gradient in each subset is minimized. The left and right subsets represent the whole gradient
255 together with the spatial component (see Fig. 1) In addition, we included a subset of the diagonal
256 patterns (that is, the top left plus the bottom right plots and the top right plus the bottom left
257 plots) in order to account for potential tilting of the gradient orientation (compare Fig. 2).
258 The null hypothesis was considered rejected when the observed C-Score was significantly
259 different from the average simulated C-Scores ($P < 0.05$). A C-score lower than the simulated
260 average represents an aggregated community, while a higher score represents a segregating
261 community. Standardized effect sizes were used to compare results meaningfully and calculate
262 probability values. The effect size is calculated as $(\text{observed C-score} - \text{simulated C-score}) /$
263 $(\text{standard deviation of simulated C-score})$. Negative standardized effect sizes indicate that the
264 observed index was less than the mean of the simulated indices while positive values indicate
265 that the observed index was greater than the mean of the simulated indices (Gotelli and
266 Entsminger, 2012).
267

268

269 3. Results

270

271 3.1. Sampling

272 We detected a total of 68 herb and grass species plus five different species of mosses and lichens
273 in the survey of the entire plots, outlining the high abundance found in our sample region. Out
274 of these herb and grass species, 47 species were found inside the 15 cm radius environment of
275 sampled *Festuca brevipila* plants (see Table S1). Species not found in the 15 cm radius around
276 samples were excluded from the species matrix prior to analysis so no zero-occurrences were
277 present in the matrices subsequently used. The majority of plant cover was attributed to the
278 grasses *Festuca brevipila* and *F. psammophila*, accounting together for up to 70% of the plant
279 canopy in a plot. Other abundant plants were *Arrhenatherum elatius*, *Carex humilis* and *Rumex*
280 *acetosella*, which are all common representatives of sunny-dry nutrient poor grassland habitats
281 (Hensen, 1997).

282 All plots showed steep gradients in pH, carbon and nitrogen content (Fig. 1, Table S2), with
283 macroplot 3 being generally richer in nutrients than macroplots 1 and 2. Plant available
284 phosphorus content was poor in all three macroplots, ranging from 8.7 mg/kg soil to 42.2
285 mg/kg. Soil C to N ratios ranged between 11:1 and 23:1. Measured pH ranged from 3.7 to 7.6,
286 encompassing four orders of magnitude in pH change. Macroplot 1 represented almost the
287 whole pH range, while macroplot 2 was more acidic and macroplot 3 more alkaline than
288 macroplot 1. Distances between samples in the plots ranged from 0.32 meters to 2.6 meters,
289 with an average of 1.56 metres.

290

291 3.2. Variance partitioning

292 From the different models tested for the MEMs, the “Nearest Neighbour” approach for
293 calculating the connectivity matrix with a concave-down weighting function yielded the lowest
294 AIC and was subsequently used for calculating the eigenvector maps. The spatial component
295 of the variation could be described by five low-rank MEMs and one high-ranking MEM,
296 pointing out that in our community spatial influences are predominantly small-scaled, that is to
297 say there is more significant spatial structure within plots and macroplots than between
298 macroplots. The variance partition attributed 17.9% of the community variation to spatially
299 independent environmental variables, from which carbon, nitrogen and pH were significant at
300 $P < 0.05$ (Table 1). The spatial component represented by the MEMs accounted for 14.5% of
301 the community variation and was highly significant (Table 1), while the spatially structured

302 environmental component (i.e. shared variation between spatial and environmental variables)
303 accounted for 4.7% of the variation. Roughly 63% of variance remained unexplained (Table 1).
304 When we tested for the effect of environmental variables ignoring spatial autocorrelation (Table
305 1, second column), all tested environmental factors except water content and microbial activity
306 were significant, indicating that spatial structure in the environment could drive some of the
307 spatial changes observed in the plant community (Table 1, compare first two columns). The
308 linear effect of the linear spatial coordinates alone accounted for 3.6% of total variation.

309

310 *3.3. Null model analysis*

311 The null model analysis revealed that the C-score was consistently higher in the sampled
312 communities than in the random ones, making the matrix overall segregated. This was also true
313 for every subset of the metacommunity we tested. This clearly shows that species associate non-
314 randomly (Table 2). PAIRS reported a list of significant plant pair interactions, which we used
315 to examine types of interactions between plants. When we tested the subsets of the community
316 matrix, we noticed that the difference in effect size was higher for the left and right subset (i.e.
317 along the environmental gradient) than for the top and bottom ones (i.e. orthogonal to the
318 environmental gradient, Table 2). The effect size represents a measure of segregation strength,
319 with larger effects sizes indicating more strongly segregating communities. The results indicate
320 that the spatially structured environment is a bigger segregating factor than the environmental
321 gradient alone, which is consistent with our variance partition results. In order to check for
322 biases in the pooling of the subsets, we also compared the effect sizes of the three macroplots
323 plus the possible two-macroplot-combinations (1 and 2; 2 and 3; 1 and 3), however, the effect
324 sizes were comparable in all three subsets (Table 2). Since some of the individual gradients
325 were not perfectly aligned with the sides of the macroplots, we also examined effect sizes of
326 cross-plot subsets (that is, all plots in the southwest – northeast axis and all plots in the southeast
327 – northwest axis). We noticed that the gradient axis oriented towards the pH causes a less
328 segregating community than the axis oriented towards carbon and nitrogen (Fig. 2, Table S2).
329 Mosses and lichens were not included in the null model analysis; however, including them did
330 not change the result (data not shown).

331 4. Discussion

332 333 4.1. Do the environmental factors that are known to determine community assembly at 334 broad scales operate at fine scales?

335 Given the steep gradients and the high species richness we found in our study area, we initially
336 expected that environmental filtering account for significantly more of community variance
337 than the spatial component. We found that the environment is on par with spatial processes
338 similar to the results found in other ecosystems (Li et al., 2011). The fact that environmental
339 filtering play a significant part in shaping plant communities is a common idea in community
340 ecology (Medinski et al., 2010; Olf and Ritchie, 1998; Payne et al., 2011; Silva and Batalha,
341 2010; Tilman and Olf, 1991; Wellstein et al., 2007). We included the environmental factors
342 that are generally considered important drivers of plant growth and distribution and that should
343 cover the majority of abiotic influences (Bardgett, 2005). Even so, we lack a complete analysis
344 of the micronutrients like Mg, Fe or Zn, and in general any aboveground environmental data
345 like temperature, rainfall distribution or wind strength (even though these macroscopic factors
346 definitely operate at scales much broader than those of our study). This might obscure some
347 patterns currently not attributed to the environmental factors. Nonetheless, given the influence
348 of key parameters like pH or phosphorus and the conservative analysis approach, it is unlikely
349 that measuring more environmental variables would significantly increase the amount of
350 variation accounted for by the environment. In fact, variables such as micronutrients generally
351 correlate well with the general parameters (e.g., C and pH) we have measured. Since every
352 environmental variable was spatially structured in our study area, it is possible that a significant
353 influence from an unmeasured variable would be reflected in the spatial eigenvectors and could
354 therefore be accounted for indirectly. Also, given the variables we measured, it is unlikely that
355 we missed out major environmental predictors of plant communities. Next to the environmental
356 part of the variation, a smaller fraction of variation was accounted for by the spatially structured
357 environment component, which suggests that the environment might exert its effect in a
358 spatially structured fashion (see below). The processes behind patterns found when analysing
359 communities oriented along the different environmental gradients via null models may account
360 for a significant part of the variation that remained unexplained after multivariate analysis.

361 Variance partitioning revealed that roughly half of the explained variance in species
362 composition is due to the spatial position of the plant species in our sampled macroplots,
363 regardless of environmental variation (Table 1). The permutation tests pointed out that the
364 spatial structure of the environment can be a major determinant, given the significance of the

365 environmental terms with and without spatial autocorrelation corrected (Table 1). This suggests
366 that the prevalent environmental filtering could determine changes in species composition via
367 its own spatial structure and/or by interacting with other processes, especially biotic interactions
368 which are expressed by stabilizing niche differences (Hall et al., 2003). The scale of our study
369 is so small that we can rule out dispersal limitation or considered it a very minor source of
370 spatial variation. Thus, we have a spatially structured effect of the environment, which could
371 also be due to biotic interactions mediated by the environment, plus much spatial variation that
372 neither the environment nor dispersal limitation can account for.

373 The remaining proportion of unexplained variation in community composition is likely to
374 represent either random variation or variation related to unmeasured variables that are not
375 spatially auto-correlated at the scales considered by our sampling design and MEM method
376 (Table 1). It might be possible that processes in the sub-meter range may be responsible for
377 parts of the unexplained variation; however, our analysis was designed to capture processes
378 taking place between our community sampling units on a scale of more than one meter.

379

380

381

382 *4.2. In which way do biotic and abiotic factors drive changes in species composition?*

383 Null model analysis confirmed that changes in species between sampling spots are not random.
384 We found that the segregation of species is higher in our studied area than expected by chance
385 (Table 2).

386 The effect sizes of different subsets we analysed were all positive, indicating that the
387 segregating effects are ubiquitous and do not necessarily correlate with spatial changes in the
388 environment, a result consistent with the multivariate results discussed two paragraphs above.
389 Given that it is a fair assumption that dispersal limitation does not play a significant role in our
390 study system, we can thus assume that negative biotic interactions (consistent with segregation)
391 can act as a structuring factor alongside the environmental filtering processes in our system,
392 alongside other potential effects attributed to small-scale environmental heterogeneity too small
393 to be addressed by the design of our study. We noticed that the effect sizes differed noticeably
394 in certain subsets of our data. These differences can be linked to some characteristics of the
395 gradient in our plots, thereby suggesting a potential effect of environmental gradients exerted
396 via biotic interactions. For example, we see that the plants in the top plots are segregating more
397 strongly, thus we can infer that biotic processes like competitive exclusion should be more
398 prevalent there. In fact, the upper part of the hills was less sandy and more densely populated

399 with generally larger plants, which implies more competition for space or light. It has also been
400 suggested that positive relationships between species are related to stressful conditions and
401 negative relationships to productive environments (Callaway et al., 2002; Walker and Chapin,
402 1987), which is in consent with our observations given that the upper hill part of our sampling
403 areas is indeed more productive due to higher resource availability (like water, nutrients and
404 sunlight).

405 Complex interactions among conflicting processes such as competition for space, optimization
406 of space utilization or demand for similar resources can facilitate exclusion (Sebastiá, 2004).
407 We found a large difference in effect size and hence segregation when comparing the left and
408 right subsets of the macroplots, which cannot be attributed just to environmental gradients, but
409 also to patchy processes which remain to be investigated. In part, patterns of variation in the
410 effect size of segregation seem to correlate with some environmental heterogeneity observed
411 within macroplots (Table 2).

412 We never detected aggregation in any heterogeneous subset of the community matrix, which
413 suggests that environmental filtering can take place mostly via niche partitioning, although care
414 must be taken when inferring these processes from co-occurrence patterns. Given the small
415 scale of our sampling design, we are not likely to find local coexistence, therefore any niche
416 partitioning will be observed as segregation of species. The scale of observation may influence,
417 how positive and negative interactions are related to biodiversity (Gotelli et al., 2010).

418

419

420 **5. Conclusion**

421

422 Overall, our data supports the hypothesis that at small scales steep environmental gradients
423 share equal importance in structuring the plant assemblage dominated by *Festuca brevipila* with
424 either spatially structured environmental effects or species spatial segregation due to negative
425 interactions or a combination of these two factors. Small scale and high resolution sampling
426 design will in the future allow teasing apart these two factors and scaling up their effects.

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430 **References**

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579 **Legends to Figures**

580

581 **Figure 1:** Sampling location and sampling design: all three macroplots were located on a hill
582 slope in a German natural reserve close to the Polish border, offering a high environmental
583 gradient within a few meters. A: A general map of Germany with the sampling area as red
584 rectangle (left) and a satellite picture of the sampling area (right) (Google, 2013). Purple
585 rectangles labelled as P1, P2 and P3 depict the location and orientation of the three macroplots.
586 B: Diagrams of the three macroplots. The spatial gradient is oriented orthogonally to the
587 environmental gradient. From the four corner plots (green), five *F. brevipila* plants were
588 sampled randomly as described in the materials and methods section (black dots). Numbers on
589 the diagrams represent the size of the respective macroplots in metres.

590

591 **Table 1:** P-Values of the RDA (redundancy analysis) based permutation tests and
592 decomposition of the total variation in the community matrix into unique environmental (soil
593 properties) and spatial (geographic position) components. Significant values are bold.
594 Important variables were selected by applying automatic stepwise model building for
595 constrained ordination methods including forward and backward selection. Values in italic were
596 dismissed in the step-wise selection process from the model. The last line (“explained
597 variation”) shows the percentage of explained variation of each component. The amount of
598 unexplained variation was 62.9%. P-values for the environmental variables in the column “env”
599 are based on partial-RDA, which accounts for spatial autocorrelation. P-values for the same
600 variables but in the column “space + env.” are based on the RDA that does not correct for spatial
601 autocorrelation, which can therefore include spatially structured environmental effects. Missing
602 values marked with a "-" are non-testable.

component	env.	space + env.	space
Carbon	<i>0.48</i>	0.01	-
Nitrogen	0.06	0.05	-
C/N ratio	<i>0.85</i>	<i>0.21</i>	-
Phosphorus	0.07	0.01	-
pH	0.01	0.01	-
microbial activity	0.04	0.02	-
water content	<i>0.51</i>	<i>0.64</i>	-
cumulative	<0.01	-	<0.01
explained variation	17.9%	4.7%	14.5%

605

606

607 **Table 2:** Null model analysis of community variation, using the C-Score index and the
608 algorithm MOD9 in PAIRS as described by Ulrich & Gotelli (Gotelli and Entsminger, 2012;
609 2007). The effect sizes and P-values of different subdivisions of the plant community matrix
610 are shown. Positive effect sizes implies a segregating community (species repel each other),
611 negative values indicate an aggregating one (species attract each other). P-Values are depicted
612 as stars: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; NS = not significant. The left table represents
613 heterogeneous subsets used for inferences on community composition, while the right table
614 represents homogenous subsets used to check the validity of our heterogeneous subset
615 assumptions.

616

community matrix	effect size	P	community matrix	effect size	P
all plots	7.25	***	macroplot 1 left	3.53	**
top plots	5.48	***	macroplot 2 left	1.85	NS
bottom plots	3.24	**	macroplot 3 left	4.41	**
left plots	2.24	*	macroplot 1 right	5.54	***
right plots	8.04	***	macroplot 2 right	1.55	NS
macroplot 1	6.40	***	macroplot 3 right	-1.03	NS
macroplot 2	2.75	*	plot 1	-1.19	*
macroplot 3	6.18	***	plot 2	0.16	NS
diagonal with gradient	6.49	***	plot 3	0.71	NS
diagonal w/o gradient	3.31	**	plot 4	0.77	NS
			plot 5	3.51	**
			plot 6	1.15	NS
			plot 7	1.14	NS
			plot 8	0.66	NS
			plot 9	-0.37	NS
			plot 10	0.00	NS
			plot 11	-0.15	NS
			plot 12	-0.58	NS

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