

1 **Soil conditions drive belowground trait space in temperate agricultural grasslands**

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21 **Abstract**

- 22 1. Plant belowground organs perform essential functions, including water and nutrient
23 uptake, anchorage, vegetative reproduction and recruitment of mutualistic soil
24 microbiota. Determining how belowground traits jointly determine dimensions of the
25 trait space and how these dimensions are linked to environmental conditions would
26 further advance our understanding of plant functioning and community assembly.
- 27 2. Here, we investigated belowground plant-trait dimensionality and its variation along
28 10 soil and land-use parameters in 150 temperate grasslands plots. We used eight
29 belowground traits collected in greenhouse and common garden experiments, as well
30 as bud-bank size and specific leaf area from databases, for a total of 313 species, to
31 calculate community weighted means (CWMs).
- 32 3. Using PCA, we found that about 55% of variance in CWMs was explained by two
33 main dimensions, corresponding to a mycorrhizal ‘collaboration’ and a resource
34 ‘conservation’ gradient. Frequently overlooked traits such as rooting depth, bud-bank
35 size and root branching intensity were largely integrated in this bidimensional trait
36 space. The two plant-strategy gradients were partially dependent on each other, with
37 ‘outsourcing’ communities along the collaboration gradient being more often ‘slow’.
38 These ‘outsourcing’ communities were also more often deep-rooting, and associated
39 with soil parameters, such as low moisture and sand content, high topsoil pH, high
40 C:N and low $\delta^{15}\text{N}$. ‘Slow’ communities had large bud-banks and were associated
41 with low land-use intensity, high topsoil pH, and low nitrate but high ammonium
42 concentrations in the soil. We did not find a substantial role of phosphorus-
43 availability as an indicator along the ‘collaboration’ gradient.

44 4. In conclusion, the ‘collaboration’ and ‘conservation’ gradients previously identified at
45 the species level scale up to community level in grasslands, encompass more traits
46 than previously described, and vary with the environment.

47

48 **Keywords:** clonal traits; environmental filtering; land-use; mycorrhiza; nitrogen;
49 phosphorus; plant economics spectrum; root traits

50 **Introduction**

51 Plant traits are of major interest as they determine plant functioning (Solbrig, 1993),
52 covary with environmental conditions (Garnier, Navas, & Grigulis, 2016), and influence
53 ecosystem functions (de Bello et al., 2010; Hanisch, Schweiger, Cord, Volk, & Knapp, 2020).
54 Nevertheless, traits may have low predictive power (Klimešová, Tackenberg, & Herben,
55 2016; van der Plas et al., 2020), because there is limited understanding which and how many
56 traits are needed in ecological studies (Shipley et al., 2016). An important step forward has
57 been the grouping of multiple traits into a limited number of syndromes, with continuous
58 variation in the form of gradients of plant strategies (Westoby, Falster, Moles, Vesk, &
59 Wright, 2002; Wright et al., 2004; Chave et al., 2009; Pierce, Brusa, Vagge, & Cerabolini,
60 2013; Díaz et al., 2016; Klimešová, Martínková, & Herben, 2018; Bergmann et al., 2020;
61 Roddy et al., 2020). For example, Diaz et al. (2016) showed that the variation in aboveground
62 traits can be captured by a ‘size’ gradient representing the size of whole plants and plant
63 organs, and an ‘economic’ gradient representing the leaf economics spectrum. A similar
64 attempt has recently addressed root traits that identified a ‘conservation’ gradient and a
65 ‘collaboration’ gradient as two independent axes of belowground plant economy (Weemstra
66 et al. 2016; Kramer-Walter et al. 2016; Bergmann et al. 2020).

67 Bergmann et al. (2020) suggested that in the root economic space the ‘conservation’
68 gradient, ranging from ‘slow’ to ‘fast’, is related to carbon conservation and determined by
69 root-tissue density and nitrogen content. In contrast, the ‘collaboration’ gradient, ranging
70 from ‘do-it-yourself’ to ‘outsourcing’ of resource uptake to fungal partners, is reflected by
71 specific root length and root diameter along with mycorrhizal colonization (Fig. 1a). Despite
72 the recent progress in the understanding of trait dimensionality, several root traits with a high
73 potential importance for plant functioning (Laliberté, 2017) were so far not integrated into the
74 existing framework. For example, high root-branching intensity can be seen as an alternative

75 to the reliance on mycorrhiza, and may be associated with specific root length for better local
76 soil exploitation (Kong et al., 2014; Freschet et al., 2020; Ding et al., 2020). Thus it may be
77 indicative of a ‘do-it-yourself’ strategy. Furthermore, rooting depth is also likely to be an
78 important source of interspecific variation as it varies considerably across biomes (Schenk &
79 Jackson, 2002), and could explain overyielding in grasslands via the mechanism of resource
80 partitioning (Mommer et al., 2010; Mueller, Tilman, Fornara, & Hobbie, 2013). As deep-
81 rooting species are able to take up nutrients and water from deeper soil layers, rooting depth
82 might be part of the ‘fast’ strategy of the conservation gradient (Fig. 1a). However, up to now
83 many of these belowground traits received much less attention, limiting a comprehensive
84 understanding of the plant-soil interface.

85 Belowground organs other than roots add another layer of complexity in terms of
86 form and function to plant-trait space. Plant structures such as rhizomes, root buds and tubers
87 play important roles in storage and vegetative reproduction (Klimešová, Martínková, &
88 Ottaviani, 2018). Species with large bud-bank size are more likely to be perennial and ‘slow’
89 growing (E-Vojtkó et al. 2017). Furthermore, although not strictly a belowground trait, the
90 root-weight ratio, i.e. the proportion of biomass allocated to roots, is a useful indicator of
91 plant investment into the uptake and storage of different resources (Reynolds & D’Antonio,
92 1996), and may be linked to rooting depth (Schenk and Jackson 2002a) and bud-bank size. It
93 remains to be tested, whether these belowground traits are aligned with the ‘conservation’ or
94 ‘collaboration’ gradient, or rather represent independent plant strategies.

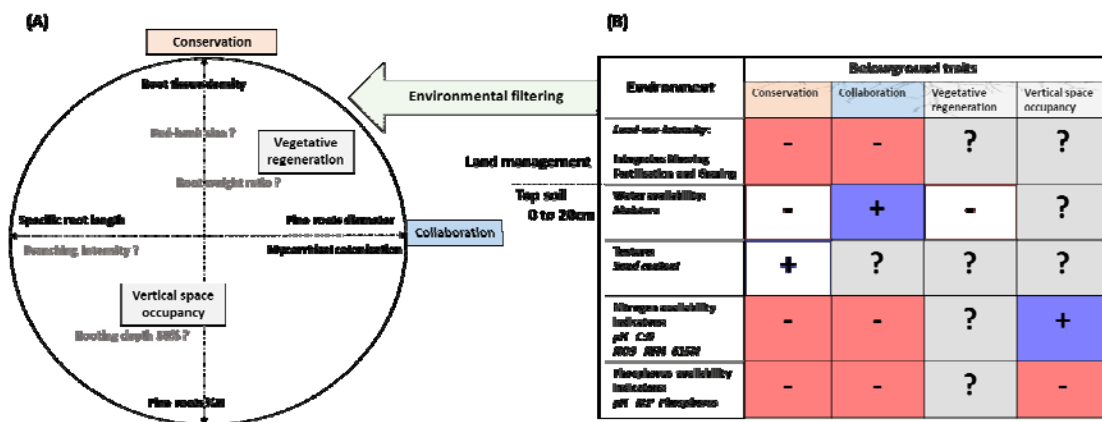
95 In grassland communities, which cover about 40% of the continents excluding
96 Greenland and Antarctica (Suttie, Reynolds, & Batello, 2005), the few studies on
97 belowground traits and their variation along environmental gradients are generally limited to
98 root morphological traits and only use a limited set of coarse environmental parameters
99 (Craine, Froehle, Tilman, Wedin, & Chapin, 2001; Prieto et al., 2015; Erktan et al., 2018).

100 Analysing the relationships between aboveground traits and various types of environmental
101 factors, such as climate, soil properties and land-use intensity, has already improved our
102 understanding of trait variation in grasslands (Garnier et al., 2007), and should also be
103 applied to belowground traits. In particular, plants may have various strategies to deal with
104 nutrient deficits and imbalances in soils. For example, it is likely that mycorrhizal
105 collaboration becomes more important at soils with limited phosphorus (P) availability (Ma et
106 al., 2020). Similarly the form of plant-available mineral soil nitrogen (ammonium versus
107 nitrate) could also select for different belowground traits, as species vary in their preference
108 for different forms of N (Weigelt, Bol, & Bardgett, 2005; Pornon, Escaravage, & Lamaze,
109 2007; Maire, Gross, Da Silveira Pontes, Picon-Cochard, & Soussana, 2009).

110 To better understand how belowground plant traits relate to environmental variation,
111 we investigated 1) how community mean values of different belowground traits align along
112 known plant-strategy gradients, and 2) how the shifts of community means along these
113 strategy gradients depend on environmental variables. Thus, we complemented traits defining
114 the gradients of the root economics space with additional traits that might represent
115 independent strategies of plant functioning. Therefore, we measured traits of plants grown in
116 pot and used database information and vegetation relevés to calculate community weighted
117 means of belowground traits for 150 grassland plots in Germany. We then assessed the
118 dimensionality of the variation in community weighted means of ten traits with principal
119 component analysis (PCA), and related the principal components to ten land-use intensity and
120 soil variables. A priori hypotheses of the relationships between traits, plant strategy
121 dimensions and environmental variables are presented in Fig. 1.

122

123 **Figure 1.** Hypothesized relationships between (A) community weighted means (CWMs)
 124 of belowground traits in grasslands potentially aligned on two plant-strategy gradients. A
 125 ‘conservation’ and a ‘collaboration’ gradient are expected as the main dimensions of plant
 126 variation, as our trait selection contains mostly traits of the root economics space. A
 127 ‘vegetative reproduction’ and ‘vertical space occupancy’ aspect could represent additional
 128 plant-strategy gradients, or be embedded within the ‘conservation’ and ‘collaboration’
 129 gradients. The two known belowground dimensions are here represented as two orthogonal
 130 axes (‘Conservation’ and ‘Collaboration’) with the traits that have previously shown to be
 131 associated with them in black font. The positions of four other traits, bud-bank size, root
 132 weight ratio, branching intensity and rooting depth 50% (grey font), are yet unknown. (B) As
 133 a result of environmental filtering, each plant-strategy gradient could be associated with
 134 different or overlapping environmental variables. The signs and colors indicate the
 135 hypothesized direction of the relationships. The references on which these hypothesized
 136 relationships are based can be found in Appendix S4.



137

138 **Methods**

139 **Data on grassland vegetation composition**

140 The plant-community data used as a baseline for Central European mesic grassland
141 vegetation originate from the ‘Biodiversity Exploratories’ project (Fischer et al., 2010). In
142 each of three regions of Germany, the Schwäbische-Alb (south-western Germany), Hainich-
143 Dün (central Germany), and Schorfheide-Chorin (north-eastern Germany), 50 grasslands
144 covering a wide range of land-use intensities were selected. From 2008 to 2019, the
145 vegetation composition of a 4 m × 4 m plot in each of the 150 grasslands was assessed
146 annually in May/June by identifying all vascular plant species and estimating their cover. To
147 align the species names between the vegetation and trait datasets, we standardized the species
148 names according to the accepted names in The Plant List (www.theplantlist.org, accessed 15
149 June 2019, using the Taxonstand R package (Cayuela, La Granzow-de Cerda, Albuquerque,
150 & Golicher, 2012). In total, 319 vascular plant species have been identified in the 150
151 grassland plots.

152 **Plant species traits**

153 We obtained mean species values for eight traits from four pot experiments that we
154 performed, and for two further traits from databases. For 291 of the 319 grassland species, we
155 were able to obtain seeds from commercial seed suppliers or botanical gardens. We then
156 performed four pot experiments to measure species traits. *Taraxacum* spp. are abundant in the
157 grassland plots, though, due to their complex taxonomy, rarely identified at the species level.
158 We here used trait values of *Taraxacum campylodes* for *Taraxacum* spp. The trait values are
159 part of a previously published dataset (Lachaise, Bergmann, Rillig, & van Kleunen, 2020)
160 and an unpublished dataset (Bergmann et al. *unpublished data*), and comprehensive
161 descriptions of the experiments are provided in Appendix S1. In brief, we did one greenhouse

162 experiment in which we grew 2659 individual plants, representing 216 species, for four
163 weeks after which we weighed the roots and analysed scanned images of the roots with
164 WinRHIZO 2017a software (Regent Instruments Inc., Canada) to determine root tissue
165 density, specific root length, fine root diameter, root weight ratio and root branching intensity
166 (Lachaise et al., 2020). We did a second greenhouse experiment using 2007 plants,
167 representing 196 species, to determine the nitrogen content of fine roots (Fine roots %N)
168 using isotope-ratio mass spectrometry. In a third greenhouse pot experiment, we determined
169 mycorrhizal colonization rate for 225 plants, representing 75 species that are among the most
170 common ones in the grasslands plots (mean cover of 65%, Appendix S3). Six weeks after
171 inoculation with spores of *Rhizophagus irregularis* (see Bergmann et al. *unpublished data*),
172 roots were harvested and washed, and the percentage of mycorrhizal colonization was
173 determined using the line-intersect method (McGonigle, Miller, Evans, Fairchild, & Swan,
174 1990). In a fourth experiment, we grew 752 plants, representing 183 species, in outdoors
175 growth-tubes to determine the depth above and below which plants have 50% of their root
176 biomass (Rooting depth 50%, see Appendix S1 or Schenk & Jackson, 2002 for the
177 calculation method) for about 16 weeks. In addition, to have an estimate of the belowground
178 regeneration potential, we extracted bud-bank size, including stem and root-derived buds
179 occurring belowground or at the soil surface, from the CLO-PLA database (Klimešová,
180 Danihelka, Chrtěk, de Bello, & Herben, 2017) for 313 of the 319 species. Finally, to also
181 have a reliable indicator of the plant communities' acquisitive side of the plant economics
182 spectrum (Allan et al., 2015; Busch et al., 2019), we extracted specific leaf area, the one and
183 only aboveground trait in our analyses, for 279 of the 319 species from the LEDA database
184 (Kleyer et al., 2008).

185 **Environmental variables of grassland plots**

186 To relate the different dimensions of variation in community weighted trait means of
187 the grassland plots to the abiotic environment, we used ten environmental variables related to
188 land-use intensity and soil conditions. The goal was to capture a relatively independent set of
189 descriptors likely to drive the belowground functioning of plants. A detailed description of
190 each variable can be found in Appendix S2. We used the land-use-intensity index (Blüthgen
191 et al., 2012), which aggregates the intensity of mowing, fertilization and grazing, and is a
192 major driver of ecosystem properties (Allan et al., 2015). We used a variety of
193 physicochemical indicators related to soil fertility of the topsoil (0-20 cm): Soil-moisture
194 content and sand content were measured to capture soil water availability and texture,
195 respectively. Soil pH was chosen, as it affects the availability of essential plant nutrients such
196 as P in soils. We used soil extractable NO_3 , NH_4 and $\delta^{15}\text{N}$ as indicators of soil nitrogen
197 availability and related processes (Robinson, 2001; Kleinebecker et al., 2014), and the C:N
198 ratio as a coarse indicator of stoichiometry and organic matter decomposability
199 (Schachtschabep, Blume, Brümmer, Hartge, & Schwertmann, 1998). We further made use of
200 resin-bag-adsorbed phosphorus and the N:P ratio to capture phosphorus availability in soil
201 (Güsewell, 2004). Because soil volume is a central element in soil fertility and root-system
202 distribution, we used data on soil bulk density to convert per-mass nutrient concentrations to
203 per-volume concentrations (Appendix S2). Few of the grassland-site descriptors were
204 measured for each of the years for which we had vegetation-composition data (i.e. for the
205 period 2008-2019). However, we tried to maximize the coverage for this period by using all
206 available census dates for these variables (see Appendix S2 for years covered) and averaging
207 the values per plot.

208 **Statistical analyses**

209 All the statistics were done using R v 4.0.1 (R Core Team, 2020).

210 *Community weighted trait means*

211 To characterize the plant communities of each of the 150 grassland plots based on
212 values of functional traits of their species, we calculated community weighted means
213 (CWMs) as

$${}_{cwm}Trait = \sum_{j=1}^S p_j Trait_j$$

214 Here p_j is the relative cover of species j in the community, $Trait_j$ is the trait value of species j ,
215 and S is the number of species in the community with available trait data. Because some plots
216 had patches of bare soil in some of the annual vegetation surveys, and because for some
217 species trait data were missing, we normalized plant cover to cumulate to 100% for all
218 species with available trait data in each plot before calculating the CWMs. As we have trait
219 data for most of the dominant grassland species, we have data for about 90% of the total
220 cover in most plots, for most traits (Appendix S3). The only exception is mycorrhizal
221 colonization, which was only available for 78 species, but, even for that trait, the average
222 cover of species included was 65% (range 32 - 87%, Appendix S3).

223 *Principal components of CWMs variation*

224 As the CWMs of several traits were correlated (Appendix S10), we performed
225 principal component analyses (PCA) to reduce the dimensionality of the data. To assess how
226 robust the resulting dimensions are to the inclusion of additional information, we performed
227 four separate PCAs. Each of these PCAs included all nine belowground traits, but they
228 differed in that we also included or excluded ${}_{cwm}Specific\ leaf\ area$, as one of the major traits
229 associated with the aboveground ‘fast’ side of the plant economics spectrum, and that we
230 included or excluded plant-functional-type information (i.e. the percentages cover of

231 graminoids, N-fixing forbs and non-N-fixing forbs). So, one PCA included CWMs of
232 belowground traits only (“Belowground PCA”), one additionally included *CWM**Specific leaf*
233 *area* (“Above-Belowground PCA”), one additionally included the proportions of Poales,
234 Fabaceae and non-Fabaceae forbs, and one included all. To increase the separation of the
235 variable loadings (the trait CWMs) on the two first axes, we performed an ‘oblimin’ rotation
236 on these axes for the Belowground PCA and the Above-Belowground PCA. To complement
237 the information provided on taxonomic or phylogenetic influence on community trait values,
238 we also looked at the ten most dominant species or taxa in the trait space formed by PC1 and
239 PC2 and the indicator species or taxa that associated with each quadrant of the two-
240 dimensional space formed by PC1 and PC2 (Appendix S15). As the proportion of plant
241 functional types were shown to be significantly related to specific plant strategies in PC1 and
242 PC2 (Appendix S6, Appendix S10), we also did three additional versions of the Above-
243 Belowground PCA, removing each plant functional type in the CWMs calculation once, to
244 evaluate how much the trait relationships are affected by the presence of the respective plant
245 functional type (Appendix S7). To compare the relationships observed at the community
246 level and at the species level, we also did the Above-Belowground PCA using trait means of
247 the species instead of CWMs (Appendix S16). For each PCA, *CWM**Root tissue density* was
248 \log_{10} transformed and for each trait or proportion of plant functional type, data was
249 standardized by subtracting the mean and dividing by the standard deviation to conform to
250 the multinormality requirements.

251 *Associations of the principal components of CWMs with environmental variables*

252 To test for associations between the principal components of CWMs of the grassland
253 plots and the environmental variables, we performed multiple regression. The PC1 and PC2
254 scores from each of the four PCAs on CWMs of the functional traits were used as response
255 variables, and the environmental variables were used as predictors. C:N, N:P, sand content,

256 NH₄, NO₃, and δ¹⁵N were log-transformed before analysis to get a more regular (less
257 clumped) distribution of the predictor values. To account for the fact that the grassland plots
258 are located in three different regions of Germany, we also included region as a predictor in
259 the models. For model reduction, backward stepwise model selection based on AIC was
260 performed using the function `step()`. This procedure selects a parsimonious set of predictors
261 while minimizing the variance inflation factor (max VIF = 3.6 for Above-Belowground
262 PCA). Because the two first axes (PC1 and PC2) of the four PCAs produced similar scores
263 for the CWMs of the grassland plots (all pairwise correlations of the PC1s were >0.98 and
264 those of the PC2s were >0.67), we present the results of the analysis of the “Above-
265 Belowground PCA” in the main text (Fig. 3 based on the PC axes of Fig. 2), and the results
266 for the other three PCAs in Appendix S8. We did the same for the PC3 to PC6 scores from
267 the Above-Belowground PCA (Appendix S13), and for each of the ten *c_{WM}Traits* (Appendix
268 S14). We further tested if the proportion of the three plant functional types, as related to the
269 trait dimensions, responded to environmental variables in a similar way, and ran the same
270 models with the proportion of plant functional types as the response variables (Appendix
271 S12).

272

273 **Results**

274 *Dimensionality of CWMs*

275 The Above-Belowground PCA (Fig. 2, Appendix S5) as well as the other three PCAs
276 (Appendix S5, S6) revealed that the two first axes generally explained about 55-60% of the
277 total trait variance, and that each of the 10 traits had intermediate to strong loadings on at
278 least one of these two axes (Appendix S9). PC1 had strong negative loadings of
279 *c_{WM}Specific root length* and *c_{WM}Branching intensity*, and strong positive loadings of

280 $C_{WM}Mycorrhizal\ colonization$, $C_{WM}Fine\ roots\ \%N$, and $C_{WM}Fine\ roots\ diameter$. PC2 had
281 strong positive loadings of $C_{WM}Bud\text{-}bank\ size$, $C_{WM}Root\ weight\ ratio$, and
282 $C_{WM}Root\ tissue\ density$, and strong negative loadings of $C_{WM}Specific\ leaf\ area$. PC1 thus
283 overall captured the mycorrhizal ‘collaboration’ gradient of the root economic space, and
284 PC2 captured the resource ‘conservation’ gradient. The traits of the two gradients of plant
285 functioning, the ‘collaboration’ and the ‘conservation’ gradients were, however, only
286 partially independent (see CWMs correlations in Appendix S10). The ‘fast’ strategy tended to
287 associate with the ‘do-it-yourself’ strategy. $C_{WM}Rooting\ depth\ 50\%$ loaded rather strongly on
288 both of these two PCs (Fig 2A; Appendix S9), suggesting that deep-rooting communities
289 were associated with the ‘outsourcing’ side of the ‘collaboration’ gradient as well as the ‘fast’
290 side of the ‘conservation’ gradient.

291 *Associations of the dimensions of CWMs with environmental variables*

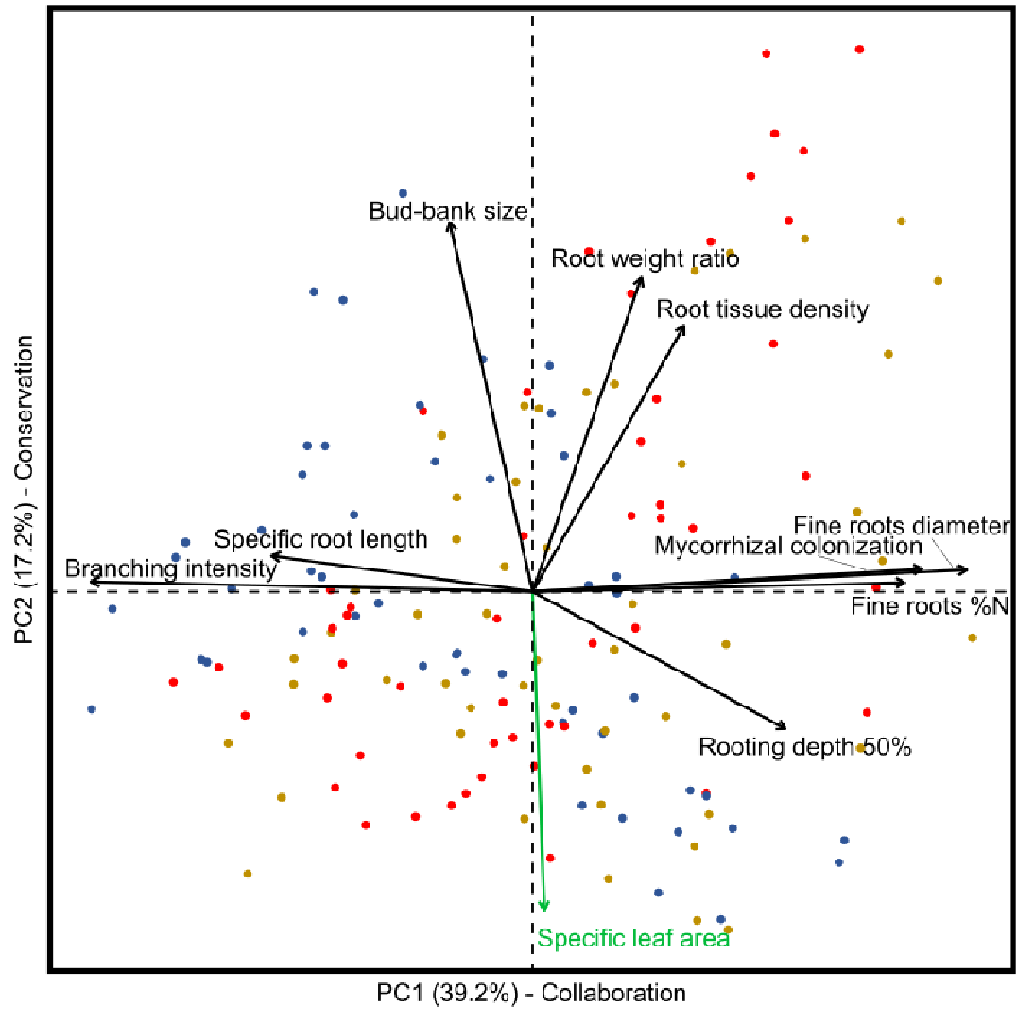
292 The position of grassland communities along the ‘collaboration gradient’ (PC1) and
293 the ‘conservation’ gradient (PC2) was significantly related to several environmental variables
294 (Fig. 3). The $\delta^{15}N$ isotopic signal, sand content and moisture of the topsoil were associated
295 with the ‘do-it-yourself’ side of the ‘collaboration’ gradient (*i.e.* had negative effects on
296 PC1). Land-use intensity and NO_3 content were retained by the model-selection procedure, as
297 associating with the ‘do-it-yourself’ side, but their effects were not significant (Fig. 3a). The
298 pH and C:N ratio, on the other hand, were associated with the ‘outsourcing’ side of the
299 ‘collaboration’ gradient (*i.e.* had positive effects on PC1; Fig. 3a).

300 Among the environmental variables, NO_3 content and land-use intensity were
301 significantly associated with ‘fast’ communities (*i.e.* had negative effects on PC2; Fig. 3b).
302 Phosphorus content was also associated with ‘fast’ communities, but this effect was only
303 marginally significant (Fig. 3b). NH_4 content and pH, on the other hand, were significantly

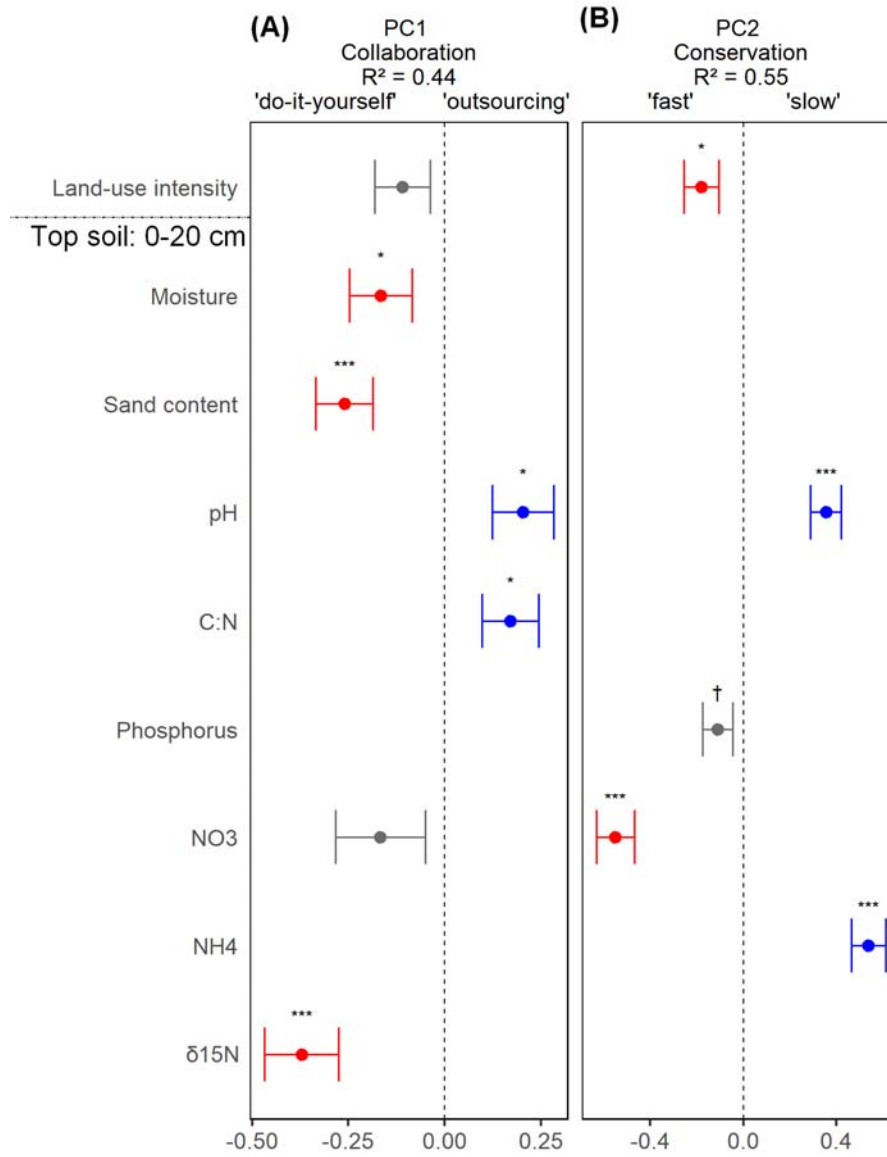
304 associated with the ‘slow’ communities (*i.e.* had positive effects on PC2; Fig. 3b). The effects
305 and the variance explained by the different models are comparable for the four PCAs, with
306 and without c_{WM} Specific leaf area and with and without the plant functional types (Appendix
307 S6).

308 **Figure 2** The two first PCs of the Above-belowground PCA, explaining 56.4% of the total
309 variance in community weighted means (CMWs). Every *c_{WM}Trait* has a strong loading on
310 either PC (Appendix S9). The subsequent PCs, including PC3 representing 14.8% of the total
311 variance (Appendix S5), mainly captured variation among the three regions, probably
312 capturing differences in regional species pool, but did not strongly relate to any
313 environmental parameter (Appendix S13). The sole aboveground trait that we included,
314 *c_{WM}Specific leaf area*, is shown in green. The scores of the 150 grassland plots used for the
315 PCA are shown in different colors for each of the three regions (red for the Schwäbische Alb,
316 brown for Hainich, blue for Schorfheide, each with N=50). PC1 is mostly characterized by
317 CWMs of traits related to the mycorrhizal ‘collaboration’ gradient of the root economic
318 space, with on the left, the ‘do-it-yourself’ strategy and on the right, the ‘outsourcing’
319 strategy. PC2 is more characterized by CWMs of traits related to the ‘conservation’ gradient
320 of a ‘root and leaf economic spectrum’, with on the top, the ‘slow’ strategy and on the
321 bottom, the ‘fast’ strategy. Bud-bank size, as a surrogate of the vegetative regeneration
322 potential is associated with the ‘slow’ strategy. Correlation coefficients between the CWMs
323 are provided in Appendix S10 and corroborate the relationships observed on PC1 and PC2.
324 The loadings onto PC1 to PC6 (90% of variance explained) are in Appendix S9. To maximize
325 the loadings of the traits characteristic of the ‘collaboration’ and ‘conservation’ gradients on
326 PC1 and PC2, an “oblimin” rotation was performed on the plot scores.

Community Weighted Means



328 **Figure 3** Estimates from linear models testing the effects of environmental variables on PCA
329 scores for (A) PC1 - ‘Collaboration’ gradient and (B) PC2 - ‘Conservation’ gradient from the
330 Above-Belowground PCA on community weighted means of traits. On the y-axis are the nine
331 environmental variables that were retained in the most parsimonious models (Region and N:P
332 were not retained). The error bars around the estimates are standard errors. Significant (* for
333 $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$) negative and positive estimates are marked in
334 red and blue, respectively. Non-significant ($p > 0.05$) estimates are marked in grey.
335 Marginally significant ($p < 0.10$) estimates are marked with †.



336

337 **Discussion**

338 We investigated the belowground trait dimensionality of grassland communities and
339 found that a ‘collaboration’ (do-it-yourself vs. outsourcing) and a ‘conservation’ (slow vs.
340 fast) gradient (*sensu* Bergmann et al. 2020) explained most of the variation in community-
341 weighted means of belowground traits. Three traits that were not considered previously in the
342 belowground trait space were largely part of these two dimensions. *CWMRooting depth 50%*
343 was associated with the ‘outsourcing’ and ‘fast’ strategies, *CWMBranching intensity* with the
344 ‘do-it-yourself’ strategy, and *CWMBud-bank size* with the ‘slow’ strategy. *CWMFine root %N*
345 was surprisingly associated with the ‘outsourcing’ strategy. Both gradients responded to
346 environmental variables related to soil conditions, and more fertile soils were generally
347 associated with the ‘fast’ and the ‘do-it-yourself’ strategies. In line with this, we also found
348 that land-use intensity was associated with the ‘fast’ strategy and tended to be associated with
349 the ‘do-it-yourself’ strategy.

350 **Trait relationships and dimensionality of belowground traits**

351 For the grasslands in our study, variation in community weighed means of
352 belowground traits tended to separate along two dimensions that largely correspond to the
353 two ecological root-trait gradients recently identified for species. PC1 related to the
354 collaboration of plants with mycorrhizal fungi. This ‘collaboration’ gradient (Bergmann et
355 al., 2020) ranged from ‘outsourcing’ communities with a high mycorrhizal colonization rate
356 and thick roots but also, surprisingly, high root nitrogen content, to ‘do-it-yourself’
357 communities with high specific root length and a high root-branching intensity. PC2 related
358 to the construction cost of roots and leaves and the vegetative regeneration potential. This
359 ‘conservation’ gradient ranged from ‘slow’ communities with high root-tissue density, high
360 root-weight ratio and large bud-banks to ‘fast’ communities with high specific leaf area.
361 *CWMRooting depth 50%* relates to both of these PCs, with deep-rooting communities being

362 ‘outsourcing’ and ‘fast’. To maximize the loadings of the traits onto one of the PC axes, we
363 used an ‘oblimin’ rotation, and as a consequence the PCs are not orthogonal to each other.
364 This also shows that the two plant-strategy gradients (i.e. PC1 and PC2) were not entirely
365 independent, as the ‘fast’ strategy and ‘do-it-yourself’ strategy partly coincide, both with PC1
366 and PC2 scores, and with the traits that are associated with each strategy (Appendix S10).
367 This is partly in accordance with recent findings of Laughlin et al. (2021) who found cold
368 climatic conditions to enhance the probability of occurrence for both ‘fast’ as well as ‘do-it-
369 yourself’ plant species in a global analysis.

370 At the community level, belowground trait relationships can differ from the one found
371 at the inter-specific level (Craine et al., 2001; Roumet, Urcelay, & Díaz, 2006; Prieto et al.,
372 2015; Schroeder-Georgi et al., 2016; Zhou, Bai, Zhang, & Zhang, 2018; Erktan et al., 2018;
373 Delpiano, Prieto, Loayza, Carvajal, & Squeo, 2020). The trait clustering we found for CWMs
374 was generally in accordance with previous findings of trait clustering among species, both for
375 trees and herbaceous plants (Weemstra et al., 2016; Kramer-Walter et al., 2016; Bergmann et
376 al., 2020). The main exception was $CWM_{Fine\ roots\ \%N}$, which in our study associated with
377 the ‘outsourcing’ side of the collaboration gradient instead of the ‘fast’ side of the
378 conservation gradient. This might be particular to our study using CWMs, as root nitrogen
379 content relates to both the ‘fast’ and the ‘outsourcing’ strategies when we do the PCA at the
380 species level (Appendix S16a), but only with the ‘outsourcing’ strategy when scaling up to
381 community level (Fig. 2). Trait-performance relationships have already been shown to differ
382 between common garden and field condition in the Biodiversity Exploratories grasslands
383 (Breitschwerdt, Jandt, & Bruelheide, 2019). The differences in relationships among species
384 traits and among community weighted means of traits could reflect the multiple constraints
385 exerted by environmental filtering on the trait values selected in a field context. More effort

386 will be required to disentangle filtering effects and phenotypic changes when traits are
387 assessed in controlled versus field conditions.

388 We found that communities with large bud-banks were on the ‘slow’ side of the
389 ‘conservation’ gradient. Previously, bud-bank size was shown to be rather independent of the
390 plant economics spectrum, as specific leaf area — a key trait in this spectrum — explained
391 less than 2% of variation in bud-bank size among 1359 herbaceous species (Klimešová et al.,
392 2016). In our study, the correlation between bud-bank size and specific leaf area of species
393 mean values was significantly negative (-0.17 , $p < 0.01$; Appendix S16b), though still weaker
394 than between the corresponding CWMs (-0.34 ; Appendix S10). Because all of our species
395 were selected based on their presence in permanent grasslands, it could be that the association
396 between bud-bank size and ‘conservation’ traits is a feature of this specific habitat.
397 Nevertheless, inclusion of other traits linked to clonality, such as clonal lateral spread, shoot
398 persistence or bud-bank depth could reveal specific clonal strategies (Herben & Klimešová,
399 2020), potentially increasing the dimensionality of belowground trait space (Ladouceur et al.,
400 2019). The smaller bud-bank size we observed in communities with the ‘fast’ strategy,
401 typical of resource rich grasslands, where competition for light might be more intense
402 (Hautier, Niklaus, & Hector, 2009) could indicate that those plants invest more in immediate
403 aboveground light-harvesting structures at the cost of future regrowth ability. In line with
404 this, we also found that low root weight ratios are indicative of ‘fast’ communities.

405 **Variation in community-trait dimensions explained by the environment**

406 The ‘collaboration’ and ‘conservation’ gradient in PCA were associated with several
407 environmental variables, partly in an overlapping and partly in a unique manner. About half
408 of the variation in PC1 and PC2 scores was explained by environmental variables. Along the
409 ‘collaboration’ gradient, the ‘outsourcing’ strategy was found on dry, non-acidic soils with a

410 low sand content and low N availability (i.e. high C:N, low $\delta^{15}\text{N}$ and marginally low NO_3),
411 and tended to be associated with a low land-use intensity (although not significantly). Along
412 the ‘conservation’ gradient, the ‘slow’ strategy was found on non-acidic soils with low P and
413 NO_3 but high NH_4 availabilities, and in sites with low land-use intensities. Hence, although
414 the ‘outsourcing’ and ‘slow’ strategies correspond to two different plant-strategy gradients,
415 they both tend to be associated with relatively unproductive soils under low land-use
416 intensity. In fact, all plots in the related upper right section of the PCA diagram originate
417 from calcareous grasslands on shallow, infertile Rendzic Leptosols that are mostly used as
418 unfertilized sheep pasture and characterized by P or NP-limitation (Klaus et al., 2011).

419 The relationships we found between the ‘collaboration’ gradient and environmental
420 variables are generally in accordance with the current knowledge in mycorrhizal ecology. For
421 example, (Hempel et al., 2013) found, that among 1752 species of the German flora,
422 obligatory mycorrhizal species tended to be positively associated with dry, non-acidic,
423 infertile habitats. In line with this, we found that drier top soils were associated with deeper
424 rooting, more mycorrhizal-associating communities (Fig. 3, Appendix S14). While
425 mycorrhiza have a well-known positive effect under water limited conditions (Augé, 2001),
426 deeper roots allow the uptake of water from deeper soil layers (Fan, Miguez-Macho, Jobbágy,
427 Jackson, & Otero-Casal, 2017). We found that ‘outsourcing’ communities were also linked
428 with lower $\delta^{15}\text{N}$ isotopic ratios of the soil. The value of $\delta^{15}\text{N}$ in the soil is the result of
429 multiple processes implicated in the nitrogen cycle and by the primary source of nitrogen in
430 the system, which can be fixation by legumes and organic or inorganic fertilisation
431 (Robinson, 2001). In previous work at the same plots, a high $\delta^{15}\text{N}$ has been linked to higher
432 plant productivity and lower species richness, potentially indicating a more open N-cycle
433 with enhanced nitrogen losses (e.g. via leaching) and the dominance of few species with a
434 ‘fast’ strategy (Kleinebecker et al., 2014). Indeed, $\delta^{15}\text{N}$ is strongly positively correlated with

435 NO₃ concentration in the soil and moderately with NH₄, moisture and land-use intensity
436 (Appendix S11). If interpreted as an indicator of more plant-available nitrogen in soils, the
437 negative relationship between δ¹⁵N and the ‘outsourcing’ strategy is in line with the finding
438 of reduced mycorrhizal colonization in response to nitrogen addition (Ma et al., 2020) and
439 with our finding that ‘outsourcing’ communities tend to be on the ‘slow’ side of the
440 ‘conservation’ gradient.

441 As arbuscular mycorrhizal fungi are known to help plants with the uptake of
442 phosphorus, we expected that communities on soils with low phosphorus content would score
443 high on the ‘collaboration’ gradient. Nitrogen addition generally decreases the degree of
444 mycorrhizal colonization in conditions of high P availability and increases it under low P
445 availability at the plot level (Ma et al., 2020). Arbuscular mycorrhizal fungi could also help
446 with nitrogen uptake under conditions of high phosphorus concentrations, with a possible
447 negative relationship between N:P and mycorrhizal colonization rates (Blanke et al., 2005;
448 Blanke et al., 2011). Soils depleted in P or in which P is not plant-available are also selecting
449 root systems with little reliance on mycorrhiza, for example by having cluster roots and
450 carboxylate exudation to mobilize inorganic phosphorus (Lambers, Bishop, Hopper,
451 Laliberté, & Zúñiga-Feest, 2012). Reliance on mycorrhiza could depend on how much
452 phosphorus is available, but also on the balance between phosphorus and nitrogen (i.e. the
453 N:P ratio). Neither the anion-exchange-resin data we used as indicator of soil phosphorus
454 availability nor the N:P ratio was related to the ‘collaboration’ gradient (though N:P was
455 marginally positively associated with *c_{WM}Mycorrhizal colonization*, Appendix S14).
456 However, low resin-phosphorus was marginally related to the ‘slow’ side of the
457 ‘conservation’ gradient. The nature of plant-available phosphorus in soil is still debated
458 (Barrow, 2021). Phosphorus is also more available in slightly acidic soils (Alt, Oelmann,
459 Herold, Schrumpf, & Wilcke, 2011). The pH of our soils ranged from acidic to slightly

460 alkaline (min. 4.5, max 7.5), with a mean of 6.5, and only 37 out of 150 plots have a pH
461 between 6.5 and 7 which is often used as an optimum to assess phosphorus availability (Penn
462 & Camberato, 2019). So, the positive effect of pH on the ‘collaboration’ gradient could
463 indicate a lower P availability at high pH values. Furthermore, organic fertiliser, applied in
464 the plots with high land-use intensity, is usually rich in P, and our land-use-intensity variable
465 thus could capture part of the P supply that is not captured by resin-P ($r=0.49$ between resin-P
466 and land-use intensity, Appendix S11). In conclusion, we did not find a decrease of
467 mycorrhizal colonization when P is more available through the effects of resin-P on
468 ‘collaboration’ gradient (Fig. 3), but we cannot rule out an effect of P availability, because of
469 the potential changes in phosphorus availability through fertilisation and pH changes. The
470 proven P-limitation of plant growth (Klaus et al 2011) in plots allocated in the upper right
471 corner of the PCA-diagram (outsourcing and slow) also points in this direction.

472 The relationships we found between the ‘conservation’ gradient and environmental
473 variables are overall in line with expectations on how soil fertility should relate to the plant
474 economic spectrum. Accordingly, high land-use-intensity and acidic soils with high
475 phosphorus and nitrate concentrations were associated with the ‘fast’ strategy. The decrease
476 in bud-bank size at higher soil fertility (Fig. 3, Appendix S14) is congruent with recent
477 findings that land-use intensity and nitrogen addition decrease total bud density and rhizome
478 biomass in temperate perennial grasslands (Qian, Wang, Klimešová, Lü, & Zhang, 2021;
479 Ottaviani et al., 2021). Disturbance and habitat-productivity indices of species have been
480 associated with an increase in specific leaf area and a decrease in bud-bank size (Herben,
481 Klimešová, & Chytrý, 2018). In contrast to the negative effect of nitrate concentration on the
482 ‘conservation’ gradient, we found a positive effect of soil ammonium concentration. Species
483 preferences for specific nitrogen forms vary with ecological strategies. Early successional
484 species, which are usually on the ‘fast’ side of the ‘conservation’ gradient, generally prefer

485 nitrate, whereas late successional species, which are usually on the ‘slow’ side, generally
486 prefer ammonium (Britto & Kronzucker, 2002; Warren, 2009). It has also been shown that
487 there might be a trade-off between nitrate and ammonium uptake in grassland species (Maire
488 et al., 2009). Some plants can also inhibit nitrification, thereby retaining NH_4 , which is less
489 prone to leaching than NO_3 (Boudsocq et al., 2012). High rates of nitrification in fertile soil
490 with high microbial activity could lead to a stronger dominance of nitrogen in form of nitrate.
491 Increase in ammonia oxidation with land-use intensity (and therefore fertilisation) has already
492 been shown in our grassland system (Stempfhuber et al., 2014). So, the positive association
493 between ‘fast’ communities and NO_3 could reflect an overall higher nitrifying activity of
494 microbial communities in fertile, nitrogen-rich soils. In conclusion, the form of nitrogen
495 available in the soil has contrasting effects on belowground traits, with ammonium being
496 more related to the ‘slow’ strategy and nitrate more related to the ‘fast’ strategy.

497 **Conclusion**

498 The dimensionality of trait syndromes and their relation to environmental variables are
499 central questions in ecology. At the grassland community level, we found an integration of
500 root branching intensity, root-weight ratio, bud-bank size and rooting depth within the
501 bidimensional ‘collaboration’ and ‘conservation’ trait space previously observed at the
502 species level. The variation of both gradients with environmental variables was partly
503 overlapping and partly independent. Indicators of high soil fertility were generally associated
504 with both the ‘fast’ and the ‘do-it-yourself’ strategies. Overall, our study shows that the
505 belowground plant-strategy gradients identified among species are also applicable to the
506 description of plant communities, and can be linked to environmental variables.

507

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524

525 **Declarations**

526 Tom Lachaise performed three of the experiments, ran the analyses and wrote the paper.
527 Joana Bergmann performed one experiment and participated in one of the other three.
528 Matthias Rillig contributed to the design of the experiments. Norbert Hölzel, Valentin Klaus
529 and Till Kleinebecker collected environmental data. Mark van Kleunen designed three of the
530 experiments, advised on data analysis and extensively revised the paper. All authors
531 contributed to revisions. The authors declare no conflicts of interest.

532 **Data availability**

533 The trait data will be archived in Dryad, and the data DOI will be included at the end of the
534 article. The environmental data is partly publicly available on BExIS and partly under an
535 embargo period of three years. It can be requested directly to the authors of the data (see
536 Appendix S2).

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