Environmental filtering vs. resource-based niche partitioning in diverse soil animal assemblages

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Abstract

Terrestrial invertebrates constitute most of described animal biodiversity and soil is a major reservoir of this diversity. In the classical attempt to understand the processes supporting biodiversity, ecologists are currently seeking to unravel the differential roles of environmental filtering and competition for resources in niche partitioning processes: these processes are in principle distinct although they may act simultaneously, interact at multiple spatial and temporal scales, and are often confounded in studies of soil communities. We used a novel combination of methods based on stable isotopes and trait analysis to resolve these processes in diverse oribatid mite assemblages at spatial scales at which competition for resources could in principle be a major driver. We also used a null model approach based on a general neutral model of beta diversity. A large and significant fraction of community variation was explainable in terms of linear and periodic spatial structures in the distribution of organic C, N and soil structure: species were clearly arranged along an environmental, spatially structured gradient. However, competition related trait differences did not map onto the distances separating species along the environmental gradient and neutral models provided a satisfying approximation of beta diversity patterns. The results represent the first robust evidence that in very diverse soil arthropod assemblages resource-based niche partitioning plays a minor role while environmental filtering remains a fundamental driver of species distribution.

Keywords: stable isotopes, trophic niche, community structure, neutral theory, soil microarthropods, oribatid mites

1. Introduction

The classical view of communities and the assembly processes forming them has historically been dominated by the approaches pioneered by the founders of niche theories. More recently classical theories have been rethought to include stochastic processes such as those related to stochastic demographic fluctuations and dispersal dynamics, which for example are the only mechanisms postulated in neutral theories (Bell, 2001; Hubbell, 2001). Stochastic processes have also been included in the more general framework of metacommunity theories (Cottenie, 2005; Leibold et al., 2004), which focus on the spatial nature of assembly processes and extend the principles of metapopulation dynamics to community ecology. For example, processes such as dispersal create spatial patterns in species distribution. These spatial patterns do not depend on spatial structure in the distribution of environmental variables although the processes generating these patterns may interact with environmentally driven processes (Smith and Lundholm, 2010). Biotic interaction, too, can create spatial patterns (e.g., segregation of competing species in fairly homogeneous environments), regardless of other spatial processes (Gotelli, 2000; Gotelli et al., 2010). Environmental gradients determine spatial patterns in species distribution by sorting species according to their environmental requirements (e.g., dry-tolerant vs. moist tolerant species) and for a long time community ecology has been synonymous with studying species distributions along such gradients (Morin, 2011).

These various processes are entangled in nature at multiple spatial scales but a key general point we analyse in this paper is that environmental filtering is one component of niche partitioning dynamics, which might or might not involve resource based niche partitioning due to competition for shared resources (Adler et al., 2013; HilleRisLambers et al., 2012; Hubbell, 2005; Kraft et al., 2014). Interestingly, the point of possible independence of environmental filtering and resource-based niche

71 partitioning has been made both by niche (HilleRisLambers et al., 2012; Kraft et al.,
72 2014) and neutral theorists (Hubbell, 2005) in spite of the fact that several ecologists in
73 practice continue to see niches in the sense of Grinnell, that is to say in terms of species
74 environmental requirements (Chase and Leibold, 2003).
75 Invertebrates constitute most of animal biodiversity and soil is a major reservoir of this
76 diversity. Soil animal community ecologists, following other animal and plant
77 ecologists (Dornelas et al., 2006; Hubbell, 2001; Ritchie, 2009), for a long time have
78 addressed taxonomically defined assemblages such as oribatid mites, collembolans or
79 nematodes to unravel the mechanisms that allow species coexistence in very diverse
80 systems (Wardle, 2002). Recently, microarthropods have also been investigated within
81 the niche-neutral debates or the more general framework of metacommunity theories
82 (Caruso et al., 2012; Lindo and Winchester, 2009; Nielsen et al., 2010; Salmon and
83 Ponge, 2012). However, in recent years studies based on stable isotopes and molecular
84 genetics have clearly shown that assemblages such as oribatid mites or collembolans
85 actually consist of species that can range in diet from being decomposers of low quality
86 organic matter to being top predators of nematodes (Heidemann et al., 2011; Maraun et
87 al., 2011; Schneider et al., 2004). This fact implies a strong bias of previous studies in
88 terms of how observed patterns can inform on underlying mechanisms. For example, if
89 we test neutral theories against niche partitioning theories, we should test these within
90 trophic levels (Hubbell, 2005), which challenges previous studies (Caruso et al., 2012;
91 Gao et al., 2014; Lindo and Winchester, 2009; Nielsen et al., 2010). In general, there is
92 little theoretical and empirical support for the hypothesis that soil animal communities
93 are structured by niche dynamics based on competition (Gao et al., 2014; Wardle,
94 2006), although several studies have shown that microarthropod communities are sorted
95 by environmental gradients (Auclerc et al., 2009; Lindo and Winchester, 2009; Salmon
96 and Ponge, 2012).

97 We addressed this general point by focusing on diverse soil oribatid mite assemblages
98 from a dry grassland using a spatially explicit sampling design that allowed us minimise
99 dispersal processes and focus on environmental filtering and niche partitioning based on
100 food resources. Instead of focusing on taxonomic assemblages, we used the stable
101 isotopes ratios $^{15}N/^{14}N$ and $^{13}C/^{12}C$, and for the first time focus community analysis on
102 trophic assemblages within which competition for shared resources could be a key
103 process. To further characterise species in terms of traits that can be related to
104 competition for resources, we quantified body size and depth distribution and then
105 defined a trait matrix. We used these data to test the hypothesis that species that were
106 closer in space and time were more dissimilar and vice-versa (limiting similarity
107 concept) than expected by chance. The assumption is that limiting similarity and/or trait
108 trade-offs should be observed if resource based niche partitioning is a mechanism
109 through which species coexist locally while competing for shared resources. Still,
110 resource-based niche partition and environmental filtering may act simultaneously.
111 Thus, species could also be sorted along environmental gradients either in relation to the
112 measured traits or not. In fact, environmental filtering and resource-based niche
113 partition could also be decoupled if competition is not taking place or is of minor
114 importance. The rationale behind the test of these hypotheses is that demonstrating a
115 clear link between trait differences and environmental distance is a key premise to
116 unravel the mechanisms that allow species coexistence in rich communities (Adler et
117 al., 2013).
118
119 **2. Materials and Methods**
120 *2.1 Study area and sampling strategy*
121 This study was conducted in dry grassland in a natural reserve in Mallnow, Lebus,
122 (Brandenburg, Germany, 52°27.778' N, 14°29.349' E). This reserve has been managed

123 by low-intensity sheep grazing for at least 500 years and is dominated by *Festuca*
124 *brevipila* (Poaceae). There are areas where grazing may not occur for one year or longer
125 and plant diversity can be very high locally (e.g., > 40 species in a 10 x 10 m plot)
126 although grasses such as *Festuca* spp. dominate the assemblage. In these areas, in April
127 and October 2012 we took soil core samples (local communities) within two
128 undisturbed plots of 15 x 15 m along the slope of a hillside, with the two plots about 20
129 m apart. The two plots represented spatial replicates of a steep soil textural gradient
130 running from the sandy-loamy soil uphill to highly sandy soil downhill. Main soil
131 parameters such as pH, water content, organic C and N varied along the gradient, in
132 some case with remarkable variation (Supplementary Material, Table S1). Sampling
133 was replicated in the two main seasons (spring and autumn). To standardise the local
134 soil arthropod community, we took soil cores (5 cm diameter, 10 cm deep) centred on
135 the grass *Festuca brevipila*, which was by far the most abundant species in the area (in
136 some case cover > 70%). Twenty randomly positioned samples per plot were collected
137 in each season (total of 80 local communities) and the position of each sample was
138 recorded in the UTM system.
139
140 *2.2 Sample processing and analysis*
141 Each soil core was cut into five 2 cm slices to quantify species depth distribution.
142 However, the soil core was the main unit of analysis and we defined the local assemblage
143 as the species inhabiting this unit. Eventually, each species was assigned a depth score
144 based on the weighted average of its depth distribution and depth was treated as a species
145 trait. The soil fauna was extracted in a Macfadyen apparatus for two weeks. All
146 arthropods were preserved in 70% ethanol and the adult oribatids morphologically
147 determined to species level (Weigmann, 2006). Body lengths were measured for each
148 individual under a dissecting microscope (Leica M 165, Wetzlar, Germany) using the

149 software LAS. Each species was assigned a size score based on the average length
150 obtained from a number of replicated measurements (mean number of measurements per
151 species = 85; median number of measurements per species = 30). Soil water content was
152 measured as the difference between the weights of fresh vs. dried soil (soil dry weight,
153 SWD), with samples collected at field capacity. Soil pH was measured in a soil-water
154 suspension, where 3 g of soil and 15 ml distilled H_2O were mixed and stirred. The
155 measurement was conducted in the supernatant until the value remained constant.
156 Organic carbon (C) and total nitrogen (N) were measured by direct combustion of 30 mg
157 of soil in a Euro EA Element Analyzer (HEKAtech GmbH, Wegberg, Germany). Mean
158 weight diameter (MWD) was calculated as the weighted sum of the proportion of soil
159 particles and aggregates in each size class (2-4 mm, 1-2 mm, 0.5-1 mm and 0.2-0.5 mm),
160 determined by dry sieving of the soil.
161
162 *2.3 Stable isotope analysis*
163 Specimens were transferred into tin capsules. Rare (e.g. *Carabodes willmanni*) or smaller-
164 sized species (e.g. *Microppia minus)* required the pooling of several individuals to reach
165 the biomass necessary to the analysis. After drying at 60°C for at least 12 h, samples were
166 reweighed and stored in a desiccator until further analysis. The same procedure was used
167 to prepare samples of nematodes, extracted from fresh soil by using a modified Baermann
168 funnel method. Soil, mosses, lichens, roots, and plant material were ground and subjected
169 to the same procedure (root and plant material 1.0 - 1.5 mg, soil 34.1 - 35.3 mg). We
170 analysed these organisms and material to obtain baseline values of different potential food
171 sources for oribatid mites (Supplementary Material). A coupled system of an elemental
172 analyzer (Euro EA 3000, Euro Vector S.p.A.: Milano, Italy) and a mass spectrometer
173 (Delta V Plus Thermo Electron; Bremen, Germany) was used to analyze the $^{13}C/^{12}C$ and
174 $^{15}N/^{14}N$ ratios (Reineking et al., 1993). The primary standard for ^{15}N was atmospheric

175 nitrogen whereas acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) served for internal
176 calibration. Vienna Pee Dee Belemnite (V-PDB) was used as a primary standard for ^{13}C.
177 See also Fischer et al. (2010), Maraun et al. (2011), Pollierer et al. (2009), and Schneider
178 et al. (2004) for further details.
179
180 *2.4 Data analysis*
181 We used stable isotopes to focus on a diverse but narrowly defined trophic assemblage.
182 We based the definition of 'relatively narrow trophic assemblage' on the concentration
183 of ^{15}N, which increases from food sources to consumers (Deniro and Epstein, 1981;
184 Peterson and Fry, 1987; Scheu, 2002). The enrichment of ^{15}N varies with diet,
185 especially in generalists, but despite this variation, an average enrichment of 3.4‰ is
186 commonly used to define trophic groups (Post, 2002). The concentration of ^{13}C is
187 usually associated with the analysis of ^{15}N because ^{13}C reflects the basal food source
188 (Deniro and Epstein, 1981; Peterson and Fry, 1987; Post, 2002). The variance of stable
189 isotope signatures reflects the dietary niche width of consumers (Bearhop et al., 2004),
190 which led some authors to define the concept of isotopic niche (Newsome et al., 2007).
191 Eventually (see results) we could define a set of 18 species that potentially competed for
192 fungal resources, and we focused our analysis on this assemblage.
193 In order to visualise and quantitatively summarise the multivariate covariation of
194 environmental variables (Organic C, N, C:N, Water, pH, Mean Weight Diametre of soil
195 particles) and major gradients, we performed a Principal Component Analysis (PCA) on
196 the correlation matrix of the variables (Legendre and Legendre 1998; Gotelli and
197 Ellison 2004). We used PCA axes as environmental correlates of species distribution to
198 eliminate collinearity in predictors (Gotelli and Ellison 2004).
199 Given the small scale of the study and all else being equal, we used a modelling strategy
200 consisting of several steps to test the general hypothesis that species closer in space and

time were more dissimilar in terms of traits related to competition for resources (limiting similarity concept): if resource based niche partitioning is a mechanism through which species coexist locally while competing for shared resources, then limiting similarity or trait trade-offs should be observed (HilleRisLambers et al., 2012; Adler et al. 2013). In order to test this hypothesis, we first used a multivariate regression approach based on RDA (Borcard et al., 2004, 1992; Legendre and Legendre, 1998) to empirically define the spatial and temporal niches of each species. We Hellinger transformed raw data to meaningfully apply RDA, which is PCA-based (Euclidean space), and ensure no inflation of the weights of rare species (Legendre and Gallagher, 2001). The spatially explicit and seasonal sampling design together with the measurement of several crucial environmental variables allowed us to model species distribution as a function of both spatial and environmental factors, and changes between the two sampled seasons. We used the well-established method of principal coordinate analysis of neighbour matrices (PCNM; Borcard and Legendre, 2002) to define a set of spatial factors that parsimoniously accounted for patterns in species distribution. The final set of PCNM vectors was defined using a multivariate extension of the Akaike information criterion (AIC; Dray et al., 2006). Environmental factors were soil water content (% dry weight), pH, organic C, total N, the C:N ratio, and the mean weight diameter of soil aggregates, used as a proxy for soil structure (Caruso et al., 2011). We used the species scores of the statistically significant axes of the RDA model to define species niches: by definition, the Euclidean distance between any two species in the vectorial space defined by RDA axes reflects predicted distances in space, seasons, and environmental conditions: the further apart any two species are in the RDA space the further apart these species are in space, time, and average environmental characteristics of the patches they colonise. We also used permutational tests to test for the effects of spatial and environmental factors, including partial effects (i.e. testing for

227 one factor while statistically controlling for other factors). Once we defined the RDA
228 model-based spatial, temporal and environmental position of species (Grinnellian
229 niche), we used body size and depth distribution together with the $^{15}N/^{14}N$ and $^{13}C/^{12}C$
230 signature to define a species trait matrix. After data standardization and calculation of
231 Euclidean distance, a trait distance matrix of species was obtained. We finally used a
232 Mantel test to test the hypothesis of a negative correlation between the trait distance
233 matrix and the distance matrix based on space, season, and environment: we expected a
234 negative correlation under the limiting similarity hypothesis because the more similar
235 species are in traits involved in competition the more distant species should be in their
236 Grinnellian niche. In practice, species minimise spatial and temporal coexistence to
237 avoid competition and at the same time can coexist locally if they differ in key traits.
238 Conversely, the closer species are in terms of spatial, temporal and environmental
239 position the less similar they should be in terms of traits involved in competition. We
240 used the R packages vegan, spacemakeR and ade4 for all multivariate analyses
241 (Chessel et al., 2004.; Dray et al., 2006; Oksanen et al., 2009).
242 We completed our analysis with a neutral model, based on the null assumption that
243 trophically similar species are not involved in resource-based niche partitioning when
244 they come together to form assemblages. To fit a general neutral model, we used the
245 formula for multiple samples and a PARI/GP code (Etienne, 2007) to estimate neutral
246 model parameters theta (diversity) and I (immigration rate). Afterwards, we used the
247 PARI/GP function urn2.gp (Etienne, 2007) to create 4999 neutral communities based on
248 the estimate parameters. We applied this approach to the following datasets: all species
249 across all trophic levels (spring and autumn, respectively), and just fungal feeders
250 (spring and autumn, respectively). The simulated communities were used to build a null
251 distribution of beta diversity values. We quantified beta diversity (BD) following
252 Legendre and De Cáceres (2013): the sum of species variances in the species by site

253 matrix (with usual correction terms for unbiased estimates of variance). Data were
254 Hellinger-transformed (Legendre and Gallagher 2001). The observed value of BD was
255 compared to the null distribution: if observed BD was within the 95% interval of the
256 simulated data sets, the neutral model could not be rejected at $p < 0.05$ (Maaß et al.,
257 2014).
258
259 **3. Results**
260 *3.1 Environmental variation*
261 PCA of environmental variables (Fig. 1) summarised more than three quarters of total
262 variation in the first two axes. Although all variables have some effect on all PCA axes,
263 PC1 (53%) described a main gradient mostly due to organic matter (organic C and total
264 N) and soil structure (Mean Weight Diameter, MWD) while PC2 (24%) mostly
265 accounted for a negative covariation between water content and C:N ratio. Consistently
266 with the construction of our sampling strategy, the gradients were maximised along the
267 up- to down-hill direction, with some variation between the two sampling plots
268 (Supplementary Material, Table S1): the gradient in organic matter and soil structure
269 was more pronounced in Plot 1 while the negative correlation between water and C:N
270 was more pronounced in Plot 2. There was no significant difference between spring and
271 autumn samples for either plots (Supplementary Material, Fig. S1). Absolute variation
272 in individual soil variables was remarkable in some case: for example, organic C
273 content ranged from 0.15 to 3.49%, total N from 0.01 to 0.26%, and pH from 4.8 to 8.9,
274 and these ranges were comparable between the two plots.
275
276 *3.2 Oribatid mite assemblage and isotopes*
277 In total, we collected 2,397 adult Oribatids of 33 species belonging to 18 families. The
278 most abundant species in both seasons were *Liebstadia pannonica, Punctoribates*

279 *punctum* and *Peloptulus phaenotus*. There were five species (*Achipteria coleoptrata*,
280 *Carabodes willmanni*, *Trichoribates novus*, *Galumna obvia*, and *Minunthozetes*
281 *semirufus*) that were present with few individuals (1 to 4) only in one of the two
282 seasons. Rarefaction curves (not shown) confirmed that the sampling effort was
283 sufficient to describe the overall richness of the oribatid community. We obtained ^{15}N
284 and ^{13}C data for 28 species (Supplementary Material, Fig. S2 and Table S3). *Microppia*
285 *minus* and *Porobelba spinosa* showed the highest ^{15}N signatures whereas *Carabodes*
286 *willmanni* had the lowest ^{15}N signature. Three species (*M. semirufus, T. vel. sarekensis,*
287 *S. sculptus*) had very similar ^{15}N signatures comparable with the root signatures while
288 mosses, lichens, and nematodes were about one trophic level below their potential
289 consumers/predators (Supplementary Material, Fig. S2 and Table S1).
290 Overall, the stable isotope analysis and relevant literature (Fischer et al., 2010; Maraun
291 et al., 2011; Pollierer et al., 2009; Schneider et al., 2004) allowed us to group the
292 oribatid mite community into five trophic groups (predators, fungal feeders/secondary
293 decomposers, decomposers, lichen feeders and species with endophagous
294 juveniles/tunnelers, see Supplementary Material). However, for *T. novus*, *Passalozetes*
295 *perforates* and *M. semirufus*, the group affiliation was not clear. We consider *P.*
296 *perforates* to be a mycophagous species and *M. semirufus* a moss feeder but definitive
297 evidence is missing. The feeding preferences of *T. novus* remain unclear.
298 Based on these data, we defined a group of 18 species (Table 1; Supplementary
299 Material, Table S2) in the broad category fungal feeder/secondary decomposers: several
300 of these species can in principle compete for shared resources. We focused our
301 modelling and hypothesis testing on this assemblage.
302
303 *3.3 Hypothesis testing*

304 The RDA showed that PCNM-based spatial factors and environmental factors (PC1 and
305 PC2 from PCA of environmental variables, see Fig. 1) could account for 31% of total
306 community variation, the total effect of these factors being statistically significant at $p <$
307 0.01 following a permutational test. However, variance partitioning showed that 21% of
308 this variation was attributable to spatial patterns in the environmental variables while
309 10% were accounted for by statistically significant (partial RDA, $p < 0.05$) spatial
310 patterns not related to environmental variation. Less than 1% of variation was
311 explainable in terms of environmental variation that was not spatially structured and this
312 variation was not statistically significant. A RDA based just on environmental factors
313 (i.e. implicitly including spatial structures) accounted for 22% of total variation, the
314 effect of the environment being significant at $p < 0.01$. To test for the factor season, we
315 extracted the residuals of the first, main RDA model and submitted these to a
316 PERMANOVA test, which showed a significant effect of season ($F_{1, 78} = 4.17$, $p <$
317 0.01).
318 Introducing the season factor in the RDA increased total explained variation to 44%. A
319 permutation test showed that the first five RDA axes were significant at $p < 0.01$ and
320 these axes were therefore retained to define the niche space (i.e., based on spatial and
321 temporal distance, which we, given our result, basically understand as the
322 environmental or Grinnellian component of a species niche). A plot of the first two
323 RDA axes (Fig. 2) and the main environmental gradients (based on PCA of
324 environmental variables) showed that the first RDA axis is driven by a gradient in
325 organic matter and soil structure. This gradient is associated with a certain species set
326 while the second axis is driven by a second gradient due to the negative covariation of
327 soil water and C:N. This second gradient is associated to a species set other than that
328 associated to the first gradient. Size and the ^{15}N signature were negatively and
329 significantly correlated with each other but scarcely correlated with the major

environmental gradients, although a positive and significant correlation was detected between ^{15}N and RDA1 (Fig. 3). After standardization, a Euclidean distance matrix was calculated from the Grinnellian niche space and correlated to the species trait distance matrix (based on ^{15}N, ^{13}C, size and depth distribution) via a Mantel test: no significant correlation was found (Fig. 4), which is inconsistent with the limiting similarity hypothesis.

None of the tested assemblages differed significantly from a neutral model for beta diversity (Supplementary Information, Fig. S3; whole assemblage, spring: $p = 0.10$; whole assemblage autumn: $p = 0.16$; fungal feeders spring: $p = 0.07$; fungal feeders autumn: $p = 0.10$, see Table S4 for the estimate of neutral model parameters). However, in all cases we observed assemblages with beta diversity higher than expected under neutrality (Fig. S3), and this trend was more pronounced in the fungal feeder group.

4. Discussion

4.1 Differences between environmental filtering and competition

In recent works investigating the role of deterministic and stochastic drivers of soil organism community structure (Beck et al., 2015; Caruso et al., 2012; Dumbrell et al., 2010; Gao et al., 2014; Lindo and Winchester, 2009; Nielsen et al., 2010) researchers contrasted environmental filtering, typically equated to niche dynamics, with spatial factors not dependent on patterns of environmental variation, sometimes called ‘pure’ spatial factors. These spatial factors are often understood as the effect of dispersal and/or demographic fluctuations in neutral assembly processes; but several ecologists, including those cited above, also recognise that these factors do not necessarily represent stochastic spatial factors (Anderson et al., 2011; Caruso et al., 2012; Smith and Lundholm, 2010). Besides the problem of the interpretation of spatial factors, a key but not often addressed aspect of this central topic is that environmental filtering may

356 imply competition for resources but does not necessarily imply resource-based niche
357 partitioning dynamics: this is a point on which niche and neutral theorists may agree
358 (HilleRisLambers et al., 2012; Hubbell, 2005), although from very different
359 perspectives. At certain scales environmental filtering is compatible with neutral
360 processes because in neutral dynamics competition for resources between species is not
361 a driver of community structure while individuals, regardless of the species they belong
362 to, must still exploit resources and fit their environment (Hubbell 2005). Different
363 species can therefore come together into a local community if they are adapted to the
364 environmental conditions of the locale, and in this sense the environment will tend to
365 select for similar species (e.g., shade-tolerant species in shaded environments).. A
366 neutrally assembled local community can therefore be environmentally filtered at
367 certain scales while being neutral at scales at which competition among species has
368 classically been postulated to structure communities (Etienne, 2007; Hubbell, 2005). It
369 is in this general framework that we interpret our results: when biotic interactions start
370 to be a fundamental driver and predictor of community structure neutral theories should
371 be abandoned. Specifically, neutral theories directly contrast with resource-based niche
372 partitioning processes. A first consideration is therefore that not all biological
373 interactions should be considered, especially multitrophic interactions, which, apart
374 from possible future developments, are usually outside the realm of application of
375 neutral theories (Hubbell, 2005, 2001). For the first time, we have focused on a soil
376 animal assemblage that was trophically defined by the use of stable isotopes of N and C.
377 In doing so, we could start from the empirically validated assumption that competition
378 for resources is a fairly valid possibility within the analysed assemblage. The small
379 scale of the study also allowed us to assume that dispersal limitation, while still a
380 possible factor given the size of our animals (Ettema and Wardle, 2002), should play a
381 minor role. As shown by the analysis of the soil, communities were sampled along steep

382 environmental gradients in a very short distance. Accordingly, we observed a strong,
383 spatially structured correlation between environmental gradients and the structure of the
384 species assemblage. We can therefore conclude that the assemblage was subjected to
385 environmental filtering. This result might imply that species living in different
386 environmental patches spatially segregate to avoid competition locally. However, by no
387 means can this result in itself be considered evidence of resource-based niche
388 partitioning, which should also explain coexistence locally. This is an observational
389 study: in order to reject non-neutral dynamics and find strong evidence of resource-
390 based niche partitioning, we should have rejected neutral prediction of beta diversity
391 and detected patterns consistent with the limiting similarity hypothesis along the
392 environmental gradient, including the local scale of the assemblage inhabiting
393 individual soil cores. Instead, neither could we reject neutral predictions of beta
394 diversity nor could we find patterns consistent with the limiting similarity hypothesis.
395 Observed beta diversity of the assemblage was higher than neutral predictions, as
396 usually expected under environmental filtering (Caruso et al., 2012; Dornelas et al.,
397 2006), but not significantly higher, with fairly high p-values in all cases but one.
398 Species more similar in terms of spatial and seasonal distribution were not more
399 dissimilar in terms of isotopic signature, size, and depth distribution. In theory, size
400 could here be related to competition if we make the classical assumption that species at
401 similar trophic positions avoid competition by differing in size: in this way competing
402 species have access to similar resources in different places (i.e., colonization of
403 differently sized soil pores; Weis-Fogh, 1948; Ritchie, 2009; Turnbull et al., 2014). The
404 local community of our study is the cylindrical soil core used as sampling unit. In this
405 relatively small locale, species that feed on similar resources and have similar size could
406 still partition space by dwelling at different average depths but species weighed mean
407 average depth was not a trait that could explain coexistence.

4.2 Niche partitioning mechanisms and competition

In spite of all the efforts we made to identify the possible dimensions along which competing species could partition their niches, none of these dimensions or their combination provided us with evidence of limiting similarities indicative of resource-based niche partitioning. In fact, the only pattern we have found is a slightly positive correlation between trophic position ($\delta^{15}N$ value) and the major environmental gradient along which the community is structured. However, the correlation seems made up by three low $\delta^{15}N$ values and one high $\delta^{15}N$ value, with the other points scattered in a fairly random manner. In any case, even if we accepted the validity of this correlation, this result would not support the limiting similarity hypothesis. We observed a significant fraction of spatial variation that was not related to environmental gradients. This variation can be due to stochastic but spatial factors such as dispersal, or it could be due to biotic interactions such as predation or competition. Predation can mediate competition by controlling the population of the more competitive species (Chase and Leibold, 2003): predators may spatially structure their prey but in the case of oribatid mites, and differently from collembolans, there is strong evidence that predation is not a strong factor controlling populations (Peschel et al., 2006). Competition and resource based niche partitioning could still play some role because we measured the traits that were most logically expected to be key traits for coexistence, but in fact we could have missed some important aspects. For example, there are limitations in the stable isotope markers we employed: the ^{13}C signature of animal fatty acids has now been demonstrated to be a finer marker for a detailed differentiation of fungal feeders (Pollierer et al., 2012; Ruess and Chamberlain, 2010) while with the method we employed we have been able to isolate a narrowly defined trophic assemblage (i.e. guild) but we might not have been able to differentiate trophic differences within this

assemblage. Natural variability in isotopic signatures may also suggest high intraspecific variability in feeding strategies. This could be especially true for different developmental stages. We are aware of data at this level for one species only (Schneider et al. 2004) and these data suggest small differences between adults and nymphs but other species could definitely vary their diet depending on developmental stage. The interesting point is that high intraspecific variability can imply broad interspecific niche overlaps at the species level, opening the way to neutral assembly processes. The same arguments apply to temporal variation in species soil depth and may imply a theoretical scenario for which levels of competition vary in space (both horizontally and vertically) and time as a function of fluctuations in population densities.

Another limit of our study is that we might not have included all the species relevant to the analysed assemblage. We focused on fungal feeder/secondary decomposer oribatid mites, which is by far the most diverse and abundant group of microarthropods together with collembolans. However, there are other fungal feeders/secondary decomposers in soil, for example collembolan species. We cannot exclude that competition for resources would have been a strong driver of an assemblage that included all the species competing for a limited set of resources.

Finally, our multivariate analysis suggested that seasonal variation is potentially a key niche dimension although our study is deficient in terms of temporal replication. Species competing for similar resources could peak at different times of the year to avoid competition, basically for the same principle for which competing species may segregate spatially. Nevertheless, only future studies will tell whether the observed temporal patterns depend on a temporal form of environmental filtering (e.g. seasonality) or resource based niche partitioning mediated by temporal fluctuations in resources and population densities, or both.

Overall, our results indicate that environmental filtering and resource-based niche partitioning can be decoupled in soil animal assemblages while the burden of the proof of resource-based niche partitioning in soil community still remains with the ecologist.

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Data Accessibility

All data (species abundances, environmental and geographical data, isotopic data, trait data) are uploaded as Supporting Information

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643 **Fig. Legends**

644 **Fig. 1** Principal Component Analysis (PCA) of the correlation matrix (z-scores) of
645 environmental variables: 77% of total variance can be summarized in the first two axes.
646 PC1 (53%) described a main gradient in organic matter (organic C and total N) and soil
647 structure (Mean Weight Diameter, MWD); PC2 (24%) described a negative covariation
648 between water content and the C:N ratio. The vectors associated with the variables are
649 based on PCA eigenvectors (i.e. variables loadings on PCA axes).

650

651 **Fig. 2** First two RDA axes based on a model including spatial vectors, environmental
652 gradient and seasons. Only species points are displayed to show which species are
653 associated with the two environmental gradients. See Table 1 for species labels. This
654 RDA model accounted for 44% of total species matrix. The RDA axis 1 is driven by a
655 gradient of organic matter and soil structure (PC1 of Fig. 1). RDA axis 2 by a contrast
656 between water content and C:N ratio (PC2 of Fig. 1);

657

658 **Fig. 3** a) correlation between size (x-axis) and species trophic position (^{15}N, y-axis) is
659 negative and statistically significant; b and c), correlation between species scores of
660 RDA 1 (y-axis; see Fig. 2) and size (panel b) or ^{15}N (panel c), on the x-axis. RDA1 is a
661 proxy for the environmental, spatial and temporal (seasonality in this case) components
662 of niche. No or weak correlation is observed in panel c and d respectively. Similar
663 figures were drawn (but now shown here) for the first five RDA axes, with the same
664 result. Each data point represents a species.

665

666 **Fig. 4** Niche distance between species is based on the species scores of the statistically
667 significant axes of an RDA (spatial vectors, seasons, and environmental variables). The
668 Euclidean distance between any two species in the vectorial space defined by RDA axes

669 reflects predicted spatial, temporal and environmental distances: the further apart any
670 two species are in this space the further apart these species are in terms of their niche.
671 This RDA-based Euclidean distance matrix was correlated to the species trait distance
672 matrix (based on ^{15}N, ^{13}C, size and depth distribution) via a Mantel test: the Fig. and test
673 show a remarkable lack of correlation, which is inconsistent with the limiting similarity
674 hypothesis.