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Fine-scale horizontal and vertical micro-distribution patterns of testate amoebae along a narrow fen/bog gradient

Vincent E.J. Jassey^{1*}, Geneviève Chiapusio¹, Edward A.D. Mitchell², Philippe Binet¹, Marie-Laure Toussaint¹, Daniel Gilbert¹

¹Laboratory of Chrono-Environment UMR-CNRS 6249, University of Franche-Comté, F-25211 Montbéliard cedex, France

²Laboratory of Soil Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2009 Neuchâtel, Switzerland

*** Corresponding author:** Vincent Jassey

E-mail address: vincent.jassey@univ-fcomte.fr

Phone: +33 381 994 693, Fax : +33 3 81 99 46 61

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Running headline: Micro-distribution of testate amoebae in *Sphagnum*

1 **Abstract**

2 The ecology of peatland testate amoebae is well studied along broad gradient from very wet
3 (pool) to dry (hummock) micro-sites where testate amoeba are often found to respond
4 primarily to the depth to water table (DWT). Much less is known on their responses to finer-
5 scale gradients and nothing is known of their possible response to phenolic compounds,
6 which play a key role in carbon storage in peatlands. We studied the vertical (0-3 cm; 3-6 cm;
7 6-9 cm sampling depths) micro-distribution patterns of testate amoebae in the same
8 microhabitat (*Sphagnum fallax* lawn) along a narrow ecological gradient between a poor fen
9 with an almost flat and homogeneous *Sphagnum* carpet (fen) and a “young bog” (bog) with
10 more marked micro-topography and mosaic of poor-fen and bog vegetation. We analysed the
11 relationships between the testate amoeba data and three sets of variables (1) “chemical” (pH,
12 Eh potential & conductivity), (2) “physical” (water temperature, altitude i.e. *Sphagnum* mat
13 microtopography & DWT) and (3) phenolic compounds in/from *Sphagnum* (water-soluble
14 and primarily bound phenolics) as well as the habitat (fen/bog) and the sampling depth.
15 Testate amoeba Shannon H' diversity, equitability J of communities, and total density peaked
16 in lower parts of *Sphagnum*, but the patterns differed between the fen and bog micro-sites.
17 Redundancy analyses (RDA) revealed that testate amoeba communities differed significantly
18 in relation to Eh, conductivity, water temperature, altitude, water-soluble phenolics, habitat,
19 and sampling depth, but not to DWT, pH, or primarily bound phenolics. The sensitivity of
20 testate amoebae to weak environmental gradients makes them particularly good integrators of
21 micro-environmental variations and has implications for their use in paleoecology and
22 environmental monitoring. The correlation between testate amoeba communities and the
23 concentration of water-soluble phenolic suggests direct (e.g. physiological) and/or indirect
24 (e.g. through impact on prey organisms) effects on testate amoebae, which requires further
25 research.

26 **Introduction**

27 Testate amoebae are abundant and diverse shelled protozoa living in a wide range of habitats
28 ranging from soils, lakes, rivers, wetlands, and moss habitats [4, 13, 62]. Owing to ecological
29 gradients and the preservation of their shells in peat and sediments, these protists are useful
30 proxies in paleoenvironmental and ecological studies of peatland and lakes [6, 11, 43]. In
31 *Sphagnum* bogs, testate amoeba community composition is generally strongly correlated to
32 surface wetness conditions (mostly assessed by the water table depth – hereafter DWT) and
33 water chemistry [3, 39, 48, 59].

34 While the relationship between testate amoebae and DWT, and a few other variables
35 such as pH are well documented along broad ecological gradient (e.g. wet pools to dry
36 hummocks, fen to bog) [26, 47], much less is known on their finer-scale responses to micro-
37 environmental gradients. Some data suggests that testate amoebae may be highly sensitive
38 even to subtle micro-environmental gradients. For example Mitchell et al. [40] studied the
39 horizontal distribution patterns of testate amoeba communities in a 40x60cm almost flat
40 mono-specific *Sphagnum* lawn and found spatial heterogeneity in the communities that was
41 significantly correlated to altitude (microtopography) (despite a very short – ca. 6cm –
42 elevation gradient). Assessing testate amoeba species-environment correlation along fine-
43 scale environmental gradients is necessary to define the practical limits (i.e. the resolution) of
44 their use as bioindicators in ecological and palaeoecological studies.

45 Another open question is the range of abiotic and biotic factors to which testate
46 amoebae respond. Although many variables have been studied, DWT almost always emerges
47 as the strongest variable despite the fact that testate amoebae are unlikely to be directly
48 influenced by the position of the water table 10 or 30 cm below the level where they live [41].
49 Still some important potential factors have not yet been studied including peat and water
50 chemistry beyond simple ions and elements. *Sphagnum* peatlands are indeed generally

51 characterized by gradients such as nutrients (nutrient-poor ombrotrophic bogs vs. rich fens),
52 hydrology (wet hollow vs. dry hummocks) and acidity [14, 22, 23, 52].

53 Recently, phenolic compounds (secondary metabolites) produced by plants have been
54 described to play an important role in the interactions of plants with their environment
55 including microorganisms [24]. For example in humus spruce forests such compounds have
56 been shown to cause the increase of several microbial communities (i.e. cellulose hydrolyser)
57 and in the decrease of others (i.e. bacteria) [56, 57]. While the production of phenolic
58 compounds by vascular plants is well documented, few studies have addressed phenols
59 production by non-vascular cryptogams such as *Sphagnum*. The role of phenolics produced
60 by vascular plants on the functioning of the bog ecosystem is established [18], as well as the
61 phenolics content gradient between knoll forest-peat bogs and peat bogs [16]. Possible effects
62 of phenolics produced by *Sphagnum* on microorganisms, including testate amoebae, are still
63 unknown. *Sphagnum* contains weakly as well as primarily bound phenolics to the cell wall
64 [61]. The unique morphology and anatomy of *Sphagnum*, allows water-soluble phenols to be
65 easily released in the *Sphagnum* surrounding environment. Thus the patterns of phenol
66 concentrations at the surface of *Sphagnum* peatlands may contribute to creating micro-
67 patterned habitats and a range of ecological niches suitable for the establishment of diverse
68 communities of organisms including testate amoebae [1, 12, 40].

69 The aims of this study are to explore (1) the species-environment relationships and (2)
70 vertical micro-distribution patterns of testate amoebae along a short ecological gradient from
71 a *Sphagnum*-dominated poor fen (for simplicity hereafter referred to here as “fen”) and a
72 vegetation with mixed bog and poor fen plant elements and a more marked micro-topography
73 (hereafter referred to as “bog”). Rather than sampling contrasted microhabitats or moss
74 species, we sampled only within macroscopically homogenous and similar *Sphagnum fallax*
75 carpets across the gradient. We assessed (1) how horizontal and vertical patterns of testate

76 amoebae community structure varied along the gradient, and (2) the relationships between the
77 testate amoeba communities and DWT, water chemistry and phenolic compound content. We
78 hypothesized (1) that the vertical patterns of community structure would be more marked in
79 the structurally more complex mixed *Sphagnum* “bog” habitat than in the more uniform poor
80 fen, despite the fact that the sampled habitats were macroscopically identical and, (2) that
81 phenolic compounds would explain a similar fraction of the community data structure as other
82 more commonly studied environmental factors (i.e. altitude, DWT, water chemistry).

83

84 **Methods**

85 **Sampling and laboratory analyses**

86 The study site was an undisturbed ombrotrophic *Sphagnum*-dominated mire [2] situated in the
87 Jura Mountains (The Forbonnet peatland, France, 46°49'35''N, 6°10'20''E) at an altitude of
88 840 m above sea level (Supplementary Fig. 1). Cold winters (on average of -1.4°C) and mild
89 summers (on average of 14.6°C) characterized the climate of the site. The annual mean
90 temperature measured at the site over a one year period from November 5th 2008 to
91 November 30th 2009 was 6.5°C and the annual precipitations were 1200 mm.

92 Samples of *Sphagnum fallax* were collected from two adjacent areas (ca. 10 m x 12 m)
93 selected in relation to their micro-topography, vegetation and assessment of sources and
94 decay of organic matter [15]. The first sampling area (coded “fen”) is a transitional
95 *Sphagnum*-dominated poor fen area, relatively flat and homogeneous, characterized by a moss
96 cover dominated by *Sphagnum fallax* and by the lack of *S. magellanicum*. Vascular plants as
97 *Eriophorum vaginatum*, *Vaccinium oxycoccus* and *Andromeda polifolia* were recorded in very
98 low abundance. *Scheuchzeria palustris* and *Carex limosa* occurred outside of the studied
99 plots. The second sampling area (coded “bog”) is an open bog area with mixed vegetation,

100 directly adjacent to the fen area. Patterns of hummocks with *S. magellanicum*, *V. oxycoccus*,
101 *E. vaginatum*, *C. rostrata* and *Calluna vulgaris*, and hollows with lawns of *S. fallax* and *A.*
102 *polifolia* characterized the sampling area. The terms “fen” and “bog” are used here for
103 simplicity and to denote the existence of a trophic gradient inferred from the vegetation.
104 However the “bog” sub-site represents a mosaic of poor-fen (lawns, hollows) and bog
105 (hummock) vegetation.

106 In each of the two sampling areas, six plots were selected in representative surfaces.
107 Among the 12 sampling plots, the maximal distance between the two most distant plots was
108 ca. 30 m. On June 26th 2008, samples of *S. fallax* were collected in each plot for the study of
109 testate amoeba communities and phenolic compounds around 10 permanent markers in each
110 plot. The goals of this sampling design were (1) to allow for multiple sampling at the site over
111 time (this study representing the T0 of a warming experiment), and (2) to obtain a composite
112 sample from each plot and avoid any bias due to spatial heterogeneity [40]. Moreover in each
113 plots, the Eh potential, the pH, the conductivity (K), the water temperature (W-Temp), the
114 depth to the water table (DWT; measured in a piezometer in the centre of each plot), and the
115 average altitude (microtopography, Alt) of the sampled plot were measured. To assess the
116 effect of microtopography on spatial distribution patterns, the average altitude (in millimeters)
117 of the 10 permanent markers was recorded in each sampling plots using an arbitrary reference
118 [40]. The values of pH and conductivity were standardized to 20°C. The conductivity caused
119 by hydrogen ions was subtracted according to Sjörs [55]. Corrected conductivity (K_{corr}) was
120 then used as a proxy for total mineral richness of the water.

121 Primarily bound (hereafter “bound”) and water-soluble phenolic (hereafter “free”)
122 compounds were extracted and quantified from lyophilized mosses. The green section (0-6
123 cm; 0 being defined as the top of the capitulum) was used for these analyses, excluding the
124 lower part where the mosses start to decay. Two methods were used to extract phenolic

125 compounds from *Sphagnum*. For free phenolics, 0.05g dry weight (DW) of *Sphagnum* was
126 ground in a mortar, mixed with 10 mL distilled water, bubbled with nitrogen and agitated on a
127 reciprocal shaker (15 rpm) for 3 hours and filtered. For bound phenolic compounds, 0.05g
128 DW of *Sphagnum* was ground in a mortar, mixed with 25 mL ethanol / distilled water (80/20
129 v/v) and warmed under reflux at 120°C for 30 minutes. This extract was filtered and
130 evaporated by using a rotary evaporator. Finally, the dry extract was dissolved in 25 mL of
131 boiling distilled water (adapted from Gallet and Lebreton [19]). The free and bound total
132 phenolic contents were quantified with the Folin-Ciocalteu reagent and were expressed in
133 mg equivalent gallic acid (A_{760}).

134 For testate amoeba analysis, the *Sphagnum fallax* samples were cut in three levels
135 (sampling depth): 0-3 cm (upper), 3-6 cm (intermediate), and 6-9 cm (lower). The samples
136 were fixed with 20 mL glutaraldehyde (2% final concentration) and stored at 4°C in the dark.
137 Testate amoebae were extracted from mosses using the following extraction method [45]:
138 each sample was shaken for 1 min on a vortex and then pressed to extract microorganisms
139 (first solution). The mosses were then soaked again with 20 mL of glutaraldehyde (2%),
140 shaken a second time on a vortex and pressed to extract *Sphagnum* leachate. The leachate was
141 left to settle for 12h, after which the supernatant was added to *Sphagnum* and the bottom to
142 the first solution. The process was repeated six times, and all fractions were combined to
143 obtain a final composite sample of 40 mL. The remaining fraction was dried at 80°C for 48h
144 and weighted to express testate amoeba density by gram dry weight (DW) of *Sphagnum*. The
145 testate amoebae were identified and counted to a total of 150 at x200 and x400 magnification
146 by inverted microscopy (OLYMPUS IX71) following Uthermöhl's method [60]. Testate
147 amoebae were identified to the species level whenever possible. Only living amoebae (active
148 only, encysted individuals were not included) were counted.

149

150 Numerical analyses

151 Total density, species richness (S), diversity index (the Shannon index H') and equitability
152 index (J) were calculated. Because the distributions of these data were not normal, non-
153 parametric Friedman tests were performed.

154 In all analyses, species that occurred in less than 2% of maximum density were
155 removed from the data set to reduce the influence of rare taxa on multivariate analyses [32].
156 We analyzed differences among sampling depths and between the fen and bog zones (nominal
157 variables) for the dominant testate amoeba species using a MANOVA test.

158 For all multivariate analyses, a Hellinger transformation was applied to stabilize the
159 variance and reduce the influence of the dominant taxa [33]. A Non-metric multidimensional
160 scaling (NMDS) was used to assess patterns of variation in testate amoeba community
161 structure along the different segments of *Sphagnum* (upper, intermediate and lower segments)
162 and between the fen and bog zones. As this analysis revealed clear differences among
163 sampling depths and between “fen” and “bog” zones ($P < 0.001$), we further explored the
164 species-environment correlations for the different sampling depths and in the two zones
165 separately as well as conducting global analyses.

166 Multiple factor analysis (MFA) was used to assess the general structure of the data and
167 to determine the relationships among the three Hellinger-transformed testate amoeba data sets
168 and the three environmental variables data sets (chemical, physical and phenolics) [17]. MFA
169 was performed in two steps. Firstly, a PCA was performed on each subset, which was then
170 normalized by dividing all its elements by the first eigenvalue obtained from its PCA.
171 Secondly, the normalized subsets were assembled to form a unique matrix and a second PCA
172 was performed on this matrix. RV-coefficient (ranging from 0 to 1) was used to measure the
173 similarity between the geometrical representations derived from each groups of variables [51].
174 RV-coefficients are then tested by permutations [29]. Euclidean distances of global PCA were

175 used in MFA to perform cluster analysis according to the Ward method, and the resulting
176 dendrogram was projected in the MFA ordination space. This analysis revealed the main
177 differences in the structure of the data described by all biotic and abiotic subsets of variables.

178 We assessed the relationships among the testate amoeba communities in the upper,
179 intermediate and lower sampling depth and the three sets of environmental variables (1)
180 “chemical” (pH, Eh potential & conductivity), (2) “physical”: (water temperature, altitude &
181 DWT) and (3) phenolic compounds (bound and free). The ordination patterns of testate
182 amoeba communities and their causal relationships to environmental data-sets were assessed
183 using redundancy analysis (RDA) [58]. The proportion of variance explained by
184 environmental variables was quantified using variance partitioning. Adjusted R^2 were used in
185 all RDA to estimate the proportion of explained variance [49]. The analysis was repeated with
186 the sampling area and sampling depth data-sets transformed to presence/absence in order to
187 reveal only testate amoeba communities differences.

188 All multivariate analyses were performed with the software R [50] using vegan [47]
189 and FactoMineR [28] packages.

190

191 **Results**

192 Environmental variables

193 The range of values for the eight measured environmental variables, minimum, maximum and
194 averages for the “fen” and “bog” areas are given in Table 1. The Eh potential and water
195 temperature were significantly higher in the “fen” area while altitude and free phenols were
196 significantly higher in the “bog” area ($P < 0.05$). Water pH, conductivity, DWT and the
197 concentration of slightly bound phenolic compounds did not differ significantly between the
198 two areas. All environmental variables, except Kcorr, pH, DWT and altitude, were

199 significantly correlated to free phenolics (Table 2) while no environmental variables were
200 significantly correlated to primarily bound phenolics.

201

202 Testate amoeba density and diversity

203 The total density of testate amoebae increased significantly with depth in the “bog” area from
204 3.2×10^4 ind.g⁻¹ DW in the upper segments to respectively 7.45×10^4 and 10×10^4 ind.g⁻¹
205 DW in the intermediate and lower segments ($P < 0.05$). By contrast there was no significant
206 difference with depth in the “fen” area (average density over the three depths: 4.34×10^4
207 ind.g⁻¹ DW).

208 A total of 28 testate amoeba taxa were identified in the 36 samples analyzed. In the
209 “bog” area, species richness did not vary among the different *Sphagnum* segments (on
210 average: 15 species), while in the “fen” area species richness significantly increased between
211 the upper segments (on average: 12 species) and the intermediate/lower segments (on
212 average: 15 species) ($P < 0.05$). In both areas, the highest diversities were measured in the
213 intermediate and lower segments ($H' = 3.3$), and the lowest diversity in the upper segments
214 (“fen”: $H' = 1.8$; “bog”: $H' = 2.5$). The equitability index also demonstrated a strong
215 dominance of some species in upper segments (“fen”: $J = 0.5$; “bog”: $J = 0.7$), while in the
216 intermediate and lower segments the communities were more balanced (both areas: $J = 0.85$).

217

218 Vertical micro-distribution

219 The NMDS ordination of samples from the two sampling areas showed that testate amoeba
220 communities differed significantly along *Sphagnum* segments in the two sampling areas (Fig.

221 1; $P < 0.001$). In the “fen” area the upper segment was clearly different from the intermediate
222 and lower segments, while in the “bog” area this difference was less marked.

223 In the “fen” area, the most abundant taxa in the upper segments were *Archerella*
224 *flavum* (on average 2.2×10^4 ind.g⁻¹ DW) and *Hyalosphenia papilio* (on average 1.5×10^4
225 ind.g⁻¹ DW) (Fig. 2 and supplementary Fig. 2). The intermediate segments were characterized
226 by an increased of the abundance of *Hyalosphenia elegans* (on average of 8.3×10^4 ind.g⁻¹
227 DW), *Nebela tincta* and *Physochila griseola* (both on average 3.5×10^4 ind.g⁻¹ DW), and a
228 significant decrease in the abundance of *A. flavum* and *H. papilio*. The lower segments were
229 characterized by the highest abundance of *P. griseola* (on average 1.07×10^4 ind.g⁻¹ DW) and
230 *H. elegans* (on average 6.5×10^4 ind.g⁻¹ DW).

231 In the “bog” area, the most abundant taxa in the upper segments were also *A. flavum*
232 (on average 1.22×10^4 ind.g⁻¹ DW), *N. tincta* (on average 3.8×10^4 ind.g⁻¹ DW), *H. papilio*
233 (on average 3.5×10^4 ind.g⁻¹ DW), and *Assulina muscorum* (on average 8×10^4 ind.g⁻¹ DW)
234 (Fig. 2 and supplementary Fig. 2). The intermediate segments were characterized by
235 significantly higher densities of *H. elegans* (on average 1.18×10^4 ind.g⁻¹ DW), *N. tincta* (on
236 average 1.0×10^4 ind.g⁻¹ DW), *Amphitrema wrightianum* (on average 9.7×10^4 ind.g⁻¹ DW)
237 and *P. griseola* (on average 7.0×10^4 ind.g⁻¹ DW) and lower density of *H. papilio*. In the
238 lower segments, the most abundant taxa were *P. griseola* (on average 2.4×10^4 ind.g⁻¹ DW)
239 and *N. tincta* (on average 9.0×10^4 ind.g⁻¹ DW).

240

241 Species-environment correlations

242 The multiple factor analysis (MFA) of the three environmental matrices and the three testate
243 amoeba data sets confirmed the existence of an overall division between “fen” and “bog”
244 areas (Fig. 3). The composition of testate amoebae community in the upper segments was

245 significantly linked to the chemical data and to testate amoeba assemblages of the
246 intermediate segments (Table 3). The testate amoeba communities from the intermediate
247 segments were significantly correlated to both chemical and phenolic data. No significant
248 correlation was found between the testate amoeba communities of the lower segment and the
249 environmental data sets. These patterns are further explored in the RDAs.

250 In the RDA ordinations (Fig. 4a, b, c and d), the two areas were clearly separated in
251 the overall analysis as well as for each of the three sampling depths. The model explained
252 51.8% (adjusted r^2) of the variability in testate amoeba data in the overall analysis and 27.5%,
253 52.7% and 41.9% (adjusted r^2) of the variability in the data for the upper, intermediate and
254 lower sections respectively. In the overall RDA, testate amoeba communities in the “fen” area
255 were related to higher values of Eh, pH and W-temp, while testate amoeba communities in the
256 “bog” area were related to higher values of phenolics, altitude and conductivity (Fig. 4a, b, c
257 and d).

258 The RDA on individual environmental variables revealed that the proportion of testate
259 amoebae data explained by each explanatory variable and the significance varied strongly
260 among variables, between the two areas, and among the three vertical positions (Table 4). In
261 the separate RDAs on the “fen” and “bog” samples all sampling depths were significant but
262 no physical or chemical variable was found significant. Free phenolics explained a high
263 proportion of variance in the upper and intermediate *Sphagnum* segments.

264 The partial RDAs showed that chemical, physical and phenolic data sets each
265 significantly explained, independently of the other two data sets, about 7% of the species data
266 variance ($P = 0.02-0.08$) in the overall RDA. The proportion of variance explained by these
267 data was however much higher in the upper two segments (16.5–34.1%) but on average lower

268 in the third segment where no significant correlation was found for the lower segment (Table
269 5).

270

271 **Discussion**

272 Testate amoeba density and diversity

273 The communities of testate amoeba were dominated by representative of the *Amphitremidae*
274 and *Hyalosphenidae*. This community composition is similar to the hummock fauna described
275 by Heal [26, 27] along a fen-bog gradient. The similarities between these surveys are not
276 surprising, and support previous studies in illustrating the cosmopolitan distribution of many
277 peatland testate amoeba morphospecies from the same habitat type [43, 64]. Density is also
278 similar to that reported in other studies on peatlands [20, 44].

279

280 Vertical micro-distribution

281 Testate amoebae reached their highest Shannon diversity and equitability in the intermediate
282 and lower *Sphagnum* segments. The density of some taxa also differed significantly between
283 the two sampling areas in some segments. The NMDS and RDA revealed contrasting vertical
284 patterns of the testate amoeba communities especially in the fen area. *Archerella flavum*,
285 *Heleopera sphagni* and *Hyalosphenia papilio* together represented between 57% (“bog”) and
286 88% (“fen”) of the total community in the upper segments, but much less in the intermediate
287 and lower segments. Thus in agreement with previous studies [25, 34, 35, 39, 54],
288 mixotrophic species largely dominated the community in the upper segments, while
289 heterotrophic species (e.g. *P. griseola* or *Hyalosphenia elegans*) occurred principally in the
290 intermediate and lower segments of *Sphagnum* in both areas.

291 The vertical micro-distribution of testate amoebae in *Sphagnum* reflects some
292 gradients such as light, temperature, oxygen, prey organisms [35, 53]. A vertical niche
293 separation among co-generic or otherwise closely related species also appeared in both
294 sampling areas (e.g. the Amphitematidae *Archerella* and *Amphitrema*, and the
295 Hyalospheniidae *Nebela*, *Hyalosphenia* and *Physochila*). This would support the idea of a
296 competitive exclusion mechanism between closely related species of testate amoebae [44].
297 Mixotrophic species preferentially colonize the uppermost segments of *Sphagnum*, where
298 their endosymbionts can photosynthesize [9, 25, 54]. Testate amoebae also need to find the
299 required material to build their test, and this requirement may be another constraint that
300 determines their vertical micro-distribution [35, 53]. For example, *Amphitrema wrightianum*
301 and *Archerella flavum*, two closely related mixotrophic taxa, have an ecological niche
302 separation along *Sphagnum* segments [25]. *A. flavum* produces a shell composed of self-
303 secreted proteinaceous material whereas *A. wrightianum* uses xenosomes (e.g. organic debris,
304 diatom frustules) [46]. This difference in shell construction explains the different vertical
305 distribution pattern between *A. flavum* (upper segments) and *A. wrightianum* (intermediate
306 segments) in the two sampling areas [43]. The source of material for test construction and the
307 availability of appropriate food thus appear as major regulators of the abundance and the
308 repartition of these species along *Sphagnum* parts [20, 25, 37]. In addition, these different
309 constraints could also be taken into account to explain some species distribution patterns
310 along micro-environmental gradients [43].

311

312 Species-environment correlations

313 Our results agree with earlier studies in identifying the fen/bog gradient as an important factor
314 shaping the structure of testate amoeba communities [5, 27, 28, 34, 37, 38, 63]. Indeed in the
315 “fen” habitat, *A. flavum*, *H. sphagni* and *H. papilio* were found in greatest abundance and

316 marked the ecological transition in *Sphagnum* upper segments. These species are typically
317 found in habitats with high (> 95%) soil water content [7, 30, 63]. Other species such as *N.*
318 *tincta* and *A. muscorum* described as xerophilous [12, 13] were more abundant in the “bog”
319 habitat. Nevertheless, DWT did not emerge as strongly correlated to testate amoeba
320 communities. The DWT gradient (ca. 3 cm) may not have been long enough to emerge as a
321 significant relationship. However other factors, including altitude, temperature, Eh,
322 conductivity, and free phenolics did explain a high proportion of the species data and all of
323 these were significantly different or nearly so between the two areas. Thus although DWT
324 almost always emerges as the strongest variable explaining testate amoeba community
325 structure in *Sphagnum* peatlands [3, 7], other variables become more important when the
326 DWT gradient is short.

327 Direct gradient analysis (RDA) with single explanatory variables revealed the
328 correlations of chemical factors (i.e. Eh and conductivity) with testate amoeba communities in
329 upper and intermediate segments. Water chemistry is known to influence testate amoebae
330 reproduction [25] and to contribute to changes in testate amoeba distribution [30, 42, 48], but
331 generally strongest correlations were reported with pH [41, 43]. Mieczan [39] demonstrated
332 that testate amoeba in the lower section (5-10 cm) were influenced by a combination of
333 chemical and physical factors (DWT and total organic carbon). Chemical factors explained a
334 high proportion of the testate amoeba data in the upper and intermediate segments, and their
335 influence decreased in lower segments. Testate amoebae from the upper segments were most
336 strongly correlated with the physical variables (i.e. altitude and water temperature) while in
337 the lowest segment, of all measured variables only water temperature and altitude were
338 significant. These results illustrate how vertical gradients lead to ecological niche separations
339 in *Sphagnum* peatlands.

340

341 Influence of phenolic compounds on testate amoeba communities

342 *Sphagnum* phenolics quantified in this work were extracted either water (free phenolics) or
343 solvent (bound phenolics) and the two methods yielded different results and patterns: bound
344 phenolics did not differ along the gradient whereas water-soluble phenolics did suggesting
345 that the amount of free phenolics may respond more strongly to micro-environmental
346 conditions (e.g. moisture content of mosses). These results also suggested that different kind
347 of phenolic compounds or phenolic concentrations characterized those extract. The
348 correlation between free phenolics and testate amoeba communities was particularly clear in
349 the upper and intermediate segments that correspond to the depth sampled for total
350 polyphenol analyses (0 – 6 cm). As the upper segment constitutes most of the biomass of
351 *Sphagnum* mosses owing to the weight of the capitulum (top 1 cm), most of the measured
352 phenols are contained in this segment. This may explain that the correlation between testate
353 amoebae and free phenols was highest in the upper segment and was also high in the
354 intermediate segment. We tentatively interpret the fact that no significant correlation was
355 observed between free or bound phenols and testate amoebae in the lower segment as an
356 indication that either the patterns of phenol concentration at that depth is not correlated with
357 that of the upper 6 cm or that the amoebae are more influenced by other aspects of water
358 chemistry closer to the water table. These results clearly call for a detailed analysis of
359 phenolics and testate amoebae at different depth, which could not be done at our site owing to
360 the limited amount of material that could be harvested in this long-term experiment.

361 Among competitive interactions, this study outlines potential chemical interaction
362 between *Sphagnum* and testate amoebae. Recently, phenolic compounds released by
363 *Sphagnum* mosses (e.g. *p*-hydroxyl phenolics) have been shown to possess antibacterial
364 activity [36]. Thus it is possible that free phenolic compounds play a role in testate amoeba
365 assemblages due to their selective positive or negative effects. Although results do not allow

366 to drawing any conclusions on a possible direct (e.g. physiological) and/or indirect (e.g.
367 through impact on prey organisms) effect of phenolics on testate amoeba communities, they
368 raise the issue of the possible role of such compounds. An experimental approach to test such
369 effects is necessary.

370

371 Conclusions

372 In this study we explored the community patterns and species-environment relationships of
373 testate amoebae living in *Sphagnum fallax* along a narrow ecological gradient from a poor fen
374 (homogeneous *Sphagnum* carpet) to a “young bog” (mosaic of poor fen and bog microsites).
375 In agreement with our hypotheses we observed differences between the two sampled habitats
376 and a vertical stratification of communities. These results illustrate how strongly microbial
377 communities respond even to short ecological gradients in *Sphagnum*-dominated peatlands.
378 The analysis of testate amoebae from three *Sphagnum* segments allowed us to explore the
379 detailed patterns of species-environment relationships at the time of sampling and showed
380 that slight environmental variations (e.g. altitude and related variables) are significant at the
381 microbial level. This study therefore confirmed that testate amoebae are sensitive to
382 environmental gradients at a very fine scale [40]. The importance of temporal patterns also
383 would deserve more attention. Indeed, the location and size of different microhabitats and
384 related communities in *Sphagnum* peatlands are not stable over time [8] and this is clearly
385 also true for testate amoeba assemblages as attested by the limited existing data on seasonal
386 patterns [62] as well as the changes documented in numerous palaeoecological records [10].
387 Understanding environmental controls on testate amoebae communities at these finer spatial
388 and temporal scales is key to improving our ability to interpret the high-resolution fossil
389 testate amoeba records in peatlands that is starting to being produced [31]. This will require

390 both further detailed descriptive studies as well as manipulative experiments using biotic
391 (phenols) and abiotic data and aiming to determine which factors influence testate amoebae
392 and what the mechanisms are.

393

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402

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574 **Tables**

575

576 Table 1. Environmental variables measured in the “fen” and “bog” sampling areas in Le
577 Forbonnet mire (French Jura) (n = 12, average ± S.E).

578

579 Table 2. Non-parametric correlation matrix of measured environmental variables along the
580 “fen”/”bog” transition of Le Forbonnet mire.

581

582 Table 3. RV-coefficients (below diagonal) and corresponding *P*-values (above diagonal)
583 among the six groups of variables used in the MFA of the Forbonnet peatland. Significant
584 coefficients appear in bold.

585

586 Table 4. Summary of RDA on testate amoebae and environmental variables from Le
587 Forbonnet mire (France): fraction of variance explained and significance of individual
588 variables taken alone.

589

590 Table 5. Summary RDA and variance partitioning on testate amoebae and environmental
591 variables data from Le Forbonnet mire (France).

592

593 **Figures**

594 Figure 1. (a) The two primary axes of the 3-dimensional NMDS ordination of testate amoebae
595 communities in the “bog” area from Le Forbonnet mire (France) (n = 18, final stress = 4.1).
596 The solution represents 75% of the variability in the data, with axes 1, 2 and 3 representing
597 respectively 43%, 18% and 13%. Samples are coded by sampling area with open symbols. (b)
598 The two primary axes of the 3-dimensional NMDS ordination of testate amoebae
599 communities in the “fen” area (n = 18, final stress = 2.4). The solution represents 84% of the
600 variability in the data, with axes 1, 2 and 3 representing respectively 55%, 19% and 10%.
601 Samples are coded by sampling area with filled symbols.

602

603 Figure 2. Distribution maps of total testate amoeba abundance and of dominant testate
604 amoeba taxa in *Sphagnum fallax* from the two sampling areas in Le Forbonnet mire (France).
605 A = upper (0-3cm) B = intermediate (3-6cm) and C = lower (6-9cm) segments. X and Y axes
606 correspond to GPS data converted into Lambert 2 references. Dot sizes are directly
607 proportional to the number of individuals per gram DW in the samples and are comparable
608 among maps.

609

610 Figure 3. Multiple factor analysis of the three testate amoeba communities (Hellinger-
611 transformed) and environmental (chemical, physical and phenolics) data sets from the
612 Forbonnet peatland. Projection of the MFA axes 1 and 2 with the result of a hierarchical
613 agglomerative clustering (grey lines), obtained by the Ward method on the Euclidean distance
614 matrix between MFA site scores, showing two main groups of sampling plots (open symbols
615 = “fen”, filled symbols = “bog”). Sampling plots are indicated by F (“fen”) or B (“bog”)
616 followed by a number.

617 Figure 4. Redundancy analyses biplots (axes 1 and 2) of testate amoeba data from Le
618 Forbonnet mire (France) in upper (a), intermediate (b) and lower (c) *Sphagnum* segments, and
619 the overall data set (d). Sampling areas are coded with open symbol for the “fen” area and
620 with filled symbol for the “bog” area. Samples are indicated as follows: circles = upper
621 segments, squares = intermediate segments, triangles = lower segments. F_phe : free
622 phenolics; B_phe : bound phenolics; W-temp: water temperature; Alt: average altitude
623 (microtopography) of the sampled plot; Kcorr: conductivity.

624

625 **Supplementary Online Material**

626

627 Supplementary Figure 1. Location of the Forbonnet peatland with inset showing the location
628 of the sampling areas.

629

630 Supplementary Figure 2. Vertical micro-distribution of selected testate amoeba taxa in the
631 two sampling areas (average \pm S.E) (circles: “bog” area; triangles: “fen” area). Asterisks
632 indicate significant differences between the sampling areas ($P < 0.05$). Different letters
633 indicate significant differences among *Sphagnum* sections ($P < 0.05$).