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Fine-Scale Horizontal and Vertical Micro-distribution Patterns of Testate Amoebae Along a Narrow Fen/Bog Gradient

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

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Introduction

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

While the relationship between testate amoebae and DWT and a few other variables such as pH are well documented along broad ecological gradient (e.g., wet

pools to dry hummocks, fen to bog) [26, 47], much less is known on their finer-scale responses to micro-environmental gradients. Some data suggests that testate amoebae may be highly sensitive even to subtle micro-environmental gradients. For example, Mitchell et al. [40] studied the horizontal distribution patterns of testate amoeba communities in a 40×60 cm almost flat mono-specific *Sphagnum* lawn and found spatial heterogeneity in the communities that was significantly correlated to altitude (micro-topography; despite a very short—ca. 6 cm—elevation gradient). Assessing testate amoeba species-environment correlation along fine-scale environmental gradients is necessary to define the practical limits (i.e., the resolution) of their use as bioindicators in ecological and palaeoecological studies.

Another open question is the range of abiotic and biotic factors to which testate amoebae respond. Although many variables have been studied, DWT almost always emerges as the strongest variable despite the fact that testate amoebae are unlikely to be directly influenced by the position of the water table 10 or 30 cm below the level where they live [41]. Still some important potential factors have not yet been studied including peat and water chemistry beyond simple ions and elements. *Sphagnum* peatlands are indeed generally characterized by gradients such as nutrients (nutrient-poor ombrotrophic bogs vs. rich fens), hydrology (wet hollow vs. dry hummocks), and acidity [14, 22, 23, 52].

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

The aims of this study are to explore (1) the species-environment relationships and (2) vertical micro-distribution patterns of testate amoebae along a short ecological gradient from a *Sphagnum*-dominated poor fen (for simplicity hereafter referred to here as "fen") and a vegetation with mixed bog and poor fen plant elements and a more marked micro-topography (hereafter referred to as "bog"). Rather than sampling contrasted microhabitats or moss species, we sampled only within macroscopically homogenous and similar *Sphagnum fallax* carpets across the gradient. We assessed (1) how horizontal and vertical patterns of testate amoebae community structure varied along the gradient and (2) the relationships between the testate amoeba communities and DWT, water chemistry, and phenolic compound content. We hypothesized (1) that the vertical patterns of community structure would be more marked in the structurally more complex mixed *Sphagnum* "bog" habitat than in the more uniform poor fen, despite the fact that the sampled habitats were macroscopically identical and, (2) that phenolic compounds would explain a similar fraction of the community data structure as other more commonly studied environmental factors (i.e., altitude, DWT, and water chemistry).

Methods

Sampling and Laboratory Analyses

The study site was an undisturbed ombrotrophic *Sphagnum*-dominated mire [2] situated in the Jura Mountains (The Forbonnet peatland, France, 46°49′35′N, 6°10′20′E) at an altitude of 840 m above sea level (Electronic Supplementary Fig. 1). Cold winters (on average of −1.4°C) and mild summers (on average of 14.6°C) characterized the climate of the site. The annual mean temperature measured at the site over a 1 year period from the 5th November 2008 to 30th November 2009 was 6.5°C, and the annual precipitations were 1,200 mm.

Samples of *S. fallax* were collected from two adjacent areas (ca. 10×12 m) selected in relation to their micro-topography, vegetation and assessment of sources and decay of organic matter [15]. The first sampling area (coded "fen") is a transitional *Sphagnum*-dominated poor fen area, relatively flat and homogeneous, characterized by a moss cover dominated by *S. fallax* and by the lack of *Sphagnum magellanicum*. Vascular plants as *Eriophorum vaginatum, Vaccinum oxycoccus*, and *Andromeda polifolia* were recorded in very low abundance. *Scheuchzeria palustris* and *Carex limosa* occurred outside of the studied plots. The second sampling area (coded "bog") is an open bog area with mixed vegetation, directly adjacent to the fen area. Patterns of hummocks with *S. magellanicum, V. oxycoccos*, *E. vaginatum, C. rostrata*,

and *Calluna vulgaris,* and hollows with lawns of *S. fallax* and *A. polifolia* characterized the sampling area. The terms "fen" and "bog" are used here for simplicity and to denote the existence of a trophic gradient inferred from the vegetation. However, the "bog" sub-site represents a mosaic of poor fen (lawns, hollows) and bog (hummock) vegetation.

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

Primarily bound (hereafter "bound") and water-soluble phenolic (hereafter "free") compounds were extracted and quantified from lyophilized mosses. The green section (0–6 cm; 0 being defined as the top of the capitulum) was used for these analyses, excluding the lower part where the mosses start to decay. Two methods were used to extract phenolic compounds from *Sphagnum*. For free phenolics, 0.05 g dry weight (DW) of *Sphagnum* was ground in a mortar, mixed with 10 mL distilled water, bubbled with nitrogen, and agitated on a reciprocal shaker (15 rpm) for 3 h and filtered. For bound phenolic compounds, 0.05 g DW of *Sphagnum* was ground in a mortar, mixed with 25 mL ethanol/distilled water (80/20 *v*/*v*) and warmed under reflux at 120°C for 30 min. This extract was filtered and evaporated by using a rotary evaporator. Finally, the dry extract was dissolved in 25 mL of boiling distilled water (adapted from Gallet and Lebreton [19]). The free and bound total phenolic contents were quantified with the Folin–Ciocalteau reagent and were expressed in mg equivalent gallic acid (A_{760}).

For testate amoeba analysis, the *S. fallax* samples were cut in three levels (sampling depth): 0–3 cm (upper), 3–6 cm (intermediate), and 6–9 cm (lower). The samples were fixed with 20 mL glutaraldehyde (2% final concentration) and stored at 4°C in the dark. Testate amoebae were extracted from mosses using the following extraction method [45]: each sample was shaken for 1 min on a vortex and then pressed to extract microorganisms (first solution). The mosses were then soaked again with 20 mL of glutaraldehyde (2%), shaken a second time on a vortex and pressed to extract *Sphagnum* leachate. The leachate was left to settle for 12 h, after which the supernatant was added to *Sphagnum* and the bottom to the first solution. The process was repeated six times, and all fractions were combined to obtain a final composite sample of 40 mL. The remaining fraction was dried at 80°C for 48 h and weighted to express testate amoeba density by gram DW of *Sphagnum*. The testate amoebae were identified and counted to a total of 150 at ×200 and ×400 magnification by inverted microscopy (OLYMPUS IX71) following Uthermöhl's method [60]. Testate amoebae were identified to the species level whenever possible. Only living amoebae (active only, encysted individuals were not included) were counted.

Numerical Analyses

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

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

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

Multiple factor analysis (MFA) was used to assess the general structure of the data and to determine the relationships among the three Hellinger-transformed testate amoeba data sets and the three environmental variables data sets (chemical, physical, and phenolics) [17]. MFA was performed in two steps. Firstly, a PCA was performed on

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

Sampling area	pH	Eh (mV)	Kcorr (µS/cm)	W-temp (°C)	DWT (cm)[a]	Altitude (m)	Bound phenolics (mg/g DW)	Free phenolics (mg/g DW)
Bog								
Mean	3.57±0.1	255±20.1	38.4±10.5	15.2±0.1	18.6±0.6	0.82±0.01	1.5±0.1	1.3±0.1
Min	3.31	177	5.6	15.0	16.3	0.78	1.20	1.1
Max	3.90	333	79.3	15.4	18.8	0.88	1.83	1.44
Fen								
Mean	3.84±0.1	338±19.0	12.6±1.6	16.5±0.5	16.3±4.1	0.75±0.03	1.4±0.2	1.1±0.0
Min	3.77	304	8.1	16.2	11.0	0.69	1.22	0.93
Max	3.95	356	19.7	17.4	19.3	0.81	1.84	1.2
P value[b]	0.15	0.03	0.06	0.03	0.21	0.03	0.56	0.03

[a] Average daily measurments over a period of 1 month

[b] Difference bog/fen (Wilcoxon test)

each subset, which was then normalized by dividing all its elements by the first eigenvalue obtained from its PCA. Secondly, the normalized subsets were assembled to form a unique matrix and a second PCA was performed on this matrix. RV-coefficient (ranging from 0 to 1) was used to measure the similarity between the geometrical representations derived from each groups of variables [51]. RV-coefficients are then tested by permutations [29]. Euclidean distances of global PCA were used in MFA to perform cluster analysis according to the Ward method, and the resulting dendrogram was projected in the MFA ordination space. This analysis revealed the main differences in the structure of the data described by all biotic and abiotic subsets of variables.

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

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

Table 2 Non-parametric correlation matrix of measured environmental variables along the "fen"/"bog" transition of Le Forbonnet mire

	Bound phenolics	Free phenolics
pH	0.16	−0.31
Eh	−0.22	−0.62*
Kcorr	0.20	0.37
W-temp	−0.24	−0.72*
DWT	0.02	0.10
Altitude	0.17	0.46

*Corrections significant at the P<0.01

Results

Environmental Variables

The range of values for the eight measured environmental variables, minimum, maximum and averages for the "fen" and "bog" areas are given in Table 1. The Eh potential and water temperature were significantly higher in the "fen" area while altitude and free phenols were significantly higher in the "bog" area (P<0.05). Water pH, conductivity, DWT, and the concentration of slightly bound phenolic compounds did not differ significantly between the two areas. All environmental variables, except Kcorr, pH, DWT, and altitude, were significantly correlated to free phenolics (Table 2) while no environmental variables were significantly correlated to primarily bound phenolics.

Testate Amoeba Density and Diversity

The total density of testate amoebae increased significantly with depth in the "bog" area from 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} DW in the intermediate and lower segments (P<0.05). By contrast, there was no significant difference with depth in the

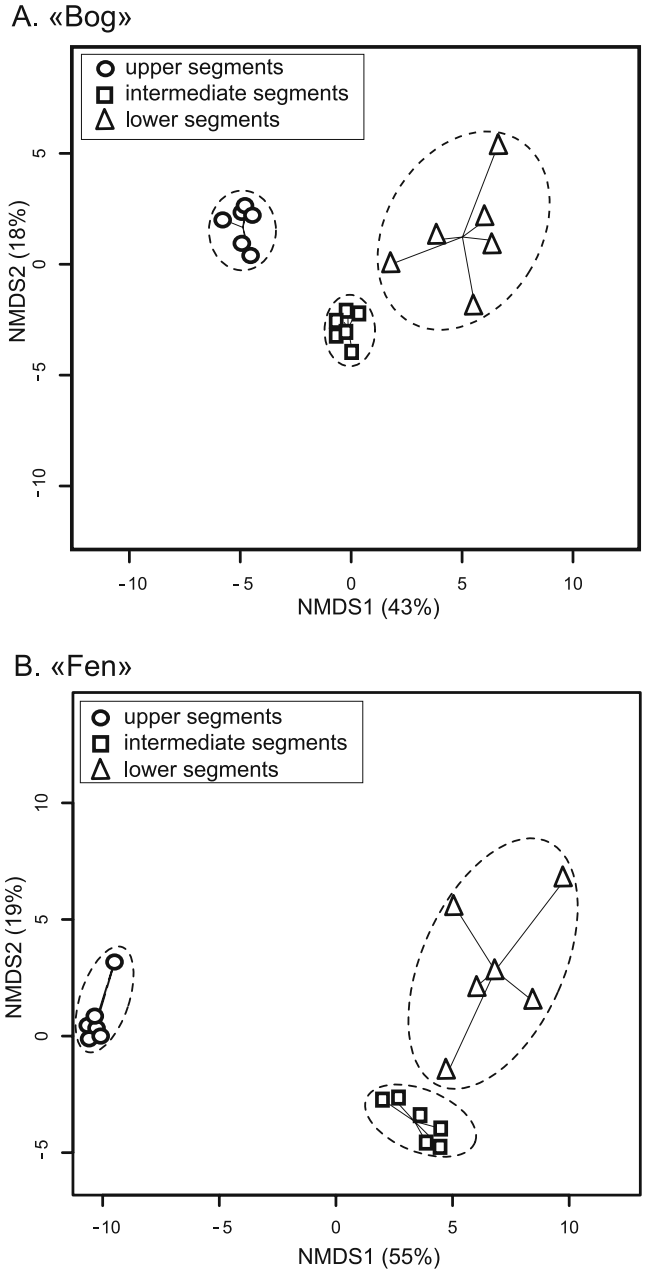

Figure 1 **a** The two primary axes of the three-dimensional NMDS ordination of testate amoebae communities in the "bog" area from Le Forbonnet mire (France; n=18, final stress=4.1). The solution represents 75% of the variability in the data, with axes 1, 2, and 3 representing, respectively, 43%, 18%,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

"fen" area (average density over the three depths, 4.34×10^4 ind.g^{-1} DW).

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

Vertical Micro-distribution

The NMDS ordination of samples from the two sampling areas showed that testate amoeba communities differed significantly along *Sphagnum* segments in the two sampling areas (Fig. 1; $P<0.001$). In the "fen" area the upper segment was clearly different from the intermediate and lower segments, while in the "bog" area this difference was less marked.

In the "fen" area, the most abundant taxa in the upper segments were *Archerella flavum* (on average 2.2×10^4 ind.g^{-1} DW) and *Hyalosphenia papilio* (on average 1.5×10^4 ind.g^{-1} DW) (Fig. 2 and Electronic Supplementary Fig. 2). The intermediate segments were characterized by an increased of the abundance of *Hyalosphenia elegans* (on average of 8.3×10^4 ind.g^{-1} DW), *Nebela tincta*, and *Physochila griseola* (both on average 3.5×10^4 ind.g^{-1} DW), and a significant decrease in the abundance of *A. flavum* and *H. papilio*. The lower segments were characterized by the highest abundance of *P. griseola* (on average 1.07×10^4 ind.g^{-1} DW) and *H. elegans* (on average 6.5×10^4 ind.g^{-1} DW).

In the "bog" area, the most abundant taxa in the upper segments were also *A. flavum* (on average 1.22×10^4 ind.g^{-1} DW), *N. tincta* (on average 3.8×10^4 ind.g^{-1} DW), *H. papilio* (on average 3.5×10^4 ind.g^{-1} DW), and *Assulina muscorum* (on average 8×10^4 ind.g^{-1} DW) (Fig. 2 and Electronic Supplementary Fig. 2). The intermediate segments were characterized by significantly higher densities of *H. elegans* (on average 1.18×10^4 ind.g^{-1} DW), *N. tincta* (on average 1.0×10^4 ind.g^{-1} DW), *Amphitrema wrightianum* (on average 9.7×10^4 ind.g^{-1} DW) and *P. griseola* (on average 7.0×10^4 ind.g^{-1} DW) and lower density of *H. papilio*. In the lower segments, the most abundant taxa were *P. griseola* (on average 2.4×10^4 ind.g^{-1} DW) and *N. tincta* (on average 9.0×10^4 ind.g^{-1} DW).

Species-Environment Correlations

The MFA of the three environmental matrices and the three testate amoeba data sets confirmed the existence of an overall division between "fen" and "bog" areas (Fig. 3).

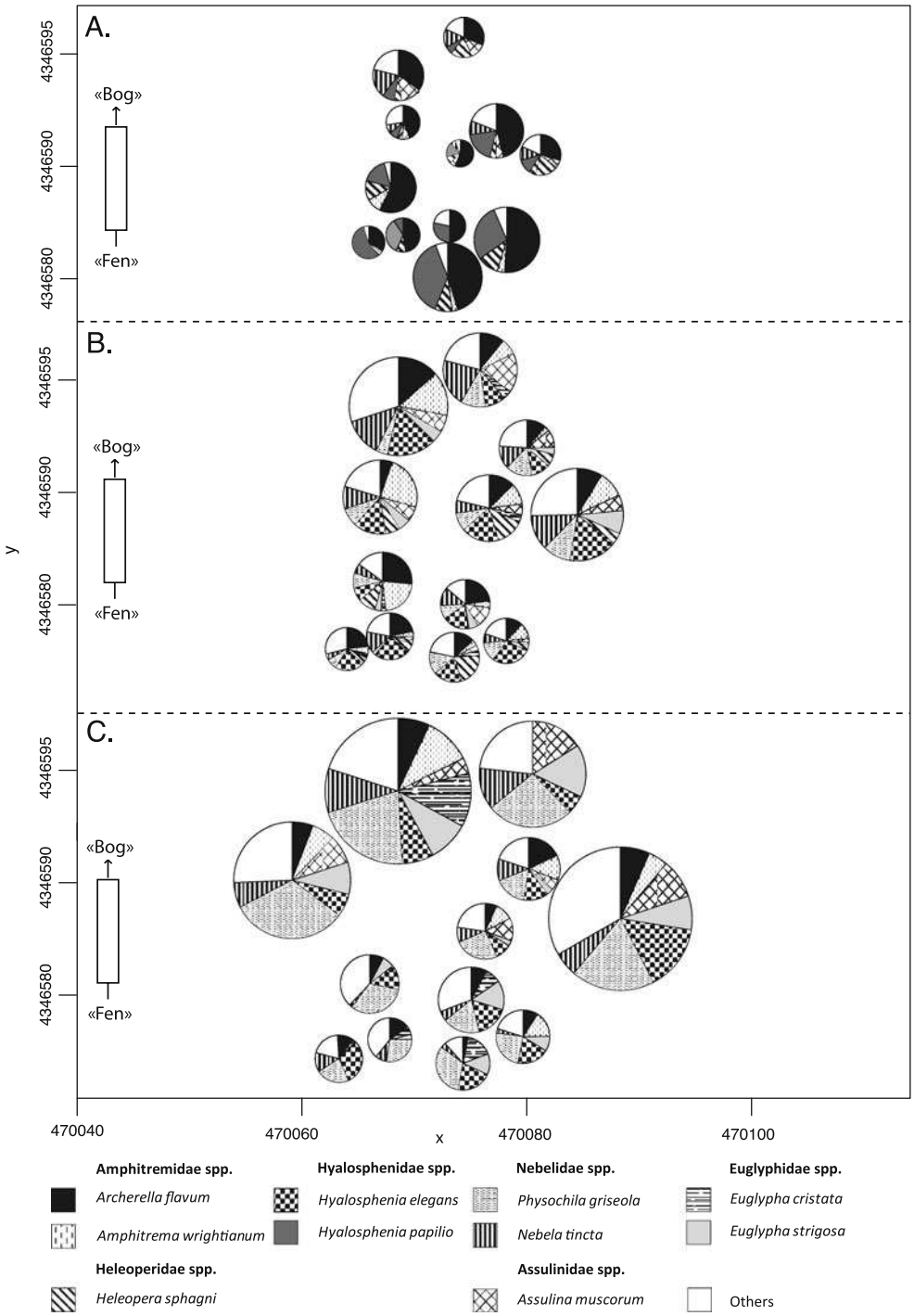

Figure 2 Distribution maps of total testate amoeba abundance and of dominant testate amoeba taxa in *Sphagnum fallax* from the two sampling areas in Le Forbonnet mire (France). *A* Upper (0–3 cm), *B* intermediate (3–6 cm), and *C* lower (6–9 cm) segments. *x*- and *y*-axes correspond to GPS data converted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

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

In the RDA ordinations (Fig. 4a, b, c, and d), the two areas were clearly separated in the overall analysis as well as for each of the three sampling depths. The model explained 51.8% (adjusted R^2) of the variability in testate amoeba data in the overall analysis and 27.5%, 52.7%, and 41.9% (adjusted R^2) of the variability in the data for the upper, intermediate and lower sections respectively. In the overall RDA, testate amoeba communities in the "fen" area were related to higher values of Eh, pH, and W-temp, while

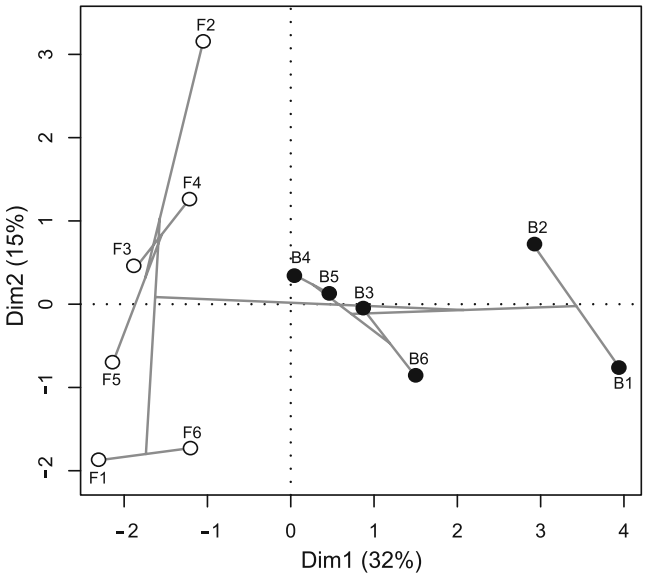

Figure 3 Multiple factor analysis of the three testate amoeba communities (Hellinger-transformed) and environmental (chemical, physical, and phenolics) data sets from the Forbonnet peatland. Projection of the MFA axes 1 and 2 with the result of a hierarchical agglomerative clustering (*grey lines*),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

testate amoeba communities in the "bog" area were related to higher values of phenolics, altitude and conductivity (Fig. 4a, b, c, and d).

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

The partial RDAs showed that chemical, physical and phenolic data sets each significantly explained, independently of the other two data sets, about 7% of the species data variance (P=0.02–0.08) in the overall RDA. The proportion of variance explained by these data was however much higher in the upper two segments (16.5–34.1%) but on average lower in the third segment where no significant correlation was found for the lower segment (Table 5).

Discussion

Testate Amoeba Density and Diversity

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

Vertical Micro-distribution

Testate amoebae reached their highest Shannon diversity and equitability in the intermediate and lower *Sphagnum* segments. The density of some taxa also differed significantly between the two sampling areas in some segments. The NMDS and RDA revealed contrasting vertical patterns of the testate amoeba communities especially in the fen area. *A. flavum*, *Heleopera sphagni*, and *H. papilio* together represented between 57% ("bog") and 88% ("fen") of the total community in the upper segments, but much less in the intermediate and lower segments. Thus in agreement with previous studies [25, 34, 35, 39, 54], mixotrophic species largely dominated the community in the upper segments, while heterotrophic species (e.g., *P. griseola* or

Table 3 RV-coefficients (below diagonal) and corresponding *P* values (above diagonal) among the six groups of variables used in the MFA of the Forbonnet peatland

	Upper segments	Intermediate segments	Lower segements	Chemical	Physical	Phenolic compounds
Upper segments	1.00	0.001	0.10	0.01	0.22	0.07
Intermediate segments	*0.78*	1.00	0.16	0.02	0.32	0.02
Lower segements	0.45	0.55	1.00	0.09	0.31	0.56
Chemical	*0.51*	*0.43*	0.34	1.00	0.57	0.14
Physical	0.21	0.24	0.26	0.05	1.00	0.82
Phenolic compounds	0.34	*0.45*	0.23	0.21	0.02	1.00

Significant coefficients are italicized

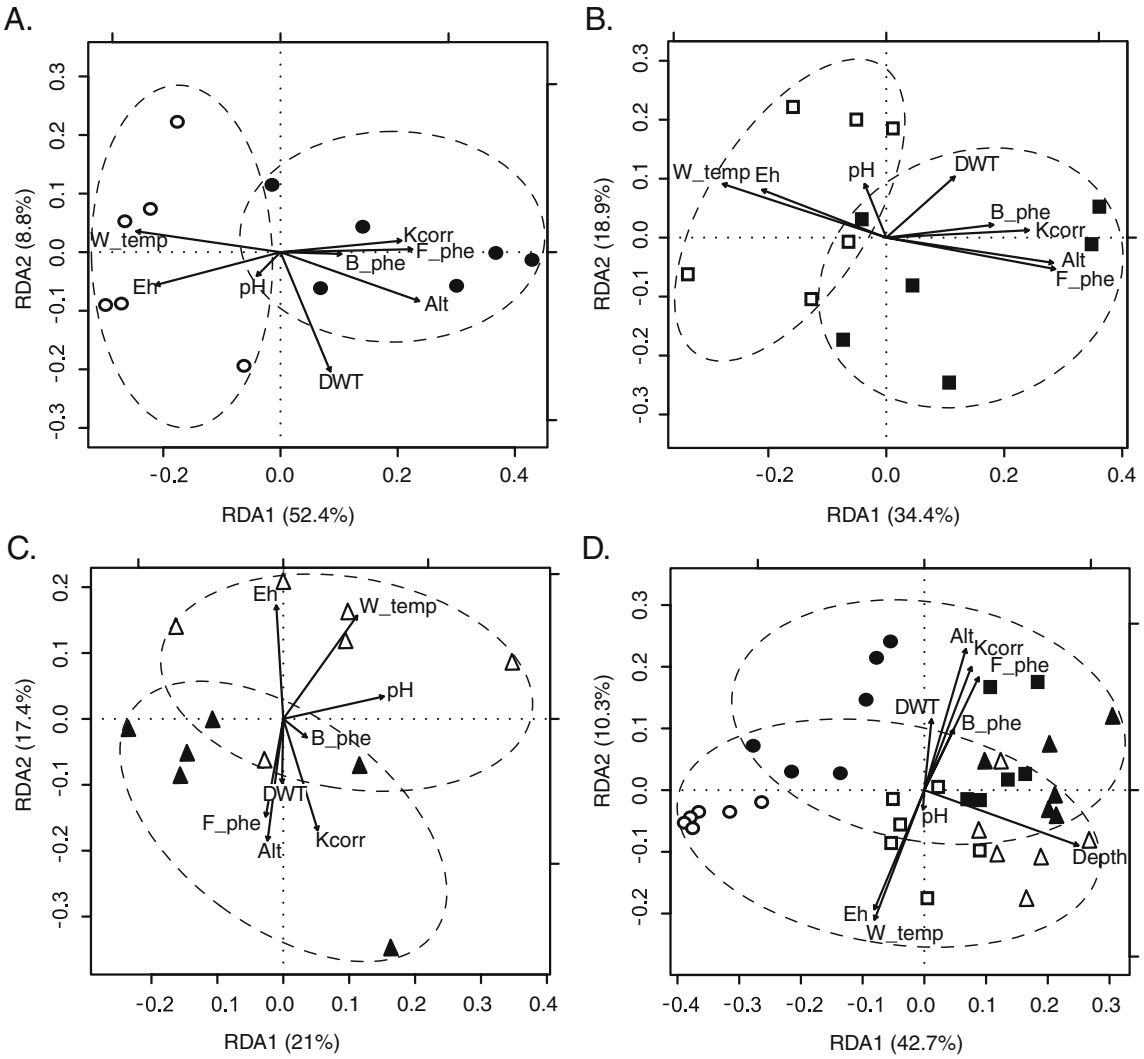

Figure 4 Redundancy analyses biplots (axes 1 and 2) of testate amoeba data from Le Forbonnet mire (France) in upper (**a**), intermediate (**b**), and lower (**c**) *Sphagnum* segments, and the overall data set (**d**). Sampling areas are coded with *open symbol* for the "fen" area and with filled symbol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

H. elegans) occurred principally in the intermediate and lower segments of *Sphagnum* in both areas.

The vertical micro-distribution of testate amoebae in *Sphagnum* reflects some gradients such as light, temperature, oxygen, and prey organisms [35, 53]. A vertical niche separation among co-generic or otherwise closely related species also appeared in both sampling areas (e.g., the Amphitrematidae *Archerella* and *Amphitrema*, and the Hyalospheniidae *Nebela, Hyalosphenia*, and *Physochila*). This would support the idea of a competitive exclusion mechanism between closely related species of testate amoebae [44]. Mixotrophic species preferentially colonize the uppermost segments of *Sphagnum*, where their endosymbionts can photosynthesize [9, 25, 54]. Testate amoebae also need to find the required material to build their test, and this requirement may be another constraint that determines their vertical micro-distribution [35, 53]. For example, *A. wrightianum* and *A. flavum,* two closely related mixotrophic taxa, have an ecological niche separation along *Sphagnum* segments [25]. *A. flavum* produces a shell composed of self-secreted proteinaceous material whereas *A. wrightianum* uses xenosomes (e.g., organic debris, diatom frustules) [46]. This difference in shell construction explains the different vertical distribution pattern between *A. flavum* (upper segments) and *A. wrightianum* (intermediate segments) in the two sampling areas [43]. The source of material for test construction and the availability of appropriate food thus appear as major regulators of the abundance and the repartition of these species along *Sphagnum* parts [20, 21, 25, 37]. In addition, these different

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

Variables	Overall RDA		Bog		Fen		Upper		Intermediate		Lower	
	%[a]	*P* value	%[a]	*P* value	%[a]	*P* value	%[a]	*P* value	%[a]	*P* value	%[a]	*P* value
Eh	7.0	0.01	1.5	0.20	0.7	0.85	23.8	0.02	10.7	0.002	5.3	0.09
Kcorr	7.0	0.01	5.1	0.10	0.3	0.77	20.9	0.01	12.4	0.02	4.9	0.11
pH	0.6	0.24	0.4	0.31	0.2	0.38	2.0	0.25	2.5	0.24	2.4	0.25
DWT[b]	0.3	0.34	0.0	0.65	5.7	0.84	1.8	0.26	0.3	0.40	1.1	0.33
W-Temp	8.1	0.005	0.4	0.44	5.7	0.84	33.6	0.005	16.2	0.01	7.8	0.03
Altitude	7.6	0.02	7.0	0.06	0.1	0.98	30.9	0.005	15.8	0.005	7.7	0.03
Bound phenolics	1.4	0.13	2.1	0.27	2.0	0.98	0.1	0.32	5.4	0.11	0.1	0.97
Free phenolics	7.0	0.02	3.2	0.16	4.4	0.79	27.1	0.02	16.9	0.005	2.3	0.25
Fen/Bog (0/1)	8.8	0.01	n.a.	n.a.	n.a.	n.a.	38.4	0.005	14.5	0.05	8.3	0.01
Upper (0/1)	36.3	0.01	35.4	0.004	47.6	0.008	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Intermediate (0/1)	4.9	0.01	3.5	0.01	3.7	0.008	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Lower (0/1)	19.9	0.01	16.3	0.001	26.9	0.007	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sample depth (1–3)	35.8	0.001	33.3	0.005	52.0	0.05	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a] Percentage of variance explained (adjusted R^2)

[b] Depth to water table

constraints could also be taken into account to explain some species distribution patterns along micro-environmental gradients [43].

Species-Environment Correlations

Our results agree with earlier studies in identifying the fen/bog gradient as an important factor shaping the structure of testate amoeba communities [5, 27, 28, 34, 37, 38, 63]. Indeed in the "fen" habitat, *A. flavum*, *H. sphagni*, and *H. papilio* were found in greatest abundance and marked the ecological transition in *Sphagnum* upper segments. These species are typically found in habitats with high (>95%) soil water content [7, 30, 63]. Other species such as *N. tincta* and *A. muscorum* described as xerophilous [12, 13] were more abundant in the "bog" habitat. Nevertheless, DWT did not emerge as strongly correlated to testate amoeba communities. The DWT gradient (ca. 3 cm) may not have been long enough to emerge as a significant relationship. However other factors, including altitude, temperature, Eh, conductivity, and free phenolics did explain a high proportion of the species data and all of these were significantly different or nearly so between the two areas. Thus although DWT almost always emerges as the strongest variable explaining testate amoeba community structure in *Sphagnum* peatlands [3, 7], other variables become more important when the DWT gradient is short.

Direct gradient analysis (RDA) with single explanatory variables revealed the correlations of chemical factors (i.e., Eh and conductivity) with testate amoeba communities in upper and intermediate segments. Water chemistry is known to influence testate amoebae reproduction [25] and to contribute to changes in testate amoeba distribution [30, 42, 48], but generally strongest correlations were reported

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

Variable RDA model	Covariables	Overall RDA		Upper		Intermediate		Lower	
		%[a]	*P* value	%[a]	*P* value	%[a]	*P* value	%[a]	*P* value
Chemical factors (Eh, pH and Kcorr)	DWT, altitude, W-temp phenolic compounds	5.2	0.08	16.8	0.09	18.4	0.02	3.9	0.28
Physical factors (DWT[b], altitude and W-temp)	Eh, pH, Kcorr phenolic compounds	7.9	0.07	34.1	0.002	16.5	0.01	8.1	0.08
Phenolic compounds (free and bounds phenolics)	Eh, pH, Kcorr DWT, altitude, W-temp	6.6	0.02	25.6	0.01	14.8	0.03	6.2	0.14

[a] Percentage of variance explained (adjusted R^2)

[b] Depth to water table

with pH [41, 43]. Mieczan [39] demonstrated that testate amoeba in the lower section (5–10 cm) were influenced by a combination of chemical and physical factors (DWT and total organic carbon). Chemical factors explained a high proportion of the testate amoeba data in the upper and intermediate segments, and their influence decreased in lower segments. Testate amoebae from the upper segments were most strongly correlated with the physical variables (i.e., altitude and water temperature) while in the lowest segment, of all measured variables only water temperature and altitude were significant. These results illustrate how vertical gradients lead to ecological niche separations in *Sphagnum* peatlands.

Influence of Phenolic Compounds on Testate Amoeba Communities

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

Among competitive interactions, this study outlines potential chemical interaction between *Sphagnum* and testate amoebae. Recently, phenolic compounds released by *Sphagnum* mosses (e.g., *p*-hydroxyl phenolics) have been shown to possess antibacterial activity [36].Thus, it is possible that free phenolic compounds play a role in testate amoeba assemblages due to their selective positive or negative effects. Although results do not allow to drawing any conclusions on a possible direct (e.g., physiological) and/or indirect (e.g., through impact on prey organisms) effect of phenolics on testate amoeba communities, they raise the issue of the possible role of such compounds. An experimental approach to test such effects is necessary.

Conclusions

In this study we explored the community patterns and species-environment relationships of testate amoebae living in *Sphagnum fallax* along a narrow ecological gradient from a poor fen (homogeneous *Sphagnum* carpet) to a "young bog" (mosaic of poor fen and bog micro-sites). In agreement with our hypotheses we observed differences between the two sampled habitats and a vertical stratification of communities. These results illustrate how strongly microbial communities respond even to short ecological gradients in *Sphagnum*-dominated peatlands. The analysis of testate amoebae from three *Sphagnum* segments allowed us to explore the detailed patterns of species-environment relationships at the time of sampling and showed that slight environmental variations (e.g., altitude and related variables) are significant at the microbial level. This study therefore confirmed that testate amoebae are sensitive to environmental gradients at a very fine scale [40]. The importance of temporal patterns also would deserve more attention. Indeed, the location and size of different microhabitats and related communities in *Sphagnum* peatlands are not stable over time [8] and this is clearly also true for testate amoeba assemblages as attested by the limited existing data on seasonal patterns [62] as well as the changes documented in numerous palaeoecological records [10]. Understanding environmental controls on testate amoebae communities at these finer spatial and temporal scales is key to improving our ability to interpret the high-resolution fossil testate amoeba records in peatlands that is starting to being produced [31]. This will require both further detailed descriptive studies as well as manipulative experiments using biotic (phenols) and abiotic data and aiming to determine which factors influence testate amoebae and what the mechanisms are.

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