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Vertical Micro-Distribution and Response to Nitrogen Deposition of Testate Amoebae in *Sphagnum*

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ABSTRACT. Previous studies have shown the existence of a vertical micro-distribution of testate amoebae in the first centimeters of *Sphagnum* and their response to nutrient enrichment. In order to test the response of testate amoebae to depth and N addition in dry moss carpets recolonizing cutover peatlands, we sampled *Sphagnum* that had received 0, 1, 3, or 10g N m^{-2} yr^{-1} for three years. The mosses were cut into three segments: 0–1cm, 1–3cm and 3–5cm and analyzed for testate amoebae. The overall diversity (22 taxa) was high considering the dryness of the site, but the species richness of individual samples was low (mean 6.6). The presence of several species characteristic of wetter conditions suggests that they have a broader tolerance than usually believed and/or have a high colonization potential. Species richness increased with depth. *Assulina muscorum* was most abundant in the top segment, while *Phryganella acropodia*, *Heleopera rosea* and *Nebela militaris* were most abundant in the deepest segment. Neither the metabolism type nor the shell characteristics significantly explained the vertical distribution of species. There was no overall response of testate amoebae to N, although one species, *Bullinularia indica*, was significantly more abundant in the fertilized than in the control plots.

Key Words. Atmospheric nitrogen pollution, community composition, micro-environmental gradients, peatland, protist ecology, protozoa, testaceans, vertical gradient.

TESTATE amoebae are a group of free-living protists that inhabit aquatic to temporarily moist habitats, ranging from lakes to soils. These amoebae are especially numerous and diverse in *Sphagnum* mosses (Meisterfeld 1978; Warner 1987) where they have been shown to be related to water availability and water chemistry (Booth 2001; Charman 1997; Charman and Warner 1992; Heal 1962; Lousier 1974; Mitchell et al. 1999; Tolonen, Warner, and Vasander 1992). In *Sphagnum*-dominated peatlands, testate amoebae are of special interest for ecologists and paleoecologists because of their relative ease of identification, their bioindicator value for hydrology and pH, and their use in the reconstruction of paleoenvironments due to the long-term preservation of their shells in lake and peat deposits (Charman 2001).

In addition to responding to broad-scale environmental gradients, such as the transition from bog to fen, testate amoebae and other soil microorganisms are also sensitive to finer-scale spatial gradients (Ettema and Wardle 2002), even when these are not macroscopically obvious (Mitchell et al. 2000a). Communities of testate amoebae also vary with depth within the first few centimeters of *Sphagnum* mosses: Mixotrophic species tend to be more abundant in the upper (green) parts of the mosses and overall diversity increases with depth in the first few centimeters (Bonnet 1958; Booth 2002; Chacharonis 1954, 1956; Heal 1962, 1964; Meisterfeld 1977; Schönborn 1963). However, the existence of this vertical gradient has not yet been demonstrated in disturbed peatlands, which, in central Europe often represent a major proportion of the total surface covered by peatlands (Joosten and Clarke 2002). In peatlands, testate amoebae respond primarily to the moisture gradient and the communities that occur in *Sphagnum* mosses in relatively dry forested peatlands with no peat accumulation (Harnisch's ''Waldmoos-Typ'') (1927) have been shown to be dominated by ubiquitous taxa such as *Assulina muscorum* and *Corythion dubium* and lacking many characteristic species of pristine mires such as *Amphitrema* spp., and large *Nebela* species (Mitchell et al. 1999).

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In the present study, our first goal was therefore to examine the community composition and vertical micro-distribution of testate amoebae in *Sphagnum* mosses from a moss carpet dominated by *Polytrichum strictum* Brid. on a cutover *Sphagnum* peat bog in the Swiss Jura Mountains. We first hypothesized (H1) that the communities of testate amoebae colonizing these dry moss carpets would be of low diversity and would be more similar to communities found in acidic forest mosses than to communities characteristic of natural and wet *Sphagnum* peatlands. The vertical distribution of testate amoebae documented in moister *Sphagnum* (Bonnet 1958; Booth 2002; Chacharonis 1954, 1956; Heal 1962, 1964; Meisterfeld 1977; Schönborn 1963) led us to hypothesize (H2) that testate amoebae would also respond to the depth gradient in the drier cutover bog studied here. Bonnet (1973) observed that 1) the limiting factor for testate amoebae living in mosses growing on trees and soils was water availability and 2) the species found in mosses of dry habitats were characterized by a relatively small size and shell morphology adapted to a thin water film. In the upper centimeters of *Sphagnum* peatlands, pore space is not a limiting factor within the size range of testate amoebae, but the level of humidity increases with depth and its variability decreases. This led us to predict (H3) that smaller species and those with a ventral aperture would be more abundant at the top of the mosses while larger ones and those with a terminal aperture should dominate lower down.

In addition to the existing natural gradients, soil organisms are increasingly exposed to direct and indirect effects of human activities. One of the most dramatic of these effects is the modification of the global nitrogen cycle, which has already clearly affected natural ecosystems (Lee 1998). Nitrogen (N) deposition was shown to cause important changes in the composition of plant and animal communities and the general functioning of nutrient-poor ecosystems, such as ombrotrophic (rain water-fed) peatlands and arctic tundra (Aerts and de Caluwe 1999; Press, Callaghan, and Lee 1998; Press et al. 1998; Shaver et al. 1998). Atmospheric N deposition may also slow or prevent the regeneration of *Sphagnum* peatlands by stimulating competing plants and/or by inhibiting *Sphagnum* growth (Berendse et al. 2001; Mitchell et al. 2002). Here too, testate amoebae have been shown to respond both to natural nutrient gradients and to experimental fertilization (Aescht and Foissner 1994; Foissner 1987, 1999; Gilbert et al. 1998a, b; Mitchell 2004; Tolonen, Warner, and Vasander 1992). As for the vertical gradient, the magnitude of the response of testate amoebae to N deposition has not been assessed in cutover bogs. The response of

testate amoebae to N and fertilizer addition in wet *Sphagnum* (Gilbert et al. 1998a, 1998b; Mitchell 2004) led us to experimentally assess the response of testate amoebae to N addition. Because many testate amoebae species characteristic of *Sphagnum* peatlands are strongly associated with conditions characterized by low pH, high water table, and low nutrients (Tolonen, Warner, and Vasander 1992), we hypothesized (H4) that, if present in the control plots, these species would disappear and be replaced by ubiquitous species such as *Assulina muscorum* and *Corythion dubium*.

In both cases (response to depth and N) we hypothesized that the responses would be species-specific (Mitchell 2004) and would also differ among groups of species sharing metabolic and/or morphological traits. More precisely, regarding the depth gradient, our goal was to test if the vertical distribution of species traits, such as the material used to build the shell and the presence or absence of symbiotic algae observed in previous studies (Bonnet 1958; Booth 2002; Buttler et al. 1996; Chacharonis 1954, 1956; Heal 1962; Meisterfeld 1978; Schönborn 1963), could also be detected in the extremely dry condition for *Sphagnum* mosses represented by secondary moss carpets dominated by *Polytrichum strictum* recolonizing a former bare peat surface. We therefore hypothesized (H5) that mixotrophic species, and those with secreted shells, or species with a combination of both traits would colonize preferentially the upper part of the vertical gradient and larger, heterotrophic species and those with agglutinated shells, or species with a combination of both traits would dominate the communities further down.

MATERIALS AND METHODS

Site description. Our study was conducted in Le Cachot Bog in the Swiss Jura Mountains (altitude 1,050 m a.s.l., 47.5°N, 6.4°E). This site is one of the few remnants of ombrotrophic *Sphagnum* bogs in the region. Former peat harvesting has both reduced the surface and affected the hydrology of the site causing changes in the vegetation (increasing shrub and tree cover), especially near the edges where a vertical peat wall strongly depressed the water table. Experimental plots were marked close to one of these peat walls, in a surface where the vegetation and surface peat had been removed in preparation for peat harvesting. The vegetation of the study area is a secondary moss carpet dominated by *Polytrichum strictum* Brid., a drought-tolerant bryophyte that colonizes the bare peat following peat mining and is also found in natural peatlands in the drier microhabitats, such as the top of large hummocks. Within the *Polytrichum* carpet, *Sphagnum fallax*, a peat moss commonly found in secondary regeneration of cut-over bogs in the region with a good tolerance for temporary desiccation, is able to establish itself (Grosvernier, Matthey, and Buttler 1995). However, in the early stages of *Sphagnum* re-colonization, such as the one studied here, the total cover of *Sphagnum* is small. The experimental area can therefore be considered to be approximately the driest possible habitat in which *Sphagnum* could grow.

Field experiment, sampling. Nitrogen was added as ammonium nitrate aqueous solutions (fine spray) every two weeks in four treatments (0 = control, 1, 3, and 10 g N m^{-2} $year^{-1}$). The plots were 30 × 40 cm in size and each treatment was replicated four times. The treatments started in spring 1993 and continued until the samples were collected in November 1995. *Sphagnum* mosses were extracted with forceps from each of the 16 plots. To avoid a border effect, no mosses were taken in the border 10 cm, thus the total sampling area in each plot was reduced to 10 × 20 cm. To obtain a representative sample of the surface while reducing the effect of spatial heterogeneity, which can be high even in macroscopically homogeneous surfaces (Mitchell et al. 2000a), mosses were taken from at least five different locations within the 10 × 20 cm sampling area of each plot.

Microscopic analyses. To assess the importance of the vertical distribution of testate amoebae within the *Sphagnum* mosses, each sample was cut into three parts (sub-samples): Capitulum (top 1 cm: subsample a), living green part (1–3 cm: subsample b) and dead brown part (3–5 cm: subsample c). The mosses were boiled in water for 5 min to separate the shells of the testate amoebae from the mosses. Shells were extracted by sieving the sample over a tea strainer (approximate mesh size 750 μm) and then concentrated by centrifugation (5 min. at 3,000 rpm). Testate amoebae were identified and counted under a phase contrast microscope at 200× and 400× magnifications. All microscopic analyzes were done by the same person (EM). Species identification was based on several guides and monographs to specific genera or species groups (Charman, Hendon, and Woodland 2000; Corbet 1973; Deflandre 1928, 1929, 1936; Foissner and Korganova 2000; Grospietsch 1958; Meisterfeld 2000a, 2000b; Ogden 1983; Ogden and Hedley 1980). The relationship between total number of individuals counted and the species richness suggests that a total count of about 100 is needed to reach a sill (not illustrated). In addition, the species richness was significantly correlated to the total count ($r = 0.413$; $P = 0.004$) for all samples, but this correlation decreased and was no longer significant when the samples with less than 100 individuals were excluded ($r = 0.277$; $P = 0.088$). Based on these observations, nine sub-samples with less than 100 individuals were rejected for further analysis, leaving 39 subsamples in the data set for which the average count was 154 individuals (min = 100, max = 273, SE = 7.2).

Numerical analyses. The species data were expressed as relative abundance [%] and then log transformed [$x' = \ln(x + 1)$] for all further analyses. Species occurring in a single sample were deleted from the data set prior to the analyses. Kruskal-Wallis tests were used to assess the overall response of species to N and depth. Mann-Whitney tests were used to compare treatments two by two. To analyze the correlation between species traits and the response of species to depth and N addition several non-parametric and parametric tests were performed. Canonical Correspondence Analyses (CCA) and partial CCAs were performed to assess the fraction of variation in the species data that was correlated with either depth, N, or the cross product depth*N used as explanatory quantitative variables (Ter Braak 1988–1992). In the partial CCA either depth or N was used in turn as a covariable and the correlation of the other variable to the residual variation was tested. Monte-Carlo tests were used to obtain a permutational probability for the correlations (999 permutations). For depth, the median depth of each subsample was used (i.e. 0–1 cm = 0.5; 1–3 cm = 2; 3–5 cm = 4). For N, the actual N load received by the plots was estimated by adding the N treatment (0, 1, 3, or 10 g m^{-2}, yr^{-1}) to the estimated background for the region (1.8 g m^{-2} yr^{-1}) (NABEL 1995). Finally the species' scores along the first and only canonical axis of the CCA were used as a metric for the response of species to depth or to N addition. If depth or N was significant in the CCA, and after checking the data distribution using a Kolmogorov-Smirnov normality test, an ANOVA was performed using the species scores to test the response of groups of species sharing metabolic and/or shell characteristics. For all analyses, the significance threshold was set at 0.05.

RESULTS

Species richness and community composition. A total of 22 testate amoebae taxa were identified in the samples from Le

Table **1**. Relative abundance of testate amoebae species in secondary *Polytrichum strictum-Sphagnum fallax* moss carpets from Le Cachot Bog in the Swiss Jura Mountains. Overall summary data for three sampling depths and four N treatment.

Taxon[a]	N	Average %	SE	CV [%][b]	Max
Archerella flavum (Archer, 1877)	1	0.0	0.0	100	0.7
Arcella arenaria Greff, 1866	1	0.0	0.0	100	0.8
Assulina muscorum Greff, 1888	35	12.9	2.3	18	61.0
Assulina seminulum (Ehrenberg, 1848)	9	1.2	0.5	44	15.8
Bullinularia indica (Penard, 1907)	27	2.3	0.5	21	10.8
Centropyxis aerophila Deflandre, 1929 var. *sphagnicola* Deflandre, 1929	1	0.0	0.0	100	1.0
Corythion dubium Taranek, 1881	38	12.9	2.4	19	65.3
Cryptodifflugia oviformis Pennard, 1890	3	0.5	0.4	80	16.0
Euglypha ciliata (Ehrenberg, 1848)	27	3.8	1.2	31	42.9
Euglypha compressa (Carter, 1864)	4	1.4	1.0	72	37.0
Euglypha rotunda Wailes 1911	8	0.8	0.3	39	7.5
Euglypha strigosa (Ehrenberg)	5	0.3	0.2	54	6.1
Heleopera rosea Penard, 1890	11	0.5	0.2	32	3.4
Heleopera sphagni (Leidy, 1874)	1	0.0	0.0	100	1.3
Hyalosphenia elegans Leidy, 1874	8	1.1	0.6	55	23.0
Hyalosphenia papilio Leidy, 1875	11	2.7	1.5	55	53.4
Nebela militaris Penard, 1890	14	0.9	0.3	31	7.9
Nebela tincta (Leidy, 1879)	38	57.3	4.3	7	93.9
Phryganella acropodia (Hertwig & Lesser, 1874)	5	0.1	0.1	54	2.7
Trigonopyxis arcula (Leidy, 1879)	1	0.0	0.0	100	1.7
Trinema enchelys (Ehrenberg, 1838)	1	0.0	0.0	100	1.7
Trinema lineare Penard, 1890	8	1.2	0.7	60	24.8

[a] Species names and authorities following Ogden and Hedley 1980 except for *Archerella flavum* (synonym: *Amphitrema flavum* Archer).
[b] Coefficient of variation CV = 100 * Average/SE.

Cachot Bog (Table 1). Despite this overall diversity, the species richness in individual samples was low (minimum = 3, maximum = 12, average = 6.6, S.E.= .33). Of the 22 species recorded, three species, *Nebela tincta*, *Assulina muscorum*, and *Corythion dubium* were most common (present in 35 or more of the 39 subsamples) and strongly dominated the communities (on average 57%, 13%, and 13% of the total count, respectively). Two other species, *Bullinularia indica* and *Euglypha ciliata*, were present in over 50% of the samples but with lower average relative abundances (2.3% and 3.8% respectively). Five other species were present in the samples with a mean percentage of over 1%: *Assulina seminulum*, *Euglypha compressa*, *Hyalosphenia elegans*, *H. papilio*, and *Trinema lineare*. The remaining 12 species were found only in few samples and usually in small numbers (Table 1). The intersample variability was high, as reflected by the standard errors, difference between average and maximum values (the minimum in all cases was 0), and coefficient of variation (Table 1).

Vertical micro-distribution and effect of N addition. Overall, only few significant differences emerged between the treatments. If a Bonferroni correction were applied to the tests on the individual species, none of the responses would be significant. These are still presented and discussed here because the likelihood of detecting significant responses would be very low given the number of species recorded and the number of replicates in the experiment. The species richness increased with depth (Kruskal-Wallis test, $P = 0.011$) and was significantly higher for the deepest level as compared to the first two levels (Mann-Whitney tests: a,c: $P = 0.007$; b,c: $P = 0.013$) (Fig. 1). The detailed responses to depth are given for the 14 most frequent species (Fig. 2). Species-specific responses appear clearly and were significant for four species. *Assulina muscorum* was relatively more abundant in the uppermost sampling depth and decreased in relative abundance with depth (Kruskal-Wallis test, $P = 0.029$). *Phryganella acropodia*, *Heleopera rosea* and *Nebela militaris* all reached their highest relative abundance in the deepest sampling depth while there were no differences between the upper two sampling depths (Kruskal-Wallis tests, $P = 0.042$, 0.025, and 0.052, respectively).

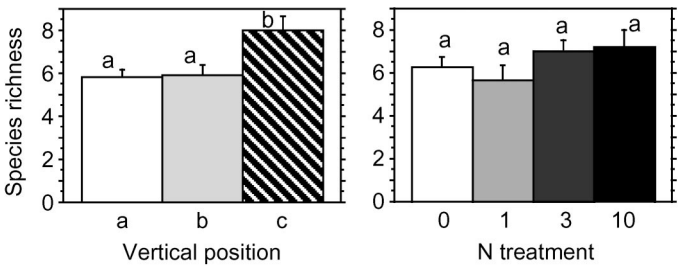

Fig. **1.** Species richness of testate amoebae in *Polytrichum* moss in a peat bog in Switzerland. **A)** along the vertical gradient (a: 0–1 cm depth, b: 1–3cm, c: 3–5cm), and **B)** in the four different N addition treatments [g m^{-2} yr^{-1}]. Bars indicate standard errors, different letters indicate significant differences (Mann-Whitney tests).

There was no change in species richness with N addition (Fig. 1). The response of species to N was less marked than with respect to depth (Fig. 3). A significant difference was found only for one species, *Bullinularia indica*, which increased with increasing N addition (Kruskal-Wallis test, $P = 0.011$). Several species had maximum relative density at intermediate N addition rates, either 1 or 3 g m^{-2} yr^{-1} but these maxima were not significantly different. *Euglypha strigosa* and *Assulina seminulum* were most abundant in the control plots (n. s.). Similarly to the results of the Kruskal-Wallis tests, in the canonical correspondence analyses (CCA) and partial CCAs, depth was significant, but nitrogen and N*depth were not. Depth explained 6.1% of the species data (P-value = 0.013). As there was only one variable in the model, the first axis is the only canonical axis and represents the depth gradient.

Correlation between the traits of species and their response to depth and N. The species characteristics and their

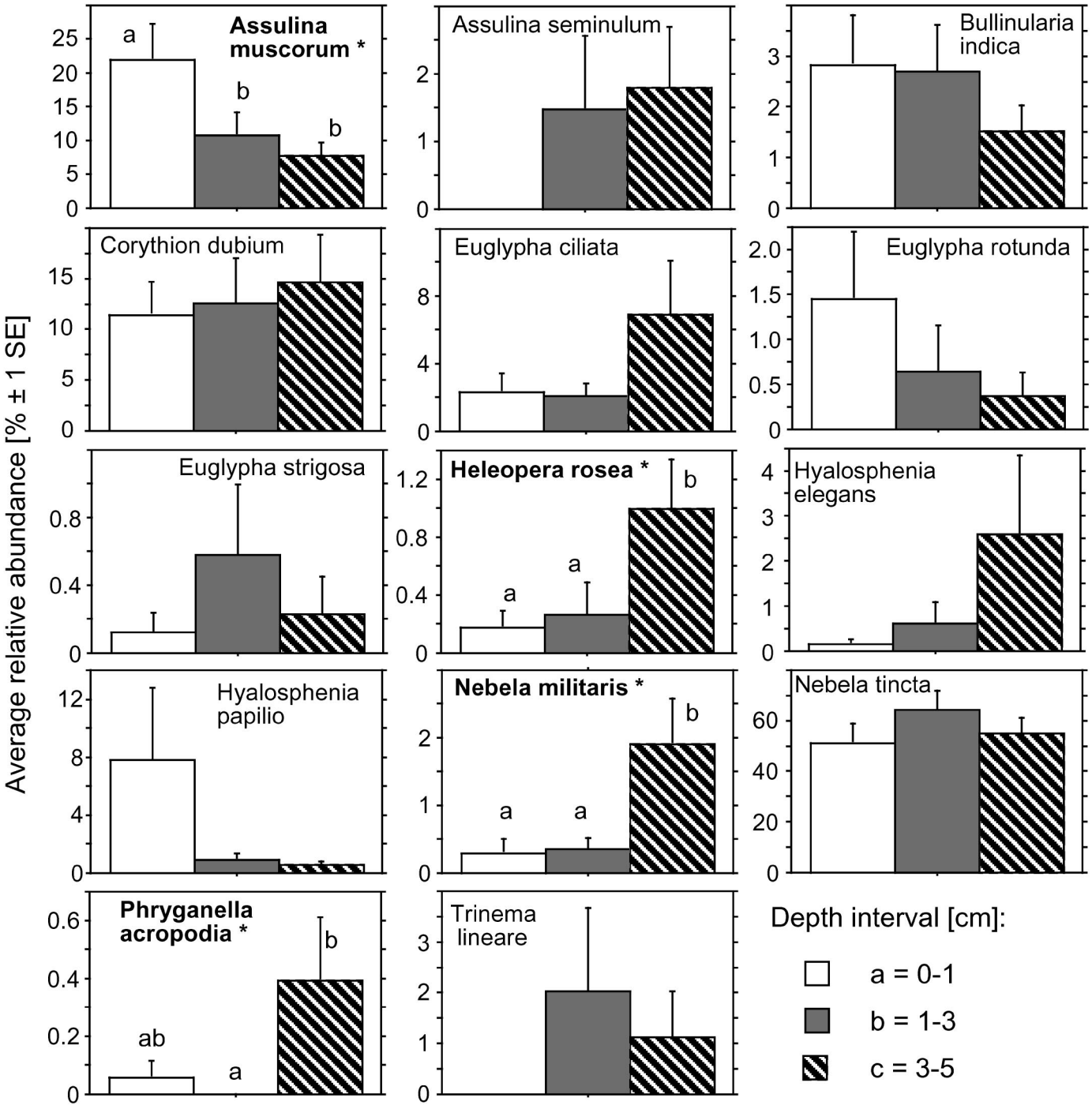

Fig. **2.** Average relative abundance of the fourteen most abundant species along the vertical gradient in *Sphagnum* moss in a peat bog in Switzerland (a: 0–1 cm depth, b, 1–3cm, c: 3–5cm]. Different letters over the bars indicate significant differences (Mann-Whitney tests). Bars indicate standard errors.

scores along the first axis of the partial CCA with N as a co-variable and depth as an explanatory variable showed some variation (Table 2). Although the score of *Hyalosphenia papilio* on the first axis of the CCA was the lowest of all (i.e. most negative correlation with depth, Table 2), the ANOVA did not reveal any significant relationship between species traits, be it size, position of the aperture, or composition of the shell and their CCA score.

DISCUSSION

Diversity. The total species diversity found in this study (22 species) is relatively high considering 1) that the plots were selected to be as similar as possible at the onset of the experiment, and 2) the history of the site and recent development of the moss carpet over the former bare peat surface. In agreement with our first hypothesis, the species richness of individual samples (average = 6.6, S.E. = 0.33) was, however, low by com-

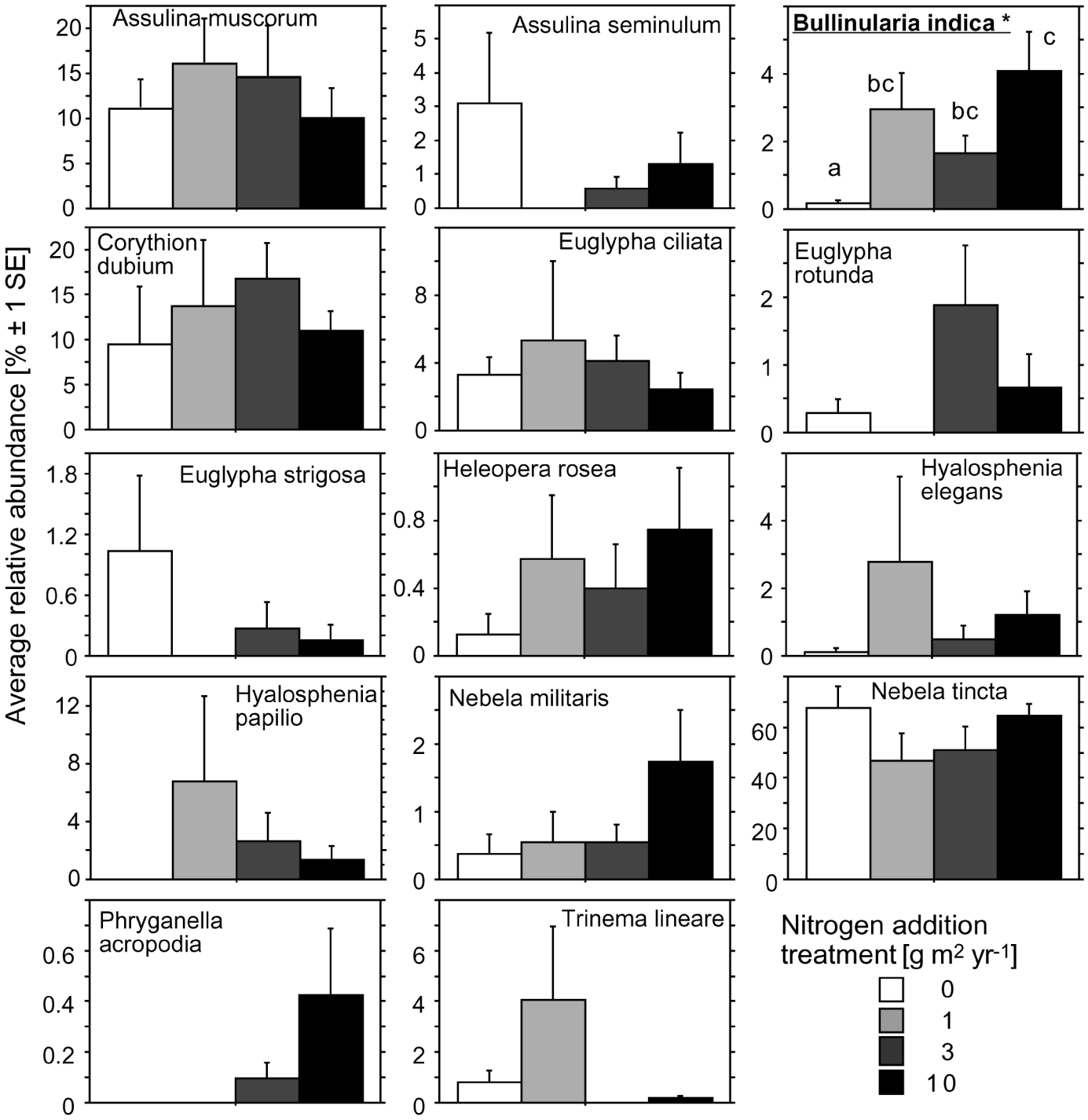

Fig. **3.** Average relative abundance for the fourteen most abundant species in the four different N addition treatments in *Sphagnum* moss in a peat bog in Switzerland [g, m^{-2}, yr^{-1}]. Different letters over the bars in indicate significant differences (Mann-Whitney tests). Bars indicate standard errors.

parison to wetter sites of the same peatland where only the top 1 cm of *Sphagnum* mosses was analyzed (Mitchell et al. 2000a) (average = 8.6, S.E. = 0.28, *t*-test $P < 0.0001$) and to comparable peatlands in the same region (Mitchell et al. 1999) (average = 16.01, S.E.= 0.67, *t*-test $P < 0.0001$). The average species richness was also lower than that observed in La Chaux-des-Breuleux Bog, another cutover peatland of the Jura Mountains with a better-developed secondary moss carpet (Mitchell et al. 2000b) (average = 9.2, S.E. = 0.29, *t*-test $P < 0.0001$). However, in the latter study the overall diversity was lower with only 16 species recorded in 19 samples, perhaps because only one sampling depth, corresponding to the lower segment of the present study (3–5 cm), was analyzed (Mitchell et al. 2000b). In secondary moss carpets developing in cutover peat bogs of the same region, Buttler et al. (1996) found 12 species in a *P. strictum–S. fallax* moss carpet in Le Cachot Bog (same site as

Table **2**. Functional traits and shell characteristic for 22 testate amoebae species found in Le Cachot Bog and species score along the first axis of the partial CCA with N (added + background) used as a covariable and the average depth of the sample used as explanatory variable.

	Metabolism[a]		Shell type[b]		Aperture position		
Species	Mixo	Hetero	Xeno	Id/pro	Terminal	Ventral	CCA score
Hyaosphenia papilio	x			x	x		−0.51
Euglypha rotunda		x		x	x		−0.49
Assulina muscorum		x		x	x		−0.25
Bullinularia indica		x	x			x	−0.20
Nebela tincta		x	x		x		−0.05
Corythion dubium		x		x		x	−0.01
Euglypha strigosa		x		x	x		0.01
Euglypha ciliata		x		x	x		0.20
Euglypha compressa		x		x	x		0.24
Trinema lineare		x		x		x	0.41
Hyalosphenia elegans		x		x	x		0.54
Heleopera rosea		x	x		x		0.55
Nebela militaris		x	x		x		0.55
Assulina seminulum		x		x	x		0.60
Phryganella acropodia		x	x			x	0.75
Cryptodifflugia oviformis		x		x		x	1.14

[a] Mixo: Mixotrophic; Hetero: Heterotrophic.
[b] Xeno: Xenosomes: Id/Pro: Idiosomes or proteinaceous.

this study), and 12 species in a *Sphagnum angustifolium* lawn that had overgrown an *Eriophorum vaginatum* (cotton grass) tussock in La Chaux d'Ablel. Finally, Jauhiainen (2002) recorded 13 species and 16 species plus an unspecified number of unidentified taxt in two sites that had been drained for 40 yrs (a fen and a bog, respectively). The lower diversity of both of these studies can be explained by the fact that in each case they represent a single sampling point only.

Community composition. The three dominant species, *Nebela tincta*, *Assulina muscorum*, and *Corythion dubium*, are commonly found in mosses outside of peatlands. For example *A. muscorum* and *C. dubium* have frequently been recorded in dry mosses in the Arctic (Beyens and Chardez 1994; Beyens, Chardez, and de Baere 1992; Van Kerckvoorde et al. 2000) and at least *C. dubium* is known from terrestrial environments in the Antarctic (Smith 1992). *Nebela tincta*, *A. muscorum*, and *C. dubium*, were also found to be ubiquitous in a wide range of peatlands in the Jura region and to have an ecological optimum for water table depth of over 50 cm (Mitchell et al. 1999). In support to our first hypothesis, the dominance of these species is in accordance with the dryness of the study site. These species were also the dominant species in another study of cutover peatland of the Jura Mountains (Mitchell et al. 2000b). By contrast, and in agreement with our hypothesis, species characteristic for oligotrophic moist to wet microsites were, predictably, rare (e.g. *Archerella flavum*, *Heleopera sphagni*) or absent (e.g. *Amphitrema wrightianum*) at this relative dry site. However, even if such species were rare, their occasional presence suggests that these species are able to tolerate a broader range of conditions than usually believed and/or that they have a relatively high colonization potential from more favorable habitats that existed within a relatively short distance (approximately 100m) in wetter parts of the peatland.

The vertical micro-distribution of testate amoeba in *Sphagnum*. The present study differs from all of the existing studies on the vertical distribution of testate amoebae in *Sphagnum* except one (Buttler et al. 1996) in that we focused on an unusual type of environment: the secondary moss communities re-colonizing bare peat surfaces. In support of our second hypothesis, this study demonstrated the existence of a vertical micro-distribution of testate amoebae in an unusual habitat for *Sphagnum* mosses, possibly the driest conditions in which these mosses can grow. Testate amoebae communities differed significantly with depth and species-specific responses were observed. However, contrary to our third hypothesis, no correlation was found between any of the species traits and their response to the depth gradient. Furthermore, although three mixotrophic species were recorded, only one, *H. papilio* was abundant enough for its response patterns to be analyzed. Although when present this species represented between 9% and 53% of the total community in the uppermost segment and was much less dominant in the lower segments (always less than 5%), this pattern was not significant because this amoeba was absent from so many samples. This lack of significant effect, which contradicts our fifth hypothesis, can clearly be attributed to the dryness and history of the study site where *Sphagnum* mosses and their testate amoebae faunas are currently re-colonizing a former bare peat surface, resulting in a high degree of patchiness in species distribution.

The findings of other authors on the vertical micro-distribution of testate amoebae in peatlands, (Bonnet 1958; Booth 2002; Buttler et al. 1996; Chacharonis 1954, 1956; Heal 1962; Meisterfeld 1978; Schönborn 1963) are summarized in Tables 3 and 4. Methodological differences, such as the sampling interval and separation or not of living and dead individuals, make comparisons difficult. To summarize the findings of Chacharonis (1954), we report in Table 3, the number of times each species reached highest abundance at each of four sampling depths from the apex to the lowest, non-photosynthetic segment analyzed. In a published abstract, Chacharonis (1956) reported species to be separated into two associations: The first association is composed of species living in the upper portion of the *Sphagnum* mosses, referred to as ''photophylic'' and containing mostly mixotrophic species, while the species of the second association live in the darker, lower portions of the mosses and lack chlorophyll and symbiotic algae (zoochlorellae) (Chacharonis 1956). Chacharonis (1954), however, provides more details in his dissertation, which differ slightly from the published summary in that, in half of the cases where it was found, *Heleopera sphagni*, a mixotrophic species that uses xenosomes to build its shell, reached its highest density in the 1–3 cm section (usually still photosynthetic) rather than in the apex (Table 3). The results from all other studies are summarized in Table 4 in which the species richness and dominant

Table **3**. Vertical microdistribution and species richness of testate amoebae observed by Chacharonis (1956) in *Sphagnum.*

Species (in bold: mixotrophic)	Segment in which the species reached high abundance*			
	Apex 0–1 cm	Upper 1–3 cm	Middle 3–5 cm	Bottom 5–7 cm
Hyalosphenia papilio	3	1		
Heleopera sphagni	2	2		
Arcella vulgaris		2		
Hyalosphenia elegans		1	2	
Nebela collaris		3	4	
Euglypha ciliata		2	3	1
Euglypha alveolata		2	4	1
Heleopera petricola			1	
Euglypha cristata			1	
Nebela dentistoma			3	1
Sphenoderia lenta			4	2
Assulina seminulum			1	1
Average species richness	3.2	5.6	7.2	5.4

* Numbers indicate the number of cases where species reached highest abundance in each specific segment. Rare species excluded. Exact depth not specified, but estimated from a figure to be 1 cm for the apex (= capitulum) and 2 cm for each of the other three sections.

species are given for each sampling depth along with a brief description of the methodologies used. The two things that stand out from this compilation are 1) an increase in species richness with depth and 2) mixotrophic species reach highest abundance in the top segment, except, in some but not all cases, for those that use xenosomes. Heal (1962) interpreted these vertical patterns as follows: mixotrophic species that do not require organic or mineral particles to build their shell are able to colonize the uppermost section of *Sphagnum* mosses. This might allow them to benefit more from their algal symbionts as they are exposed to more photosynthetic active solar radiation than if they were living deeper down. In fact, although some mixotrophic testate amoebae are well able to prey on other organisms, none of them seems to be able to survive in total darkness (Schönborn 1965). Over a century ago, Penard (1902) had already observed that mixotrophic species are never seen without their symbionts, and he could find no evidence of predation in *Amphitrema* species (Penard 1902). The algal symbionts thus appear to be vital for the amoeba, and represent more than just a diet complement. However, it does not seem that any research has been done on this subject in the last 40 yrs. Species that are mixotrophic and that also use xenosomes (foreign particles) to build their shells may find more appropriate building material deeper down in the moss carpet while still receiving enough light. If true, their maximum abundance should not be in the uppermost segment, but just below the capitulum of the *Sphagnum* mosses, a position that represents a compromise between the absolute sunlight requirements of the algal symbiont and their own requirements for shell construction. However, the existing data only partly support this hypothesis as in some cases all mixotrophic species, regardless of their shell type, are most abundant in the top (Meisterfeld 1977, 1978), while other studies suggest an optimum lower down in the mosses for mixotrophic species using foreign material to build their shell (Booth 2002; Buttler et al. 1996; Heal 1962) (Table 4), or as Chacharonis (1954) observed in the first study on this topic, *H. sphagni* may reach maximum abundance in the uppermost segment or in the second segment (Table 3). However, it must be noted that only Meisterfeld (1977, 1978) carefully separated the living amoebae from the dead (empty shells). In all other studies either this was not done or it was not mentioned in the methods. It is therefore possible that the observed patterns are partly due to the inclusion of empty shells in the counts, which would artificially increase the numbers of mixotrophic species in lower parts of the mosses.

Beyond the question of mixotrophy vs. vertical position, an interesting pattern of niche separation among congeneric species appears from the existing studies on vertical micro-distributions. This pattern suggests that competitive exclusion may occur between some closely related species of testate amoebae. In the following list of paired species the first has regularly been found to reach highest density or relative density at the top of the mosses while the second one is usually more abundant lower down: *Amphitrema flavum-A. wrightianum*/*A. stenostoma*; *Assulina muscorum-A. seminulum*; *Hyalosphenia papilio-H.elegans*. Interestingly, all combinations of mixotrophic and heterotrophic species are covered in this list. Other couples, such as *Euglypha rotunda*/*E. ciliata*, or groups of three species (candidates could be found among the *Euglypha* or *Nebela* species) may be added to this list. This vertical niche separation corresponds to a pattern of horizontal niche separation along the wet to dry gradient observed in Russian peatlands (Bobrov, Charman, and Warner 1999). Bobrov et al. (1999) observed that the larger forms, such as *Trigonopyxis arcula* var *major* and *Assulina seminulum* were found in wetter habitats than the closely related and smaller *Trigonopyxis minor* and *Assulina muscorum*. Furthermore, they also noted that spined forms were consistently found in wetter habitats than glabrous ones. In the upper centimeters of *Sphagnum* peatlands, pore space is not a limiting factor within the size range of testate amoebae, but the level of humidity increases with depth and its variability decreases. This led us to predict that smaller species and those with a ventral aperture would be more abundant at the top of the mosses while larger ones and those with a terminal aperture should dominate lower down, but our data support neither of these hypotheses as we found no significant correlation between species traits and their response to the depth gradient. The variability in our data was too high for the observed traits to be significant (Fig. 2). Furthermore, with respect to moisture, the common occurrence of xerophile species (e.g. *Assulina muscorum*) together with hygrophile or hydrophile species (e.g. *Hyalosphenia papilio*) has long been a puzzle for ecologists studying testate amoebae. The vertical micro-distribution of heterotrophic species probably reflects several abiotic and biotic factors, such as their requirements for space, humidity, and the abundance of prey and predators organisms.

Response of testate amoeba to N in *Sphagnum*. Although there are fewer data on the response of testate amoebae to N than to the vertical gradient in *Sphagnum*, descriptive and experimental evidence show that testate amoebae respond to nutrient gradients and experimental fertilization in *Sphagnum* peatlands and other ecosystems. In the only existing study on the preferences of testate amoebae for a range of chemical variables, testate amoebae were shown to exhibit different preferences for peat N content ranging from 0.5–2.0 [% dw^{-1}] (Tolonen, Warner, and Vasander 1992). In a N-enrichment experiment similar to ours in the French Massif Central, microbial communities reacted to N, PKCa, and NPKCa additions in a *Sphagnum*-dominated peatland (Gilbert et al. 1998a, 1998b). One of the conclusions of these studies was that the biomass of testate amoebae decreased with N addition. Similarly, in an arctic wet sedge tundra, the combined addition of N and P reduced the density of testate amoebae, although, for most species the effects of N and P addition were not significant and none at all were significant when a Bonferroni correction was applied to the p-values (Mitchell 2004). Beyond *Sphagnum* peatlands,

Table **4**. Summary of the existing data on the vertical micro-distribution of testate amoebae in *Sphagnum* mosses with the exception of Chacharonis (1954). Methodology, species richness at different sampling depths, position at which the main species reached their highest abundance, and main conclusions of the study.

Reference	Methodology	Living and dead separated?	Species richness (depth range in cm)		
			Top	Middle	Bottom
Bonnet 1958	Three segments analyzed: top 1 cm, 2–3 cm, 3 cm to base (depth not specified). Natural peatland.	no, or not specified	16	33	38
Heal 1962	Three segments analyzed: 0–2 cm, 2–4 cm, 4–6 cm	no, or not specified	8		22
Schönborn 1963	Using Jung's (1936) moisture classification*. Categories I and II. Two segments: upper, green part (0–7 cm—estimated from a figure) and the lower part (to an unspecified depth)	11	n.a.	11	
Schönborn 1963	Same as above, but Jung's moisture categories III and IV	no, or not specified	5	n.a.	9
Schönborn 1963	Same as above, but Jung's moisture categories V to VII		0	n.a.	8
Schönborn 1963	Ditto, Jung's moisture category VIII		0	n.a.	6
Meisterfeld 1977	*Sphagnum recurvum* 1 cm sections from 0 to 7 cm. Jung's moisture category III	yes	Increasing with depth, 4 (0–2 cm), 5 (2–4 cm), 7 (4–5 cm), 6 (5–6 cm), 5 (6–7 cm)		
Meisterfeld 1977	*Sphagnum recurvum* 1 cm sections from 0 to 10 cm. Jung's moisture category IV	yes	Increasing with depth, 8–11 (0–2 cm), 13–14 (3–6 cm), 16–18 (6–8 cm), 22–25 (8–10 cm)		
Meisterfeld 1978	*Sphagnum sp.* 1 cm sections from 0 to 8 cm. Jung's moisture category IV	yes	Increasing with depth, 4 (0–3 cm), 13–17 (4–8 cm)		
Meisterfeld 1978	*Sphagnum sp.* 1 cm sections from 0 to 8 cm. Jung's moisture category VII	yes	Increasing with depth, 3–4 (0–3 cm), 6–8 (4–8 cm)		
Buttler et al. 1996	1 cm increment paleoecological study.	no		n.a.	
Booth 2002	Upper (photosynthetic) part of mosses (1.5 to 12 cm long depending on the cases, on average 4.5 cm) compared to lower (brown) part (usually 2–5 cm long)	no	13*	n.a.	20*
This study	*Sphagnum fallax* cut into 3 segments: 0–1 cm, 1–3 cm, 3–5 cm. Very dry conditions (cut-over bog site dominated by *Polytrichum strictum*) equivalent to Jung's category VIII.	no	5.8	5.9	8.0

§ Species names abbreviated with the first four letters of the genus and the species. When applicable, optimal depths (in cm) are given in brackets. Mixotrophic species are highlighted in bold.

Jung's moisture categories: I = submerged: >95% moisture; II = partly submerged, partly emerged, >95% moisture; III = very wet, water drips from mosses, >95% moisture; IV = wet, water can be extruded by squeezing the mosses slightly in the hand, approximately 95% moisture; V = half-wet, water can be extruded by squeezing the mosses strongly in the hand, approximately 90% moisture; VI = moist, a small amount of water can be extruded by squeezing the mosses strongly in the hand, approximately 85% moisture; VII = half-dry, only a few drops of water can be extruded by squeezing the mosses strongly in the hand, approximately <80% moisture; VIII = dry, no water can be extruded by squeezing the mosses strongly in the hand: <50% moisture.

* Estimated from a figure.

Table **4**. Extended.

Species reaching highest abundance:§			
Top	Middle	Bottom	Other observations
Assu musc, ***Hyal papi, Amph flav,*** *Nebe marg*	—	*Nebe pena, Nebe dent*	Mixotrophic species more abundant in upper part
Assu musc, ***Hyal papi, Amph flavu***	*Nebe tinc*	***Amph wrig***	1) Mixotrophic species, especially *Hyal papi* more abundant in upper part. 2) Species using xenosomes more abundant at the bottom. 3) the combination of mixotrophy and xenosomes is important in determining species' optimal position
Plac spin, Hyal papi, *Diff baci, Diff glob*	n.a.	*Hyal eleg, Nebe tinc, Hele petr*	
Amph flavu, Amph sten, Hele spha, Hyal papi	n.a.	*Assu musc, Assu semi, Hyal eleg, Nebe coll, Nebe tinc, Nebe milli, Nebe mino*	1) All mixotrophic species were restricted to the upper segment, although two of theses, *Amph sten* and *Hele spha*, use foreign particles to build their shell. 2) *Nebe tinc* was also abundant at top but more so at bottom. 3) No living testate amoebae were recorded in the top segment in the three driest moisture classes).
none	n.a.	*Assu musc, Cory dubi, E. compr, Nebe coll, Nebe tinc, Nebe mili*	
none	n.a.	*Assu musc, Eugl comp, Phry hemi, Trig arcu, Bull indic, Cent orbi*	
Hyal papi, Hele spha, *Cycl arce* (0–1 cm)		*Eugli cili* (4–5 cm)	
Hyal papi, Hele sph, Plac spin (0–1 cm)	*Nebe tinc* (2–8 cm)	*Crip ovif* (6–7 cm), *Wail ebor* (4–5 cm), *Nebe dent* (6–9 cm), *Nebe tinc* (2–8 cm), *Quad symm* (8–10 cm)	All mixotrophic species were restricted to the upper segment
Hyal papi		*Nebe tinc, Nebe coll* (5–6 cm), *Eugl stri* (6–8 cm)	
none	*Nebe tinc* (2–4 cm)	*Nebe coll* (4–7 cm), *Eugl stri* (6–7 cm), *Hele petr* (6–7 cm), *Nebe mili* (6–7 cm), *Cory dubi* (7–8 cm)	*Hyalosphenia papilio*, the only mixotrophic species was restricted to the upper segment
Hyal papi	***Hele spha***		
Amph flav, Hyal papi, Hele spha, *Assu musc, Assu semi, Arce arto, Nebe cari, Nebe tinc*	n.a.	***Amph wrig,*** *Nebe pena, Pseu fasc, Sphe lenta, Trac dent, Hyal eleg, Hele sylv*-type, *Nebe gris, Nebe coll, Nebe mili*	
Assu musc, ***Hyal papi, Amph flav*** (one sample only)	none	*Phry acro, Hele rose, Nebe mili*, ***Hele spha*** (one sample only)	

a reduction in biomass and density of testate amoebae, as well as species-specific responses, were reported in a spruce forest soil (Aescht and Foissner 1994), and a number of other responses to various environmental stresses and gradients have also been reported outside *Sphagnum* peatlands (Foissner 1987; Foissner 1999).

In this study, testate amoebae responded more clearly to the micro-environmental gradient in the top 5 cm of the mosses

than to the addition of N, even at the relatively high dose of 10 g m^{-2} yr^{-1} over two years, which is more than five times the background level experienced in the region (NABEL 1995). Contrary to our fourth hypothesis, there was no overall response of testate amoebae to N, although one species, *B. indica*, was significantly more abundant in the fertilized than in the control plots. This result does not support our hypothesis that ubiquitous species would be favored by N addition and is also in contradiction with the response of *B. indica* to peat N content in Finland where it had the second lowest preference (weighted average for N% d.w. 0.6 ± 0.13) (Tolonen, Warner, and Vasander 1992). Further research is clearly needed to address more specifically the response of *B. indica* and other testate amoebae species to N deposition and nutrient gradients under different background N loading and soil N content.

Although our results do not show many clear and significant differences, they do not allow us either to formerly rule out any possible effect of N deposition on testate amoebae communities. The few existing studies rather suggest that, in addition to their total biomass, or contribution to the total microbial biomass, the species composition of communities of testate amoebae may be affected by atmospheric N deposition. Thus, in accordance with most studies on environmental change, the reaction of testate amoebae to N pollution was species specific, although as in many field studies, few or no significant responses to the treatments were observed.

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