

Horizontal Distribution Patterns of Testate Amoebae (Protozoa) in a *Sphagnum magellanicum* Carpet

E.A.D. Mitchell,¹ D. Borcard,² A.J. Buttler^{1,3} Ph. Grosvernier,¹ D. Gilbert⁴ J.-M. Gobat,¹

¹ Department of Plant Ecology, Institute of Botany, University of Neuchâtel, Rue Emile Argand 11, CH - 2007 Neuchâtel, Switzerland

² Département de sciences biologiques, Université de Montréal, case postale 6128, Succursale Centre Ville, Montréal, Québec, H3C 3J7, Canada

³ Université de Franche-Comté, UMR 6565 du CNRS, UFR des Sciences et Techniques, 16, Route de Gray, F-25030 Besançon, France

⁴ Swiss Federal Institute for Forest, Snow and landscape Research (WSL/FNP), Antenna Romande, c/o EPFL, 1015 Lausanne, Switzerland

⁵ GEOTOP, Université du Québec à Montréal case postale 8888, Succursale Centre-Ville, Montréal, Québec, H3C 3P8, Canada

Abstract

The distribution of soil microorganisms is generally believed to be patchy and to reflect habitat heterogeneity. Despite this general rule, the amount of existing data on species distribution patterns is scarce. Testate amoebae (Protozoa; Rhizopoda) are an important component of soil microbial communities and are increasingly used in ecological and paleoecological studies of *Sphagnum*-dominated peatlands, but data on the spatial structure of communities are completely lacking. This is an important aspect since quantitative models used for paleoecological reconstruction and monitoring are based on species assemblages. We explored the distribution patterns of testate amoebae distribution in a macroscopically homogeneous *Sphagnum* carpet, down to a scale of several centimeters. Distributions maps of the species and spatially constrained sample groups were produced. Multivariate and individual spatial autocorrelations were calculated. The importance of spatial structure was quantified by canonical correspondence analysis. Our ultimate goal is to find the finest resolution of environmental monitoring using testate amoebae. The distribution patterns differed among species, resulting in a complex spatial structure of the species assemblage in a whole. Spatial structure accounted for 36% of the total variation of species abundance in a canonical correspondence analysis constrained by spatial variables. This structure was partly correlated to altitude (microtopography) at a very fine scale. These results confirmed the existence of significant broad- and fine-scale spatial structures within testate amoebae communities that could in part be interpreted as effects of ecological gradients. This shows that, on a surface area of 0.25 m², ecological conditions which look uniform from a macroscopic point of view are not perceived as such by *Sphagnum*-inhabiting organisms. Therefore, testate amoebae could prove very useful to monitor fine-scale ecological processes or disturbances. Studies of the species' spatial distribution patterns in combination with autoecological studies are needed and should be included in the toolbox of biomonitoring itself.

Introduction

In natural ecosystems, the distribution of living organisms is most often heterogeneous and reflects to some extent the heterogeneity of soil resources and of the physical and chemical characteristics of the environment [11]. Species interactions or past (historical) processes may also cause nonrandom distributions of organisms [4, 5]. Soil microorganisms reflect edaphic conditions, e.g., soil type, soil moisture regime, and soil pore distribution [10], and are indirectly influenced by vascular plant roots and by the activity of larger organisms such as small invertebrates [16]. However, despite these established relationships, spatial heterogeneity of living organisms—especially microorganisms—and ecological conditions may be difficult to assess in soils and mosses where gradients are often not visible. Furthermore, the scale of spatial heterogeneity will depend on the kind of organism studied. These issues also raise fundamental and technical problems involving the scale at which measurements are to be made [31].

Among the different groups of microorganisms there is an increasing interest in the study of testate amoebae (Protozoa; Rhizopoda), especially in peatlands. Testate amoebae (also called testaceans) are a group of protozoa living in lakes, rivers, peatlands, mosses, and soils. They are used in ecological and palaeoecological studies of *Sphagnum*-dominated peatlands and other wetlands [e.g., 34, 3, 9, 26]. Testate amoebae are useful in such studies because they are minute in size (10 to 300 μm) and very abundant [17, 36], and their shells are diagnostic to the species level and are well preserved in the peat. In addition, most species living in *Sphagnum*-dominated peatlands are cosmopolitan in their distributions, and the number of testate amoebae species in a given place is larger than the number of moss and vascular plants species [27]. Furthermore, they are sensitive to microenvironmental conditions such as moisture content and water chemistry [24, 35, 26], react rapidly to environmental changes [7, 13, 14], and their ecological preferences appear to be comparable in different geographical regions, at least for some species [9]. In paleoecological studies, the species'

ecological preferences are used to infer past conditions from subfossil communities using quantitative models [9, 26].

Testate amoebae also play a key role in the microbial trophic network in *Sphagnum*-dominated peatlands, where they are one of the dominant taxa in terms of biomass. They prey on a wide range of organisms, including bacteria, protozoa, microalgae, fungi, and micrometazoa, and may also ingest senescent or dead organisms [15]. Finally, the different species can be classified into several functional groups according to the presence or absence of symbiotic zoochorellae, their feeding habits, their size, and their vertical position on the mosses [25, 32]. However, the spatial and temporal limits of their use as biomonitors need to be assessed. The usefulness of the testate amoebae communities as indicators starts only at the point where the magnitude of their response to ecological fluctuations exceeds their random spatial and temporal fluctuations, and this point still needs to be determined.

The aim of this study is to explore the distribution patterns of testate amoebae in a macroscopically homogeneous *Sphagnum* carpet, down to a scale of several centimeters. Finding significant spatial structures at such a fine scale would indicate that a potential for bioindication for testate amoebae indeed exists at a resolution much finer than what can be expected from the vegetation. Our ultimate goal is to find the finest resolution of environmental monitoring using testate amoebae. Using several statistical tools, we compared the distribution and spatial autocorrelation patterns of different species and the distribution pattern of communities.

Materials and Methods

Sampling Site and Strategy

Sphagnum moss samples were collected in November 1997 in a homogeneous *Sphagnum magellanicum* carpet in the center of an ombrotrophic raised bog in the Swiss Jura mountains (Le Cachot bog, altitude 1050 m a.s.l., 47.5°N, 6.4°E). The site was chosen for its macroscopic uniformity, i.e., no ecological gradient could be inferred from the topography (maximum height difference from the lowest to the highest point: 6.6 cm) or the vegetation pattern. The vegetation of the sampling site was dominated by *Vaccinium*

oxycoccus (estimated cover: 20%), *Eriophorum angustifolium* (10%), *Carex nigra* (2%), and *Andromeda polifolia* (1%). The dominant moss is *Sphagnum magellanicum* (>90%) with scattered *Sphagnum angustifolium* (5%), *Sphagnum rubellum* (1%), and *Aulaacomnium palustre* (<1%). Because of the vertical structure of the vegetation, the total adds to more than 100%. *Scirpus caespitosus* and *Pinus rotundata* were also present in the vicinity.

A systematic sampling strategy with a random component was applied. A 40 cm × 60 cm plot was subdivided into 96 subplots 5 cm × 5 cm in size. In each subplot the position of a first sample unit was determined randomly. From the first sample unit of each subplot a second sample unit was taken at a random angle and at a random distance ranging from 0.5 cm to 3.5 cm. This sampling strategy resulted in a total of 192 sample units and aimed at a regular coverage of the sampling area (for mapping purposes) while maintaining a necessary randomness in the ultimate choice of the sampling locations. At each sampling spot, a single *Sphagnum* moss was carefully extracted from the carpet with forceps and stored in a stoppered vial. The sample units were numbered by rows, starting from the upper left-hand corner of the sampling plot. A subset of 65 sample units was selected, starting with unit 1 and with a step of 2, 3, and 4, thus resulting in the following sequence: unit 1, 3, 6, 10, 12, 15, 19, etc. To assess the effect of microtopography on spatial distribution patterns, the altitude (in millimeters) of each of the 192 sampling spots was recorded using an arbitrary reference.

Each *Sphagnum* specimen was cut at 2 cm, a length corresponding to about one year's growth, which, because of the continuous growth of *Sphagnum* mosses, contained mostly living microorganisms. The mosses were dried at 45°C and weighed, and testate amoebae were extracted and identified following Warner [38]. All testate amoebae shells (live and dead) were identified and counted under a phase contrast microscope at 200× and 400× magnification. Biovolumes of each species were estimated by assuming geometrical shapes and converted to carbon using the conversion factor $1 \mu\text{m}^3 = 0.11 \times 10^{-6} \text{ mgC}$ [39].

Data Analysis

Distribution Maps. To visualize the species' distribution patterns, we drew distribution maps for the most frequent species' abundance, and for the total number of species and total number of individuals.

Spatially Constrained Clustering of Samples. To detect limits in the testate amoebae communities, we computed a spatially constrained complete linkage clustering of samples using the Steinhaus similarity index [22]. Of all possible links between samples, a subset of 177 was selected using the Delaunay triangulation. Among these, three links between distant border samples were deleted, leaving 174 possible links for the spatially constrained clustering. All computations were done using the R-package for data analysis [23]. The spatially constrained clustering and all further analyses were done using logtransformed abundance data [$y' = \ln(y + 1)$].

All-Directional Correlograms. To test for spatial structure in the data, we computed autocorrelograms. At the community level, we

calculated a multivariate all-directional Mantel correlogram [33, 29] based on a similarity matrix of the sample units. We used the Steinhaus similarity index [22]. Univariate correlograms based on Moran's *I* index [28] were computed for the most frequent species. For all correlograms the number of classes was determined according to Sturge's rule:

$$\text{Number of classes} = 1 + 3.3 \times \log(m) \quad (1)$$

where *m* is the number of distances in the triangular matrix without the diagonal (2,080 in our case). The resulting number was 11.95, rounded up to 12. All computations were done using the R-package for data analysis [23].

Canonical Correspondence Analysis. To assess the importance of the spatial structure, we computed a canonical correspondence ordination (CCA) of the multivariate data set. The spatial matrix was made of 40 variables covering a large range of spatial scales, and generated by taking the principal coordinates of a matrix of Euclidean distance truncated to retain only the closest distances among samples [Borcard D, Legendre P. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices, in preparation]. Significant variables were selected by a procedure of forward selection of explanatory variables. The variables and axes were tested for significance using a Monte-Carlo permutation test (999 permutations). Interpolation maps were produced using the resulting significant canonical axes (sample scores which are linear combinations of the explanatory variables; [20]). The program CANOCO [33] was used for CCA and interpolation maps were produced using the Mac Gridzo program with the following gridding parameters: 6 points squared inverse distance interpolation algorithm with no radial search and a grid size of 1 cm.

Results

General Observations

A total of 21 testate amoebae species were identified in the sample (Table 1). Among these, nine species are present in very few sample units (1–3) and usually in low numbers. The most frequent species are in decreasing order: *Nebela tinctoria* var. *major*, *Hyalosphenia papilio*, *H. elegans*, *Assulina muscorum*, *Pseudodifflugia gracilis*, and *A. seminulum*. The number of testate amoebae species increases with the total number of individuals in the sample unit. A minimum of about 100 individuals appears to be necessary in order to reach a plateau (Fig. 1), but the number of species only drops sharply below 50 individuals.

We found an average of 5.8×10^3 testate amoebae individuals per g dry weight (Table 1), a figure comparable with results of previous studies. Heal [18] found (for living individuals) 18.6×10^3 testate amoebae individuals per g dry weight, but his figure is an average over May–July, a period where testate amoeba abundance may be two to three times

Table 1. Summary table for 21 testate amoebae species in 65 sample units of *Sphagnum magellanicum* (2 cm length) from Le Cachot Bog, Swiss Jura

	n	Abundance ^a			Relative abundance (%) ^b			No./g (dry weight)			Biovolume ^c (μm^3)	Carbonbiomass ($\mu\text{g/g}$ (dw)) ^d		
		Mean \pm SD	Max	CV (%)	Mean \pm SD	Max	C.V. (%)	Mean \pm SD	Max	CV (%)		Mean \pm SD	Max	CV (%)
<i>Amphitrema flavum</i>	3	0.05 \pm 0.21	1	458	0.05 \pm 0.27	1.8	503	2.5 \pm 11.7	58	459	1.47 $\times 10^4$	0.004 \pm 0.019	0.09	459
<i>Arcella</i> sp.	1	0.02 \pm 0.12	1	806	0.02 \pm 0.16	1.3	806	0.9 \pm 7.3	59	806	1.50 $\times 10^4$	0.002 \pm 0.012	0.10	806
<i>Assulina muscorum</i>	55	8.29 \pm 11.4	61	137	12.5 \pm 13.7	60	109	530 \pm 715	4207	135	1.58 $\times 10^4$	0.918 \pm 1.239	7.29	135
<i>Assulina seminulum</i>	41	2.32 \pm 3.03	15	130	3.79 \pm 5.76	33	152	148 \pm 172	641	115	7.00 $\times 10^4$	1.142 \pm 1.317	4.94	115
<i>Bullinularia indica</i>	28	0.49 \pm 0.64	3	130	0.96 \pm 1.82	10	189	33.5 \pm 46.7	210	139	1.19 $\times 10^6$	4.374 \pm 6.098	27.4	139
<i>Centropyxis aerophila</i> var. <i>sphagnicola</i>	1	0.02 \pm 0.12	1	806	0.02 \pm 0.17	1.4	806	0.7 \pm 5.4	43	806	6.67 $\times 10^4$	0.005 \pm 0.039	0.32	806
<i>Centropyxis laevigata</i>	39	2.66 \pm 5.65	37	212	3.79 \pm 6.49	34	171	177 \pm 331	2242	187	3.45 $\times 10^5$	6.701 \pm 12.55	85.1	187
<i>Corythion dubium</i>	18	1.09 \pm 4.34	34	397	1.66 \pm 6.62	45	398	62.7 \pm 241	1858	384	2.67 $\times 10^4$	0.184 \pm 0.707	5.45	384
<i>Euglypha compressa</i>	50	7.26 \pm 15.9	121	218	8.25 \pm 11.9	77	144	486 \pm 1091	8462	225	7.79 $\times 10^4$	4.162 \pm 9.350	72.5	225
<i>Euglypha laevis</i>	1	0.03 \pm 0.25	2	806	0.01 \pm 0.10	0.8	806	2.4 \pm 19.4	156	806	9.24 $\times 10^3$	0.002 \pm 0.020	0.16	806
<i>Euglypha strigosa</i>	8	0.38 \pm 1.90	15	494	0.75 \pm 2.85	19	381	41.4 \pm 264	1974	594	3.21 $\times 10^4$	0.146 \pm 0.869	6.97	594
<i>Heleopera sylvatica</i>	2	0.08 \pm 0.44	3	577	0.03 \pm 0.16	1.2	589	4.6 \pm 25.9	159	567	2.36 $\times 10^5$	0.119 \pm 0.673	4.13	567
<i>Hyalosphenia elegans</i>	55	17.2 \pm 27.6	145	161	17.6 \pm 16.4	59	93	1185 \pm 1845	9380	156	4.33 $\times 10^4$	5.650 \pm 8.794	44.7	156
<i>Hyalosphenia minuta</i>	2	0.05 \pm 0.28	2	597	0.04 \pm 0.31	2.5	719	2.9 \pm 16.8	118	585	3.60 $\times 10^3$	0.017 \pm 0.100	0.70	585
<i>Hyalosphenia papilio</i>	56	15.3 \pm 19.9	102	130	20.9 \pm 21.3	74	102	1073 \pm 1387	6190	129	1.25 $\times 10^5$	0.425 \pm 0.549	2.45	129
<i>Nebela griseola</i>	1	0.03 \pm 0.25	2	806	0.01 \pm 0.10	0.8	806	1.6 \pm 12.7	103	806	7.15 $\times 10^4$	0.022 \pm 0.175	1.41	806
<i>Nebela militaris</i>	3	0.06 \pm 0.30	2	487	0.06 \pm 0.32	2.3	518	5.1 \pm 24.9	155	489	3.08 $\times 10^4$	0.040 \pm 0.196	1.22	489
<i>Nebela tinctoria</i>	57	17.1 \pm 28.4	180	166	16.0 \pm 15.1	69	95	1233 \pm 2174	14063	176	9.63 $\times 10^4$	4.183 \pm 7.366	47.6	176
<i>Nebela tinctoria</i> var. <i>major</i>	29	2.20 \pm 5.13	28	233	2.82 \pm 6.05	31	214	175 \pm 493	3158	282	2.64 $\times 10^5$	1.853 \pm 5.223	33.4	282
<i>Phryganella acropodia</i>	3	0.63 \pm 4.84	39	767	0.22 \pm 1.50	12	673	39.5 \pm 306	2468	776	1.79 $\times 10^4$	1.146 \pm 8.889	71.7	776
<i>Pseudodiffugia gracilis</i>	42	9.09 \pm 14.8	78	163	10.6 \pm 14.6	73	138	592 \pm 904	4149	153	5.39 $\times 10^4$	1.166 \pm 1.780	8.17	153
Total	65	89.8 \pm 72.7	334	81				5797 \pm 4800	21655	83		32.26 \pm 30.18	167	94

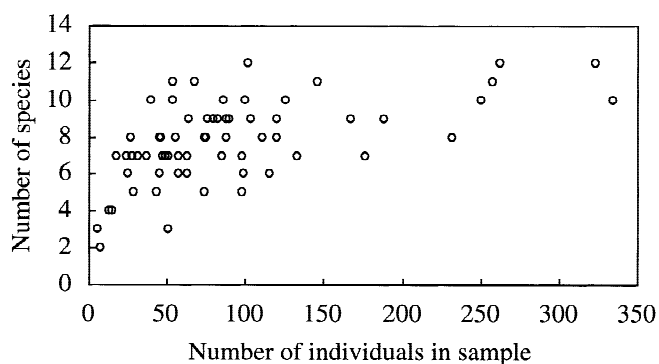
Mean number of species per sample \pm 1 standard deviation (S.D.) = 7.7 \pm 2.3; min = 2; max = 12; Coefficient of variation (C.V.) = 30%.

^a Number of individuals in the top 2 cm of the *Sphagnum* moss.

^b Relative abundance = 100 \times abundance/total number of individuals in the sample.

^c Biovolumes were estimated by assuming geometrical shapes.

^d Carbon-biomass was calculated from biovolumes using $1\mu\text{m}^3 = 0.11 \times 10^{-6}$ mgC [39].



higher than in autumn when our samples were taken. Fantham and Porter [12] recorded $0.8\text{--}7.0 \times 10^3$ testate amoebae individuals per g wet weight, which, following Heal [18], can be converted to $16\text{--}140 \times 10^3$ testate amoebae individuals per g dry weight, assuming 95% moisture content. Warner [37] found $100\text{--}400 \times 10^3$ testate amoebae individuals per g dry weight (calculated from $10\text{--}40 \times 10^3$ testate amoebae individuals per g wet weight—living and dead shells—and 90% average water content). However, direct comparison is difficult because of methodological differences between studies, mainly the depth range of the samples. Warner [37] might have counted many empty shells, as his samples extended down to the upper limit of the decomposing horizon; therefore, his figure may be over-

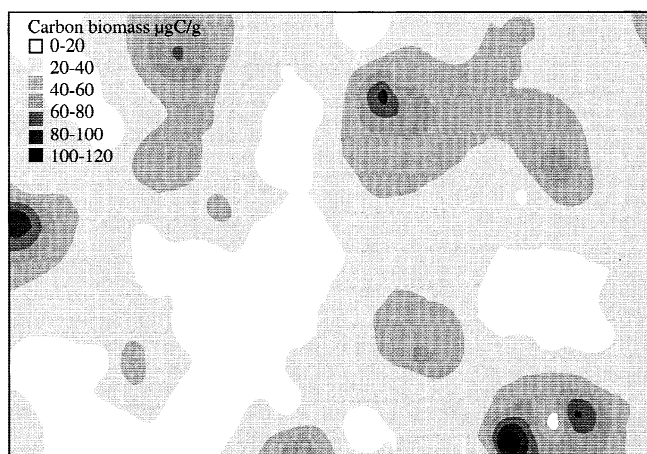


Fig. 2. Interpolated map of testate abundance carbon biomass (μgC per g dry weight). The maps were produced with the following gridding parameters: 6 points inverse distance gridding algorithm with no radial search, and a grid size of 1 cm.

estimated. Furthermore, testate amoebae are more abundant lower in the *Sphagnum* carpet than in the top part [25], and this may explain the relatively low numbers we recorded. Although we also counted empty shells, their proportion in the upper two centimeters is very low [17] and is unlikely to have significantly affected our estimations.

Testate amoeba biomass is quite uniform over the surface (Fig. 2). Only in five small areas, mostly single sample units, is testate amoebae biomass higher than the average figure of $32.3 \mu\text{g}$ carbon biomass per g dry weight. The only other study where testate amoebae carbon biomass was estimated [13] was carried out in a wetter, more minerotrophic peatland and results are expressed as mgC per ml. Therefore, a direct comparison of data is impossible.

The most abundant species (*H. elegans* and *H. papilio*) are characteristic for very wet microhabitats in the center of ombrotrophic bogs in the Jura mountains [26]. However, *H. elegans* is also abundant in *Sphagnum* lawn, and *H. papilio* may also be abundant in slightly minerotrophic *Sphagnum*. The next two most abundant species, *N. tinctoria* and *A. muscorum*, are ubiquitous in *Sphagnum* peatlands and are also abundant in other habitats such as forest mosses and humus. The variability (coefficient of variation) of both abundance and percentage abundance is important for all species, but lowest for the most frequent species.

Distribution Maps

The distribution maps (Fig. 3) of testate amoebae species' total number and total number of individuals suggest spatial heterogeneity. This is also visible on the distribution maps of the most frequent species on which the variability of species' abundance (also attested by the high coefficients of variation in Table 1) appears clearly. Furthermore, spatial distribution pattern also varies among species. This pattern contrasts with that observed for biomass (Fig. 2).

Spatially Constrained Clustering

The spatially constrained clustering of sample units (Fig. 4) shows the pattern of successive groups corresponding to different similarity levels. Interestingly, faunal differences are important between several pairs of sample units lying only a few centimeters apart and the opposite occurs with distant sample units sharing similar communities. The comparison of the maps in Fig. 3 and the clustering reveals which species is most abundant in the different groups. Two main groups appear: a first group containing the sample units with high

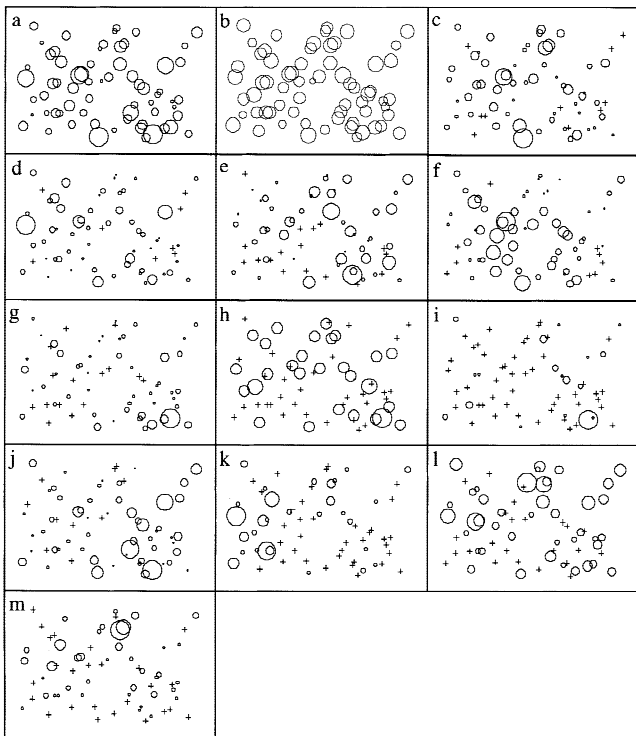


Fig. 3. Distribution map of testate amoebae in 65 *Sphagnum magellanicum* samples from a 40 × 60 cm surface in Le Cachot bog, Swiss Jura. Dot sizes are directly proportional to the number of individuals in the samples but are not comparable among maps. Crosses indicate the absence of the species in the sample unit. (a) Total number of testate amoebae individuals. (b) Total number of testate amoebae species. (c–m) Distribution maps for individual species: (c) *Assulina muscorum*, (d) *Nebela tinctoria*, (e) *Pseudodiffugia gracilis*, (f) *Hyalosphenia papilio*, (g) *Euglypha compressa*, (h) *Bullinularia indica*, (i) *Corythion dubium*, (j) *Hyalosphenia elegans*, (k) *Nebela tinctoria* var. *major*, (l) *Assulina seminulum*, (m) *Centropyxis laevigata*.

abundance of *Hyalosphenia papilio* and a second group, divided into several subgroups, with high abundance of *Pseudodiffugia gracilis*, *Nebela tinctoria*, and *Assulina muscorum*.

Spatial Autocorrelation

Significance levels follow the Holm [19] correction: to reach global significance, at least one individual value must be significant at a p -level of $\alpha/\text{number of classes}$, i.e., here $0.05/12 = 0.00417$; further values are significant at p -levels of 0.05/11, 0.05/10, etc.). The community level Mantel correlogram (Fig. 5) shows that two sample units closer than about 10 cm were significantly more similar than expected under the hypothesis of random distribution, and that samples lying about 45 to 50 cm apart are significantly more differ-

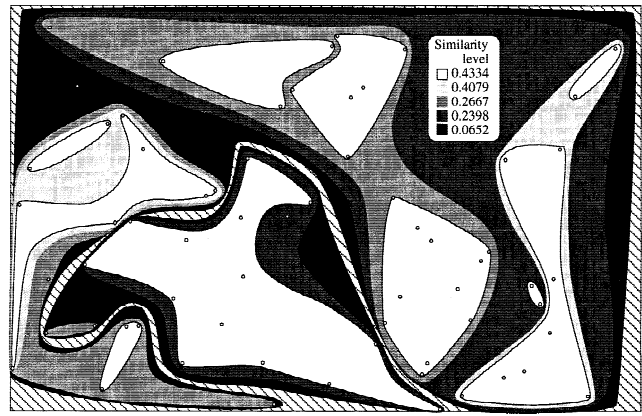


Fig. 4. Spatially constrained clustering of 65 samples from Le Cachot bog based on testate amoebae communities. See text for details.

ent. Total abundance and species richness also seem to show a positive, albeit nonsignificant, autocorrelation at short distances. Total abundance has a negative autocorrelation for sample distances of about 20 cm.

The Moran correlograms computed for the most frequent species reveal interspecific differences in spatial autocorrelation (Fig. 5). Only three species, *Euglypha compressa*, *Hyalosphenia elegans*, and *Nebela tinctoria* var. *major*, have a pattern similar to the community-level Mantel correlogram, with a positive autocorrelation for close samples and a negative one for samples about 50 cm apart. *Euglypha strigosa* and *Nebela tinctoria* have a positive autocorrelation for close samples (not significant for *N. tinctoria*) but no significant negative autocorrelation for distant samples. For *Assulina muscorum* and *Centropyxis laevigata* the pattern is similar, but no significant autocorrelation was found in the first distance class. A third pattern appears for *Hyalosphenia papilio*, for which autocorrelation is positive for samples closer than about 10 cm, negative for samples at distances of about 25 to 40 cm, and again positive for samples at distances of about 50 to 55 cm. Finally, four species, *Pseudodiffugia gracilis*, *Assulina seminulum*, *Bullinularia indica*, and *Corythion dubium*, have no significant autocorrelation at all.

Canonical Correspondence Analysis with Spatial Constraint

In the canonical correspondence analysis (CCA), 10 of the 40 spatial variables were selected as significant in the forward selection procedure and the first four axes are significant ($P < 0.05$, Monte-Carlo test, 999 permutations; Table 2). *A posteriori*, the first two canonical axes are significantly cor-

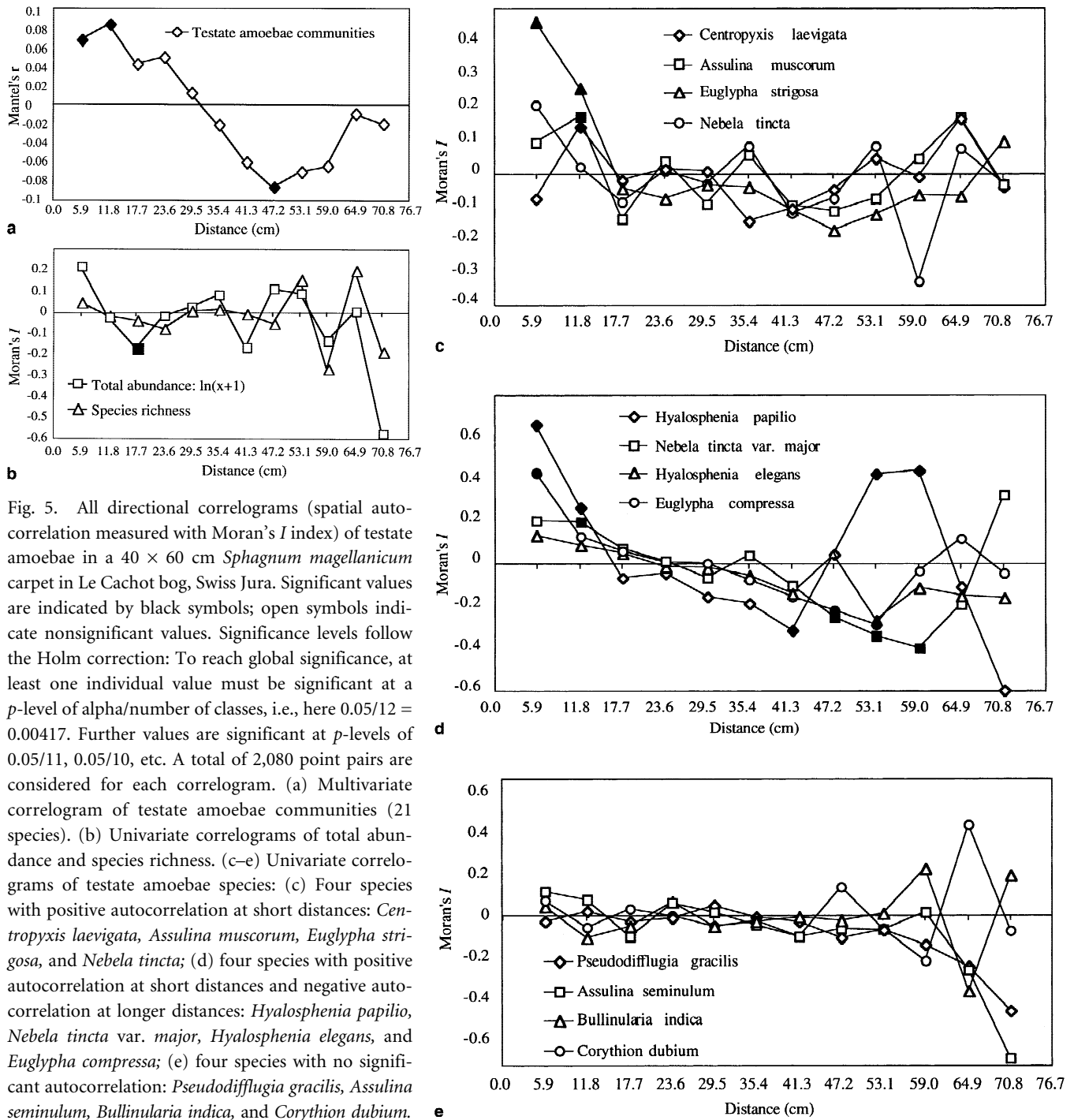


Fig. 5. All directional correlograms (spatial autocorrelation measured with Moran's I index) of testate amoebae in a 40×60 cm *Sphagnum magellanicum* carpet in Le Cachot bog, Swiss Jura. Significant values are indicated by black symbols; open symbols indicate non-significant values. Significance levels follow the Holm correction: To reach global significance, at least one individual value must be significant at a p -level of $\alpha/\text{number of classes}$, i.e., here $0.05/12 = 0.00417$. Further values are significant at p -levels of 0.05/11, 0.05/10, etc. A total of 2,080 point pairs are considered for each correlogram. (a) Multivariate correlogram of testate amoebae communities (21 species). (b) Univariate correlograms of total abundance and species richness. (c–e) Univariate correlograms of testate amoebae species: (c) Four species with positive autocorrelation at short distances: *Centropyxis laevigata*, *Assulina muscorum*, *Euglypha strigosa*, and *Nebela tincta*; (d) four species with positive autocorrelation at short distances and negative autocorrelation at longer distances: *Hyalosphenia papilio*, *Nebela tincta* var. *major*, *Hyalosphenia elegans*, and *Euglypha compressa*; (e) four species with no significant autocorrelation: *Pseudodifflugia gracilis*, *Assulina seminulum*, *Bullinularia indica*, and *Corythion dubium*.

related with the altitude (i.e., microtopography; axis 1 negatively correlated, $r = 0.46$, $P < 0.0001$; axis 2 positively correlated, $r = 0.47$, $P < 0.0001$). These correlations can also be seen by comparing the interpolated maps of microtopography (Fig. 6) and the canonical axes (Fig. 7). This means that the most important spatial patterns of the testate amoeba assemblage are conditioned by their reaction to ecological factors linked to the "altitude" of the *Sphagnum* carpet, i.e., microtopography.

Discussion

Microorganisms are not directly perceptible to the human observer. The testate amoebae studied live in the water film on the surface of the *Sphagnum* mosses. The mosses form carpets, with usually only one or two different species. *Sphagnum* species have ecological preferences regarding water table depth and water chemistry and values of these variables can be inferred from them [1]. In addition to the

Table 2. Summary of the canonical correspondence analysis.

Canonical axes	1	2	3	4
Eigenvalue	0.100	0.074	0.052	0.036
% of total variation	10.3	7.5	5.3	3.7
<i>P</i>	0.001	0.001	0.007	0.050
Species–environment correlation	0.85	0.83	0.72	0.71

35.8% of the total variation of the species matrix is explained by the 10 spatial variables. Total inertia = 0.978, sum of canonical eigenvalues = 0.350.

moss species, the microtopography provides some information on possible ecological gradients (moisture regime, water holding capacity, micro- and mesoporosity, water chemistry). However, inferring ecological conditions from the vegetation or the microtopography can be misleading [6]. Therefore, the macroscopic vegetation pattern is only a rough indication of microenvironmental conditions and is not *a priori* a good integrator of testate amoebae community requirements.

Several authors have studied the composition of testate amoebae communities across different microhabitats in North American and European peatlands. Early studies focused on faunal aspects [31, 17, 18, 25]. In such studies the testate amoebae faunas from contrasted habitats such as hummocks and hollows were compared and clear faunal differences were found. More recently the species' ecological preferences were determined quantitatively and characteristic species for specific microhabitats have been established [37, 36, 8, 9, 26]. Balik [2] studied testate amoebae communities in two ecotones (transition from either spruce or beech litter to soil covered by mosses) along 50-cm transects with a sampling interval of 1 cm. In both examples he found characteristic testate amoebae faunas corresponding to the different soil covers and a higher diversity at the contact between litter and mosses. All these studies focused on contrasted microhabitats where gradients were visible and thus significant differences in testate amoebae communities highly likely. In such situations, the random spatial and temporal fluctuations of communities are negligible compared to the magnitude of their response to ecological gradients. It may also be argued that such clear gradients are visible without the help of testate amoebae and their usefulness is therefore limited. Testate amoebae could be valuable indicators at a finer scale, but the limit of their use will be determined by the relative importance of random fluctuations as compared to their response to ecological gradients. Furthermore, the importance of random fluctuations is likely to depend on the species. Studying the spatial structure of testate amoebae

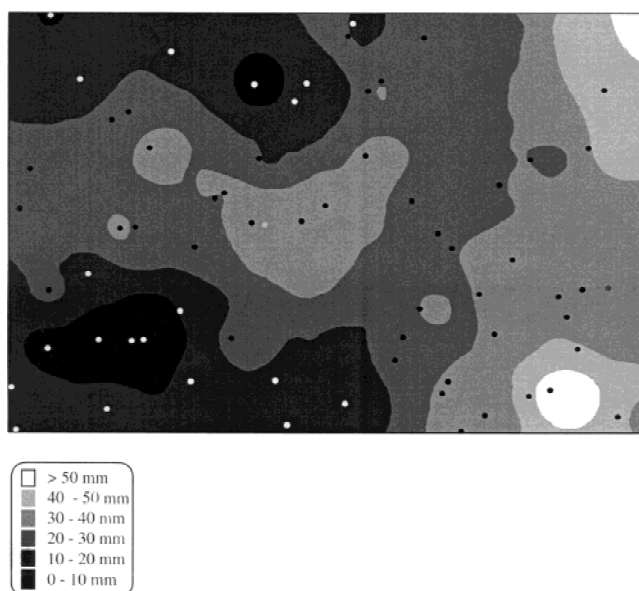


Fig. 6. Interpolated map of the microtopography (altitude in millimeters above an arbitrary level). Gridding parameters as for Fig. 2.

communities and the comparison of individual species' pattern can help to select the best indicator species.

Our study focused on the upper two centimeters of the *Sphagnum* mosses. Lower down the profile the abundance and diversity of testate amoebae species increase in relation to microenvironmental gradients of, e.g., light and moisture [25]. Therefore the horizontal structure is likely to vary with respect to depth. Microenvironmental conditions are probably less variable at greater depth and therefore testate amoeba communities may be more uniform.

The different analyses agree in suggesting that the area sampled is not perceived by testate amoebae as absolutely uniform. Interestingly, this heterogeneity would not have been clear from the pattern of total testate amoeba biomass, a result that clearly calls for the study of microbial communities at the species level. It can be argued that if totally uniform ecological conditions on a surface of 0.25 m² exist at all, such situations are very rare in *Sphagnum*-dominated peatlands. Ombrotrophic raised bogs are characterized by a complex vegetation of *Sphagnum* hummocks, lawns, and hollows. The transition from one microtopographic situation to another usually takes place within a few decimeters. Finding a surface, even relatively small, with no clear ecological gradients is difficult and where this situation exists, invisible gradients may still be present at the scale of testate amoebae.

The community-level Mantel correlogram revealed posi-

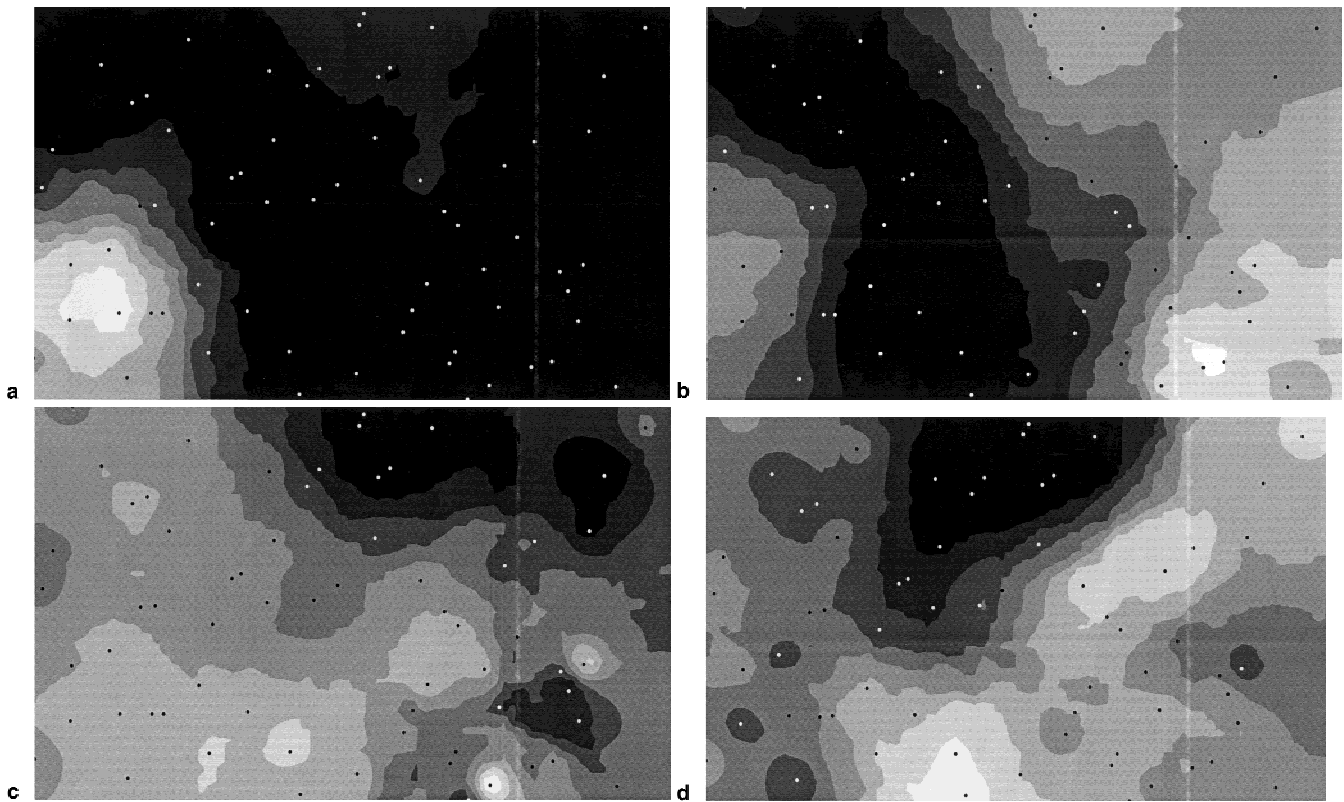


Fig. 7. Interpolated maps of the four significant ($P \leq 0.05$) canonical axes of the CCA constrained by spatial variables. (a) axis 1; (b) axis 2; (c) axis 3; (d) axis 4 (see text for details). Gridding parameters as for Fig. 2.

tive autocorrelation for distances up to about 15 cm and negative autocorrelation for distances between 45 and 50 cm. Such a combination is characteristic for spatial gradients [21], although such a gradient was not visible on a macroscopic scale in the field. However, the result does not allow exclusion of a periodicity (patchy distribution) with an inter-patch distance of about 1 meter. This pattern would be consistent with that of one of the most abundant species, *H. papilio*, which seems to show a patch-like distribution with smaller patches (50 cm). The negative autocorrelation of total abundance for distances between 15 and 20 cm suggests the existence of patches with high abundance, although the positive autocorrelation for short distances is not significant in this case. Short distance structures reveal contagious phenomena in the case of positive autocorrelation and avoidance in the case of negative autocorrelation. Both may relate to either to relationships with external environmental factors (such as food availability or the distribution of the water film) or to biotic processes such as intra- or interspecific relationships. But little is known of the trophic links within microbial communities or species interactions among testate amoebae species. The size range of testate amoebae species

(10 μm to over 150 μm) alone tends to indicate complex trophic links. Indeed, the larger species have been reported to prey on the smaller [15].

In the CCA with spatial constraint, 10 variables and 4 canonical axes are significant. These axes represent broad-scale (axes 1 and 2) as well as finer-scale (axes 3 and 4) structures in the testate amoeba assemblages. The fact that both axes 1 and 2 are significantly correlated to altitude not only shows that a relationship exists between amoebae and one or several ecological factors linked to microtopography, but also that this link translates into measurable spatial patterns in the amoeba assemblage. Moreover, our results show that significant spatial patterns can be detected at even finer scales (axes 3 and 4). This means that testate amoebae could indeed become useful tracers of processes acting at very fine spatial scales, provided that these patterns can be linked to ecological variables responsible for their generation.

Altitude may affect testate amoebae indirectly through the chemistry of capillary water and the moisture regime. In higher microtopographic situations such as hummocks, pH, and ion concentrations are usually lower than in hollows [16] and the moss species are also different. However, even

in less contrasted microhabitats such as monospecific carpet of *Sphagnum* mosses, the higher spots are more likely to dry out during summer drought, especially in lawn or pool species which do not form hummocks resistant to desiccation [30]. Moisture may affect testate amoebae directly, whereas water chemistry and moisture may affect the organisms (bacteria, microalgae, other protozoa, micrometazoa, fungi) on which they prey.

We therefore think that testate amoebae can provide information on the water regime and water chemistry at a finer scale than can mosses and, because the number of species is larger, the precision is likely to be higher. Furthermore, since testate amoeba assemblages also display broad-scale structures, they may be useful to monitor changes in peatland functioning related to climate change, atmospheric pollution, or nutrient input on a wide geographical scale.

However, before our results are used for management purposes we need fine-scale analyses of testate amoebae communities together with ecological variables measured at a very fine scale. Furthermore, a comparative study of several groups of organisms covering a wide range of sizes and biological types would allow selection of useful bioindicator groups. In such studies, a common methodology is needed to define the limits of the usefulness of each group in terms of spatial scale, ecological sensitivity, and speed of reaction to environmental change. The complexity of spatial distribution patterns has to be taken into account when using testate amoebae and other organisms in experiments or as biomonitors. Replications and a careful sampling design are therefore essential. Studies of the species' spatial distribution patterns in combination with autoecological studies in different habitats are needed to select the most useful species for biomonitoring and paleoecological reconstruction. Ultimately, our results show that techniques allowing very fine assessment of spatial structures may also be included in the toolbox of biomonitoring itself.

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References

1. Andrus RE (1986) Some aspects of *Sphagnum* ecology. *Can J Bot* 64:416–426
2. Balik V (1996) Testate amoebae communities (Protozoa, Rhizopoda) in two moss–soil microecotones. *Biol (Bratislava)* 51:125–133
3. Beyens L, Chardez D (1990) Evidence from testate amoebae for changes in some local hydrological conditions between c. 5000 BP and c. 3800 BP on Edgeøya (Svalbard). *Polar Res* 5:165–169
4. Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. *Ecology* 73:1045–1055
5. Borcard D, Geiger W, Matthey W (1995) Oribatid mite assemblages in a contact zone between a peat-bog and a meadow in the Swiss Jura (Acari, Oribatei): Influence of landscape structures and historical processes. *Pedobiol* 39:318–330
6. Bridgham SD, Pastor J, Janssens JA, Chapin C, Malterer T (1996) Multiple limiting gradients in peatlands: A call for a new paradigm. *Wetlands* 16:45–65
7. Buttler A, Warner BG, Matthey Y, Grosvernier P (1996) Testate amoebae (Protozoa: Rhizopoda) and restoration of cut-over bogs in the Jura, Switzerland. *New Phytol* 134:371–382
8. Charman DJ, Warner BG (1992) Relationship between testate amoebae (Protozoa: Rhizopoda) and microenvironmental parameters on a forested peatland in north-eastern Ontario. *Can J Zool* 70:2474–2482
9. Charman DJ, Warner BG (1997) The ecology of testate amoebae (Protozoa: Rhizopoda) in oceanic peatlands in Newfoundland, Canada: Modelling hydrological relationships for paleo-environmental reconstruction. *Ecoscience* 4:555–562
10. Cowling AJ (1994) Protozoan distribution and adaptation. In: Darbyshire JF (ed) *Soil Protozoa*. CAB International, Oxon, UK, pp 5–42
11. Ettema CH, Coleman DC, Vellidis G, Lowrance R, Rathbun SL (1998) Spatiotemporal distributions of bacterivorous nematodes and soil resources in a restored riparian wetland. *Ecology* 79:2721–2734
12. Fantham HB, Porter A (1945) The microfauna, especially the Protozoa, found in some Canadian mosses. *Proc Zool Soc Lond* 115:97–174
13. Gilbert D, Amblard C, Bourdier G, Francez A-J (1998) The microbial loop at the surface of a peatland: Structure, function and impact of nutrient input. *Microb Ecol* 34:83–93
14. Gilbert D, Amblard C, Bourdier G, Francez A-J (1998) Short-term effect of nitrogen enrichment on the microbial communities of a peatland. *Hydrobiol* 373/374:111–119
15. Gilbert D, Amblard C, Bourdier G, Francez A-J, Mitchell EAD (2000) Le régime alimentaire des thécamoebiens. *Année Biol*, in press
16. Gobat J-M, Aragno M, Matthey W (1998) Le sol vivant. *Bases*

- de pédologie, biologie des sols. Presses polytechniques et universitaires romandes, Lausanne
17. Heal OW (1962) The abundance and microdistribution of testate amoebae (Protozoa: Rhizopoda) in *Sphagnum*. *Oikos* 13:35–47
 18. Heal OW (1964) Observations on the seasonal and spatial distribution of testaceans (Protozoa: Rhizopoda) in *Sphagnum*. *J Animal Ecol* 33:395–412
 19. Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Statist* 6:65–70
 20. Legendre P (1990) Quantitative methods and biogeographic analysis. In: Garby DJ, South GR (eds) *Evolutionary Biogeography of the Marine Algae of the North Atlantic*, NATO ASI Series, Vol. G22. Springer-Verlag, Berlin, pp 9–34
 21. Legendre P, Fortin M-J (1989) Spatial pattern and ecological analysis. *Vegetatio* 80:107–138
 22. Legendre P, Legendre L (1998) *Numerical Ecology*, 2nd English edition. Elsevier, Amsterdam
 23. Legendre P, Vaudor A (1991) The “R” package—Multidimensional analysis, spatial analysis. Département de sciences biologiques, Université de Montréal
 24. Lousier JD (1974) Response of soil testacea to soil moisture factors. *Soil Biol Biochem* 6:235–239
 25. Meisterfeld R (1977) Die horizontale und vertikale Verteilung der Testaceen (Rhizopoda: Testacea) in *Sphagnum*. *Arch Hydrobiol* 79:319–356
 26. Mitchell EAD, Buttler A, Grosvernier P, Rydin H, Hoosbeek MR, Greenup A, Saarinen T (1999) Ecological patterns of testate amoebae (Protozoa) on peatlands in the Jura mountains, Switzerland and France. *Ecoscience* 6:565–576
 27. Mitchell EAD, Buttler A, Grosvernier P, Rydin H, Hoosbeek MR, Greenup A, Saarinen T (2000) Relationships among testate amoebae (Protozoa), vegetation and water chemistry in five *Sphagnum*-dominated peatlands in Europe. *New Phytol* 145:95–106
 28. Moran PAP (1950) Notes on continuous stochastic phenomena. *Biom* 37:17–23
 29. Oden NL, Sokal RR (1986) Directional autocorrelation: An extension of spatial correlograms to two dimensions. *Syst Zool* 35:608–617
 30. Rydin H (1985) Effect of water level on desiccation of *Sphagnum* in relation to surrounding *Sphagna*. *Oikos* 45:374–379
 31. Schneider DC (1994) *Quantitative Ecology: Spatial and Temporal Scaling*. Academic Press, New York
 32. Schönborn W (1962) Zur Ökologie der sphagnikolen, bryokolen und terrikolen Testaceen. *Limnol* 1:231–254
 33. Sokal RR (1986) Spatial data analysis and historical process. In: Diday E (ed) *Data Analysis and Informatics, IV*. North-Holland, Amsterdam
 34. ter Braak CJF (1988–1992) CANOCO—an extension of DECORANA to analyse species–environment relationships. *Vegetatio* 75:159–160
 35. Tolonen K (1986) Rhizopod analysis. In: Berglund BE (ed) *Handbook of Holocene Palaeoecology and Palaeohydrology*. John Wiley, Chichester, pp 645–666
 36. Tolonen K, Warner BG, Vasander H (1992) Ecology of Testaceans (Protozoa: Rhizopoda) in Mires in Southern Finland: I. Autecology. *Arch Protistenkd* 142:119–138
 37. Warner BG (1987) Abundance and microdistribution of testate amoebae (Rhizopoda, Testacea) in *Sphagnum* peatlands in Southwestern Ontario, Canada. *Arch Protistenkd* 133:173–189
 38. Warner BG (1990) Testate Amoebae (Protozoa). In: Warner BG (ed) *Methods in Quaternary Ecology*, Geoscience Canada, Reprint Series 5. Geological Association of Canada, St. John’s, pp 65–74
 39. Weisse T, Muller H, Pinto-Coelho RM, Schweizer A, Springmann D, Baldringer G (1990) Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. *Limnol Oceanogr* 35:781–794