

Changes in Testate Amoebae (Protists) Communities in a Small Raised Bog. A 40-year Study

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Summary. We analyzed the testate amoebae communities from two sets of moss samples taken forty years apart (1961 and 2001) in the same locations of a peat bog of the Swiss Jura Mountains. Peat cutting and lateral drainage of Le Cachot bog have caused a clear increase in tree cover, especially near the edges. Changes affecting herbaceous plants, mosses, or soil organisms can be subtle, and may easily be overlooked. We hypothesized that we would see changes in the dominant *Sphagnum* species and the structure of testate amoebae communities living in the mosses. More specifically, we hypothesized that the frequency of bryophyte and testate amoebae species indicative for dry conditions would increase and that the frequency of species indicative for wet conditions would decrease. The mean testate amoebae species richness per sample decreased from 11.9 to 9.6, but the overall species richness was identical (33 species) in both years. Three species increased significantly in mean relative abundance: *Nebela tinctoria* s. l. (+97%), *Bullinularia indica* (+ 810%), and *Cyclopyxis eurystoma* (+ 100%; absent in 1961), while two species decreased significantly: *Assulina muscorum* (- 63%) and *Euglyphis compressa* (-93%). The testate amoebae communities clearly differed among microhabitat types (hummocks, lawns, hollows), but no overall significant change in the community was detectable between the two sampling dates (Mantel test). These results could signify that changes at the microscopic level had already taken place by 1961 and no further overall significant changes in micro-environmental conditions took place during the 40-year period of this study. This would agree with the faster response time usually attributed to microorganisms and would also imply that the bushes and trees may be poor predictors of the response of microorganisms if they are themselves in a non-equilibrium stage. Other possible causes for the lack of overall differences are discussed.

Key words: autogenic succession, drainage, long-term changes, Protozoa, *Sphagnum*-dominated peatland, testaceans.

INTRODUCTION

In Central Europe many peatlands have been damaged or destroyed, mainly through peat extraction, drainage for cultivating crops or for forestry. Today, in many European countries, the conservation of the remaining peatlands has become a priority. The two main

aims of such conservation measures are the preservation of biodiversity and of the carbon pool and sequestration functions of peatlands (Grünig 1994, Raeymaekers 2000, Joosten and Clarke 2002).

An important challenge for the management of the remaining peatlands is the effect human activities have had and continue to have on their hydrology. The surface of most remaining peatlands is significantly smaller than it was originally resulting in a lowering of the water table starting in the periphery and extending towards the central part of the mires (Freléchoux *et al.* 2000a). Such effects are especially dramatic in the case of small

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raised bogs, which are quite common in mountainous areas of Central Europe. The drainage of peatlands causes changes in the vegetation and biogeochemical functioning. Peatlands often become overgrown with trees (pine, spruce, or birch) and from C sinks become C sources as decomposition rates increase. Although peatlands in some cases are capable of recovering from the damage, this process takes long time (Chapman *et al.* 2003). In a conservation perspective, although small wetlands can play a vital role in the persistence of local population of wetland-associated organisms (Gibbs 1993), even preserving the remaining peatlands may not suffice to stop the loss of unique species related to the wettest habitats because of the gradual modification of small raised bogs through autogenic vegetation succession due to change in the hydrology (Freléchoux *et al.* 2000b). The challenge here is that such changes happen slowly and can easily go unnoticed unless, as is rarely the case, a good monitoring program is established. For protistologists, a further challenge is that there is much less available data than for other taxonomic groups, especially plants and animals.

A number of peatlands have been studied around the middle of the 20th century. In some cases, especially when the author of these early studies is still active, it is possible to go back and collect new data to study the changes that have taken place. Several such studies have recently been published, but none of them have included microorganisms. Gunnarsson and others observed changes in pH, conductivity and plant communities in two Swedish mires. The observed changes were interpreted either as autogenic succession, or indirect human impact through acid precipitation and increased N availability (Gunnarsson *et al.* 2000, 2002). Chapman and Rose investigated changes in the vegetation of a peatland in the UK over a period of 28 years and found important shifts in the vegetation that were interpreted as being due to afforestation in the periphery of the bog as well as changes in the use of the bog itself (Chapman and Rose 1991).

We analyzed the changes in the testate amoebae communities from a small raised bog of the Swiss Jura Mountains, Le Cachot bog, over a period of forty years, between 1961 and 2001. Generations of scientists from the Neuchâtel region have studied the ecology, paleoecology, vegetation, arthropod and protist communities of peatlands of the Jura Mountains since the mid 19th century (Lesquereux 1844). See Matthey and Borcard (1996) for a list of some of these studies. One of these scientists, Dr Willy Matthey had kept *Sphagnum* samples

he had collected in 1961 and was able to lead the second author through the mire to the same spots to sample a second series of samples in 2001. This made it possible for us to assess how much testate amoebae communities have changed in this site over a period of forty years.

Like most peatlands of the region, as a consequence of peat cutting, the surface of Le Cachot peatland has been strongly reduced over the years and its edges are constituted of more or less eroded peat extraction walls. The resulting lateral drainage causes changes in the vegetation on the bog with a strong increase in tree cover, especially near the edges of the peatland. Vegetation changes however are not instantaneous and, although the site has not suffered from peat extraction in recent decades and is now protected by law, recent comparative studies have shown a significant increase in tree cover and reduction of small water bodies - many of which were the result of peat extraction activities - over the second part of the 20th century (Matthey 1998, 2000, 2001). Changes in tree cover are relatively easy to see and less likely to be overlooked than more subtle changes such as those affecting herbaceous plants, mosses, or soil organisms. *Sphagnum* species have relatively well-defined preferences along the water table depth gradient (Vitt and Slack 1984; Rydin and McDonald 1985; Rydin 1986, 1993; Vitt 2000). Testate amoebae also respond well to this gradient (Tolonen 1986; Charman and Warner 1992, 1997; Tolonen *et al.* 1992; Warner and Chmielewski 1992; Charman 1997; Bobrov *et al.* 1999; Mitchell *et al.* 1999; Booth 2001, 2002). Hence we hypothesized that we would see changes in the dominant *Sphagnum* species and the structure of testate amoebae communities living in the mosses. More specifically, we hypothesized that the frequency of bryophyte and testate amoebae species indicative for dry conditions would increase and that the frequency of species indicative for wet conditions would decrease.

MATERIALS AND METHODS

Site description, site history, and field sampling

La Cachot bog is located in the Jura Mountains, northwest Switzerland (47.5°N, 6.4°E), at an altitude of 1050 m a. s. l. The climate of the region is favorable for peatland development: The annual mean precipitation is 1446 mm and 36% of the precipitation falls between June and September. The mean annual temperature is 4.7°C and ranges between 9.7 and 13.3°C between June and September although it may fall below 0°C at any time of the year. The relative humidity is

usually almost 100% in the evening so the area is often foggy in the morning. Winter temperatures can be extremely cold during windless nights (the record is almost -40°C) due to the frequent inversion of air masses caused by the closed basin topography of the valley (karst landscape).

Traditional peat harvest for heating was practiced until the end of World War II and caused the loss of approximately 90% of the original surface of raised bogs in the region (Grünig 1994). Peat extraction has now stopped and in places natural regeneration is taking place (Matthey 1996). At present, Le Cachot bog is delimited in several places by 1 to 2 meter-high vertical peat walls that are slowly eroding. The bog surface located near these walls is dry because of the lateral drainage due to the modified topography. The peat extraction process had also created some pools, many of which are now being re-colonized by the vegetation. As a result, their number and surface area has been decreasing substantially (Matthey 1998, 2000, 2001). The lateral drainage has clearly been beneficial to trees, mainly pine (*Pinus rotundata*) and birch (*Betula pubescens*), and to ericaceous shrubs (e.g. *Vaccinium uliginosum*, *V. myrtillus*, *V. vitisidaea*, and *Calluna vulgaris*). This site, like other comparable ones in the region, nevertheless has a high conservation value for the many rare species of plants and animals that still thrive on it, as well as for other animals, not necessarily linked to peatlands, but that may benefit from the food, water or protection it offers (Matthey and Borcard 1996).

Dr Willy Matthey collected a number of *Sphagnum* samples in 1961 in different microhabitats of Le Cachot bog. A total of 23 samples were available for this study. In September 2001, Dr Willy Matthey lead the second author to the study site to collect *Sphagnum* samples from, as close as possible to the same spots where the 1961 samples had been taken. The general characteristic of the sites were recorded at the same time (Table 1). All mosses were air-dried and preserved in envelopes until extractions could be done. The relative proportion of different bryophytes was estimated in each sample to the nearest 5%. All bryophytes were identified to the lowest taxonomic level. Taxonomy follows Tutin *et al.* (1964-1980) for vascular plants, Isoviita (1967) for *Sphagnum*, and Smith (1980) for other mosses.

Testate amoebae extraction and analysis

The top 3 cm of the mosses was used for testate amoebae extraction. The mosses and about 75 ml of water were boiled for 10 min to detach the tests from the mosses. The samples were filtered through a tea strainer first then through a 300 µm mesh, then through a 150 µm mesh and finally over a 20 µm filter. The fraction between 150 µm and 20 µm was then back sieved and preserved in 5 ml vials with glycerol. Microscopic slides were prepared and amoebae tests were identified and counted at 200× and 400× magnification. The following taxonomic references were used for identification (Deflandre 1929, 1936; Grospietsch 1958; Ogden and Hedley 1980; Ogden 1983; Lüftenegger *et al.* 1988; Charman *et al.* 2000). For each sample a minimum total of 150 individuals were counted. One or more entire slides were counted for each sample. The rotifer *Habrotrocha angusticollis* was also counted but treated separately in the numerical analyses. Both living and dead amoebae are included in the counts. Given that only the top 3 cm were counted and that *Sphagnum* mosses usually grow at least 1cm a year, we consider the counts to be representative of the mean testate amoebae community over a 3-year period.

Data analyses

The following grouping of species were used: (1) *Nebela tinctoria* s. l., includes *Nebela tinctoria*, *Nebela tinctoria* var. *major*, *N. collaris*, *N. bohémica* and *N. parvula*; (2) *Centropyxis aerophila* s. l. includes *Centropyxis aerophila* and *Centropyxis cassis*; (3) *Phryganella acropodia* s. l. includes *P. acropodia* and *Pseudodiffugia gracilis*; (4) *Phryganella* sp. includes *Phryganella paradoxa* and similar taxa such as some small *Diffugia* species. In addition, *Heleopera sylvatica* was excluded from the data because it appeared that this species might in some cases have been mistaken for other similar species (at least in lateral view) such as some *Diffugia* species. For six samples from 1961 the available material was very limited and the total count was less than 100. These samples were therefore excluded from both the 1961 and 2001 data sets, leaving 17 samples for each year and 34 in total. As a consequence of this, three rare species, *Centropyxis cassis* var. *spinifera*, *Trinema complanatum*, and *Diffugia bacillifera* were lost from the data. Because entire slides were counted, in some 2001 samples the total number of individual counted was much higher than the target of 150 to 200 individuals. As a result there was a significant difference between the mean total count between the 2001 and 1961 samples. Such a difference could cause a bias in the data if very rare species were found in the samples with a higher count. As the limited amount of material available from 1961 did not allow us to increase the counts for these samples we randomly reduced the count of four samples from 2001 (samples 8, 9, 14 and 23) to bring the total below 300 individuals. This was sufficient to reduce the mean total count difference between the two sets of samples and make it no longer significant.

We compared the species richness, Shannon-Wiener diversity and evenness indices between the two data sets using t-tests after checking for normality of the data. The multivariate community data of bryophytes and testate amoebae were analyzed using Mantel tests to assess if there was a significant difference in community structure between the 1961 and 2001 samples (Legendre and Legendre 1998). For each of these analyses we used two similarity matrices: (1) a species × samples matrix and (2) a binary variable represented the two sampling dates. A Steinhaus similarity coefficient was used for the species percentage data and a simple matching coefficient was used for the binary variable (Legendre and Legendre 1998). The possible influence of rare testate amoebae species on the result was assessed by removing successively the species that occurred (1) only once (5 species), (2) once or twice (9 species), or (3) up to three times (13 species) in the overall data set. These tests were performed using the R package free software (Casgrain and Legendre 2004). To illustrate in reduced space the structure of the multidimensional testate amoebae data we performed a detrended correspondence analysis (DCA) using the software CANOCO (Ter Braak 1988-1992).

RESULTS

Micro-habitat types

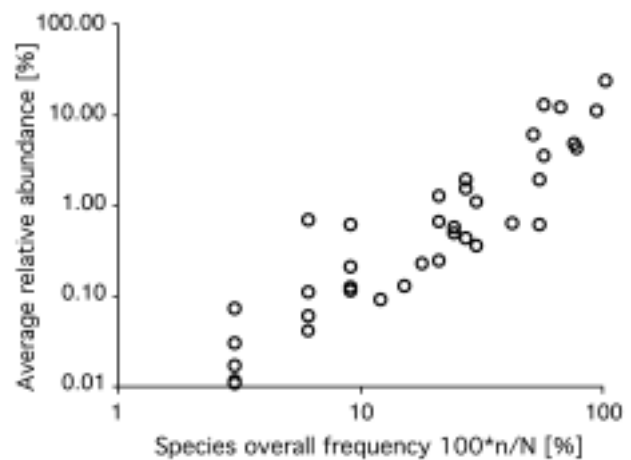
A brief description sampling sites is given in Table 1. We identified four micro-habitat types: hummocks, lawns, hollows, and non-*Sphagnum* mosses (usually growing on very dry hummocks or in closed pine forests). At

Table 1. Description of the 17 sampling sites from Le Cachot bog used in the study.

Sample Nr.	1961	Site descriptions	2001
1	Small hollow		Lawn on the edge of a <i>Pinus rotundata</i> forest
4	Hollow near a <i>Sphagnum fuscum</i> hummock		Lawn approximately 20 m from the edge of a <i>Pinus rotundata</i> forest
5	Hummock, not far from sample 4		Hummock in an open <i>Pinus rotundata</i> forest
6	Hollow <i>Pinus rotundata</i> forest		Small hollow in an open
7	Lawn of an open <i>Pinus rotundata</i> forest		Lawn with tussocks on the edge
8	Small hollow not far from the <i>Pinus rotundata</i> forest edge		Lawn with low bushes on the edge of an open <i>Pinus rotundata</i> forest
9	Lawn with <i>Andromeda polifolia</i> on the side of a hollow		Hollow in an open <i>Pinus rotundata</i> forest
10	Lawn		Wet lawn
11	Small hollow in an open <i>Pinus rotundata</i> forest		Low, wet hummock in a pool
12	Hummock near a <i>Pinus rotundata</i> tree		Lawn in open <i>Pinus rotundata</i> forest
13	Small cushion with <i>Polytrichum strictum</i> in heath vegetation		Lawn, no <i>Sphagnum</i>
14	Hummock in <i>Vaccinium</i> heath a <i>Pinus rotundata</i> forest, no <i>Sphagnum</i>		Opening with low bushes in
15	Lawn		Lawn in an open <i>Pinus rotundata</i> forest
17	Hummock at the edge between the pine forest and the open <i>Sphagnetum magellanici</i> open bog community		Lawn with tussocks on the edge of an open <i>Pinus rotundata</i> forest
21	<i>Pinus rotundata</i> forest		Closed <i>Pinus rotundata</i> forest
22	<i>Pinus rotundata</i> forest with <i>Polytrichum strictum</i>		Closed <i>Pinus rotundata</i> forest
23	<i>Pinus rotundata</i> forest		Closed <i>Pinus rotundata</i> forest

**Fig. 1.** Changes in the dominant moss type and microtopography of 17 sampling sites in Le Cachot bog between 1961 and 2001. Hollows, lawns and hummocks all were dominated by *Sphagnum* mosses.

most sites the microtopography either did not change or seemed to have evolved towards drier conditions between 1961 and 2001. As all the sites sampled in 1961 were dominated by *Sphagnum* mosses, the total number of possible transitions was 12. Of these 12 possible transitions, only seven were actually observed (Fig. 1). The microhabitat status of seven sites did not change. Of those that changed, seven became drier (four hollows became lawns and three hummocks lost their *Sphagnum* cover) while only three became wetter (one lawn became a hollow and two hummocks became lawns). However, this trend towards drying was not significant (paired sign test, $P = 0.3438$).

**Fig. 2.** Relationship between the frequency of testate amoebae species and their overall mean relative abundance.

Bryophytes

Overall our samples contained 14 species of bryophytes in 1961 and 12 species in 2001. There was no significant difference in the species richness of individual samples between the two sampling periods ($2.12 \pm \text{S.E. } 0.30$ in 1961; $2.06 \pm \text{S.E. } 0.23$ in 2001). Three species increased both in terms of frequency and relative abun-

Table 2. Number of presences and average relative abundance of 14 bryophyte species in 17 samples from Le Cachot. Swiss Jura Mountains, in 1961 and 2001.

	Number of presences			1961		Relative abundance [%]		Change 1961-2001	
	1961 n	2001 n	Change	Average	SE	2001 Average	SE	Absolute [%]	
<i>Aulacomnium palustre</i>	3	2	-1	0.9	0.5	0.6	0.1	-0.3	-33
<i>Mylia anomala</i>	1	1	0	0.3	0.3	0.3	0.1	0.0	0
<i>Pleurozium schreberi</i>	2	5	3	0.6	0.4	9.4	2.3	8.8	1500
<i>Polytrichum strictum</i>	5	3	-2	3.8	2.6	12.4	3.0	8.5	223
<i>S. angustifolium</i>	1	5	4	4.1	4.1	22.9	5.6	18.8	457
<i>S. capillifolium s. l.</i>	3	3	0	12.6	7.7	2.9	0.7	-9.7	-77
<i>S. contortum</i>	2	0	-2	7.6	6.0	0.0	0.0	-7.6	-100
<i>S. cuspidatum</i>	2	1	-1	11.8	8.1	4.7	1.1	-7.1	-60
<i>S. fallax</i>	3	2	-1	10.9	7.3	9.7	2.4	-1.2	-11
<i>S. fuscum</i>	3	3	0	10.9	6.5	13.5	3.3	2.6	24
<i>S. magellanicum</i>	5	8	3	11.8	6.6	17.6	4.3	5.9	50
<i>S. quinquefarium</i>	1	0	-1	5.6	5.6	0.0	0.0	-5.6	-100
<i>S. rubellum</i>	2	1	-1	7.1	5.5	0.6	0.1	-6.5	-92
<i>S. subsecundum</i>	3	1	-2	12.1	7.5	5.3	1.3	-6.8	-56

dance between 1961 and 2001: *Pleurozium schreberi*, *Sphagnum angustifolium*, and *S. magellanicum*. In addition to these, *Polytrichum strictum*, and *S. fuscum* increased in relative abundance but either decreased or did not change in frequency. Seven species decreased in relative abundance between 1961 and 2001: *S. capillifolium*, *S. cuspidatum*, *S. fallax*, *S. rubellum*, *S. subsecundum*, *S. quinquefarium* and *S. contortum*, the latter two being totally absent from the 2001 samples (Table 2). There was no overall significant difference in bryophyte community structure between the two years as assessed by a Mantel test between a similarity matrix of a binary site variable and a similarity matrix of bryophyte relative abundance ($P = 0.491$; 999 permutations).

Testate amoebae

A total of 6795 testate amoebae were identified and counted in this study (3641 in 2001 and 3154 in 1961). The mean count per sample was 200 (SE: 8.3). Overall a total of 37 taxa were recorded. The overall mean species richness per sample was 10.8 (SE: 0.5). The overall mean relative abundance of testate amoebae taxa was positively correlated with their frequency in the samples (Fig. 2). The four most abundant taxa *Nebela tinctoria s. l.*, *Hyalosphenia papilio*, *Amphitrema flavum*, and *Assulina muscorum* together made up 64.5% of the total count. The eight most abundant taxa accounted for 84.7% the total count (Table 3). The rotifer *Habrotrocha angusticollis* was observed in 18 samples and its mean

relative density was 1.2 % (SE: 0.3) of the total testate amoebae count (Table 3).

The total species richness of individual sampling periods was identical (33 species) in 1961 and 2001, although four of the 37 taxa recorded were absent from each set of samples. The mean species richness per sample however was significantly lower in 2001 than 1961 (means and SE in 1961 and 2001 respectively 11.9 ± 0.7 and 9.6 ± 0.7 , paired t-test $p = 0.042$, unpaired t-test $p = 0.022$; Table 3). However, the diversity and evenness indices were not significantly different between 1961 and 2001 (Table 4).

Overall the relative abundance of 21 taxa increased between 1961 and 2001 while that of 16 taxa decreased. However, due to the variability in the data few of these differences were significant. Three taxa, *Nebela tinctoria s. l.* (+ 97%), *Bullinularia indica* (+ 810%), and *Cyclopyxis eurystoma* (+ 100%; absent in 1961) increased significantly between 1961 and 2001, while two other species significantly decreased: *Assulina muscorum* (- 63%) and *Euglypha compressa* (- 93%). Despite these differences, neither the changes in relative abundance of the species nor the change in rank of relative abundance could be related to their known preferences for water table depth or pH in the region (Mitchell *et al.* 1999) (data not shown). The rotifer *Habrotrocha angusticollis* was more frequent in 2001 ($n=13$) than 1961 ($n=5$). The relative abundance (% of the total testate amoebae count) of this species increased significantly from 0.43 (SE 0.20) in 1961 to 1.92 (SE 0.49) in

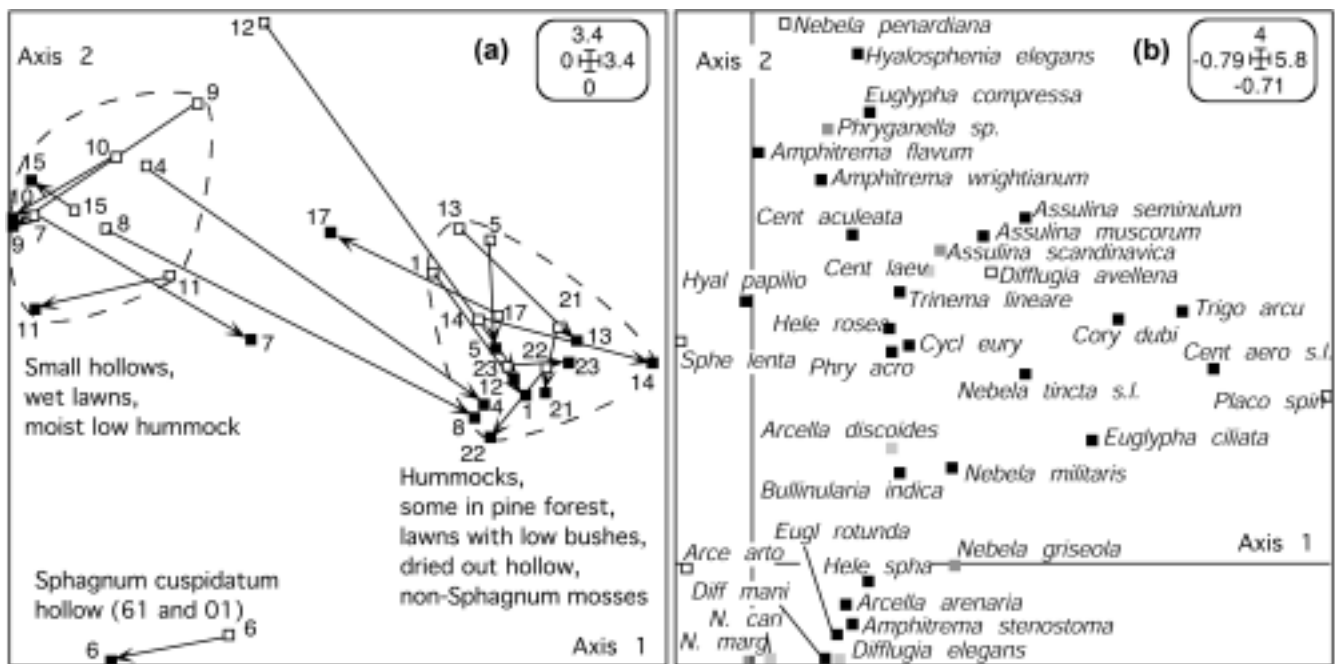


Fig. 3. Scatter diagram of a DCA of testate amoebae data with representation of samples (a) and species (b). Axes 1 and 2 explain 21.5 and 12.8% of the total variation in the species data. Samples from 1961 are represented by open squares and samples from 2001 by black squares. Arrows connect each pair of samples. Groups of samples are outlined in dashed lines and the types of micro-habitats sampled are summarized. Rare species that occurred in a single sample, two and three samples are represented respectively by open squares, light gray squares and dark gray squares.

2001 (paired t-test $p = 0.013$, unpaired t-test $p = 0.009$; Table 3).

The position of samples in the first two axes of the DCA revealed which samples shared similar testate amoebae communities and how similar or dissimilar pairs of samples were between 1961 and 2001 (Fig. 3). Most samples were clustered into two major groups corresponding to (1) samples taken mostly in moist lawns and (2) samples taken mostly in hummocks, forests and non-*Sphagnum* mosses. In addition some samples were projected in between these two groups and the pair of samples no 6, representing a wet *Sphagnum cuspidatum* hollow in 1961 and 2001 stood out as being a group of their own. For each sampling sites arrows linking pair of samples illustrate the changes in testate amoebae communities over the 40-year period. In most cases the 1961 and 2001 samples were quite similar but some pairs of samples underwent important changes (e.g. samples 4, 7, 8, 9, 12, and 17). Interestingly, while some of these suggest drier conditions in 2001 than in 1961 (samples 4, 7, and 8), others seem to have become wetter (samples 9, 12, and 17).

The position of testate amoebae in the ordination shows which species were associated with the different groups of samples and the known ecology of the species in the region (Mitchell *et al.* 1999) can be used to interpret the ordination space. Species indicative for low water table, such as *Corythion dubium*, *Nebela tincta*, *Nebela militaris*, *Trigonopyxis arcula*, and *Euglypha ciliata* have high scores on the first axis of the ordination. Species characteristic of bog pools such as *Nebela carinata*, *Nebela marginata*, and *Amphitrema stenostoma* have low scores on both axis 1 and 2, in agreement with the position of samples 6 (*Sphagnum cuspidatum* hollow). Other species, such as *Amphitrema flavum*, *Amphitrema wrightianum*, and *Hyalosphenia papilio*, usually found in bog hollows but also abundant in moist lawns had low scores on axis 1 but higher scores on axis 2.

Despite the changes observed for some species between 1961 and 2001, no overall significant difference was found between the two data sets: The Mantel tests did not reveal any overall significant differences between the two data sets (999 permutations

Table 3. Relative abundance of testate amoebae and the rotifer *Habrotrochoa angusticollis* in moss samples from Le Cachot bog collected in 1961 and 2001.

	Relative abundance										n 1961 N=17	n 2001 N=17	Change					
	Overall		1961		2001		Change		t-tests					P-value		Relative abundance rank		
	Average	SE	Average	SE	Average	SE	%1*	SE	Paired	Unpaired				Overall	1961	2001	Change	
<i>Amphitrema flavum</i>	12.70	3.18	16.08	4.79	9.31	4.18	-42	4.18	0.106	0.294	3	3	3	0	22	13	9	-4
<i>Amphitrema stenostoma</i>	1.67	0.65	1.13	0.86	2.21	0.97	95	0.97	0.322	0.414	11	13	9	-4	9	3	6	3
<i>Amphitrema wrightianum</i>	1.38	0.95	2.28	1.88	0.47	0.33	-80	0.33	0.362	0.347	12	10	20	10	7	4	3	-1
<i>Arcella arenaria</i>	0.68	0.26	0.23	0.14	1.13	0.49	381	0.49	0.098	0.088	16	23	14	-9	14	4	10	6
<i>Arcella artocrea</i>	0.01	0.01	0.00	0.00	0.02	0.02	100	0.02	0.332	0.325	36	34	32	-2	1	0	1	1
<i>Arcella discoides</i>	0.04	0.03	0.00	0.00	0.09	0.06	100	0.06	0.203	0.194	33	34	26	-8	2	0	2	2
<i>Assulina muscorum</i>	11.82	2.35	17.25	3.84	6.39	2.07	-63	2.07	0.015	0.018	4	2	5	3	31	17	14	-3
<i>Assulina scandinavica</i>	0.13	0.09	0.22	0.18	0.04	0.04	-82	0.04	0.342	0.327	27	24	31	7	3	2	1	-1
<i>Assulina seminulum</i>	3.78	1.29	5.45	2.37	2.12	0.96	-61	0.96	0.131	0.202	8	6	10	4	19	13	6	-7
<i>Bullinularia indica</i>	0.47	0.18	0.09	0.07	0.85	0.34	810	0.34	0.050	0.039	21	28	16	-12	9	2	7	5
<i>Centropyxis aculeata</i>	0.27	0.12	0.37	0.21	0.16	0.14	-57	0.14	0.419	0.395	23	20	23	3	7	5	2	-3
<i>Centropyxis aerophila</i> s. l.	1.15	0.70	0.48	0.22	1.83	1.39	283	1.39	0.360	0.344	13	17	11	-6	10	5	5	0
<i>Centropyxis laevigata</i>	0.12	0.10	0.04	0.04	0.20	0.20	403	0.20	0.449	0.438	29	30	21	-9	2	1	1	0
<i>Corythion dubium</i>	4.59	1.18	3.95	1.43	5.23	1.91	32	1.91	0.620	0.595	7	7	6	-1	26	16	10	-6
<i>Cyclopyxis eurystoma</i> s. l.	0.10	0.05	0.00	0.00	0.20	0.09	100	0.09	0.052	0.044	30	34	22	-12	4	0	4	4
<i>Diffflugia avellana</i>	0.02	0.02	0.04	0.04	0.00	0.00	-100	0.00	0.332	0.325	35	31	34	3	1	1	0	-1
<i>Diffflugia elegans</i>	0.73	0.69	1.46	1.39	0.00	0.00	-100	0.00	0.309	0.301	14	12	34	22	2	2	0	-2
<i>Diffflugia manicata</i>	2.06	1.23	1.91	1.60	2.21	1.93	15	1.93	0.507	0.907	10	11	8	-3	9	6	3	-3
<i>Euglypha ciliata</i>	5.26	1.13	3.57	1.03	6.95	1.97	95	1.97	0.055	0.137	6	8	4	-4	25	13	12	-1
<i>Euglypha compressa</i>	0.38	0.15	0.71	0.27	0.05	0.03	-93	0.03	0.030	0.020	22	14	30	16	10	8	2	-6
<i>Euglypha rotunda</i>	0.51	0.29	0.37	0.17	0.65	0.56	76	0.56	0.644	0.634	20	21	19	-2	8	5	3	-2
<i>Heleopera rosea</i>	0.70	0.43	0.57	0.30	0.83	0.83	45	0.83	0.783	0.773	15	16	17	1	7	6	1	-5
<i>Heleopera sphagni</i>	0.61	0.27	0.27	0.24	0.94	0.49	249	0.49	0.250	0.225	19	22	15	-7	8	2	6	4
<i>Hyalosphenia elegans</i>	6.50	2.67	9.60	4.68	3.40	2.52	-65	2.52	0.287	0.252	5	5	7	2	17	9	8	-1
<i>Hyalosphenia papilio</i>	14.14	3.74	11.65	4.43	16.64	6.10	43	6.10	0.282	0.512	2	4	2	-2	19	12	7	-5
<i>Nebela carinata</i>	0.06	0.05	0.02	0.02	0.11	0.11	360	0.11	0.461	0.450	32	32	25	-7	2	1	1	0
<i>Nebela griseola</i>	0.12	0.08	0.09	0.06	0.15	0.15	69	0.15	0.719	0.708	28	29	24	-5	3	2	1	-1
<i>Nebela marginata</i>	0.65	0.52	0.13	0.13	1.17	1.02	805	1.02	0.333	0.321	18	27	13	-14	3	1	2	1
<i>Nebela militaris</i>	2.08	0.62	2.75	1.01	1.41	0.69	-49	0.69	0.338	0.282	9	9	12	3	18	10	8	-2
<i>Nebela pennadana</i>	0.01	0.01	0.02	0.02	0.00	0.00	-100	0.00	0.332	0.325	37	32	34	2	1	1	0	-1
<i>Nebela tinctoria</i> s. l.	25.88	4.27	17.44	4.37	34.31	6.88	97	6.88	0.026	0.047	1	1	1	0	34	17	17	0
<i>Phryganella acropodia</i> s. l.	0.65	0.14	0.60	0.16	0.71	0.24	18	0.24	0.703	0.708	17	15	18	3	18	10	8	-2
<i>Phryganella</i> sp.	0.23	0.20	0.43	0.39	0.02	0.02	-95	0.02	0.318	0.309	25	18	33	15	3	2	1	-1
<i>Placocista spinosa</i>	0.03	0.03	0.00	0.00	0.06	0.06	100	0.06	0.332	0.325	34	34	29	-5	1	0	1	1
<i>Sphenodeia lenta</i>	0.08	0.08	0.15	0.15	0.00	0.00	-100	0.00	0.332	0.325	31	26	34	8	1	1	0	-1
<i>Trigonopyxis arcuata</i>	0.24	0.10	0.41	0.18	0.08	0.08	-81	0.08	0.133	0.106	24	19	27	8	6	5	1	-4
<i>Trinema lineare</i>	0.14	0.07	0.21	0.12	0.07	0.07	-67	0.07	0.356	0.328	26	25	28	3	5	4	1	-3
Average total count	200	8.3	186	8.0	214	14.0	15	14.0	0.103	0.085					18	5	13	8
Total species richness	37		33		33		0											
Average sp richness	10.8	0.5	11.9	0.7	9.6	0.7	-19	0.7	0.042	0.022								
<i>Habrotrochoa angusticollis</i> #	1.2	0.3	0.43	0.20	1.92	0.49	350	0.49	0.013	0.009								

- percentage of the total testate amoebae count; * - calculated as $100 \times a/b$ (where a = 2001, and b = 1961 relative abundance).

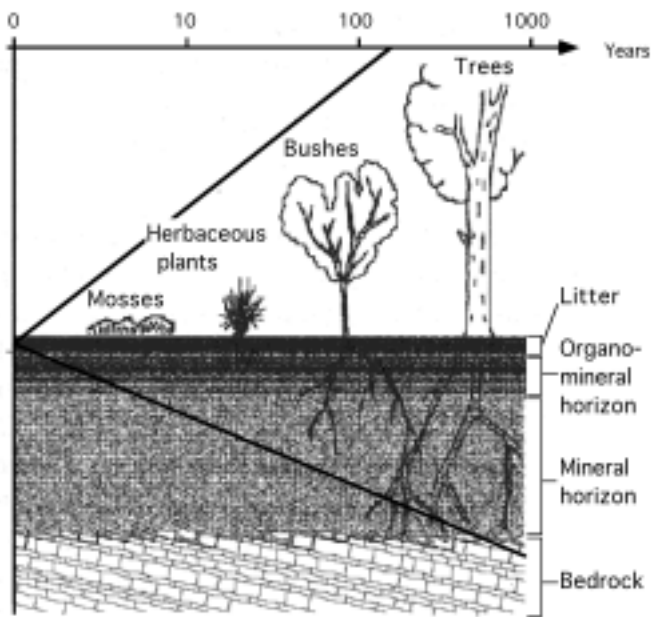


Fig. 4. Comparative response time of different plant strata and soil horizons to changes in the environment. (Modified from “Le Sol vivant”, Jean-Michel Gobat, Michel Aragno and Willy Matthey, Presses polytechniques et universitaires romandes, copyright 2003). By analogy, the response time of different soil organisms is expected to increase with size.

p-value = 0.096). Removing rare species occurring in a single, two or three samples did not influence the result.

DISCUSSION

The most significant changes that can be observed at the landscape level are the growth of trees, mainly pines (*Pinus rotundata*) but also locally birch (*Betula pubescens*), at the periphery of the bog and the infilling through natural succession of many small water bodies. Indeed, Matthey (1998, 2000, 2001) recorded a decrease in the number of open water bodies from 43 to 24 and a reduction of the overall surface from 309 m² to 176 m² over the same time period. With such dramatic changes at the macroscopic level we expected to see major changes in the bryophyte communities and also, at the microscopic level, in the testate amoebae communities, but only few significant changes were detected and therefore our data provided little support to our hypothesis.

No overall significant change was found between 1961 and 2001 in the bryophyte communities despite

some changes in absolute and relative abundance of some species. For example, the dramatic increase in *Pleurozium schreberi* could be seen as a clear indication that in several sampling sites conditions were drier in 2001 than in 1961. Furthermore, species such as *Sphagnum cuspidatum* and *S. subsecundum*, typically associated with wet hollows were among those that decreased most dramatically. One of the species that decreased, *S. capillifolium*, is usually found in dry conditions, but this species was replaced by *Polytrichum strictum*, *Pleurozium shreberi*, or *S. angustifolium* three species that are often associated with equally or more dry conditions. However, in some cases the changes may suggest shift to wetter conditions, for example for sample number 22 where *Polytrichum strictum* disappeared and was replaced by *S. magellanicum*.

Our results show a significant reduction in testate amoebae diversity, but no changes in the Shannon-Wiener diversity or equitability. Although the overall species richness was exactly the same (33 species in both years), the mean value per sample decreased significantly from 11.9 to 9.6. Furthermore five species either increased or decreased significantly. However no clear interpretation can be drawn from these effects. For example, while *Nebela tinctoria s. l.* increased *Assulina muscorum* decreased, but as both species are indicators of dry conditions in peatland of the region (Mitchell *et al.* 1999) these contradictory responses cannot be interpreted as an indication of changes in hydrology. A better taxonomic resolution for the *Nebela tinctoria s. l.* species complex might have allowed further interpretation. Furthermore, despite the significant response of some of the dominant species, the Mantel test revealed no overall significant difference was found between 1961 and 2001 at the community level. Several tentative explanations can be given for the overall low level of response of testate amoebae.

The first possible explanation related to the sampling protocol: As the sampling sites were not permanently marked, it may be that, for some sites at least, the 2001 sampling spot did not correspond exactly to the one of 1961. However, it must be pointed out that Dr Willy Matthey knows the Le Cachot bog very well, having studied it in detail continuously since the early 1960s. Nevertheless, although for many sampling sites he was able to point to the exact spot where the moss sample had been taken in 1961, in a few cases he was not able to locate the spot within less than 1 m and, in these cases, a representative sampling site for the general area was selected. For this reason, both paired and unpaired test

Table 4. Diversity indices calculated from the testate amoebae data from Le Cachot bog.

Sample No	Species richness			Shannon-Wiener diversity			Shannon-Wiener equitability		
	1961	2001	Change	1961	2001	Change	1961	2001	Change
1	17	6	-11	0.71	0.18	-0.53	0.25	0.10	-0.15
4	14	12	-2	0.63	0.76	0.13	0.24	0.31	0.07
5	8	14	6	0.53	0.89	0.37	0.25	0.34	0.08
6	15	13	-2	0.91	0.87	-0.05	0.34	0.34	0.00
7	11	10	-1	0.56	0.48	-0.08	0.23	0.21	-0.02
8	15	12	-3	0.76	0.71	-0.04	0.28	0.29	0.01
9	12	8	-4	0.70	0.39	-0.31	0.28	0.19	-0.09
10	13	5	-8	0.69	0.37	-0.32	0.27	0.23	-0.04
11	11	13	2	0.70	0.50	-0.20	0.29	0.20	-0.10
12	9	11	2	0.48	0.61	0.13	0.22	0.25	0.04
13	10	7	-3	0.76	0.65	-0.10	0.33	0.34	0.01
14	13	7	-6	0.84	0.73	-0.11	0.33	0.38	0.05
15	13	12	-1	0.61	0.48	-0.13	0.24	0.19	-0.05
17	11	7	-4	0.79	0.58	-0.21	0.33	0.30	-0.03
21	8	8	0	0.63	0.38	-0.25	0.30	0.18	-0.12
22	8	11	3	0.48	0.78	0.30	0.23	0.33	0.10
23	15	8	-7	0.82	0.61	-0.21	0.30	0.29	-0.01
Mean	11.9	9.6	-2.3	0.68	0.59	-0.09	0.28	0.26	-0.02
SE	0.7	0.7	0.7	0.03	0.05	0.67	0.01	0.02	0.67
t-test P-values									
Paired			<u>0.042</u>			0.105			0.389
Unpaired			<u>0.022</u>			0.102			0.463

Table 5. Comparison of meteorological measurements from Neuchâtel, Switzerland between a three and a five year period preceding the 1961 and 2001 sampling of *Sphagnum* mosses in Le Cachot bog, detail for the main growing period (May to September).

	Five year period				t-test P-value	Three year period				t-test P-value
	5.1957-9.1961		5.1997-9.2001			5.1959-9.1961		5.1999-9.2001		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Minimum temperature [°C]	11.8	2.1	13.0	2.0	<u>0.045</u>	11.9	2.1	13.1	2.0	0.109
Maximum temperature [°C]	21.5	2.5	22.0	2.5	0.487	21.8	2.6	22.1	2.4	0.719
Precipitation [mm]	85	38	93	31	0.368	85	47	91	27	0.644
Mid-day relative humidity [%]	69.6	4.5	69.8	4.6	0.882	68.3	4.7	69.8	3.1	0.296

results are given. As testate amoebae show heterogeneous horizontal distribution patterns even in apparently homogeneous surfaces in response to micro-environmental gradients (Mitchell *et al.* 2000), and any uncertainty in the location of the 1961 sampling sites can be expected to have an important effect on the data. In this respect this study can be viewed as a test for the limit of using testate amoebae as biomonitors in *Sphagnum* peatlands. Their micro-distribution patterns may make it impossible to simply return to a given sampling spot years or decades after an initial study unless the initial

sampling sites were marked permanently. A paleoecological approach on a series of short cores taken in different parts of the peatland, or the same sampling spots would eliminate this problem and also offer the opportunity to see how testate amoebae communities changed between 1961 and 2001. However, one challenge of this approach will be to obtain exact dates for the different sampling levels.

The second possible explanation is that the climate changed over the 40-year period in a way that counteracted the effect of drainage and vegetation succession.

We used to nearest reliable meteorological survey station, in Neuchâtel (487 m a.s. l. - n. b. approximately 600 m lower than the studied peatland which is located at 1050 m a.s. l.) to determine if the climate (parameters analyzed: monthly average of minimum and maximum temperatures, precipitation and relative humidity) had changed between a three and a five-year period preceding the two sampling periods. Three to five years corresponds to the approximate time covered by mosses sampled for testate amoebae. The climate record suggests that for the growing period (May through September) of the five-year period preceding the 1961 sampling the average minimum temperature was warmer than for the same period preceding the 2001 sampling. These observations are in agreement with the long-term climatic observations for Switzerland where a clear general warming is due mostly to an increase in minimum (i.e. night) temperature while a slight but significant reduction in maximum temperatures is observed (Rebetez 2001, Rebetez *et al.* 2003). However, the trend for warmer minimum temperatures was not significant when only the three-year preceding the samplings were compared (Table 5). By contrast no significant difference was observed for the same time periods for precipitation and relative humidity (Table 5). If we accept that the trends in climatic data measured at Neuchâtel, at a much lower elevation than Le Cachot bog (487 m vs. 1050 m), are representative for the studied site, we consider it unlikely that changes in climate could have compensated for the effects of drainage and vegetation changes at the bog surface.

The third possible explanation is that if indeed major changes in micro-environmental conditions did occur, the testate amoebae did not respond to them. This would be in contradiction with (a) results from multiple studies on the ecology of testate amoebae (Tolonen 1986; Warner 1990; Charman and Warner 1992, 1997; Tolonen *et al.* 1992, 1994; Warner and Chmielewski 1992; Charman 1997; Mitchell *et al.* 1999; Booth 2001, 2002), (b) their fine-scale distribution patterns in relation to micro-topography (Mitchell *et al.* 2000), and (c) the observed rapid changes in testate amoebae communities during peatland regeneration (Buttler *et al.* 1996).

The fourth possible explanation, and the one we consider most likely, is that most of the changes at the microscopic level had already taken place when the 1961 samples were taken and no overall significant changes in micro-environmental conditions took place during the 40-year period covered by this study. This

would agree with the faster response time usually attributed to microorganisms (Foissner 1987, 1999). This would also agree with the observed differential response times of different plant strata and soil horizons to environmental change (Gobat *et al.* 2003) (Fig. 4). This interpretation also implies that the bushes and trees are poor predictors of the response of microorganisms if they are themselves in a non-equilibrium stage. A series of short peat cores in this or comparable bogs would probably be the best way to test this hypothesis.

Despite the lack of overall clear change between the two sampling dates, the testate amoebae clearly differed among the microhabitat types as attested by the position of wet pool, lawn and hummock samples in the ordination space (Fig. 3). Furthermore, most of the sample pairs for which marked differences between 1961 and 2001 appeared in the DCA (i.e. samples 4, 7, 8, 12, and 17) indeed corresponded to observed changes in the microhabitat and *Sphagnum* species (Table 1). These observations confirm the well-established responsiveness of testate amoebae to the major ecological gradients that exist in *Sphagnum*-dominated peatlands.

Opportunities to carry out studies such as this one are few, owing to the small number of ecological studies that were done several decades ago. An invaluable asset to alleviate some of the uncertainties of such studies would be to have permanently marked sampling sites, but these do not always exist.

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