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Relationships among testate amoebae (Protozoa), vegetation and water chemistry in five *Sphagnum* -dominated peatlands in Europe

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SUMMARY

To study the relationships between groups of organisms and the degree to which these relationships are consistent across major climatic gradients, we analysed the testate amoeba (*Protozoa*) communities, vegetation and water chemistry of one peatland in five countries: Switzerland, The Netherlands, Great Britain, Sweden and Finland, as part of the BERI (Bog Ecosystem Research Initiative) project. The relationships between the different data sets and subsets were investigated by means of detrended correspondence analysis, canonical correspondence analysis and Mantel permutation tests. The comparison of data on vegetation and testate amoebae showed that inter-site differences are more pronounced for the vegetation than for the testate amoebae species assemblage. Testate amoebae are a useful tool in multi-site studies and in environmental monitoring of peatlands because: (1) the number of species in *Sphagnum*-dominated peatlands is much higher than for mosses or vascular plants; (2) most peatland species are cosmopolitan in their distributions and therefore less affected than plants by biogeographical distribution patterns, thus differences in testate amoeba assemblages can be interpreted primarily in terms of ecology; (3) they are closely related to the ecological characteristics of the exact spot where they live, therefore they can be used to analyse small-scale gradients that play a major role in the functioning of peatland ecosystems. This study revealed the existence of small-scale vertical gradients within the vegetation and life-form niche separation in response to water chemistry. The deep-rooted plants such as *Carex* spp. and *Eriophorum* spp. are related to the chemistry of water sampled at or near the ground water table, whereas the mosses are not. Testate amoebae were

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shown to be ecologically more closely related to the chemistry of water sampled at or near the water table level and to the mosses than to the deep-rooted plants.

Key words: peatland, vegetation, testate amoebae, water chemistry, biogeography, microenvironmental gradients.

INTRODUCTION

Hydrodynamics and hydrochemistry are determinant factors for peatland development (Bridgham *et al.*, 1996). Peatlands are characterized by gradients of nutrients (eutrophic–oligotrophic), hydrology (ombrogenous–geogenous) and acidity. The relationships to environmental gradients are well known for vegetation (Glaser *et al.*, 1990), especially for *Sphagnum* (Clymo, 1973; Andrus, 1986; Rydin, 1993), and for animal communities, especially invertebrates (Borcard & Vaucher-von Ballmoos, 1997). These different groups of organisms respond to, and can be indicative for, these variables. However, less is known about the degree to which the relationships between these structuring groups are consistent across major climatic gradients, and to what extent they react to environmental differences.

In conservation biology there is growing interest in relationships in species diversity of high-order taxa, but the results are highly variable (Prendergast *et al.*, 1993; Prendergast & Eversham, 1997). Studies of species composition among such taxa could reveal the degree to which they are dependent on one another and the same or different environmental variables, and help to explain the variability in species diversity. Very few studies of community composition include species from different high-order taxa (e.g. phytosociological relevés compared with analyses of diatoms; de Sloover *et al.*, 1996).

Among the different groups of microorganisms, the testate amoebae (Protozoa; Rhizopoda) are both abundant and diverse in *Sphagnum* mosses (Warner, 1987). Their well defined ecological preferences in relation to important ecological variables governing peatlands (such as water table depth and the concentration of ions) have long been recognized (for review see Tolonen, 1986) and have made them useful in ecological studies (Charman & Warner, 1992; Tolonen *et al.*, 1992, 1994). In addition, testate amoebae produce shells which are well preserved in peat and allow paleoenvironmental reconstruction (Warner & Charman, 1994; Mitchell, 1995; Buttler *et al.*, 1996). Testate amoebae have been shown to be sensitive to micro-habitat variation (i.e. a mire's surface with the transition from a *Sphagnum* lawn to hummock) and to macro-scale variation between geographical regions (Warner, 1987; Mitchell *et al.*, 1999). Furthermore, they are small (10–300 μm long) and most abundant in the upper few centimetres of the moss carpet, in the living part of *Sphagnum* mosses and in the poorly decomposed litter below. Recent studies on the effect of nitrogen fertilization on microorganisms in a *Sphagnum*-dominated peatland have shown that testate amoebae have a central position in the microbial trophic network and react rapidly to environmental changes (Gilbert *et al.*, 1998a,b).

In this study we examined the relationships between three independently collected data sets (testate amoebae, vegetation and water chemistry) originating from five *Sphagnum*-dominated peatlands across Europe. Our aims were (1) to improve understanding of the factors affecting testate amoebae communities; and (2) to determine the scale at which testate amoebae are sensitive to environmental factors, thus improving the precision of inferences that may be drawn from amoebae in ecological and paleoecological studies.

MATERIALS AND METHODS

Study sites

We chose five sites of representative lawn communities in ombrotrophic or near-ombrotrophic peatlands, in the major climates where *Sphagnum* peatlands occur in Europe (Fig. 1, Table 1). These sites were chosen as they allowed delivery of CO_2 needed in the BERI project (Hoosbeek *et al.*, 1995).

Boreo–nemoral (*southern Sweden*). The Kopparås Mire, in the boreo–nemoral zone of southern Sweden, is mainly open with scattered, small trees of *Pinus sylvestris*. The mire is weakly minerotrophic, but smaller spots are ombrotrophic. The water supplies are both soligenous and, to a lesser extent, topogenous. The mire surface slopes slightly (1:164) towards the south-south west. The lawn vegetation of the area studied is dominated by *Eriophorum angustifolium*, *Calluna vulgaris*, *Andromeda polifolia*, *Narthecium ossifragum* and *Scirpus caespitosus*. Predominant peat mosses are *Sphagnum magellanicum*, *S. papillosum* and *S. rubellum*.

Continental–boreal (*Ilomantsi*, *eastern Finland*). The Salmisuo Mire, Ilomantsi, Finland is mainly open with scattered, small trees of *P. sylvestris*. Most parts are weakly minerotrophic, but small ombrotrophic spots exist. The water supply is both soligenous and, to a lesser extent, topogenous. The mire surface slopes slightly (1:200) towards the west. The vegetation is dominated by graminoids such as *Eriophorum vaginatum* and *Carex pauciflora* and, to a lesser extent, by dwarf shrubs such as *A. polifolia*, *Empetrum nigrum*, *Chamaedaphne calyculata* and *Vaccinium oxycoccos*. Scattered shoots of *Carex*

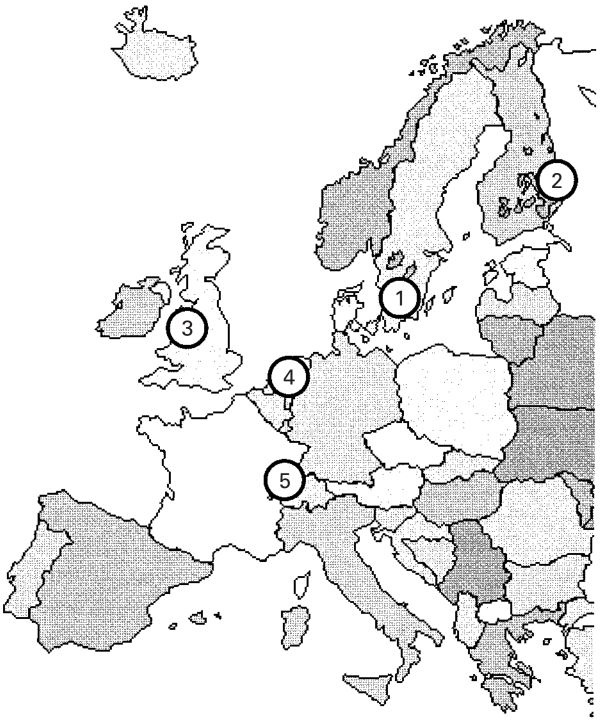

Fig. 1. Map of Europe showing the location of the five sites. 1, Kopparås mire, south Sweden; 2, Salmisuo mire in Ilomantsi, eastern Finland; 3, Roudsea bog, north-west England; 4, peat from Dwingelo mire transplanted to Wageningen; 5, La Chaux-des-Breuleux, Swiss Jura.

lasiocarpa can also be found. On lawn communities, the dominant peat mosses are *Sphagnum balticum* and *S. papillosum* with the subdominants *S. magellanicum* and *S. rubellum*. Scattered hummocks are dominated by *S. angustifolium*, *S. fuscum*, *Polytrichum strictum* and *Aulacomnium palustre*.

Nemoral, strongly Atlantic (*north-west England*). At Roudsea, on the edge of the English Lake District National Park, the mire surface slopes slightly towards the west. The site has been drained, but the drainage ditches were blocked and secondary vegetation is well established. Most of the surface is covered by lawn communities with isolated hollows. The vegetation is dominated by *E. vaginatum*, *S. caespitosus*, *Erica tetralix* and *S. papillosum*.

Nemoral, mild Atlantic (*The Netherlands*). The site is a small (150 × 50 m) peat area in the State Forestry Dwingeloo, province of Drenthe. The mire developed in a depression (part of a late glacial brook system in and along which pingos arose) and rests on loamy sand and boulder clay. Up to 1955 the site was used by local farmers for peat cutting. Today the vegetation consists of a mosaic of secondary successional stages including pools, carpets and hummocks. Common species are *E. tetralix*, *V. oxycoccos* and *S. magellanicum*.

Subalpine (*Jura, Switzerland*). La Chaux-des-Breuleux lies in the subalpine zone in the Jura mountains. The mire developed on the bottom of a shallow valley, on impermeable marl deposits. The mire was drained and peat was mined until the end of World War II. The mire surface slopes slightly (1 : 200) towards the south. The drainage ditches are colonized by *Sphagnum fallax*, *Carex rostrata*, *E. angustifolium* and *Potentilla palustris*. Between the ditches a bog vegetation has re-established through a natural regeneration process (Grosvernier *et al.*, 1995) and a mosaic of lawn, hummocks and depressions is now well developed with *E. vaginatum*, *Carex nigra* and *V. oxycoccos*. The dominant mosses are *S. fallax* and *P. strictum*.

Vegetation analysis

At each site, 20 plots (10 in Britain), 1 m in diameter, were randomly selected in a visually homogeneous part of the mire. The vegetation was analysed using two methods: point-intercept measurements and classical vegetation relevés. The point-intercept method (Buttler, 1992) was chosen in order to obtain a quantitative, objective measure of abundance for

Table 1. *Location, climatic data and nitrogen deposition at the five sites*

Country	Name, location and height above sea level of field site	Climate*	Nitrogen deposition†	Water table depth (mm)‡
Sweden	Kopparås mire, 57° 7.5′ N, 14° 30′ E, 225 m	16°C, −2°C, 800 mm, 80–120 d snow	Medium	102
Finland	Salmisuo in Ilomantsi, 62° 47′ N, 30° 56′ E, 155 m	16°C, −10°C, 650 mm, 150–200 d snow	Low	106
Britain	Roudsea bog, 54° 14′ N, 3° 1′ W, 5 m	13°C, 1°C, 1800 mm, < 20 d snow	Medium	106
Netherlands	Dwingelo, 52° 50′ N 6° 40′ E, 13 m	18°C, 3°C, 750 mm, < 10 d snow	High	92
Switzerland	La Chaux-des-Breuleux, 47° 13′ N, 7° 3′ E, 1000 m	15°C, −5°C, 1390 mm, 80–120 d snow	Medium	324

*Mean daily temperature in warmest and coldest month, annual precipitation, number of days with snow cover.

†Low: < 1 g m^{-2} yr^{-1}; medium: 1–3 g m^{-2} yr^{-1}; high > 3 g m^{-2} yr^{-1}.

‡Average over 3 yr, 1996–98 weekly measurements of 20 plots during the growing season.

the species. We chose a homogeneous subplot (35 × 22.5 cm) with a square grid of 2.5 cm pitch which gave a total of 150 points. At each point a sharp needle (1 mm diameter) was lowered vertically into the vegetation. As the needle was lowered, the contacts of plant species with the tip of the needle were noted. Frequencies expressed as percentages were calculated for each species, giving a quantitative measurement of cover. The vegetation relevés were made on the entire plots of 0.79 m^2 to obtain a general description and a better assessment of rare species. In this method the percentage cover of all species was estimated by eye. All data were collected at the peak biomass period. Authorities for plant species follow Corley *et al.* (1981) for mosses, Grolle (1983) for hepatics, and Tutin *et al.* (1964–80) for vascular plants.

Water samples

Water samples were collected from all plots at the five sites using Millipore soil-moisture samplers (Rhizon, Eijkelkamp BV, The Netherlands). The samplers were inserted in the moss carpet as close as possible to the water table, and connected to pre-evacuated glass bottles. The water samples were taken at the beginning of the 1996 growing season and in spring, summer and autumn, 1997 and analysed for dissolved organic carbon (DOC), pH, total N and P, and major cations and anions (Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, NO_3^-, Cl^-, SO_4^{2-}) at Wageningen Agricultural University, following Buurman *et al.* (1996). The three data sets from 1997 were used to check for seasonal variations. Similarity matrices (Euclidian distance on centred-reduced data) were significantly correlated (Mantel permutation tests between pairs of matrices, $P < 0.001$). Therefore, although the concentrations of different chemical species varied during the year, the similarity between pairs of samples remained comparable.

Testate amoebae sampling and analyses

At the beginning of the 1996 growing season 5–10 *Sphagnum* mosses were extracted from each plot. Each moss was cut at 3 and 5 cm depth, and testate amoeba shells were extracted from the 3–5 cm section following Warner (1990). Moss samples were boiled in water to detach the amoebae, and filtered (mesh diameter 300 μm). The shells were then concentrated by centrifugation and the samples stored in vials. All shells living and dead were identified and counted by the same person (E. M.) to a minimum number of 150. Frequencies expressed in percentage were calculated for each species. Nomenclature follows Tolonen (1986).

Numerical analyses

(1) Data on vegetation (point-intercept and cover-estimation relevés) and testate amoebae were first submitted to separate detrended correspondence analyses (DCA: CANOCO Program, ter Braak, 1988–92) to show variations of species assemblages in relation to the sites. Both analyses were carried out using the same option in the program: detrending by 26 segments, no data transformation.

(2) Partial canonical correspondence analyses (CCA: CANOCO Program) were used to determine how much of the variation in the data sets for testate amoebae and vegetation (point-intercept data only) was explained by the water chemistry data. The significance of the water chemistry variables was tested by means of a Monte Carlo permutation test in the forward selection procedure. No data transformation was applied. Four samples out of 90 (one from Switzerland and three from The Netherlands) with extreme values, detected by CANOCO as having an extreme influence on the regressions, were deleted. Five dummy variables (coded 0/1) representing the five sites were used as covariables, and permutations were limited within these five blocks. Testate amoebae and plant species occurring only once in the data set were omitted in order to reduce the noise in the data set and thus reduce the total variation in the matrix. This reduced the number of testate amoeba species to 40. The point-intercept vegetation data were subdivided into three sets: all plants, 31 species; deep-rooted vascular plants (*Carex* spp., *Eriophorum* spp., *E. tetralix*, *C. vulgaris*, *E. nigrum*, *S. caespitosus*, *N. ossifragum* and *Rhychospora alba*, 11 species); mosses, lichens and liverworts (hereafter cryptogams, 12 species).

(3) Mantel permutation tests (Mantel, 1967) were performed between pairs of similarity matrices (Legendre & Fortin, 1989) to test the overall relationships between all combinations of the following four data sets: deep-rooted vascular plants (point-intercept data only); cryptogams (point-intercept data only); testate amoebae; water chemistry. The Steinhaus similarity index was used for the amoeba and vegetation matrices (transformed to a distance matrix for comparison) and the Euclidean distance for the water chemistry matrix (Legendre & Legendre, 1998). These computations were made using the R package for data analysis (Legendre & Vaudor, 1991). The permutations were restricted within five blocks representing the sites. As for the CCA analyses, testate amoebae and plant species occurring only in one sample were deleted.

RESULTS

Water chemistry

From the water chemistry data (Table 2), the five sites could be classified along two main large-scale

Table 2. *Water chemistry of the five sites*

Chemistry	Sweden	Finland	Britain	Netherlands	Switzerland
pH	4.73	4.43	4.47	4.72	4.80
Ca^{2+}	2.29	0.30	1.53	0.49	0.34
Mg^{2+}	0.88	0.13	0.84	0.55	0.12
Na^{+}	3.93	0.35	6.95	3.84	0.39
K^{+}	0.62	0.29	0.54	1.68	1.25
NH_4^{+}	0.03	0.00	0.46	0.92	0.00
Fe^{3+}	0.85	0.24	0.75	1.76	0.23
Al^{3+}	0.13	0.02	0.27	0.06	0.01
NO_3^{-}	1.47	0.38	1.55	0.13	0.18
Cl^{-}	8.43	0.00	11.6	7.98	1.21
SO_4^{2-}	21.5	0.03	16.6	0.00	0.01
N_{tot}	0.33	0.14	1.14	1.50	0.14
P	0.00	0.01	0.09	0.01	0.01
DOC*	21.5	18.1	41.3	39.1	15.4

Mean concentrations (mg l^{-1}, except pH) of major cations, anions and elements in the water collected from the five sites. $n = 20$, except for Britain, $n = 10$.
*Dissolved organic carbon.

Table 3. *Mean percentage cover of plant species in the five sites*

Plant species	Sweden	Finland	Britain	Netherlands	Switzerland
Andromeda polifolia	3.9	6.5	3.9	0.1	0.2
Calluna vulgaris	2.7		6.5	0.3	0.2
Carex nigra					2.9
Carex pauciflora		1.0			
Drosera rotundifolia	5.7	0.7		0.8	
Erica tetralix	0.1		12.9	4.9	
Eriophorum angustifolium	17.7		0.3	2.0	0.9
Eriophorum vaginatum	2.6	9.3	13.9	0.1	2.7
Narthecium ossifragum	3.3		<0.1		
Rhynchospora alba	1.3			0.1	
Scheuchzeria palustris		1.6			
Scirpus cespitosus	0.4	0.2	2.2		
Vaccinium oxycoccos	2.4	3.6	0.2	4.4	1.3
Aulacomnium palustre				0.3	1.2
Polytrichum strictum					57.5
Sphagnum balticum	2.8	63.4			
Sphagnum fallax				0.2	88.5
Sphagnum magellanicum	80.5	1.9		96.3	
Sphagnum papillosum	10.4	34.9	78.5	0.8	
Sphagnum rubellum	1.7			<0.1	
Number of vascular plant species	13	12	9	11	9
Number of cryptogam species	6	6	4	8	6
Total number of plant species	19	18	13	19	15
Total number of plant species in the point-intercept relevés	18	12	13	15	8

Only species with cover $\geqslant 1\%$ in any site are included. Total number of species includes all species recorded.
The following species were found at lower abundance: *Betula nana*, *B. pubescens*, *Calliergon stramineum*, *Carex lasiocarpa*, *C. limosa*, *C. rostrata*, *Cephalozia connivens*, *Cladonia* sp., *Empetrum nigrum*, *Equisetum palustre*, *Hypnum cupressiforme*, *Odontoschisma sphagni*, *Picea abies*, *Pinus sylvestris*, *Pleurozium schreberi*, *Polytrichum commune*, *Potentilla erecta*, *Rubus chamaemorus*, *Sphagnum capillifolium*, *S. fuscum*, *S. majus*, *S. pulchrum*, *S. tenellum*.

gradients: from oceanic (Britain, Sweden and The Netherlands) to continental (Finland and Switzerland), as reflected by Na, Cl and Mg, and from low atmospheric pollution (Finland, Switzerland and Sweden) to high (The Netherlands and Britain), as reflected mainly by increasing total N and NH_4^{+} levels. These gradients add to the large-scale climatic gradients (Table 1).

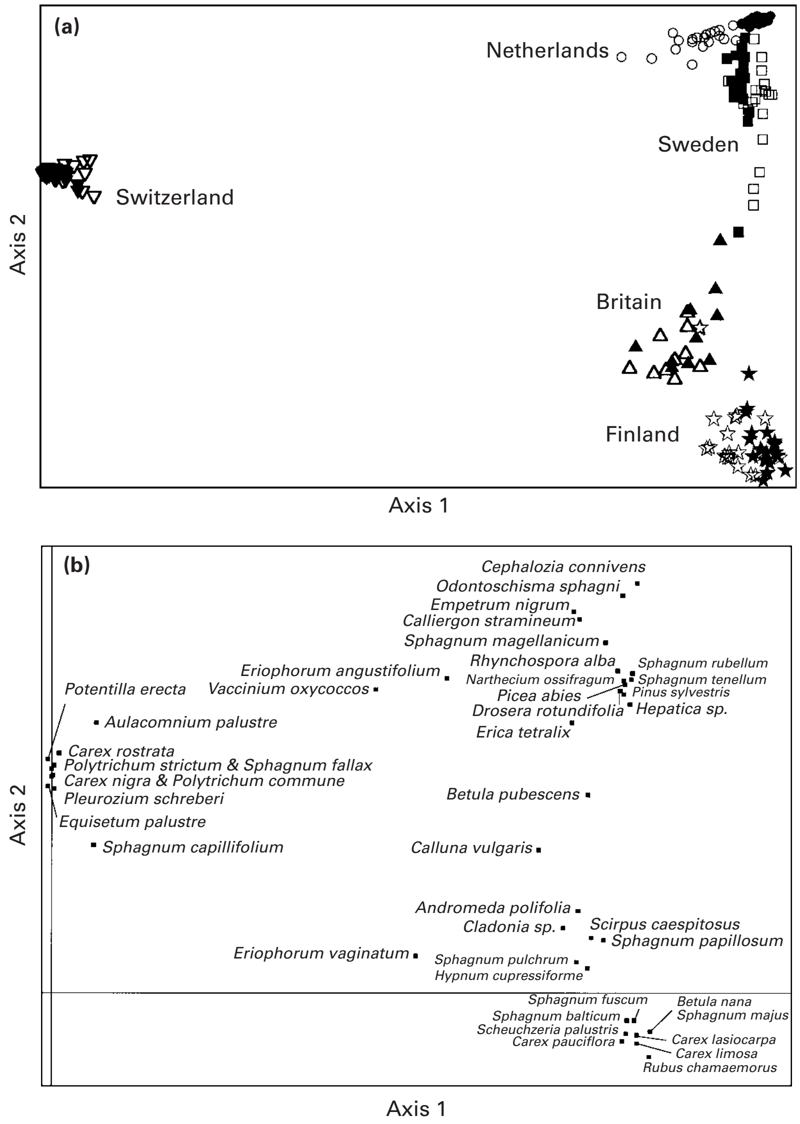

Fig. 2. (a) Detrended correspondence analysis (DCA) scatter diagram of point-intercept (open symbols) and cover-estimation (filled symbols) relevés of vegetation for the five sites. Samples from the different sites are represented by symbols: Switzerland, triangles base uppermost; Finland, stars; Netherlands, circles; Sweden, squares; Britain, triangles apex uppermost. Axes 1 and 2 represent 60% of the total variance (eigenvalues 0.949 and 0.708, respectively, total inertia = 2.781). (b) DCA scatter diagram of the plant species.

Vegetation

A total of 44 plant species was recorded. Plant species present at all sites are *E. vaginatum*, *V. oxycoccos* and to a lesser extent *A. polifolia*. The different sites clearly differ in vegetation composition (Table 3). Most species were present in only one or two sites. The total number of species was low in the British and Swiss sites (13 and 15 species), and almost equal in the other three sites (18 and 19 species). The number of species in the point intercepts was lower or equal to that of the cover estimations in the relevés, but most of the species absent from the point intercepts were either rare or of little interest in the context of this study (seedlings of *Picea*, *Betula*, etc.).

The position of point-intercept and cover-estimation relevés in the DCA scatter diagram revealed an almost complete separation of sites in the species assemblages (Fig. 2a). The within-site variation was much smaller because plots were placed in homogeneous areas within the sites. The Netherlands and Sweden are close together in the scatter diagram, reflecting their many common species, often with similar abundance, especially for *S. magellanicum* (Table 3). Similarly, Britain and Finland shared a high percentage of *S. papillosum* and *E. vaginatum*, and the two groups overlapped on the ordination. Switzerland was far off from the other four countries, which reflects the abundance of three species (*S. fallax*, *P. strictum* and *C. nigra*) rare or absent from the other sites.

Testate amoebae

A total of 54 species were recorded. Each site appeared to have either several characteristic species (usually rare in Sweden, The Netherlands and Britain), or different frequencies of some common species, as compared with other sites (Table 4). Total species number also varied between the sites. The Swedish site showed the highest number of species with 40; the Swiss site was the poorest (16 species); and the three other sites had 29–31 species. These differences resulted in a partial separation of the different sites on the DCA scatter diagram of testate amoebae data (Fig. 3a). As in the vegetation ordination, Switzerland was distinct from the other four sites, but the overlap among the other sites was much higher than in the vegetation ordination. Britain and Finland were well separated in the testate amoebae ordination but not in the vegetation ordination. Three species showed highly contrasting relative abundance between the two countries: *Nebela tincta* var. *major* which was very abundant in the British site (29%) and *Hyalosphenia elegans* and *Hyalosphenia papilio* which were very abundant in the Finnish site (14% each).

Testate amoebae, vegetation and water chemistry: multivariate analyses

In general, the significant water chemistry variables explained only a small part of the total variance (2–6%, Table 5). Nitrate, calcium and sulphate were found to be significant in the CCA of testate amoebae data, and these variables explained 6% of the total variance. In the CCA of all plants and that of deep-rooted plants, DOC was found to be significant, explaining 4 and 6% of the total variance, respectively. In the CCA of cryptogams, iron was significant and explained 4% of the total variance. The percentage variance explained by all chemistry variables was highest for deep-rooted plants (17%), almost equal for all plants and testate amoebae (13%) and lowest for cryptogams (10%). The importance of the site effect for the different data subsets varied: it was greatest for the cryptogams (89%), high for all plants (79%), low for deep-rooted vascular plants (57%), and lowest for testate amoebae (51%). Interestingly, the data subsets which were found to have a low site effect also had a higher fraction of their variance explained by the water chemistry.

Mantel tests with limited permutations (Fig. 4) revealed significant relationships between the water chemistry and deep-rooted vascular plants, and between the water chemistry and testate amoebae. Furthermore, the relationship between the cryptogam and testate amoebae data was marginally significant ($P = 0.07$). For the other three pairs of data sets, the relationships were clearly not significant.

DISCUSSION

Important floristic differences exist between the five sites, but these are due in part to plant distribution patterns. Many of the species occurring only in one or two sites are absent or rare in the other countries. The oceanic species *N. ossifragum* was found only in Britain and Sweden. *Rubus chamaemorus* is a northern species, the English Lake District and The Netherlands are outside the range of *Betula nana*, and *E. tetralix* is not indigenous to Switzerland. However, most species (*Carex limosa*, *C. lasiocarpa*, *C. nigra*, *R. alba*, *P. strictum*, *S. fallax*, *S. papillosum*, *S. tenellum*) are absent from some sites while they do occur in the region, suggesting that the sites differ in their ecology.

By contrast, differences in assemblages of testate amoebae are less pronounced among the sites studied across Europe. Interestingly, testate amoebae appear to reflect the pollution gradient (axis 2, Fig. 3a) from the least polluted site (Finland), through the intermediate site (Sweden) and the more polluted sites in Britain and The Netherlands. This suggests that the existing differences could well reflect ecological differences between the sites, and testate amoebae may be less dependent than plants on differences in distribution pattern. For instance, *Corythion dubium* and *N. tincta* are very abundant at the Swiss site but much rarer at the other sites. These species are very common in mosses and humus, and are therefore not typical peatland species (Chardez, 1965). Their abundance in the Swiss samples is related to the history of the site where both plant and animal communities have not yet fully recovered from the peat exploitation. On the other hand, several species occurring only in Sweden are characteristic for very wet microhabitats such as minerotrophic pools (*Difflugia elegans*, *D. microstoma*, *Euglypha cristata*, *Nebela dentistoma*, *N. gracilis*, *Sphenoderia fissirostris*) (Jung, 1936; Mitchell *et al.*, 1999). Three species present only in the Dutch site (*Arcella vulgaris*, *Centropyxis eurystoma* and *Difflugia globulosa*) are characteristic for minerotrophic and wet conditions. Among the species occurring only in Britain, *Hyalosphenia subflava* is an indicator of dry conditions (the site was drained until recently), and *Nebela flabulellum* could represent an exception to the general rule that peatland testate amoebae are cosmopolitan – this species has been found only in oceanic peatlands on both sides of the northern Atlantic (Charman & Warner, 1997). However, there are not yet sufficient data on distributions of testate amoebae to interpret with confidence the negative evidence of species distribution patterns.

To some extent the different groups of organisms behave similarly: the Swiss site differs from the others both in amoeba composition and in vegetation. The reason might be its successional status. On the other hand, in accordance with suggestions

Table 4. *Mean frequency (%) of the testate amoebae species in the five sites*

Testate amoebae species	Sweden	Finland	Britain	Netherlands	Switzerland
Amphitrema flavum	1.1	9.3	0.5	0.1	
Arcella arenaria	0.1	4.9	3.0	1.1	
Arcella artocrea	<0.1	1.0		0.1	
Arcella sp.			1.4	0.7	
Assulina muscorum	5.8	5.5	9.2	10.4	8.6
Assulina seminulum	6.0	1.9	1.1	4.3	1.0
Bullinularia indica	0.1	0.7	0.9	3.8	5.7
Corythion dubium	1.0	1.0	8.7	8.4	28.3
Difflugia leidyi	2.1	8.4	4.0	1.6	
Euglypha ciliata	0.4	2.5	0.1		6.3
Euglypha compressa	5.1	2.7	9.7	10.8	
Euglypha laevis	1.4	0.9	3.7	1.6	2.8
Euglypha strigosa	6.6	0.6	6.8	16.5	1.5
Heleopera rosea	2.8				<0.1
Heleopera sphagni	10.6	4.6	0.1	0.2	
Heleopera sylvatica	3.3	0.7	9.1	0.4	
Hyalosphenia elegans	7.9	13.7	<0.1	30.3	
Hyalosphenia papilio	6.2	13.8		0.5	2.0
Nebela flabellulum			5.9		
Nebela griseola	21.5	5.6	1.3	2.5	
Nebela militaris	0.7	2.1	1.1	0.1	0.4
Nebela tincta	3.2	6.4	0.5	1.0	42.0
Nebela tincta var. *major*	6.3	0.8	28.3	3.6	1.0
Phryganella acropodia	2.7	11.3	1.1	0.4	
Trigonopyxis arcula		0.3	2.3	0.2	
Trinema sp.	1.5				
Total number of testate amoebae species	40	30	31	29	16

Only species with abundance ⩾1% in any site are included. Total number of species includes all species recorded. The following species were found at lower abundance: *Arcella vulgaris*, *Assulina scandinavica*, *Centropyxis aculeata*, *C. aerophilla sphagnicola*, *C. eurystoma*, *Corythion pulchellum*, *Cryptodifflugia oviformis*, *Difflugia bacillifera*, *D. elegans*, *D. globulosa*, *D. manicata*, *D. microstoma*, *Euglypha cristata*, *E. rotunda*, *Hyalosphenia minuta*, *H. subflava*, *Nebela carinata*, *N. dentistoma*, *N. gracilis*, *N. marginata*, *Phryganella paradoxa*, *Placocista spinosa*, *Sphenoderia fissirostris*, *Testacea* sp1, *Testacea* sp2, *Testacea* sp3, *Trinema enchelys*, *T. lineare*.

from other studies (Prendergast *et al.*, 1993; Prendergast & Eversham, 1997) we find that organism groups are not well correlated, the inter-site differences being more marked for the vegetation than for the testate amoeba communities, and the relative positions of the sites on the first two axes of the DCA being different. Several explanations may be suggested for this observation.

- Biogeography: although the five sites differ in their ecology, the importance of vegetation differences is due in part to large-scale plant distribution patterns. By comparison, the testate amoeba fauna is less variable between sites – most peatland species being cosmopolitan in their distributions, biogeographical aspects appear less important.
- Phenology: testate amoebae are able to encyst during periods of unfavourable environmental conditions such as drought or frost, whereas plants remain exposed during the whole growing season. This difference could in part explain the greater inter-site differences in the vegetation. It suggests also that the vegetation and the testate amoebae reflect different ecological factors and may be used as complementary biomonitors in peatlands.
- Vertical gradients: testate amoebae are very sensitive to microsite conditions. The samples were all taken at the moss surface and therefore differences in testate amoebae faunal composition are indicative of different conditions at that level (3–5 cm deep). By contrast, the vegetation data include moss species which, like testate amoebae, are dependent on microsite conditions, together with vascular plants which can be rooted at different depths depending on the species. For example, at the Swiss site, *V. oxycoccos* occurs together with *C. rostrata* in some plots, but these two plants differ markedly in their ecology.

These observations support the use of testate amoebae in experimental studies of mires which cover a wide geographical area. Of particular interest is the greater diversity of testate amoebae as compared to cryptogams: 54 species versus 19 in our data set.

The fraction of variance explained in the CCA is very low for all data sets, but this is not surprising because the plots were chosen to be as similar as

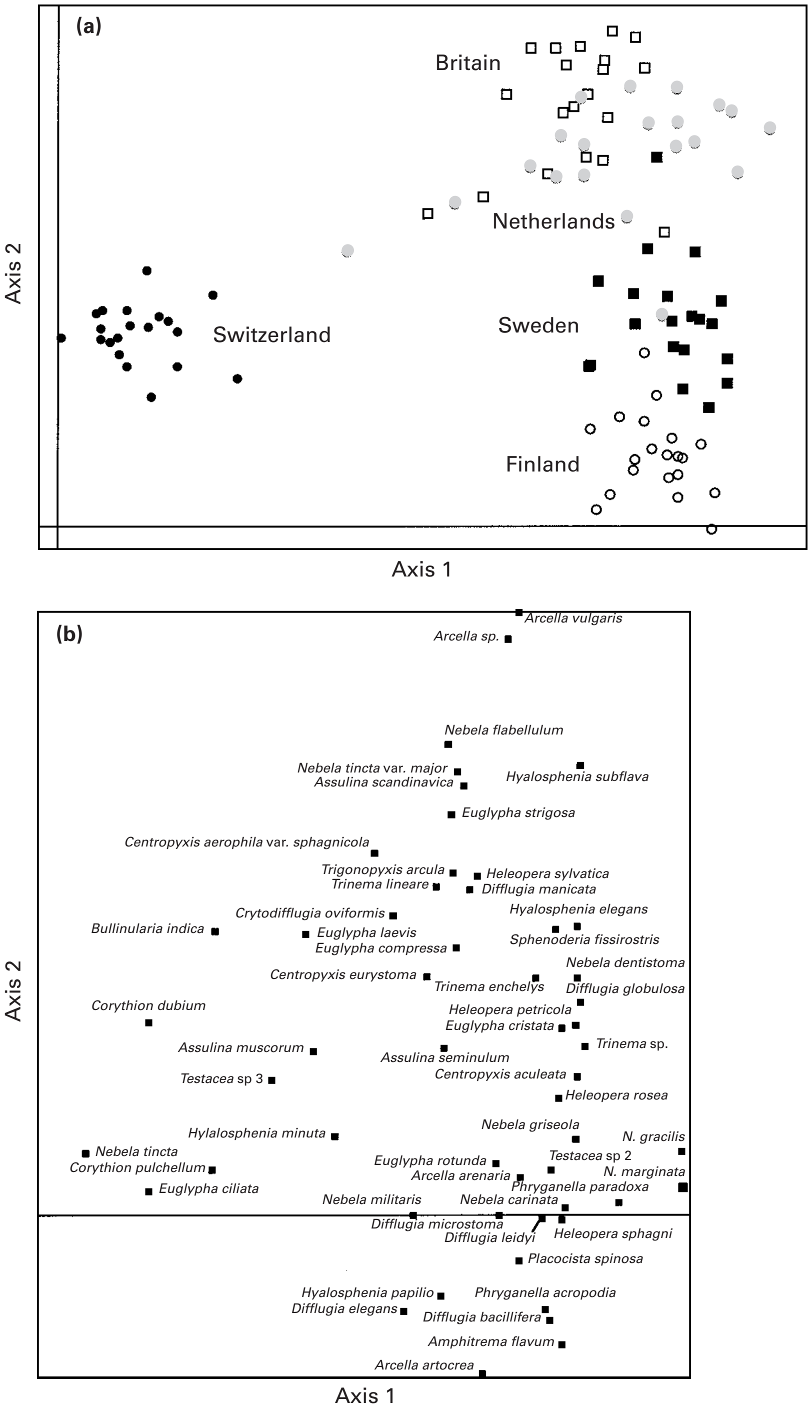

Fig. 3. (a) Detrended correspondence analysis (DCA) scatter diagram of testate amoebae samples from the five sites. Samples from the different sites are represented by symbols: Switzerland, closed circles; Finland, open circles; Netherlands, grey circles; Sweden, closed squares; Britain, open squares. Axes 1 and 2 represent 36% of the total variance (eigenvalues 0.655 and 0.285, respectively, total inertia = 2.645). (b) DCA scatter diagram of the testate amoebae species.

possible and therefore the within-site differences are minimal. The water samples were taken at the level of the water table (average depth *c.* 10 cm except for Switzerland, *c.* 30 cm) where most vascular plants have their roots, and the testate amoebae were sampled near the moss surface (3–5 cm deep). This explains the results of the CCA analyses and the Mantel tests which reveal a small-scale vertical gradient: water chemistry explains a higher fraction of the variance for deep-rooted plants and testate amoebae as compared to cryptogams. Even if non-destructive measurement of the physical and chemical properties within the moss layer is difficult, it is worthwhile as some of the processes governing peatland ecosystems take place there. Furthermore, the chemical variables measured vary seasonally, and different organisms are likely to be affected differently by the water chemistry according to their

Table 5. *Summary of the partial canonical correspondence analyses of testate amoebae and vegetation data from the five sites*

Analysis	Cryptogams	All plants	Deep-rooted plants	Testate amoebae
Sum of all eigenvalues (*a*)	2.325	2.590	2.323	2.468
Sum of all eigenvalues after fitting covariables (*b*)	0.256	0.547	1.001	1.218
Site effect (%) (*c*)*	89	79	57	51
Sum of all canonical eigenvalues if all variables are accepted in the model (*d*)	0.026	0.074	0.166	0.161
Percentage of variance explained by all variables (*e*)†	10.2	13.5	16.6	13.2
Significant variables	Fe	DOC	DOC	Ca, NO_3, SO_4
Sum of all significant canonical eigenvalues (*f*)	0.006	0.019	0.064	0.070
Percentage of variance explained by the significant variables (*g*)‡	2.3	3.5	6.4	5.7
Significance of axis 1 and correlation of significant variable(s)	0.037 $r(\mathrm{Fe}) = 0.73$	0.040 $r(\mathrm{DOC}) = 0.60$	0.010 $r(\mathrm{DOC}) = 0.61$	0.001 $r(\mathrm{Ca}) = 0.15$; $r(\mathrm{NO_3}) = 0.06$; $r(\mathrm{SO_4}) = 0.10$
Significance of axis 2 and correlation of significant variable(s)	–	–	–	0.01 $r(\mathrm{Ca}) = 0.11$; $r(\mathrm{NO_3}) = 0.23$; $r(\mathrm{SO_4}) = 0.18$
Significance of axis 3 and correlation of significant variable(s)	–	–	–	ns $r(\mathrm{Ca}) = -0.10$; $r(\mathrm{NO_3}) = -0.14$; $r(\mathrm{SO_4}) = 0.45$

Five dummy variables representing the sites were used as covariables.
*Calculated as: $c = 100\ (a-b)/a$.
†Calculated as: $e = 100\ (d/b)$.
‡Calculated as: $g = 100\ (e/b)$.

physiological characteristics and phenology. For example, *E. vaginatum* grows and flowers very early in the growing season and also has an early senescence, whereas *C. nigra* has a later phenology.

The Mantel tests reveal interesting relationships between the different subsets of data (Fig. 4). Deep-rooted vascular plants are significantly related to water chemistry, but cryptogams are not. For testate amoebae the situation is somewhat different: they are related to water chemistry and also (marginally significantly) to the mosses in which they live, but not to plants rooted as much as 40 cm below the surface. Thus testate amoebae reflect the chemistry of the ground water and to a lesser extent the botanical composition of the moss carpet in which they live. Lacking roots, mosses are more directly affected by their immediate surroundings as compared to vascular plants. The growth of *Sphagnum* mosses depends mainly on the ecological condition at the level of the capitulum. Even subtle micro-environmental changes such as shading can affect *Sphagnum* growth (Buttler *et al.*, 1998). In addition, although water can reach the capitula by capillarity, *Sphagnum* mosses modify the chemical characteristics of water by active uptake and exchange of cations, thus reducing the nutrient supply to vascular plants (Clymo & Hayward, 1982; Van Breemen, 1995). *Sphagnum* mosses therefore create a chemical gradient which adds to the gradient due to chemical differences between rain water and that of the water table. Thus a marked vertical ecological (mainly trophic) gradient appears within the acrotelm, the aerobic upper layer of *Sphagnum* peatlands. Deep-rooted plants and mosses may respond to different factors and can be considered as two different functional groups. Such differences are more marked in relatively dry situations. This is most obvious in the Swiss site where the deep-rooted plants (*E. vaginatum*, *E. angustifolium*, *C. nigra* and *C. rostrata*) have their roots in mineralized peat formed when the peatland was drained and cut. Soil chemical properties at this depth differ from those closer to the surface.

These results bring to light an aspect of peatland ecosystems that is often overlooked: the existence of vertical as well as horizontal ecological small-scale gradients below the surface. This relates to the crucial distinction between ombrotrophic (rainwater-fed) and minerotrophic (influenced by surrounding mineral soil) conditions of mires: deeply rooted species may be exposed to minerotrophic conditions while the bryophytes at the

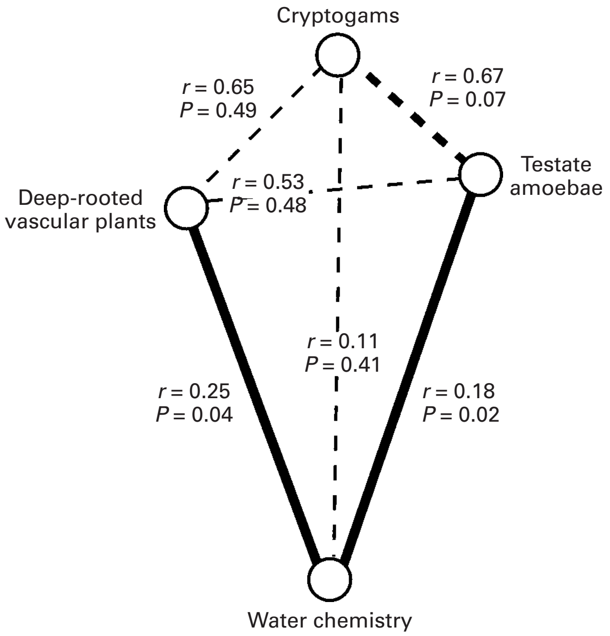

Fig. 4. Schematic representation of relationships between the four data sets based on the Mantel statistics. The line lengths are proportional to the Mantel statistic (short lines indicate strong relationships). Broad, unbroken lines, significant relationships ($P<0.05$); broad, dashed lines, marginally significant relationships ($0.1>P>0.05$); narrow, dashed lines, non-significant relationships ($P>0.1$).

surface may undergo ombrotrophic conditions. For the plants this is an excellent case of life-form niche separation (Grubb, 1977), enabling resource acquisition from different sources. At or near the surface, the importance of small-scale ecological gradients for the growth of mosses has recently been established in Swiss peatlands (Grosvernier *et al.*, 1995, 1997; Buttler *et al.*, 1998), but what happens below the surface is also important for the functioning of peatland ecosystems.

From a global perspective, a good understanding of below-ground processes is crucial for predictions of the response of peatlands to environmental change (Lee, 1998). The results of a recent study on the effect of nitrogen deposition on the microbial loop in a French peatland (Gilbert *et al.*, 1998a,b) show that testate amoebae are one of the dominant groups in terms of biomass, that they react quickly to nitrogen fertilization, and that they appear to play a key role in the cycling of matter and energy. Therefore testate amoebae could be an invaluable bioindication tool for ecologists.

From a modelling perspective, Peterson *et al.* (1998) have recently stressed the importance of process and structure variations across the spatial and/or temporal scales in order to understand ecological resilience ('a measure of the amount of change or disruption that is required to transform a system from being maintained by one set of mutually reinforcing processes and structures to a different set of processes and structures'). These authors argue that understanding the interactions between scaling of species and scaling of ecological processes should be a central goal of ecology, especially in the context of global change. Given the importance of peatlands in the global carbon cycle, the modelling of peatland–atmosphere exchanges is crucial for global change scenarios; recognition of the vertical gradients in peatlands may assist in better modelling of their functioning.

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