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Ecology of testate amoebae (Protists) in *Sphagnum* peatlands of eastern Poland: Vertical micro-distribution and species assemblages in relation to environmental parameters

Tomasz Mieczan*

Department of Hydrobiology, University of Life Sciences, Dobrzańskiego 37, Lublin 20-262, Poland

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Abstract – The testate amoebae communities living among different species of mosses in *Sphagnum* peatlands in eastern Poland were studied. Sampling was done on a monthly basis from April to November 2005–2007. To assess the importance of the vertical distribution of testate amoebae within the mosses, each sample was cut into two parts: the upper living part (1–5 cm) and the lower dead part (5–10 cm). The highest species richness occurred in hollows dominated by *Sphagnum angustifolium, Sphagnum flexuosum* and *Sphagnum palustre*. Lower numbers of taxa were observed in hummocks dominated by *Sphagnum magellanicum*, *Polytrichum strictum* and *Polytrichum commune*. There was a distinct horizontal micro-zonation of the abundance of testate amoebae occurring among *Sphagnum* mosses, but only a small difference ascertained among *Polytrichum*. The number of testate amoebae was significantly greater in the deeper samples. The results demonstrated that depth to water table, pH and total organic carbon can strongly regulate the abundance and taxonomic composition of testate amoebae.

Key words: Biodiversity / classification / micro-distribution / protists / wetlands

Introduction

Testate amoebae (Protozoa: Rhizopoda) are common inhabitants of moist soils, lakes and peatlands (Tolonen *et al*., 1992). They produce a decay-resistant test, or shell, that protects the cell from desiccation. The shell may be proteinaceous, siliceous, or calcareous and may incorporate extraneous materials such as mineral grains, fungal hyphae and diatoms (Odgen and Hedley, 1980). The morphology of tests is usually unique, allowing species-level identification. In addition, testate amoebae produce shells which are well preserved in peat and allow paleo-environmental reconstruction (Mitchell *et al*., 2000a). Their well-defined ecological preferences in relation to important ecological variables in peatlands have made them useful in ecological studies (Charman, 1997). Testate amoebae are excellent indicators of calcite precipitation in lakes (Casper and Schönborn, 1985) and heavy metal pollutants in peatlands (Nguyen-Viet *et al*., 2007). Previous studies have shown that the abundance of each taxon, and hence the structure of communities, are controlled by a set of environmental variables. Moisture conditions have often been identified as the most important factor controlling testate amoebae community composition in peatlands (Charman and Warner, 1997; Mitchell *et al*., 2000a). Numerous ecological studies dealing with the responses of recent species to the water level gradient in *Sphagnum* peatlands provide a good basis for the usage of testate amoebae as indicators of past hydrological changes, and also as recent indicators of hydrological changes during restoration (Buttler *et al*., 1996; Booth, 2002; Kishaba and Mitchell, 2005; Opravilová and Hájek, 2006). According to Bobrov *et al*. (1999), Mitchell *et al*. (2000b), Booth (2002) and Mieczan (2007a, 2007b) the composition of testate amoebae communities is primarily controlled by the moisture regime, and to a lesser extent by pH. Opravilová and Hájek (2006) found that nutrient status is a secondary control on amoebae communities. Even temperature can co-determine the distribution pattern of the testacean fauna (Schönborn, 1962). Testate amoebae require a minimum temperature at a specific time of the year to reproduce successfully. In addition, factors such as light, oxygen and food availability may also affect testate amoebae communities (Charman *et al*., 2000). The ecology of most species is still rather poorly known although there have been a number of recent studies in several regions of the world, including New Zealand (Charman, 2001), north-western Poland (Lamentowicz and Mitchell, 2005),

*Corresponding author: tomasz.mieczan@up.lublin.pl

Alaska (Payne *et al.*, 2006), and in France (Nguyen-Viet *et al.*, 2007). Several authors have studied the vertical micro-distribution of testate amoebae in peatlands. One of the main observations has been that mixotrophic species preferentially colonize the uppermost part of mosses, where their endo-symbionts can photosynthesize, whereas heterotrophic species are found in the lower part of the mosses (Heal, 1962; Mitchell and Gilbert, 2004). Micro-distribution of testate amoebae taxa has been observed along the *Sphagnum* stem, and this spatial variation has been attributed primarily to gradients of light, temperature, oxygen and food (Meisterfeld, 1977). Vertical micro-distribution is also a gradient from mostly live testate amoebae in the aerobic, upper portions and mostly empty test in the more anaerobic, lower portions (Booth, 2002). However the vertical micro-distribution of testate amobae has not been studied with respect to individual moss species. These comparisons can provide insights into the ecology of testate amoebae, and may guide the collection of more representative calibration datasets. However, information concerning the relationships between environmental factors and the seasonal occurrence of testate amoebae is almost completely lacking (but see Heal, 1964; Warner *et al.*, 2007). More needs to be learned about the sensitivity of these microorganisms to change (either human-induced or natural), if testate amoebae are to be used as bioindicators of human pollution and environmental disturbance. Whilst important gaps remain in our understanding of the relationships between testate amoebae and seasonal changes (Warner *et al.*, 2007), the aims of this study were (i) to examine the community structure and vertical micro-distribution of testate amoebae in *Sphagnum*-dominated peatlands; (ii) to improve our understanding of factors affecting the distribution of moss testate amoebae communities; and (iii) to analyze the seasonal changes of testate amoebae communities.

Materials and methods

Study site

The study was performed in peatlands located in the western part of the Polesie Lubelskie (Eastern Poland, 51° N, 23° E). Its borders encompass the most precious parts of Poleski National Park, including lakes and floodplains, as well as swamps and peatlands, which survived until now in relatively unaltered conditions. It is one of the most natural region in Poland that was not covered by the last glaciation. The mean air monthly temperatures of January and July were −4.1 °C and 17.9 °C, respectively. Mean annual rainfall is *ca.* 551 mm. The raised and transitional bogs selected for this study were considered to be representative of the bogs of the region and contained a broad diversity of habitats. All of these sites are oligotrophic and are dominated by peat mosses (*Sphagnum* spp.) and vascular plants characteristic of nutrient-poor peatlands. The vegetation of peatlands is characterized by the presence of a number of rare species, such as *Scheuchzeria palustris* L., *Drosera anglica* Huds., *Drosera intermedia* Hayne, *Salix myrtilloides* L. and *Salix lapponum* L. The vegetation is also dominated by graminoids such as *Eriophorum vaginatum* (L.), *Carex acutiformis* Ehrhart, *Carex gracilis* Curt. Mosses are present, but less abundant. Bryophytes include species such as *Sphagnum angustifolium* (C.C.O. Jensen ex Russow), *Sphagnum cuspidatum* Ehrh. ex Hoffm., *Sphagnum flexuosum* Dozy and Molk., *Sphagnum magellanicum* Bird., *Polytrichum strictum* Menzies ex Brid., and *Polytrichum commune* Hedw. (Table 1).

Field sampling and laboratory analyses

Samples for testate amoebae analysis were collected from five peatlands. The micro-sites sampled in this study include hummocks, lawn and hollows. Eight samples were collected from the studied peatlands once a month from April to November from 2005 to 2007. A total of 160 samples were taken. Each sample was packed into a cylindrical plastic container (10 cm in diameter), which was driven into the moss carpet and cut with the knife. To assess the importance of the vertical distribution of testate amoebae within the mosses, each sample was cut into two sub-samples: the living green part and the dead brown part. All samples were storted in a cooler and transported within one day to the laboratory. Testate amoebae were isolated from moss samples using standard methods (Hendon and Charman, 1997), by boiling and sieving the samples through nested sieves of 250 μm and 10 μm plankton net. The sample was mounted in glycerol, and slides were scanned until a total of at least 150 testate amoebae were identified and counted at × 500 magnification. The relative abundance of each taxon was calculated as a percentage of total individuals counted. The abundance of microorganisms was expressed as number of individuals per gram of plant material (ind.g^{-1}). Specimens were identified to the lowest possible taxonomic level. The following literature was used for species/ecophenotypes identification: Odgen and Hedley (1980), Charman *et al.* (2000) and Clarke (2003). Various environmental variables (*e.g.*, depth to water table – DWT, pH and conductivity) were measured at each sampling site on the day of sample collection. Depth to water table was measured in the hole where the sample was removed after a period of 5–10 min. Depth of water table was measured within a centimetre measure. Zero level was marked by the top parts of the peatmosses. Temperature, pH and conductivity were determined using a JENWAY 3405 electrode. Total organic carbon (TOC) was determined using the PASTEL UV and the remaining environmental data ($N\text{-}NO_3$, $P\text{-}PO_4$, P_{tot}) were analysed in the laboratory (Hermanowicz *et al.*, 1976).

Data analyses

Diversity analysis (Shannon Wiener diversity index, $\log_{10}$-based) was performed using the Multivariate

Table 1. Main characteristics of the five peatland sites sampled in this study.

Site name	Location	Area (ha)	Moss species sampled	Habitat and dominant vascular plants
Blizionki/Lejno (B)	51°25.093′N, 23°4.124′E	71.73	*Sphagnum magellanicum* Bird., *Sphagnum flexuosum* Dozy & Molk., *Polytrichum strictum* Menzies ex Brid.	Hummock with *Drosera anglica* Huds., Hollow, *Sphagnum* carpet with *Carex limosa* L.
Durne Bagno (DB)	51°22.344′N, 23°12.303′E	213.2	*Sphagnum angustifolium* (C.E.O. Jensen ex Russow)	Hummock with *Ledum palustre* L., *Vaccinum uliginosum* L., Hollow, *Sphagnum* carpet with *Carex limosa* L.
Długie (D)	51°23.044′N, 23°11.201′E	694.93	*Sphagnum magellanicum* Bird., *Polytrichum strictum* Menzies ex Brid., *Polytrichum commune* Hedw.	Hummock with *Ledum palustre* L., Hollow, *Drosera anglica* Huds., *Drosera intermedia* Hayne, *Sphagnum* carpet with *Carex limosa* L.
Moszne (M)	51°23.090′N, 23°8.122′E	205.16	*Sphagnum flexuosum* Dozy & Molk., *Sphagnum cuspidatum* Ehrh. ex Hoffm., Sphagnum *magellanicum* Bird., *Sphagnum palustre* L., *Polytrichum strictum* Menzies ex Brid., *Polytrichum commune* Hedw.	Hummock with *Ledum palustre* L., *Andromeda polifolia* L., *Drosera anglica* Huds., *Drosera intermedia* Hayne, Hollow, *Sphagnum* carpet with *Eriophorum vaginatum* L., *Carex acutiformis* Ehrhart., *Carex gracilis* Curt.
Krugłe Bagno/ Jelino (J)	51°24.099′N, 23°9.116′E	19.7	*Sphagnum magellanicum* Bird (C.E.O. Jensen ex Russow), *Sphagnum angustifolium* (C.E.O. Jensen ex Russow)	Hummock with *Ledum palustre* L., Hollow, *Sphagnum* carpet with *Eriophorum vaginatum* L.

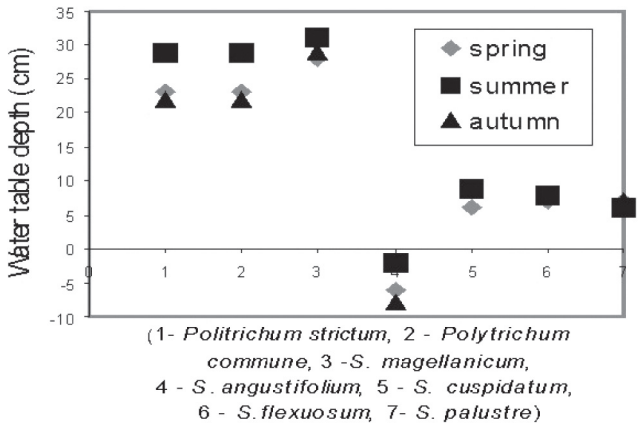

Fig. 1. Seasonal patterns of the depth to water table (DWT) in the studied peatlands (average values for period April–November 2005–2007).

Statistical Package MVSP (Kovach Computering Services; MVSP, 2002). Principal component analyses (PCA) was used to analyze relationships among the continuous environmental variables. Detrended correspondence analysis (DCA), which only uses species data to constrain the ordination (indirect gradient analysis), was used to analyze species assemblage differences between lower and upper assemblages. To examine the relationship between the community structure of testate amoebae and environmental parameters, a Canonical correspondence analysis (CCA) was applied to the species data using MVSP (2002). Logarithmic transformation [ln (x + 1)] was performed on species data to normalize the distribution. A Monte Carlo test was used to test the significance of each axis. All tests were done using 999 permutations and significance threshold of $P < 0.05$. On the resultant plot, the arrows representing the physico-chemical variables indicate the direction of maximum change of that variable, and the length of each arrow is proportional to the rate of change.

Results

Depth to the water table fluctuated during the year, and ranged from −8 to 32 cm (Fig. 1). Water pH fluctuated from 2 to 4. In turn, conductivity was significantly differentiated, attaining 30 $\mu S.cm^{-1}$ to 125.5 $\mu S.cm^{-1}$. The highest conductivity occurred in summer, but was lower in spring or autumn. In all peatlands examined, water temperature reached its highest value in summer (14.3–18.3 °C), and decreased in autumn (1.3–2.3 °C). The concentration of total organic carbon fluctuated between 31.4 mg $C.dm^{-3}$ in summer and 97.5 mg $C.dm^{-3}$ in autumn. Nutrients reached their highest values at high pH micro-sites; the concentration of nutrients were the highest during the spring and autumn periods, and considerably lower in summer. Only Lejno peatlands had a higher concentration of nutrients in summer (Table 2).

A total of 45 testate amoebae taxa were identified (Table 3). The highest numbers of testate amoebae taxa (28–43 taxa) occurred in hollows dominated by *Sphagnum angustifolium, Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*. Lower numbers of taxa (5–15)

Table 2. Physical and chemical characteristics of water in the studied peatlands (average values for the period April–November 2005–2007); * spring – average values from period April–June, summer – average values from period July–August, autumn – average values from period September–November. Temp. = water temperature, Conduct. = conductivity, P_{tot} = total phosphorus, TOC = total organic carbon.

Site name	Season*	pH	Temp. (°C)	Conduct. ($\mu S.cm^{-1}$)	N-NO_3 (mg $NO_3.dm^{-3}$)	PO_4^{3-} (mg $PO_4.dm^{-3}$)	P_{tot} (mg $P.dm^{-3}$)	TOC (mg $C.dm^{-3}$)
Blizionki/Lejno	spring	3.8	8.5	45.4	0.398	0.014	0.186	65.2
	summer	3.16	17.5	124.45	0.566	0.077	0.116	76.5
	autumn	3.5	1.3	85.3	0.273	0.042	0.557	97.5
Durne Bagno	spring	4.3	7.6	108.1	1.366	0.262	0.672	50.25
	summer	5.64	17.1	125.5	1.338	0.089	0.216	39.2
	autumn	3.71	2.3	85.5	0.922	0.324	0.749	58.2
Długie	spring	3.2	7.3	39.8	0.600	0.100	0.236	55.2
	summer	3.2	17.3	45.1	0.299	0.288	0.320	66.9
	autumn	3.4	2.1	45.3	0.662	0.122	0.365	66.2
Moszne	spring	3.3	7.9	50.53	0.583	0.081	0.404	80.5
	summer	2.3	14.3	75.36	0.647	0.123	0.197	74.0
	autumn	3.9	2.2	51.4	0.259	0.101	0.358	82.9
Krugłe Bagno/Jelino	spring	4.02	8.2	30.35	0.445	0.046	0.134	41.8
	summer	4.4	18.3	39.5	0.221	0.048	0.099	31.4
	autumn	4.1	1.3	29.5	0.378	0.08	0.402	42.75

were observed in hummocks dominated by *Sphagnum magellanicum*, *Polytrichum strictum* and *Polytrichum commune*. The most frequent taxa were *Assulina muscorum*, *Arcella discoides* type, *Hyalosphenia papilio, Hyalosphenia elegans* and *Archerella flavum*. Sixteen testate amoebae taxa had frequencies $< 5\%$. The species distribution pattern also showed a higher variation in acid habitats, from with *Hyalosphenia papilio* in wet habitats to *Assulina muscorum* assemblages in the drier microhabitats. The data set for statistical analyses was composed of 160 samples, 40 taxa, and 8 environmental variables. Five minor (less abundant) taxa were omitted in the statistical analyses. The first and second axes captured 53% of the total variation of species data in the PCA. Sampling sites from lawn and hollows were clearly separated along the first axis that represented 42% of the total species variation. This axis was closely correlated with DWT and pH (Fig. 2). The DCA showed that species composition of testate amoebae differed between the living green part (1–5 cm) and the dead brown part (5–10 cm). The first axis had the highest percentage variance of species data (18%). The second axis added 9% of variance (Fig. 3). The number of testate amoebae taxa increased with depth ($P = 0.002$) (Table 3). *Assulina muscorum, Hyalosphenia papilio* and *Hyalosphenia elegans* were relatively more abundant in the uppermost, photosynthetic part of the mosses, and decreased in relative abundance with depth. *Arcella discoides* and *Archerella flavum* were relatively more abundant in the deeper samples. The number of taxa and their abundances differed between *Sphagnum angustifolium*, *Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*; however, only a slight difference was ascertained between the abundance of testate amoebae among *Polytrichum strictum, Polytrichum commune* and *Sphagnum magellanicum*. In the micro-environment dominated by *Polytrichum*, in the living green part and the dead brown part, *Assulina muscorum* occurred in the highest numbers (Table 3). The diversity analysis revealed a mean Shannon-Wiener diversity index (H) of 2.45. The highest diversity was measured in *Sphagnum cuspidatum* ($H = 2.8$), and the lowest diversity was observed in *Polytrichum strictum* ($H = 0.65$). The species diversity, minimal in the uppermost 5-cm layer of sphagnum moss (mean Shannon index = 1.0), remained at approximately the same level in the deeper layers (2.3–2.8). The species richness alone differed significantly in the uppermost and lowermost layers (Mann-Whitney test with Bonferroni correction for multiple comparisons; $P < 0.05$). The total density of testate amoebae varied between 9 and 23.000 ind.g^{-1} of moss in the samples. The greatest abundance of testate amoebae was ascertained at the micro-sites dominated by *Sphagnum flexuosum* (> 23 000 ind.g^{-1}), and the lowest was observed at sites dominated by *Polytrichum* (9–12 ind.g^{-1}). The ordination also separated the sampling habitats quite well. Hummock sites dominated by *Sphagnum magellanicum, Polytrichum commune* and *Polytrichum strictum* mostly had high scores on the first axis. Hollows and lawn sites colonized by *Sphagnum angustifolium*, *Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre* were negatively correlated with the first axis (Fig. 4). In the CCA of testate amoebae data, four environmental variables were significant: DWT, pH, TOC and P_{tot}. Together, these variables explained 44% of the variation in the species data. Water table depth and TOC were correlated positively with the first axis, whereas pH and P_{tot} were correlated with the second axis. Water table depth and TOC appeared as dominant controls on the distribution patterns in both the upper and lower assemblages,

Table 3. Taxonomic composition and taxon frequency (% of samples) of the testate amoebae found in *Sphagnum*-dominated peatlands; *S. ang.* – *Sphagnum angustifolium*, *S. cusp.* – *Sphagnum cuspidatum*, *S. flex.* – *Sphagnum flexuosum*, *S. mag.* – *Sphagnum magellanicum*, *S. pal.* – *Sphagnum palustre*, *P. str.* – *Polytrichum strictum*, *P. comm.* – *Polytrichum commune*, U – upper assemblages 0–5 cm, L – lower assemblages 5–10 cm. Taxonomy follows Charman *et al.* (2000).

Taxon	Species abbreviation	Moss species													
		S. ang.		*S. cusp.*		*S. flex.*		*S. mag.*		*S. pal.*		*P. str.*		*P. comm.*	
		U	L	U	L	U	L	U	L	U	L	U	L	U	L
Amphitrema wrightianum (Archer, 1877)	Amph wr	15	30	18	15	18	15	-	-	8	12	-	-	-	-
Arcella catinus type (Penard, 1980)	Arc cat	-	2	-	-	-	2	-	-	-	-	-	-	-	-
Arcella disoides type (Ehrenberg, 1843)	Arc dis	49	78	46	73	46	84	-	-	23	81	-	-	-	-
Arcella sp.	Arc sp	1	8	1	8	1	13	-	-	1	8	-	-	-	-
Arcella vulgaris (Ehrenberg, 1830)	Arc vul	18	31	4	56	4	56	-	6	9	65	-	-	-	-
Archerella flavum (Archer, 1877)	Arch fl	45	69	33	59	46	61	-	4	33	42	-	-	-	-
Assulina muscorum (Greeff, 1888)	Ass musc	8	2	5	2	8	2	67	57	8	2	75	72	81	80
Assulina seminulum (Ehrenberg, 1848)	Ass sem	2	1	-	-	1	1	-	-	-	-	-	-	-	-
Centropyxis aculeata type (Ehrenberg, 1830)	Cen ac	6	20	7	29	7	31	4	12	7	31	4	4	4	6
Centropyxis aerophila (Deflandre, 1929)	Cen ar	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Centropyxis platystoma type (Penard, 1890)	Cen pl	-	3	-	3	-	4	-	-	2	1	-	-	-	-
Corythion dubium (Taranek, 1881)	Cor dub	-	2	4	7	4	7	4	7	4	6	-	-	-	-
Corythion-Trinema type	Cor-typ	-	3	3	3	3	2	3	2	3	2	4	6	4	4
Cryptodifflugia oviformis (Penard, 1890)	Cry ov	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Cyclopyxis arcelloides type (Leidy, 1879)	Cycl arc	2	2	6	5	6	3	-	-	-	-	-	-	-	-
Cyphoderia ampulla (Ehrenberg, 1840)	Cryp amp	2	2	2	2	2	3	-	-	-	-	-	-	-	-
Difflugia elegans (Penard, 1980)	Dif el	-	4	3	4	1	4	-	-	-	-	-	-	-	-
Difflugia globulosa (Dujardin, 1841)	Dif gl	-	3	-	3	1	1	-	-	-	-	-	-	-	-
Difflugia leidyi (Wailes, 1912)	Dif le	-	2	-	2	1	2	-	-	1	2	-	-	-	-
Difflugia sp.	Dif sp	-	2	-	2	-	10	-	7	2	6	-	-	-	-
Euglypha ciliata (Ehrenberg, 1848)	Eug cil	-	4	7	23	4	19	-	8	4	19	-	-	-	-
Euglypha compressa (Carter, 1864)	Eug com	-	2	6	6	-	2	-	-	-	2	-	8	8	9
Euglypha rotunda type (Wailes, 1911)	Eug rot	-	2	-	-	-	-	28	26	-	-	6	12	9	12
Euglypha sp.	Eug sp	-	2	-	2	1	2	9	12	-	-	-	4	-	-
Euglypha strigosa (Ehrenberg, 1872)	Eug st	-	3	-	3	-	3	-	-	-	3	-	5	-	5
Euglypha tuberculata type (Dujardin, 1841)	Eug tub	-	2	2	12	2	12	33	31	2	8	-	-	-	-
Heleopera petricola (Leidy, 1879)	Hel pet	3	3	3	26	5	19	-	-	9	3	-	-	-	-
Heleopera rosea (Penard 1890)	Hel ros	3	3	3	3	3	3	-	-	3	3	-	-	-	-
Heleopera sphagnii (Leidy, 1874)	Hel sph	3	3	3	3	6	3	-	-	6	3	-	-	-	-
Hyalosphenia elegans (Leidy, 1874)	Hel ele	32	30	46	43	63	49	16	9	63	56	-	-	-	-
Hyalosphenia ovalis (Wailes, 1912)	Hya ov	15	21	12	11	8	6	-	-	8	9	-	-	-	-
Hyalosphenia papilio (Leidy, 1875)	Hya pap	33	28	23	19	25	20	-	-	34	27	-	-	-	-
Hyalosphenia subflava (Cash and Hopkinson, 1909)	Hya sub	2	-	2	2	-	2	-	-	-	5	-	-	-	-
Nebela bohemica (Taranek, 1881)	Neb boh	3	-	3	-	3	-	-	-	3	-	-	-	-	-
Nebela carinata (Leidy, 1876)	Neb car	1	1	2	2	1	2	-	-	1	2	-	-	-	-
Nebela collaris (Ehrenberg, 1848)	Neb col	4	1	4	3	4	3	-	-	3	3	-	-	-	-
Nebela flabellulum (Leidy, 1874)	Neb fla	1	1	1	1	1	1	-	-	1	1	-	-	-	-
Nebela griseola type (Penard, 1911)	Neb gris	4	4	4	4	4	5	-	-	-	5	-	-	-	-
Nebela militaris (Penard, 1890)	Neb mil	2	3	4	5	4	5	3	3	-	4	-	-	-	3
Nebela parvula (Cash and Hopkinson, 1909)	Neb par	2	3	2	3	2	3	-	-	2	3	-	-	-	-
Nebela sp.	Neb sp	-	1	-	1	-	1	-	-	-	1	-	-	-	2
Nebela tincta (Leidy, 1879)	Neb tin	2	1	2	1	-	1	-	-	-	1	-	-	-	-
Nebela vitraea type (Penard, 1899)	Neb vit	1	1	1	1	-	1	1	1	-	1	-	-	-	-
Placocista spinosa type (Carter, 1865)	Plac spin	3	18	3	18	4	19	7	8	4	11	-	-	-	-
Trigonopyxis arcula (Leidy, 1879)	Trig arc	4	2	6	3	-	5	-	-	-	5	-	-	-	-
Total species number 45		28	43	33	39	32	41	11	15	26	34	4	7	5	8

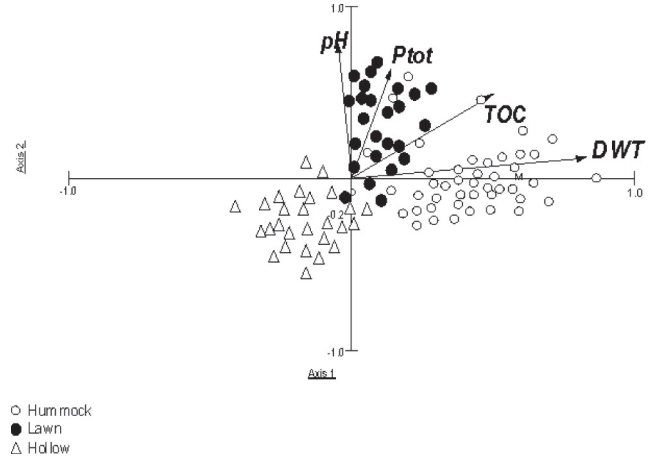

Fig. 2. PCA ordination plot of testate amoebae samples (log-transformed data).

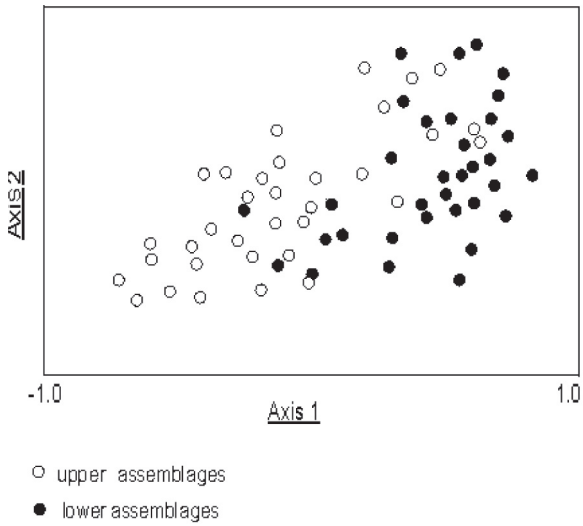

Fig. 3. Sample plot of detrended correspondence analysis (DCA) of testate amoebae data.

and the total variance explained by each CCA was similar. Monte Carlo permutation tests (Ter Braak, 1988) on these variables showed that DWT and concentrations of TOC were significant at $P < 0.001$. These two factors captured 23% of total variation of species data. The pH and P_{tot} played a less significant role. The relationships between each of these parameters and the species ordination were significant ($P < 0.01$) and explained 21% of the variation. The CCA ordination showed that the species could be separated into three groups. The first group included species associated with high DWT and TOC values (*i.e.*, dry conditions), and with low pH (*Assulina muscorum, Euglypha rotunda* type, *Euglypha tuberculata* type, *Nebela tincta, Corythion-Trinema* type). The second group included species that were associated with low DWT values (wet conditions) and low pH (*Hyalosphenia elegans, Archerella flavum, A. wrightianum, Nebela carinata, Arcella discoides*). The third group included species associated

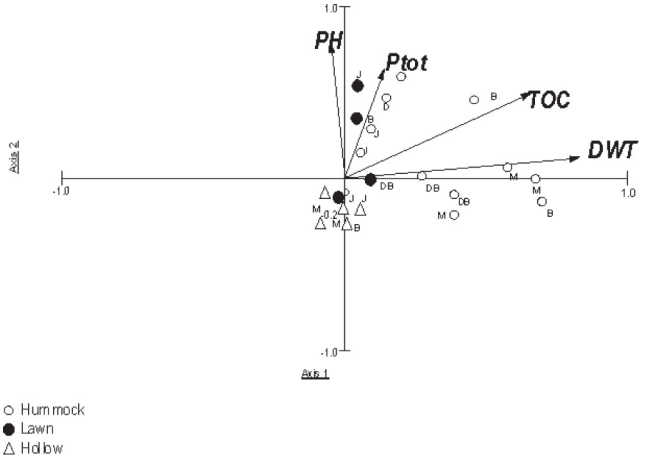

Fig. 4. Bi-plots of canonical correspondence analysis (CCA) of testate amoebae data from *Sphagnum*-dominated peatlands with representation of samples and environmental variables. Samples data were log-transformed, and rare species were downweighted.

with high pH conditions, mid-range DWT and higher concentrations of P_{tot} (*Centropyxis aculeata, C. aerophila, Nebela bohemica*) (Figs. 5A and 5B). Analysis of testate amoebae data and environmental variables revealed seasonal changes in environmental conditions and species composition. The eigenvalues of the first and second axis were 0.041 and 0.033, respectively. These two axes captured 12% of the total variation of species data. Samples in summer and autumn clustered together, but spring samples were plotted separately in the ordination diagram. Depth of the water table, pH, TOC, P_{tot} spring and autumn had significant regression coefficients. Differences in testate amoebae were primarily explained by microzonation, which is closely related to wetness. Water chemistry and season were secondary factors to explain testate amoebae distribution (Fig. 6).

Discussion

The similarity of testate amoeba assemblages between our own study sites, and with other European sites (Jauhiainen, 2002; Lamentowicz and Mitchell, 2005) is not surprising given the cosmopolitan distribution of many taxa. The highest numbers of taxa occurred in hollows dominated by *Sphagnum angustifolium, Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*. The low diversity of testate amoebae in *Polytrichum* is probably the consequence of the low moisture in this micro-environment. The number of taxa increased, however, with the drop in pH, and together with the increase in moisture conditions. This compares well to other studies (Booth, 2002; Mazei *et al.*, 2007). Considerable variation in the vertical distance spanned by the upper and lower assemblages existed between microsites, which is probably a result of differences in growth form and species of mosses. The lower assemblages had a significantly higher taxonomic richness than the upper assemblages.

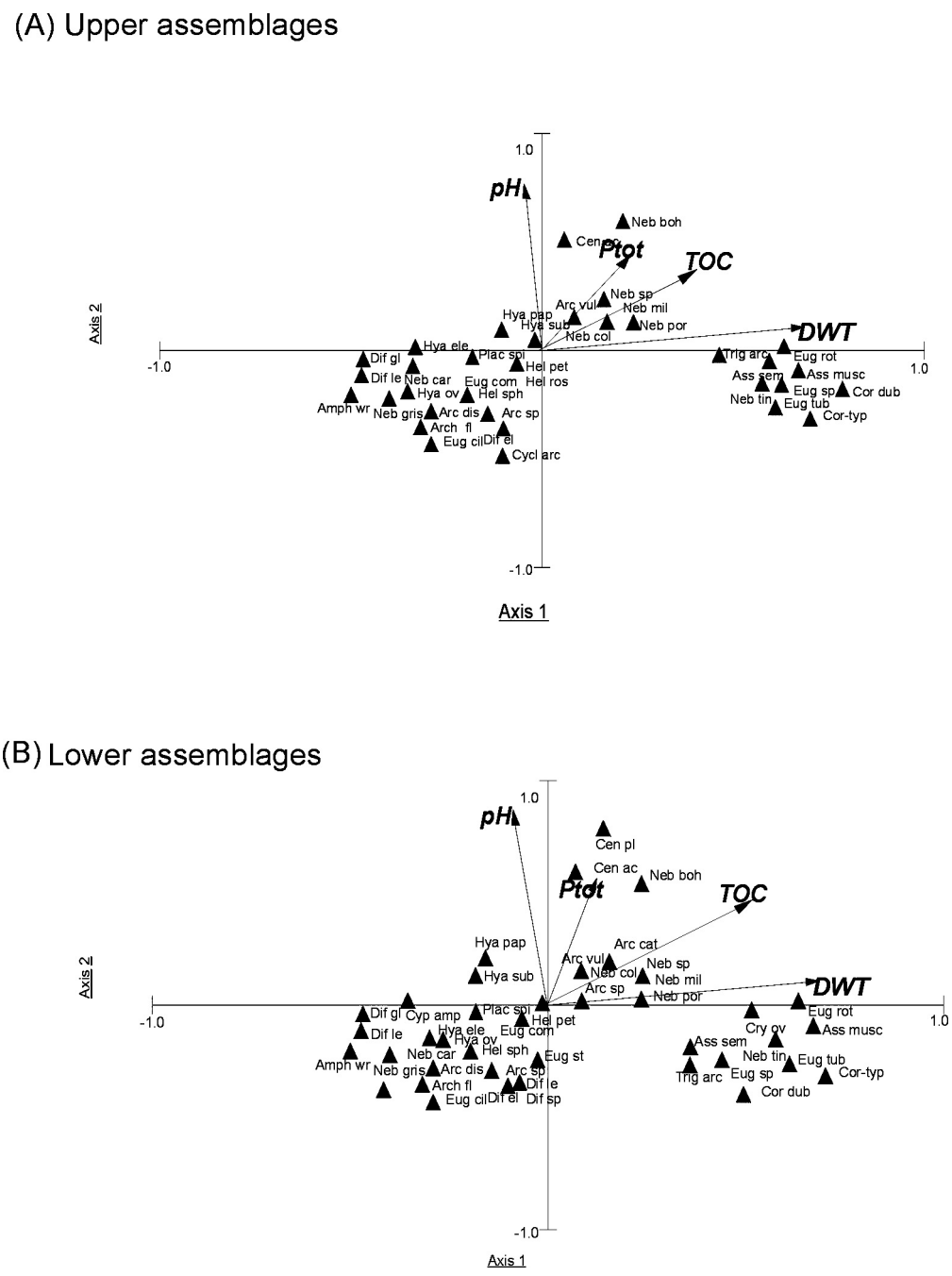

Fig. 5. Bi-plots of canonical correspondence analysis (CCA) of testate amoebae and environmental variables in (A) upper assemblages, and (B) lower assemblages. Abbreviations for testate amoebae are shown in Table 3.

Several factors probably contributed to this pattern. According to Booth (2002) and Mazei *et al.* (2007), some taxa occur predominantly in the upper portions of the mosses, and these taxa eventually become incorporated into the lower assemblage by vertical transport, and/or growth and senescence of the moss. Likewise, a distinct increase in moisture in the lower parts of the mosses may be the result of the abundance in testate amoebae.

There was a strong relationship between testate amoebae communities and both water table depth and pH. There was also a significant influence of total phosphorus on the occurrence of testate amoebae. In *Sphagnum* peatlands, testate amoebae communities were related to NO_3^- (Mitchell *et al.*, 2000a), and to a combination of physical and chemical variables such as moisture, pH, nitrogen, and dissolved organic carbon (Tolonen *et al.*, 1994; Jauhiainen, 2002). I found that the influence of total phosphorus increased with the increase in pH. In previous research on testate amoebae in relation to the chemical environment, many of the significant explanatory variables were nutrients (Tolonen *et al.*, 1994; Mitchell *et al.*, 2000b). It seems that nutrients have an indirect influence

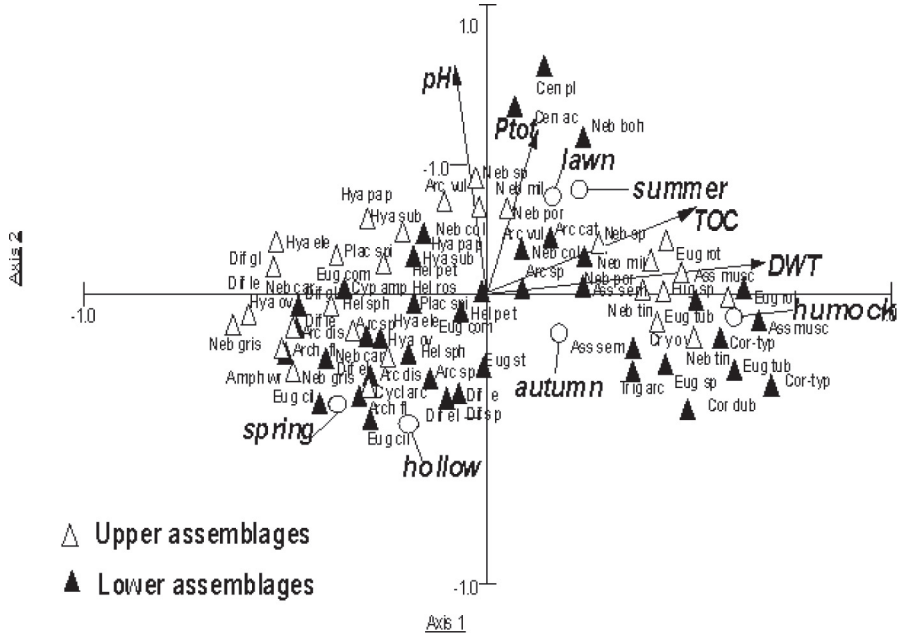

Fig. 6. Bi-plot of canonical correspondence analysis (CCA) of testate amoebae data including all the sampling stations, seasons and environmental variables. Abbreviations for testate amoebae are shown in Table 3.

on the prevalence of testate amoebae through the control food abundance (mainly bacteria, fungi, or protists). The CCA showed that DWT and pH were dominant factors controlling the distribution patterns of the upper and lower assemblages. These factors, however, showed distinct seasonal changes. During the spring period, those species characteristic of environments with considerable moisture were recorded in high numbers (*e.g.*, *Hyalosphenia elegans, Arcella discoides* type, *Placocista spinosa*). These species are typically found in habitats with high soil water content (Gilbert *et al.*, 2000; Warner *et al.*, 2007). In the other seasons, pH has a significant influence on the occurrence of these micro-organisms. A distinct increase in the number of testate amoebae was also observed by Heal (1964). Likewise, the study carried out by Warner *et al.* (2007) determined a seasonal change in the testate amoebae assemblages. The present study shows a significant relationship between testate amoebae and type of microenvironment. In hollows dominated by *Sphagnum angustifolium, Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*, amoebae species characteristic of wet conditions (*Arcella discoides* type, *Arcella vulgaris, Amphitrema wightianum* and *Archerella* flavum) were recorded in high numbers, whereas hummocks dominated by *Sphagnum magellanicum* and *Polytrichum strictum* were colonized mostly by testate amoebae located at the dry end of the water table gradient (*Assulina muscorum, Euglypha rotunda* type and *Euglypha tuberculata* type). Taxa with symbiotic zoochlorellae were more common at the top of the stem, and agglutinate taxa were more common on lower portions of the stem (Meisterfeld, 1977). A vertical micro-distribution in community of testate amoebae was clear among the *Sphagnum* moss (*Sphagnum angustifolium*, *Sphagnum cuspidatum, Sphagnum flexuosum* and *Sphagnum palustre*). On the other hand, the distribution pattern was blurred in dry environments dominated by *Polytrichum strictum*, *Polytrichum commune* and *Sphagnum magellanicum*.

In summary, the vertical distribution patterns of testate amoebae in this study were similar to those observed in various studies in Europe (Heal, 1962; Buttler *et al.*, 1996). There was a distinct horizontal micro-zonation of testate amoebae occurring among *Sphagnum* moss, but only a small difference ascertained among *Polytrichum*. The number of testate amoebae taxa significantly increased in the deeper samples. The results suggested that depth of water table, pH and total organic carbon can strongly regulate the abundance and taxonomic composition of testate amoebae. The influence of DWT and TOC were particularly clear in the lower parts of the mosses, and the number of testate amoebae was significantly greater in the deeper samples.

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