

Distributions of Testate Amoebae and Ciliates in Different Types of Peatlands and Their Contributions to the Nutrient Supply

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(Accepted August 5, 2011)

Tomasz Mieczan (2012) Distributions of testate amoebae and ciliates in different types of peatlands and their contributions to the nutrient supply. *Zoological Studies* 51(1): 18-26. The influence of plant communities on the structure, abundance, and biomass of testate amoebae and ciliates were investigated in bog and fens in eastern Poland. Samples were collected in belts of *Sphagnum*, *Phragmites*, *Carex*, *Utricularia*, and *Calliergonella*. Sampling was done on a monthly basis from Apr. to Nov. 2009. Comparisons of species numbers, abundances, and biomass levels of testate amoebae and ciliates between *Sphagnum* mosses did not show statistically significant differences. In carbonate fens, the average species numbers, abundances, and biomass levels of testate amoebae and ciliates for *Sphagnum*, *Calliergonella*, and *Utricularia* were higher than those for *Phragmites* and *Carex*. Based on differences in plant stem structure, 2 groups of habitats were distinguished. The 1st group consisted of 2 vegetated zones with a sparse stem structure (*Phragmites* and *Carex*), while the 2nd group consisted of plant species with a decidedly more-complicated structure (*Sphagnum*, *Calliergonella*, and *Utricularia*). The results demonstrated that water table depth, pH, and concentrations of total phosphorus and total organic carbon strongly regulated the taxonomic composition and abundances of protozoa. Rates of excretion of ammonia-nitrogen and phosphate-phosphorus proportionally decreased with an increase in body weight. In experiments dominated by small protozoa, excreted amounts were significantly higher than in experiments dominated by higher taxa. Average net excretion rates per protozoon of nitrogen ranged 1.0×10^{-5} - 3.72×10^{-5} $\mu\text{g/h}$ and of phosphorus ranged 6.5×10^{-6} - 1.2×10^{-5} $\mu\text{g/h}$.
<http://zoolstud.sinica.edu.tw/Journals/51.1/18.pdf>

Key words: Wetlands, Protozoa, Nitrogen, Phosphorus, Excretion

Peatlands are generally characterized by rich biodiversity and also play key roles in preserving the stability of ecological relationships in particular regions (Flessa et al. 1998). At the same time, they belong to the fastest disappearing and most endangered ecosystems in Europe. This is especially disquieting in combination with progressive climate warming (Flessa et al. 1998, Robson et al. 2005, Watters and Stanley 2006). Although ecological research on carbon dynamics, and plant and animal communities (e.g., copepods, nematodes, and insect larvae) of peatlands is well known (Walsh 1995, Wardle 2006, Watters and Stanley 2006), in contrast, in the whole of Europe

and worldwide, very little is known about the microorganisms and their roles in the functioning of these ecosystems. Testate amoebae and ciliates are good indicators of a variety of environmental variables including the hydrology, pH, and nutrient status (Mitchell et al. 2000 2004, Gilbert and Mitchell 2006, Nguyen-Viet et al. 2007, Mieczan 2007 2009a b). Studies by many authors (Mitchell et al. 2000, Mazei et al. 2007, Mieczan 2009a b) reported significant relationships between numbers of protozoan species and microhabitat types. In hollows of raised-bogs, it was noted that there is a decidedly higher species diversity and abundance of protozoa, compared to hummocks. However,

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research on the occurrence of protozoa (particularly ciliates) in carbonate fens is lacking. Until recently, only a few studies described the ecology of testate amoebae in fens (Payne and Mitchell 2007, Jassey et al. 2010, Lamentowicz et al. 2010, Payne 2011). On the other hand, studies concerning ciliates in raised, ombrotrophic bogs suggest an obvious qualitative and quantitative diversity among individual plant species (Mieczan 2009a). Thus, it seems that a similar differentiation should be expected in the case of protozoa occurring in others types of peatland microhabitats connected with patches of different plant species

Wilkinson (2008) suggested that testate amoebae, even if only a minor fraction of the total microbial biomass, could be responsible for a large proportion of nutrient recycling in peatland communities. One role of protozoa is utilization of organic particles and the regeneration of soluble inorganic matter. A major portion of nutrients is excreted within short time intervals due to rapid growth rates of testate amoebae and ciliates compared to larger zooplankton (Dolon 1997). The role played by ciliates in removing nutrients is relatively thoroughly studied in lake ecosystems (Ejsmont-Karabin et al. 2004). However, research on excretion of nitrogen and phosphorus by testate amoebae and ciliates in peatlands is lacking. Research by many authors showed that nitrogen is a factor limiting production in peatbog ecosystems (Watters and Stanley 2006, Kooijman and Paulissen 2006). The fact that protozoan distributions in peatland ecosystems seem to be of an extraordinarily mosaic character, in terms of both abundances and species structures, suggests that a similar mosaic may be expected in the case of nutrient dynamics. Since protozoa may reach extremely high abundances in peatbog ecosystems, they may have a significant role in nutrient dynamics. Still, the question of

excretion rates by protozoa in peatlands remains unanswered. Summing up, the current study was designed to test the hypothesis that protozoan communities in peatland ecosystems play major roles in nutrient cycling, deficiencies of which can be clearly observed, particularly in ombrotrophic peatlands.

The present study had 3 aims: 1) to describe testate amoeba and ciliate diversity; 2) to examine relationships between environmental variables and protozoa; and 3) to experimentally determine rates of N and P excretion by protozoa in relation to their body weights, the ambient temperature, and pH.

MATERIALS AND METHODS

Study site

The study area comprised 3 peatlands: a raised bog at Durne Bagno, a poor fen at Jelino-Krugle Bagno, and a rich carbonate fen at Bagno Bubnów (Polesie National Park, eastern Poland, 51°N, 23°E). The peatlands selected for this study represent various vegetation types. In the raised bog and poor fen, the vegetation is dominated by *Eriophorum vaginatum* (L.), *Carex acutiformis* Ehrhart., *Car. gracilis* Curt., *Sphagnum angustifolium* (C.C.O. Jensen ex Russow), *S. cuspidatum* Ehrh. ex Hoffm., and *S. magellanicum* Bird. The carbonate fen is colonized by *Phragmites australis* (Car.), *Car. acutiformis* Ehrhart, *Calliergonella cuspidata* (Hedw.), and *Utricularia* sp. (Table 1).

Field sampling and chemical analyses

Fieldwork was conducted monthly from Apr. to Nov. 2009. Sampling sites were chosen to achieve the highest diversity of microhabitats.

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

Peatland	Location	Area (ha)	Type of peatland	Dominant plants
Durne Bagno	51°22.344'N, 23°12.303'E	213.2	Raised bog	<i>Eriophorum vaginatum</i> , <i>Carex acutiformis</i> , <i>Sphagnum angustifolium</i> , <i>S. cuspidatum</i> , <i>S. magellanicum</i> , <i>S. palustre</i>
Krugle Bagno/Jelino	51°24.099'N, 23°9.116'E	19.7	Poor fen	<i>S. magellanicum</i> , <i>S. angustifolium</i> , <i>Car. acutiformis</i>
Bagno Bubnów	51°22.364'N, 23°15.303'E	2308.6	Rich fen	<i>Phragmites australis</i> , <i>Car. acutiformis</i> , <i>Calliergonella cuspidata</i> , <i>Car. davalliana</i> , <i>Comarum palustre</i> , <i>Utricularia</i> sp.

The total dataset consisted of 168 samples from 6 sites. During each sampling occasion, 3 samples were collected from each microhabitat. In the raised bog and poor fen, microbial communities were examined among different *Sphagnum* species (SA, *S. angustifolium*; SC, *S. cuspidatum*; and SM, *S. magellanicum* Bird) (with 72 total samples). In the carbonate fen, testate amoebae and ciliates were collected in belts of *P. australis* (PH), *Car. acutiformis* (CR), *Utricularia* sp. (UT), and *Cal. cuspidata* (CA) (with 96 total samples). In each type of microhabitat, water was sampled using a Plexiglas corer (1.0 m long, with an inside diameter of 50 mm). Four subsamples, of about 0.5 L each, were pooled into a calibrated vessel to form a composite sample (2 L), which was concentrated using a 10- μ m plankton net. The 1st sample was analyzed live. One liter of water was immediately preserved with Lugols solution (at a final concentration of 0.2%), allowed to settle in a glass column for over 24 h in the laboratory, and then concentrated to 30 ml. Finally, 0.1 ml of the concentrated sample was counted using a microscope at 400-1000x magnification. Abundances of testate amoebae and ciliates were determined using the Utermöhl method (Utermöhl 1958). Morphological identification of testate amoebae and ciliates was mainly based on works by Foissner and Berger (1996), Charman et al. (2000), and Clarke (2003). Biovolumes of testate amoebae and ciliates were estimated by assuming geometric shapes and converting to carbon using the following conversion factor: 1 $\mu\text{m}^3 = 1.1 \times 10^{-7}$ $\mu\text{g C}$ (Gilbert et al. 1998).

In each plot, temperature, conductivity, pH, dissolved oxygen (DO), total phosphorus (P_{tot}), total nitrogen (N_{tot}), and total organic carbon (TOC) were measured. Physical and chemical analyses were performed according to standard methods for hydrochemical analyses (Golterman 1969). Temperature, conductivity, pH, and DO were assessed at the sites with a multiparametric probe (Hanna Instruments, Woonsocket, USA); TOC was analyzed by a multiparametric UV analyzer (Secomam, Ales Cedex, France); P_{tot} by a colorimetric method; and N_{tot} by the Kjeldahl method.

Laboratory experiments

As a result of preliminary research carried out in 2008, very high numbers of microorganisms were found in peatbogs and fens, which made it possible to immediately use them for laboratory

experiments, without needing to cultivate them in order to acquire sufficient numbers. Surface water samples were taken from individual microenvironments in spring and summer 2009. In an effort to define the rate of nutrient excretion by protozoa, in the laboratory, microorganisms were washed with deionized water and condensed by filtration (through a 4- μ m mesh size). Next, protozoan (~6000) individuals were transferred to a watch glass containing 100 ml of deionized water (experiment no. 1), and then the watch glass was filled with peatbog water previously filtered through a 0.2- μ m filter (experiment no. 2). Concentrations of NH_4^+ and PO_4^{3-} were analyzed using a spectrophotometric method (APHA 1985) before removing the microorganisms and again 5 h after their removal. After 5 h, to determine the excretion rate by protozoa, the water was filtered through a 4- μ m-mesh filter, and the number of protozoa was again counted with an inverted microscope. The experiment was carried out at 3 pH values (of 4, 5, and 7) which had been observed in peatbog ecosystems, and at a medium temperature of 14-18°C noted during sample collection. Biovolumes of microorganisms were estimated by multiplying the numerical abundances by mean cell volume measurements using appropriate geometric formulae (Sherr et al. 1983). The experiment was repeated twice during the vegetative season: in spring when groups of protozoa were dominated by small forms (< 60 μm), and in late summer when larger species dominated (> 100 μm). Three replicates were used for each pH level. Rates of excretion were calculated as differences in P and N concentrations between the samples containing protozoa and the control. Protozoa were not fed during these experiments, and nutrient excretion rates of starved protozoa are ~30% lower than those for fed ones (Taylor 1986, Dolon 1997).

Data analyses

The significance of differences between mean density and biomass values of testate amoebae and ciliates was verified by an analysis of variance (ANOVA). Ordination methods were used to examine the general structure of the protozoan data and test links between the protozoa and environmental data. A detrended correspondence analysis (DCA), an unconstrained indirect method, was used to measure and illustrate gradients indicated by the protozoa. Because the length of the gradient was > 2 standard deviations (SDs), a canonical correspondence analysis (CCA),

a method which assumes unimodal species-environmental relationships (Ter Braak 1988-1992), was used. A diversity analysis (i.e., the Shannon-Wiener diversity index) was performed using the Multivariate Statistical Package (MVSP 2002). Similarities of protozoa communities among the peatlands were compared using the Euclidean distance measure.

RESULTS

Environmental variables

The water table depth (DWT) was highly variable among sites and samples, ranging 20-55 cm (ANOVA, $F = 26.5$, $p = 0.001$). Statistically significant differences among the studied peatlands were found in pH, conductivity, P_{tot} , N_{tot} , and TOC (ANOVA, $F = 30.21-31.22$, $p = 0.001$). Among the studied peatlands, the highest average pH value (pH 7.6) was noted in the rich fen with the lowest in the bog and poor fen (pH 3.2-4.5). TOC concentrations were highest in the bog and poor fen; however the remaining parameters (conductivity, P_{tot} and N_{tot}) were highest in the carbonate rich fen. In the bog and poor fen, chemical properties of the water were similar between micro-sites ($p > 0.05$). In the rich fen, chemical properties of the water significantly differed between micro-habitats (ANOVA, $F = 29.4$, $p = 0.0012$). The highest conductivity and concentrations of P_{tot} , N_{tot} , and TOC were noted in belts of *Utricularia* and *Calliergonella* (Table 2).

Protozoan diversity and density: general results

In total, 29 testate amoeba and 19 ciliate taxa were identified. The highest numbers of testate amoeba and ciliate taxa occurred in the bog and poor fen (with respective totals of 23 and 15 taxa). A lower number of taxa (16) was observed in the rich fen. A comparison of species numbers, abundances and biomass levels of testate amoebae and ciliates among *Sphagnum* mosses did not show significant differences ($p = 0.560$). These differences were significant for micro-habitats in the carbonate fen (ANOVA, $F = 31.4$, $p = 0.001$). The highest species numbers (11-16) were found in belts of *Utricularia* and *Calliergonella*, and the lowest richness levels (6-9) were observed in micro-habitats dominated by *Typha*, *Phragmites*, and *Carex*. Samples were moderately diverse with Shannon diversity 'H' values ranging from 3.2 in *Sphagnum* to 2.1 in *Typha* stands. In the studied peatlands, numbers and biomass levels of protozoa significantly differed among the studied stands, with the lowest numbers in *Phragmites* and *Carex* micro-habitats and the highest numbers in *Utricularia* and *Calliergonella*. In general, compositions of ciliates were similar among *Sphagnum* mosses, *Calliergonella*, and *Utricularia*. However testate amoeba communities from the rich fen differed from the others (Figs. 1, 2). The most abundant testate amoeba taxa in the mosses were *Assulina muscorum* and *Euglypha tuberculata* type, and the most abundant ciliate taxon was *Chilodonella uncinata*. In the carbonate, rich fen, 2 groups of habitat were generally favored by testate amoebae and ciliates. Plant beds

Table 2. Physical and chemical characteristics of water in the investigated peatlands (average values for the period Apr.-Nov. 2009 ± standard deviation). SA, *Sphagnum angustifolium*; SC, *S. cuspidatum*; SM, *S. magellanicum*; PH, *Phragmites australis*; CR, *Carex acutiformis*; UT, *Utricularia* sp.; CA, *Calliergonella cuspidata*; DWT, depth to water table; TN, total nitrogen; TP, total phosphorus; TOC, total organic carbon

	Microhabitat	DWT (cm)	pH	Dissolved oxygen (mg/L)	Conductivity ($\mu\text{S/cm}$)	TN (mg/L)	TP (mg/L)	TOC (mg/L)
Raised bog	SA	17 ± 5	3.3 ± 1	8.3 ± 3.3	29 ± 6.4	1.121 ± 0.02	0.222 ± 0.11	54 ± 11
	SC	15 ± 7	3.2 ± 1	10.1 ± 3.1	27 ± 8.2	1.13 ± 0.23	0.239 ± 0.11	56 ± 8
	SM	19 ± 6	3.6 ± 1.5	9.2 ± 3.1	32 ± 8.7	1.332 ± 0.25	0.251 ± 0.03	59 ± 7
Poor fen	SA	9 ± 4	4.5 ± 1	7.9 ± 3.3	48 ± 5.3	1.263 ± 0.06	0.241 ± 0.111	53 ± 13
	SC	11 ± 6	5.2 ± 2	8.9 ± 2.1	45 ± 4.5	1.53 ± 0.23	0.269 ± 0.06	49 ± 11
	SM	5 ± 2	4.6 ± 2	9.2 ± 2.1	48 ± 6.9	1.531 ± 0.28	0.275 ± 0.06	46 ± 15
Rich fen	PH	49 ± 3	7.9 ± 1	8.3 ± 2.1	321 ± 17	2.111 ± 0.78	0.311 ± 0.12	15 ± 2
	CR	41 ± 3	8.2 ± 1	8.5 ± 2.3	311 ± 23	2.112 ± 0.98	0.290 ± 0.16	14 ± 4
	UT	20 ± 5	7.2 ± 1	6.9 ± 1.8	421 ± 25	1.563 ± 0.96	0.368 ± 0.18	23 ± 4
	CA	22 ± 6	7.1 ± 1.5	6.7 ± 1.8	399 ± 31	1.468 ± 0.48	0.378 ± 0.13	21 ± 6

with a “simple” structure (*Phragmites* and *Carex*) were distinctly predominated by testate amoebae (*Hyalosphenia elegans*) and ciliates (*Strombidium viride*). Testate amoebae (*Arcella discoides*, *Arc. vulgaris*, *Centropyxis aculeata*, and *Cen. aerophila*) and small ciliates (of the Scuticociliatida) showed significant connections with beds possessing a decidedly complex structure (*Utricularia* and *Calliergonella*).

Correlations among testate amoebae, ciliates, and environmental variables

The DCA showed that the species composition of protozoa clearly differed between the bog and fen (Fig. 3). The 1st 2 DCA axes explained 20% of the total protozoan variability. Results for all sites showed that axis 1 was significantly correlated with DWT, P_{tot} , and TOC, whereas axis 2 was correlated with pH ($p < 0.05$). Sites were

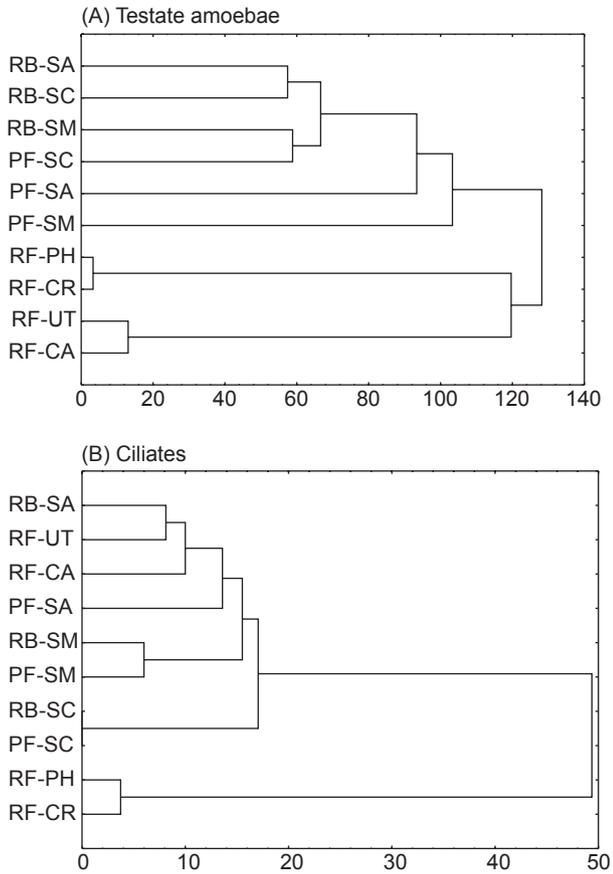


Fig. 1. (A, B) Similarity between testate amoeba and ciliate communities in the investigated peatlands. RB, raised bog; PF, poor fen; RF, rich fen; SA, *Sphagnum angustifolium*; SC, *S. cuspidatum*; SM, *S. magellanicum*; PH, *Phragmites australis*; CR, *Carex acutiformis*; UT, *Utricularia* sp.; CA, *Calliergonella cuspidata*.

separated into 2 main types of habitats: mosses-*Sphagnum* and vascular plants. In the canonical correspondence analysis, all variables (DWT, pH, and P_{tot} and TOC concentrations) together explained 40% of the variance ($p < 0.001$). The CCA revealed that the proportion of testate

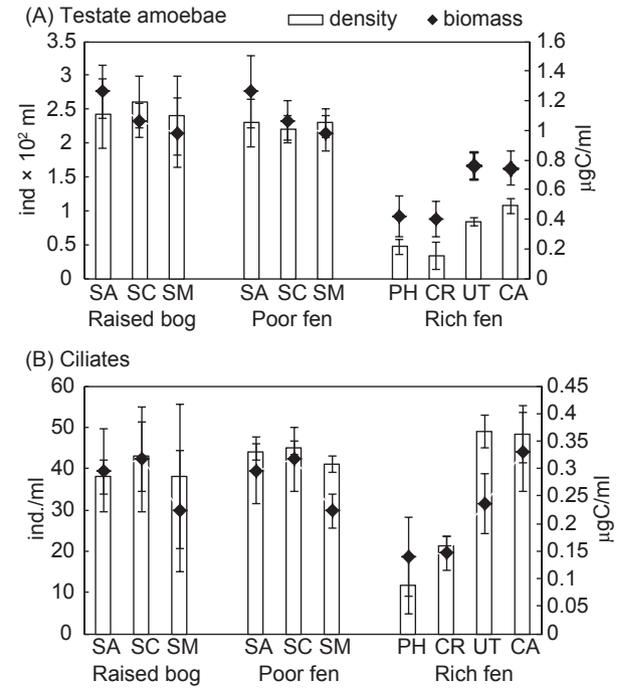


Fig. 2. (A, B) Average (Apr.-Nov. 2009) density and biomass of testate amoebae and ciliates within plant patches in the investigated peatlands. SA, *Sphagnum angustifolium*; SC, *S. cuspidatum*; SM, *S. magellanicum*; PH, *Phragmites australis*; CR, *Carex acutiformis*; UT, *Utricularia* sp.; CA, *Calliergonella cuspidata*.

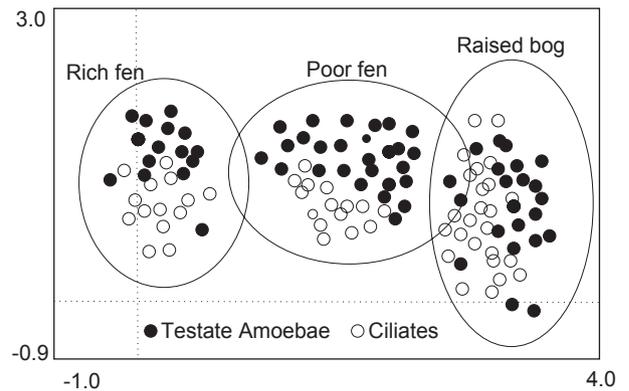


Fig. 3. Detrended correspondence analyses (DCAs) of protozoan samples (log-transformed data).

amoeba and ciliate data explained by each explanatory variable and its significance strongly varied among variables and between the bog and fen. Microsites without *Sphagnum* were usually characterized by a low water level, a low pH, and a higher concentration of TOC. More-abundant taxa in these habitats included *Ass. muscorum*, *Eug. tuberculata* type, *Nebela tincta*, *Corythion-Trinema* type, *Chi. uncinata*, *Colpidium colpoda*, and *Paramecium bursaria*. The 2nd group included species that were associated

with a higher water level and high pH (*Archerella flavum*, *Arc. wrightianum*, *Hyalosphenia elegans*, *Neb. carinata*, *Cinetochilum margaritaceum*, and *Codonella cratera*). The 3rd group included species associated with a high water level and pH conditions and a higher concentration of P_{tot} (*Arc. vulgaris*, *Arc. discoides*, *Cen. aculeata*, *Cen. aerophila*, *Colpoda steinii*, *Disematostoma tetraedricum*, *Holosticha pullaster*, *Strombidium viride*, and the *Stylonychia mytilus*-complex) (Fig. 4).

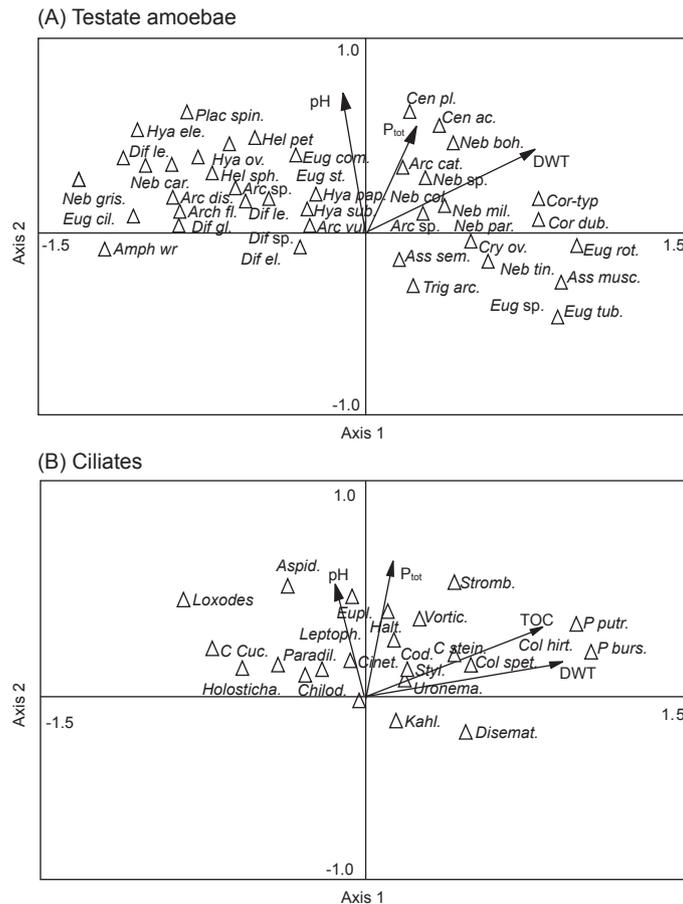


Fig. 4. Biplots of the canonical correspondence analysis (CCA) of testate amoeba and ciliate data from investigated peatlands with representation of environmental variables. Species data were log-transformed, and rare species were down-weighted. DWT, depth of water table; P_{tot} , total phosphorus; pH, water reaction; TOC, total organic carbon. Testate amoebae: *Amph wr.*, *Amphitrema wrightianum*; *Arc cat.*, *Arcella catinus* type; *Arc dis.*, *Arcella discoides* type; *Arc vul.*, *Arcella vulgaris*; *Arc sp.*, *Arcella* sp.; *Arch fl.*, *Archerella flavum*; *Ass musc.*, *Assulina muscorum*; *Ass sem.*, *Assulina seminulum*; *Cen ac.*, *Centropyxis aculeata* type; *Cen pl.*, *Centropyxis platystoma* type; *Cor dub.*, *Corythion dubium*; *Cor-typ.*, *Corythion-Trinema* type; *Cry ov.*, *Cryptodiffugia oviformis*; *Dif el.*, *Diffugia elegans*; *Dif gl.*, *Diffugia globulosa*; *Dif le.*, *Diffugia leidy*; *Dif sp.*, *Diffugia* sp.; *Eug cil.*, *Euglypha ciliata*; *Eug com.*, *Euglypha compressa*; *Eug rot.*, *Euglypha rotunda* type; *Eug st.*, *Euglypha strigosa*; *Eug tub.*, *Euglypha tuberculata* type; *Eug sp.*, *Euglypha* sp.; *Hel sph.*, *Heleoptera sphagnii*; *Hel pet.*, *Heleoptera petricola*; *Hya ele.*, *Hyalosphenia elegans*; *Hya ov.*, *Hyalosphenia ovalis*; *Hya pap.*, *Hyalosphenia papilio*; *Hya sub.*, *Hyalosphenia subflava*; *Neb boh.*, *Nebela bohemica*; *Neb car.*, *Nebela carinata*; *Neb col.*, *Nebela collaris*; *Neb gris.*, *Nebela griseola* type; *Neb mil.*, *Nebela militaris*; *Neb tin.*, *Nebela tincta*; *Neb sp.*, *Nebela* sp.; *Plac spin.*, *Placocista spinosa* type; *Trig arc.*, *Trigonopyxis arcuata*. Ciliates: *Aspid.*, *Aspidisca* sp.; *Chilod.*, *Chilodonella uncinata*; *Cinet.*, *Cinetochilum margaritaceum*; *Cod.*, *Codonella cratera*; *Col. hirt.*, *Coleps hirtus*; *Col. spet.*, *Coleps spetai*; *C. cuc.*, *Colpoda cucullus*; *C. stein.*, *Colpoda steinii*; *Disemat.*, *Disematostoma tetraedricum*; *Eupl.*, *Euplotes* sp.; *Halt.*, *Halteria grandinella*; *Holosticha.*, *Holosticha pullaster*; *Kahl.*, *Kahlilembus attenuotus*; *Leptoph.*, *Leptopharynx costatus*; *Loxodes.*, *Loxodes* sp.; *Oxytr.*, *Oxytricha* sp.; *Paradil.*, *Paradileptus elephantinus*; *P. burs.*, *Paramecium bursaria*; *P. putr.*, *Paramecium putrinum*; *Stromb.*, *Strombidium viride*; *Styl.*, *Stylonychia mytilus*-complex; *Uronema.*, *Uronema* sp.; *Vortic.*, *Vorticella companula*.

Phosphorus and nitrogen excretion by protozoa

The protozoa excreted measurable amounts of ammonia-nitrogen (N-NH₄), and phosphate-phosphorus (P-PO₄), and there were no effects of pH on excretion rates of ammonia or phosphate. Concentrations of N-NH₄ and P-PO₄ were significantly higher after a 5-h exposure, the same as the control (ANOVA, $F = 21.2$, $p = 0.0011$). Some significant additional data proving the existence of dependence between individual size classes of protozoa and the amount of excretion were also ascertained. In experiments in which small protozoa were dominant, amounts excreted were significantly higher (ANOVA, $F = 22.0$, $p = 0.0012$). Rates of excretion decreased proportionally to an increase in body weight. It was also noted that in deionized water and prefiltered peatbog water, amounts excreted were similar and showed no statistically significant difference (Tables 3, 4).

DISCUSSION

Community structure in relation to environmental parameters

Numbers of identified taxa of testate amoebae and ciliates were comparable to other studies examining peatlands (Payne and Mitchell 2007, Mieczan 2009a b, Jassej et al. 2010). In the present study, water levels, pH, and TOC, were deciding factors constraining communities of protozoa in peatlands. This compares well to other studies (Tolonen et al. 1994, Velho et al. 2003, Payne and Mitchell 2007, Mieczan 2009a b). There was also a significant influence of total phosphorus on the occurrence of protozoa. In previous research on testate amoebae in relation to the chemical environment, many of the significant explanatory variables were nutrients (Mitchell 2004). Moreover, it was demonstrated that the occurrence of testate amoebae in minerotrophic fens in Greece was significantly influenced by hydrological factors (Payne and Mitchell 2007). The autecology of

Table 3. Excretion rates (μg protozoa \times h) of ammonia and phosphate in laboratory experiments (average value \pm S.D.)

Protozoa/ size	Dry weight \times cell (μg)	Control		Protozoa (after 5 h)		Excretion rate (μg N-NH ₄ \times cell \times h)	Excretion rates (μg P-PO ₄ \times cell \times h)
		N-NH ₄ (mg/L)	P-PO ₄ (mg/L)	N-NH ₄ (mg/L)	P-PO ₄ (mg/L)		
Filtered peatbog water							
< 60 μm	15,221	1.211 \pm 0.211	0.265 \pm 0.026	2.061 \pm 0.238	0.455 \pm 0.112	2.8 $\times 10^{-5}$	6.5 $\times 10^{-6}$
> 100 μm	40,150	1.200 \pm 0.217	0.260 \pm 0.056	1.600 \pm 0.212	0.650 \pm 0.026	1.3 $\times 10^{-5}$	1.3 $\times 10^{-5}$
Deionized water							
< 60 μm	16,221	0	0	0.720 \pm 0.243	0.111 \pm 0.043	3.72 $\times 10^{-5}$	2.4 $\times 10^{-6}$
> 100 μm	43,200	0	0	0.300 \pm 0.111	0.360 \pm 0.111	1.0 $\times 10^{-5}$	1.2 $\times 10^{-5}$

Table 4. Relationships (Pearson's correlations coefficients) of the rate of excretion with individual body weights of protozoa, pH, and temperature

Experiments	r	p	r	p
Filtered peatbog water				
Body weight	-0.58	0.01	-0.62	0.01
pH	ns	ns	ns	ns
Temperature	0.43	0.05	0.41	0.05
Deionized water				
Body weight	-0.63	0.01	-0.64	0.01
pH	ns	ns	ns	ns
Temperature	0.41	0.05	0.38	0.05

ns, not significant.

many species living in investigated peatlands corresponds well to published data (Mitchell et al. 2000, Opravilová and Hájek 2006, Jassey et al. 2010). In the wettest microhabitats with low-pH species such as *Arcella vulgaris*, *Archerella flavum*, *Arch. wrightianum*, *Hyalosphenia elegans*, *Neb. carinata*, *Cinetochilum margaritaceum*, and *Codonella cratera* were present, and in the driest ones are species such as *Assulina muscorum*, the *Corythion-Trinema* type, the *Euglypha tuberculata* type, *Neb. tinctoria*, *Chilodonella uncinata*, *Colpidium colpoda*, and *Paramecium* sp. The genus *Centropyxis* is often reported as characteristic of high-pH habitats, i.e., calcium-rich fens. The results are in keeping with the recognized moisture preferences of these species. Opravilová and Hájek (2006) reported that species compositions of both the vegetation, involving vascular plants and bryophytes, and moss samples characterize testatean assemblages better than even long-term measured water-chemistry data. In the present study, species richness levels and abundances of protozoa were similar between different species of mosses. The lack of any statistically significant difference in protozoan abundances may be related to the fact that all moss species were situated in sphagnum hollows with waters of similar physical and chemical properties. On the other hand, in a rich fen, both the abundance and species diversity among the protozoa clearly varied among individual plant species. It was observed in the present study that species diversity and abundance, and the biomass of protozoa increased in the most architecturally complex habitats of *Utricularia* and *Calliergonella* beds. According to Mieczan (2008), more-structurally complex plants provide a more-attractive environment for protozoa by better providing food and refuge.

Nutrient excretion

Significant differences between protozoan size and excretion intensity suggest their particularly vital role in bogs. For an average population of 1000 protozoa in 1 ml of water in peatbogs, the average net excretion rate of nitrogen was 0.58 μg (as N-NH_4)/d and of phosphorus was 0.22 μg (as P-PO_4)/d (data presented in Table 3). The obvious prevalence of small forms in such peatbogs means that during the vegetative period (from Apr. to Nov.), these microorganisms can supply ca. 139 μg N-NH_4 and 53 μg P-PO_4 /d. Obviously, these are minimum excretion volumes compared to those

that may be noted in field conditions where the abundance of food is relatively high. A significant effect of a cell's size on the rate of nitrogen and phosphorus excretion was also observed by other authors. However, their works examined lake and sea ecosystems (Taylor 1986, Dolan 1997). No significant relationship between the excretion volume and pH was detected. Similar observations were noted by Dolan (1997). On the other hand, studies carried out by Liu et al. (2007) revealed an increase in phosphate excretion at pH 6.8-8. Ciliates are considered major consumers of bacterial production in aquatic ecosystems. However, recent studies showed that testate amoebae are also able to consume a large fraction of bacterial populations in peatbogs (Mieczan 2007). The consumption of bacteria constitutes a major portion of nutrient regeneration in peatbogs. Due to their abundance and relatively high weight-specific excretion rates, protists probably account for a large portion of nutrient regeneration in a variety of peatbog ecosystems (hypothesis 2).

These results show that vascular plant, moss, testate amoeba, and ciliate communities respond differently to ecological gradients. Factors which most highly affect their occurrences are probably water depth, pH, P_{tot} , and TOC. In accordance with the 1st hypothesis, factors limiting the occurrence of these microorganisms are the complexity of the plant cover, groundwater level, and trophic parameters. Statistically significant differences between the size of the protozoa and the intensity of excretion suggest that they have a key role in nutrient cycling in bogs, which confirms the 2nd hypothesis.

Acknowledgments: This work was partially financed by project N N304 209837 from the Ministry of Science and Higher Education, Warsaw, Poland. I would like to thank L. Błędzki for methodical comments on the laboratory experiments. I am also grateful to M. Marzec and M. Niedźwiecki for conducting the chemical analyses.

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