

# Co-Occurrence of Ciliates and Rotifers in Peat Mosses

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## Abstract

The aim of this study was to examine relations between the density and species richness of ciliates and rotifers in 6 peatlands (2 raised bogs, 2 poor fens, 1 typical fen, and 1 base-rich fen), in Polesie National Park in southeastern Poland. Their relations with selected chemical parameters were also analyzed. The peatlands differed in pH, conductivity, total phosphorus, nitrates, and total organic carbon concentrations. The study showed that the microbial communities are most strongly dependent on concentrations of total organic carbon, nitrates, phosphates, and total phosphorus. It seems that nutrients and total organic carbon have an indirect influence on the abundance of ciliates and rotifers, through the control of food abundance (mainly bacteria). Additionally, ciliates are most abundant in spring and autumn, when rotifers are infrequent. This suggests that rotifers, as competitors, could be the main regulators of ciliate abundance in surface water of peatlands.

**Keywords:** ciliates, rotifers, peat mosses

## Introduction

Peatlands are often described as intermediate between terrestrial and aquatic habitats. However, various types of peatlands can be distinguished, depending on their hydrology, concentrations of nutrients, pH, and dominant vegetation [1]. Peatlands, and especially *Sphagnum*-dominated peatlands, were for a long time erroneously believed to be devoid of microbial life. In reality, despite the successful use of *Sphagnum* as surgical dressings and menstrual pads, *Sphagnum* mosses and peatlands are home to a high diversity and density of microorganisms [2-4]. In *Sphagnum* bogs, microbial community composition is generally strongly correlated to pH, concentrations of nutrients, temperature, and oxygen concentration [4]. Mitchell et al. [5] suggest that the availability of water is the major factor limiting the occurrence of microorganisms in peatlands. In a

taxonomic approach to the ecology of peatlands, micro-metazoans and invertebrates are usually studied independently, and the focus is usually on the taxonomy of the species restricted to peatlands [6]. By contrast, little or no attention has been given to the abundance of ciliates. Ciliates were studied extensively by Groliere [7-9] in *Sphagnum*-dominated peatlands in France. In the 1990s, 2 papers focused on the distribution of moss-dwelling ciliates in eastern Antarctica [10, 11]. The ecology and spatial distribution of ciliates were studied also in small water bodies with low pH and high humic matter content [12, 13]. Those studies showed that the increase in acidity causes a decrease in abundance, biomass, diversity, and species richness of ciliates. Similarly, a small number of studies have dealt with rotifer communities in peatland ecosystems. They have been limited, so far, to bog ponds [14-20]. However, rotifers are known from various microhabitats associated with peatlands [18, 19, 21, 22]. Besides, some research has been conducted on the co-occurrence of ciliates and rotifers

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Table 1. Physical and chemical characteristics of the water of investigated peatlands (average values  $\pm$  SD for April–November 2007).

	T [°C]	pH	Conductivity [ $\mu$ S·cm <sup>-1</sup> ]	O <sub>2</sub> [mgO <sub>2</sub> ·dm <sup>-3</sup> ]	P <sub>tot</sub> [mgP·dm <sup>-3</sup> ]	N-NO <sub>3</sub> [mgNO <sub>3</sub> ·dm <sup>-3</sup> ]	TOC [mgC·dm <sup>-3</sup> ]	PO <sub>4</sub> <sup>3-</sup> [mgPO <sub>4</sub> ·dm <sup>-3</sup> ]	N-NH <sub>4</sub> [mgN·dm <sup>-3</sup> ]
BB (1)	17.44 $\pm$ 5.67	6.52 (2,5,6**; 3,4*) $\pm$ 0.65	524.00 (3,5**; 6*) $\pm$ 34.51	7.42 $\pm$ 1.01	0.46 $\pm$ 0.02	0.13 (6*) $\pm$ 0.00	16.30 (2,5,6**) $\pm$ 3.35	0.23 $\pm$ 0.00	1.81 $\pm$ 0.51
DB (2)	15.10 $\pm$ 7.09	3.80 (1**) $\pm$ 0.93	95.60 (3**) $\pm$ 15.77	8.26 $\pm$ 0.89	0.34 $\pm$ 0.01	0.19 $\pm$ 0.01	64.38 (1**) $\pm$ 26.46	0.25 $\pm$ 0.04	1.14 $\pm$ 0.05
J (3)	15.79 $\pm$ 7.29	4.70 (1*) $\pm$ 1.35	37.71 (1,2,4**) $\pm$ 12.03	7.82 $\pm$ 1.06	0.27 (5*) $\pm$ 0.07	0.24 $\pm$ 0.01	25.59 (5,6**) $\pm$ 2.18	0.37 $\pm$ 0.27	1.21 $\pm$ 0.09
L (4)	16.24 $\pm$ 8.43	4.63 (1*) $\pm$ 0.65	137.01 (3**) $\pm$ 51.01	8.45 $\pm$ 1.55	0.45 $\pm$ 0.02	0.31 $\pm$ 0.03	55.96 $\pm$ 16.77	0.97 $\pm$ 0.35	1.15 $\pm$ 0.02
M1 (5)	16.06 $\pm$ 7.92	3.87 (1**) $\pm$ 0.77	73.16 (1**) $\pm$ 17.64	7.94 $\pm$ 1.42	0.76 (3*) $\pm$ 0.10	0.32 $\pm$ 0.01	99.00 (1,3**) $\pm$ 43.49	0.56 $\pm$ 0.30	1.32 $\pm$ 0.18
M2 (6)	16.30 $\pm$ 7.81	3.47 (1**) $\pm$ 0.53	83.98 (1*) $\pm$ 29.88	7.99 $\pm$ 1.20	0.32 $\pm$ 0.02	0.35 (1*) $\pm$ 0.03	94.13 (1,3**) $\pm$ 40.04	0.25 $\pm$ 0.03	1.28 $\pm$ 0.12

TOC = total organic carbon. Significantly different peatlands are marked with appropriate numbers (\*\* for  $P \leq 0.01$ ; \* for  $P \leq 0.05$ ).

in ecosystems with *Sphagnum* moss. In the last few years, research on microbial communities in *Sphagnum* was conducted by Mitchell et al. [5] and Gilbert D. et al. [23]. Those studies showed that microbial groups are dominated by heterotrophic bacteria, while ciliates and rotifers account for only a small proportion of total biomass. Nonetheless, rotifer and ciliate communities are important links in the processes that take place in aquatic ecosystems. Ciliates play an important role in the food web of various aquatic ecosystems [12, 24, 25]. These protozoans can forage on particles that are not eaten by larger zooplankton and thus are an important source of food for larger planktivores, including rotifers [12, 25]. Similarly, the rotifers found in *Sphagnum* mats seem to be important in the processes taking places in these ecosystems, because rotifers are important elements of phosphorus and nitrogen cycles [3].

The aims of this study were:

- (1) to analyze relations between the density and species richness of ciliates and rotifers in 6 peatlands, most of them dominated by *Sphagnum*
- (2) to assess the influence of abiotic factors on their communities

## Materials and Methods

### Study Area

Our research was conducted in 6 peatlands in Polesie National Park in southeastern Poland. They are unique peatlands – some of few in Europe left after the Saalian glaciation [26]. The studied peatlands include a typical fen (L = Lejno), 2 poor fens (J = Jelino; M1 = Moszne 1), 2 raised bogs (DB = Durne Bagno; M2 = Moszne 2), and a topogenic base-rich fen on calcareous gytja (BB = Bagno

Bubnów). The peatlands differed in respect of vegetation. The typical fen (L) was dominated by *Sphagnum flexuosum* Dozy & Molk., poor fens (J and M1) by *S. magellanicum* Bird., and M1 additionally by *Polytrichum strictum* Menzies., while raised bogs by *S. angustifolium* (C. Jens. ex Russ.) in DB, as well as *S. flexuosum* Dozy & Molk., *Sphagnum cuspidatum* Ehrh. ex Hoffm. The base-rich fen (BB) was colonized by emergent vegetation: *Phragmites australis* (Car.), *Typha latifolia* L., and *Carex acutiformis* Ehrhart.

The peatlands differed in pH (3.4–6.5) and conductivity (12.03–524.00  $\mu$ S cm<sup>-1</sup>), as well as in concentrations of total phosphorus (0.27–0.76 mgP·dm<sup>-3</sup>), nitrates (0.13–0.35 mg NO<sub>3</sub>·dm<sup>-3</sup>), and total organic carbon (16.30–99.00 mgC·dm<sup>-3</sup>). The differences were statistically significant (Table 1). In the base-rich fen (BB), the highest values of pH (6.5) and conductivity (524.00  $\mu$ S·cm<sup>-1</sup>) were recorded. Both Moszne peatlands (M1 and M2) were distinguished by a markedly higher concentration of nitrates and total organic carbon than the other peatlands, while the typical fen (L), was the richest in phosphates.

### Ciliate and Rotifer Sampling

Moss samples for this study were taken from April to November 2007 in monthly intervals. Samples of ciliates were collected from various species of mosses (*S. angustifolium*, *S. cuspidatum*, *S. flexuosum*, *S. magellanicum*, *S. palustre*, *P. strictum*, and *P. commune*). Eight samples were collected from the studied peatlands once a month. A long knife was used to cut the plants out of the vegetation. Each sample was packed into a 150-ml cylindrical plastic container. All samples were stored in a cooler and transported within 1 day to the laboratory. Microorganisms were iden-

tified in 4 subsamples, each equal to 5% of the original sample. The abundance of microorganisms was expressed per 1 g of dry weight of plant material. In order to determine ciliates, 4 samples were preserved with Lugol solution. Ciliates were enumerated and identified with an inverted microscope at 400-1000 × magnification [27]. Taxonomic identifications of ciliates were based on the keys in Foissner et al. [28], as well as Foissner and Berger [29].

Samples of rotifers were collected based on the methods of Peters, Koste, and Westheide [30]. Moist moss was collected in 150-ml containers. Quantitative samples were prepared by weighing 10 g of moist moss, which was next washed 10 times with distilled water (50 ml of water each time), and the water was then filtered through a plankton net (mesh size 30 µm). Next, the residue was preserved with 4% formalin. The remaining moss constituted a qualitative sample in which live rotifers were identified.

For each quantitative sample (30 ml), rotifers were counted in 6 subsamples, in 1-ml Kolkwitz chambers. The mean number of rotifers from 6 chambers was calculated on the basis of 1 g of dry weight of mosses (DW).

### Water Sampling

When collecting the samples for ciliate and rotifer identification, physicochemical parameters of water also were measured near the sampled moss. We measured water temperature [°C], pH, electrolytic conductivity [ $\mu\text{S}\cdot\text{cm}^{-1}$ ], dissolved oxygen [ $\text{mgO}_2\cdot\text{dm}^{-3}$ ], total phosphorus [ $\text{mgP}\cdot\text{dm}^{-3}$ ], phosphates [ $\text{mgPO}_4\cdot\text{dm}^{-3}$ ], nitrates [ $\text{mgNO}\cdot\text{dm}^{-3}$ ], and total organic carbon [ $\text{mg}\cdot\text{dm}^{-3}$ ].

### Statistical Analysis

On the basis of the collected biological material, we assessed species richness, density, and dominance structure of ciliates and rotifers, and analyzed seasonal variation in their abundance. One-way ANOVA and the *post hoc* Tukey test were used to assess the significance of differences in species richness of ciliates and rotifers between the peatlands, while the Kruskal-Wallis test and multiple comparisons of mean ranks test for differences in abundance of ciliates and rotifers between the peatlands. The Wilcoxon signed-rank test was used to verify the significance of differences in density and species richness between ciliates and rotifers. One-way ANOVA and the Tukey test were employed to assess the significance of differences in temperature, pH, and oxygen content between the peatlands, whereas the Kruskal-Wallis test and multiple comparisons of mean ranks test, to investigate the significance of differences in the other physicochemical properties of water. Before selection of the statistical methods, in all cases we assessed normality by the Shapiro-Wilk test, and the equality of variances by the Levene test. To analyze the relations between density and species richness of the two groups of organisms and abiotic factors (physicochemical properties of water), Pearson's correlation coefficients were calculated. We used Statistica 8.0 software to perform all the analyses. MVSP 3.1 software was used to perform canonical cor-

respondence analysis (CCA) to assess the relations between the occurrence of ciliates and rotifers and the studied peatlands, as well as physicochemical properties of water in the peatlands.

## Results

### Species Composition

In all the studied habitats, during the whole study period, rotifers were more diverse than ciliates. Differences in species richness between these groups were significant (Wilcoxon signed-rank test,  $Z=4.92$ ,  $p=0.001$ ).

#### *Ciliates*

In the studied peatlands, during the whole study period, we recorded 51 ciliate species of 13 orders. The number of taxa was the highest (35) in spring and lowest in summer (17). In individual peatlands in spring, the number of taxa ranged from 7 taxa in L (typical fen) up to 19 in M1 (poor fen), in summer from 3 in J (poor fen) up to 10 in L (typical fen), and in autumn from 3 in M2 (raised bog) up to 17 in DB (raised bog). Considering the whole study period, the number of ciliate species was the highest in L (typical fen, 28), while the lowest in BB (base-rich fen, 11).

#### *Rotifers*

During the whole study period, in all the studied habitats we recorded 74 taxa of rotifers of 4 orders. The number of taxa was the highest in spring (48), while in summer and autumn their species richness was similar (37 and 35 taxa, respectively). In individual peatlands, during the whole study period, the number of taxa ranged from 21 in M2 (raised bog) to 39 in L (typical fen). In all seasons of the year, the smallest number of rotifer taxa was recorded in M2 (raised bog, from 7 taxa in summer up to 17 in spring). The highest species diversity of rotifers was recorded in spring in L (typical fen, 27), in summer in J (poor fen, 23), and in autumn in DB (raised bog, 25).

### Abundance and Domination Structure

In all the studied habitats, during the whole study period rotifers were much more abundant than ciliates (Fig. 1). Differences in abundance between these groups were significant (Wilcoxon signed-rank test,  $Z=5.57$ ,  $p=0.001$ ).

#### *Ciliates*

The mean abundance of ciliates during the whole study period was 38 individuals per 1 g (DW). The highest mean abundance of ciliates during the whole study period was recorded in DB (raised bog). It was high also in both Moszne peatlands (M1 and M2), while the lowest in J (poor fen) and BB (base-rich fen). The highest abundances of ciliate communities were noted in spring and autumn with the dominance of Cyrtophorida, Suctorida, or Hymenostomatida,

whereas in summer we observed a remarkable decrease in ciliate abundance. At this time Oligotrichida, Prostomatida and/or Haptorida dominated (Figs. 1, 2). In spring it ranged from 7 ind. g<sup>-1</sup> in J (poor fen) to 92 ind. g<sup>-1</sup> in DB (raised bog), in summer from 5 ind. g<sup>-1</sup> in BB (base-rich fen) to 38 ind. g<sup>-1</sup> in M2 (raised bog), and in autumn from 28 ind. g<sup>-1</sup> in J (poor fen) to 67 ind. g<sup>-1</sup> in M1 (poor fen).

### Rotifers

The mean rotifer density for the whole study period was markedly lower than that of ciliates, and reached 534 g<sup>-1</sup> (DW). In individual peatlands, during the entire study period the mean density of rotifers was the highest in DB (raised bog), while the lowest in M2 (raised bog) and BB (base-rich fen). The highest abundance of rotifers in BB, J (poor fen), and L (typical fen), were noted in summer with the dominance of Bdelloidea. In DB (raised bog), M1 (poor fen), and M2 (raised bog) the maximal values were noted in autumn with the dominance of Bdelloidea and Ploimida (Figs. 1, 3). In individual peatlands, rotifer abundance varied in spring (Fig. 1) from 11 g<sup>-1</sup> in J (poor fen) to 1,664 g<sup>-1</sup> in M1 (poor fen), in summer from 24 g<sup>-1</sup> in M2 (raised bog) to 1,475 g<sup>-1</sup> in DB (raised bog), and in autumn from 18 g<sup>-1</sup> in L (typical fen) to 2,182 g<sup>-1</sup> in DB (raised bog).

### Influence of Abiotic Factors

The analyzed environmental factors played an important role in rotifer and ciliate communities. We showed that as water temperature increases, rotifer abundance increases, while ciliates become less abundant. Ciliate species richness was positively correlated with the concentration of phosphates ( $r=0.649$ ,  $p=0.005$ ). Ciliate species number was negatively correlated with temperature ( $r=-0.613$ ,  $p=0.009$ ). This is confirmed by the highest number of ciliate species in spring and autumn, and the lowest in summer. This dependence is visible also on the CCA diagram (Fig. 4), where many ciliate species are concentrated in its left part, opposite to the temperature vector, which indicates that they prefer cold waters.

Results of CCA show that the first axis explains 36.73%, while the second axis explains 22.91% of the total variation (Fig. 4). In the diagram, the nitrate vector is the longest. It is correlated with the first ordination axis, which is reflected in its small slope. The vector's direction indicates that the concentration of nitrates increases on the diagram from the left to the right-hand side of the ordination space. M2 (raised bog) and M1 (poor fen), with the highest concentrations of nitrates and organic carbon, are located on the right-hand side of the diagram (Table 1). Also, some ciliate species are associated with nitrate-rich habitats:

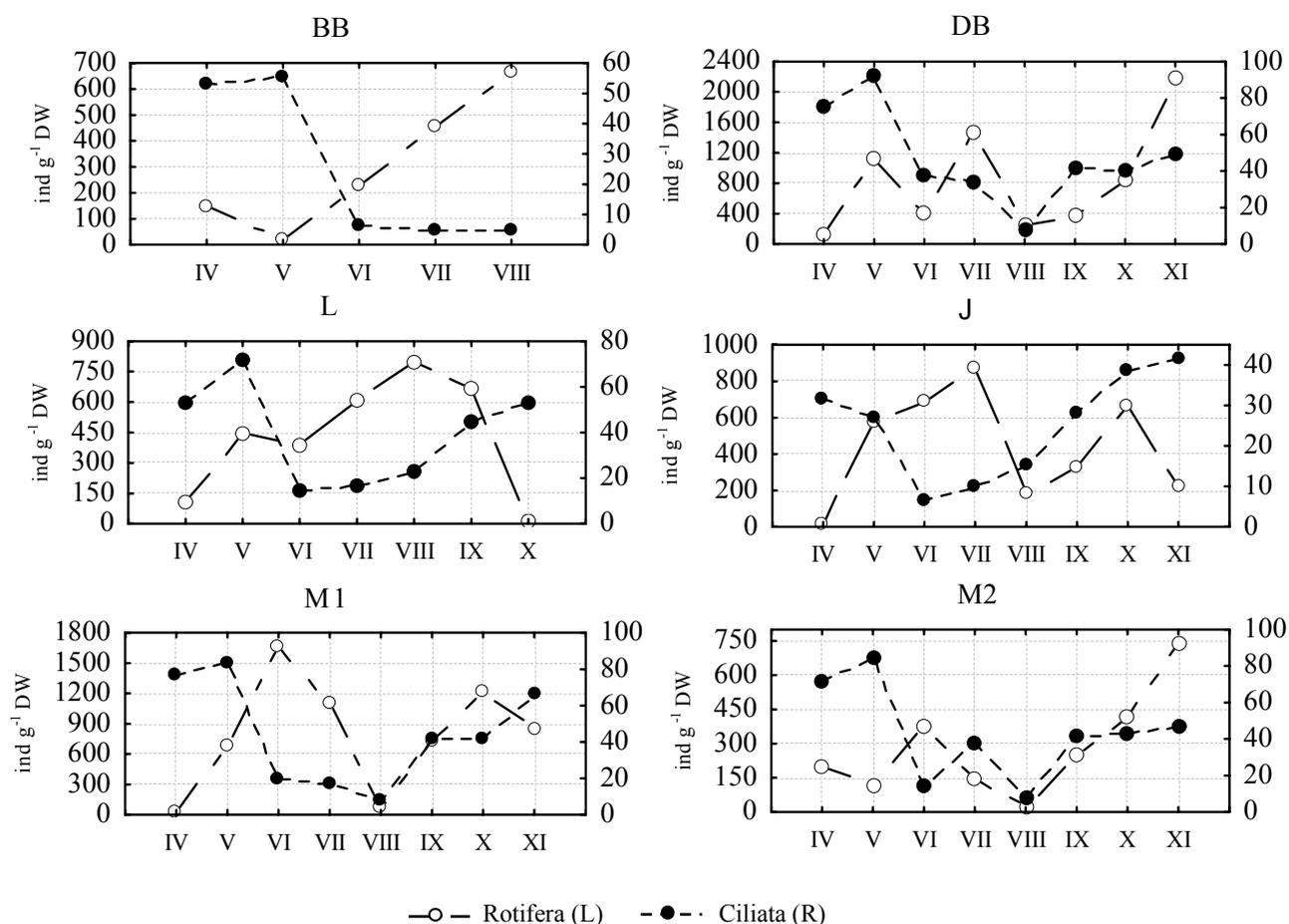


Fig. 1. Seasonal changes in abundance of ciliates and rotifers in investigated peatlands, April–November 2007.

*Paradileptus elephantinus*, *Stentor coeruleus*, and *Loxophyllum meleagris*, and to a lesser extent *Paramecium putrinum* and *Euplotes* sp. The habitats that are poor in nitrogen compounds are located on the right-hand side of the ordination space. DB (raised bog) was distinguished by a low level of nitrates, but a rather high total organic carbon concentration, while BB (base-rich fen) had low levels of nitrates and total organic carbon, but was distinguished by a much higher pH and conductivity. Some rotifer species are strongly associated with DB (raised bog): *Lepadella patella* and *Collotheca wiszniewski*. That bog was not inhabited by the ciliate and rotifer species that markedly preferred the environmental conditions of BB (base-rich fen). The ciliate species *Stentor amethystinus* and *Colpoda steinii*, as well as the rotifer species *Lecane opias*, *Keratella cochlearis*, and *Lecane subtilis*, are associated with the peatland that was richest in phosphorus compounds (typical fen L).

### Discussion

The similar taxonomic composition of ciliates and rotifers in our study sites and in other European sites is not surprising, given the cosmopolitan distribution of many taxa [4, 7-9, 23]. However, their abundances were higher than those found in the ombrotrophic or near-ombrotrophic peatlands in the Swiss Jura Mountains, eastern Finland, the Netherlands, southern Sweden, and northwestern England [5]. These differences may be a result of the differences in pH seen in the peatlands investigated in this study. In the literature, data on ciliate communities in peatlands are lacking. The density of rotifers was higher in rich fens than in bogs. This is in agreement with a previous study [32]. This trend is probably related to the differences in moisture content and pH between bogs and fens. The present study has revealed a remarkable relationship between the abundance of ciliates and rotifers. The decrease in abundance of cili-

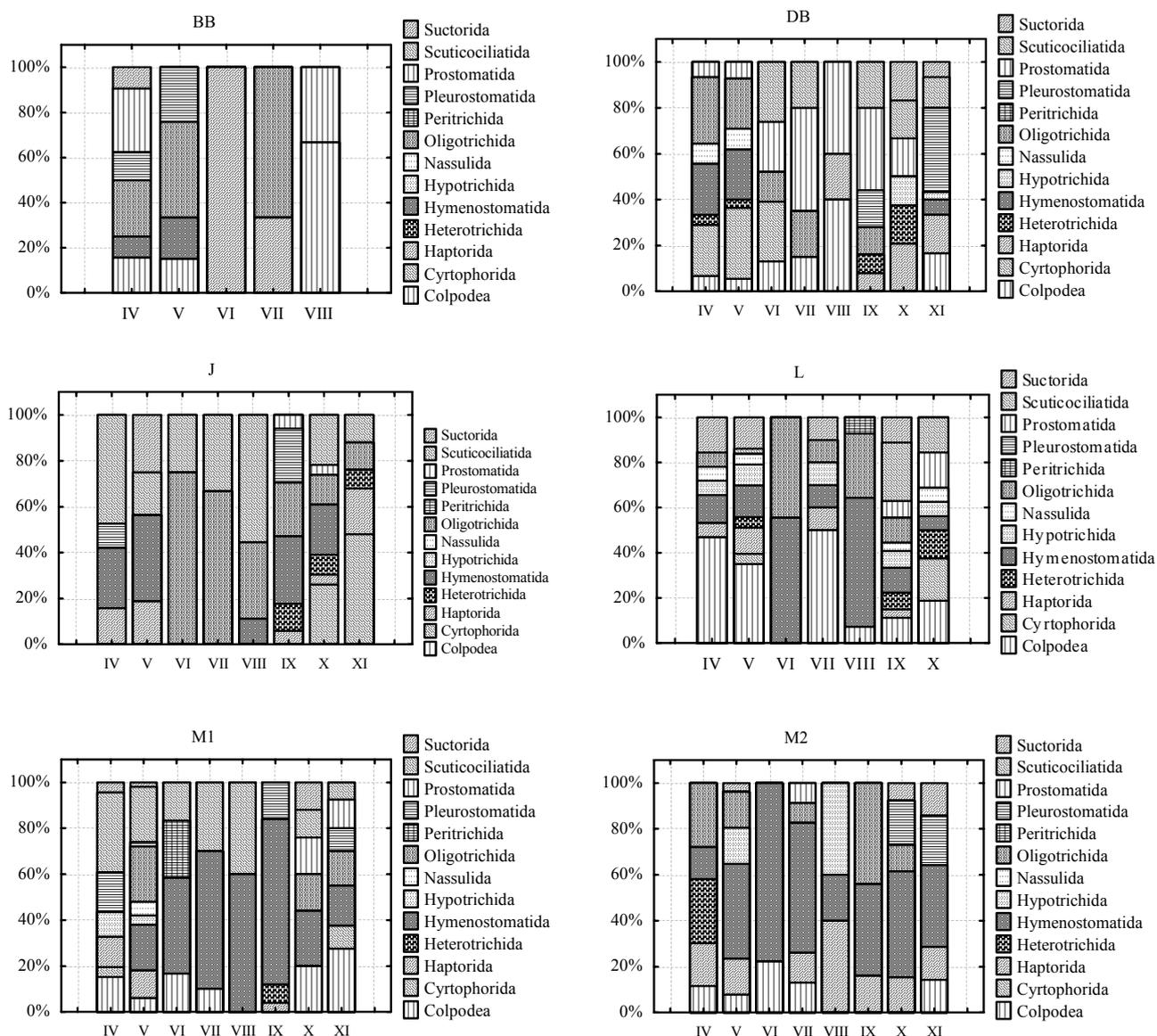


Fig. 2. Dominance structure of ciliates of investigated peatlands, April-November 2007 (% in total numbers).

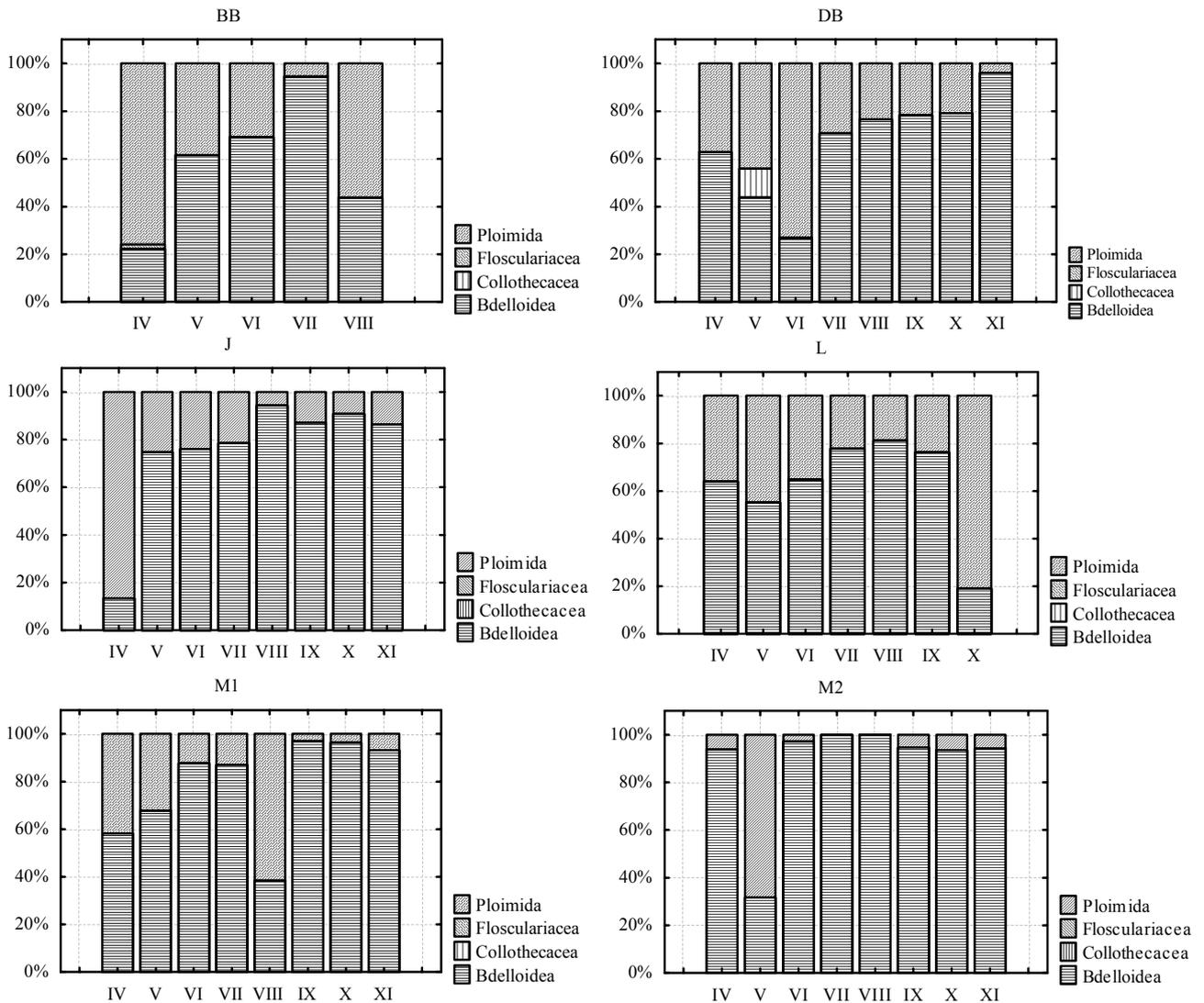


Fig. 3. Dominance structure of rotifers of investigated peatlands, April–November 2007 (% in total numbers).

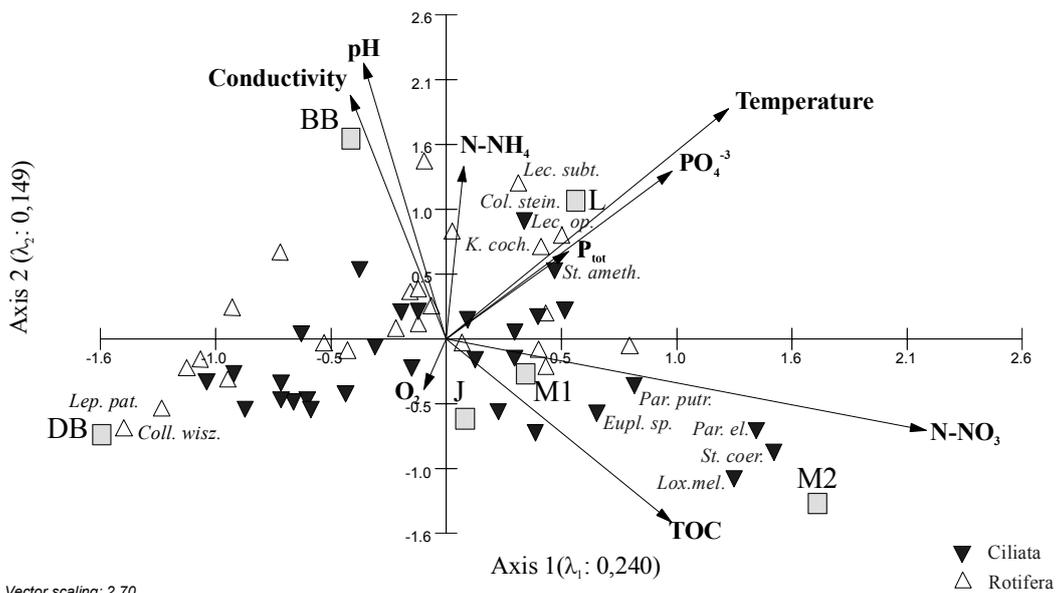


Fig. 4. Canonical Correspondence Analysis (CCA) ordination diagram showing the relationship between ciliates, rotifers, and environmental variables.

ates in summer coincided with the peak of rotifer abundance. This may be due to both their thermal preferences and to losses due to foraging of larger organisms that feed on ciliates (e.g. rotifers).

In all the studied habitats, the abundance and species richness of rotifers were much higher than those of ciliates. Similar results were reported by Mitchell et al. [5], who found that ciliates and rotifers had only small contributions to the microbial communities of studied peatlands, but rotifers were much more abundant than ciliates.

The BB (base-rich fen), characterized by nearly neutral pH (ca. 6), high conductivity, and colonization by emergent vegetation, was dominated by Oligotrichida (32% of the total catch). It is a characteristically dominant group in lakes with well-developed vegetation. In the densely vegetated Lake Biandantang, this group of ciliates accounted for 39% of the total catch [33]. A characteristic feature of ciliate communities in L (typical fen) was the dominance of Colpepoda. Its dominance was also observed on Gough and Marion Islands [11]. Ciliate communities of all the other peatlands were dominated by Hymenostomatida, Prostomatida, and Scuticociliatida. Many of the ciliate species found in the analyzed peatlands were also observed in lakes of varied trophic status [33-37]. This is probably associated with the wide ecological tolerance of those microorganisms [37]. According to Grolie [7-9] some of the ciliates recorded in our study are ubiquitous (*Cyclidium*, *Paramecium*, *Prorodon*, *Spirostomum*, *Spathidium*, and *Vorticella*), whereas others, such as *Bryometopus* and *Climacostomum*, are specific to peatlands.

Among rotifers, in all the studied habitats, bdelloids were most abundant. Their dominance in aquatic ecosystems with a low pH has been reported by many researchers [5, 21]. *Sphagnum* acidifies its habitat, so rotifer diversity is limited to the species that tolerate a low pH [38]. Bdelloids tolerate a wide range of ambient pH [21, 39]. Such a high tolerance may be associated with their mode of reproduction, i.e. obligatory parthenogenesis [38, 39], and, consequently, with its colonization strategy [19, 22].

Ciliate and rotifer communities differed between the studied peatlands. This is associated with differences in water chemistry of the peatlands. In this study, no significant correlations were found between water pH and the density and species richness of both ciliates and rotifers. By contrast, research conducted in acidic dystrophic lakes shows that along with an increase in water acidity, a decrease is observed in ciliate species richness and abundance [40]. Some authors suggest that the level of total dissolved carbon may be a significant factor affecting animal communities in peatlands [21]. It also seems that concentrations of nutrients may significantly influence the communities of both ciliates and rotifers. In this study, we detected a significant positive correlation between ciliate species richness and the concentration of phosphates. Also, CCA revealed that some ciliate species are strongly associated with the peatlands of Moszne, which are rich in nitrates and total organic carbon, while some ciliate and rotifer species are associated with peatland rich in phosphates (L, typical fen). It seems that nutrients have an indirect influ-

ence on the abundance of protozoans and small metazoans through the control of food abundance (mainly bacteria, fungi, or other protists). But data on relationships between both rotifer and ciliate communities in *Sphagnum* peatlands and water chemistry parameters remain insufficient.

## Conclusions

1. In the studied peatlands, rotifers were more abundant and diverse than ciliates. Both groups of microorganisms were dominated by taxa that tolerate a wide range of low pH.
2. The abundance of ciliates and rotifers varied seasonally. The highest numbers of ciliates were recorded in spring and autumn, whereas rotifers were most abundant in summer. This relationship between ciliates and rotifers, demonstrated in the seasonal cycle, may indicate that ciliates lack food in summer because then they are outcompeted by rotifers.
3. This study showed that the microbial community can be more strongly determined by concentrations of total organic carbon, nitrates, phosphates, and total phosphorus than by water pH. It seems that nutrients and total organic carbon have an indirect influence on the abundance of ciliates and rotifers through the control of food abundance (mainly bacteria).

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