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SEASONAL PATTERNS OF TESTATE AMOEBAE AND CILIATES IN PEATBOGS VS. BACTERIA AND FLAGELLATES ABUNDANCE

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Abstract. The seasonal development of testate amoebae and ciliates was studied in two peatbogs with different pH values. The values of numbers of testate amoebae were seasonally changeable. The highest numbers of testate amoebae were found in spring and summer with the dominance of *Areclla vulgaris*, *Nebela barbata* and *Cucurbitella* sp. The density during the autumn was the lowest. The highest numbers of ciliate communities were noted in spring and autumn during the mass development of small Scuticociliatida and Colpodea. The density throughout the summer was the lowest. Generally, in the present studies the abundance of testate amoebae was correlated with the abundance of bacteria and heterotrophic flagellates. The density of bacterivorous Colpodea, Scuticociliatida and Cyrtophorida correlated positively with the density and biomass of bacteria. In low pH peatbog relations between microbial loop components were stronger.

Key words: peatbog, Sphagnum, microbial loop, testate amoebae, ciliata

INTRODUCTION

Peatbogs are often described as being intermediate between terrestrial and aquatic ecosystems. *Sphagnum*-dominated peatbogs are often characterized by strong microtopographic patterning, and studies have clearly shown that testate amoebae and other organisms are distributed along these topographic gradients [Mitchell *et al*. 2000]. These patterns are strongly linked to hydrologic and climatic conditions, and probably have implications for small-scale energy and nutrient dynamics [Bridgham *et al.* 1996, Bobrov *et al.* 1999, Mitchell *et al.* 2003]. These ecosystems are likely to be colonized by different microbial communities. Ciliates, flagellates and bacteria constitute the "microbial loop" which is a distinct and important element of the trophic food web in aquatic ecosystems [Kalinowska 2004]. In peatbog the testate amoebae are both abundant and di-

verse. They could, thus have a dominant role in trophic pathways [Mitchell *et al.* 2000]. These microorganisms are significant consumers of bacteria, flagellates and algae; they also participate in transformations of the organic matter and biogenes. Their short generation times make them useful indicators of environmental changes [Lamentowicz and Mitchell 2005]. In recent years the structure of microbial communities in *Sphagnum* have been studied by Mitchell *et al.* [2003]. They indicate that heterotrophic organisms dominated the microbial communities and together represented from 78% to 97% of the total microbial biomass. Little has been known about the seasonal dynamics of testate amoebae and ciliata dwelling *Sphagnum*. In world ecological studies there was noted only three publications regarding seasonal abundance of microorganisms living amongs mosses from *Sphagnum* genus [Heal 1964, Gilbert *et al.* 1998, Mieczan 2007]. Understanding seasonal patterns of abundance and biomass of microorganisms are potentially important to our understanding of nutrient and energy dynamics, as well as our understanding of species-environment relationships. The aims of this study was therefore to follow the seasonal abundance of testate amoebae and ciliates in two peatbogs with different pH values and relationships between the abundance of the main groups of microorganisms forming the microbial loop.

MATERIAL AND METHODS

The composition and abundance of microorganisms in two peatbogs ("Durne Bagno" – DBP (pH = 5.5) and "Lejno" – LP (pH = 4.1) of the Poleski National Park (Eastern Poland) were studied. Conductivity reached the highest values in DBP (86 ±34.8 µS cm^{-1}), however the remaining factors (P_{tot}, P-PO_4, N-NO_3^-) in LP. Only content of ammonia-nitrogen reached the highest values in DBP. The chemical characteristics of these peatbogs are summarized in Table 1.

Table 1. Hydrological, physical and chemical characteristic of water in investigated peatbogs (average values for period May – October 2009, mean ±SD)

Peatland	Location	WTD mm	pH	Conductivity uS cm^{-1}	N-NO_3 mg N L^{-1}	N-NH_4 mg N L^{-1}	PO_4 mg PO_4 L^{-1}	TP mg P L^{-1}	TOC mg C L^{-1}
Lejno	51°25.093´N, 23°4.124´E	58	4.1^{a*} ±0.61	61.2^{a**} ±24.4	0.091^{b} ±0.02	0.053^{ab} ±0.02	0.188^{b} ±0.03	0.25^{c**} ±0.12	$77.2^{n.s.}$ ±8.4
Durne Bagno	51°22.344´N, 23°12.303´E	40	5.5^{ab*} ±0.65	86.1^{ab**} ±34.8	0.031^{a} ±0.02	0.100^{b} ±0.01	0.09^{a} ±0.05	0.02^{a**} ±0.01	$57.5^{n.s.}$ ±9.05

WTD – water table depth; means significantly different between peatlands do not share the same letters (Duncan test analysis: P ≤ 0.01**, P ≤ 0.05*, n.s. – not significant, ±SD)

In LP the vegetation is dominated by graminoids such as *Eriophorum vaginatum* (L.); *Carex acutiformis* Ehrhart. *Carex gracilis* Curt. and *Sphagnum palustre* L.

In DBP the vegetation is dominated by *Phragmites australis* (Car.), *Equisetum limosum* (L.)*, Sphagnum palustre* L. and *Sphagnum squarrosum* Pers. Microbial communities were examined in the center of peatbogs. The site was chosen for its macroscopic uniformity, i.e., no ecological gradient could be inferred from the topography (maximum height difference from the lowest to the highest point: 5 cm) or vegetation pattern (the vegetation of the sampling site was dominated by *Sphagnum*). From April to October 2013 from each peatbog twice a month, twenty eight samples were collected by washing 10 g of a wet mass of plant material (capitulum, living green and dead brown parts) in 50 ml of distilled water. All microorganisms were identified in four subsamples, each equal to 5% of the original sample. The abundance of microorganisms were calculated on 1 g wet weight of the plant material. The abundance and biomass of the bacteria were determined by means of an epifluorescent microscope with the use of DAPI – 4′6-diamidino-2-phenylindole according to the methods Porter and Feig [1980]. 10 ml of water were preserved in formaldehyde up to the final concentration of 2% and kept in darkness at temperature of 4°C. Four preparations were made from each sample. The sub-samples of 2 ml were condensed on polycarbon filters coloured with irgalan with pore diameter of 2 µm. Filters with the material condensed in such a way were placed on the basic glass and the bacteria were counted from 250 fields of view randomly chosen on the filter. Flagellates were determined using prymulin, according to the method of Caron [1983]. 10 ml of water were collected to the dark sterilized bottles. The samples were preserved in formalin up to the final concentration of 2% and they were kept in darkness at temperature of 4°C. Four preparations were made from each sample. The sub-samples of 10 ml were condensed on coloured polycarbon filters with the diameters of the opening of 1µm. Filters with the material condensed in such a way were placed on the base glass and the flagellates were counted from 150–200 fields of view randomly chosen on the filter. In order to determine the density and biomass of testate amoebae and ciliates, four samples were preserved with Lugols salution. The samples with testate amoebae and ciliates were condensed by the sedimentation method. Three subsamples of 50 ml volumes were settled for at least 24 h in plankton chambers. Testate amoebae (live and dead) and ciliates were enumerated and identified with an inverted microscope at 400–1000 × magnification. Biovolumes of each community were estimated assuming geometric shapes and converting to carbon using the following conversion factor: heterotrophic bacteria: 1 $\mu m^3 = 5.6 \times 10^{-7}$ mg C; flagellates: 1 $\mu m^3 = 2.2 \times 10^{-7}$ mg C; ciliates and testate amoebae: 1 $\mu m^3 = 1.1 \times 10^{-7}$ mg C [Gilbert *et al*. 1998].

Twice a month, the water samples for chemical analyses were taken simultaneously with *Sphagnum* samples. Conductivity and pH were determined *in situ* using the electrode JENWAY 3405, TOC was determined using the PASTEL UV and the remaining factors were analysed in the laboratory, according to Hermanowicz *et al*. [1976].

All data collected were analyzed statistically by means of GLM and CORR procedures of SAS Programme. The significance of differences between mean density and biomass values of testate amoebae and ciliates were verified by means of ANOVA. One-way ANOVAs with post-hoc Bonferroni tests were run on abundance and biomass data to separately assess the protozoan variability caused by the sides and season. Correlation coefficients in time were calculated between pairs of variables in order to determine the relationships between particular components of the microbial loop.

RESULTS

The heterotrophic bacteria showed clear seasonal variations. In LP the numbers of bacteria was very low in August and reached a maximum in September and October. The dynamics and density of heterotrophic bacteria in DBP reaching peak values in the early spring and summer and at the end of autumn. In August the numbers of bacteria was the lowest (Fig. 1a). In investigated peatbogs the peak of flagellates was noted in spring (May) and autumn (September and October) (Fig. 1b). Main effects ANOVA run on protozoa species abundance data did not shown any statistically significant difference between the sites ($p > 0.05$). One-way ANOVA with post-hoc Bonferroni test on abundance of protozoans was a statistically significant difference between season ($F = 29.1$, $p = 0.0138$) and between peatbogs with different pH values ($F = 22.1$, $p = 0.0015$). The highest abundance of testate amoebae in LP were noted in June with the dominance of *Arcella vulgaris*. In DBP the max was noted in May with the dominance of *Nebela barbata*. The density during the autumn was the lowest. The dynamics of ciliate communities in studied peatbog were quite similar, reaching peak values in May and October during the mass development of small bacterivorous Scuticociliatida and Colpodea, which constituted about 90% of the total density of ciliates. The density throughout June was the lowest for all sites (Fig. 1 c–d). Biomass of heterotrophic bacteria was very low in August, and reached its maximum in October. Peaks of the HF biomass were noted in spring (May) and autumn (October). The biomass of testate amoebae was characterized by changes and the highest peaks in July in the LP, while in DBP the highest peak occurred in May. The highest biomass of ciliata communities were noted in May and October. The biomass of ciliates during the late April was the lowest. Generally, the abundance of testate amoebae and ciliates were correlated with the abundance of bacteria and heterotrophic flagellates (from $r = 0.42$, $p \leq 0.05$ to $r = 0.56$, $p \leq 0.01$). However, the number of significant correlations between the main groups of microorganisms forming the microbial loop was different amongst peatbog. In LP the relations between microbial loop components were stronger. Bacterial density and biomass correlated positively with the density and biomass of testate amoebae and bacterivorous Colpodea ($r = 0.58$, $r = 0.63$ and $r = 0.53$,

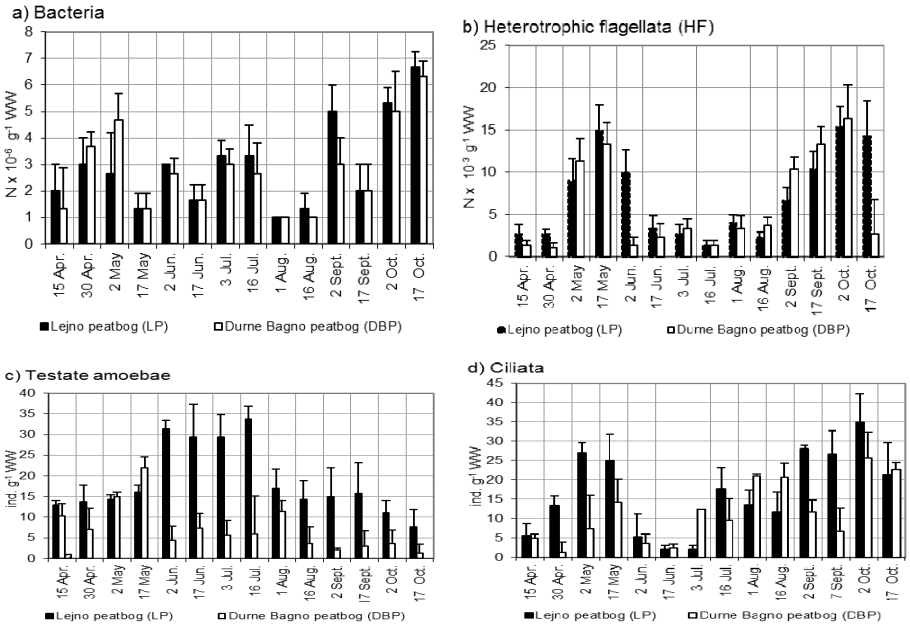

Fig. 1. Seasonal changes of the density of bacteria, heterotrophic flagellates, testate amoebae and ciliates in investigated peatbogs

$r = 0.46$, $p \leq 0.01$, respectively). The abundance of nanoflagellates (HF) correlated positively with the density and biomass of testate amoebae ($r = 0.28$, $r = 0.37$, $p \leq 0.05$). In DBP, there was a significant and positive correlation between bacterial density and small-sized bacterivorous Scuticociliatida ($r = 0.35$, $p \leq 0.05$). Testate amoebae density and biomass correlated with the abundance of bacteria and HF ($r = 0.53$, $r = 0.54$, $p \leq 0.01$ and $r = 0.29$, $r = 0.42$, $p \leq 0.05$, respectively).

DISCUSSION

The abundance of testate amoebae were seasonally variable in all of the studied peatbogs. The highest abundance occurred during spring and summer and could have resulted from higher water table depth. According to Lamentowicz and Mitchell [2005] testate amoebae community positively correlated with WTD. Spring and summer peaks have also been noted by Heal [1964] and Gilbert *et al.* [1998]. In all of the studied peatbogs the density during the autumn was the lowest. At this time water table depth was the lowest and the concentrations of nutrients were the highest. Similar relationships were found in New Zealand [Charman 1997] and in north-western Poland in the Tuchola Pinewoods

region [Lamentowicz and Mitchell 2005]. Maximum densities of ciliates have often been observed during mid or late summer [Simek *et al.* 1990]. In addition to high late-summer densities, the concentrations of ciliates reached a peak in all studied peatbogs in spring and autumn. Spring and autumn peaks of ciliates coincided with the higher concentrations of total organic carbon in studied peatbogs. Larsson [1978] observed that highest densities of ciliates were always found at the time of the bacterial maximum. Testate amoebae have been described as able to grow on diverse food sources, including bacteria, ciliates or ever rotifers. Generally, in the present study the abundance of testate amoebae and ciliates were correlated with the abundance of bacteria and heterotrophic flagellates. In low pH peatbog relations between microbial loop components were stronger. Gilbert *et al.* [1998] documented that the biomass of testate amoebae is significantly related to the biomasses of heterotrophic flagellates and ciliates. In studied peatbogs the density of bacterivorous Colpodea, Scuticociliatida and feeding on bacteria, flagellates and algae Cyrtophorida correlated positively with the density and biomass of bacteria. Kankaala *et al.* [1996] noted close correlations between the biomass of heterotrophic flagellates and the biomass of ciliates. In world ecological studies was noted only two publication regarding abundance of heterotrophic flagellates and their ecological role in peatbogs environments [Gilbert *et al.* 1998, Mieczan *et al.* 2015]. The studies conducted by Gilbert *et al.* [1998] indicate that biomass of HF are weakly correlated with bacteria. It is probable that, as has been observed in lakes ecosystems, HF have a diet that consists mainly of bacteria. Testate amoebae and ciliates abundance and biomass were significantly greater in low pH peatbog. A slight proportion of bacteria may be explained by the fact that acidity negatively affects microbial community. Several authors have suggested that acidity negatively correlated with microbial communities in lakes and decomposition in peatlands [McKinley and Vestal 1982].

CONCLUSIONS

The relations demonstrated between bacteria, HF and testate amoebae may point to an important process of matter and energy flow from bacteria to higher trophic levels. Consuming much of bacterial productions, testate amoebae become an important link between bacteria and metazoans. However the number of significant correlations differed among peatbogs with different pH values. In seasonal cycle in low pH peatbog the relationships were stronger.

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SEZONOWA ZMIENNOŚĆ AMEB SKORUPKOWYCH I ORZĘSKÓW W EKOSYSTEMACH TORFOWISKOWYCH VS. OBFITOŚĆ BAKTERII I WICIOWCÓW

Streszczenie. Sezonową zmienność obfitości ameb skorupkowych i orzęsków analizowano w dwóch torfowiskach o zróżnicowanym odczynie wód. Obfitość ameb skorupkowych wykazywała istotną zmienność sezonową. Największe liczebnośći tych organizmów występowały wiosną i latem z wyraźną dominacją *Areclla vulgaris*, *Nebela barbata* and *Cucurbitella* sp. W sezonie letnim notowano natomiast ich najmniejsze liczebności. Największa liczebność orzęsków występowała wiosną i jesienią, z wyraźną dominacją drobnych Scuticociliatida i Colpodea. Latem natomiast liczebność tych mikroorganizmów była najmniejsza. Liczebności ameb skorupkowych i orzęsków korelowały zarówno z liczebnością bakterii, jak i wiciowców. Liczebność bakteriożernych Colpodea, Scuticociliatida i Cyrtophorida wykazywała istotne, pozytywne korelacje z liczebnością i biomasą bakterii. W torfowisku o niższym odczynie wód zarówno liczba, jak i siła korelacji były większe.

Słowa kluczowe: torfowisko, Sphagnum, pętla mikrobiologiczna, ameby skorupkowe, orzęski