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Original article

The impact of climate warming on the diurnal dynamics of the microbial loop: Ice cover vs. lack of ice cover on dystrophic lakes

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ABSTRACT

One of the effects of warming is earlier retreat of the ice cover or a complete lack of ice cover on water bodies in the winter. However, there is still no information on how climate warming affects the 24-hour dynamics of the planktonic microbial loop in winter. The aim of this investigation was to assess the diurnal dynamics of the taxonomic composition and abundance of microbial communities in experimentally reproduced conditions (samples from under the ice, +2, +4 and +8 °C) and to analyse the relationships between components of the microbial loop in relation to physical and chemical parameters. Samples were taken in winter from three dystrophic reservoir. The biological and physicochemical parameters in the water were analysed at the start (day 0), 15 and end of the experiment (day 30) over a 24-hour cycle. The increase in temperature caused an increase in the numbers of predators (particularly testate amoebae and ciliates) and a reduction in the body size of individual populations. During the period with ice cover, marked dominance of mixotrophic testate amoeba *(Hyalosphenia papilio)* and ciliates (*Paramecium bursaria*) was observed, while the increase in temperature caused an increase in the proportion of bacterivorous ciliates (*Cinetochilum margaritaceum)*.

1. Introduction

Climate change models indicate that in the coming decades the temperature may rise between 2 °C and 8 °C (IPCC, 2007). Currently, a wide range of changes caused by global warming can be observed, especially in shallow lake ecosystems (Moss, 2012). The effects of global warming may include very hot periods in the summer or a complete lack of ice cover on water bodies during the winter (Cao et al., 2015; Audet et al., 2017; Nandini et al., 2019). The results of previous studies indicate that in natural lake ecosystems the share of smaller species increases in planktonic invertebrate communities, because the higher temperature causes an increase in mortality, mainly through pressure from top predators (Heckmann et al., 2012; Shurin et al., 2012; Rall et al., 2012; Zingel et al., 2018; Reczuga et al., 2018). According to Ohlberger (2013), warming-induced responses in average body size are not only determined by changes in individual growth and development rates, but also mediated through size-dependent feedbacks at the population level, as well as by competitive and predatory interactions within the community. Additionally, body-size structures can stabilize the strength of interactions across food webs and constitute an adaptive mechanism for effective use of food resources (Heckmann et al., 2012). On the other hand, the increase in temperature increases the competitive ability of small species compared to large ones through physiological adaptations to higher temperatures (Brown et al., 2004; Foster et al., 2013; Zingel et al., 2018). As dystrophic lakes are generally shallow, they react more rapidly to temperature changes. The lack of ice cover during the winter leads to repeated wind mixing, and thus to changes in the biotic and abiotic conditions prevailing in the lakes. Moreover, these water bodies have high concentrations of dissolved organic matter, which is utilized by heterotrophic organisms in decomposition processes. The activity of this process is significantly influenced by water temperature (Brown et al., 2004; Saad et al., 2013). There has been research on dystrophic lakes investigating the effect of temperature increase on the phenology of phytoplankton and zooplankton (Nicolle et al., 2012; Bertilsson et al., 2013; Lopez et al., 2019). This study showed an earlier peak in the abundance of planktonic algae and water fleas in experiments with elevated temperature. Beall et al. (2016) anal-

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ysed bacteriocoenoses and algae during and after ice cover in Lake Erie. They showed that earlier retreat of the ice cover causes an increase in the abundance of planktonic algae, especially diatoms, and an increase in the share of smaller-sized algal cells, which may have consequences for food web functioning. The results of research on the 24-hour cycle conducted in peatland ecosystems indicate that temperature, DOC (dissolved organic carbon) and chlorophyll *a* concentrations show dynamic changes, and these differences are particularly evident between day and night (Mieczan and Tarkowska-Kukuryk, 2013). Thus it seems that in dystrophic lakes as well we can expect substantial differences in these parameters over a 24-hour period, and thus also considerable variation in the qualitative and quantitative structure of planktonic microorganisms. Cruaud et al. (2020) showed that during the winter, the low water temperature, in combination with other prevailing conditions such as reduced light availability, created various environmental niches for potential methanotrophic Gammaproteobacteria. The presence of this group in winter suggests active carbon, iron, and nitrogen cycling under ice. According to Bertilsson et al. (2013), during the period with ice cover the microbial biomass is much lower than during the ice-free period. Whereas bacterial production tends to be lowest in winter months, the abundance of bacterivorous ciliates and heterotrophic flagellates is stable throughout the year (Bertlisson et al., 2013). This has led to the hypothesis that bacteria loss via microeukaryote predation may be most significant during the winter months, despite apparently low consumption rates at cold temperatures. In fact, bacterivory can occasionally exceed bacterial production in the winter (Nixdorf and Arndt, 1993). Knowledge of not only seasonal changes but also the 24-hour dynamics of the taxonomic composition and abundance of microorganisms is particularly important for a better understanding of the circulation of matter and energy in the ecosystem, as well as for understanding the interrelationships between species and environment. In this study, the response of individual components of the microbial loop to temperature changes in a 24-hour cycle during the winter was determined by simulating a period of ice cover and a period when the water body does not freeze over. We established, that warming and ice-free conditions will lead to: (H1) increases in the abundance of all groups of organisms (including heterotrophs and phycoflora); (H2) increases in the abundance of bacterivorous groups, while mixotrophs will decrease; (H3) change in the size structure of microbial communities in dystrophic reservoir.

2. Materials and methods

2.1. Study area

An experimental mesocosm study was carried out in three dystrophic lakes (Moszne 1, Moszne 2 and Krugłe Bagno) located in Poleski National Park (eastern Poland, 51° N, 23° E). The area of these lakes ranged from 0.7 to 1.5 ha, and their depth did not exceed 1.5 m. The littoral zone was formed mainly of *Sphagnum* and covered with other plants characteristic of peatlands: *Sphagnum angustifolium* (C.C.O. Jensen ex Russow), *Sphagnum cuspidatum* Ehrh. ex Hoffm., *Polytrichum* sp., *Eriophorum vaginatum* (L.), *Carex acutiformis* Ehrhart. and *Carex gracilis* Curt. and *Equisetum limosum* (L.). Secchi disc visibility was very low and ranged for 0.32 m to 0.47 m. In winter temperature ranged from 0 °C do 4 °C. Concentrations of dissolved organic carbon (DOC) in the lakes were very similar and ranged from 5.5 to 6.0 mg L^{-1}. Concentrations of nutrients were not significantly different between lakes. Concentrations of total phosphorus (TP) ranged from 0.60 to 0.71 mg L^{-1}, and concentrations of ammonia nitrogen ($N\text{-}NH_4$) ranged from 1.090 to 1.095 mg L^{-1}.

2.2. Field experiment

In three investigated lakes, experiments were conducted using temperature modelling, including two groups of treatments (control with no manipulation and treatments with simulation of temperature changes) with three replicates each (Fig. 1). The 'mesocosm' experiments were conducted in January and February 2018. The control treatment (CT) consisted of mesocosms with ice cover present on the lakes; this treatment was not subject to an experimental increase in temperature. Each treatment lasted for 30 days. In each month of the study, the water temperature was always 2° C, 4 °C and 8 °C higher than in the control sample (in which the water temperature was not modified, i.e. it was the 'natural' temperature for the micro-habitat). The first treatment was subjected to a 2 °C increase in temperature relative to the control sample (+2°C), the second involved a 4 °C increase in temperature compared to the control (+4°C), and in the third the temperature was increased by 8 °C compared to the control (+8°C). Microbial communities were examined *in situ* in polyethylene enclosures (80 L each, 45 cm × 45 cm, 40 cm deep), which were placed in the three lakes. For each experimental treatment in each of the reservoirs, each enclosures (control and +2 °C, +4°C and +8 °C relative to the temperature of the water in the control sample) were gently filled with surface water (Table 1). Thermal manipulation was carried out using a temperature modification system equipped with electric heaters located in each of the experimental treatments under 10 cm under water surface. In each month of the study (January and February), each of the experimental variants was carried out in triplicate within each lake (three replicates of each variant). At the beginning (one day after construction of the experimental setup to allow its stabilization), on day 15 and end of the experiment in each month, abundance of individual components of the microbial loop over a 24-hour cycle was determined. To determine changes in the number of microorganisms, in each of the lake and in each of the experimental variant, samples were taken four times a day: at 4 am, at 12 noon, at 7–8 pm, and at 12 midnight. Three samples were taken at each time of day from each variant. Samples from under the ice for biological, physical and chemical analyses of the water were taken after drilling through a layer of ice about 4 cm thick with a small hand drill. A layer of ice was present at the start of each variant of the experiment, but it retreated as the temperature rose from day to day. Samples were taken each time from just under the ice and from the bottom of mesocosms (CT) and from surface water and from the bottom of mesocosms +2 °C, +4°C and +8°. Then the 0.5 L samples were combined into one sample (1L) for analysis. From the mesocosms that were not covered with ice, i.e. those with simulated climate warming (+2°C, +4°C, +8°C), water was sampled using a plexiglass corer (length 1.0 m, Ø50 mm) placed in each of the experimental enclosures. The tube was filled with water, and its lower end was closed with the bung. Next the tube was raised vertically, the lower bung was removed, and the water samples were collected using a 0.5 L syringe fitted with a rubber tube. The volume of water extracted from the plexiglass corer ranged from 400 to 500 mL.

The abundance of bacteria, heterotrophic flagellates (HF), testate amoebae and ciliates was measured on days 0, 15 and 30 of the experiment. The volume of water extracted from the plexiglass corer ranged from 400 to 500 mL. In each study period 3 replicate samples were collected from each enclosure. Then the 3 replicate samples from each experimental treatment were mixed (vol/vol) together and the integrated sample was treated as representative for the mesocosm.

For chlorophyll *a* concentration (as an indicator of phytoplankton biomass), 1000-mL water samples were filtered through Whatman GF/C filters. Chlorophyll *a* was determined by

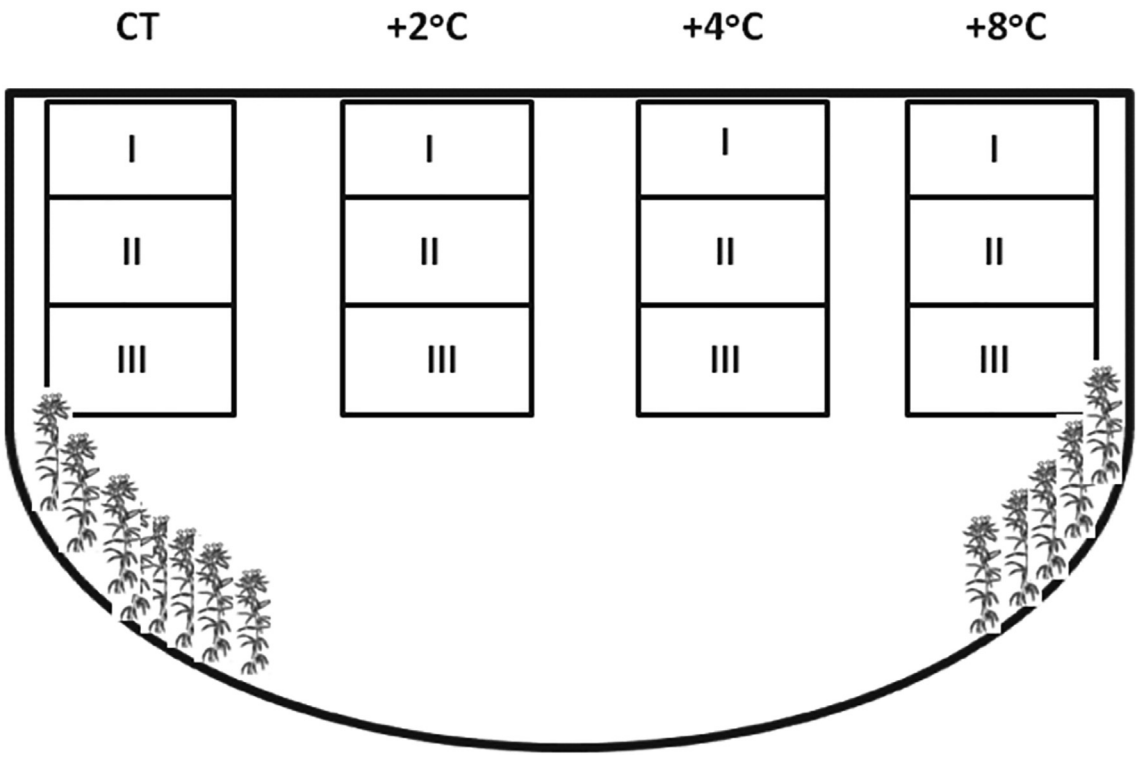

Fig. 1. Experimental setup with manipulations: CT – control treatment, warming treatments: +2°C, +4°C, +8°C.

Table 1
Temperature (°C) in different variants of mesocosms (S- start of experiments (0 day), E – end of experiments (after 30 days).

Treatments	winter	
	S	E
Control	+2 ± 1	+2 ± 1
+2°C	+2 ± 1	+4 ± 1
+4°C	+2 ± 1.5	+8 ± 2.6
+8°C	+2 ± 1	+10 ± 2.4

spectrophotometry following extraction with ethanol (Golterman, 1969).

To assess bacterial abundance and biomass, 10 mL of water was collected from each experimental treatment, preserved with formaldehyde up to a final concentration of 2%, and kept in darkness at 4 °C. Samples for quantitative analysis were kept refrigerated from 4 to 16 h. Prior to enumeration, the samples were stained for 10 min in the dark with 4́6-diamino-2-phenylindole (DAPI) according to Porter and Feig (1980). Duplicate subsamples of 2 mL were condensed on polycarbonate filters stained with Irgalan black (0.2 μm pore diameter). The proportion of active bacteria with intact membranes (MEM+) was analysed using LIVE/DEAD BacLight Bacterial Viability Kits with two stains, SYTO 9 and propidium iodide (PI), according to Schumann et al. (2003). SYTO 9 labels all bacteria with intact and damaged membranes, while PI penetrates bacteria with damaged membranes. A mixture of the two stains was added (1:1, 0.15% final concentration of both) for a 1 mL subsample, followed by incubation for 15 min at room temperature in the dark, filtration through a 0.2-μm-pore-size black polycarbonate membrane filter, and enumeration by epifluorescence microscopy with a Nikon Eclipse TE200 microscope at 1500× magnification.

To assess the abundance and biomass of heterotrophic flagellates (HF), 10 mL of water was taken from each experimental treatment and placed in dark sterilized bottles. The samples were preserved in formalin up to a final concentration of 2% and kept in darkness at 4 °C. Both abundance and biomass of flagellates were determined with primuline solution (Caron, 1983). Four slides were prepared from each sample. Sub-samples were condensed on 0.8-μm-pore-size black Nucleopore filters and enumerated by epifluorescence microscopy at 1500× magnification.

Testate amoebae and ciliate samples were fixed with Lugol's solution (4% Lugol's iodine (v/v)) and their abundance and community composition were determined using Utermöhl's method (Utermöhl, 1958). The samples (3 samples of 500 mL each) were sedimented for 24 h in a cylinder stoppered with Parafilm, and then the upper volume of 400 mL was gently removed. Ciliates were counted under an inverted microscope at 600× magnification. Morphological identification of the testate amoebae and ciliates was based mainly on works by Foissner et al. (1999), Charman et al. (2000) and Clarke (2003).

To estimate the biomass, the lengths and widths or diameter of at least 20 specimens were measured via microscopy, and then assuming them to have geometrical shapes, the biovolume of each species/group was calculated. Biomass of microbial community were estimated by assuming geometric shapes and converting to carbon using the following conversion factors: heterotrophic bacteria – 1 $\mu m^3 = 0.56 \times 10^{-6}$ μg C; flagellates – 1 $\mu m^3 = 0.22 \times 10^{-6}$ μg C; ciliates and testate amoebae – 1 $\mu m^3 = 0.11 \times 10^{-6}$ μg C (Gilbert et al., 1998).

2.3. PPMR: predator–prey mass ratio

The predator–prey mass ratio is an important parameter capturing the complex patterns of feeding links among species and individuals in a simplified way (Nakazawa et al., 2011). PPMR includes both the body size of individuals and their biomass and is therefore crucial for understanding the functioning of food webs.

In our study we analysed the relationships between predators and decomposers:

$$\text{PPMR} = \frac{\text{mean biomass of predators}}{\text{mean biomass of prey}}$$

predators: testate amoebae + ciliates + heterotrophic flagellates
prey: bacteria
We used ln (y + 1)-transformed data to calculate this ratio.

2.4. Physical and chemical variables

The physical and chemical properties of the water were analysed at the start (day 0), on day15 and at the end of the experiment (day 30), four times each day, in each mesocosm. The following parameters were analysed in each experimental treatment: temperature, conductivity, pH, dissolved oxygen (DO), chlorophyll *a*, total phosphorus (TP), ammonia nitrogen (N-NH_4) and dissolved organic carbon (DOC). Temperature, conductivity, pH and DO were assessed with a multiparametric probe (Hanna Instruments). Physical and chemical parameters were determined according to standard methods for hydrochemical analyses (Golterman, 1969). TP was determined by the colorimetric method and N-NH_4 by the Kjeldahl method. Samples for nutrient analyses were immediately filtered through Wathman GF/F filters (0.45 μm). Dissolved organic carbon (DOC) analysis was performed using a wet potassium persulfate digestion with O/I Corporation Model 700 TOC analyzer.

2.5. Numerical analyses

As neither the physicochemical parameters nor the biological parameters differed statistically significantly between lakes, averages for the three lakes are shown in the tables and figures. Furthermore, as no statistically significant differences were noted between days of the experiment (day 15 vs. 30), the figures show averages for the entire length of the experiment. The mean densities and biomass of the various groups of microorganisms were compared between experiments and between time periods (begin vs.end) using two-way repeated measures ANOVA. The significance level was set at $P < 0.05$. The response of microbial groups to shifts in temperature in lakes was assessed using linear regressions. Assumptions for normality of the data were previously tested. The analysis was performed using STATISTICA 7.0 software. Detrended Correspondence Analysis (DCA) was used to measure the variance gradient of microbial data and then to perform PCA (principal components analysis) and RDA (redundancy analysis). PCA was performed to describe temperature trends and associations between microbial communities and environmental data. RDA was performed to describe the relationships between the abundance of testate amoebae and ciliates and the environmental data. The proportion of variance explained by explanatory variables was calculated using variance partitioning. The significance of the model and of each explanatory variable included in the model was tested using 999 permutations. The analyses were performed using CANOCO 5.0 software (Ter Braak, 1988–1992).

3. Results

3.1. Environmental variables

Water temperature showed significant variation between experimental variants (ANOVA, $F_{1.33} = 63.0–64.2$, $P < 0.001$), with the highest noted in the +8 °C variants and the lowest in the ice-covered control treatment (CT), where the water temperature did not exceed 2 °C (Table 1). As the temperature in the experimental variants increased (+2°C, +4°C and +8 °C), there was a substantial increase in pH, conductivity, nutrients and chlorophyll *a* ($F_{1.33} = 62.11$, $P < 0.001$) (Table 2). This pattern was particularly pronounced in the +8 °C variant. The highest concentration of dissolved oxygen was noted in the +8 °C treatment, and the lowest in the CT. An increase in the concentration of chlorophyll *a* was noted in the +8 °C mesocosms (from 8.12 μg L^{-1} to 19.2 μg L^{-1}) (Table 2). In the case of the concentration of nutrients, the highest increase was observed in the content of N-NH_4^+, which reached > 2.7 mg l^{-1} in the experimental variant with the highest temperature (+8°C) (Table 2). Significantly higher Chl-a content was observed at noon, compared to the night and morning ($F_{1.33} = 62.71$, $P < 0.001$). The highest DOC concentration was recorded in the +4 °C and +8 °C experimental variants and the lowest under the ice, at 6 mg C L^{-1} (Fig. 2a). The 24 h dynamics of concentrations of dissolved organic carbon were similar in all experimental variants. The highest concentration was recorded at noon, with a slight increase at night and a relatively pronounced increase in the early morning ($F_{1.33} = 71.70$, $P < 0.001$) (Fig. 2b). The maximum concentration of TP was recorded in the morning (0.256 mg L^{-1} in CT, 0.394 mg L^{-1} in +8 °C). The pH, dissolved oxygen, conductivity and N-NH_4 did not show significant differences in diurnal dynamics ($P > 0.05$).

3.2. Diurnal dynamics of microbial loop components

The highest average bacterial abundance and biomass were recorded in the +2 °C and +4 °C experimental variants ($7.4 \pm 2.1 \times 10^6$ cells mL^{-1} and 0.92 ± 0.32 μg C mL^{-1}, respectively), with significantly lower values found in the CT and in the +8 °C variants in all reservoirs ($5.4 \pm 1.1 \times 10^6$ cells mL^{-1} and 0.52 ± 0.21 μg C mL^{-1}) ($F_{1.34} = 46.19$, $P \leq 0.001$) (Fig. 3a). Irrespective of the experimental variant, metabolically active bacteria had the largest share. Bacterial abundance underwent fairly substantial changes in the 24 h cycle in each of the experimental variants. In the CT, the lowest numbers were recorded in the evening and at night, while the maximum density occurred in the morning and evening ($6.2–6.5 \pm 1.0–1.3 \times 10^6$ cells ml^{-1}). Bacterial biomass and metabolic activity underwent similar changes. In the other experimental variants, the highest bacterial abundance and biomass were recorded in the morning ($6.2 \pm 2.2 \times 10^6$ cell. mL^{-1} and 0.59 ± 0.14 μg C mL^{-1}), with a slight increase observed at night (Figs. 3a, 5a). The proportion of metabolically active bacteria increased in the evening (Fig. 4). The highest average abundance and biomass of flagellates were found in the +2 °C and +4 °C variants ($2.7–2.9 \pm 1.1 \times 10^3$ cells mL^{-1} and $0.03–0.04 \pm 0.01$ μg C mL^{-1}). Significantly lower abundance and biomass of flagellates were recorded in the CT and in the case of the 8 °C temperature increase ($1.3–1.4 \pm 0.3 \times 10^3$ cells mL^{-1} and $0.02–0.03 \pm 0.01$ μg C mL^{-1}) ($F_{1.21} = 26.32$, $P \leq 0.001$). In the 24 h cycle, the greatest changes in both the abundance and biomass of flagellates occurred in experimental variants subjected to a temperature increase, with the highest number recorded in the morning ($3.2 \pm 0.8 \times 10^3$ cells mL^{-1} and 0.05 ± 0.02 μg C mL^{-1}) ($F_{1.31} = 37.52$, $P \leq 0.001$). In the CT, on the other hand, an approximately twofold increase in abundance was observed at 12 noon ($2 \pm 0.3 \times 10^3$ cells ml^{-1}), while in the evening and at night a gradual decrease was observed ($F_{1.14} = 15.23$, $P \leq 0.005$) (Fig. 3b). Biomass underwent similar changes (Fig. 5b). In the case of testate amoebae, both species number and abundance reached significantly higher values in the variants with elevated temperature and were lowest in the CT. Cyst forms were found to dominate in the CT, accounting for > 80% of the total number of amoebae. There was also a distinct 24-hour cycle of changes in the density and biomass of testate amoebae. Irrespective of the experimental variant, these organisms had significantly higher density and biomass in the evening ($4.3–4.4 \pm 1.1–1.2 \times 10^2$ cells mL^{-1} and $0.91–1.1 \pm 0.1–0.2$ μg C mL^{-1}) ($F_{1.41} = 19.12$, $P > 0.05$) (Figs. 3c, 5c). In the control variants, *Arcella*

Table 2
Changes in physical and chemical parameters in four experimental mesocosms (S- start of experiments (0 day), E – end of experiments (after 30 days).

Parameters/mesocosms	pH		O_2 mgO_2 L^{-1}		Cond. μS cm^{-1}		TP mg L^{-1}		N-NH_4 mg L^{-1}		Chl. *a* mg L^{-1}		DOC mg L^{-1}	
	S	E	S	E	S	E	S	E	S	E	S	E	S	E
Control	3.11	3.33	3.0	4.2	26.2	32.1	0.172	0.167	1.095	1.096	8.12	9.26	6.0	6.0
+ 2 °C	3.11	6.81	8.0	6.2	26.2	36.1	0.172	0.864	1.095	1.162	8.12	14.2	6.1	6.9
+ 4 °C	3.11	6.81	8.0	6.1	26.2	37.2	0.172	0.163	1.095	1.981	8.12	14.9	6.2	7.5
+ 8 °C	3.00	7.88	8.0	6.1	26.2	37.2	0.172	1.388	1.095	2.736	8.12	19.2	6.0	8.6

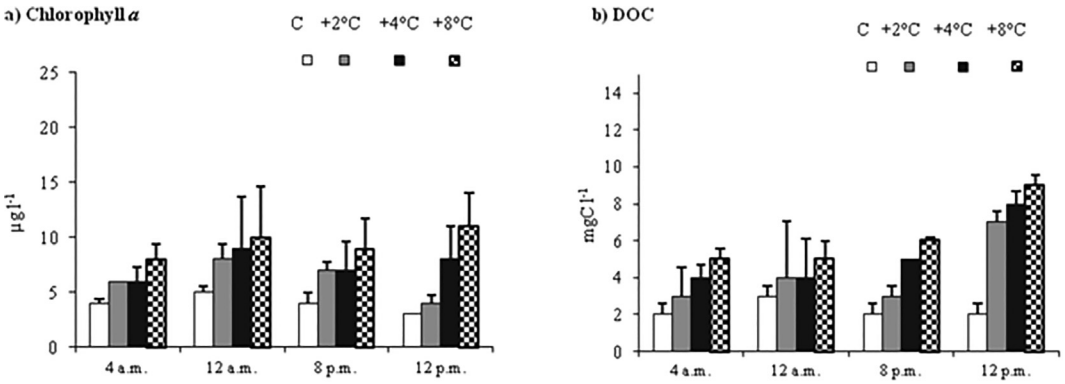

Fig. 2. a-b. Diurnal dynamics of the concentrations of chlorophyll *a* and dissolved organic carbon (DOC) in investigated mesocosms (T SD - standard deviation).

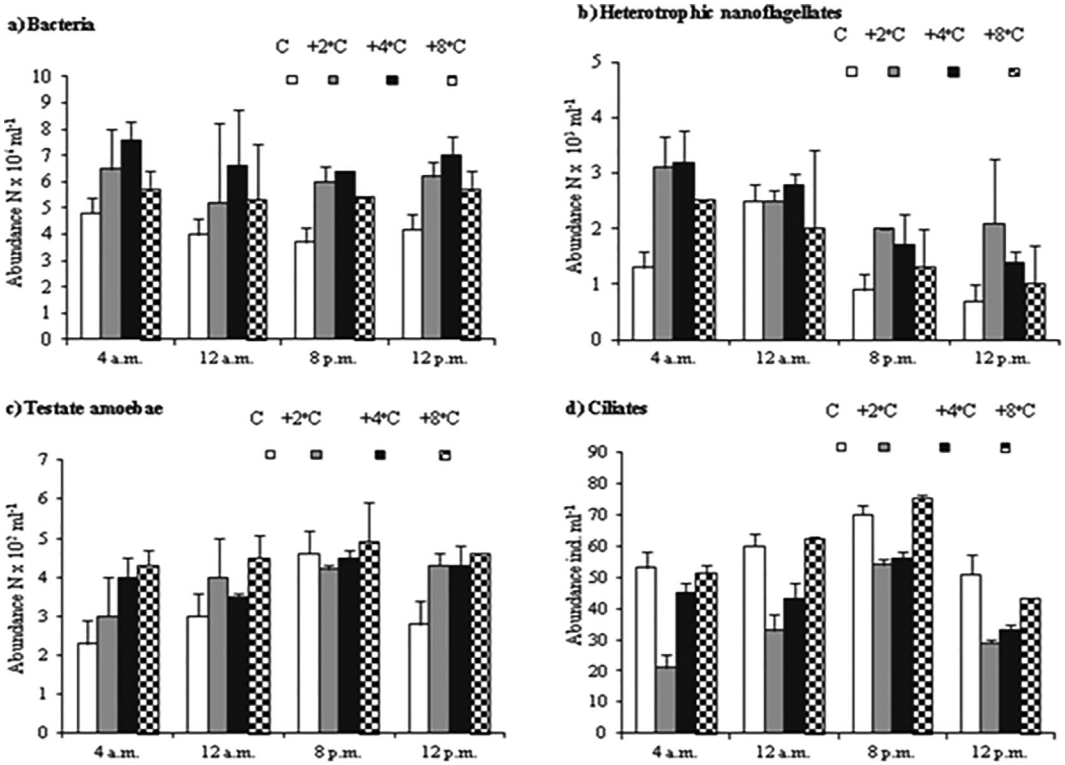

Fig. 3. a-d. Diurnal dynamics of the density of bacteria, heterotrophic flagellates, testate amoebae and ciliates in investigated mesocosms (T SD - standard deviation).

vulgaris attained the largest share at that time, while in the experimental variants subjected to a temperature increase, the share of species of the genus *Hyalosphenia* increased. The lowest abundance and biomass values were observed at night ($1.8–2.0 \times 10^2$ cells mL^{-1} and 0.5–0.7 μg C mL^{-1}). A total of 21 ciliate taxa were found in the experimental variants. The richest taxonomic composition was recorded in the +8 °C experimental variants (18 taxa), while the lowest diversity was found in the CT (3 taxa). Individual experimental variants also differed in the mean abundance and biomass of ciliates. The highest abundance was found in samples under the ice and in the +8 °C variants (54–59 ± 11 cells mL^{-1}), while the average abundance in the remaining experimental treatments was only about half as high. A distinct 24-hour cycle of changes in the density and biomass of ciliates was observed as well. In all

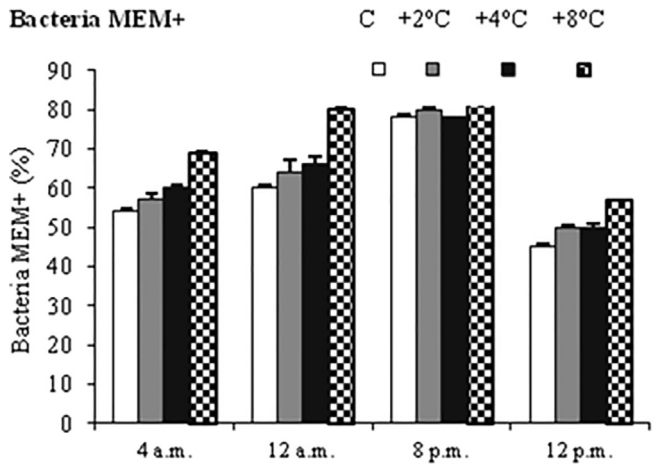

Fig. 4. Diurnal dynamics of the percentage contribution of active cells (MEM+) to the total numbers of bacteria in investigated mesocosms (T SD - standard deviation).

experimental variants, significantly higher ciliate density was observed in the evening (from 56 to 75 ± 12 cells mL^{-1}), while biomass was higher in the early morning (>0.6 ± 0.2 μgC mL^{-1}) ($F_{1.15} = 13.07$, $P \leq 0.005$) (Figs. 3d, 5d). The small bacterivorous *Cinetochilum margaritaceum* dominated in the first case and the large mixotrophic *Paramecium bursaria* in the second. At the same time, in the CT *Paramecium bursaria* attained a > 90% share in the total number of ciliates. The lowest abundance and biomass were observed at night. Irrespective of the time, the increase in temperature was accompanied by a marked decrease in the share of large bacterial and protozoan cells in the total abundance of these organisms (Table 3). This was particularly pronounced in the +8 °C variant, where at the start of the experiment these cells constituted 70% of the total numbers, and at the end of the experiment only 30% (Fig. 6a-d). In the experimental treatments with increased temperature, there was a significantly higher proportion of the smallest cells for all tested groups of organisms ($F_{1.21} = 26.32$–33.21, $P \leq 0.001$). The trophic structure of testate amoeba and ciliates also varied between mesocosms. Mainly bacterivorous and mixotrophic taxa were dominant in the CT, while the proportion of predatory and omnivorous taxa increased in other mesocosms. The testate amoeba community in the CT was characterized by high abundance of bacterivorous *Arcella vulgaris*, while in other mesocosms there was an increased share of mixotrophic species of testate amoeba (e.g. *Hyalosphenia papilio*). The reverse tendency was observed in the community of planktonic ciliates, as mixotrophic species (e.g. *Paramecium bursaria*) dominated in the CT, while the proportions of bacterivorous and omnivorous species increased in the other mesocosms. In the protozoan community, mixotrophic taxa were dominant in the CT (70% of the total abundance), while bacterivorous taxa dominated in the other mesocosms, accounting for 55% to 80% of the total number of protozoa.

3.3. Correlations between microbial loop components

The degree of correlation between groups of organisms was markedly varied depending on the experimental treatment. In the control treatments, the abundance of ciliates was correlated with DOC and abundance of MEM+ bacteria and heterotrophic flagellates (from $r = 0.29$, $P \leq 0.05$ to $r = 0.59$, $P \leq 0.01$). Along with the increase in temperature, the strength of the correlations between DOC and bacteria and between protozoa and bacteria increased (from $r = 0.31$, $P \leq 0.05$ to $r = 0.59$, $P \leq 0.01$). Different patterns were found for the number and strength of correlations in the components of the microbial loop in the 24 h cycle. In the +4 °C and +8 °C experimental variants there were far more statistical relationships between all components of the loop, and they were more

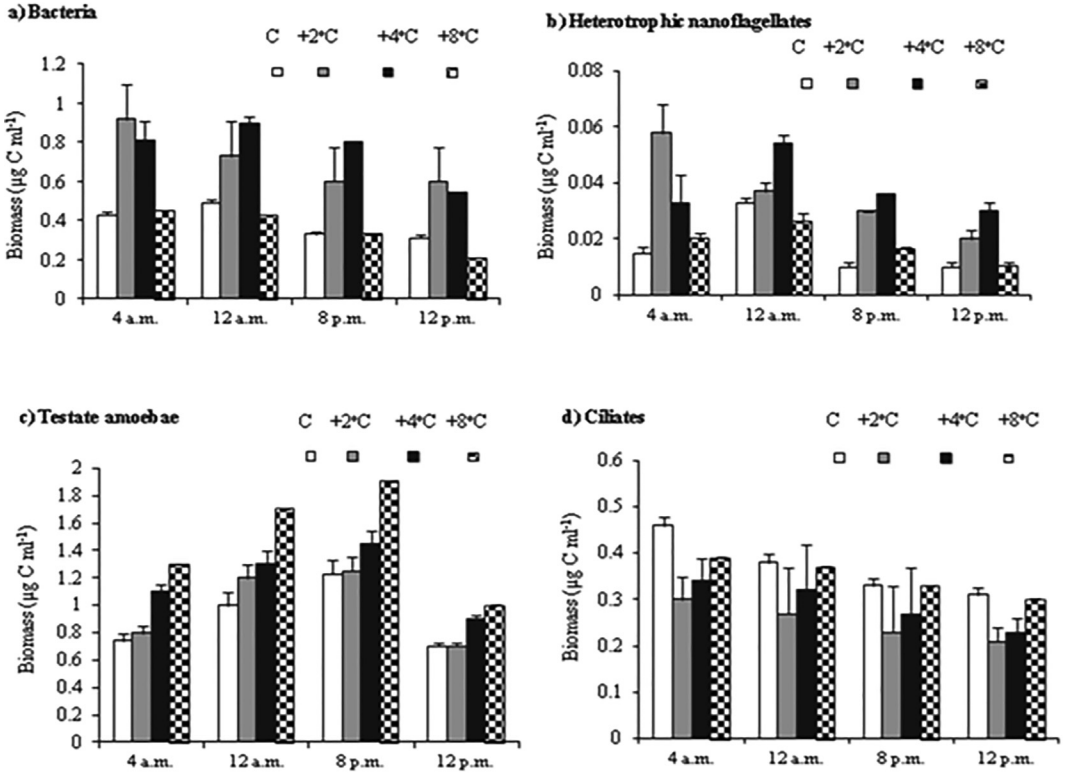

Fig. 5. a-d. Diurnal dynamics of the biomass of bacteria, heterotrophic flagellates, testate amoebae and ciliates in investigated mesocosms (T SD - standard deviation).

Table 3
Multiple linear regression relating size structure of microbial communities to increase in water temperature.

Microbial communities	Size	adj. R^2	*n*	*P*
Bacteria	1→<0.5 μm	0.49	67	≤0.01
Heterotrophic flagellates	40→<20 μm	0.36	30	≤0.01
Testate amoeba	51→<20 μm	0.25	30	≤0.01
Ciliates	151→<50 μm	0.41	30	≤0.01

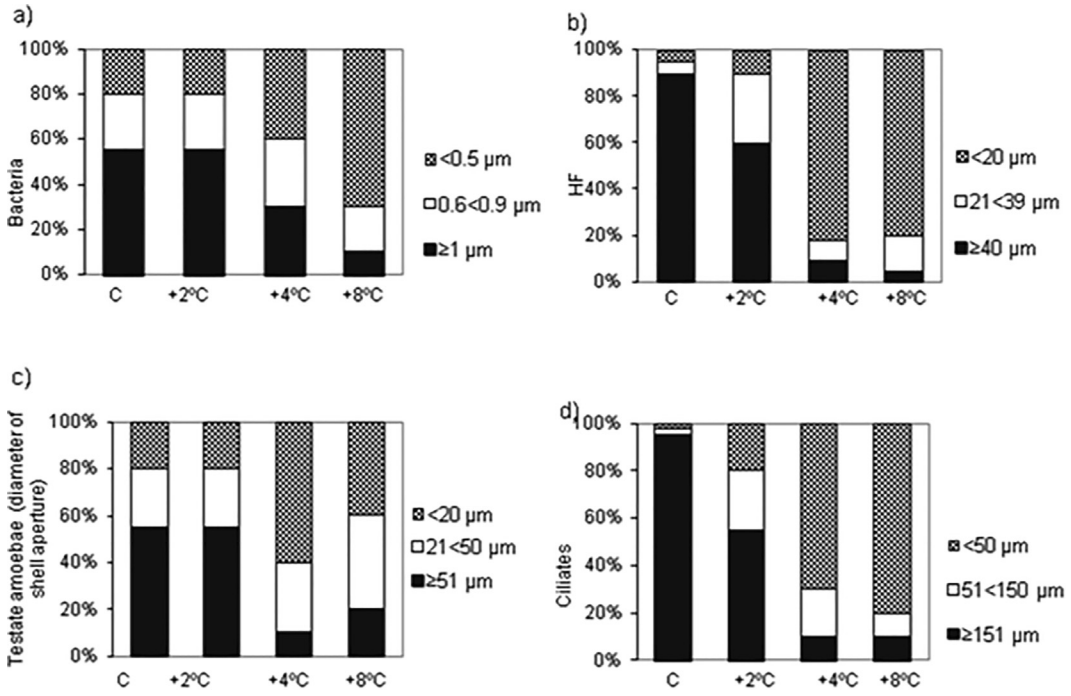

Fig. 6. a-d. Changes in size structure of (a) Bacteria, (b) Heterotrophic flagellates, (c) Testate amoebae and (d) Ciliates in four experimental mesocosms (percentage contribution to the total numbers of microorganisms).

significant. The highest number of significant correlations occurred in the evening. At that time, the abundance and biomass of metabolically active bacteria was most strongly correlated with DOC (from $r = 0.58$, $P \leq 0.01$), while in the evening a clearly positive correlation was found between bacterial abundance and protozoa ($r = 0.59–0.69$, $P \leq 0.01$) (Table 4). In the investigated lakes, PPMR decreased by 26–32% in the experimental treatments with increased temperature relative to the control ($P < 0.05$). We found a significant relationship between PPMR and water temperature ($r = 0.46$, $P < 0.001$) in studied habitats.

3.4. Ordination analyses and correlations between microbial loop components and environmental parameters

Microbial communities were significantly affected by temperature in investigated lakes (Fig. 7a-d). Although, the clearest response was observed for ciliates ($r^2 = 0.7925$), heterotrophic flagellates ($r^2 = 0.8144$) and testate amoebae ($r^2 = 0.6845$). The PCA analysis showed that abundances of microbial communities are closely related to temperature gradient. On the triplot microbial samples are separated into three groups: 1) control, 2) samples collected in mesocosms + 2 °C and +4 °C and 3) samples collected in mesocosms +8 °C. The first group corresponds with higher abundances of testate amoebae, second and third group with relatively high abundances of bacteria, heterotrophic flagellates and ciliates (Fig. 8). The results of PCA suggest that under higher temperature abundances of bacteria, flagellates and ciliates are related to pH, DOC, N-NH_4 and Chl-a . In studied lakes, the full RDA model significantly explained 60.9% of the variance in testate amoebae composition ($P < 0.05$), the fraction of variance explained by temperature was 19.5% ($P = 0.02$), pH 17.8% ($P = 0.014$), DOC 15.2% ($P = 0.014$) and TP explained 8.4% ($P = 0.02$) of the variance. N-NH_4, chlorophyll-*a* and O_2 were not significant in the model ($P > 0.05$) (Fig. 9). RDA performed for ciliates showed that the abundance of ciliate species were significantly affected by four environmental variables. In full RDA model significantly explained 84.7% of the ciliates variance. Temperature explained 22.1% ($P = 0.002$) of the variance, N-NH_4 24.6% ($P = 0.014$), TP 26.6% ($P = 0.014$) and O_2 explained 11.4% ($P = 0.002$) of the variance of ciliates composition (Fig. 10). DOC, pH and chlorophyll-*a* were not significant in the model ($P < 0.05$).

4. Discussion

4.1. Temperature and microbial food web function

The functioning of the microbial food web clearly differed between the period with ice cover and the treatments simulating gradual climate warming. This was reflected in a change in the dominance structure of top predators, as well as in a reduction in the body size of individual populations as the temperature rose, especially in the +8 °C variants. The size structure of bacteriocoenoses changed as well. Bacteria > 1 μm dominated in the control sample, while the proportion of small cells < 0.5 μm increased with the rise in temperature. This change in the size

Table 4
Linear correlation coefficients between microbial loop components in the investigated mesocosms (Key: B – bacteria, B MEM+ - bacteria MEM+, HF – heterotrophic flagellates, TA – testate amoebae, C – ciliates, DOC – dissolved organic carbon, $P \leq 0.01$.

	C						+2°C						+4°C						+8°C					
	DOC	B	B MEM+	HF	TA	C	DOC	B	B MEM+	HF	TA	C	DOC	B	B MEM+	HF	TA	C	DOC	B	B MEM+	HF	TA	C
4am																								
DOC	-						-	0.31						0.42						0.59				
B	0.28	-					0.41	-					0.34	-					0.45	-				
B MEM+	0.29		-				0.50		-						-						-			
HF				-				0.36		-				0.37		-				0.47		-		
TA					-			0.51			-			0.53									-	
C	0.37			0.35		-		0.51				-		0.57						0.59				-
Colpodea		0.52	0.36	0.34																				
Cyrtophorida		0.23							0.33						0.33						0.39			
Nassulida		0.33																						
Oligotrichida		0.27	0.29	0.33				0.28	0.29					0.25						0.29				
Scuticulociliatida		0.51																			0.56			
12 noon																								
DOC	-						-	0.31						0.42						0.51				
B	0.28	-					0.41	-					0.34	-					0.45	-				
B MEM+	0.29		-				0.54		-						-						-			
HF				-				0.36		-						-				0.47		-		
TA					-			0.51			-												-	
C	0.37			0.35		-		0.51				-								0.55				-
Colpodea		0.59	0.36	0.34																				
Cyrtophorida		0.23							0.33												0.49			
Nassulida		0.33																						
Oligotrichida		0.27	0.29	0.33				0.28	0.29					0.25						0.29				
Scuticulociliatida		0.25																			0.56			
7-8 pm																								
DOC	-						-	0.31						0.42						0.59				
B	0.28	-					0.41	-					0.34	-					0.45	-				
B MEM+	0.29		-				0.58		-						-						-			
HF				-				0.36		-				0.37		-				0.47		-		
TA					-			0.56			-			0.53									-	
C	0.37			0.37		-		0.56				-		0.59						0.67				-
Colpodea		0.69	0.36	0.39																				
Cyrtophorida		0.63							0.33						0.33						0.37			
Nassulida		0.33																						
Oligotrichida		0.27	0.21	0.38				0.28	0.36					0.25						0.29				
Scuticulociliatida		0.59																			0.33			
12 midnight																								
DOC	-						-	0.31						0.42						0.59				
B	0.28	-					0.41	-					0.34	-					0.45	-				
B MEM+	0.29		-				0.44		-						-						-			
HF				-				0.36		-				0.37		-				0.47		-		
TA					-			0.54			-			0.53									-	
C	0.37			0.35		-		0.54				-		0.51						0.59				-
Colpodea		0.52	0.36	0.34																				
Cyrtophorida		0.23							0.33						0.33						0.37			
Nassulida		0.33																						
Oligotrichida		0.27	0.29	0.33				0.28	0.29					0.25						0.29				
Scuticulociliatida		0.55																			0.43			

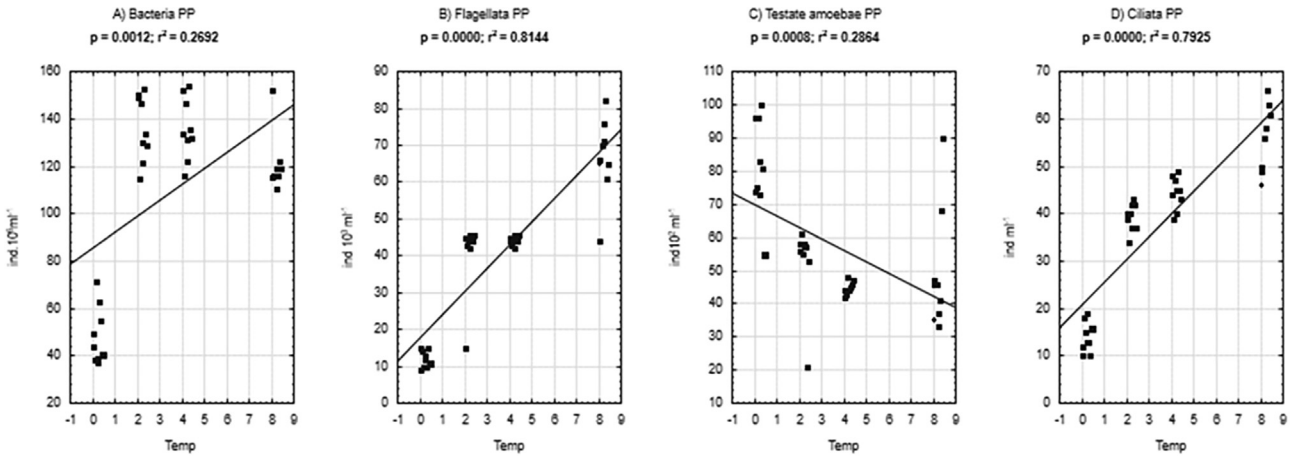

Fig. 7. a-d. Linear regressions of the abundance of microbial communities to temperature in peat investigated lakes for the polled dataset.

structure may be due to the activation of defence mechanisms against increasing predation pressure. Similar patterns have been observed in other lake ecosystems (Heckmann et al., 2012). Furthermore, as demonstrated by Kiørboe et al. (2002), resource availability is often patchy under ice (e.g. phytoplankton blooms are restricted to thin layers). Chemotactic behaviour may be a useful bacterial adaptation to efficiently exploit of organic matter and inorganic nutrients. The increase in temperature caused a systematic decrease in large-sized mixotrophic protists in favour of heterotrophic protists. These patterns are thus consistent with the metabolic theory of ecology (Brown, 2004). Others components (such as substantial increases in chlorophyll *a*) suggest that the microbial food web would be overall more autotrophic relative to ice-covered controls. Intensive development of phytoplankton is most likely a consequence of the increased concentration of biogenic compounds together with the increase in temperature (Forget et al., 2009; Bertilsson et al., 2013). Also, increased grazing on bacteria (by ciliates and flagellates) is known to promote bacterial metabolic activity, and then activity in carbon cycling (Reczuga et al., 2018). These changes might promote a loss of C from the system that could be compensated for by the increase in C-fixing phycoflora. Indeed, warming-induced declines in mean body size within a given community have been reported in numerous ecosystems (Yvon-Durocher et al., 2011; Jassey et al., 2013; 2015; Mulot et al., 2017). In the experimental variants subjected to a temperature increase, the concentrations of total organic

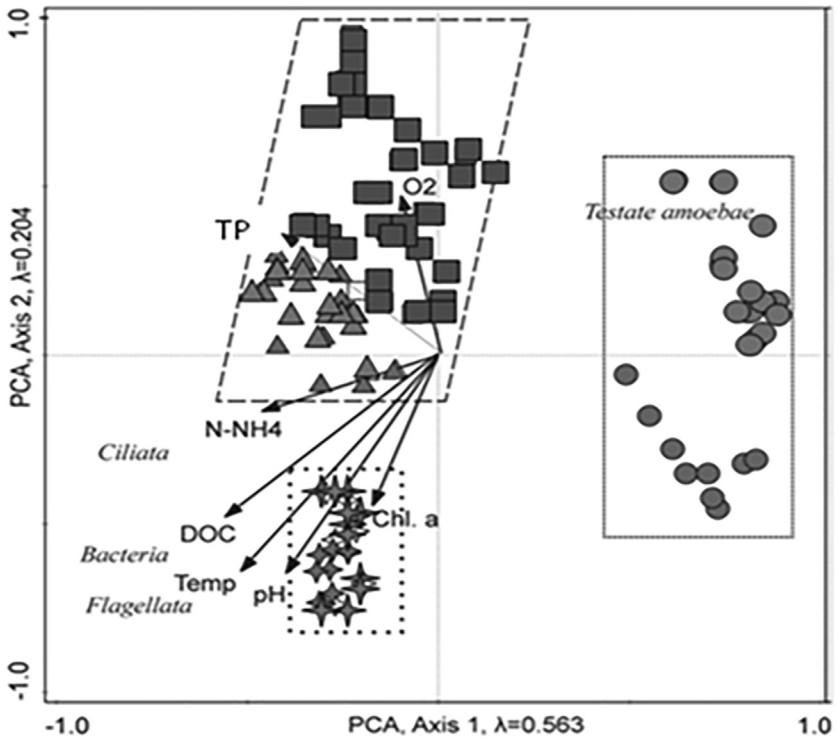

Fig. 8. PCA triplot showing microbial communities, samples and environmental variables. Samples collected in studied mesocosms are marked with geometric figures: grey circles-control, grey squares-mesocosm +2 °C, grey triangles-mesocosm +4 °C, grey stars-mesocosm +8 °C.

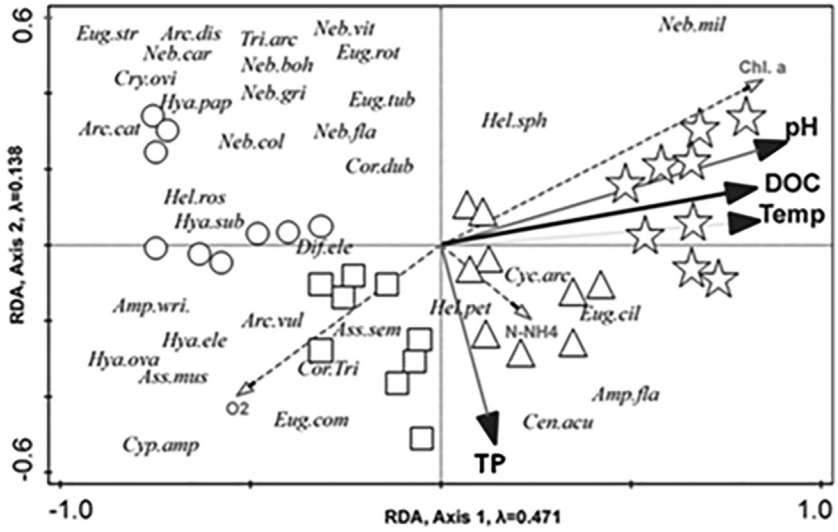

Fig. 9. Redundancy analysis biplots showing testate amoebae species and environmental variables. Solid arrows indicate significant variables based on Monte Carlo permutation test ($p < 0.05$). Species codes: Amp.fla-Amphitrema flavum, Amp.wri-Amphitrema wrightianum, Arc.cat-Arcella catinus, Arc.dis-Arcella discoides, Arc.vul-Arcella vulgaris, Ass.mus-Assulina muscorum, Ass.sem-Assulina seminulum, Cen.acu-Centropyxis aculeata type, Cor.dub-Corythion dubium, Cor.Tri-Corythion-Trinema type, Cry.ovi-Cryptodifflugia oviformis, Cyc.arc-Cyclopyxis arcelloides type, Cyp.amp-Cyphoderia ampulla, Dif.ele-Difflugia elegans, Dif.lei-Difflugia leidyi, Eug.cil-Euglypha ciliata, Eug.com-Euglypha compressa, Eug.rot-Euglypha rotunda, Eug.str-Euglypha strigosa, Eug.tub-Euglypha tuberculata, Hel.pet-Heleoptera petricola, Hel.ros-Heleoptera rosea, Hel.sph-Heleoptera sphagnii, Hya.ele-Hyalosphenia elegans, Hya.ova-Hyalosphenia ovalis, Hya.pap-Hyalosphenia papilio, Hya.sub-Hyalosphenia subflava, Neb.boh-Nebela bohemica, Neb.car-Nebela carinata, Neb.col-Nebela collaris, Neb.fla-Nebela flabellulum, Neb.gri-Nebela griseola type, Neb.mil-Nebela militaris, Neb.fla-Nebela vitraea type, Tri.arc-Trigonopyxis arcula. Samples collected in studied mesocosms are marked with geometric figures: circles-control, squares-mesocosm +2 °C, triangles-mesocosm +4 °C, stars-mesocosm +8 °C.

carbon and nutrients, which may have indirectly affected the abundance of food (particularly bacteria), were several times higher. This may explain the exceptional species richness and abundance of ciliates in these experiments. Such relationships have also been observed in various types of peatlands and eutrophic lakes, in mesocosm systems in which the concentrations of biogenic compounds were manipulated (Mieczan et al., 2015; Zingel et al., 2018). Buosi et al. (2011) have also observed that the species richness of protozoa increased with habitat fertility. Such pronounced changes in ciliate communities are probably linked to their life cycle and structure. Their generation times are short (mean 20–72-h), and they are only protected from the envi-

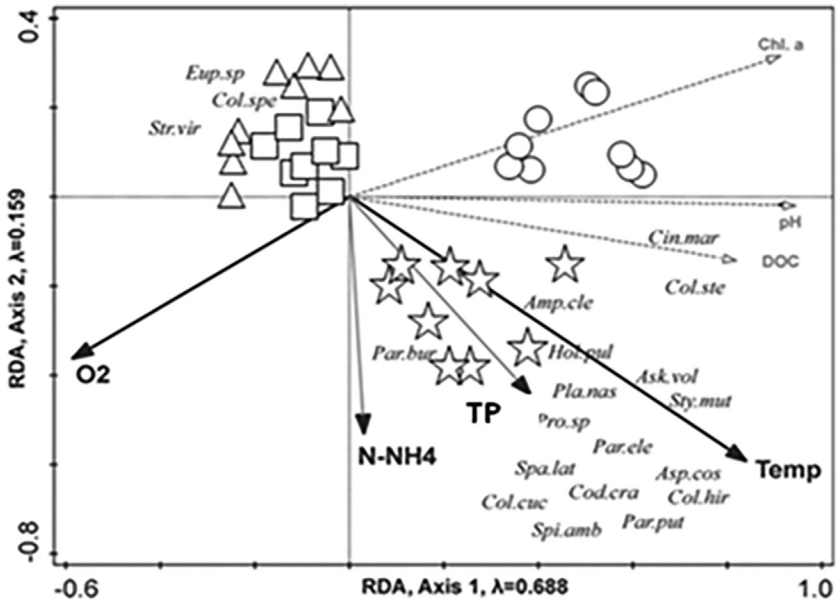

Fig. 10. Redundancy analysis biplots showing ciliate species and environmental variables. Solid arrows indicate significant variables based on Monte Carlo permutation test ($p < 0.05$). Species codes: Ask.vol-Askenasia volvox, Col.cuc-Colpoda cucullus, Col.ste-Colpoda steinii, Lac.olo-Lacrymaria olor, Par.ele-Paradileptus elephantinus, Pla.nas-Plagiopyla nasuta, Spa.sen-Spathidium sensu lato, Spi-amb-Spirostomum ambiguum, Par.bur-Paramecium bursaria, Par.put-Paramecium putrinum, Cin.mar-Cinetochilum margaritaceum, Asp.cos-Aspidisca costata, Eup.sp-Euplotes sp., Hol.pul-Holosticha pullaster, Sty.myt-Stylonychia mytilus-Komplex, Cod.cra-Codonella cratera, Str.vir-Strombidium viride, Amp.cle-Amphileptus claparadei, Col.hir- Coleps hirtus, Col.spe-Coleps spetai, Pro.sp-Prorodon sp. Samples collected in studied mesocosms are marked with geometric figures: circles-control, squares-mesocosm +2 °C, triangles-mesocosm +4 °C, stars-mesocosm +8 °C.

ronment by a delicate cell membrane. Therefore, the potential response time to environmental factors is fast (Andersen et al., 2013). On the other hand, an increase in water temperature positively correlates with the bacterial reproduction rate (Beall et al., 2016), thereby improving food conditions for protozoa. During the period with ice cover, nearly exclusive dominance of the ciliate species *Paramecium bursaria* was observed. At the same time, there was an increase in the chlorophyll *a* concentration in this variant compared to the +2 °C treatment, but it was lower than at +4 °C and +8 °C. In the control treatment, where abundance of *Paramecium* was at its highest, we observed an increase in the chlorophyll *a* concentration in the water. This increase in the chlorophyll concentration is most likely due to the presence of endosymbiotic algae of the genus *Chlorella* in cells of *Paramecium*. When this genus of protozoa is under the ice, it probably tends towards a more heterotrophic mode of life and feeds mainly on bacteria, because light conditions are less favourable in ice-covered water bodies, and the development of phytoplankton may be difficult. Microscopic observations showed that *Paramecium* was filled with tiny algal cells. At the same time, other mixotrophic ciliate species were found in the CT, including *Limnostrombidium viride*, as well as obligate phototroph flagellates (*Cryptomonas*), which could also have affected chlorophyll *a* levels. Perhaps these species use endosymbiotic algae as a potential source of energy. Mixotrophy can therefore be an effective adaptive mechanism during shortages of other potential food resources. Mixotrophs are known to be dominant in peat pools in other geographic locations, such as Rancho Hambre in Tierra de Fuego (Lara et al., 2015; Küppers et al., 2016). However, mixotroph abundance is correlated with increasing bacterial biomass, suggesting that mixotrophs are also strongly dependent on ingested bacteria, possibly for one or more essential nutrients (Jones, 2000). The increased chlorophyll *a* and nutrients concentration in the CT may also be the effect of excretion by protozoa, which enriches the water with mineral forms of nitrogen and phosphorus. According to Mieczan (2012), the average net excretion rate of nitrogen was 0.58 $\mu g\ l^{-1}$ per day (as $N\text{-}NH_4$) and that of phosphorus was 0.22 $\mu g\ l^{-1}$ per day (as $P\text{-}PO_4$) for an average population of 10,000 protozoa in the water. The clear prevalence of small forms in the mesocosms means that during the study period, these microorganisms can supply about 139 μg $N\text{-}NH_4$ and 53 μg $P\text{-}PO_4$.$\mu g\ l^{-1}$. Obviously, these are minimal excretion volumes compared to those noted in field conditions, where abundance of food is relatively high. Therefore, they may affect microbial communities through bottom-up regulation. As the temperature increased, the share of *Paramecium bursaria* decreased, while that of the amoeba species *Hyalosphenia papilio* increased. Research by Jassey et al. (2012) in peatland ecosystems has shown that *H. papilio* can selectively consume ciliates. This species more readily consumed large mixotrophic taxa, mainly *Playtorya sphagni* and *Paramecium bursaria*, than the dominant small taxa of the genus *Uronema*. It seems, therefore, that this sharp decline in the numbers of *Paramecium* could have been due to grazing by testate amoebae.

4.2. Diurnal dynamics of the microbial loop

Dynamics of the abundance showed marked variation in the 24 h cycle, with the highest density of protozoa observed in the evening and another increase in the early morning. This trend was particularly evident in the +4 °C and + 8 °C variants. The chlorophyll *a* concentration was also lowest at these times, while the bacterial density was relatively high. In these variants, the maximum abundance of ciliates coincided with an increase in the abundance of flagellates. Thus far, however, there is a lack of comparative data on the 24-hour dynamics of individual components of the microbial loop in dystrophic lakes in winter. However, as demonstrated by Šimek and Straskrabova (1992) in low-fertility environments, the rate of consumption of bacteria by ciliates in the afternoon is nearly twice as high as at night and in the early morning. It seems that the increase in ciliate density in the evening may have been associated with the abundance of available food. The highest DOC concentrations were also recorded in the evening. Dissolved organic carbon probably indirectly contributed to the

increase in the abundance and biomass of protozoa. In the present study, the DOC concentration during the 24-hour cycle was highly varied. There are no comparative data available from similar ecosystems, but it seems that such marked differences between times of day may be due to the presence of large biomass of *Sphagnum* mosses in the experimental treatments, which may be a major source of organic matter. Moreover, microscopic observations showed that *Sphagnum* mosses were inhabited by diatoms, which may also have been a significant source of organic matter. Numerous studies show that abundance of bacteria is positively correlated with concentrations of dissolved organic carbon in water, and thus can also affect the abundance of protozoa (Schumann et al., 2003; Chróst and Siuda, 2006; Tranvik et al., 2009; Jassey et al., 2011a,b; Özen et al., 2013). The relationships between DOC and bacterial abundance and between concentrations of DOC and chlorophyll *a* ($r = 0.43$) were particularly pronounced in the experimental variants simulating an increase in water temperature, while in the control treatments they showed only a slight correlation. This is probably because the chemical composition and availability of DOC are more significant in the development of heterotrophic bacteria than its quantity. According to Chróst and Siuda (2006), the particles that make up DOC refractors are available only after enzymatic decomposition. On the other hand, the increase in the number of ciliates in the morning was due to the development of *Paramecium bursaria* and could have been caused by changes in algal density (a significant increase in chlorophyll *a* content was observed at that time). In the samples subjected to a temperature increase, the increased density of flagellates in the morning was probably caused by low pressure from ciliates. Abundance and biomass peaks of flagellates were also observed during the day in the control samples, which may also be linked to light availability. The low numbers in the evening and at night may indicate consumption of these organisms by amoebae and ciliates. The decrease in the size of heterotrophic flagellate populations may be due to changes in their life strategy. Many micro- and macroorganisms shift in winter from a primarily pelagic to a benthic life stage (Bertilsson et al., 2013). Thus it is possible that lower numbers of flagellates are a consequence of this life strategy. It seems that in these water bodies there is a very specific kind of microbial loop, in which a trophic system of bacteria and ciliates and/or testate amoebae dominates, while the share of flagellates in bacterial consumption is marginalized. This also seems to be confirmed by the change in the metabolic activity of bacteria as the abundance of amoebae and ciliates increased. Irrespective of the experimental treatment, the proportion of metabolically active bacterial cells decreased as the abundance of these protozoa increased, especially in the morning. According to Del Giorgio et al. (1996) and Lew et al. (2019), the rate of consumption of metabolically active bacteria increases fourfold compared with the consumption of inactive or dead bacteria. On the other hand, an increase in the proportion of inactive or dormant bacteria may also be one of the mechanisms protecting against pressure from potential predators (Jonesa and Lennona, 2010; van Vliet, 2015).

5. Conclusions

Simulated climate warming was shown to cause a change in the qualitative and quantitative structure of the microbial loop. This is reflected in an increase in the abundance of top predators (mainly ciliates and testate amoebae) and a reduction in the body size of individual populations at the highest temperature. The higher temperature increased the abundance of autotrophic organisms, including 'facultative' mixotrophs, and at the same time increased the proportion of bacterivorous and omnivorous taxa. The latter effect is most likely the result of feedback: more phytoplankton means more organic matter, and thus more substrate for bacterioplankton, which is a potential source of food for organisms from higher trophic levels. The highest number of significant correlations occurred in the evening. At that time, the number and biomass of bacteria were most closely correlated with concentrations of dissolved organic carbon, and in the evening a clear positive correlation was observed between abundance of bacteria and heterotrophic protists. In the 24 h cycle, all components of the microbial loop were strongly correlated with the concentrations of phosphates and organic carbon. These results seem to confirm the hypothesis that temperature may significantly influence the 24-hour dynamics of the abundance of individual components of the microbial loop, as well as the number and strength of correlations between bacteria and protists.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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