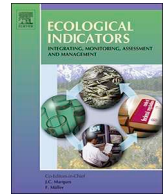




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## Experimental warming and precipitation reduction affect the biomass of microbial communities in a *Sphagnum* peatland

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### ABSTRACT

Due to their unique flora, hydrology and environmental characteristics, peatlands are precious and specific habitats for microorganisms and microscopic animals. Their microbial network structure and their biomass are crucial for peatland carbon cycling, through primary production, as well as decomposition and mineralization of organic matter. Wetlands are one of the ecosystems most at risk from anthropogenic activities and climate change. Most recent scenarios of climate change for Central Europe predict an increase in air temperature and a decrease in annual precipitation. These changes may disturb the biodiversity of aquatic organisms, and the peat carbon sink. Considering the above climatic scenarios, we aimed to: i) assess the response of microbial community biomass to warming and reduced precipitation through the lens of a manipulative experiment in a peatland ecosystem ii) predict how global warming might affect microbial biodiversity on peatlands exposed to warmer temperatures and decreased precipitation conditions. Additionally, we wanted to identify ecological indicators of warming among microorganisms living in *Sphagnum* peatland. The result of a manipulative experiment carried out at Rzecin peatland (W Poland) suggested that the strongest reduction in microbial biomass was observed in heated plots and plots where heating was combined with a reduction of precipitation. The most pronounced changes were observed in the case of the very abundant mixotrophic testate amoeba *Hyalosphenia papilio* and cyanobacteria. Shifts in the *Sphagnum* microbial network can be used as an early warning indicator of peatland warming, especially a decrease in the biomass of important phototrophic microbes living on the *Sphagnum capitula*, e.g. *Hyalosphenia papilio*.

### 1. Introduction

Peatlands have become increasingly important model ecosystems in ecology, biodiversity and climate interaction research (Bardgett and Wardle, 2010). They host rare and often endangered plants, as well as ecosystem-specific bird and amphibian species currently threatened by the climate crisis. Moreover, those peat-forming ecosystems are sensitive to water table decreases (Malmer et al., 1994; Bardgett and Wardle, 2010) triggered by intense human activity such as drainage,

agriculture, peat exploitation and forestation. The consequences are uncontrolled fires, drought, increases in greenhouse gas emissions and significant biodiversity decline (van Diggelen et al., 2006; Lamentowicz et al., 2013). Changes (e.g. deforestation or pollution) in landscape surrounding pristine peatland both directly and indirectly affect peatland organisms such as microbes and plants (Erwin, 2009; Lamentowicz et al., 2015). In particular, fens are vulnerable to the slightest hydrological disturbance (van Diggelen et al., 2006, 2015). Various disturbances cause a shift in the composition and abundance of vegetation,

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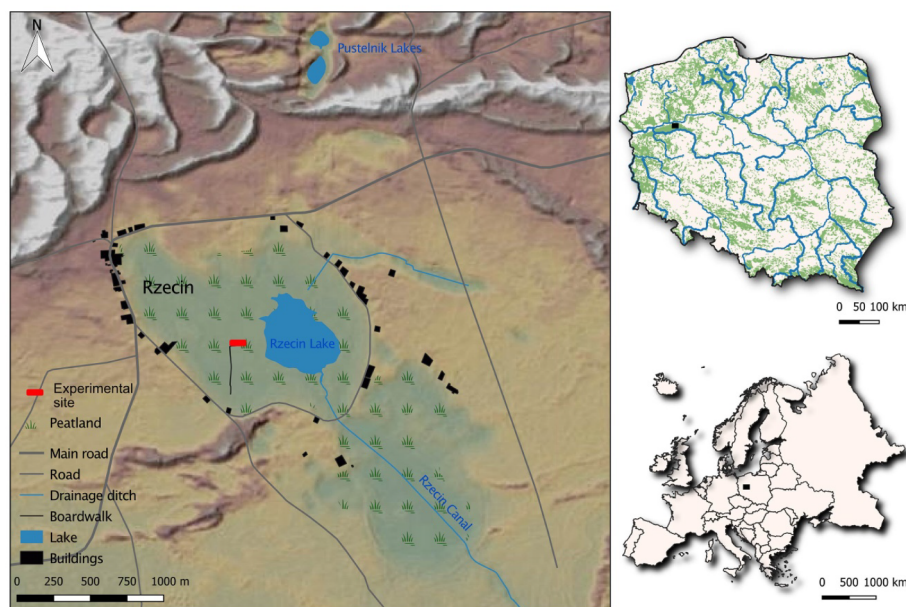


Fig. 1. Location of the “WETMAN” project experimental site in the Rzecin peatland map (52°45′ N, 16°18′ E, 59 m a.s.l.).

which may lead to a degradation of the wetland ecosystem.

Peatlands cover only 3% of the Earth’s area, but at the same time they store 1/3 of global soil carbon (C), which is the equivalent of half the carbon present in the atmosphere in the form of carbon dioxide (Limpens et al., 2008; Dise, 2010). Hence, the rapid warming and the decline in precipitation, together with an decrease in the ground water table depth, may lead to considerable carbon dioxide emissions to the atmosphere from those ecosystems (Gorham, 1991; Dise, 2010; Gallego-Sala et al., 2018). Warming of boreal and subarctic zones is expected to be the most intensive where most peatlands are located (Gorham, 1991; IPCC, 2014). The influence of possible climate changes on particular groups of organisms living in *Sphagnum*, as well as interactions between the main primary producers, consumers and decomposers in peatlands has recently begun to be studied. Peatlands accumulate carbon when input through photosynthesis exceeds carbon losses through autotrophic and heterotrophic respiration (Delarue et al., 2011; Jassey et al., 2015; Bragazza et al., 2016; Buttler et al., 2015). Currently, the majority of peatlands are protected areas with various forms of conservation being applied, but still climate changes affect their functioning (Untenecker et al., 2017). The consequences of global warming could make management and restoration of peatlands even more complicated (Erwin, 2009). For this reason, understanding and awareness of early ecosystem disturbance indicators could be useful in peatland monitoring and protection.

Jassey et al. (2011a) performed a laboratory experiment testing the influence of temperature on microbes living in *Sphagnum* and suggested that not only testate amoeba, but also other wetland group biomasses (cyanobacteria, microalgae, fungi, and ciliates) can be reliable indicators of climate changes. Furthermore, the functioning of the biotic and abiotic components of peatland depends on microbial predators – testate amoebae – which play a key role in the microbial network (Jassey et al., 2013a,b; Jassey et al., 2014; Reczuga et al., 2018). Moreover, above- and below-ground interactions between plants and microorganisms affect carbon fixing in dynamic peatland ecosystems (Bardgett and Wardle, 2010; Jassey et al., 2014; Robroek et al., 2015). The majority of microscopic organisms have short life cycles and fast reproduction rates, so they respond very rapidly to environmental changes. In particular, the response of various microbial groups to precipitation reduction and warming is not yet well recognized, with

most of studies being focused on a single group living in *Sphagnum*, e.g. testate amoeba (Andersen et al., 2013).

The majority of experimental studies have been focused on the influence of one environmental variable. Manipulations have been carried out in laboratories and in most cases the goal of the performed analyses has been to estimate the abundance and diversity of species, but not microbial biomass, which provides more relevant information about ecosystem functioning and is more comparable in the case of different types of wetland. The study by Jassey et al. (2015) suggested that within the bryophyte microbial food web only the total microbial biomass and mixotrophic testate amoeba biomass decreased due to warming. Moreover, after summarizing their passive warming and laboratory experiment, the authors found that mixotrophic testate amoeba might be important drivers in carbon fixing and that more research should be conducted to disentangle the effect of manipulation with temperature from moisture (Jassey et al., 2015). A new study of microbial networks has also suggested that the natural and experimental moisture gradients negatively affect the abundance of larger microbial consumers such as testate amoeba (Reczuga et al., 2018). However, impacts of decreased precipitation manipulation, which might have a significant impact on peatland ecosystem, have never been tested together with warming.

Considering the above intriguing problems, we designed a field experiment to explore the effect of peat warming and decreased precipitation on peatland microbial communities with special attention to testate amoeba. The further aim of the experimental work was to identify the best microbial indicators of climate change in a *Sphagnum* peatland. We aimed to: i) assess the response of microbial community biomass to warming and reduced precipitation through the lens of a manipulative experiment in a peatland ecosystem ii) predict how global warming might affect microbial biodiversity on peatlands exposed to warmer temperatures and decreased precipitation conditions because of the fact that previous studies have recorded a vertical difference in microbial communities structure (Meisterfeld, 1977; Mitchell and Gilbert, 2004; Song et al., 2016) and its response (Reczuga et al., 2018), we hypothesized that i) the upper (top) and lower sections of living microbial communities would respond differently to manipulation and ii) phototrophic microbes dwelling on the top of *Sphagnum* would respond clearly to the manipulation.

## 2. Materials and methods

### 2.1. Study site

The study site was the Rzecin peatland (Fig. 1) located between the dunes of Noteć Forest in Western Poland. The peatland covers ca. 86 ha together with the lake and surrounding grasslands and it is part of protected area of Nature 2000 European Union program.

The site is surrounded by pine forests and small farms, but anthropogenic pressure is considered not very intensive (Juszczak and Augustin, 2013; Juszczak and Augustin, 2013). A paleoecological study in Rzecin peatland showed that in the last 200 years anthropogenic and natural disturbances have occurred in the area, which changed the water table level and nutrient concentrations in the peat. These disturbances affected the lake basin and led to the development of the floating mat (Lamentowicz et al., 2015; Milecka et al., 2017) and triggered the development of a floating *Sphagnum* vegetation mat. The flora of Rzecin peatland is rich, characterized by a high diversity of habitats, various plant communities as well as rare and endangered species. Therefore, Rzecin peatland is precious for the region and for Poland (Wojterska, 2001). Vegetation analyses of wetland have shown the presence of 127 vascular plant species, including ones rare or endangered in Poland (*Carex limosa*, *C. dioica*, *Liparis loeselii*, and *Utricularia intermedia*) (Wojterska, 2001).

The study site has an average annual precipitation of 575 mm, with many cloudy days (Woś, 2010). The ground water table depth was quite high according to Juszczak & Augustin (2013), with a mean around  $-5.6$  (cm), a minimum value equal to  $-15.9$  (cm) and a maximum of  $3.6$  cm in pools.

### 2.2. Experimental design

The experimental setup was constructed in the central part of a poor fen. This part is homogenous in terms of plant species composition and structure and it is dominated by *Sphagnum* spp. (*Sphagnum angustifolium*, *S. fallax*, and *S. teres*), *Aulacomnium palustre* with vascular plants *Oxycoccus palustris*, *Carex limosa*, *C. rostrata*, *Eriophorum angustifolium* and *Drosera rotundifolia*. A wooden boardwalk was installed in 2014 from the northern part of the experimental area to avoid trampling and to facilitate access to the site and experimental plots exposed for manipulation.

The experimental design consisted of four treatments in blocks: warmed – manipulation with raised temperature only (W), warmed + reduced precipitation – (W + RP), reduced precipitation – (RP) and control – without any manipulation (C) (Rastogi et al., 2019, Fig. 2). Every block consisted of three plots (70 cm × 70 cm) considered as replicates.

The experimental manipulation of air and consequently peat

temperature was achieved by the application of 8 infrared radiators (400 W each). Details can be found in Rastogi et al. (2019). Meanwhile, precipitation was reduced through use of a retractable curtain (rain sensor-controlled), working only at nights in the period from March/April to November/December in order to not overshadow the plots during the daytime. The aim was to reach a minimum 30% reduction of precipitation in the growing season (Emmett et al., 2004). A moving platform was installed above the plots to enable non-invasive sampling of vegetation and ground water. Every block was equipped with a heated rain gauge (TPG-124-H24, ASTER, Poland) and thermohygrometers installed 50 cm above the peat surface (HC2S3, Campbell Scientific, USA). Peat temperature was measured by T-107 thermistors (Campbell Scientific, USA) at 2, 5 and 10 cm depth.

### 2.3. Field study and sampling

The first *Sphagnum* sampling campaign was carried out in August 2014, three months after construction of the experimental setup to allow its stabilization, but before starting the manipulation in the blocks: C, W, W + RP and RP. The second sampling was carried out one year later: in August 2015. All samples were taken using the moving platform and wooden boardwalk to avoid disturbances in the experimental plots. 20 *Sphagnum angustifolium* shoots were taken from 10 points (permanently marked), randomly distributed in every plot, and cut in 0–3 cm upper segments with capitulum and 3–8 cm lower shoot. Material was immediately preserved in 2.5% solution of glutaraldehyde and stored in a dark cold room at the temperature of 4 °C. After six cycles of filtration on a 300 µm mesh-sized net (Gilbert et al., 1998; Jassey et al., 2011a; Reczuga et al., 2018) the *Sphagnum* was dried at 65 °C for 48 h, and weighed to obtain the dry mass of *Sphagnum* of every sample (90 °C). The samples were then analyzed in 3-milliliter Utermöhl chambers under an inverted microscope. Organisms were identified at the species level in the case of testate amoeba and with low taxonomic resolution for other soil communities. We counted and measured cyanobacteria, microalgae, ciliates, rotifers and nematodes using 200× and 400× magnification (Gilbert et al., 1998; Jassey et al., 2011a). Microbial organisms were identified using appropriate taxonomic keys (Radwan et al., 2004; Mazei and Tsyganov, 2006; Lynn, 2008). Spores and hyphae were counted and measured (length and width) to quantify fungi. To estimate the biomass, the lengths and widths or diameters of at least 20 specimens were measured, then assuming them to have geometrical shapes, the biovolume of each species/group was calculated. The biovolume was converted to biomass expressed as microgram of carbon per *Sphagnum* dry mass gram (µg C per gram of dry mass) according to conversion factors given by Gilbert et al. (1998) and used in other research (Jassey et al., 2011b; Reczuga et al., 2018). This method allows comparable carbon estimations to be obtained. The average air temperature at 50 cm above the ground, peat

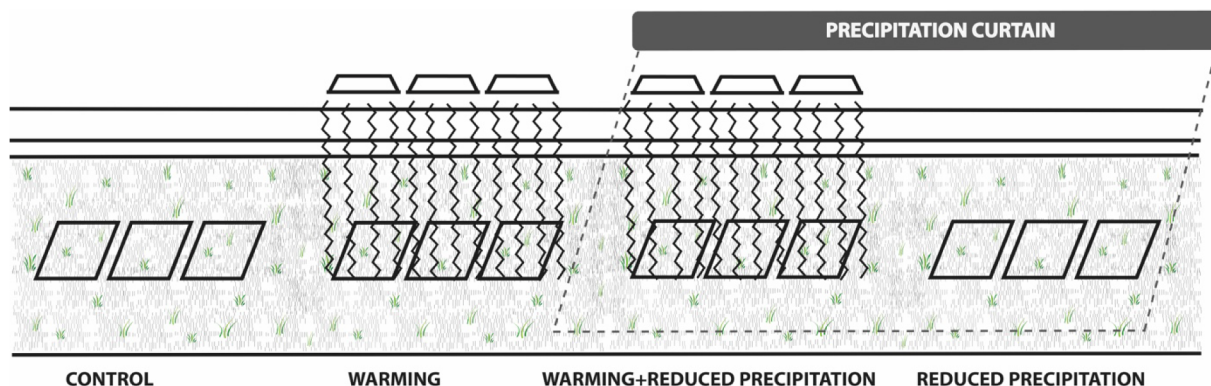


Fig. 2. Experimental setup with manipulations: warmed - the active warming (W), warmed + reduced precipitation – increased temperature and precipitation decreased with curtain (RP + W), reduced precipitation - only decreased precipitation (RP) and control block – without manipulation (C).

**Table 1**

The average of air temperature – air temp., peat temperature on depth of 2 cm – peat temp. and rain in blocks (C, W, W + RP, RP), before manipulation in August 2014 and during the manipulation in August 2015. Below, the averages of nutrients concentrations (pH level, conductivity, chlorides, nitrates phosphates, sulfates, magnesium, calcium, sodium) are presented for each block (C, W, W + RP, RP) in August 2014 and during the manipulation in August 2015.

|  | C            | W            | W + RP       | RP           |
|--|--------------|--------------|--------------|--------------|
| August 2014  |              |              |              |              |
| air temp. [°C]                                     | 16.6         | 16.6         | 16.7         | 16.7         |
| peat temp. [°C]                                    | 18.1         | 18.3         | 18.8         | 18.9         |
| rain [mm]  | 12.0         | 12.0         | 12.0         | 12.0         |
| Rain (Jan.-Aug.) [mm]                              | 342.1        | 342.1        | 342.1        | 342.1        |
| pH   | 4.35 ± 0.4   | 4.21 ± 0.36  | 4.22 ± 0.42  | 4.34 ± 0.47  |
| conductivity [µS/l]                                | 55.61 ± 4.71 | 51.99 ± 3.44 | 50.19 ± 8.32 | 57.58 ± 4.05 |
| August 2015  |              |              |              |              |
| air temp. [°C]                                     | 20.7         | 21.1         | 21.2         | 20.8         |
| peat temp. [°C]                                    | 19.4         | 19.6         | 20.6         | 19.7         |
| rain [mm]  | 31.1         | 31.4         | 0.2          | 0.5          |
| Rain (Jan.-Aug.) [mm]                              | 270.1        | 276.3        | 192.1        | 209.3        |
| pH level   | 4.51 ± 0.05  | 4.47 ± 0.09  | 4.37 ± 0.16  | 4.61 ± 0.05  |
| conductivity [µS cm <sup>-1</sup> ]                | 66.9 ± 6.6   | 65.4 ± 2.3   | 63.1 ± 2.7   | 63.4 ± 1.4   |
| chlorides [mg Cl l <sup>-1</sup> ]                 | 3.6 ± 0.9    | 2.5 ± 2.2    | 3.1 ± 0.9    | 3.0 ± 0.4    |
| nitrates [mg NO <sub>3</sub> l <sup>-1</sup> ]     | ta           | ta           | 0.012 ± 0.02 | 0.03 ± 0.01  |
| phosphates [mg P-PO <sub>4</sub> l <sup>-1</sup> ] | 0.37 ± 0.20  | 0.11 ± 0.10  | 0.12 ± 0.10  | 0.13 ± 0.04  |
| sulfates [mg SO <sub>4</sub> l <sup>-1</sup> ]     | 0.23 ± 0.13  | 0.28 ± 0.30  | 0.39 ± 0.30  | 0.45 ± 0.30  |
| calcium [mg Ca l <sup>-1</sup> ]                   | 3.38 ± 0.61  | 3.66 ± 0.66  | 3.18 ± 1.4   | 3.22 ± 0.70  |
| magnesium [mg Mg l <sup>-1</sup> ]                 | 0.54 ± 0.1   | 0.53 ± 0.12  | 0.43 ± 0.08  | 0.45 ± 0.17  |
| sodium [mg Na l <sup>-1</sup> ]                    | 2.54 ± 0.70  | 2.31 ± 0.50  | 1.75 ± 0.45  | 1.43 ± 0.41  |

\*ta - trace amounts < 0.001.

temperature at the depth of 2 cm and precipitation total in all blocks (C, W, W + RP, RP), before manipulation in August 2014 and during the manipulation in August 2015, are given in Table 1. During sampling, we also collected water from the *Sphagnum* carpet using perforated steel needles with a syringe.

The water analyses included pH level, conductivity, chloride, nitrate, phosphate, and sulfate concentrations and mineral contents, e.g. magnesium, calcium. Samples of *Sphagnum* were collected in 0.5-L polypropylene bags. Two replicates were collected for each site. Electrical conductivity (EC) and pH were measured on water extracted from the mosses samples using a portable multi-parameter probe Professional Plus multi-parameter instrument (YSI, USA). The water samples extracted from the mosses were filtered through 0.45-µm filters (Whatman, UK). Elemental analysis of calcium (Ca), magnesium (Mg), sodium (Na) and chlorides (Cl), was performed using the inductively coupled plasma optical emission spectrometer Agilent 5100 ICP-OES (Agilent, USA). A simultaneous axial and radial view of plasma was obtained by a synchronous vertical dual view (SVDV) using dichroic spectral combiner (DSC) technology. The following common conditions were applied: radio frequency (RF) power 1.2 kW, nebulizer gas flow 0.7 L min<sup>-1</sup>, auxiliary gas flow 1.0 L min<sup>-1</sup>, plasma gas flow 12.0 L min<sup>-1</sup>, charge-coupled device (CCD) temperature -40 °C, viewing height for radial plasma observation 8 mm, accusation time 5 s, three replicates. The calibration was performed using standard analytical solutions (Merck, Germany). The content of nutrients (total phosphorus (TP), orthophosphorus (PO<sub>4</sub>), nitrate nitrogen (NO<sub>3</sub>) and (NO<sub>2</sub>) nitrites nitrogen), chlorides (Cl), and sulphates (SO<sub>4</sub>) was analyzed using an ion chromatograph Basic IC plus 883 (Metrohm).

The average of nutrient and mineral concentrations in respective blocks (C, W, W + RP, RP), during the manipulation in August 2015 are also shown in Table 1.

#### 2.4. Numerical analyses

Before further data analyses, the Shapiro-Wilk test was performed to test the normality of the distribution of particular organisms' biomass. Prior to the subsequent analyses, ln(y + 1) transformation was used (Jongman et al., 1995). Comparisons of the microbial groups' biomass between treatments (C, W, W + RP, RP) were tested with ANOVA in R

(version 3.5.1) with RStudio (version 1.1.423) (R Core Team, 2018; R StudioTeam, 2018).

The biomass of testate amoeba (TA) species in treatment blocks (C, W, W + RP, RP) was compared using Kruskal-Wallis Rank Sum tests. Comparison of the biomass from before manipulation (2014) with the biomass after the manipulation (2015) was done using the U man-Whitney test for TA, *Hyalosphenia papilio* and *Nebela tinctoria* using the raw data. A co-occurrence network of biomass of the main TA species and the biomass of morpho-ecological groups was built using Spearman's correlation coefficient. The Spearman's correlation coefficient matrix was then used to build the correlation network plot (Package *spaa*, version 0.2.2 by Zhang, 2016).

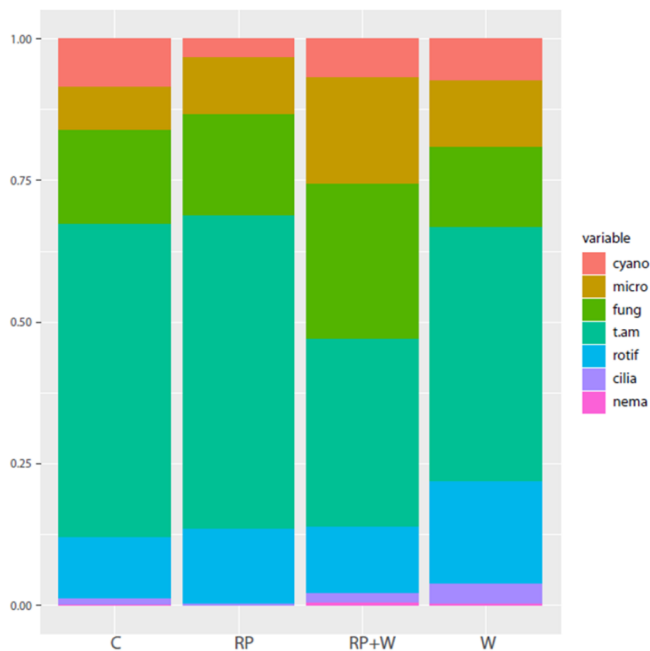
To test the relationships between selected TA species or the biomass of particular microbial groups (dependent variable) and temperature (explanatory variable), linear regression was used. The data analyses, tests, boxplots, correlations and linear regressions were carried out in R (version 3.5.1) with RStudio (version 1.1.423) (R Core Team, 2018; R StudioTeam, 2018).

Furthermore, multivariate redundancy analyses (RDA) were carried out because of the length of the data gradient. All multivariate analyses were performed in Canoco ver. 5 (Lepš and Šmilauer, 2003) and were used to test the effect of treatment and physiochemical factors on groups and species distribution. The statistical significance of the RDA models and explanatory variables were tested using 999 permutations.

### 3. Results

#### 3.1. Climate conditions and effect of climate manipulation on temperatures and precipitation

August 2015 was warmer and wetter than August 2014. Average air temperature was 16.6 °C and 20.7 °C in these months in 2014 and 2015, respectively (Table 1). Although the precipitation total, cumulated from January, reached 342 mm at the end of August 2014, this month was relatively dry with a precipitation total equal only to 12 mm. Contrastingly, the sum of precipitation from January to August 2015 did not exceed 271 mm, even though the monthly sum of precipitation in August was about 31.1 mm. As an effect of manipulation, the air temperature of heated plots was 0.4 °C higher at W and 0.5 °C at W + RP



**Fig. 3.** The proportion of particular groups biomass (cyano – cyanobacteria, micro – microalgae, t.am – testate amoeba, rotif – rotifera, cilia – ciliates, nema – nematodes) in manipulated blocks: warmed - the active warming (W), warmed + reduced precipitation – increased temperature and decreased precipitation with curtain (RP + W), reduced precipitation - only decreased precipitation (RP) and control plots – without manipulation (C) of upper – top *Sphagnum* segment.

sites; whereas peat temperature was 0.2 °C, 1.2 °C and 0.3 °C higher at W, W + RP and RP sites, respectively, in August 2015. The sum of precipitation cumulated for the first half of the year was reduced from around 273 mm to 201 mm (27%) at the manipulated blocks, while, during August 2015, the precipitation was reduced by nearly 99% (from 31 to 0.5) (Table 1).

### 3.2. Microbial groups biomass along manipulation variants

The proportion of groups in the upper *Sphagnum* layer varied in manipulation and control plots (Fig. 3). During August 2015, the contribution of TA in terms of the total microbial biomass in the *Sphagnum* upper layer was the lowest in both warmed and warmed with reduced precipitation blocks (W + RP). In mixed W + RP manipulation plots the contributions of microalgae and fungi biomass were higher in comparison to other variants and control plots (Fig. 3).

Although the total biomass of groups was lower in plots exposed to warming and decreased precipitation (W + D), this difference was not statistically significant ( $F = 2.8$ ;  $p = 0.06$ ). The biomass of particular groups of cyanobacteria, microalgae, testate amoebae, and rotifers showed diverse and different distributions along treatments (Fig. 4). The biomass of microalgae differed significantly between the treatments ( $F = 8.82$ ;  $p < 0.05$ ), with the lowest biomass occurring in control plots (C) and higher in manipulated plots, especially in RP + W (Fig. 4). The rotifer biomass was the lowest in control and warmed blocks, and the highest in plots with reduced precipitation ( $F = 4.31$ ;  $p < 0.05$ ) (Fig. 4). The testate amoeba biomass was higher in W and W + RP ( $F = 3.16$ ;  $p < 0.05$ , Fig. 4). Biomass of fungi, nematodes and ciliates did not differ significantly between the treatments ( $p > 0.05$ ). Biomass of microalgae, fungi and nematodes was higher in lower *Sphagnum* segments in all blocks (Fig. 4). In the upper (top) *Sphagnum* segment, mostly cyanobacteria, testate amoebae and rotifers had higher biomass than in lower *Sphagnum* shoots (Fig. 4).

### 3.3. Testate amoeba biomass

The Rzecin peatland TA community was characterized by a high abundance of mixotrophic species (e.g. *Hyalosphenia papilio*, *Archerella flavum*, *Heleopera sphagni*). At the beginning of the experiment and during the manipulation, TA species biomass revealed significant differences between upper and lower *Sphagnum* segments. The contribution of dominant *Hyalosphenia papilio* in terms of total TA biomass was higher in the upper *Sphagnum* segment, while the biomass of *Hyalosphenia elegans*, *Euglypha compressa*, *Nebela tincta*, *N. collaris* and *Physochila griseola* was higher in the lower segment of *Sphagnum* stems ( $p < 0.05$ ) (Fig. 5).

After the manipulation, *Hyalosphenia papilio* as well as the total biomass of testate amoeba had significantly decreased, especially in warmed (W) ( $p < 0.05$ ) and warmed with reduced precipitation blocks (RP + W) ( $p < 0.05$ ) (Fig. 6). Unlike *Hyalosphenia elegans*, which had increased in lower *Sphagnum* segments in manipulated blocks ( $p < 0.05$ ) (Fig. 6).

The dominant species (*Hyalosphenia papilio*, *Nebela tincta*) and total TA biomass showed significant differences between manipulation treatments in the top *Sphagnum* layer with a significant decrease after manipulation (2014 vs. 2015) in blocks with mixed manipulations RP + W (0.05) (Fig. 7).

### 3.4. Soil ecosystem network

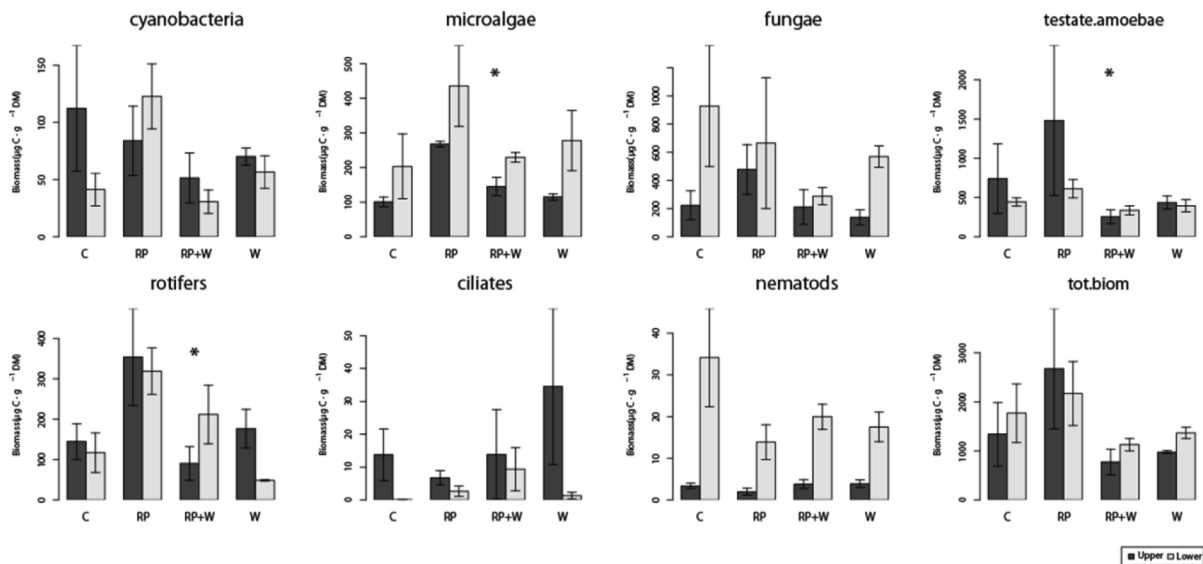
The dominant *Hyalosphenia papilio* biomass showed positive correlations with whole TA and with cyanobacteria biomass (Fig. 8). *Hyalosphenia papilio* biomass was negatively correlated with *Nebela tincta*, *N. collaris*, *Heleopera sylvatica*, *Hyalosphenia elegans*, *Euglypha strigosa*, *Physochila griseola* and fungi biomass. *Nebela tincta*, *N. collaris*, *Heleopera sylvatica*, *Hyalosphenia elegans*, *Euglypha strigosa*, and *Physochila griseola* were positively correlated with each other and fungal biomass. Nematode biomass was positively correlated with microalgae, *Hyalosphenia elegans* and *Physochila griseola*. Ciliate biomass was negatively correlated with *Hyalosphenia elegans* and *Physochila griseola* (Fig. 8).

### 3.5. Communities vs environment

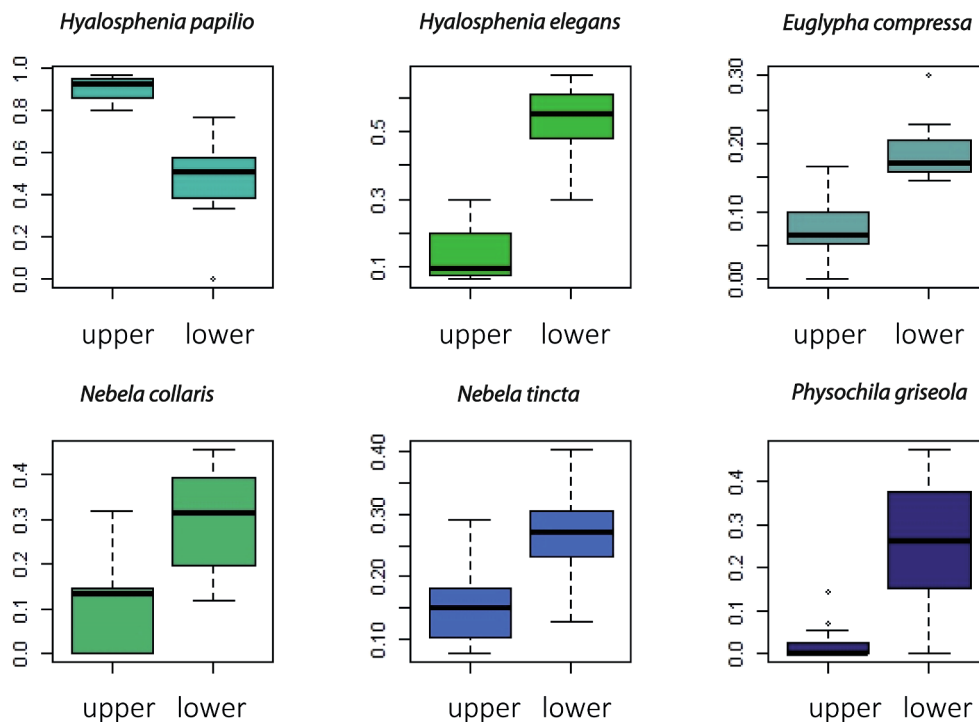
The redundancy analysis (RDA) model of precipitation, peat temperature, pH level, Ca ions concentration and conductivity explained 44.9% of the variance ( $p = 0.005$ ) in microbial groups biomass in the upper *Sphagnum* segment (Fig. 9). Testate amoeba, cyanobacteria as well as total microbial biomass were negatively related to the peat temperature. Nematode and ciliate biomass can be related to higher precipitation, in contrast to rotifers, fungi and microalgae which show a negative correlation with precipitation totals (Fig. 9).

Redundancy analysis (RDA) showed that manipulation affected TA distribution and that it was significantly affected by the treatment, *Sphagnum* segment, water conductivity and Ca concentration (the 44.3% variance was explained by the model at  $p < 0.001$ ) (Fig. 10). The *Hyalosphenia papilio*, *Corythion dubium*, and *Assulina muscorum* biomass increased in the upper *Sphagnum* segment where the water conductivity was higher, and the temperature of the peat was lower. Only one species – *Heleopera rosea* – showed a positive relationship with the temperature and calcium ions concentration. Biomass of much less abundant species, such as *Hyalosphenia elegans*, *Euglypha strigosa*, *Euglypha compressa*, *Heleopera sylvatica*, *Heleopera sphagni*, *Physochila griseola*, *Nebela tincta* and *N. collaris*, was positively correlated with lower *Sphagnum* stems (Fig. 10).

The total biomass of testate amoeba ( $R^2 = 0.23$ ,  $p < 0.05$ ), and biomass of *Hyalosphenia papilio* ( $R^2 = 0.15$ ,  $p < 0.05$ ), cyanobacteria ( $R^2 = 0.4$ ,  $p < 0.05$ ), microalgae ( $R^2 = 0.23$ ,  $p < 0.05$ ), and nematodes ( $R^2 = 0.1$ ,  $p < 0.05$ ) were significantly negatively related to temperature (Fig. 11). A significant positive relationship with peat temperature at 2 cm depth was found in the case of *Archerella flavum*



**Fig. 4.** Biomass of cyanobacteria, microalgae, fungi, testate amoebae, rotifers, ciliates, nematodes and total biomass (tot.biom) in the manipulation blocks: the active warming (W), warmed + reduced precipitation (RP + W), only reduced precipitation (RP) and control plots – without manipulation (C). Presented separately for the top (upper) and deeper *Sphagnum* segment (lower). Significant differences (p value < 0.05) marked with an \*.



**Fig. 5.** The biomass of testate amoeba species: *Hyalosphenia papilio*, *Hyalosphenia elegans*, *Euglypha compressa*, *Nebela collaris*, *Nebela tinctoria* and *Physochila griseola* distribution in upper and lower *Sphagnum* segments. Values given in among of carbon for gram of dry mass of *Sphagnum* ( $\mu\text{g C/g DM}$ ).

( $R^2 = 0.15$ ,  $p < 0.05$ ) (Fig. 11).

#### 4. Discussion

The consequences of recent Earth surface warming and its effects on various ecosystems, especially on carbon stocks such as peatlands, is an urgent issue in the global biology research (Vegas-Vilarrúbia et al., 2012; Amesbury et al., 2017; Binet et al., 2017; Bjorkman et al., 2018; Nolan et al., 2018; Steffen et al., 2018). Climate warming affects not only vegetation but also microbes and other soil organisms that play a key role in peatland functioning (Tsyganov et al., 2011; Jassey et al., 2013a,b; Bragazza et al., 2016). Carbon fixation is driven by

plant–microbe interaction, which is not yet understood (Jassey et al., 2014; Morrien et al., 2017; Gao et al., 2018). Therefore, the understanding of the functioning of peatland microbes under different climate change scenarios (e.g. via field experimental studies) remains a high-priority task (Geisen et al., 2017, 2018). Moreover, finding an ecological indicator of warming and decreased precipitation, which can be used for peatland monitoring needs different experimental approaches supported by long-term ecological studies (Lamentowicz et al., 2016). Previous investigations suggest that the diversity and abundance of microbial predators (e.g. testate amoeba), or even entire food-webs, can be an indication of critical transitions in peatlands (Jassey et al., 2018; Karimi et al., 2017, 2016; Reczuga et al., 2018).

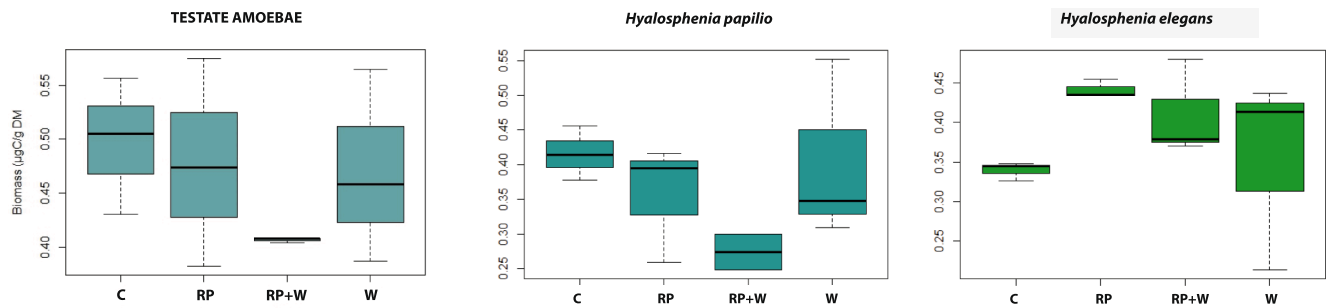


Fig. 6. The biomass of testate amoeba group and species: *Hyalosphenia papilio* in upper *Sphagnum* segment, and *Hyalosphenia elegans*, distribution in lower segment along manipulated blocks: the active warming (W), increased temperature and decreased precipitation with curtain (RP + W), reduced precipitation (RP) and control plots – without manipulation (C). Values given in among of carbon for gram of dry mass of *Sphagnum*.

In our manipulative experiment we observed a considerable shift in the biomass of cyanobacteria, microalgae, testate amoeba and nematodes, most possibly caused by warming. Furthermore, we showed a significant response of mixotrophic testate amoebae, e.g. *Hyalosphenia papilio*, that also negatively reacted to warming. We can state that those phototrophic organisms were dynamically reacting to subtle changes. Based on our results, cyanobacteria and mixotrophic testate amoebae can be regarded as early warning indicators of warming. However, they represent different parts of the food-web – *H. papilio* is the microbial top predator grazing mostly on microalgae, but also on cyanobacteria and ciliates (Jassey et al., 2012; Mulot et al., 2017; Singer et al., 2019). Our results are in agreement with Jassey et al. (2015) that found that five years of consecutive summer passive warming with peaks of +2 to +8 °C led to a 50% reduction in the biomass of the dominant mixotrophs, the mixotrophic testate amoebae (MTA). This reduction of MTA biomass in the field was linked to a significant reduction of net C uptake (–13%) of the entire *Sphagnum* bryosphere (Jassey et al., 2015).

In Rzecin experiment *H. papilio* was the most abundant species in the testate amoeba population inhabiting the surface of *Sphagnum* capitula where the process of carbon fixation takes place. Accumulation of the biomass occurs mainly in the *Sphagnum* capitulum that is responsible for carbon fixation (Bengtsson et al., 2016). In the case of the Rzecin experimental site, hydrology was relatively stable because the setup was constructed on the floating *Sphagnum* mat. Our experimental design with temperature manipulation in stable hydrological conditions suggest that decrease of *H. papilio* as species living on the top of *Sphagnum* may be considered as a ecological indicator of warming in peatlands.

Several paleoecological investigations have also revealed the loss of mixotrophic TA that was triggered by climate change or drainage (Diaconu et al., 2017; Kołaczek et al., 2018). We observed a similar pattern in our warming experiment after one year of the warming/

precipitation reduction treatment – this suggests that the rate of change and sensitivity of mixotrophic amoeba and cyanobacteria to temperature increases is really high and crossing the hydrological tipping point might be easier than we suppose (Lamentowicz et al., 2019). As a consequence of warming, the part of the food-web in the *Sphagnum* capitulum is modified and phototrophs are detached from the network. As an effect of warming, the shift is very subtle in the short term; however, it might have cumulative consequences in the long-term perspective. During the last 200 years, peatlands have been exploited and drained in cooler conditions, and now global warming overlaps with the effects of drought that additionally affect the hydrology and their carbon balance (Swindles et al., 2019). We can expect that future warming will affect microbial communities located in the lower moss parts through the cascading effect connected with the microbial biomass changes in the upper section of *Sphagnum* (Wardle et al., 2002; Valencia et al., 2018). In the deeper peat that was tested for example in the case of the SPRUCE experiment where increasing NDVI with elevated temperature at the SPRUCE site, associated with an increase in the relative abundance of shrubs and a decrease in forb cover was observed (Griffiths et al., 2017; McPartland et al., 2019; Jiang et al., 2018a,b). Additionally the clear abundance shift in cyanobacteria was identified. The top *Sphagnum* part (capitulum) is the most sensitive to the applied treatments (being the most exposed) – therefore, considering the effort required to estimate the biomass of microbes, the top part might be sufficient to identify the treatment effect (Reczuga et al., 2018). Similarly to other studies we found stronger mixotrophic and cyanobacteria decrease in top *Sphagnum* layer. The differences between the response of the upper and lower *Sphagnum* segments to manipulations support the findings of other authors in that vertical distribution of microbial communities should be considered when particular hypotheses are constructed for the experimental research (Mitchell and Gilbert, 2004; Mieczan, 2009; Mieczan, 2010; Jassey et al., 2013; Song et al., 2016).

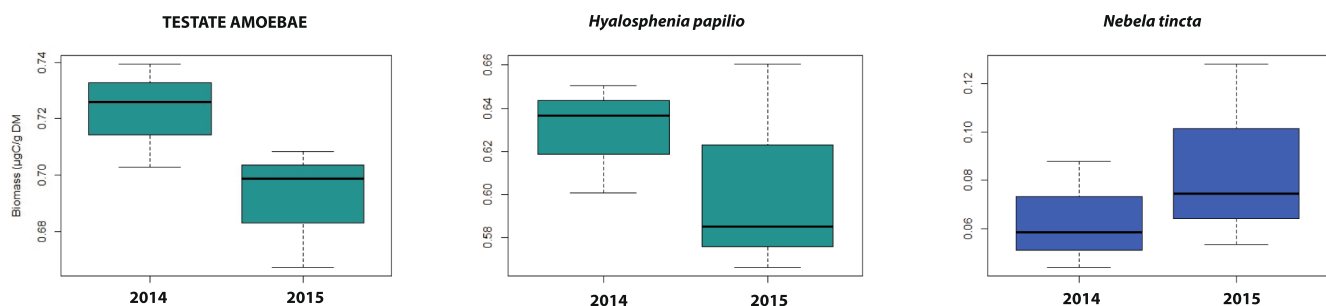


Fig. 7. The biomass of testate amoeba group and particularly *Hyalosphenia papilio* and *Nebela tinctoria* distribution before 2014 and after 2015 manipulation in plots with mixed manipulations RP + W in upper *Sphagnum* segment. Values given in carbon per gram of dry mass of *Sphagnum*.

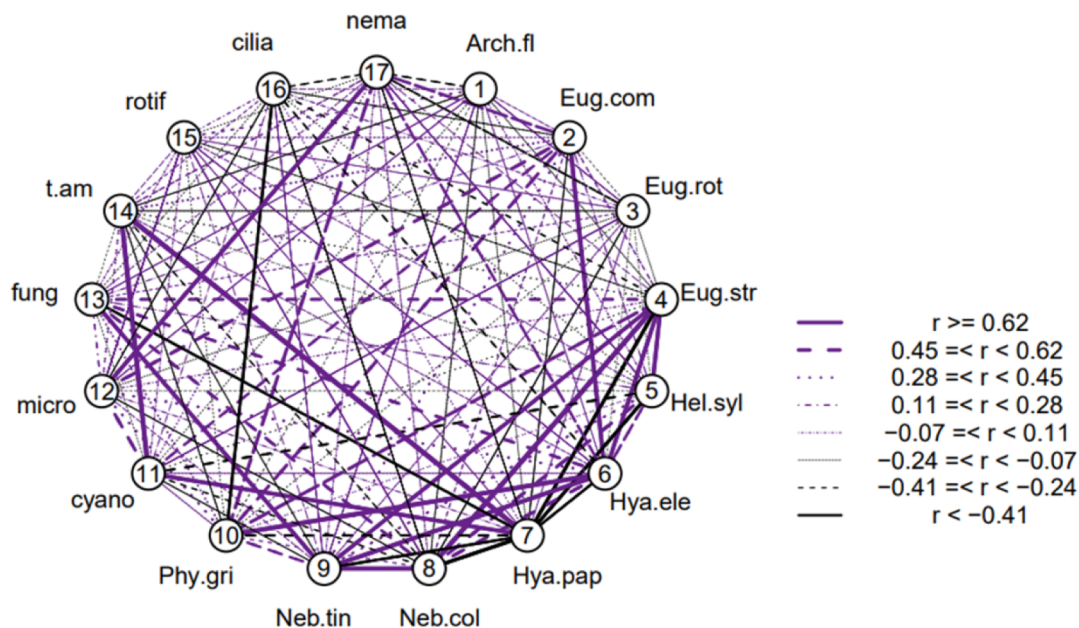


Fig. 8. Microbial network with main testate amoeba species, based on Spearman correlation coefficients. Legend: cyano – cyanobacteria, micro – microalgae, t.am – testate amoebae, rotif – rotifers, cilia – ciliates, nema – nematodes, Arch.fl – *Archerella flavum*, Eug.com – *Euglypa compressa*, Eug.rot – *Euglypha rotunda*, Eug.str – *Euglypha strigosa*, Hel.syl – *Heleopera sylvatica*, Hya.ele – *Hyalosphenia elegans*, Hya.pap – *Hyalosphenia papilio*, Neb.col – *Nebela collaris*, Neb.tin – *Nebela tinctoria*, Phy.gri – *Physochila griseola*.

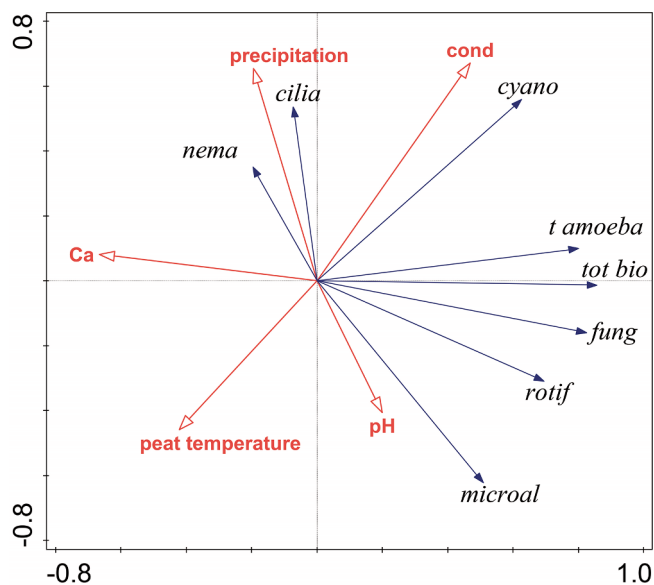


Fig. 9. Redundancy analyses (RDA) of microbial groups biomass (cyano – cyanobacteria, micro – microalgae, t.am – testate amoeba, rotif – rotifera, cilia – ciliates, nema – nematodes) of upper *Sphagnum* segment with peat temperature, precipitations, conductivity (cond), pH level and Ca ions concentration.

Previous studies have shown that hydrological conditions affect peatland communities. These studies examined the natural hydrological gradients (Lamentowicz et al., 2010; Swindles et al., 2014; Amesbury et al., 2016) and experimental changes to water table depth (Marcisz et al., 2014; Reczuga et al., 2018). To our knowledge, none of these studies, however, assessed the effects of experimentally reduced precipitation. Although a decrease in precipitation can negatively affect wetlands and their inhabitants, the rate of the response might depend on their surface area and their main supply of water. Considering the effect of reduced precipitation separately, in our experiment, we did not observe a strong negative influence on the analysed groups. Presumably in a such a *Sphagnum* mat with a moderately high water table level and

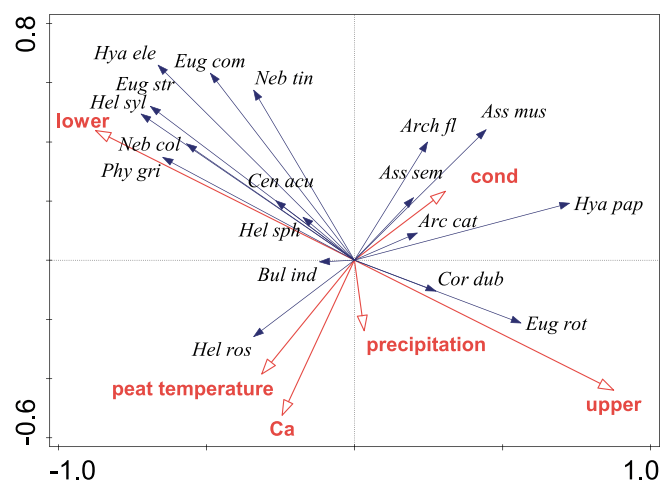


Fig. 10. Redundancy analyses (RDA) of testate amoeba species biomass of upper *Sphagnum* segment along peat temperature, precipitation, conductivity (cond), pH level and calcium ions (Ca) concentration. Legend: Arch.fl – *Archerella flavum*, Ass.mus – *Assulina muscorum*, Ass.sem – *Assulina seminulum*, Arc.cat – *Arcella catinus*, Bul.ind – *Bulinularia indica*, Cen.acu – *Centropyxis aculeata*, Cor.dub – *Corythion dubium*, Eug.com – *Euglypa compressa*, Eug.rot – *Euglypha rotunda*, Eug.str – *Euglypha strigosa*, Hel.sph – *Heleopera sphagni*, Hel.syl – *Heleopera sylvatica*, Hel.ros – *Heleopera rosea*, Hya.ele – *Hyalosphenia elegans*, Hya.pap – *Hyalosphenia papilio*, Neb.col – *Nebela collaris*, Neb.tin – *Nebela tinctoria*, Phy.gri – *Physochila griseola*.

bottom up water supply caused by capillary forces of thick floating carpet, even a 27% reduction of precipitation was not efficient enough to decrease the organism’s biomass, especially in lower *Sphagnum* segments.

In our study, lower segment communities were less affected by manipulations, although the biomass of some groups, such as microalgae and rotifers (mostly represented by bdelloids), increased in RP manipulated blocks. In an experimental study by Reczuga et al. (2018), the lower *Sphagnum* segment microbial communities were also less disturbed by drought. In contrast, the top *Sphagnum* segment is more



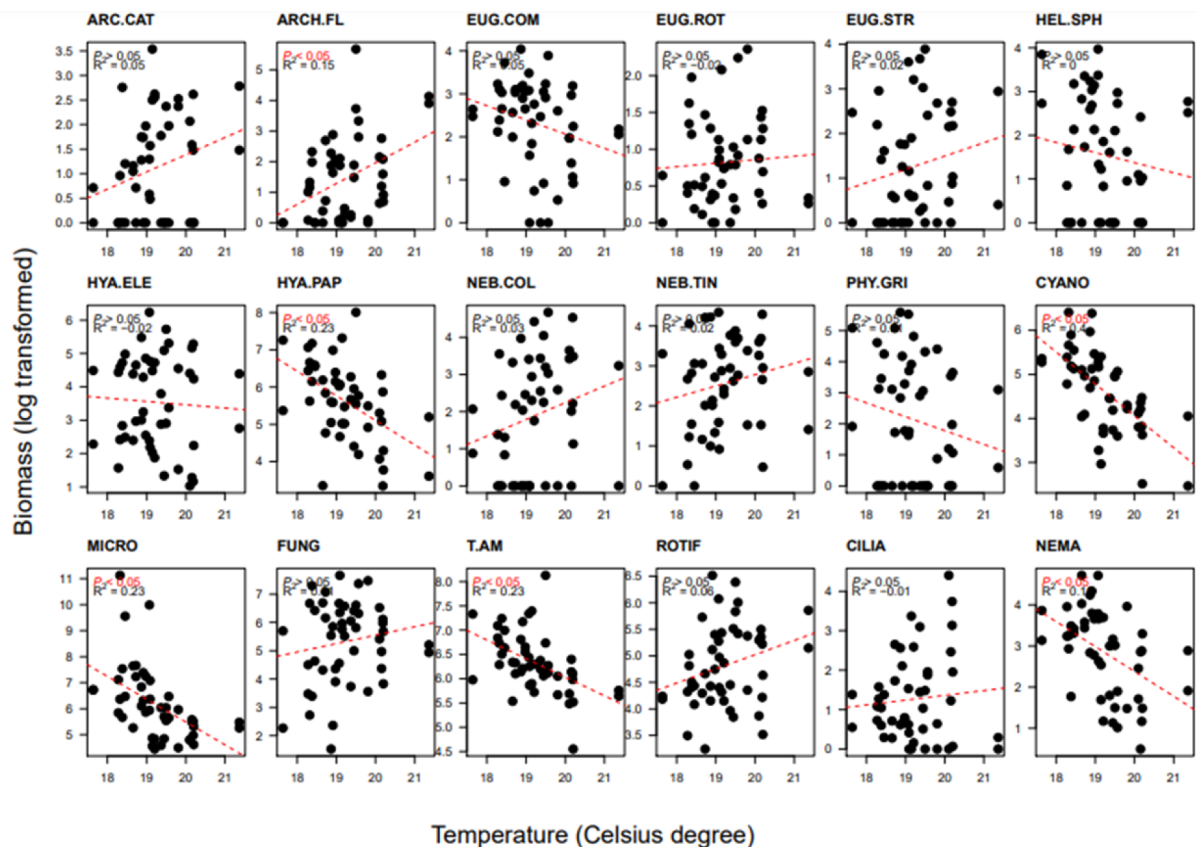


Fig. 11. Linear regression models of testate amoeba (labels – Fig. 10) and other groups biomass (cyano – cyanobacteria, micro – microalgae, t.am – testate amoeba, rotif – rotifera, cilia – ciliates, nema – nematodes) along peat temperature (°C) in experimental site.

disturbed by water table fluctuations, and also it is the preferred habitat for mixotrophs and autotrophs (Mieczan, 2009; Mieczan, 2010; Jassey et al., 2013a,b; Song et al., 2016). Therefore, in the top segment the manipulation could favour drier condition species or very tolerant species of bdelloids and testate amoeba, such as *Nebela tinctoria* that feeds mostly on fungi (Jassey et al., 2012). It is possible that a reduction of precipitation could affect the communities over a longer time scale, especially in a small peatland supplied only by rain water, e.g. ombrotrophic bogs. In the case of our study, the negative influence of reduced precipitation was only observed on ciliates in upper *Sphagnum* segments or in the case of mixed treatments (RP + W).

The testate amoeba community shifted from mixotrophic species such as *Hyalosphenia papilio* and *Archerella flavum* to communities with a higher biomass of species from genus *Nebela*, *Euglypha* and *Physochila*, characteristic for drier peatlands. Potentially in less humid and warmer environment conditions this species winning the competition with previously strong dominants. The microbial network showed links between cyanobacteria and *Hyalosphenia papilio*. A similar relationship has also been recorded in experimental manipulation with warming using open-top chambers (OTC) in peatlands in France (Jassey et al., 2013a,b). A long-term experiment on a blanket bog in a subarctic zone showed that summer passive warming with open top chambers (OTC) in Siberia reduced testate amoeba diversity and abundance (Tsyganov et al., 2012). Our study showed that even subtle warming of the natural peatland reduced microbial biomass, especially in the case of dominant testate amoeba *Hyalosphenia papilio*. Similar results have been presented by observational research (Lamentowicz et al., 2013), OTC experiments (Lamentowicz et al., 2016; Bengtsson et al., 2016) as well as experimental micro- and mesocosm manipulations (Jassey et al., 2011a; Koenig et al., 2017). According to Jassey et al. (2015), mixotrophic testate amoebae such as *Hyalosphenia papilio* play an important functional role as a primary producer and predator and have an influence

not only on the microbial food web, but also on the peatland carbon cycle. The shifts in peat food-web and photosynthetic carbon assimilation as well as heterotrophic respiration may lead to additional climate warming due to release of stored carbon (Jassey et al., 2015).

## 5. Conclusions

Our research is the next step to better understand climate-related critical transitions in *Sphagnum* peatlands. Identifying the tipping points in the biosphere is a topical issue as a result of the progress of recent global warming (Lenton et al., 2008; Scheffer, 2010; Scheffer et al., 2012; Vanacker et al., 2015; Jiang et al., 2018a,b; Moore, 2018; Munson et al., 2018; van der Hel et al., 2018). Therefore, novel experiment-based data are crucial to better predict changes in the soils where microbes play an important role in carbon accumulation.

In the experimental study with the active warming, we found the strongest effect on cyanobacteria and testate amoeba was visible in the case of mixed manipulations of reduced precipitation combined with warming and warming alone. The climate change scenario with higher peat surface temperature affected cyanobacteria and testate amoeba, especially *Hyalosphenia papilio* biomass in the upper *Sphagnum* segment. We can state that the response of phototrophs biomass can be used as an ecological indicator of climate change in peatlands. Additionally an decrease of significant primary producers and mixotrophs in microbial loop as the consequence of global warming can affects the carbon accumulation potential of *Sphagnum* peatlands.

## 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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