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Effects of Fires on Microbial and Metazoan Communities in Peatlands

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Abstract: The increase in the frequency of peatland fires is due to both global climate change and deliberate human activity. The primary aim of the study was to investigate the structure of microbial communities and nematodes before and after a peat bog fire, as well as to analyse the relationships between food web components. Hydrological and physicochemical parameters were analysed during the period before the fire (2018 and 2019) and after the fire (2020 and 2021—2, 4, 8, and 12 months after the fire). The fire clearly modified the physicochemical properties of the peat bog, increasing the temperature, pH, conductivity, and concentrations of biogenic compounds and organic matter. It also caused a pronounced deterioration in oxygen conditions. The fire clearly modified the qualitative and quantitative structure and functioning of microbial food webs. This was reflected in a decrease in the species number and abundance of testate amoebae, with various groups of microbes showing pronounced fluctuations during the study period. The functioning of food webs in peatlands after fires is still very little understood, although an understanding of the functioning of these habitats, which increasingly undergo this type of catastrophe due to global climate change, is crucial.

Keywords: climate changes; warming; microbial loop; bacteria; protozoa; nematodes

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1. Introduction

Recent decades have seen an increasing frequency of fires in various types of ecosystems, including peatlands [1]. In some parts of the world (such as the western United States), their frequency has increased nearly fourfold in the last 20 years [2]. This increase is due both to climate change (gradually rising temperatures, deterioration of hydrological conditions, and changes in land use) and deliberate human activity and burning of certain areas, particularly agricultural areas [3,4]. This phenomenon has significant short-term and long-term effects on the functioning of individual ecosystems, mainly through changes in the taxonomic composition and abundance of vegetation and microbial communities [5,6]. As a consequence, fires affect the dynamics of microbiological processes, especially the carbon cycle, primarily reducing the accumulation of carbon and contributing to the release of substantial quantities of CO_2 into the atmosphere [7,8]. However, very little is known of the effect of fires on the functioning of microbial communities on a longer time scale. Thus far, most studies have described communities of organisms during the period of a few months after the fire [8–13]. These studies have mainly concerned bacteria or fungi in forest or peatland ecosystems, while there is nearly a complete lack of studies on protozoa (testate amoebae and ciliates) and nematodes and their responses to peatland fires. In peatland ecosystems, bacteria are the main decomposers, which can contribute to greenhouse gases emission. Testate amoebae, ciliates, and nematodes are significant consumers of algae, bacteria, and flagellates, and they participate in the decomposition of organic matter and the cycling of nutrients [12]. Knowledge of fires on microbial and metazoan communities and trophic relationships is important in predicting the response of biocoenoses to the increasingly dynamic phenomena resulting from global climate change. Previous research on long-term variation in testate amoebae in connection with fires has been carried out using a peat profile from the Linje nature reserve in western Poland. However, this was a paleo-ecological study and focused on reconstructing the hydrological changes and fires that took place over the last 2000 years. Fires have been shown to cause changes in the structure of communities of testate amoebae, manifested as an increased number of xerophilic species [12]. The effects of environmental disturbances caused by fires on microbial communities can be positive, negative, or neutral. This largely depends on the species specificity of these communities [13]. Research by Hart et al. [14] and Certini [15] indicates that fires can affect groups of soil organisms, e.g., by changing the environmental parameters of the soil (pH, concentrations of biogenic compounds, pools of organic matter, or temperature). In addition, they decrease the biomass of plants and can indirectly affect communities of soil microorganisms [16]. Lauber et al. [17] showed that fires affect soil pH and reduce the abundance of fungi. Therefore, it seems that microbial communities can be a good bioindicator of changes in the environment and can provide information on how peatland ecosystems will respond to such disturbances in the future. Microbial communities play an important role in carbon and nitrogen cycle processes, in part through decomposition [18]. These communities are subject to the direct effects of fires through habitat disturbances [15], as well as their indirect effects through changes in physical, chemical, and biological habitat parameters. These changes, especially losses of organic matter and declines in its quality, changes in the composition of vegetation, or changes in albedo, have consequences for peatland organisms [19]. Meta-analyses by Pressler et al. [20] also indicate that fires most often reduce the abundance of fungi, bacteria, and arthropods, and also adversely affect their species richness. According to Barreiro et al. [21], microbes and fungi taking part in the C cycle are more sensitive to changes caused by fires than bacteria and microbes taking part in the N cycle. Qin and Liu [22] showed that the species richness and abundance of bacteria and fungi markedly decrease six months after a fire, with fungi showing greater sensitivity to fires than bacteria. Contrasting data are presented by Brown et al. [23], who indicate that fires have a more pronounced effect on bacteria during the first year after a fire. However, there is still a lack of information on the response of protozoan communities to peatland fires, especially the role of these organisms in the functioning of the food web.

The objectives of this study were to (1) compare the structure of bacteria, flagellates, testate amoebae, ciliates, and nematodes before and after a peat bog fire, (2) analyse interactions between food web components before and after the fire, and (3) assess the effect of physical and chemical factors on the microbial community and nematodes before and after the fire.

2. Materials and Method

2.1. Study Site

The study on the effects of a fire on the functioning of microbial communities (bacteria, heterotrophic flagellates, ciliates, and testate amoebae) and nematodes was conducted in a transitional peat bog located by Lake Piaseczno (Łęczna-Włodawa Lakeland, eastern Poland, 51° N, 23° E) (Figure 1). The peat bog was partially degraded in the 1960s and 1970s by intensive drainage, which lowered the water level and led to the mineralization of the peat. In April 2020, there was a fire on the bog, which covered nearly the entire area of the ecosystem, causing a total restructuring of the vegetation. Before the fire the bog was dominated by *Sphagnum* and brown mosses, and *Phragmites australis* and *Drosera* sp. grew there as well. After the fire this vegetation was destroyed, and after approximately 12 months, birch trees and isolated clumps of sedges began to appear.

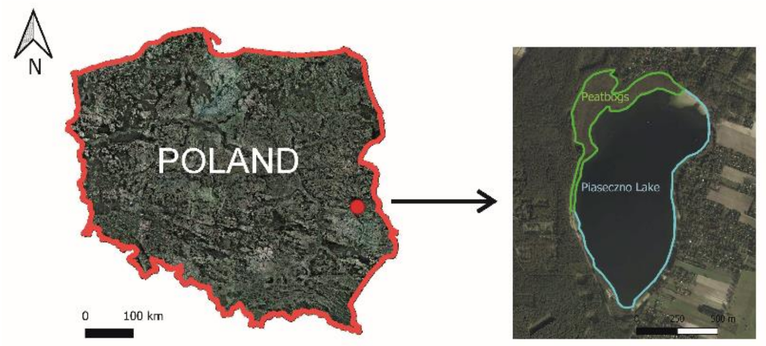


Figure 1. Location of the study area.

2.2. Abiotic Variables

Analyses of hydrological (surface water level) and physical and chemical factors were carried out in the years 2018–2021—once a month from April to November before the fire (2018–2019) and subsequently 2, 4, 8, and 12 months after the fire, which took place in April of 2020. Samples were collected from two study sites: The edge zone of the bog and the central zone. From April to November, at each site, the following were analysed according to Golterman [24]: Temperature, conductivity, pH, dissolved oxygen (DO), chlorophyll *a*, P_{tot}, N_{tot}, N-NH_4, N-NO_3, P-PO_4^{3-}, and total organic carbon (TOC). Temperature, conductivity, pH, and DO were assessed with a multiparameter probe (YSI 556 MPS). P-PO_4^{3-} was determined by the colorimetric method, N-NH_4 with an FIA analyser, P_{tot} by the colorimetric method, and N_{tot} by Kjeldahl's method. Total organic carbon (TOC) was analysed using the O/I Corporation Model 700 TOC analyser.

2.3. Microbial Communities and Nematodes

Microbial communities (bacteria, flagellates, ciliates, and testate amoebae) and nematodes were analysed for four years—once a month from April to November before the fire (2018–2019) and subsequently 2, 4, 8, and 12 months after the fire. Samples were taken from two study sites: The edge zone of the bog and the central zone. In each month (April–November), from each site, three samples of surface water were taken with a plexiglass corer (length 1.0 m, Ø50 mm). The size of each sample (the amount collected by the corer) was 400–500 mL. For each group of micro- and macro-organisms in each of the study months, three replicate samples were mixed together, and the integrated sample was treated as representative of the site. The biomass and abundance of bacteria were determined using the DAPI (4′6-diamidino-2-phenylindole) reagent, according to Porter and Feig [25]. For quantitative analysis of bacteria, 10 mL of water was filtered using polycarbonate filters dyed with Irgalan black (pore diameter 0.2 μm). The biomass and abundance of heterotrophic flagellates (HF) were determined according to Caron [26]. For quantitative analysis of flagellates, 10 mL of water was filtered using polycarbonate filters (0.8 μm pore size). The abundance of protozoa (testate amoebae and ciliates) and nematodes was determined by Utermöhl's method [27]. Samples for analysis of these organisms (3 samples; whole sample = 500 mL) were sedimented for 24 h in a cylinder, 400 mL of water was poured off, and 100 mL of water together with the sedimented organisms were analysed in plankton chambers using an inverted microscope at 600 × magnification. The biomasses of the analysed groups of organisms were determined by converting their biovolume to carbon using the following conversion factors: For heterotrophic bacteria, $1\ \mu\text{m}^3 = 5.6 \times 10^{-7}\ \mu\text{gC}$; for flagellates, $1\ \mu\text{m}^3 = 2.2 \times 10^{-7}\ \mu\text{gC}$; and for ciliates, testate amoebae, and nematodes, $1\ \mu\text{m}^3 = 1.1 \times 10^{-7}\ \mu\text{gC}$ [28]. Taxonomic identification of the investigated group of organisms was based on works by Porter and Feig [25], Caron [26], Foissner et al. [29], and Clarke [30].

2.4. Data Analyses

Basic descriptive statistics were calculated for both the abundance and biomass of the analysed groups of organisms at each sampling time. The results were presented in bar plots. The exact Poisson test was used to compare the average abundance of organisms at different times, assuming that the abundances are subject to the Poisson distribution. Biomasses of organisms were compared using the non-parametric Kruskal–Wallis test with post-hoc pairwise comparisons using the Wilcoxon rank sum test. For both non-parametric and parametric analyses, the assumed significance level was 0.05. Correlations between the abundance of individual groups of organisms and the physicochemical parameters of the water before and after the fire were analysed, and the results were presented in correlograms. In the next step of the statistical analysis, multivariate methods were used. Ward's algorithm of minimum variance was used for clustering, and the Bray–Curtis index was used as a similarity measure. The results of the classification were presented graphically in the form of a combination of dendrograms and heatmaps. Interdependencies between species composition and physicochemical factors were analysed by direct ordination, using redundancy analysis (RDA). The results were presented on ordination triplots. All statistical analyses were performed in the open-source software R, version 4.1.0 [31], using the following packages: corrplot (ver. 0.9), ggdendro (ver. 0.1.22), ggplot2 (ver. 3.3.5), MASS (ver. 7.3-54), stats (ver. 4.1.0), and vegan (ver. 2.5-7).

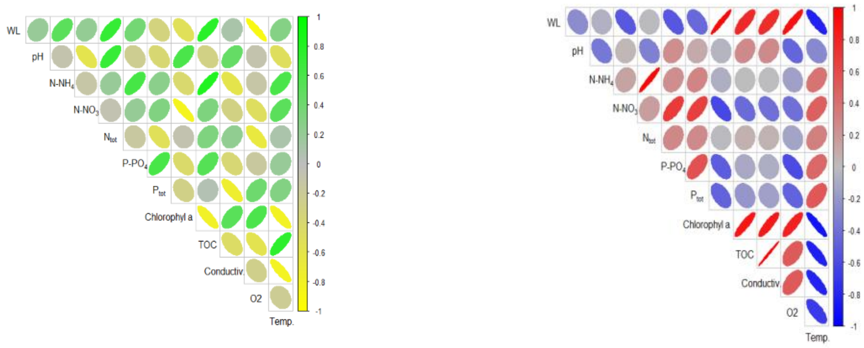
3. Results

3.1. Abiotic Variables

The average water level in the *Sphagnum* hollows before the fire was 7 cm, while after the fire, it did not exceed 6 cm. This level persisted for two more years. Statistically significant differences between years (before and after the fire) were shown for temperature, pH, conductivity, P_{tot}, N_{tot}, and TOC (ANOVA, $F_{1 \cdot 33} = 62.11–66.21$, $p < 0.001$). Values for pH, conductivity, biogenic compounds, and TOC were significantly higher after the fire. On the other hand, after the fire, there was a decrease in oxygen content, with the lowest values recorded 4 months after the fire (Table 1). Before the fire, the strongest correlations were between oxygen concentrations and the water level and between TOC and NH_4, while after the fire, there was an increase in the strength of the correlations between the water level and concentrations of chlorophyll *a*, TOC, and biogenic compounds (Figure 2a,b).

Table 1. Changes in physical and chemical parameters. Key: Water level (WL), temperature (Temp.), conductivity (Cond.), reaction (pH), dissolved oxygen (O_2), total phosphorus (Ptot), phosphates ($P\text{-}PO_4{}^{3-}$), total nitrogen (N_{tot}), ammonia nitrogen ($N\text{-}NH_4$), nitrite nitrogen ($N\text{-}NO_3{}^{-}$), and total organic carbon (TOC).

Parameters		**Before Fire**		**After Fire**			
		2018	**2019**	**2020–2 Months**	**2020–4 Months**	**2020–8 Months**	**2021–12 Months**
WL	(cm)	7	7	1	1	3	5
Temp.	(°C)	15	16	23	21	16	16
Cond.	($\mu S\ cm^{-1}$)	87	79	90	138	160	190
O_2	($mg\ O_2\ L^{-1}$)	8	8	3	2	3	8
pH		4	4.3	6	7.6	8.5	6
$N\text{-}NH_4$	($mg\ NH_4\ L^{-1}$)	0.124	0.132	0.221	0.734	0.124	0.264
$N\text{-}NO_3$	($mg\ NO_3\ L^{-1}$)	0.193	0.194	0.312	0.470	0.207	0.223
N_{tot}	($mg\ N\ L^{-1}$)	1.567	1.884	1.676	1.852	1.567	1.631
$P\text{-}PO_4$	($mg\ PO_4{}^{3-}\ L^{-1}$)	0.078	0.021	0.039	0.112	0.033	0.033
Ptot	($mg\ P\ L^{-1}$)	0.137	0.136	0.237	0.341	0.211	0.235
Chlorophyll a	($mg\ L^{-1}$)	11.00	11.00	1.00	2.00	5.00	8.00
TOC	($mg\ C\ L^{-1}$)	15.0	15.0	21.0	46.0	58.0	65.0



(**a**) (**b**)

Figure 2. Correlogram between all related physical and chemical parameters before (**a**) and (**b**) after fire. The ellipse angulation and the colour intensity are proportional to the Pearson correlation coefficient: Positive correlations are drawn in green/red whereas the negative correlations are in yellow/blue.

3.2. Microbial Communities

Poisson's test was used to compare the average abundances of organisms at individual sampling times. The analysis indicated statistically significant differences for some sampling times. The results of the comparisons are shown on a bar plot (Figures 3a–e and 4a–e), on which different letters above the bars indicate statistical differences. Comparison of the distribution of biomass at different times was based on non-parametric tests. The results indicated that the distribution of biomass before and after the fire differed significantly. Only in the case of nematodes was there no significant difference in the distribution of biomass between sampling times. An exception was the period 2 months after the fire, when the distribution was significantly different from all the others.

Significantly higher abundance and biomass of bacteria were recorded before the fire ($7.5 \pm 2.2 \times 10^6$ cells mL^{-1} and 0.93 ± 0.36 µg C mL^{-1}, respectively). After the fire, there was a sharp decline in the abundance and biomass of bacteria to $0.5 \pm 0.01 \times 10^6$ cells mL^{-1} and 0.13 ± 0.002 µg C mL^{-1}. A significant increase in the abundance and biomass of bacteria was noted 8 months after the fire, reaching $5.5 \pm 2.1 \times 10^6$ cells mL^{-1} and 0.53 ± 0.20 µg C mL^{-1}, respectively, after which bacterial abundance and biomass remained at a similar level. The abundance and biomass of heterotrophic flagellates were also markedly higher before the peat bog fire ($2.8 \pm 1.1 \times 10^3$ cells mL^{-1} and 0.03 ± 0.01 µg C mL^{-1}). The lowest abundance and biomass of heterotrophic flagellates were recorded just after the fire and during the first 4 months after it ($1.8 \pm 0.8 \times 10^3$ cells mL^{-1} and 0.02 ± 0.01 µg C mL^{-1}). In total, 24 taxa of testate amoebae were recorded in the study area; there were 22 taxa before the fire, but no living forms of testate amoebae immediately after the fire. Testate amoebae—only four species—were observed 4 months after the fire. Species similarity was highest before the fire and again 8 months after the fire. In the community of ciliates, 18 taxa were recorded during the entire study period: 18 before the fire and 6 after the fire. In the case of ciliates, analysis of species similarity indicated the highest taxonomic similarity before the fire and 4 and 8 months after the fire (Figure 5a). The dominant amoeba taxa before the fire were *Hyalosphenia papilio* and *Arcella discoides*. After the fire, *Assulina muscorum* and *Cryptodifflugia oviformis* had the highest share in the total abundance of amoebae. After the fire, the abundance and biomass of testate amoebae decreased significantly: 4.1–4.2 ± 1.1–1.2 × 10^2 cells mL^{-1} and 0.91–1.1 ± 0.1–0.2 µg C mL^{-1} before the fire and $2.1 \pm 0.2 \times 10^2$ cells mL^{-1} and 0.51 ± 0.1–0.2 µg C mL^{-1} after the fire. Ciliate abundance and biomass showed marked fluctuations in different years. Before the fire, the abundance and biomass of ciliates in the peat bog averaged 55 ± 11 cells mL^{-1} and $>0.6 \pm 0.2$ µgC mL^{-1}, while just after the fire, there was a marked decrease in their abundance and biomass, to 6 ± 2 cells mL^{-1} and 0.1 ± 0.05 µgC mL^{-1}, and 12 months after the fire the corresponding values were 85 ± 11 cells mL^{-1} and $>0.9 \pm 0.3$ µgC mL^{-1}.

The dominant structure of ciliates was very different before and after the fire. In the years 2018–2019, scuticociliates and peritrichids had the highest share in the total abundance of ciliates, whereas after the fire, *Paramecium putrinum* and *Cyaenomorpha* spp. were dominant (Figure 5b).

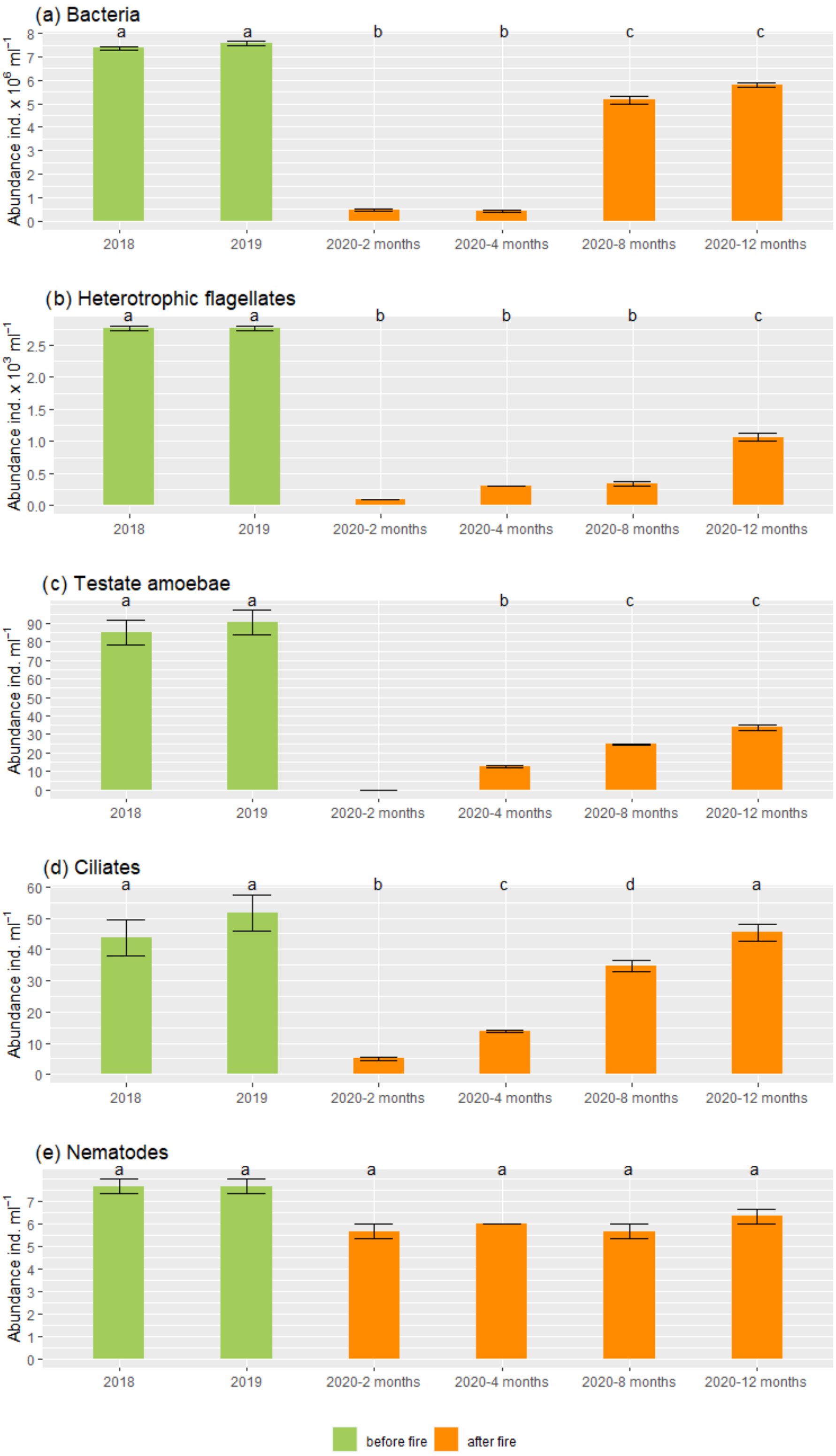


Figure 3. Barplot with error bars for abundance of (**a**–**e**) before and after fire. Different letters above the bars indicate statistically significant difference.

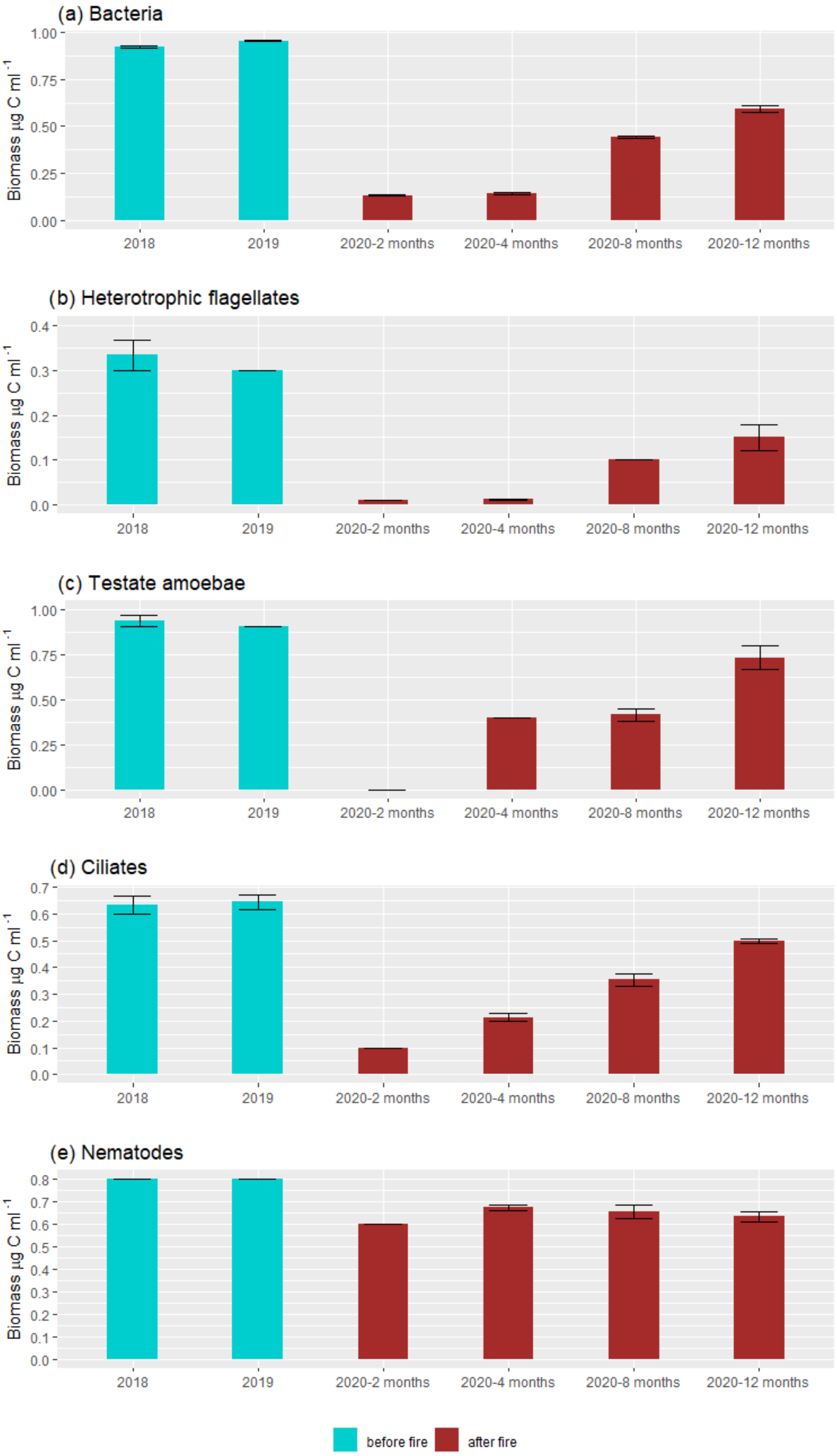
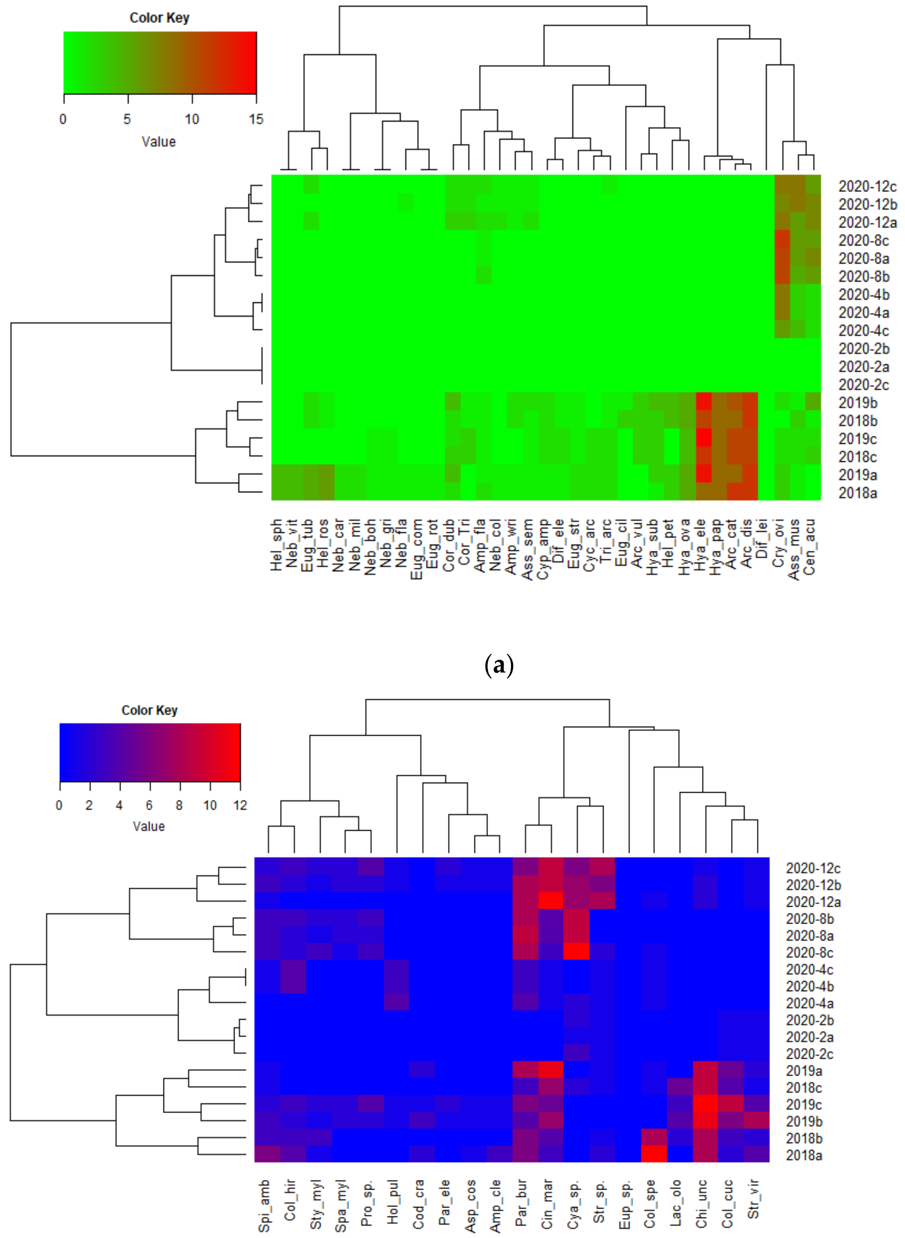


Figure 4. Barplot with error bars for biomass of (**a**–**e**) before and after fire.



(**a**)

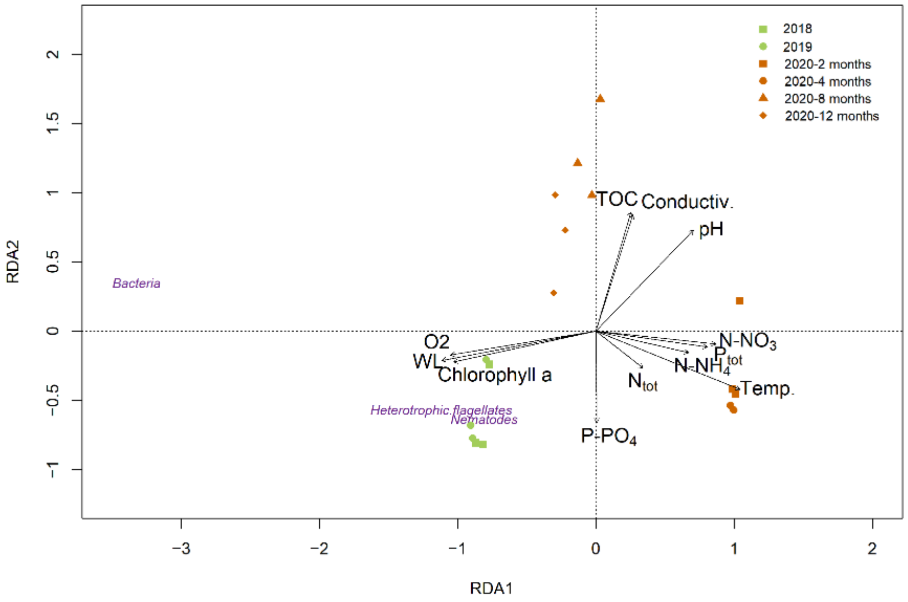
(**b**)

Figure 5. Dendrogram and heatmap of hierarchical cluster analysis of species composition of (**a**) testate amoebae and (**b**) ciliates based on Brey's coefficient and Ward method. The letters a, b, c (right edge of the graph) indicate the number of the measurement during the period.

The abundance and biomass of nematodes showed no significant differences before and after the fire. Before the fire the average values were 8 ± 4 ind ml^{-1} and >0.8 ± 0.2 µgC mL^{-1}. Just after the fire, there was a slight decrease in the abundance and biomass of these organisms (to 6 ± 0.3 ind mL^{-1} and 0.6 ± 0.05 µgC mL^{-1}), but after 4 months, the abundance and biomass of nematodes reached values similar to those from before the fire.

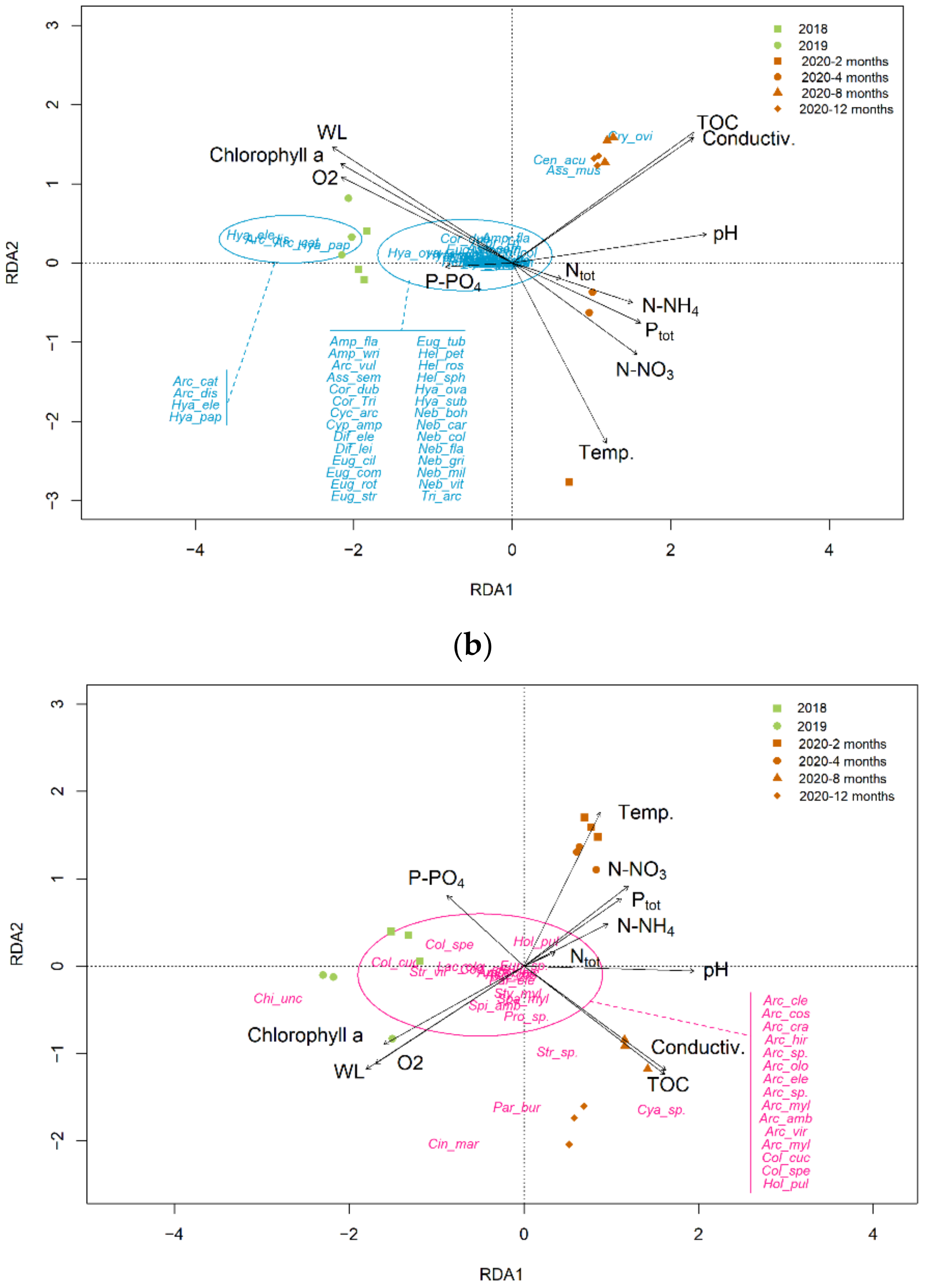
3.3. Redundancy Analysis (RDA) and Correlations

Redundancy analysis was carried out to determine the direct relationships between the abundance of individual groups of organisms and the physicochemical properties of the habitat before and after the fire. In the case of bacteria, flagellates, and nematodes, all environmental variables together explain 98.62% of the variation. The first constrained axis (RDA1) explains 92.2% of the variance, while the second (RDA2) explains 6.12%. The communities of these organisms before the fire showed significant relationships with the water level, chlorophyll *a*, and TOC. After the fire, the influence of temperature and concentrations of biogenic compounds increased (Figure 6a). For communities of testate amoebae, the first and second constrained axes (RDA1 and RDA2) explain 75.67% and 11.32% of the variation, respectively, while the first and second unconstrained axes (PC1 and PC2) represent 3.45% and 1.19% of the variation, respectively. Before the fire, the occurrence of testate amoebae was most influenced by the water level, chlorophyll *a*, and O_2, while after the fire, the influence of organic matter increased (Figure 6b). On the RDA biplot, the presence of *Assulina muscorum* and *Cryptodifflugia oviformis* shows significant relationships with TOC and conductivity, whereas the water level, chlorophyll *a*, and concentrations of PO_4^{3-} mainly determined the occurrence of *Hyalosphenia papilio* and *Arcella discoides*. The environmental variables included in the environment explain 92.64% of the variation in the ciliate community composition across sampling times. The first two RDA axes accounted for 66.6% of the explained variation (RDA1 = 38.86%; RDA2 = 27.74%). The first two unconstrained axes explained 3.13% (PC1) and 2.19% (PC2), respectively. Before the fire, the water level, chlorophyll *a*, and O_2 had the greatest influence on the occurrence of ciliates (mainly *Chilodonella uncinate* and *Colpoda cucculus*), while after the fire, the influence of the concentration of biogenic compounds, temperature, and TOC increased. These parameters influenced the occurrence of taxa such as *Cyanomorpha* spp., *Stylonychia mytilus*, and *Paramecium bursaria* (Figure 6c).



(a)

Figure 6. *Cont.*

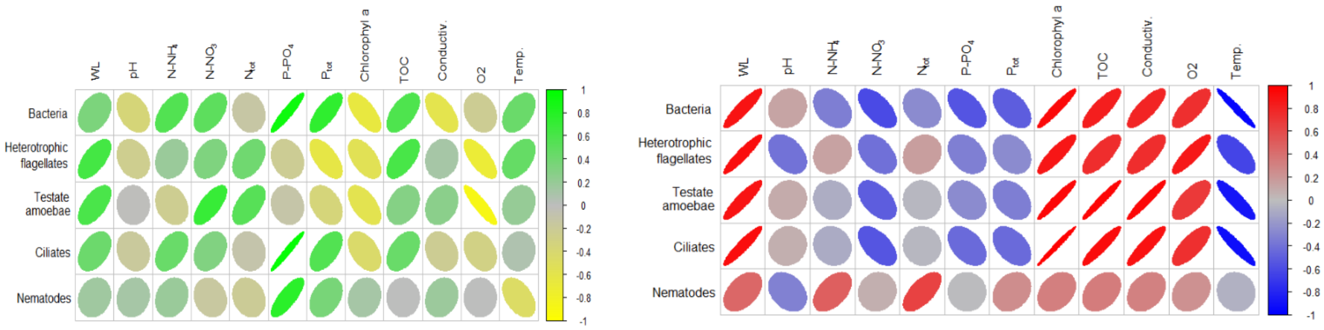


(**b**)

(**c**)

Figure 6. Redundancy analysis (RDA) triplots showing correlations between the studied organisms and environmental variables in investigated peatbog; (**a**) bacteria, heterotrophic flagellates, nematodes; (**b**) testate amoebae; (**c**) ciliates.

Correlations between individual groups of organisms and the physicochemical properties of the water were very different before and after the fire. Before the fire, the strongest positive correlations were noted between bacteria and ciliates and the phosphate concentration in the water (r = 0.92–0.97). A negative correlation was noted between oxygen concentrations in the water and the abundance of testate amoebae. After the fire, the strongest positive correlations were noted between the abundance of bacteria, flagellates, testate amoebae, and ciliates and concentrations of chlorophyll *a*, oxygen, TOC, and electrical conductivity (r = 0.75–0.95). At the same time, there was a negative correlation between these groups of organisms and the water temperature (from r = −0.64 to r = −0.96) (Figure 7a,b).

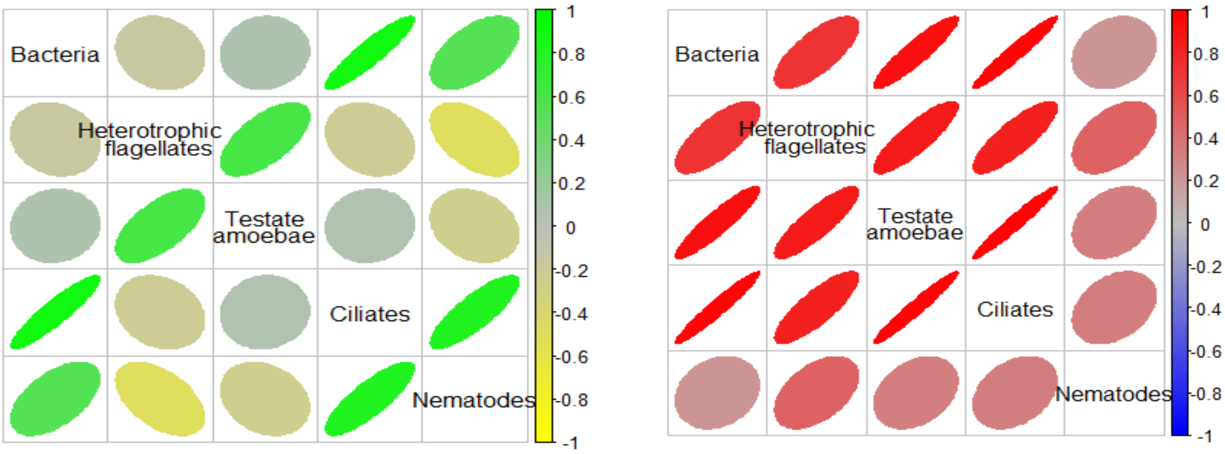


(**a**) (**b**)

Figure 7. Correlogram between all related physical and chemical parameters and microbial and nematode communities before and (**a**,**b**) after fire. The ellipse angulation and the colour intensity are proportional to the Pearson correlation coefficient: Positive correlations are drawn in green/red whereas the negative correlations are in yellow/blue.

3.4. Relationships between Food Web Components in the Peat Bog

In general, the numbers of correlations between trophic groups of organisms before and after the fire were very different. Before the fire, the strongest positive correlations were noted between ciliates and bacteria (r = 0.91) and between nematodes and ciliates (r = 0.81). After the fire, the number and strength of these correlations increased. The strongest positive correlation was observed between bacteria and protozoa, i.e., testate amoebae, and ciliates (r = 0.91–0.96), and a somewhat weaker correlation between heterotrophic flagellates and bacteria (r = 0.71). There were no significant relationships between nematodes and the other analysed groups of organisms (Figure 8a,b).



(**a**) (**b**)

Figure 8. Correlogram between microbial and nematode communities before and (**a**,**b**) after fire. The ellipse angulation and the colour intensity are proportional to the Pearson correlation coefficient: Positive correlations are drawn in green/red whereas the negative correlations are in yellow/blue.

4. Discussion

4.1. Effects of Fire on Environmental Factors

The peat bog fire caused significant changes in the physicochemical properties of the peat bog water. Immediately after the fire (2 to 6 months), there was a marked increase in the concentration of total organic carbon and biogenic compounds. This may indicate significant destabilization of habitat conditions resulting from a large amount of decomposing plant biomass as well as decomposition of various taxonomic groups of micro- and macro-organisms sensitive to high temperatures. A similar phenomenon has been observed following forest fires [16]. At the same time, there was a marked increase in pH from acidic to strongly alkaline, as well as an increase in water mineralization and a deterioration of oxygen conditions. The low oxygen concentrations are likely due to its consumption in the decomposition of the large amounts of organic matter arising after the fire. At the same time, there was an increase in water temperature relative to the period before the fire, which may also have negatively affected oxygen conditions. This temperature increase in the peat bog water was additionally caused by the nearly complete destruction of the vegetation and exposure to sunlight. A gradual stabilization of habitat conditions in the peat bog was observed eight months after the fire, when there was a decrease in the concentrations of biogenic compounds and water mineralization. The increase in the concentrations of biogenic compounds may be not only the effect of fires but also the influence of the immediate catchment area. Their source (especially phosphates) was most likely the waters of the catchment dominated by agriculture and tourism infrastructure, which can feed the peat bog. Kruk [32] reported similar observations in a raised bog in the Masurian Lake District.

4.2. Effects of Fire on Micro- and Macroorganisms

The analysed communities of organisms showed distinctly varied responses to the fire. The abundance of microorganisms declined dramatically immediately after the fire. This may have been due not only to the effect of the high temperature on peat bog microorganisms, but also to changes in the composition of organic matter [33] and/or an increase in the number of substances toxic to microorganisms [34]. Then, after eight months, there was a sudden increase in the abundance of bacteria, which was likely linked to changes in the temperature and C:N:P ratio. Similar observations were reported by Barreino and Diaz-Raviña [21]. Widden and Parkinson [34] found that bacteria diversity was closely related to the N:P and C:P ratios. These results suggest that bacteria communities may be limited by the imbalances of C, N, and especially P in afforested ecosystems, which provides evidence of linkages between bacteria, plants, and soil. At the same time, four months after the fire, there was only a slight increase in the abundance of bacteria; this may be not only the direct consequence of the fire but also the result of increased predation pressure—mainly the increase in the abundance of flagellates and ciliates during this period. In the first two months after the fire, testate amoebae showed the greatest decline in abundance. The results of our study correspond with the research of other authors, mainly the significant relationships between communities of protozoa and concentrations of nitrogen compounds, pH, and water level [10]. The high tolerance of bacteria for the increasing pH after the fire in comparison with protozoa may explain the decline in the abundance of protozoa. At the same time, a decrease in plant biomass after a fire may reduce soil water uptake and evapotranspiration, thereby increasing soil moisture [35]. In time, the decrease in the abundance of vegetation may increase the exposure of the substrate to sunlight, adversely affecting hydrological conditions [36]. Our study is the first to investigate the response of heterotrophic flagellates to peat bog fires. This group had markedly higher numbers before the fire, showing a marked decline after the fire. It seems that this decrease is due not only to the higher temperature, but also to the increase in the concentrations of biogenic compounds. Mieczan et al. [37] showed that the abundance of flagellates decreases as concentrations of biogenic compounds increase in peatland ecosystems. Riemann [38] showed significant correlations between the concentrations of biogenic compounds and the abundance of heterotrophic flagellates; however, that study was conducted in lake ecosystems. At the same time, our study showed an increase in the abundance of ciliates, which may have controlled the abundance of flagellates; research by Mieczan et al. [37] showed that it is mainly ciliates that are able to control flagellate abundance. It is likely that the decrease in the water level and the increase in pH and concentrations of biogenic compounds were responsible for the change in the dominance structure of protozoa—mainly the decline in the abundance of testate amoebae and the increase in the abundance of ciliates. Similar relationships were observed in peat bog ecosystems subjected to simulated eutrophication [37]. After the fire, there was also a restructuring of the taxonomic composition of testate amoebae, with a marked increase in the proportions of *Assulina muscorum* and *Cryptodifflugia oviformis*. It seems that these species may be good bioindicators of changes taking place after fires. A paleoecological study carried out by Marcisz et al. [12] showed that they were more frequent in dry habitats where fires had taken place. These species are relatively undemanding in terms of habitat requirements and are able to survive conditions of long-term drought [39]. Research by Turner and Swindles [40] suggests that *Hyalosphenia subflava* and *Trigonopyxis arcula* may be indicators of changes in peatlands resulting from fires. However, this was not the case in our study. The number of species decreased as pH increased and the water level decreased. According to Payne and Mitchell [41], the water table level and pH correlated with testate amoebae in peatlands in Greece. The proportion of live forms of testate amoebae decreased after the fire, which may have been linked not only to the high temperature during and after the fire, but also to the marked deterioration in oxygen conditions. Research by Mazei et al. [42] showed that a decrease in oxygen content in peatland ecosystems causes a 50–65% reduction in the abundance of testate amoebae as well as an increase in the proportion of empty shells. At the same time, ciliates were observed to regain their abundance relatively quickly, and after approximately eight months, they reached numbers close to those recorded before the fire. Their abundance showed a marked increase together with the increase in pH, the concentration of biogenic compounds, and organic matter. These relationships have also been shown in other peatland ecosystems, in a study modelling the concentrations of biogenic compounds [37]. In addition, most ciliate species are euthermic and show high tolerance for temperature changes, and an increase in temperature causes an increase in their abundance [43]. It also seems that the diversity and abundance of ciliates may be significantly influenced by the nature of the microhabitat, mainly its vegetation. This is particularly evident in microhabitats with water a year after a fire, in which Calliergonella begins to develop. Studies by Walsh [44] and Kuczyńska-Kippen [45] have shown that plants with a ‘dense’ stem structure (more structurally complex) offer better food supply and refuge. In the present study, concentrations of total organic carbon and total phosphorus were several times higher among patches of Calliergonella and may have indirectly affected the abundance of potential food for ciliates. The markedly higher abundance of protozoa in the clump of Calliergonella may have additionally been linked to the rapidly developing periphyton here, which made the habitat more diversified. The quantitative structure of ciliates before the fire was mainly dominated by Colpodea and Oligotrichida, while after the fire, Scuticociliatida and cyanomorphs had the largest share. These taxa have a broad tolerance range and are often associated with oxygen-deficient habitats [46]. The most stable organisms were the nematodes. Studies of this group of organisms show that the long-term effect of fires on them is minor [47]. In addition, fire has been shown to have a much less pronounced effect on nematodes than on microorganisms. This is most likely explained by the distinct differences in the morphology, physiology, and ecology of these taxonomic groups. Nematodes respond much more slowly to sudden environmental disturbances. According to Grygoruk et al. [48], in the Biebrza wetlands located in eastern Poland, the most abundant were Daphnia sp., Nematoda, and Chironomidae.

4.3. Effects of Fire on Food Webs

The strongest positive correlations before the fire were between ciliates and bacteria and between nematodes and ciliates. After the fire, the number and strength of correlations increased. The strongest correlation was between bacteria and protozoa (amoebae and ciliates), and there was a slightly weaker correlation between heterotrophic flagellates and bacteria. Therefore, it seems that after the fire it was mainly ciliates that were reducing the abundance of bacteria and flagellates; the abundance of ciliates was 2–3 times higher than that of testate amoebae, and furthermore, ciliates are believed to graze on other microorganisms [37]. After the fire, a transformation of the trophic structure of protozoa towards the dominance of omnivorous and mixotrophic taxa was observed. This change may be an adaptive response to survival in the unfavourable environmental conditions appearing after the fire. The literature contains no data at all on the role of protozoa in the food web functioning after a fire. According to research by Hansen and Christoffersen [47], flagellates mainly prefer large bacterial cells, whereas mainly small forms were dominant in the peat bog in the present study. At the same time, larger forms of flagellates (over 15 µm) were dominant both before and after the fire. Such large forms feed not only on bacteria but also on algae and other protozoa [49]. Thus, this cell size may have allowed them to acquire not only bacteria, but also other groups of organisms as an alternative food source in a habitat undergoing enormous environmental stress. Knowledge of fire effects and of trophic relationships is important in predicting the response of biocoenoses to the increasingly dynamic phenomena resulting from global climate change.

In conclusion, the fire modified the physical and chemical parameters in the water of the peat bog, increasing the temperature, pH, water mineralization, and concentrations of biogenic compounds and organic matter, and decreasing the oxygen concentrations. Our study shows that fire significantly influences the moisture regime of peatlands and vegetation changes compared to typically drier habitats. The fire distinctly modified the qualitative and quantitative structure and the functioning of microbial food webs, and thus influenced carbon cycling. This was reflected in the increase in the abundance of ciliates and the decrease in the species number and abundance of testate amoebae. The functioning of food webs after fires is still very little understood, but it is important for an understanding of the functioning of peatlands, which are increasingly susceptible to fires due to global climate change.

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