

## Article

# The Effect of Peatland Restoration on Ciliate Communities: Long-Term Analyses

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**Abstract:** Peatlands are extremely valuable ecosystems of enormous biological diversity, mainly due to their specific geological and hydrological conditions. One of the most important threats facing these habitats is overgrowth resulting from intensifying succession processes. Due to the fact that peatlands are endangered or vanishing, attempts are made to restore their original natural assets. The aim of the present study was to determine the effect of active conservation of peatland ecosystems, in the form of removal of birch trees, on communities of ciliates. The subject of the research was the Durne Bagno peat bog, located in Polesie National Park in eastern Poland. Analysis of the results of long-term research (nine years) showed that active conservation measures affect the physical, chemical, and hydrological properties of peatlands. In the habitat where birch trees were cut down, the water level, temperature, and chlorophyll *a* concentration in the vast majority of cases were higher than in the area with *Betula* L. Changes in habitat conditions are reflected in the taxonomic composition and abundance of ciliates. In the habitat from which birch trees were removed, ciliate abundance was higher than in areas where birch remained. The total number of taxa in the habitats was the same, but there were seasonal differences. The most taxa were recorded in spring and summer and the fewest in autumn. Thus, analysis of the results indicates that ciliates are an appropriate indicator group of species for biomonitoring of peatland restoration.

**Keywords:** ciliates; peatland; taxonomic composition; interactions



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## 1. Introduction

Peatlands occupy about 3% of the world's land surface and accumulate about 25–30% of the carbon accumulated in ecosystems [1,2]. They play an important role in research on ecology and biological diversity, due to their unique flora, hydrology, and environmental characteristics [3]. Unfortunately, these ecosystems are also among the most endangered, largely due to human impact. Draining of peatland ecosystems for agriculture, agricultural pollution, peat extraction, afforestation, and acid rain are only some of the threats resulting from human activity [4]. Effective conservation of these ecosystems depends mainly on a good understanding of the history of the ecosystem [5]. Long-term studies conducted in peatlands provide a more complete understanding of the functioning of these ecosystems [6]. Accumulation and emission of carbon from peatlands plays an important role in the balance of this element, which is an individual trait dependent on the ecohydrology of the peatland [7]. This is why it is so important to support the preservation of accumulated carbon in peatlands, as well as the capture and accumulation of carbon from the atmosphere by peatlands in the future. Changes in water conditions in peatlands accelerate succession processes and encroachment of trees or shrubs, including *Betula* [8,9]. This has consequences for the functioning of the peatland ecosystem, as encroachment of birch causes the

unfavourable phenomenon of oxygenation of the peat deposit and also disturbs the water regime [10,11]. Therefore, it is extremely important to conduct restoration measures in these ecosystems [11,12]. Restoration of degraded peatland can restore the stable circulation of greenhouse gases and prevent the decomposition of carbon already accumulated in the peat [13]. This in turn can affect microbial communities, causing changes in the functioning of the microbial loop due to the changing concentrations of nutrients in the environment [9]. Moreover, it is possible to influence the organisms inhabiting peatlands, which have varied responses to both biotic and abiotic factors [14]. A very important limiting parameter is the depth of the water table. Lowering of the groundwater level increases the consumption of oxygen, used for decomposition of matter, thereby negatively affecting the activity of microbial communities in peat [15].

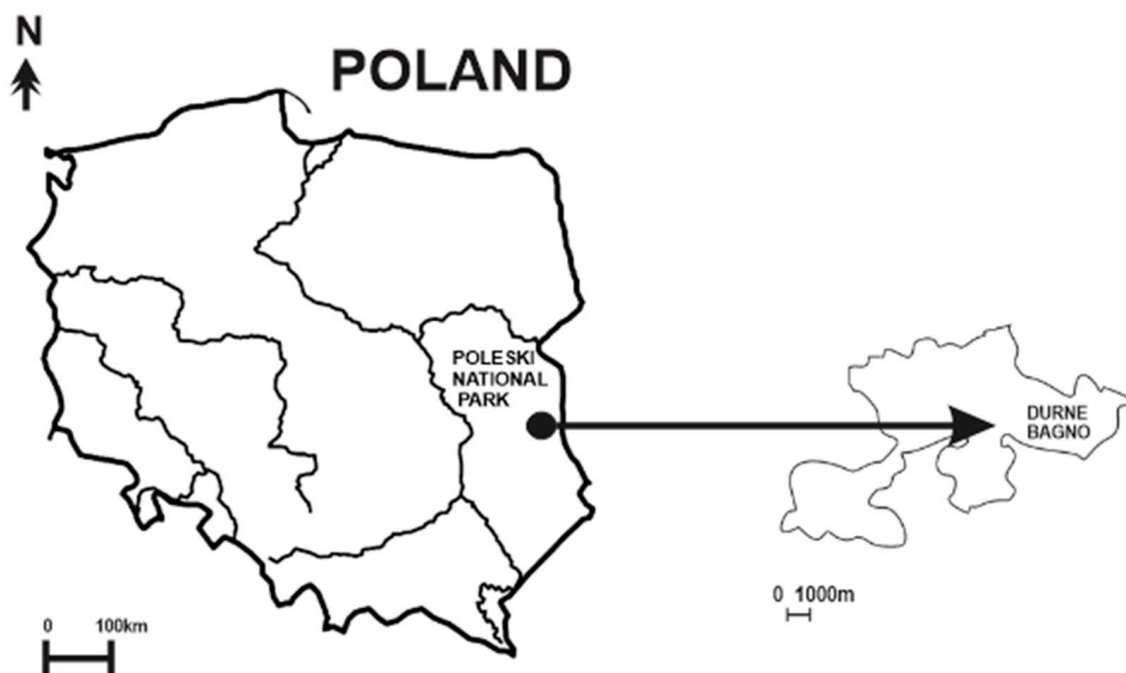
Previous research conducted by Mitchell et al. (2003), Kexin et al. (2007), Nguyen-Viet et al. (2007), Wilkinson and Mitchell (2010), and Mieczan et al. (2012) indicates that ciliates can achieve considerable numbers in peatland ecosystems [16–20]. These protozoa are important consumers of bacteria, flagellates, and algae and are also involved in the mineralization of organic matter and the circulation of biogenic compounds [21]. Attention has also been drawn to the main factors affecting the functioning of this group of protozoans, i.e., water depth and pH [16,18]. Their fast reaction time makes them useful indicators of environmental changes [22]. Previous research has concerned the effect of restoration measures on the species diversity of protozoa [23–26]. However, the functioning of ciliates in peatland ecosystems subjected to long-term restoration processes and seasonal changes are not sufficiently understood. It is crucial to understand the consequences of restoration measures and seasonal abundance patterns. Therefore, the aim of the study was to determine the effect of long-term restoration measures on ciliate communities in peatland ecosystems. Based on a nine-year study, we attempted to learn how restoration measures (removal of birch) affect the hydrological and physicochemical parameters of peatlands which can determine the occurrence of ciliates—their diversity, abundance, and taxonomic structure. The main objective of this project is to analyse in detail the impact of active conservation of peatland ecosystems, in the form of removal of birch trees, on communities of ciliates. In the proposed analysis, we intend to assess the research hypotheses:

1. Restoration causes temporary destabilization of environmental parameters.
2. Ciliates can be an excellent group of indicator species useful in monitoring the effects of peatland restoration.

## 2. Materials and Methods

### 2.1. Study Site

Restoration procedures involving cutting down birch trees (*Betula* L.), which have colonized the central part of wetland areas and the lag zone in massive numbers, were carried out in the Durne Bagno peat bog (Polesie National Park, 51° N, 23° E) (Figure 1). In the 1960s and 1970s, intensive drainage procedures were carried out in the Polesie Lubelskie region, lowering the groundwater and surface water level and leading to the drainage and degradation of peatlands. This resulted in the intensification of succession processes and overgrowth—mainly by common reed or birch. In the 1990s, restoration measures were initiated to improve the hydrological conditions in Polesie National Park and inhibit the overgrowth of the peatlands. Active conservation measures in the Durne Bagno peat bog have been carried out since 2006, primarily involving the removal of trees or shrubs (including birch growing on parts of the bog). The sites where birch trees were removed constituted study samples, while sites where birch trees were not removed constituted control samples. At the sites where birch trees were removed, the dominant taxa are currently *Eriophorum vaginatum* (L.), *Carex acutiformis* Ehrhart., *Carex gracilis* Curt., *Sphagnum angustifolium* (C.C.O. Jensen ex Russow), *Sphagnum cuspidatum* Ehrh. Ex Hoffm. and *Polytrichum* sp.



**Figure 1.** Location of the peatland under study.

### 2.2. Abiotic Variables

Hydrological (surface water level) and physicochemical parameters were determined in the years 2014–2022. Peatland restoration procedures involving birch removal were begun in 2014 and repeated systematically every year for 9 years. The biomass was moved outside the peatland. Water samples for physicochemical and biological analysis were collected from two sampling sites with an area of 250 m<sup>2</sup>. Once a month, from April to November, water samples were collected from the zone from which birch was systematically removed (B−) and the zone where birch was not removed (B+). Each time, the following physical and chemical parameters of the water were analysed: temperature, conductivity, This is not necessarily the case. This is an overview map pH, dissolved oxygen (DO), chlorophyll *a*, P<sub>tot</sub>, N<sub>tot</sub>, N-NH<sub>4</sub>, N-NO<sub>3</sub>, P-PO<sub>4</sub><sup>3−</sup>, and total organic carbon (TOC) [27]. Temperature, conductivity, pH, and DO were analysed using a multi-parameter meter (YSI 556MPS). The P-PO<sub>4</sub><sup>3−</sup> level was determined by the colorimetric method, N-NH<sub>4</sub> with an FIA analyser, P<sub>tot</sub> by the colorimetric method, and N<sub>tot</sub> by Kjeldahl's method. The chlorophyll concentration was measured by spectrophotometric analysis following preparation of extracts [10]. Total organic carbon (TOC) analysis was performed using wet potassium persulfate digestion with the O/I Corporation Model 700 TOC analyser.

### 2.3. Ciliata Communities

In the years 2014–2022, from April to November, analyses of ciliate communities were performed. Samples were taken once a month from two sites: from the zone from which birch was systematically removed (B−) and the zone where birch was not removed (B+). Water for analysis of ciliates was sampled using an acrylic glass tube (length 1.0 m, Ø50 mm). Three samples were taken from each site every month. Then, a syringe with a rubber tube was used to obtain 400–500 mL of peatland water. In laboratory conditions, ciliate abundance was determined by the Utermöhl method [27]. Each time, three samples of ciliates (one sample = 500 mL) were left to settle for 24 h, and then the upper 400 mL was gently removed. To determine the density of ciliates, three samples were preserved with Lugol's solution. In addition, live samples that were not stained with Lugol's solution were analysed. Morphological analysis of ciliates was performed using keys by Foissner and Berger (1996) and Foissner et al. (1999) [28,29]. The Shannon–Wiener index was calculated

to determine the taxonomic diversity of each habitat. It was estimated from the number of taxa and the evenness of occurrence of individual taxon in a given habitat (Kacprzak and Kotlarz, 2017) [30].

#### 2.4. Data Analyses

A regression framework was used to analyse changes in ciliate abundance data. To account for the over-dispersed nature of the count data, the negative binomial (NB) regression method was used to examine the relationships between ciliate abundance (as the response variable) and the contributing factors. The independent variables of interest were a binary variable indicating the presence (B+) or absence (B−) of *Betula* sp. and an eight-level categorical predictor to represent the impact of months (to determine seasonality patterns). The negative binomial model was fitted using the `glm.nb` function in the MASS R package. The Mann–Whitney U test was implemented to determine the effect of the site on the water quality parameters. A nonparametric test with post-hoc pairwise comparisons (Kruskal–Wallis H) was performed to determine the differences in the physicochemical parameters between the sampling sites (B+ and B−) and months. Pearson correlation coefficients were used to study the correlation structure between ciliate abundance data and the physicochemical properties of the water. Agglomerative hierarchical classification was used to determine the similarity of the species composition at each sampling site and in each month or year. Ward’s algorithm of minimum variance was used for clustering, and the Jaccard index was used as a similarity measure for the clustering of species. Clustering of sites (and months/years) was performed using the UPGMA method and the Euclidean metric. The results of the classification were presented graphically in the form of a combination of dendrograms and heatmaps. The variability gradients indicated by ciliates were measured using detrended correspondence analysis (DCA). Redundancy analysis (RDA) was used to explore the relationships between the density of species and environmental variables [31]. Automatic forward selection of environmental variables (Monte Carlo permutation test) was used to determine the significant variables. The analysis was carried out for each habitat and sampling month and for habitat and year. The results were presented on ordination triplots. All ordination analyses were performed using the `vegan` R package. A significance level of 0.05 was assumed for all analyses.

### 3. Results

#### 3.1. Abiotic Variables

The water level in the habitats in the years 2014–2022 was varied. In the B− part of the Durne Bagno peat bog, the water level in most cases was higher than at site B+. The results clearly indicate that the restoration measures initially caused minor differences in the depth of the water table between the sites. Exceptions were the years 2019 and 2020, in which the water level at site B+ reached 23–43 cm and 4–45 cm, which were significantly higher than at site B− (Tables 1 and 2).

At site B−, the temperature was higher than at site B+. In the vast majority of analyses, the pH was slightly higher in the B− habitat. In addition to the increase in pH, increased values were noted for biogenic substances, conductivity, and oxygen concentration. The concentration of chlorophyll *a* content was higher at site B− (the highest concentration, 37 mg L<sup>−1</sup>, was recorded in 2015) (Tables 1 and 2).

The statistical analysis, based on the Kruskal–Wallis test by ranks (H), comparing the distribution of the variables, revealed differences in the physicochemical parameters of the water depending on the site. For most parameters (water level, biogenic substances, chlorophyll *a*, electrolytic conductivity, temperature, and TOC), H was much higher at site B− than at site B+. In the case of pH, however, the value was higher at the site where birch was not removed (B + H = 40.81; B − H = 14.08) (Table 3).

**Table 1.** Hydrological, physical, and chemical parameters of water in investigated peatbog (B−).

Size	B−								
Parameters/ years	2014	2015	2016	2017	2018	2019	2020	2021	2022
Water level (cm)	4–25	11–24	11–23	14–27	14–27	14–27	2–23	2–15	34–56
Temp. °C	4–22	2–21	2–17	4–16	3–19	4–16	3–23	3–20	6–21
pH	7–9.6	7–9.8	8–9.8	8–9.8	7–9.8	7–9	6–9.8	6–9.2	7–9.2
Cond. ( $\mu\text{S cm}^{-1}$ )	180–321	78–278	78–278	98–278	98–278	98–232	98–340	320–498	202–343
O <sub>2</sub> (mg L <sup>−1</sup> )	5–9	5–9	7–9	8–11	7–11	7–11	7–11	8–11	3.4–9.1
NH <sub>4</sub> <sup>+</sup> (mg L <sup>−1</sup> )	0.124–0.970	0.124–0.970	0.124–0.890	0.124–0.970	0.124–0.970	0.124–0.894	0.124–0.970	0.124–0.970	0.022–0.343
NO <sub>3</sub> <sup>−</sup> (mg L <sup>−1</sup> )	0.112–0.470	0.112–0.470	0.114–0.431	0.157–0.431	0.14–0.431	0.157–0.431	0.114–0.431	0.114–0.431	0.023–0.343
N <sub>tot</sub> (mg L <sup>−1</sup> )	1.456–1.852	1.456–1.952	1.456–1.884	1.456–1.884	1.456–1.884	1.466–1.884	1.456–3.456	1.466–3.456	1.267–1.884
PO <sub>4</sub> <sup>3−</sup> (mg L <sup>−1</sup> )	0.021–0.112	0.021–0.132	0.011–0.169	0.011–0.169	0.011–0.169	0.011–0.169	0.011–0.169	0.011–0.169	0–0.102
P <sub>tot</sub> (mg L <sup>−1</sup> )	0.136–0.636	0.082–0.636	0.023–0.346	0.023–0.346	0.023–0.3460	0.023–0.346	0.090–0.346	0.090–0.346	0.029–1.087
Chlorophyll <i>a</i> (mg L <sup>−1</sup> )	9–27	5–37	2–19.04	2–19.04	2–19.04	2–19.04	2–23.04	2–23.04	5.93–110.23
TOC (mg L <sup>−1</sup> )	15.9–37	15.9–37	18.5–34	18.3–34	18.3–34	18.5–34	18.3–34	18.5–36.2	17.8–27.5

**Table 2.** Hydrological, physical, and chemical parameters of water in investigated peatbog (B+).

Site	B+								
Parameters/ years	2014	2015	2016	2017	2018	2019	2020	2021	2022
Water level (cm)	6–13	8–23	11–23	14–27	14–27	23–43	4–45	12–19	34–46
Temp. °C	3–23	2–21	2–16	4–16	3–14	4–16	3–24	3–24	6–20
pH	6–9	7–9	8–9	8–9.8	7–9.8	8–14	8–9.8	6–9.2	3–9
Cond. ( $\mu\text{S cm}^{-1}$ )	180–321	180–321	78–278	98–278	98–278	98–230	98–332	280–390	225–314
O <sub>2</sub> (mg L <sup>−1</sup> )	5–8	4–12	7–9	8–11	7–11	7–11	7–11	8–11	4.3–7.9
NH <sub>4</sub> <sup>+</sup> (mg L <sup>−1</sup> )	0.124–0.894	0.124–0.894	0.124–0.923	0.124–0.970	0.124–0.970	0.124–0.894	0.124–0.970	0.124–0.970	0.011–0.778
NO <sub>3</sub> <sup>−</sup> (mg L <sup>−1</sup> )	0.112–0.470	0.028–0.314	0.015–0.161	0.112–0.431	0.112–0.431	0.157–0.431	0.114–0.431	0.114–0.431	0.018–0.300
N <sub>tot</sub> (mg L <sup>−1</sup> )	1.466–1.884	1.466–1.884	1.466–1.884	1.456–1.884	1.456–1.884	1.466–1.884	1.466–3.456	1.466–3.456	1.531–2.456
PO <sub>4</sub> <sup>3−</sup> (mg L <sup>−1</sup> )	0.021–0.112	0.001–0.033	0.003–0.029	0.011–0.169	0.011–0.169	0.011–0.169	0.011–0.169	0.011–0.169	0.01–0.112
P <sub>tot</sub> (mg L <sup>−1</sup> )	0.136–0.636	0.005–0.211	0.011–0.522	0.023–0.346	0.023–0.346	0.023–0.346	0.090–0.346	0.090–0.346	0.010–0.260
Chlorophyll <i>a</i> (mg L <sup>−1</sup> )	9–27	9–27	2.3–8	2–16.04	2–19.04	2–19.04	2–23.04	2–23.04	4.76–23.59
TOC (mg L <sup>−1</sup> )	15.9–37	13.2–24	13.2–21.9	18.3–34	18.3–224.0	18.3–34	18.3–34	18.5–36.5	15.6–26.2

**Table 3.** Statistical analysis—Kruskal–Wallis test (p—probability; B−, the zone from which birch was systematically removed; B+, the zone from which birch was not systematically removed).

	Kruskal–Wallis H		Statistical Significance (p)	
	B−	B+	B−	B+
WL	7.95	4.82	0.33	0.68
pH	14.08	40.81	0	0
N-NH <sub>4</sub>	41.26	40.81	0	0
N-NO <sub>3</sub>	36.41	19.62	0	0
N <sub>tot</sub>	44.16	36.33	0	0
P-PO <sub>4</sub>	26.26	23.5	0	0.01
P <sub>tot</sub>	41.83	18.76	0	0
Chl a	2.2	21.17	0.01	0
TOC	26.94	18.42	0	0.01
Cond.	20.32	8.47	0	0.29
T	47.52	43.47	0	0

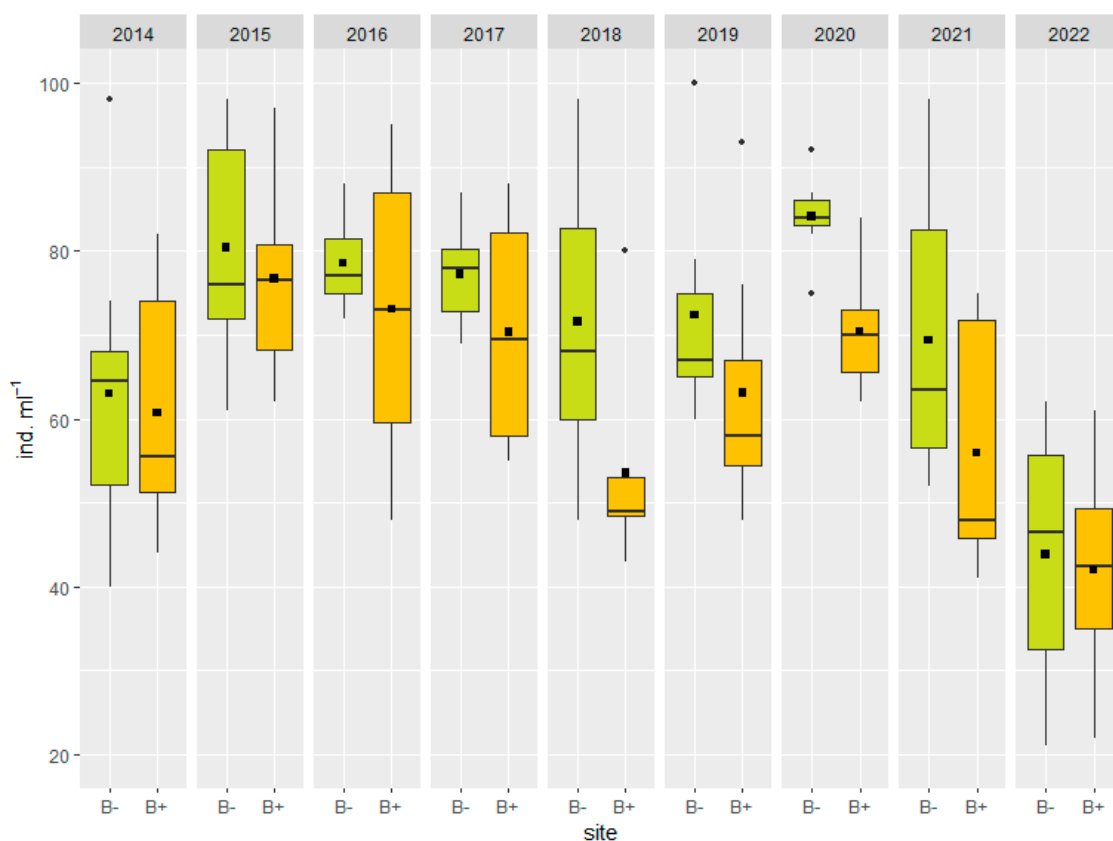
A comparative analysis of the physical and chemical parameters in the B− and B+ habitats was carried out. The results of the Mann–Whitney U Test indicate that there were no differences (in the central tendency) in the physicochemical parameters between the two habitats.

### 3.2. Microbial Communities

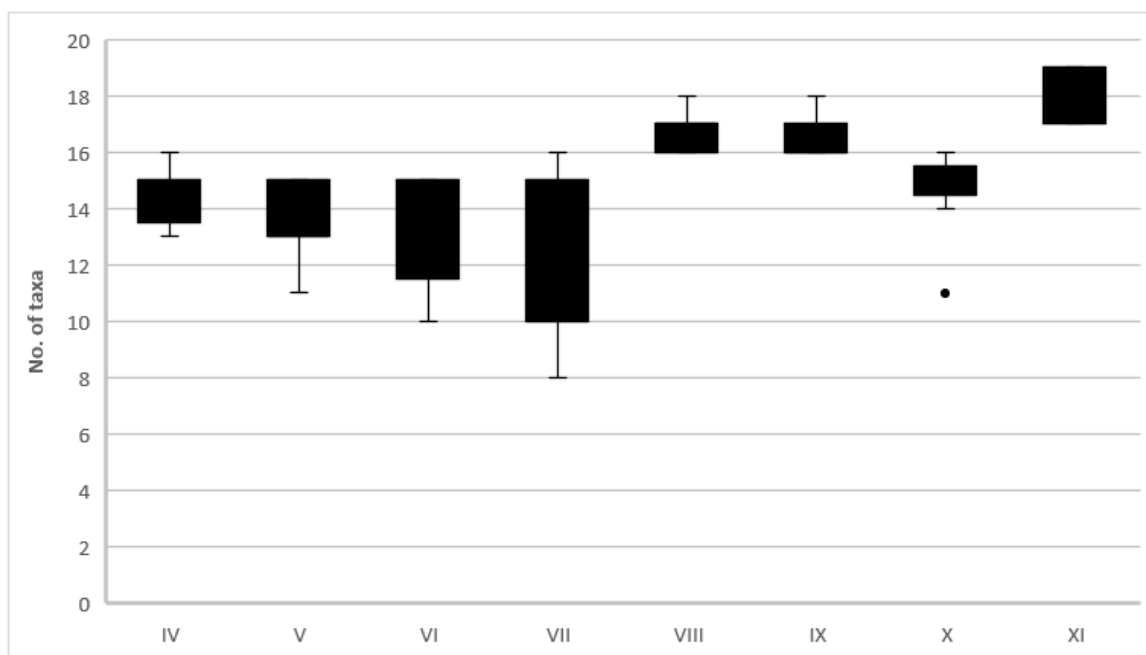
Ciliate abundance was markedly varied at the study sites. The highest abundance was recorded at site B− in 2015 (92 ind. mL<sup>−1</sup>). Abundance of ciliates was lowest (55 ind. mL<sup>−1</sup>) in 2022. In the B+ habitat, the highest ciliate abundance was noted in 2016 (87 ind. mL<sup>−1</sup>) and the lowest in 2022 (35 ind. mL<sup>−1</sup>) (Figure 2). At site B− as well as at the site where restoration measures were not carried out, the highest ciliate abundance was attained by the following species: *Cinetochilum margaritaceum* (at B−, a total of 769 ind. mL<sup>−1</sup>; at B+, 1035 ind. mL<sup>−1</sup>). The least numerous species at site B− was *Spathidium sensu lato* (54 ind. mL<sup>−1</sup> in total), while at site B+, the least abundant species was *Colpoda cucullus* (42 ind. mL<sup>−1</sup>).

There were 20 ciliate taxa recorded in the study area. The number of taxa showed marked seasonal variation. *Coleps spetai*, *Strombidium viride*, *Cinetochilum margaritaceum*, *Paramecium bursaria*, *Colpoda cucullus*, and *Chilodonella uncinata* were more frequent in spring and summer, while the remaining taxa were more abundant in summer and autumn. The Shannon–Wiener index was markedly higher at site B− (0.69) than at site B+ (0.43), which indicates that the area with active conservation had higher seasonal taxonomic diversity in the years 2014–2022 than site B+ (Figures 3 and 4).

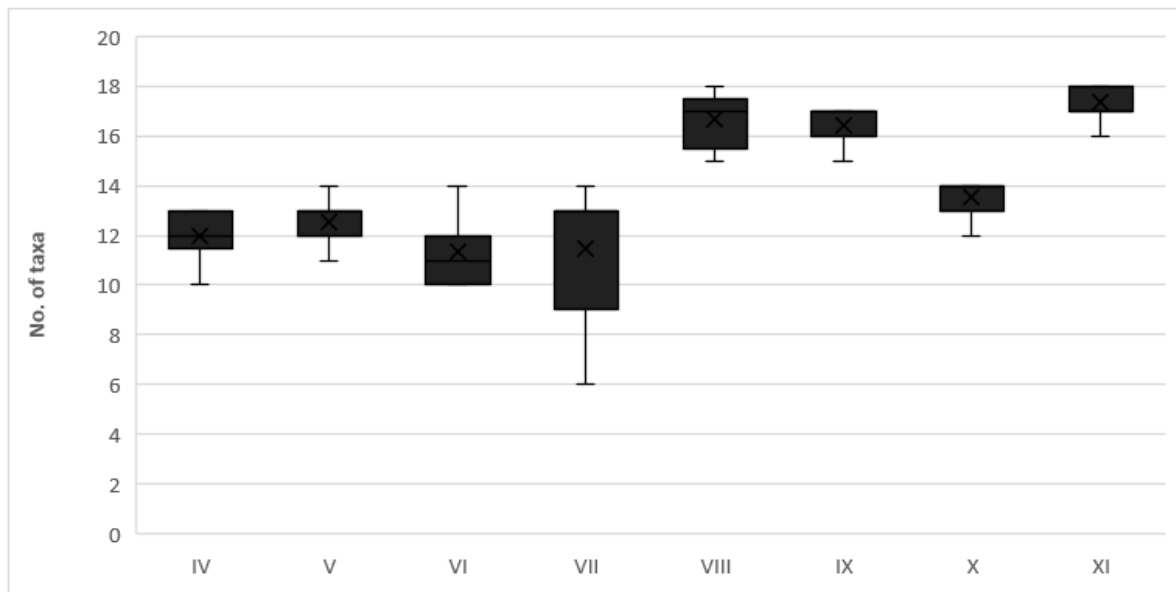
The dominance structure was markedly varied in the years 2014–2022. At site B−, in 2014 and 2015, the dominant taxa were *Strombidium viride* (26%, 27%) and *Cinetochilum margaritaceum* (12%, 17%). In the following year, in addition to *Strombidium viride* (23%), *Aspidisca costata* was a dominant taxon (16%). In 2017–2020, there were three dominant taxa: *Strombidium viride* (20%, 17%, 20%, 12%), *Cinetochilum margaritaceum* (12%, 13%, 15%, 15%), and *Aspidisca costata* (10%, 11%, 15%, 15%). In 2021, *Coleps hirtus* (24%) was dominant, and in 2022, *Strombidium viride* became dominant once again (24%) (Figure 5). At site B+, in 2014 and 2015, the dominant ciliate taxa were *Chilodonella uncinata*, *Amphileptus cleparedei*, and *Cinetochilum margaritaceum*. In 2016 and 2017, *Cinetochilum margaritaceum* accounted for 22% and 17% of ciliates. In 2018 and 2019, three dominant taxa were identified: *Strombidium viride*, *Cinetochilum margaritaceum*, and *Spirostomum ambigum*. In subsequent years the following taxa were dominant: in 2020, *Spathidium sensu lato*, *Spirostomum ambigum*, and *Coleps spetai*; in 2021, *Strombidium viride*; and in 2022, *Paramecium bursaria* (Figure 6).



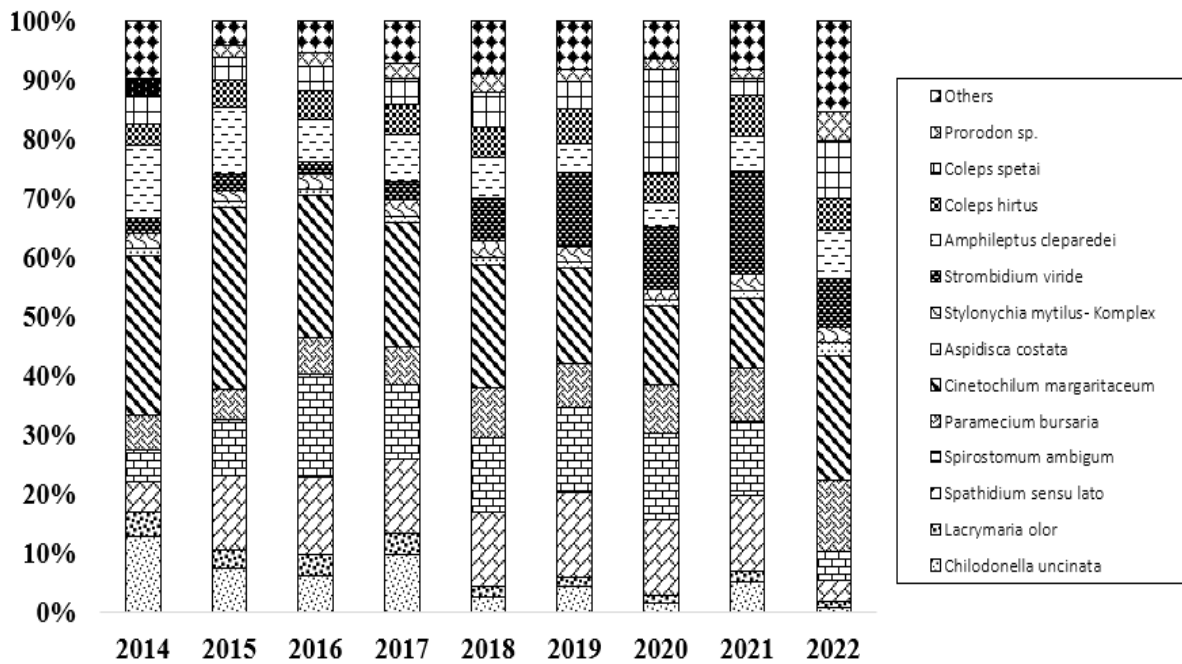
**Figure 2.** Changes in number regarding total abundance of *Ciliata* (B- (green color): habitat from which birch was removed, B+ (orange color): habitat continuously overgrown by birch, ind. mL<sup>-1</sup>: abundance of *Ciliata*).



**Figure 3.** Average seasonal changes in number of taxa of *Ciliata* in B-, from 2014 to 2022 (No. of taxa—number of taxa, IV–XI: months).



**Figure 4.** Average seasonal changes in number of taxa of *Ciliata* in B+, from 2014 to 2022 (No. of taxa: number of taxa, IV–XI: months).



**Figure 5.** Dominance structure of *Ciliata* found in B-.

The similarity analysis indicated the occurrence of seasonal taxonomic differences between the study sites (B- and B+). The highest similarity was noted in the area colonized by birch, especially in the years 2014–2016, while considerable differences in species were observed at the site where birch was removed, especially in 2018–2022 (Figure 7).



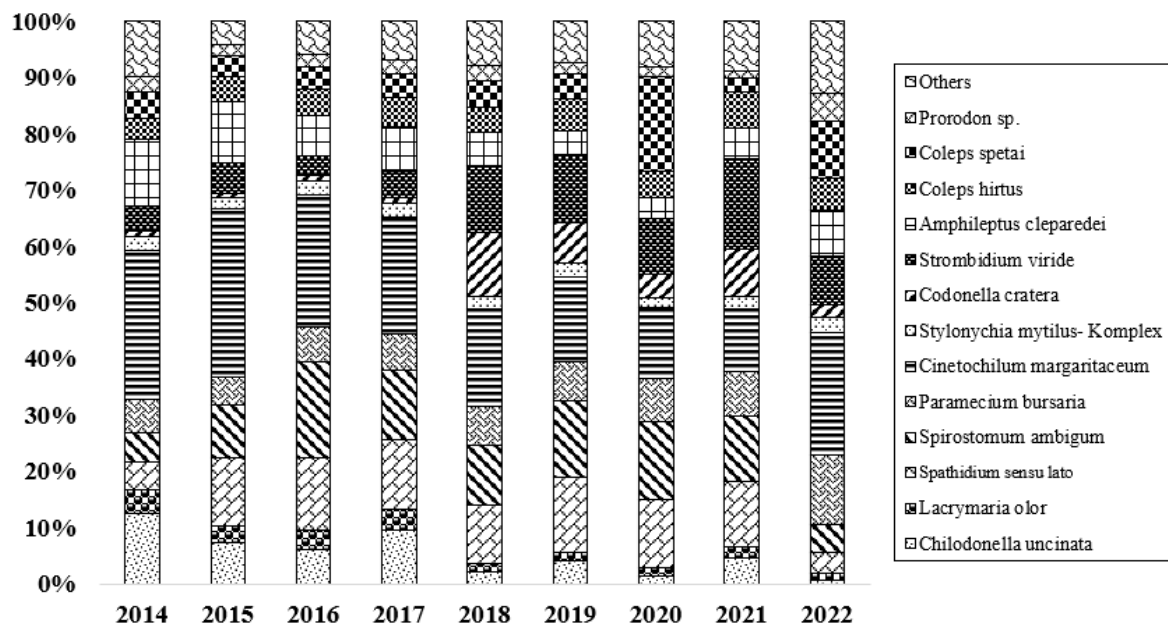


Figure 6. Dominance structure of Ciliata found in B+.

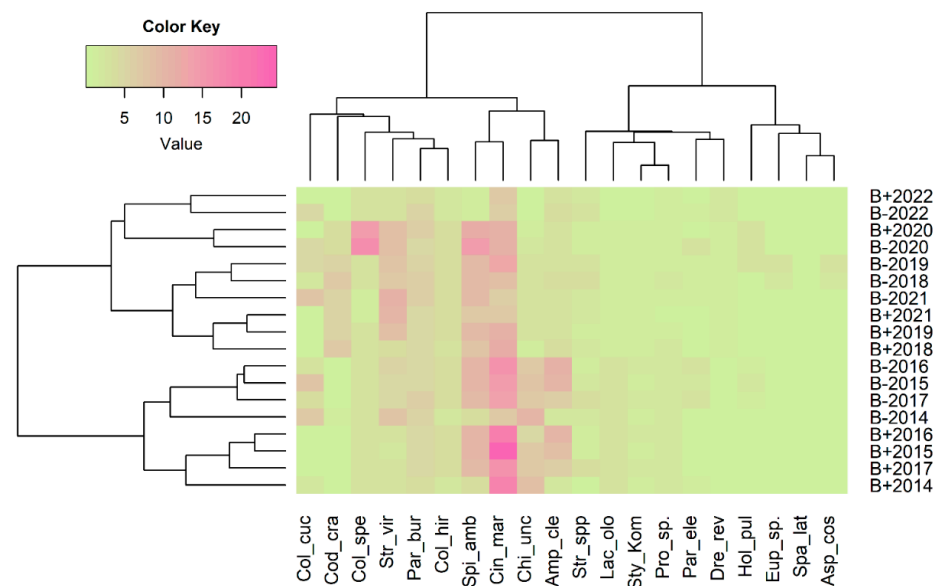


Figure 7. Index of faunistic similarity of ciliates between sites for each year (Col\_cuc—*Colpoda cucullus*; Cod\_cra—*Codonella cratera*, Col\_spe—*Coleps spetai*; Str\_vir—*Strombidium viride*; Par\_bur—*Paramecium bursaria*; Col\_hir—*Coleps hirtus*; Spi\_amb—*Spirostomum ambigum*; Cin\_mar—*Cinetochilum margaritaceum*; Chi\_unc—*Chilodonella uncinata*; Amp\_cle—*Amphileptus cleparedei*; Str\_spp—*Strombidium spp.*; Lac\_olo—*Lacrymaria olor*; Sty\_Kom—*Stylonychia Mytilus-Komplex*; Pro\_sp—*Prorodon sp.*; Par\_ele—*Paradileptus elephantinus*; Dre\_rev—*Drepanomonas revoluta*; Hol\_pul—*Holosticha pullaster*; Eup\_sp.—*Euplotes sp.*; Spa\_lat—*Spathidium sensu lato*; Asp\_cos—*Aspidisca costata*).

It follows from the log linear model of ciliate abundance that the presence of birch can significantly affect the number of ciliates. Based on our estimates, the ciliate community abundance was predicted to decrease by a factor of 0.859 when B+ was compared to B– and other variables were assumed to be constant. There was a significant change in the mean score over the seasons, with the highest score in the fourth quarter. Specifically, November showed the highest abundance among sampling months, with an expected count that is 1:407-times as high than during April (given the other predictor variables in the model are

held constant). At the other extreme, expected counts decrease by a factor of 0.917 when June is compared to April and the other variables are kept constant.

At site B−, in spring and summer, the bacterivorous and algivorous order Scuticociliatida—*Cinetochilum margaritaceum*—was dominant. In autumn, the most common were representatives of Cyrtophorida—*Chilodonella uncinata* (Figure 8). At site B+, in spring, the order Prostomatida was the most common and in summer, bacterivorous and algivorous Scuticociliatida—*Cinetochilum margaritaceum* as well as Pleurostomatida—*Amphileplus Celparedei*. In autumn, Scuticociliatida and Pleurostomatida were most frequently recorded (Figure 9).

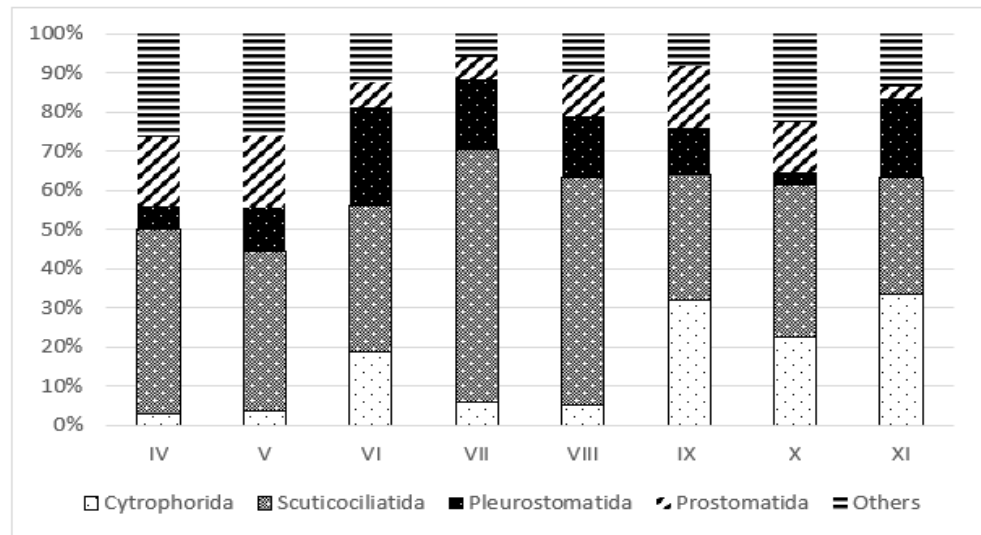


Figure 8. Seasonal changes in percentage of contribution of dominant orders of Ciliata in B− (IV–XI: months).

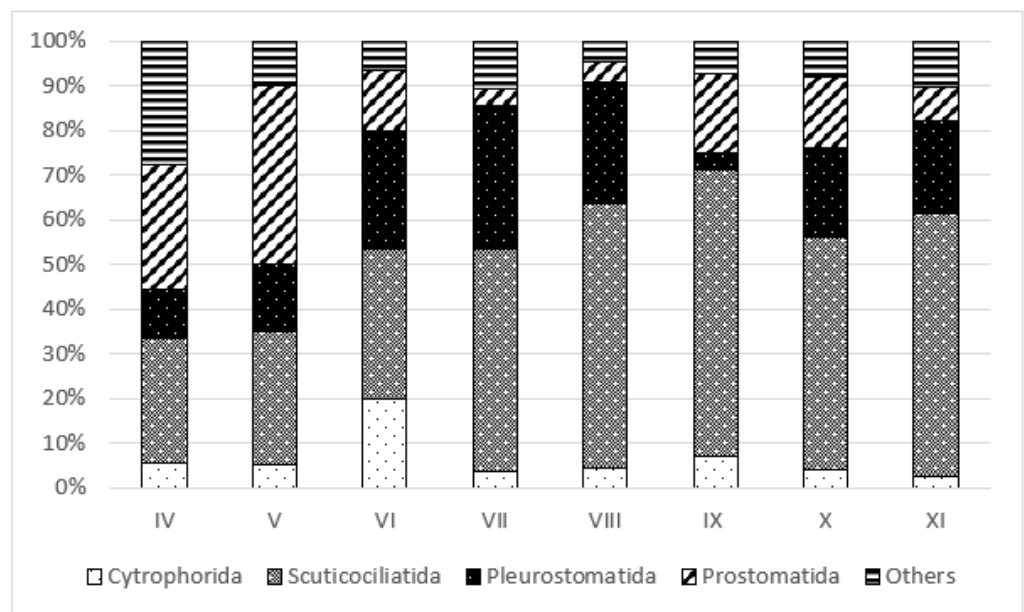


Figure 9. Seasonal changes in percentage of contribution of dominant orders of Ciliata in B+ (IV–XI: months).

### 3.3. Trophic Structure

Bacterivorous ciliates accounted for the largest proportion of ciliates in the B− habitat, with slightly fewer algivorous and predatory ciliates. Omnivorous and mixotrophic ciliates had a small share (Figure 10). Similar results were observed in the B+ habitat. Bacterivorous

ciliates accounted for nearly 50% of identified organisms, while 15–20% were algivorous and predatory ciliates, and 12% were omnivorous and mixotrophic (Figure 11).

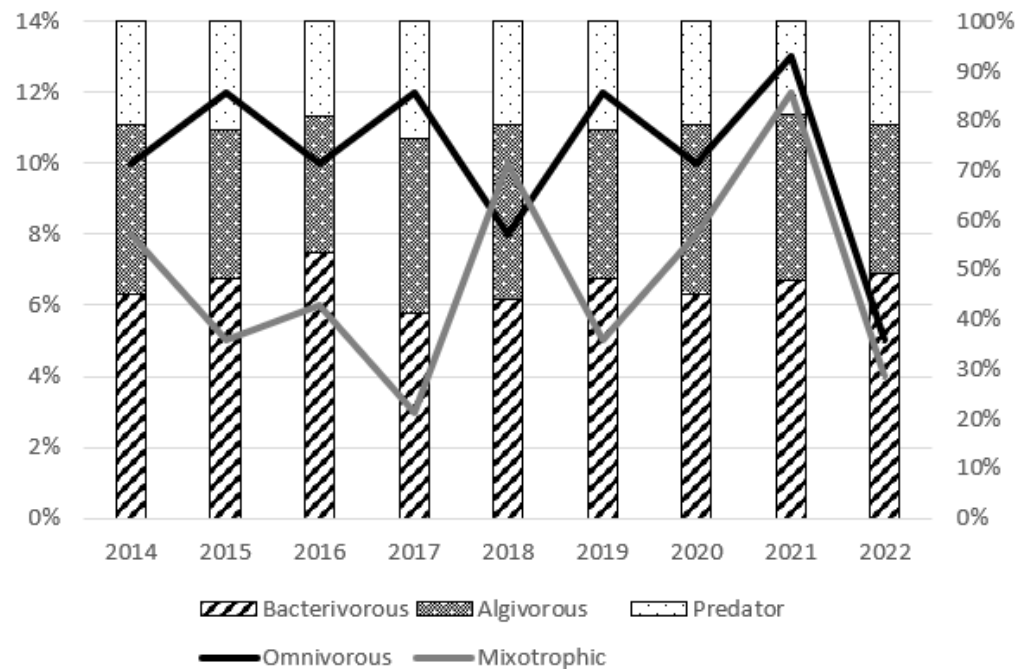


Figure 10. Trophic structure of ciliates in the B- habitat.

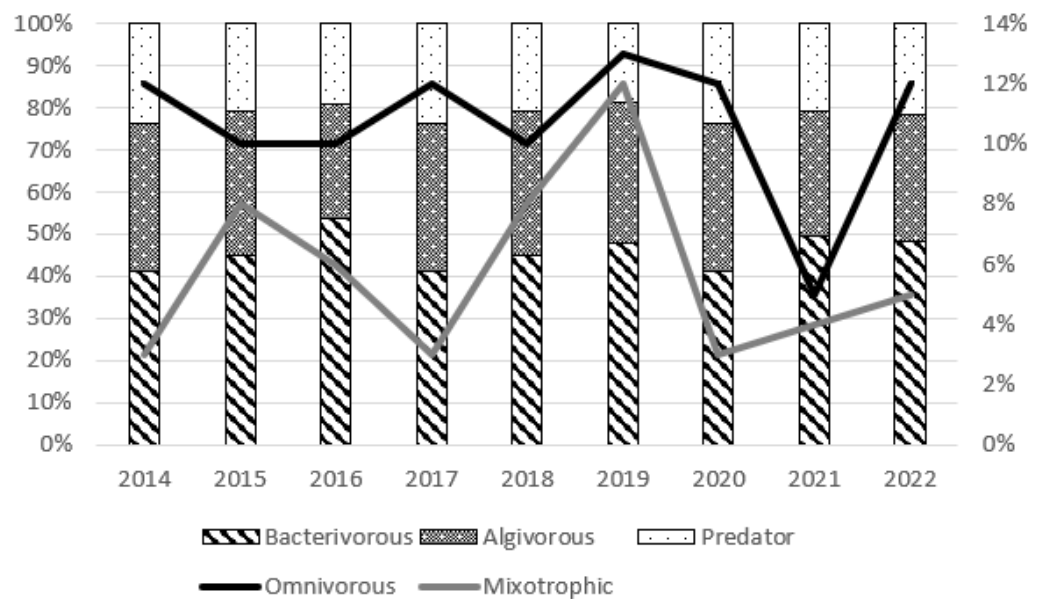
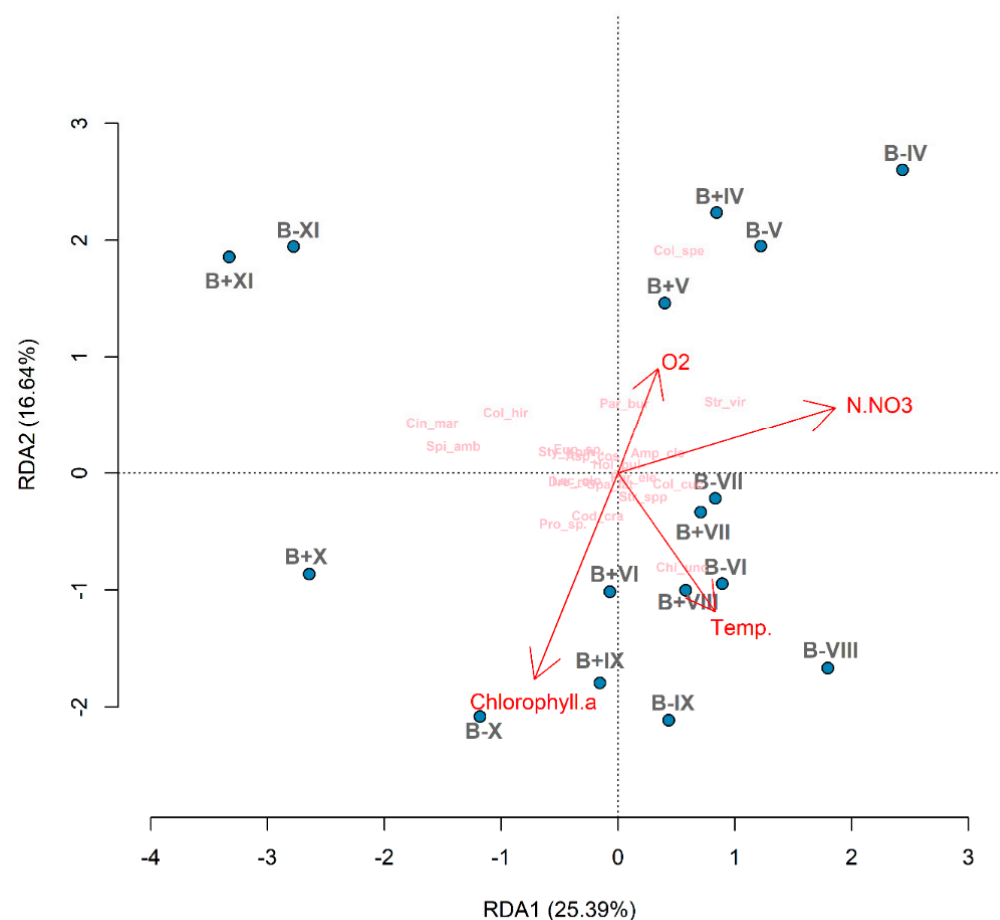


Figure 11. Trophic structure of ciliates in the B+ habitat.

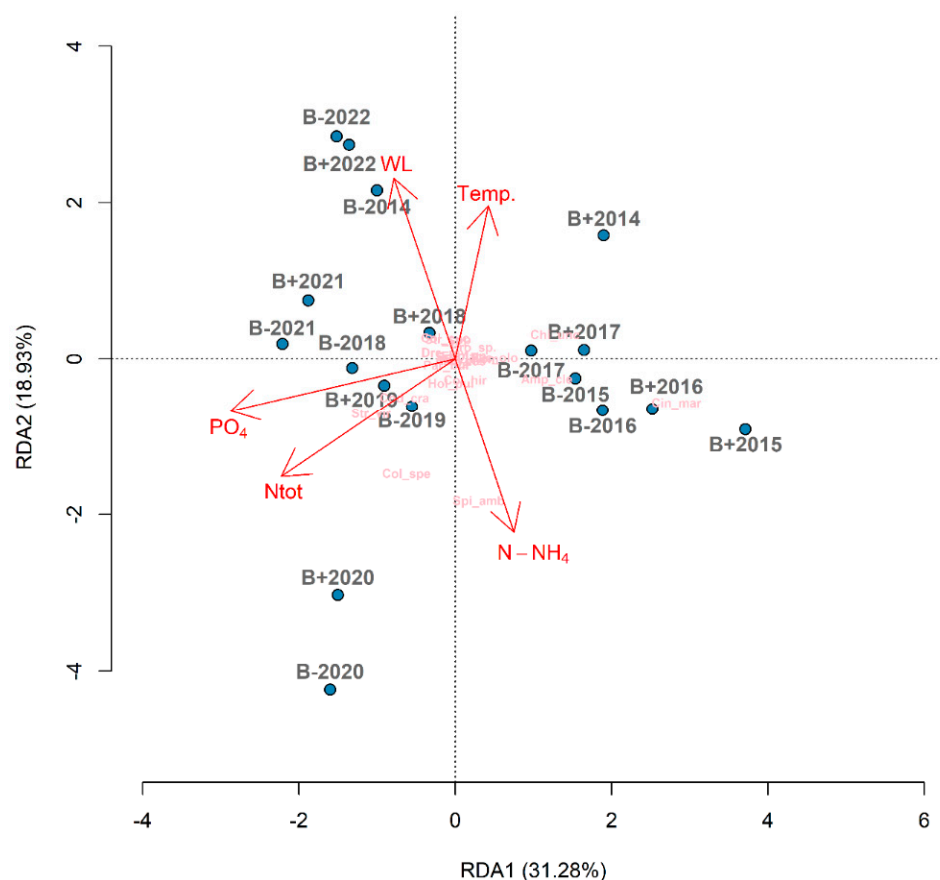
### 3.4. Redundancy Analysis (RDA) and Correlations

Environmental variables were standardized prior to RDA analysis. All environmental variables included in the analysis explained 91.48% of the variation in the ciliate community composition across the sites. The analysis showed that N-NO<sub>3</sub>, temperature, chlorophyll *a*, and oxygen were significant factors determining the occurrence of ciliates. At site B-, chlorophyll *a* had the greatest impact and was the parameter determining the occurrence of this group. At site B+, the decisive parameters were N-NO<sub>3</sub>, temperature, and oxygen. The RDA analysis also showed that the occurrence of *Chilodonella uncinata* was mainly associated with water temperature, while the O<sub>2</sub> and N-NO<sub>3</sub> concentrations mainly determined

the occurrence of *Strombidium viride* and *Paramecium bursaria*. In the years 2014–2022, environmental factors were observed to have pronounced differentiating effects on the community of protozoa. In 2022, irrespective of the sampling site (B+, B−), the occurrence of ciliates was mainly influenced by the water level (WL), while in the earlier part of the study period, i.e., from 2014, it was biogenic substances (P-PO<sub>4</sub>, N-NH<sub>4</sub>, N<sub>tot</sub>) that played the major role. These factors were most important in determining the occurrence of *Spirostomum ambiguum*, *Coleps spetai*, and *Cinetochilum margaritaceum*. The correlation analysis indicates that at site B−, the factors having the strongest positive effect on ciliate abundance were pH, biogenic substances, and electrolytic conductivity. Factors negatively correlated with ciliate abundance at this site were oxygen concentration and temperature. At site B+, ciliate abundance was positively correlated with the water level, biogenic substances, and electrolytic conductivity and negatively correlated with chlorophyll *a* and temperature. The number and strength of correlations varied between years (Figures 12 and 13).



**Figure 12.** Redundancy analysis (RDA) biplot for habitats, showing environmental variables and months of research. Arrows indicate significant parameters in the Monte Carlo permutation test at  $p < 0.05$ . B−: habitat from which birch was removed, B+: habitat continuously overgrown by birch, IV–XI: months, Temp = water temperature, O<sub>2</sub> = dissolved oxygen, Chl-a = chlorophyll *a*, N-NO<sub>3</sub> = nitrate nitrogen.



**Figure 13.** Redundancy analysis (RDA) biplot for habitats, showing environmental variables and months of research. Arrows indicate significant parameters in the Monte Carlo permutation test at  $p < 0.05$ . B–: habitat from which birch was removed, B+: habitat continuously overgrown by birch, IV–XI: months, WL = water level, Temp = water temperature, N-NH<sub>4</sub> = ammonium nitrogen, N<sub>tot</sub> = nitrogen total, P-PO<sub>4</sub> = dissolved orthophosphates.

#### 4. Discussion

##### 4.1. Effect of Restoration on Environmental Factors

Economic exploitation of peatland areas (e.g., peat extraction, afforestation, and agricultural activity) is a factor negatively affecting peatland ecosystems in Europe. These activities have led to a reduction in the abundance of individual species of flora and fauna. However, this is not the only threat facing peatland ecosystems. Human activity can affect the physicochemical and hydrological parameters of peatlands, e.g., by lowering the water level, which amplifies the succession process. This leads to overgrowth, initially by scrub communities and then by swamp forests. For this reason, hydrological conditions play an extremely important role in the proper functioning of peatland ecosystems [32]. Restoration measures in the peat bog that was the subject of the present study initially caused minor differences in hydrological conditions (especially in 2016–2018), but in 2019–2022, a higher water level was noted at site B–. Similar patterns were observed by Mieczan and Tarkowska-Kukuryk, who noted a marked increase in the surface water level following active conservation measures involving the removal of shrubs from a carbonate fen [26]. Mieczan et al., in a study of the effect of removal of reed beds in peatlands, also noted a significant increase in the water level and a reduction in the rate of succession [25]. Similar patterns were observed by Andersen et al. [32]. In addition, Glińska-Lewczuk et al. observed a slight increase in the water level following active conservation, but drew attention to the effect of climatic conditions on the results [33]. The authors demonstrated a specific hydrological and thermal regime. The water levels were lowest in autumn and highest during the wet, cool summer. Grzywna, in an analysis of 26-year research conducted in

Polesie Lubelskie, also showed that meteorological conditions significantly influenced the water levels in peatland areas [34]. Słowińska and Słowiński reported a significant effect of climate change, including an increase in temperature, on the hydrological parameters of peatlands [35]. Degrott et al. (2021) also reported a significant association between the increase in temperature caused by climate warming and the water level in peatlands [36]. A similar relationship was shown in our analyses.

The pH of the water in the habitats ranged from 6 to 9.8. In most measurements, the pH was only slightly higher in the B− habitats. Research by Gilbert et al. confirmed that an increase in the concentrations of biogenic substances is correlated with the water pH in the environment [37]. Mieczan and Tarkowska-Kukuryk observed markedly higher water pH following active conservation measures [25]. They also noted that the increase in pH accompanied a higher concentration of biogenic substances, which was observed in the present study as well. According to Scheffer, plant mass can temporarily destabilize environmental conditions [38]. Hence, mowed vegetation may increase the concentrations of biogenic substances and the water pH [39]. However, in research by other authors, this increase was much greater than in the present study, most likely due to the remaining plant mass [25]. In the present study, birch was completely removed from the peat bog, so the differences in parameters were smaller than in research by other authors.

#### 4.2. Effect of Restoration on the Composition and Abundance of Ciliates

The relationship between taxonomic diversity and the concentration of biogenic substances is not obvious [40]. Burkholder (2001) showed that an increase in the concentration of biogenic substances can have a limiting effect on the taxonomic diversity of protozoa [41]. In our research conducted in the years 2014–2022, the taxonomic composition was similar at sites B+ and B−. The number of ciliate taxa did not differ; 20 taxa were recorded in each year. What changed was the dominance of taxa in individual years. Research by Wassen et al. indicates that a gradual decrease in the amount of nutrients in the environment could cause significant changes in the taxonomic structure [42].

The results of the ordination analyses indicate that the main factors determining the occurrence of protozoa in the study area were the concentrations of biogenic substances and chlorophyll *a*. Thus, higher abundance of ciliates was recorded at site B−. Bauer et al. Samuelsson et al. and Gong et al. also demonstrated this relationship [3–45]. In 2022, however, despite the increased content of biogenic substances, ciliate abundance was lower. The lower abundance of this group of protozoa in those years may have also depended on the presence of species from higher trophic levels, which controlled their numbers [46]. The main group of organisms influencing ciliate abundance in peatland ecosystems is rotifers [47,48]. Jurgens et al. found that the abundance of ciliates can be controlled by larger, predatory species [49]. Mieczan et al., however, showed that the abundance of these taxa can also be controlled by other predatory ciliates [50]. Thus, *Paradileptus elephantinus*, *Amphileptus cleparedei*, and *Prorodon* sp. may have controlled the abundance of other taxa [28].

In the B− habitat, as the content of biogenic substances decreased, so did the abundance of ciliates. Bauer et al., Pereira et al. and Buosi et al. also showed a strong relationship between abundance and the availability of nutrients [43,51,52]. Such correlations were also observed by Mieczan and Tarkowska-Kukuryk and by Mieczan et al. [26,50]. In the present study, a significant relationship was also observed between water temperature and ciliate abundance. At site B−, the temperature was higher than at site B+. Correlation analysis showed that temperature had the smallest effect on the abundance of ciliates at sites B+ and B−. Temperature can thus be a factor which contributes significantly to the growth and reproduction of ciliates [24,52–55]. According to Ju et al., water temperature also largely determines the distribution of protozoa, by influencing access to food and oxygen conditions [56]. Chróst and Siudy showed that temperature influenced the abundance of bacteria, which may also indirectly influence ciliate abundance by increasing food resources [57].

According to Lukić et al. the growth rate of organisms and their abundance is controlled by abiotic factors such as temperature and resources such as food [58].

The correlation analysis indicates that at site B−, the factors with the greatest positive effect on the abundance of ciliates were pH, biogenic substances, and electrolytic conductivity. Ciliate abundance was found to be negatively correlated with oxygen concentrations and temperature. At site B+, ciliate abundance was positively correlated with the water level, biogenic substances, and electrolytic conductivity and negatively with chlorophyll *a* and temperature. The negative correlation of ciliate abundance with temperature is particularly interesting. According to Chróst and Siuda and Mieczan et al., higher temperature should increase the reproduction rate of bacteria, which can be food for bacterivorous ciliates [50,57].

#### 4.3. Effect of Restoration on Functional Groups of Ciliates

Ciliates are a key element of aquatic food webs. Ciliates perform the function of consumers—of bacteria, cyanobacteria, and algae [59,60]. On the other hand, they are food for Metazoa, mainly copepods and cladocerans [61]. Ciliates can be a food base for other ciliates and can feed on other ciliates [62]. In addition, they can significantly influence primary production in the aquatic environment [60]. The diversity of this monophyletic group of unicellular eukaryotes contributes to the stability and dynamics of the ecosystem. In view of the influence of ciliates on the ecosystem [60,63], trophic groups of ciliates in the areas undergoing restoration procedures were analysed. The results of previous research suggest that the community of these protozoa is regulated by two main routes: manipulation of environmental conditions (abiotic factors) and changes in the level of interactions between organisms [64–66]. Abundance of ciliates was higher in the B− habitat than in the B+ habitat. Among them were bacterivorous ciliates, whose numbers increased in the spring and summer. At this time, higher temperatures were observed, which favour the reproduction and growth of bacterial communities [67]. It is likely that the increase in the abundance of these microbes increased the food base, which was favourable to the development of bacterivorous ciliates during this period [68]. Research by Andersen (2013) showed a significant relationship between the abundance of bacterivorous ciliates and the abundance of bacteria [32]. In our study, the proportion of algivorous ciliates was smaller than that of bacterivorous species but greater than that of predatory species, at both site B− and site B+. According to Gaiser (2005), high concentrations of biogenic substances, especially nitrogen and phosphorus, favour the development of algae [68]. In our study, the concentrations of nitrogen and phosphorus at site B− were greater than at site B+, and this is most likely why the abundance of algivorous ciliates was higher at this site. Some ciliate species can perform the function of predators and control the abundance of other species in this taxonomic group [69]. In our study, predatory ciliates were dominant in autumn. They can potentially act on several levels in the trophic structure, mainly as primary predators, but also as consumers [70,71]. Predatory *Paradileptus elephantinus*, *Amphileptus cleparedei*, and *Prorodon* sp. were dominant during this period and may have controlled the abundance of other taxa as their potential prey [28]. The last group performing a significant trophic function was mixotrophic ciliates. Mixotrophy is the occurrence of phagotrophy and phototrophy in the same organism. A phagotroph consumes phototrophs, such as flagellates, and leaves them intact, thus deriving organic matter from them [72,73]. Although most ciliates are heterotrophic, many species are mixotrophic [74]. Mixotrophic ciliates can develop in both an oxygen-rich habitat and in water with a low oxygen level [75,76]. The abundance of mixotrophic ciliates is significantly influenced by seasonality. In the present study, mixotrophic ciliates were present in low numbers at both site B− and site B+. Their abundance was lowest in summer and highest in autumn. Similar results were obtained by Mieczan (2002, 2018) and Weisse et al. (2016) [22,60,76]. In these conditions, mixotrophic ciliates can be an important source of food for organisms from higher trophic levels [77,78].

## 5. Conclusions

Our study showed that restoration causes temporary destabilization of environmental parameters. Ciliate communities are excellent indicators of environmental conditions due to seasonal reactions to fluctuating values of parameters such as the concentrations of biogenic substances, oxygen, or chlorophyll *a*. The RDA results showed that ciliates at site B+ were strongly dependent on the concentration of N-NO<sub>3</sub>, oxygen, and chlorophyll *a* and on the water temperature, while at site B−, chlorophyll *a* was the limiting parameter. The RDA analysis also showed that the occurrence of *Chilodonella uncinata* was associated mainly with the water temperature, while the concentrations of O<sub>2</sub> and N-NO<sub>3</sub> mainly determined the occurrence of *Strombidium viride* and *Paramecium bursaria*. In addition, the restoration processes were clearly reflected in the abundance of ciliates. Thus, it can be concluded that ciliates can be an excellent group of indicator species useful in monitoring the effects of peatland restoration.

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