Phytoplankton communities of temporary ponds under different climate scenarios: Experiments on vernal pool microcosms.

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Abstract

Temporary water bodies, especially vernal pools, are the most sensitive to climate change, yet the least studied aquatic environments. Their functioning largely depends on the phytoplankton communities structure. This study aimed to determine how temperature and photoperiod length (simulating inundation in different parts of the year under six climate scenarios) affect the succession and the structure of phytoplankton communities soon after inundation. For longer photoperiods and at lower temperatures in vernal pool microcosms (simulating a cold spring after a warm snowless winter), the phytoplankton community evolved into chlorophytes and cryptophytes. At short photoperiod (inudation in winter, followed by freezing of the water surface) the communities evolved into the euglenoids. Medium temperatures and long photoperiods (late inundation during cool spring) promoted the development of chlorophytes, with high total phytoplankton abundance as well as species richness and diversity. The lack of cyanobacteria dominance, suggests that they will not be the leading group in vernal pools in the temperate zone with progressive global warming. Our study shows that climate change will result in the seasonal shifts of the species abundance or even in their disappearance, and finally in strong changes in the biodiversity and food web of aquatic ecosystems in the future.

Introduction

A major feature of temporary waters is their cyclic nature, with recurring watery and dry phases. In some cases, this cycle is very regular: vernal pools fill with water every late winter and desiccate with the onset of summer. However, it is not clear what will happen if this natural cycle is shifted as a result of climate change. Will a plankton community of vernal pool keep

its character when the watery phase starts in the early summer, or will there be an absolutely new microcosm established?

Climate change has a huge impact on aquatic ecosystems, which is particularly relevant in the case of biotic interactions, like boundary shifts, behavioural and physiological adaptations, or changes in phenology and community structure [1–3]. The specific effects of climate change in water ecosystems (e.g., increase in solar radiation or rainfall, decrease in wind speed) will vary among regions and water body types [2]. While one of the most deleterious components of climate change for freshwater environments is global warming [4], knowledge on its effect on the quantitative and qualitative changes in aquatic communities is not balanced over different types of ecosystems [5]. Numerous studies of climate change impact have primarily focused on larger and permanent water bodies, such as lakes, sea, and oceans (e.g., [2,6–9]). There is still a lack of knowledge about the effects of global warming on the functioning of small water bodies - especially temporary ones - which are the most sensitive to climate change among aquatic environments [10–12].

Temporary water bodies are an extremely valuable yet at the same time poorly studied type of surface water [13]. As one of the types of small water bodies, they are, however, perfect sites for a broad range of ecological research and the monitoring of global environmental change. They play vital roles in the human-transformed landscape, constituting significant biodiversity hotspots and taking part in flood control, ground water recharge, the recycling of nutrients, and toxicant removal [14–17]. In the era of water deficits caused by global climatic changes, their role in stabilising local groundwater balance is especially important [16].

Among all types of temporary waters, snow-fed vernal pools of the temperate climatic zone seem to be one of the most sensitive to climate change. They are usually shallow, small, and ephemeral water ecosystems with a dry period recurring in the late spring every year and

lasting until the next year's snow thaws [13]. Due to their size and drying cycle, they respond rapidly to environmental changes [18] and are characterised by a greater fluctuation of abiotic factors in comparison to larger water ecosystems [19]. Moreover, the functioning of such ecosystems to a large degree depends on the labile structure and function of phytoplankton communities, being an important component and primary producer group [20]. Any changes at the base of the aquatic foodweb are instantly translated into the shape of the whole ecosystem [21].

After each dry period, phytoplankton communities are in fact formed *de novo*, mostly through secondary succession from resting cells preserved in the bottom sediments. The process of recolonisation after the inundation of ponds is crucial for the future structure of communities, but the factors affecting its course and the shaping of algal communities at the beginning of the hydroperiod are still poorly known. Phytoplankton react quickly to changing environmental conditions [22], due to their short life cycles; thus, it is a useful model group for research into the influence of environmental variables on the course of such succession. Furthermore, the phytoplankton of temporary ponds is dominated by fast-growing singlecelled r-strategists and opportunists, which are adapted to unstable conditions in rapidly changing environments [13,20,23]. Their course of succession is thus possible to trace over a relatively short period of time since the core of the phytoplankton community should be established shortly after inundation. Its structure is subsequently altered by the response of particular species to biotic factors, e.g., macrophytes [21,24] and filtrators [20], as well as by abiotic factors, like temperature, light, pH, and nutrients [24,25].

Temperature is one of the most important climate-related abiotic factors, which can strongly influence the phytoplankton community the onset of the hydroperiod [23,26,27]. Global warming is known to cause changes in phytoplankton community dynamics [28–30], species

composition and abundance [31,32], favouring those species that are best adapted to changing conditions. A number of studies (none of them conducted on temporary waters, however) have generally indicated decreasing plankton diversity, increasing small-sized picophytoplankton abundance, and cyanobacteria blooms as the most evident effects of global warming in water ecosystems [23,27,32–34].

Shifts in temperature due to climate change largely interact with another climatic factor determining the functioning of phytoplankton communities: light conditions, on which the photosynthesis of algae largely depends. The light climate – especially in temperate areas of higher geographic latitudes – primarily depends on the length of the day (photoperiod); as such, it is highly related to seasonality, making its influence on phytoplankton difficult to disentangle from other climatic conditions (especially temperature). Consequently, this interaction is largely neglected in the literature. As a result, species specificity of microalgal light and temperature requirements is not well recognised. Some studies have dealt with the effects of temperature and/or photoperiods in controlled conditions, focusing on selected taxonomic groups only – e.g. diatoms, cyanobacteria and chlorophytes. Most of the studies concentrated on particular species, e.g. *Cryptomonas* sp. and *Dinobryon* sp. [35], *Alexandrium* sp. [36], *Nannochloropsis* sp. and *Tetraselmis* sp. [37], *Spirulina platensis* [38], *Stephanodiscus minutulus* and *Nitzschia acicularis* [39] or *Thalassiosira* sp. [40], and their response to the different temperature and/or photoperiod (light intensity). There is a lack of knowledge about the influence of these two factors on the whole phytoplankton communities in terms of time, especially from temporary water bodies, a lack that is particularly important in light of the ongoing climate change.

This study was aimed to fill this gap. Our objective was to determine to what degree and in what way photoperiod length and temperature (as a reflection of different climate scenarios) affect the process of secondary succession of algae and the subsequent structure of

phytoplankton communities at the onset of the hydroperiod. Based on our data collected in the field, we expected that conditions prevailing immediately after the inundation of ponds should determine the course of phytoplankton secondary succession and shape the subsequent structure of their communities. Hence, following the predictions made by climate change scenarios, we assumed that the shape of the vernal pool phytoplankton community will significantly differ if the start of the water phase is shifted over time (resulting in an inundation under atypical day length conditions) or accompanied by altered temperatures.

To test this assumption, we conducted experiments under controlled laboratory conditions using a microcosm array, testing for the influence of temperature and photoperiod length on the whole phytoplankton communities. Our general hypothesis is that these two factors significantly influence the phytoplankton community structure at the initial stage of succession and this alteration translates into the shape of communities later in the season. We treat particular combinations of temperature and photoperiod as an equivalent of different climatic scenarios, with photoperiod length reflecting the day length when inundation is shifted towards winter or late spring. Our detailed hypotheses are: 1) particular phytoplankton species and taxonomic groups initiate succession depending on different combinations of temperatures and photoperiod lengths (reflecting particular climate scenarios), so that the succession sequence differs between the climate scenarios; 2) regardless of the time factor, particular algal groups and species respond differently to each photoperiod and temperature combination (to different climate scenarios); 3) cyanobacteria dominate with higher temperatures and longer photoperiods; and 4) the vernal pool phytoplankton diversity decreases with increasing temperature (with climate warming).

Results

There were no significant differences between the three repetitions of the experiment with respect to the number of taxa (Chi = 7.5226; P_{adj} = 0.0697), the abundance of phytoplankton (Chi = 0.8938; P_{adj} = 0.9211) and the values of the Shannon-Weaver diversity index (Chi = 1.5505; P_{adj} = 0.9211), Appendix S1 (Figs 1A-1C).

Taxonomic richness in particular experimental treatments

In total, 198 phytoplankton taxa from 8 taxonomic groups were identified. The most taxon-rich groups were chlorophytes (80 taxa), euglenoids (43 taxa) and diatoms (34 taxa). Less numerous with respect to taxonomic richness were cyanobacteria, cryptophytes, dinoflagellates, xanthophytes and chrysophytes (21, 12, 4, 3, 1 taxa respectively), see Appendix S2.

A significant influence of photoperiod length on the total number of taxa was found (Chi = 64.6387; $P_{adj} < 0.0001$): the longer the photoperiod, the higher the number of phytoplankton taxa (at the photoperiod 0, 8, 16 and 24 h the maximum numbers of taxa were respectively: 27, 34, 45 and 50), see Appendix S1 (Fig. 2A) and Appendix S3. Also, the temperature was a factor that significantly affected the number of taxa (Chi = 30.5327; $P_{adj} < 0.0001$), Appendix S1 (Fig. 3A). The greatest taxonomic richness, regardless of photoperiod length, was always observed at 16°C. At this temperature, when the photoperiod was long (16 and 24h), chlorophytes were the most taxon-rich group, while at shorter photoperiods, diatoms and euglenoids were always the two groups with the highest number of taxa (see Appendix S3).

During the experiment, the total number of taxa did not significantly change over time (Chi = 6.0532; P_{adj} = 0.1952) (Fig. 4A in Appendix S1). As a consequence, there was no influence of photoperiod or temperature on the changes in taxonomic richness over time (interaction time x photoperiod: P_{adj} = 0.2793; time x temperature: P_{adj} = 0.4003). In the initial stage of succession (first and/or second week of investigations), the most numerous were always

the representatives of diatoms (the most frequent were: *Eunotia bilunaris, Hantzschia amphioxys, Nitzschia palea, Pinnularia mesolepta, Pinnularia viridis, Stauroneis anceps* f. *gracilis, Stauroneis phoenicentron*) and euglenoids (the most frequent was *Trachelomonas volvocinopsis*), regardless of the photoperiod or temperature (see Appendix S2). In the subsequent weeks, the representatives of chlorophytes became the most numerous in the taxa and their participation in the qualitative structure of phytoplankton increased, especially for the photoperiods of 16 h and 24 h.

Changes in Shannon-Weaver diversity index

The mean overall values of Shannon-Weaver diversity index did not change significantly over the time of the experiment when all of the treatments were compared (Chi = 7.8728; P_{adj} = 0.1927) (Fig. 4C in Appendix S1). Nevertheless, significant differences in phytoplankton diversity were found when treatments conducted under particular temperature regimes were compared (Chi = 11.1664; P_{adj} = 0.0112): at 16°C, the highest values of the index were noted and the clearly lowest values were observed at 25°C, Fig. 3C in Appendix S1. On the other hand, the photoperiod length did not significantly differentiate the treatments with respect to the values of Shannon-Weaver diversity index (Chi = 2.40896; P_{adj} = 0.4920), Fig. 2C in Appendix S1. Nonetheless, both photoperiod and temperature significantly influenced the dynamics of the phytoplankton diversity changes over time (interaction with time: $P_{adj} < 0.0001$ in case of both factors). The values of the index varied between 0.013 and 2.795 (data in Appendix S4). The highest mean value was noted at the 8 h photoperiod and temperature of 16°C in the first week of the experiment, while the lowest mean value was found at the 0 h photoperiod and a temperature of 25°C in the last week (Figs 1A-1C). At the temperature of 4°C, a relatively slight fluctuation over time in the values of Shannon-Weaver diversity index was found, when compared to other thermal conditions. The highest mean values of the index

at the temperature of 4°C were noted in the last week of investigations at the 0, 8 and 16 h photoperiods, and in the 4th week at the 24 h photoperiod. However, at 16°C and 25°C, the opposite was true: the highest index values were found in the first week at the 0, 8 and 16 h photoperiods, but the highest values were found in the 5th week (at 16°C) and 2nd week (at 25°C) for the 24 h photoperiod.

Effect of temperature and photoperiod on phytoplankton abundance

Photoperiod and temperature significantly influenced the total phytoplankton abundance (respectively: Chi = 81.2682; $P_{adj} < 0.00001$ and Chi = 7.4751; $P_{adj} = 0.0238$), Figs 2B and 3B in Appendix S1. As expected, in all of the temperature treatments, the longer the photoperiod was, the higher the average total phytoplankton abundance was. In the case of temperature, under almost all light conditions, the highest values of abundance were noted at 16ºC, see Appendix S5. At the 0 h photoperiod, the abundance was 4916 indiv. mL^{-1} (domination of cyanobacteria, chlorophytes or cryptophytes), at 8 h it was 11,580 indiv. mL^{-1} (chlorophytes or diatoms dominated) and at 24 h the maximum abundance reached 15,128 indiv. mL^{-1} (cryptophyte domination). Only at the 16 h photoperiod was the highest phytoplankton abundance found at 4ºC (15,493 indiv. mL^{-1} with cryptophyte domination).

Changes in phytoplankton abundance over time

The total phytoplankton abundance changed significantly over the course of the experiment (Chi = 25.8363; $P_{adj} = 0.0001$), Fig. 4B in Appendix S1. In general, the abundance was increasing until the fourth week of the experiment, and then decreased. There was no significant impact of photoperiod and temperature on these changes in abundance over time (interaction time x photoperiod: $P_{adj} = 0.0991$; time x temperature: $P_{adj} = 0.2237$). Despite this, some clear trends were observed (see Appendix S5). As expected, the numbers of phytoplankton individuals were lowest at the 0 h photoperiod compared to the other photoperiod lengths and

remained stable during consecutive samplings, regardless of temperature. At the 8 h photoperiod, the total abundance of phytoplankton gradually increased over time and consistently reached the highest values in the last week of the experiment. At the 16 h photoperiod, the abundance increased over time until the third week (with the domination of cryptophytes at 4 and 25°C and chlorophytes at 16°C), before decreasing. At the 24 h photoperiod, the abundance increased until the 3rd or 4th week (with a domination of cryptophytes at 4°C and chlorophytes at 16°C), before decreasing. The highest fluctuations in the total phytoplankton abundance over time were observed at the photoperiods of 16 h and 24 h.

Chlorophytes, diatoms and cryptophytes dominated quantitatively, Figs 2A-2C. The share of diatoms and euglenoids in the first two weeks of the investigation was always the highest; in the following weeks, they were replaced by chlorophytes and/or cryptophytes, regardless of photoperiod and temperature. The share of chlorophytes increased over time at 4°C and at all photoperiods until the 3rd or 4th week, before decreasing. At 16°C, the share of chlorophytes increased over time until the 4th week and then decreased for the 0, 16 and 24 h photoperiods. At 25°C, the share of chlorophytes increased at the 0 and 8 h photoperiods throughout the research period, while at the 16 and 24 h photoperiods, this decreased in the last week.

The greatest share of chlorophytes (above 80% of the total phytoplankton abundance) was always noted at the 8 h photoperiod, especially at 16°C (almost 100%), Figs 2A-2C. The share of cryptophytes was the highest at the 16 and 24 h photoperiods, especially at 4°C and 25°C, but their abundance varied over time.

Response of phytoplankton taxonomic groups to particular experimental treatments

According to statistical analysis, the abundance of the following phytoplankton taxonomic groups (see Appendix S6) significantly changed over time, regardless of photoperiod and temperature: cyanobacteria (Chi = 31.7312; $P_{adj} < 0.0001$), chlorophytes (Chi = 44.0866; $P_{adj} < 0.0001$), euglenoids (Chi = 16.8724; $P_{adj} = 0.0102$), and cryptophytes (Chi = 28.5262; $P_{adj} < 0.0001$). The abundance of cyanobacteria increased until the 3rd week of investigations and then decreased, the abundance of chlorophytes and cryptophytes increased until the 4th week and then decreased, while euglenoids had a peak of abundance in the first week and their abundance decreased in the following weeks.

Photoperiod had a significant impact on the following phytoplankton groups: cyanobacteria (Chi = 37.4673; $P_{adj} < 0.00001$), chlorophytes (Chi = 63.0565; $P_{adj} < 0.0001$), diatoms (Chi = 61.8816; $P_{adj} < 0.0001$), cryptophytes (Chi = 84.6732; $P_{adj} < 0.0001$), dinoflagellates (Chi = 10.9841; $P_{adj} = 0.0354$) and xanthophytes (Chi = 15.9802; $P_{adj} = 0.0046$), see Appendix S6. Diatoms clearly had the highest abundance at the 16 h photoperiod compared to the other photoperiod lengths, while cyanobacteria, chlorophytes and cryptophytes clearly had the highest abundance for the photoperiod of 24 h.

The temperature significantly influenced the abundance of cyanobacteria (Chi = 19.4109; $P_{adj} = 0.0004$), euglenoids (Chi = 12.8766; $P_{adj} = 0.0096$), diatoms (Chi = 19.8323; $P_{adj} = 0.0003$), dinoflagellates (Chi = 11.0995; P adj = 0.019) and chlorophytes (Chi = 8.4337; P adj = 0.0590; P = 0.0147), see Appendix S6. All of these groups had the highest numbers of individuals at 16ºC (except dinoflagellates, reaching the highest abundances at 25ºC).

The interaction of time and photoperiod was only significant in the case of chlorophyte ($P_{adj} < 0.0001$) and xanthophyte ($P_{adj} = 0.0492$) abundance. The interaction of time with

temperature had no significant impact on the abundance of any taxonomic group (in the case of chlorophytes, the model was significant before the Holm's adjustment was performed: $P = 0.0206$; $P_{adj} = 0.0902$).

The relations mentioned above are reflected by the results of the PRC analysis (Fig. 3). According to the initial analysis, throughout the experiment, the communities could evolve in three general directions, hereafter referred to as three community types: (1) towards chlorophyte and/or cryptophyte dominance; (2) increasing diatom and cyanobacteria abundance; and (3) domination of euglenoids-xanthophytes-chrysophytes-dinoflagellates. This partitioning was illustrated by the first canonical axis of RDA analysis (eigenvalue = 0.412, significant at $F = 118.614$, $P < 0.001$), which was subsequently used for the analysis of trends by the means of PRC (Fig. 3, right side of the graph).

According to the resulting graph, particular experimental treatments grouped nicely with respect to the photoperiod lengths (marked with colours at Fig. 3). The treatments with the longest (24h, red colour) and the second-longest (16h, green) photoperiods displayed very similar patterns. Their phytoplankton communities were quickly dominated by chlorophytes and cryptophytes (type 1). In the case of those in which temperature was the highest, however, there was a visible turning point after the second week of the experiment and the communities turned towards the domination of diatoms and cyanobacteria (type 2). Communities in the lines with the 8h photoperiod slowly and gradually transformed from type 3 communities towards type 2. Treatments from the control groups, kept in the dark for the entire period, corresponded to type 3, with no visible change over time. No such clear pattern grouping the experimental lines was visible if the treatments were arranged with respect to temperature (the same line patterns, Fig. 3), except for those kept at the highest temperature (solid lines, Fig. 3). Although the trajectory of changes was different for each of these lines, all of them seemed to aim at the diatoms/cyanobacteria as their final community type.

Species-level response of phytoplankton communities to experimental conditions

The species-level CCA analyses were performed on the 41 most dominant and frequent species (Fig. 4). The majority of such species belonged to chlorophytes, diatoms and cryptophytes (respectively: 22, 8 and 7 taxa). The model showed that changes in the structure of phytoplankton communities at the species level were significantly influenced by both temperature ($P = 0.001$; $F = 4.65$) and photoperiod length ($P < 0.001$; $F = 7.43$). According to the results, changes in abundance over the time of the experiment for some chlorophytes (*Spirogyra* sp., *Chlamydomonas sp.* 2) and cryptophytes (*Cryptomonas marssonii*, *Chroomonas minuta*) were positively associated with both photoperiod and temperature, while diatoms (*Nitzschia hungarica, Nitzschia palea, Navicula* sp., *Stauroneis anceps* f. *gracilis*) were negatively affected by these parameters. The dynamics of a large group of species (mostly chlorophytes: *Oedogonium* sp., *Uronema intermedium, Uronema confervicolum, Haematococcus pluvialis, Monoraphidium griffithii, Schroederia setigera, Planctococcus sphaerocystiformis, Phacotus lenticularis, Pseudosphaerocystis lacustris, Chlamydomonas passiva, Tetraspora gelatinosa, Chlorogonium elongatum* var. *aculeatum*) was positively correlated with photoperiod length and negatively with temperature. Changes in the abundance of diatoms (*Hantzschia amphioxys, Eunotia bilunaris, Navicula minima*) and the chlorophyte *Chlorogonium elongatum* were negatively correlated with photoperiod length only. Moreover, the abundances of *Cryptomonas phaseolus, Chlamydomonas* sp. and *Euglena* sp. increased over time in treatments at higher temperatures. The whole model was significant: $P < 0.001$; $F = 6.117$; significance of the first canonical axis: $P < 0.001$; $F = 7.396$; eigenvalue of the first axis: 0.238; second axis: 0.146.

Discussion

Our results suggest that, with progressive climate changes in the temperate climate zone, the highest species richness of phytoplankton (especially chlorophytes) would be observed under scenarios predicting late spring inundation with mild temperatures (16°C). This is in contrast to experiments conducted on communities forming permanent ponds where the phytoplankton taxonomic richness increased with warming [41]. However, in parallel to our results [42] showed, that diatom species richness was reduced by increased temperature. Our findings underline threats to vernal pools associated with global warming: medium temperatures and long photoperiods promote species richness in this type of temporary pool.

Interestingly, in the first and/or second week of our investigations, the most numerous were always the representatives of single-celled diatoms, independent of photoperiod and temperature. Among the diatoms, there were epontic (adopted to firmly attaching to substratum, e.g. *Eunotia bilunaris* [43]) benthic (living on, in, or near the bottom: *Hantzschia amphioxys*, *Pinnularia mesolepta*, *Pinnularia viridis*, *Stauroneis anceps* f. *gracilis*, *Stauroneis phoenicentron*) as well as epontic and benthic species (*Nitzschia palea*). Benthic and/or epontic forms are known to be typical for shallow water bodies [44,45], where they often become a part of the phytoplankton communities as a result of intensive water mixing. According to [43], *Eunotia bilunaris* and *Stauroneis phoenicentron* are common in periodic waters, including temporary ponds. In the first week/weeks of the experiments, the frequent appearance of euglenoids (especially *Trachelomonas volvocinopsis*) was also observed, which is typical for ponds enriched in organic matter [46]. In the subsequent weeks, the number of diatoms and euglenoids taxa decreased under all climatic scenarios, and representatives of chlorophytes became the most numerous, especially at the 16 and 24 h photoperiods.

As expected, species richness in the investigated samples was very high (198 taxa in total), which is characteristic for small water bodies as a consequence of favourable environmental conditions (rapid heating of water, large quantity and availability of nutrients, favourable light supply, quick succession processes) and shallow depth (enrichment of phytoplankton communities by originally benthic species). On the other hand, unstable environmental conditions associated with high fluctuations of the physical and chemical parameters of water, may also promote the exceptionally high species richness in ephemeral habitats [13].

The total phytoplankton abundance changed significantly over the duration of the experiment, regardless of the photoperiod and temperature. The abundance increased until the 4th week of the study (which was mostly connected with changes in the abundance of chlorophytes and cryptophytes) and then decreased. Frequent changes in the phytoplankton abundance are characteristic for the small water bodies. They are dominated by small-sized single cell microalgae, fast-growing r-strategists (e.g. cryptophytes and chlorophytes with a short life cycles), which are, according to our research, adapted to living in unstable and rapidly changing environmental conditions, and mainly prevailing in temporary ponds [20].

Small algal species, dominating in our experiments, are known to be at a selective advantage at high temperatures and low nutrient concentrations, and also tend to dominate phytoplankton communities under these conditions [47]. As a consequence, increasing water temperature favours the development of small algal species [34]. Thus, from the global perspective, we would expect to see domination of small-sized freshwater phytoplankton in the future, as an effect of warming [48]. According to another study [41] however, phytoplankton communities in the warmed treatments are dominated by larger species. Our findings seem to bring a compromise to these two opposing claims: we showed species-specific differences in responses to warming among small-sized microalgae, suggesting that we should not generalise

findings when taking into account only the size of the species or their life strategies. This is of a great importance in the context of trophic interactions in water ecosystems. Small-celled algal species play a major role as food resources for zooplankton, so their presence in water environments provides a survival benefit for small animals (especially crustaceans), which is especially important in the era of progressive global warming.

Chlorophytes, diatoms, euglenoids and cryptophytes were the major groups, which dominated quantitatively. Interestingly, similarly to the results of phytoplankton qualitative analysis regarding the successive sequence of taxonomic groups, the share of diatoms (e.g. common in periodic waters *Nitzschia hungarica* and *Eunotia bilunaris* [43]**)** and euglenoids (mainly *Trachelomonas volvocinopsis*) in the total phytoplankton abundance in the first couple of weeks was always the biggest. In the following weeks, they were replaced by chlorophytes and/or cryptophytes, regardless of the photoperiod and temperature. These findings were inconsistent with one of the research hypothesis, because the successional sequence of phytoplankton groups was the same in various combinations of photoperiod and temperature: diatoms and euglenoids were replaced by chlorophytes and/or cryptophytes, despite different climatic scenarios. In line with our results, [28] showed that climate change did not appear to affect the successional pattern of phytoplankton taxonomic groups in general. Similarly, [49] confirmed that the changes in phytoplankton community dynamics in the experiments with warming was slight. On the other hand, some studies [50] showed that temperature may influence the germination of algae resting stages and modify the succession. However, it is not consistent with our results on the level of the initial successional sequence of dominating phytoplankton groups.

Previous experimental data reveal that nutrient limitation plays an important role in the algal succession. Similarly to our observations, [28] demonstrated that the initial dominance of

diatoms was found in all of the climate scenarios, but they decreased from the start of the experiment. This was explained as a result of the low availability of silicate in combination with relatively high sinking rates of diatoms, because silicate depletion is known to increase sinking rates. In the case of euglenoids (especially species of the *Trachelomonas* genus), which also dominated at the first weeks of experiments, they are known to prefer high concentrations of organic matter, nitrogen and phosphorus [51]. Some of these compounds could be found in sufficient quantities in the initial stages of the experiment and were released into the water from sediments right after the inundation of the aquariums. That might be the reason for the high abundance of euglenoids in the first week of the investigations. On the other hand, representatives of this phytoplankton group are known for their ability to survive in unfavourable environments [52], like temporary ponds. Similar to our results, [53] also described the dominance of euglenoids in phytoplankton abundance was described in the earlier stages of community development of the mining lake; however, it was explained by the high values of water colour. This could also explain the highest abundance of euglenoids and diatoms in the first week of our study and seems to be the most likely reason. The water was turbid in the first days of our experiment as a result of mixing of the sediments after inundation, which can also be observed in the field in vernal pools immediately after thawing, followed by surface runoff.

Poor light conditions gave a competitive advantage to diatoms and mixotrophic euglenoids over other algae that need more light to photosynthesise from the beginning of the succession. Thus, the phytoplankton groups which are able to survive at low light availability (at least for a period of time) initiated the phytoplankton succession. Our statistical analysis confirmed that photoperiod did not affect the abundance of euglenoids. Moreover, they are known for their fast reproduction [52], additionally explaining their initial dominance and rapid decline after the first week of the investigations, regardless of photoperiod. Diatoms, similar to mixotrophic euglenoids, are known to survive under low light intensity [54,55], but only for a

period of time. Their ability to survive prolonged darkness and adaptation to high turbulence is associated with a reduction of metabolism [3], so they are successful competitors in deep mixing conditions. Moreover, diatom taxa in our samples were originally epontic or benthic, so they probably entered phytoplankton communities via the water mixing.

Despite the fact that photoperiod and temperature did not affect the dynamics of the total phytoplankton abundance over time, these factors (especially photoperiod) significantly influenced changes in abundance of one of the major groups - chlorophytes – throughout the experiment. The PRC analysis relating to the courses of the phytoplankton succession showed that communities clearly evolved into chlorophytes and cryptophytes dominance at longer photoperiods (16 and 24 h) and at low or medium temperatures (4 and 16°C) at the same time. These findings suggest that, with progressive climate changes in the temperate zone, the domination of cryptophytes and chlorophytes in phytoplankton communities will be observed under scenarios predicting a late spring inundation with lower temperatures and a dry, snowless winter. According to [49], cryptophytes were more abundant in lower temperatures than at higher ones, similar to our results. Moreover, [56] demonstrated, that chlorophytes also showed a trend to perform better at lower temperatures in mixed phytoplankton communities in controlled laboratory experiments. However [57] and [22] stated that microalgal chlorophytes prefer warm waters.

Our study showed that climate change will result in seasonal shifts of species abundance or in their disappearance. Most of the dominating phytoplankton taxa belonged to chlorophytes. Among them, many species (*Haematococcus pluvialis*, *Monoraphidium griffithii*, *Schroederia setigera*, *Planctococcus sphaerocystiformis*, *Phacotus lenticularis, Pseudosphaerocystis lacustris, Chlamydomonas passiva, Tetraspora gelatinosa, Chlorogonium elongatum* var. *aculeatum* and filamentous *Oedogonium* sp., *Uronema intermedium, Uronema confervicolum*)

increased their abundances over time at long photoperiod lengths and lower temperatures. These findings suggest that the warming will not favour these species and they could even be in danger of extinction in some climate zones. On the other hand, in the temperate climate zone with progressive climate changes, a further increase in chlorophytes could be expected under scenarios predicting a dry, snowless and cold winter/spring with a late spring/summer inundation. Some other species in our experiment, like pennate diatoms (*Hantzschia amphioxys, Eunotia bilunaris, Navicula minima, Nitzschia hungarica, Nitzschia palea, Navicula* sp., *Stauroneis anceps* f. *gracilis*) and the flagellated chlorophyte *Chlorogonium elongatum* preferred a short photoperiod. In line with our results, [40] reported that a short photoperiod favours larger diatom taxa. According to [55], diatoms have an advantage in regions with a prolonged absence of irradiance. Thus, a large portion of the year in Polar Regions and winter seasons in the temperate climate zone seem to favour the development of these species because of the short photoperiod. This situation will change in the future, if vernal pools inundate later in the season (lack of snow cover and thus no surface runoff connected with thawing) or will not freeze during winter due to progressive warming.

Temperature did not significantly influence time changes in the abundance of the majority of taxonomic groups present in our experiment (except chlorophytes - this relation was not significant after applying Holm's correction, though). However, it did affect the abundance of particular species: the number of individuals of *Cryptomonas phaseolus, Chlamydomonas* sp. and *Euglena* sp. increased more dynamically in treatments with higher temperatures. Thus, under climate change scenarios, assuming an increase in temperature after the inundation of the ponds (dry winter, hot and rainy spring/summer season in the temperate zone), these species will have a competitive advantage over other taxa. Changes in abundance over time in some of the species were positively affected by both photoperiod and temperature; these were the chlorophytes: *Spirogyra* sp., *Chlamydomonas sp.* 2 and cryptophytes: *Cryptomonas marssonii*

and *Chroomonas minuta*. These species will dominate the communities of temporary pools if their water phases shift from the winter/spring to late spring/summer inundation (under scenarios predicting dry winter and hot spring/summer with heavy showers in the temperate zone). The development of such species favoured by warming could be further stimulated by the release of phosphorus from the bottom sediments induced by climate changes [58]. Such an indirect influence of global warming may thus enhance the domination of species favoured by shifts in colonisation patterns.

Changes in the abundance of some diatoms over time (*Nitzschia hungarica, Nitzschia palea, Navicula* sp., *Stauroneis anceps* f. *gracilis*) were negatively affected by photoperiod and temperature. According to our results, these diatom species and those mentioned before are the pioneering components of phytoplankton communities, dominating at the onset of the hydroperiod in vernal pools under natural conditions (poor light conditions due to initial turbidity, freezing and short day and often low temperature). Such conditions were observed under recent climatic scenarios, typical for vernal pools in the temperate zone: a cold and snowy winter, when ponds fill with water from transient snowmelts in February and then the surface freezes. Such environmental settings were represented in our experiments in treatments with low temperatures and no light (simulating freezing of the water surface) or an 8 h photoperiod (early inundation but no freezing). Thus, the negative relation between the dynamics of these species and photoperiod length as well as temperature shows that they are under strong competitive pressure when inundation occurs later in the season or winters become warmer. Therefore, with the progressive global warming, a number of vernal diatom species could be in danger of extinction in some regions of the world.

Our results showed that the temperature 16ºC (regardless of the time factor) favours the most abundant phytoplankton groups: chlorophytes, diatoms and euglenoids. As a result, the

total phytoplankton abundance was also highest at this temperature; this was in contrast to our assumptions that phytoplankton abundance would be the highest in treatments with the highest temperature. This result could be caused by the fact that the microalgae in our experiment came from the sediments of the temperate climate vernal pool, usually desiccating before the average water temperature exceeds 16ºC. Thus, dormant stages of species adapted to the lower temperatures of the spring season prevail in the sediments. Under global warming scenarios predicting an increase in spring temperatures and heat wave frequencies, not only the pioneering species at the beginning of the water phase will be threatened. Entire algal communities, including the species dominating in the late phases of the hydroperiod, will be out of their temperature optima. Such a disturbance will vastly influence functioning of the whole ecosystem, which is largely dependent on the phytoplankton and invertebrate filterfeeders [20]. On the other hand, if climate changes in the central European temperate zone cause the local lowering of summer temperatures, as predicted by some scenarios, these algae should also dominate in early summer if the hydroperiod extends.

Interestingly, despite finding that cyanobacteria were favoured by a long photoperiod (24 h), as we expected, the results of our experiments disprove the hypothesis that this group will dominate in vernal pools with an increase in water temperature. Contrary to expectations, in our study, cyanobacteria as a group did not dominate the phytoplankton communities and did not achieve very high abundances, even at the highest water temperatures combined with long photoperiods. This is surprising and inconsistent with many other studies (conducted on permanent water bodies), which showed that cyanobacteria have a high growth potential at elevated temperatures [59–63] ranging from 20 to 35ºC [22], and they usually increase their abundance and biomass with global warming [27,33,64]. According to [65] and [66] climate warming may also favour cyanobacteria indirectly, by enhancing the eutrophication of freshwater environments [28,67]. An experimental studies upon the climate changes in a shallow subtropical lake [68] showed,

that phytoplankton community structure was more affected by nutrient enrichment than by temperature increase. While [27] found that warming in combination with high nutrient concentrations reduce the abundance of cyanobacteria. Our results showed that thermal conditions indeed significantly affected the abundance of cyanobacteria, but the optimal temperature for this group in the experiment was 16ºC and not higher. Moreover, we found that changes in the abundance of species dominating among cyanobacteria (*Chroococcus* sp. and *Hyella* sp.) over time were not significantly associated with temperature. These findings suggest that with progressive climate changes in the temperate zone, a higher abundance of cyanobacteria will be observed under scenarios predicting a dry, snowless winter and a mild, rainy end of the spring with medium temperatures.

There could be various reasons for the small share of cyanobacteria in the total phytoplankton abundance and composition, even at high water temperatures. Collecting data in the field from the same vernal pool, we also found a relatively low abundance of the bluegreen algae; thus specific conditions in our experiment (e.g. no external source of nutrients) should not be the explanation. Temporary waters are highly susceptible to rapid heating and cooling, not just seasonally, but also daily or even hourly, due to their small area and depth [13]. High fluctuations in temperature, together with frequent water mixing, seem to provide unfavourable conditions for cyanobacteria. On the other hand, cyanobacteria in phytoplankton communities could lose in competition with the species that are typical for small water bodies, like euglenoids or fast growing single-celled chlorophytes, cryptophytes and originally benthic diatoms, which tolerate low water temperatures, water mixing, lower nutrient concentrations and/or are characteristic for the initial stage of the succession. Moreover, in the temperate zone, cyanobacteria are known to dominate the freshwater communities during the summer season, when ephemeral freshwater bodies (vernal pools especially) are often already dry. According to our study, vernal pools seem to be inhabited by specific cyanobacteria species, which prefer

lower water temperatures and tolerate unstable environmental conditions. The effects of warming can vary even between cyanobacteria genera [27]. Nonetheless, their dominance as a group in vernal pools may not occur, as can be seen from our research.

In line with our initial hypothesis, the phytoplankton species diversity decreased with higher temperatures (with climate warming), which was in accordance with many earlier findings [23,69–73]. However, some other experimental studies showed the exact opposite pattern: the Shannon Diversity Index increased with warming [41] or changes in temperature did not affect the diversity index [74]. In our study, the lowest values of Shannon Diversity Index were observed at 25°C, while the highest values were noted at 16°C. These results suggest that the highest portion of the present species diversity will be conserved under scenarios predicting elongation of the water phase and the lowering of summer temperatures, according to predictions of models for central Europe [40,75,76]. The temperature and photoperiod also had a great impact on the phytoplankton diversity changes over time. In the course of succession, higher temperatures seem to favour the decline in species diversity, especially when the water phase starts earlier in the season, as in the case of climate change scenarios predicting a shift towards warm and rainy winters in the temperate zone.

Our findings suggest that global warming will strongly affect phytoplankton community structure and dynamics in vernal pools, but mainly at the species level. The initial successional sequence of dominating phytoplankton groups (both at the level of qualitative and quantitative structure) was not affected by temperature and photoperiod, so climate change should not influence the communities in this way. Diatoms and euglenoids initiated the successional process and were quickly replaced by chlorophytes and cryptophytes, regardless of the climate scenarios. Photoperiod and temperature also did not affect the total number of phytoplankton species and abundance over time. However, we found that photoperiod and temperature

influenced the abundance of particular taxonomic groups. At long photoperiods and lower temperatures (dry winter and cooler spring/early summer with late spring inundation in the temperate zone in the future, according to some predictions), the phytoplankton community evolved into chlorophytes and cryptophytes. In short photoperiods (recently, the winter season) the communities evolved into euglenoids, xanthophytes, chrysophytes and dinoflagellates.

Regardless of time, the temperature of 16ºC and long photoperiod (cooler late spring in temperate zone in the future) were the best conditions for the studied phytoplankton communities. Such thermal conditions favoured the development of the major phytoplankton group (chlorophytes), the total phytoplankton abundance, species richness and diversity. It therefore follows that communities in vernal pools seem to be adapted to cooler temperatures (probably due to the high and frequent fluctuations of temperature) and often long photoperiods, conditions which are currently prevailing at the end of the vernal pool hydroperiod in the late spring.

The lack of cyanobacteria dominance in the investigated communities suggests that they may not be the leading group in the vernal pools of the temperate zone in the onset of summer with progressive global warming. At the same time, chlorophytes seemed to be the major group and the one that is most sensitive to climate changes among all phytoplankton groups. We showed that they were also the most diverse and abundant in dominant species. It is well known that they often dominate freshwater phytoplankton communities, thus playing a basic role in the functioning of many aquatic ecosystems. Individual species within chlorophytes and other phytoplankton groups responded very differently to changes in temperature and photoperiod. Our study indicated a group of species that may be favoured by global warming, while on the other hand showing that the abundance of the most dominant of species (chiefly chlorophytes) declined at higher water temperatures. For short photoperiods coupled with low temperatures

(simulating natural conditions before the present climate changes), diatom species that are almost absent from other treatments dominated during the succession, so they could be in danger of extinction in warming winters. Therefore, climate warming may result in the seasonal changes of some species abundance or even in their disappearance, and hence significant changes in the biodiversity and foodweb of aquatic ecosystems in the future.

Species dominating in our study were mostly opportunists (especially chlorophytes and cryptophytes), which is characteristic of temporary ponds [13]. On the other hand, communities of small, periodic waters are also inhabited by specialists that are typical only for these environments (as in our research, e.g. *Nitzschia hungarica*, *Stauroneis phoenicentron*). They are adapted to unique environmental conditions and, at the same time, are rare or absent from other water ecosystems. Phytoplankton in our study was also enriched by originally benthic species (some diatoms and chlorophytes), which is characteristic for ponds due to their small area and depth. As a result, the overall species richness was very high, proving that vernal pool microcosms are valuable models for investigations into phytoplankton community changes under global warming. Our experiments underline the fundamental importance of temporary waters as local biodiversity hotspots and their high value for a broad range of ecological research studies and monitoring in the era of global climate change.

Methods

To start the experiment, we collected a sample of bottom sediments from a temporary pond located in Western Poland (52°29'02"N; 16°37'08"E). This is one of the vernal pools forming a cluster of ponds in this area (see [75] for a map and some basic parameters) and is usually inundated in February (length of the day: 9h; mean temperature: -1.1 ºC) with water from thawing snow. The water phase (maximum depth: 1.2 m) lasts for an average of four months and the pond desiccates completely in late May/June (length of the day: 16 h; mean

temperature: 15,1 ºC). The sediment sample was collected in August, from the dry bottom of the pond, which was covered at this time by monocots (mainly *Agrostis stolonifera*). The sample was formed of a series of ca. 40 subsamples of the top 6 cm layer of sediments collected at random places scattered evenly over the surface of the pond.

After being transported to the laboratory, the sediments were sieved using a 5 mm soil sifter, before being mixed and homogenised. The resulting material (36 L of sediments) was divided into three parts which were used for subsequent repetitions of the experiment. For each repetition, we used 12 glass aquaria, each filled with 1 L of sediments and 10 L of deionised water as a substitute for the melted snow water or rainwater. The aquaria were stored for five weeks in three rearing rooms with constant temperatures (4, 16 or 25ºC), with four aquaria in each room. Three aquaria in each set were equipped with a 6500 K, 900 lm cold light source set for an 8, 16 or 24 h photoperiod. One aquarium in each room was left in the dark as a control (under 4ºC it was meant as a simulation of conditions prevailing under ice, when the vernal pools freezes after initial inundation). The sediments for the three subsequent repetitions were stored dry in the dark at +4°C.

The experimental design of our study aimed to simulate vernal pool environments under six climate scenarios (one present/recent and five future) according to prediction models for central Europe [76–78]: (1) a cold, snowy winter: ponds fill with water from transient snowmelts in February and then the surface freezes – a scenario typical for vernal pools, still occurring some 10-20 years ago (treatment: 4ºC, photoperiod 0 h); (2) a mild, wet winter: pools inundate in February as a consequence of snow thawing or rains but do not freeze (treatment: 4ºC, photoperiod 8 h); (3) a very short, snowy or rainy winter followed by a sudden increase in temperature (spring in February scenario; treatment: 16ºC, photoperiod 8 h); (4) a dry, snowless winter/spring and cold spring with rains at the onset of summer – ponds fill with rainwater later in the season (4ºC, 16 h); (5) a dry winter and mild, rainy end of the spring – as above, but with

higher temperatures (16ºC, 16 h); and (6) a dry winter with a hot spring/summer with heavy showers (25ºC, 16 h). All of the treatments conducted using a 24h photoperiod were considered as a point of reference, since such light conditions are exotic for the temperate climate zone. They show the sole influence of temperature when access to light is unlimited and might be considered as a proxy of changes in the functioning of temporary ponds in polar regions (taxonomical structure of the phytoplankton communities is different, however).

From each aquarium, weekly samples (1 L) for phycological analyses were collected. Based on the samples, temporal changes in phytoplankton were observed over five weeks (for each repetition and each combination of temperature and photoperiod). In total, 12 experimental combinations of temperature and photoperiod were compared, each in 3 replicates of 5 samplings, producing 180 samples altogether. Water samples for phycological analyses were fixed with Lugol solution. Samples were sedimented in the laboratory and concentrated up to a volume of 5 mL, and then preserved with formalin. Phycological investigations were conducted under a light microscope (at 200x, 400x and 1000x magnification). Phytoplankton cells were counted in a Fuchs-Rosenthal chamber (height: 0.2 mm, area: 0.0625 mm^2). Unicellular microalgae and colonies were treated as individual units. In the case of trichomes, the length of the individual was standardised. The standard length of the individual was considered as 100 μm. In the case of species forming colonies (e.g. *Aphanocapsa* sp., *Aphanothece* sp.), a cover area of 400 μm^2 was classified as a unit. Since the abundance in particular taxa was far from fitting any classical distribution type, we used nonparametric tests to analyse the data. The Kruskal-Wallis rank sum test was used to check for differences in the total number of taxa, the abundance of phytoplankton and the values of Shannon-Weaver diversity index between the treatments, sampling events and replicates of the experiment. Interactions between the treatments and time were analysed using a linear model with a permutation test using

Anscombe's method [79]. To account for type I error due to multiple tests being conducted, Holm's correction was used to adjust the reported P-values.

Particular species, characterised by individual preferences and adaptations, respond to environmental factors with different dynamics. Slower or more rapid changes in abundance over the time of observation are reflected in the sequence of changes in the community structure. To analyse patterns of such changes on the level of particular taxonomic groups, Principal Response Curve (PRC) analysis was used. Patterns on the level of particular species were more complex, so Canonical Correspondence Analysis (CCA) with an interaction between time and treatment was used instead.

PRC was conducted based on partial redundancy analysis [80]: first, Redundancy Analysis (RDA) was conducted on the log-transformed data on the abundance of particular taxonomic groups. Sampling time indicators were used as covariables and the interactions between the treatment levels and sampling times were used as explanatory variables. The Monte Carlo permutation test (5000 permutations) was used to test the significance of particular variables as well as first and second canonical axis. Next, canonical scores of explanatory variables from the first canonical axis were extracted and used to draw response curves for each treatment. The resulting graph was supplemented by the diagram of the first RDA axis, enabling interpretation of the direction of departure from the community composition in the reference treatment (4ºC and darkness).

In canonical analyses, records of species with low frequencies are often overemphasised, unduly influencing the results of ordination[80–82]. To avoid such bias and reduce the noise caused by the stochastic occurrence of some rare species, CCA analysis was conducted only on data of the most numerous and dominant taxa. As such, we considered taxa with a high frequency (occurring in more than 5% of samples, i.e. at least 10 samples) and high abundances (reaching abundances of at least 200 cells per ml in all the samples). In total, 41 species passed these

criteria and were included in the CCA analyses (22 chlorophytes, 8 diatoms, 7 cryptophytes, 2 cyanobacteria and 2 euglenoids). Interactions between particular treatments and samplings (time x photoperiod; time x temperature) were used as explanatory variables and their statistical significance in the model was assessed using the Monte Carlo permutation test (5000 permutations); the same test was performed on the first canonical axis as well as on the whole model. To avoid pseudoreplication, all of the permutation tests were restricted to blocks of data representing the particular time series analysed (cyclic shifts were used) [81,82].

All of the canonical analyses (PRC, RDA, and CCA) were conducted using the Canoco 4.56 software package [81,82]. The remaining analyses were conducted in R 4.0.2 [83] under RStudio 1.3.1056 using 'coin' and 'lmPerm' packages [79,84]. We considered p = 0.05 as a threshold determining statistical significance.

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Author contributions

S.C. and B.G. designed the study. S.C. performed qualitative and quantitative analyses of phytoplankton. B.G. performer statistical analyses. S.C. wrote a first draft of the manuscript, and both authors contributed critically to the drafts and gave final approval for publication.

Additional information

The authors declare no competing interests.

Figure legends:

Figs 1A-1C. Temporal changes in mean values of Shannon-Weaver diversity index (Shannon_H) for phytoplankton in particular experimental treatments (A – temp. 4°, B – temp. 16°C, C – temp. 25°C); 1-5: sampling weeks.

Figs 2A-2C. Temporal changes in percentage contributions of phytoplankton groups to the total mean phytoplankton abundance in particular experimental treatments (A – temp. 4°C, B – temp. 16°C, C – temp. 25°C); 1-5: sampling weeks.

Fig 3. Results of PRC analysis illustrating changes over time in the structure of phytoplankton communities in particular experimental treatments.

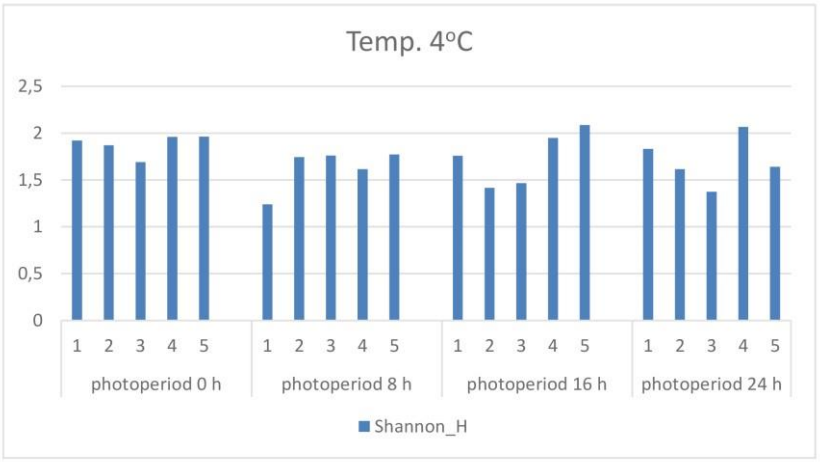
Phytoplankton taxonomic groups/Abbreviations: Cyanopr – Cyanoprokaryota/Cyanobacteria; Chloroph – Chlorophyta (chlorophytes); Cryptop – Cryptophyta (cryptophytes); Chrysop –

Chrysophyceae (chrysophytes); Bacillar - Bacillariophyceae (diatoms); Euglen - Euglenophyta (euglenoids); Dinoph – Dinophyceae (dinoflagellates); Xanthoph - Xanthophyceae (xanthophytes); Treatments: L1 – photoperiod 0 h (control), L2 – photoperiod 8 h, L3 – photoperiod 16 h, L4 – photoperiod 24 h; T1 – temperature 4°C, T2 – temperature 16°C, T3 – temperature 25ºC.

Fig. 4. Results of CCA analysis illustrating the phytoplankton species distribution over time and environmental variables (time*temp – temperaturę over time, time*light – photoperiod lenght over time). Dominatind taxa/Abbreviation of **Cyanobacteria**: Chrooc1 – *Chroococcus* sp. 1; Hyella - *Hyella* sp.; **Chlorophytes:** Chla1 – *Chlamydomonas* sp. 1; Chla2 – *Chlamydomonas* sp. 2; Chlapas – *Chamydomonas passiva* Skuja; Chlorac – *Chlorogonium elongatum* var. *aculeatum* (Pascher) L.Péterfi; Chlorel – *Chlorogonium elongatum* (P.A. Dangeard) Francé; Chlorop1 – filamentous chlorophyte 1; Chlorop2 – filamentous chlorophyte 2; Clostac – *Closterium acerosum* Ehrenberg ex Ralfs; Haemat – *Haematococcus pluvialis* Flotow; Mongr - *Monoraphidium griffithii* (Berkeley) Komárková-Legnerová; Oedogon - *Oedogonium* sp.; Phaclen – *Phacotus lenticularis* (Ehrenberg) Diesing; Plansph - *Planctococcus sphaerocystiformis* Korshikov; Pseudla – *Pseudosphaerocystis lacustris* (Lemmermann) Nováková; Schrose – *Schroederia setigera* (Schröder) Lemmermann; Sphagel - *Sphaerellopsis gelatinosa* Korshikov (Gerloff); Spirog – *Spirogyra* sp.; Tetrdim – *Tetradesmus dimorphus* (Turpin) M.J.Wynne Tetrage – *Tetraspora gelatinosa* (Vaucher) Desvaux; Ulothrix – *Ulothrix* sp.; Uroncon – *Uronema confervicola* Lagerheim; Uronint – *Uronema intermedium* Bourrelly; **Diatoms:** Eunbil - *Eunotia bilunaris* (Ehrenberg) Schaarschmidt; Hantzam – *Hantzschia amphioxys* (Ehrenberg) Grunow in Cleve & Grunow; Nav1 – *Navicula* sp. 1; Nav2 – *Navicula* sp. 2; Navmin – *Navicula minima* Grunow in Van Heurck; Nitzhun – *Nitzschia hungarica* Grunow; Nitzpal – *Nitzschia palea* (Kützing) W.Smith; Stauran – *Stauroneis anceps* f. *gracilis* (Ehrenberg) F.Hustedt; **Cryptophytes:** Chromin – *Chroomonas minuta* (Skuja) Bourrelly;

Crypter – *Cryptomonas erosa* Ehrenberg; Cryptma – *Cryptomonas marssonii* Skuja; Cryptov – *Cryptomonas ovata* Ehrenberg ; Cryppha – *Cryptomonas phaseolus* Skuja; Rhodmin – *Rhodomonas minuta* Skuja; Rhodten – *Rhodomonas tenuis* Skuja; **Euglenoids:** Euglen – *Euglena* sp.; Trachv – *Trachelomonas volvocinopsis* Svirenko.

Fig. 1A.



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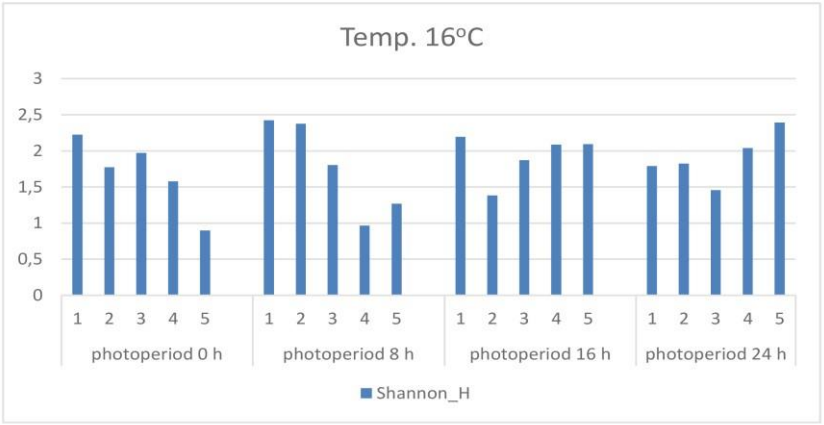

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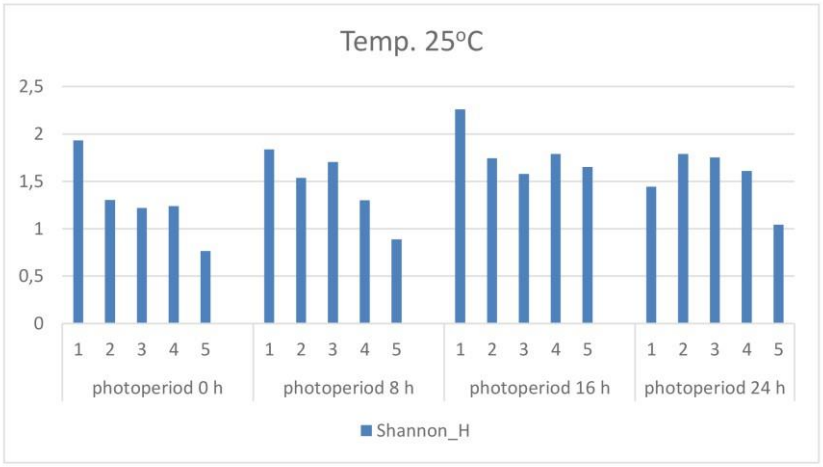

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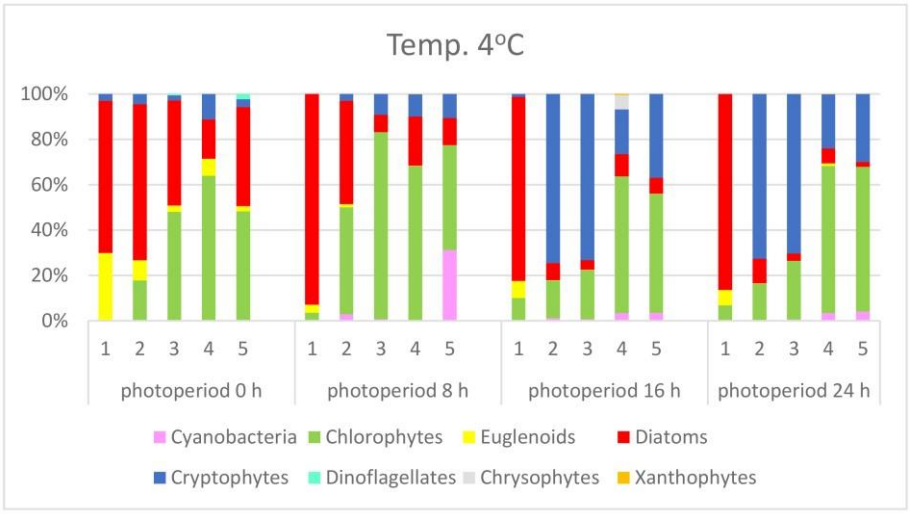

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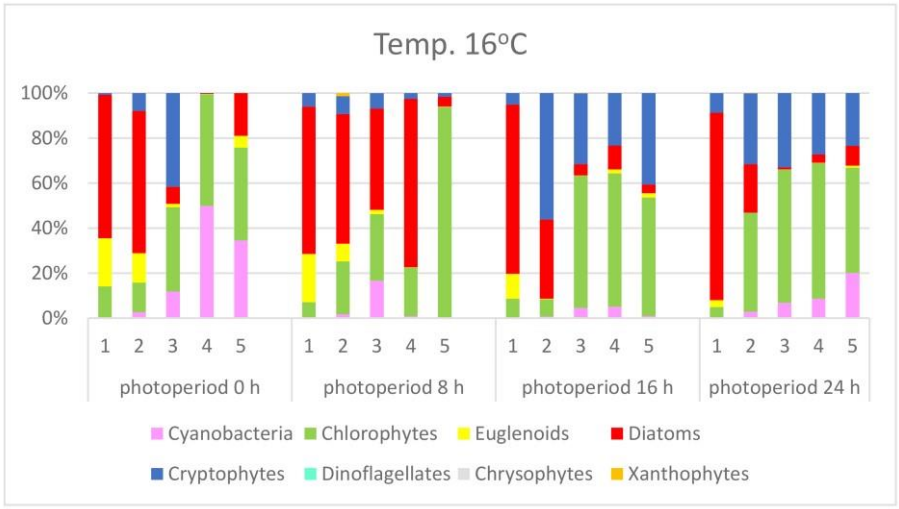

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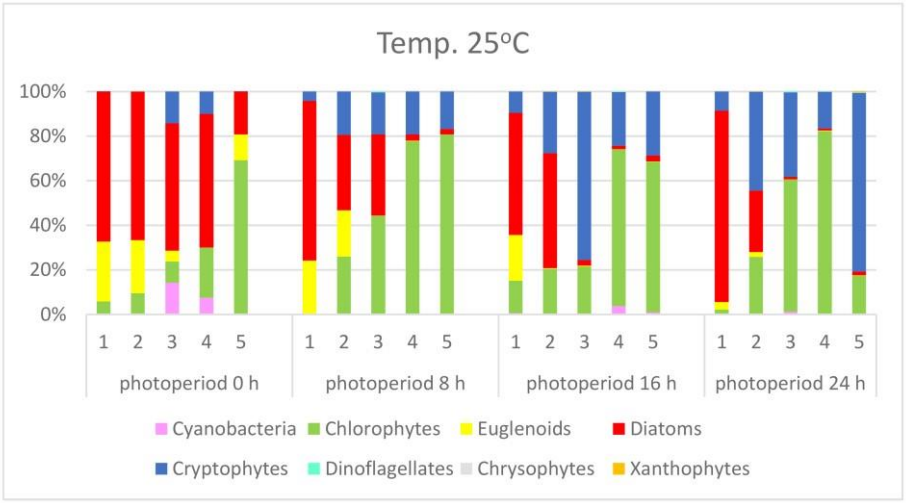
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Euglenoids
Diatoms
Cryptophytes
Dinoflagellates
Chrysophytes
Xanthophytes

Fig. 3.

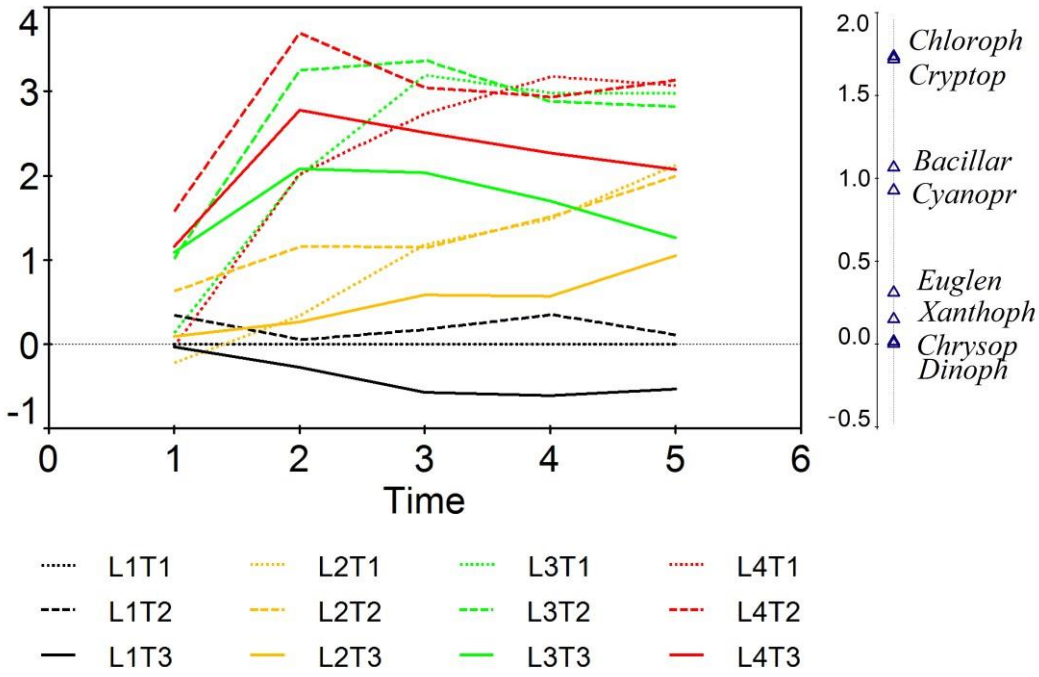
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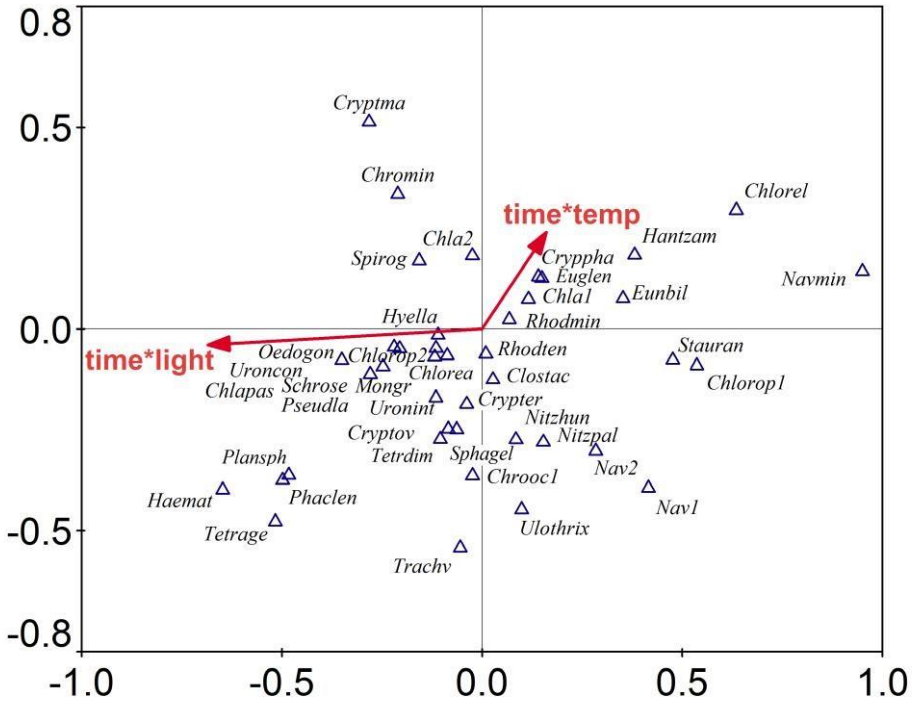
Fig. 4.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Uroncon
Chlapas
Schrose
Pseudla
Mongr
Uronint
Chlorea
Chlorop2
Cryptov
Tetrdim
Plansph
Haemat
Phaclen
Tetrage
Trachv
Rhodten
Clostac
Crypter
Sphagel
Chrooc1
Nitzhun
Nitzpal
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Chlorel
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Nav1