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Nutrient Loading, Temperature and Heat Wave Effects on Nutrients, Oxygen and Metabolism in Shallow Lake Mesocosms Pre-Adapted for 11 Years

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Abstract: Global changes (e.g., warming and population growth) affect nutrient loadings and temperatures, but global warming also results in more frequent extreme events, such as heat waves. Using data from the world's longest-running shallow lake experimental mesocosm facility, we studied the effects of different levels of nutrient loadings combined with varying temperatures, which also included a simulated 1-month summer heat wave (HW), on nutrient and oxygen concentrations, gross ecosystem primary production (GPP), ecosystem respiration (ER), net ecosystem production (NEP) and bacterioplankton production (BACPR). The mesocosms had two nutrient levels (high (HN) and low (LN)) combined with three different temperatures according to the IPCC 2007 warming scenarios (unheated, A2 and A2 + 50%) that were applied for 11 years prior to the present experiment. The simulated HW consisted of 5 °C extra temperature increases only in the A2 and A2 + 50% treatments applied from 1 July to 1 August 2014. Linear mixed effect modeling revealed a strong effect of nutrient treatment on the concentration of chlorophyll a (Chl-a), on various forms of phosphorus and nitrogen as well as on oxygen concentration and oxygen percentage (24 h means). Applying the full dataset, we also found a significant positive effect of nutrient loading on GPP, ER, NEP and BACPR, and of temperature on ER and BACPR. The HW had a significant positive effect on GPP and ER. When dividing the data into LN and HN, temperature also had a significant positive effect on Chl-a in LN and on orthophosphate in HN. Linear mixed models revealed differential effects of nutrients, Chl-a and macrophyte abundance (PVI) on the metabolism variables, with PVI being particularly important in the LN mesocosms. All metabolism variables also responded strongly to a cooling-low irradiance event in the middle of the HW, resulting in a severe drop in oxygen concentrations, not least in the HN heated mesocosms. Our results demonstrate strong effects of nutrients as well as an overall rapid response in oxygen metabolism and BACPR to changes in temperature, including HWs, making them sensitive ecosystem indicators of climate warming.

Keywords: climate change; extreme events; ecosystem processes; lake restoration

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1. Introduction

The Earth is facing a rapid climate warming and population growth [1]. This has implications for lake ecosystem functioning and entails an increased risk of eutrophication, reflecting both increasing external and internal nutrient loading and a shift in top-down control that reduces the zooplankton grazing pressure on phytoplankton [2–4]. This is especially the case for shallow lakes that are presumed to be the most common and vulnerable lake type to global warming [2,5,6]. It should be added that the predicted increase of the world population will demand higher food production and with this trend comes increasing eutrophication [2–4].

Several studies have shown positive effects of temperature on both gross primary production (GPP) and ecosystem respiration (ER), often accompanied, however, by reduced net ecosystem production (NEP = GPP − ER) [7–10], reflecting that ER theoretically is more sensitive to temperature than GPP. Accordingly we may expect an increasing degree of heterotrophy in shallow lake ecosystems with increasing temperatures [9,11], as also demonstrated experimentally in a recent cross-European shallow lakes experiment [12]. In contrast, elevated nutrient concentrations (eutrophication) increase metabolic rates, but often the impact is greater on GPP than on ER, leading to an increase in NEP and organic matter accumulation in the sediment [13,14]. Accordingly, Scharfenberger et al. [12] found that higher nutrient availability not only resulted in significantly higher GPP and ER, but also in a significantly lower ER to GPP ratio, indicating that high-nutrient systems are likely to have a lower risk of becoming net heterotrophic than those with lower nutrient concentrations. In addition, a cross-system analysis has revealed that ER and GPP are more coupled in oligotrophic lakes than in eutrophic lakes [12]. However, the interacting effects of temperature and trophic state on productivity/oxygen metabolism are generally not well understood [15,16]. It is, however, well-established that bacterioplankton production (BACPR) increases with temperature (e.g., [17–21]), but BACPR is also highly sensitive to changes in lake trophic state and typically increases with increasing eutrophication [17,18,22], which is facilitated further at high temperatures [18].

Global warming not only increases average temperatures, it also enhances the frequency of extreme events such as heat waves [23–25]. Shallow lakes may be particularly sensitive to heat waves, as their average water temperature will increase more rapidly than in deep lakes [26] and this may lead to a catastrophic shift if metabolism imbalances occur. Thus, some shallow lake studies have shown sudden oxygen depletion and fish kills during extreme climate event including heat waves [27] or when phytoplankton blooms undergo intensive bacterial degradation, often in warm, calm weather [28].

In the present study, we focused on nutrients, oxygen and oxygen metabolism and BACPR before, during and after a simulated 1-month summer heat wave in shallow lakes. We used data from the longest running mesocosm experimental facility on climate effects on shallow lakes [29], where different temperatures crossed with low and high nutrient levels are employed. We heated the already long-term heated mesocosms an extra 5 °C during July, to elucidate how lakes in a future warmer world would respond to heat waves. We hypothesized that (1) nutrients would have a strong stimulating effect on GPP, ER, NEP and BACPR, (2) higher temperatures would increase GPP, ER and BACPR but decrease NEP, with the strongest effects at high nutrient concentrations where the nutrient constraints on productivity would be lower and (3) a HW in the warmed mesocosm hosting an already temperature-stressed lake ecosystem might result in sudden dramatic shifts due to imbalances in the metabolism that may lead to sudden changes in oxygen and perhaps have major detrimental impacts on the fish population.

2. Materials and Methods

The experiment was conducted in lake mesocosms established in August 2003 in Jutland, Denmark, for studying the effects of climate change at contrasting nutrient levels [29]. In brief, the facility includes 24 outdoor flow-through mesocosms (diameter 1.9 m, water depth 1 m, retention time of about 2.5 months). At the start of the experiment in 2003,

a 20 cm layer of a mixture of washed and nutrient-rich sediment from a nearby freshwater pond was added to each mesocosm (see details in Liboriussen et al. [29]). The mesocosm facility has three temperature treatments: unheated (A0, abbreviations are provided in Table 1), treatment A2 (A2) according to the International Panel on Climate Change's (IPCC) predicted temperature increase [24] and treatment of A2 + 50%, which is A2*1.5 (A2+). The A2 and A2+ mesocosms are warmed by heating elements placed about 10 cm above the sediment. To avoid stratification, the water column is fully mixed by paddles [29]. The water temperature in A0 mesocosms fluctuates with the air temperature, while the two heated treatments are 2–4 °C (A2) and 3–6 °C (A2+) warmer than the A0 mesocosms depending on the season based on future climatic projections for Central Jutland, Denmark downscaled to monthly resolutions using 1961–1990 as the reference period [30].

Table 1. Abbreviations used.

Abbreviation	Definition	Abbreviation	Definition
A0	Ambient treatments	**HN**	High nutrient treatments
A2	Ambient + 2–4 °C treatments	**IPCC**	Int. Panel on Climate Change
A2+	Ambient + 3–6° C treatments	**LN**	Low nutrient treatments
BACPR	Bacterioplankton Production	**NEP**	Net Ecosystem Production
Chl-a	Chlorophyll-a	**NEP20**	NEP standardized to 20 °C
DO	Dissolved Oxygen	**NO_3**	Nitrite+nitrate
DO%	DO percent saturation	**PO_4**	Orthophosphate
ER	Ecosystem Respiration	**O_2**	Oxygen concentration
ER20	ER standardized to 20 °C	**O_2%**	Oxygen percent saturation
GPP	Gross Primary Production	**PAR**	Photosynthetic Active Radiation
GPP20	GPP standardized to 20 °C	**PVI**	Plant Volume Inhabited
HW	Heat wave	**TCA**	Trichloroacetic acid
HW0	pre- and post- HW period	**TEMP**	Temperature in the water
HW1	HW period	**TP**	Total phosphorus
HW2	Ambient summer period	**TN**	Total nitrogen

The temperature treatments are crossed with two nutrient treatments (low (LN) and high (HN)), i.e., six treatments for each run with four replicates. LN only receives nutrient input from tap water, while HN receives additional nutrients weekly (7 mg P m^{-2} d^{-1} and 27.1 mg N m^{-2} d^{-1}). The two nutrient loadings mimic conditions in clear water and turbid water shallow lakes, respectively [31]. Accordingly, submerged macrophytes generally dominate in the LN mesocosms, whereas the HN mesocosms are mainly turbid with abundant phytoplankton. Temperature is measured continuously in all mesocosms. Oxygen is recorded approximately 0.4 m below the surface using a two-wire oxygen probe (OxyGuard International A/S, Birkerød, Denmark) [29].

Our study was conducted in summer 2014, i.e., 11 years after the initiation of the mesocosms experiment. During July, a heat wave (HW) was simulated in A2 and A2+ by increasing the water temperature by 5 °C, while the A0 mesocosms remained unheated. Hence, during the heat wave the heated mesocosms were about 7–9 °C (A2) and 9–12 °C (A2+) warmer than in A0. As for the long-term temperature treatments, the water temperatures in A2 and A2+ were continuously adjusted during the heat wave following the temperature variation in A0. Thus, we studied the effects of a heat wave occurring in shallow lakes by the end of this century, when lakes are expected to be warmer, and compared the results with the present-day situation.

Water for nutrient analysis and chlorophyll-a (Chl-a) was collected 2 times before, 6 times during and 2 times after the HW. Sampling was conducted with a 1 m long tube sampler (covering the whole water column) at 6 randomly selected sites and pooled. A 100–1000 mL subsample was filtered through GFC filters and the Chl-a concentration

was determined on a spectrophotometer after extraction with ethanol [32]. Standard methods were used for determination of chemical variables [33]. Macrophyte abundance was quantified as percent volume of the water column inhabited by plants (plant volume inhabited, PVI, %). Percentage cover and height of the submerged plants were assessed, allowing estimation of the proportion of the water column occupied by submerged plants (for further details, see Davidson et al. [34]. Data are presented in [35]).

Gas transfer velocities for O_2 were measured in three mesocosms in December 2009 [36] and corrected for the water temperature [37]. There were only little variations in the k_{O_2} estimates based on the recovery of artificial reduction in O_2 in three of the mesocosms (0.0080, 0.0086 and 0.0100 m h^{-1} at 20 °C) [36]. As the mixing in all mesocosms are done with the same set-up (paddles), we assumed similar piston velocities and used an average of these three estimates for all mesocosms (k_{O_2} = 0.0088 m h^{-1} at 20 °C). Wind speed alters piston velocity; however, at low wind speed (<3 m s^{-1}) the effect is negligible [38], which was the case in our study as the mesocosms are relatively well sheltered from the wind.

GPP and ER was estimated using the 30 min interval records of dissolved oxygen (DO), light intensity and water temperature according to the method of [39,40] (see Jeppesen et al. [41] for details) seen in Equation (1):

$$\left(\frac{dDO}{dt}\right) = \left(K_2 \cdot 1.0241^{(T_w-20)} \cdot (DO_{sat} - DO_t)\right) - \left(p_{20} \cdot 1.07^{(T_w-20)}\right) + \left(\pi_{20} \cdot 1.035^{(T_w-20)} \cdot \frac{I_t}{\eta + I_t}\right)$$
$$(O_2\ change) = (Air\ water\ exchange) - (Temperature\ dependent\ respiration) + (Light\ and\ temperature\ dependent\ production) \quad (1)$$

where: T_W: water temperature in °C, DO_{sat}: dissolved oxygen saturation concentration in mg L^{-1}, DOt: dissolved oxygen concentration in mg L^{-1}, I_t: light intensity—Photosynthetic Active Radiation (PAR) mol m^{-2} 30 min^{-1}

Constants ρ_{20}, π_{20} and η were estimated using the secant non-linear method, PROC NLIN, in the statistical software SAS 9.3. DO_{sat} was represented as the DO percent saturation (DO%), calculated as a function of T_W [42]:

$$DO_{sat} = 14.652 - 0.41022 \cdot T_W^1 + 0.0079910 \cdot T_W^2 - 0.000077774 \cdot T_W^3. \quad (2)$$

PAR was calculated as 45% of global radiation, which was obtained from a national meteorological station 40 km from the experimental site. Net ecosystem production (NEP) was estimated as NEP = GPP − ER.

Bacterioplankton production was measured using the approach of Fuhrman and Azam [43], with minor changes. Triplicate samples (subsample of depth-integrated samples from the various mesocosms) were incubated in Jena flasks in the different mesocosms with 12.5 nM ^{3}H-methylthymidine for 15–60 min, depending on the lake water temperature. The reaction was stopped by adding formalin. Less than 2 h after sampling, 7–10 mL of each sample was filtered on a 25-mm cellulose acetate filter (0.2 µm) and rinsed 8 times with 1 mL of ice-cold 5% trichloroacetic acid (TCA) solution. The radioactivity retained on the filters was counted within 48 h after sampling. Bacterial production was calculated from ^{3}H-methylthymidine incorporation using the following conversion factors: 2×10^9 cells (nmol thymidine)$^{-1}$ [44].

Linear mixed effect models were used to explore linkages between metabolism variables and treatments and/or environmental variables. We conducted analyses on the full data set and tested for significant treatment effects (temperature, nutrient and HW treatments). Treatment effects were included in the model as fixed effects and mesocosms as a random effect [45]. When needed, a variance structure was added (varFixed or varPower) to deal with the variance heterogeneity [46].

To elucidate the effect of the HW, we followed the method used in a study of HW effects on GHG in the same HW experiment [47]. In the models, HW had three levels: HW0, the pre- and post-HW period (i.e., June and August in A0, A2 and A2+); HW1, the HW period (July in A2 and A2+); HW2, the "Ambient summer" period (July in the A0),

the latter because A0 was not heated the extra 5 °C during the HW period but A2 and A2+ were. Starting with a full model including treatments and environmental variables, model selection was made in a backward stepwise fashion using the likelihood ratio involving removal of the term of least significance until all remaining covariables were significant [45]. When HW was found to be significant in the mixed models, the variations among the different levels of the HW treatments were tested using Tukey's post-hoc test.

We also analyzed the data from HN and LN mesocosms separately to explore treatment effects that potentially are masked by nutrient effects, including only temperature and HW treatment effects. All mixed models were checked for normality and homogeneity of variance by visual inspection of plots of residuals against fitted values. The significance of the models was assessed by comparison with a null model using the likelihood ratio. To reduce variance heterogeneity in the data and to meet the assumptions of linear mixed effect models, the metabolism variables were log-transformed before the analysis. These statistical analysis were performed using the R package "nlme" and the function "lme" [48] in the R software (version 3.1.1) [49].

We also conducted linear mixed regression models on the full dataset using Chl-a, TP, TN and PVI as explanatory variables (fixed factors) and mesocosms as random factors. Furthermore, repeated measurements on mesocosms were considered in the covariance structure used (applying the procedure MIXED in SAS).

3. Results

Time series of water temperature oxygen concentration (O_2) and percent saturation (O_2%) are shown in Figure 1 and boxplots of the same variable plus photosynthetic active radiation (PAR) are shown for the different treatments and periods in Figure 2. Linear mixed effect model results are given in Table 2. The maximum of the 24 h daily mean water temperatures during the HW were 22.8 °C in A0, 30.2 °C in A2 and 31.5 °C in A2+ (Figure 1), while the corresponding minimum values were 14.2, 19.7 and 17.9 °C, respectively. The period with the HW was unusually warm (see A0 curve in Figure 1), and warmer than the periods before and after the HW. In addition, there was a sudden drop in water temperature in mid-July during a rainy and cold period, a period that also experienced less irradiation (Figure 1). The highest temperature in A2 and A2+ during the artificial HW was substantially (3.6 °C) higher than the values recorded on a single day in the previous years of the experiment in these mesocosms, being 26.6 and 27.9 °C, respectively (authors' unpublished results).

The water temperature decline had implications for O_2 and O_2%, as both decreased substantially in all mesocosms during the rainy/cold period, not least in the HNs; for both HN and LN, larger decrease occurred in the heated mesocosms than in the ambient mesocosms. During this period, the 24 h mean oxygen percentage in LN dropped to an average of 8.0, 5.1 and 5.8 mg O_2 L^{-1} in A0, A2 and A2+, respectively, and to 8.2, 4.5 and 4.5 mg O_2 L^{-1} in HN (Figure 1), while the same figures for minimum concentrations at night averaged 7.0, 3.3 and 4.3 mg O_2 L^{-1} in LN and 6.3, 2.2 and 2.1 mg O_2 L^{-1} in HN, being as low as 0.6 and 0.9 mg O_2 L^{-1} in two of the heated (A2+) HN mesocosms (data not shown).

Overall, maximum and minimum O_2 and O_2% showed larger day-to-day variations in HN than in LN over the study period (Figure 1), and the water was mostly supersaturated with O_2 in both LN and HN, but with higher maximum percentages in HN. Thus, O_2 and O_2% were strongly positively related to the nutrient level (Table 2, Figures 1 and 2). The 24 h daily mean O_2 values in A0, A2 and A2+ for the entire study period were 10.4, 9.9 and 9.2 mg L^{-1}, and 13.0, 11.5 and 11.7 mg L^{-1} in LN and HN, respectively. For O_2%, the figures were 112, 116 and 111% in LN and 138, 134 and 141% in HN. However, we found no significant temperature or HW effect on O_2, and O_2% using the full data set (Table 2). When LN and HN data were separately analyzed, we found a positive HW effect on O_2, but this was not significant when taking into account the warming effect of the natural HW in A0 (Table 2).

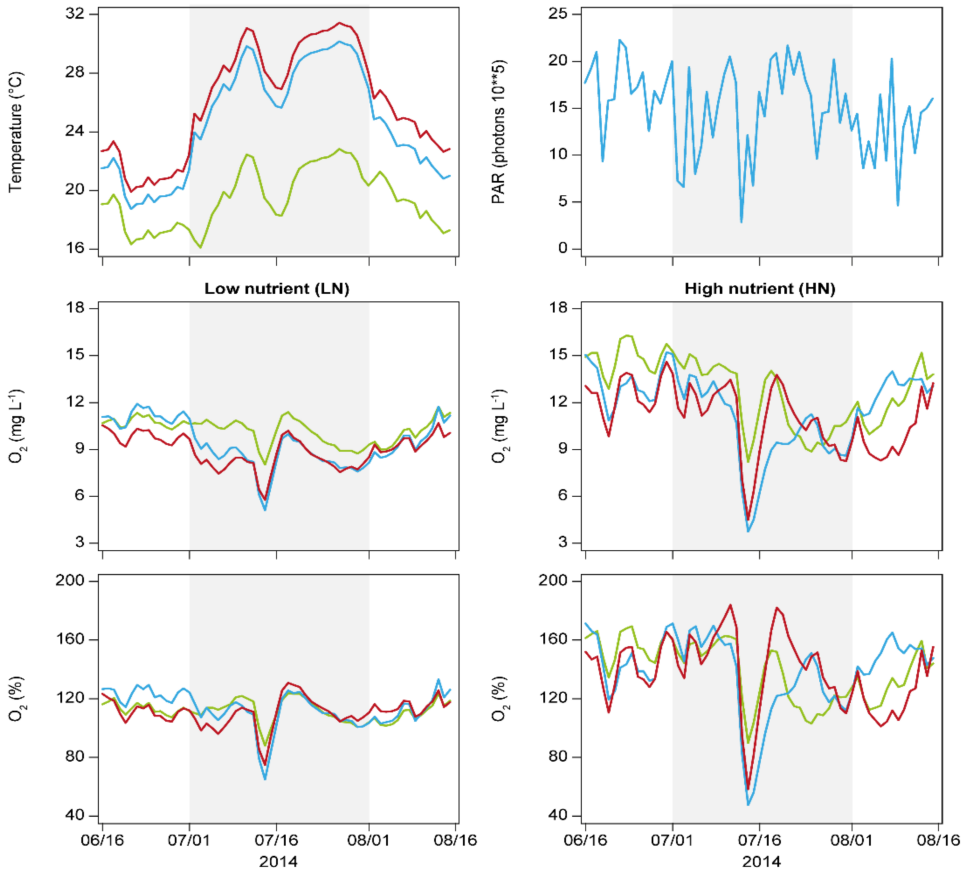

Figure 1. Changes in 24 h mean water temperature, irradiation (PAR), oxygen concentration (O_2) and oxygen saturation (O_2 (%)) in the low and high nutrient mesocosms before, during and after the heat wave. The three temperature scenarios are: A0 (green), A2 (blue) and A2+ (red). Variation—see Figure 2. Grey indicates the experimental heat wave period.

Table 2. Results from the linear mixed effect models used to test the effects of nutrient, temperature and heat wave (HW) treatments on chlorophyll a (Chl-a), total phosphorus (TP), orthophosphate (PO_4), total nitrogen (TN), nitrite+nitrate (NO_3) oxygen concentration (O_2) and percentage (O_2%)—ns, not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Significant HW indicates that the HW treatment (HW1, i.e., July in A2 and A2+) differed significantly from the non-HW period (i.e., HW0: A0, A2 and A2+ in June and August). The sign between brackets indicates whether the effect on the response variable was positive or negative. "0": the HW1 treatment did not differ significantly from the HW2 treatment, i.e., the effect of the HW did not differ from the effect of the A0 summer temperature (see "Materials and Methods" and Audet et al. [47]).

Data Set	Treatment	Chl-a	TP	PO_4	TN	NO_3	O_2	O_2%
Full	Nutrient	*** (+)	***(+)	*** (+)	*** (+)	ns	*** (+)	*** (+)
	Temperature	ns	ns	ns	ns	ns	ns	ns
	Heat wave	* (+)0	ns	*** (+)0	** (+)0	ns	ns	ns
Low Nutrient	Temperature	* (+)	ns	* (-)	ns	ns	ns	ns
	Heat wave	*** (+)0	ns	ns	ns	ns	** (+)0	ns
High Nutrient	Temperature	ns	ns	** (+)	ns	ns	ns	ns
	Heat wave	ns	ns	ns	ns	ns	ns	ns

Boxplots of the nutrient concentrations and Chl-a for the six treatments and three periods are shown in Figure 3 and test results are given in Table 2. The figure indicates substantial differences in TP, TN, PO_4 and Chl-a (but not in NO_3) between HN and LN. Accordingly, the linear mixed effect models on the full dataset revealed a strong nutrient effect on Chl-a, TP, TN and PO_4 but not NO_3, while no significant temperature treatment

effects were recorded. We found a significant HW effect on Chl-a, TN and PO_4, all showing an increase, but not more than in A0, reflecting the fact that the temperature in A0 during this period also was higher than before and after the HW due to a natural HW during July (Table 2).

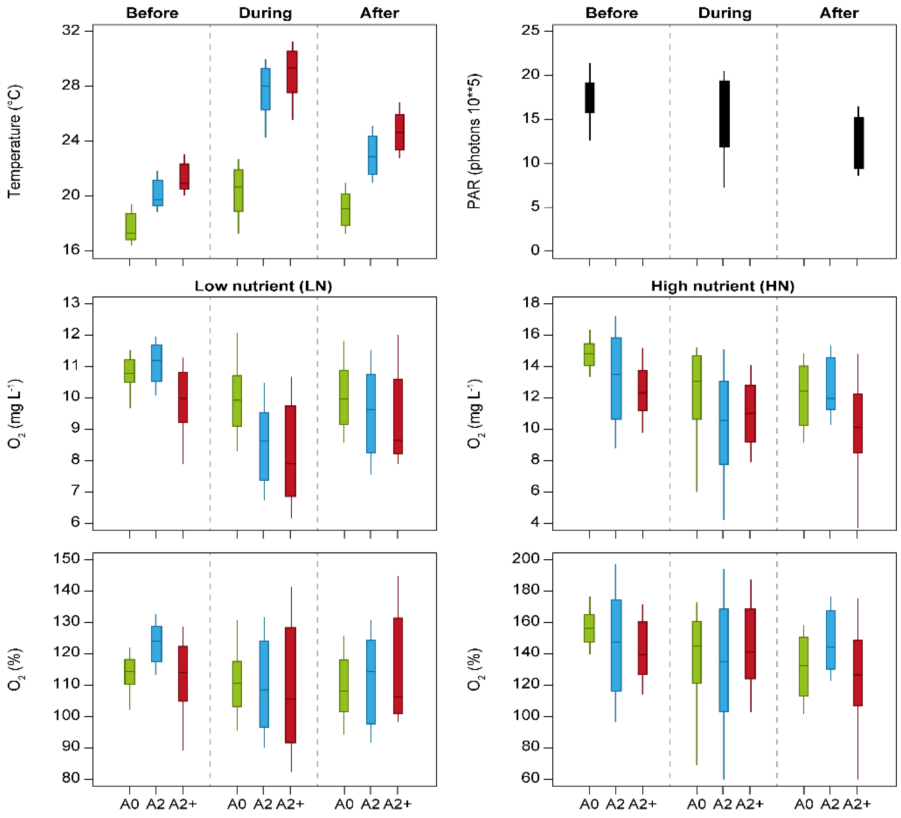

Figure 2. Box plot (10%, 25%, 75% and 90% percentiles) of 24 h mean temperature, oxygen concentration (O_2) and oxygen percentage (O_2 (%)) in the low (LN) and high (HN) nutrient mesocosms before, during and after the heat wave (the 4 replicates pooled). The three temperature scenarios are: A0 (green), A2 (blue) and A2+ (red). Also shown is the irradiation (PAR) in the three periods (upper right). Note the different scale on the y-axes of the LN and HN treatments.

After analyzing LN and HN data separately, we found increasing Chl-a and decreasing PO_4 with increasing temperature in LN, while PO_4 increased in HN (Table 2). No temperature treatment effect was recorded for the other variables. During the HW, Chl-a was higher in LN, but not significantly so when the warming effect of the natural HW in A0 was considered (Table 2).

Time series (24 h means) of GPP, ER and NEP are shown in Figure 4, while boxplots of the same variables and BACPRO for the six treatments and three periods are given in Figure 5 and linear mixed effect models' results are in Table 3. On the full dataset, the models revealed a significant positive effect of nutrients on GPP, ER, NPP and BACPR. For the entire study period in LN, GPP values were 5.6, 8.4 and 6.6 g O_2 m^{-2} 24 h^{-1} in A0, A2 and A+, respectively, and 9.7, 10.9 and 13.3 g O_2 m^{-2} 24 h^{-1} in HN, respectively. For ER, the figures were 5.2, 7.8 and 6.2 g O_2 m^{-2} 24 h^{-1} in LN and 8.5, 10.9 and 12.0 g O_2 m^{-2} 24 h^{-1} in HN. Accordingly, NEP was 0.4, 0.6 and 0.4 g O_2 m^{-2} 24 h^{-1} in LN and somewhat higher in HN: 1.2, 1.0 and 1.3 g O_2 m^{-2} 24 h^{-1}. The figures for BACPR were 6.6, 9.6, 14.8 cells $\times$ 10^7 mL^{-1} h^{-1} in LN and 35.6, 88.6 and 55.0 cells $\times$ 10^7 mL^{-1} h^{-1} in HN.

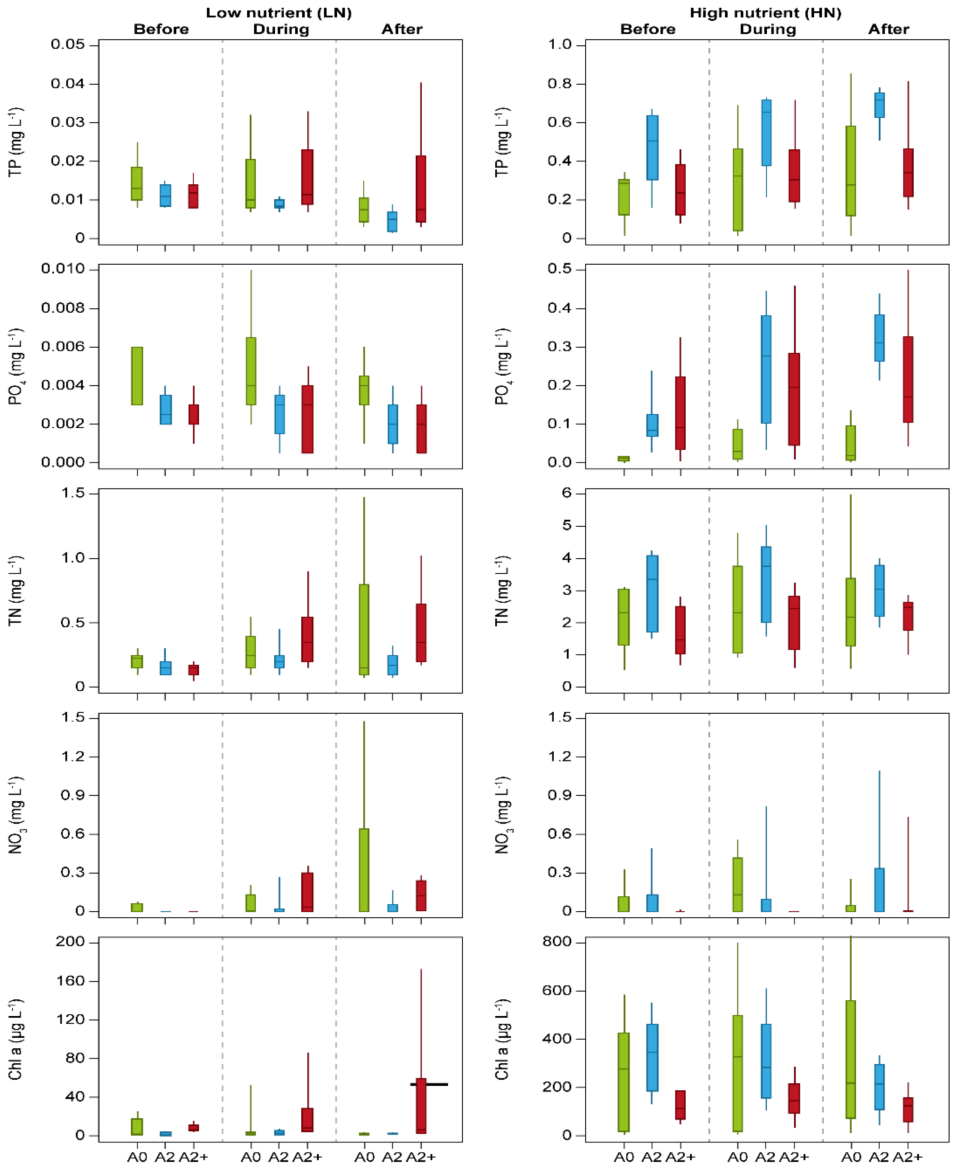

Figure 3. Box plot (10%, 25%, 75% and 90% percentiles) of total phosphorus (TP), phosphate (PO_4), total nitrogen (TN), nitrite+nitrate (NO_3) and chlorophyll a (Chl a) in the low and high nutrient mesocosms before, during and after the heat wave (the 4 replicates pooled). The three temperature scenarios are: A0 (green), A2 (blue) and A2+ (red). Note the different scale on the y-axes of the LN and HN treatments.

We found a significant positive effect of temperature on ER and BACPR, but not on GPP and NEP (Table 3). The HW had a positive effect on GPP, ER and BACPR and a negative effect on NEP compared with the pre/post HW period, but when taking the changes in the A0 mesocosms into account, only the HW effect on GPP and ER was significant (Table 3). When dividing the data into low and high nutrient concentrations, temperature had a significant positive effect on GPP, ER and BACPR in both LN and HN, while NEP was not significantly related to temperature (Table 3). In the LN mesocosms, there was a positive HW effect on GPP, ER and BACPR compared with the pre/post HW period, but when considering the changes in A0, only the HW effect on GPP and ER was significant. In HN, there was a positive HW effect on GPP, ER and BACPR compared with the pre/post heat wave period, but when considering the changes in A0, no HW effect was found (Table 3).

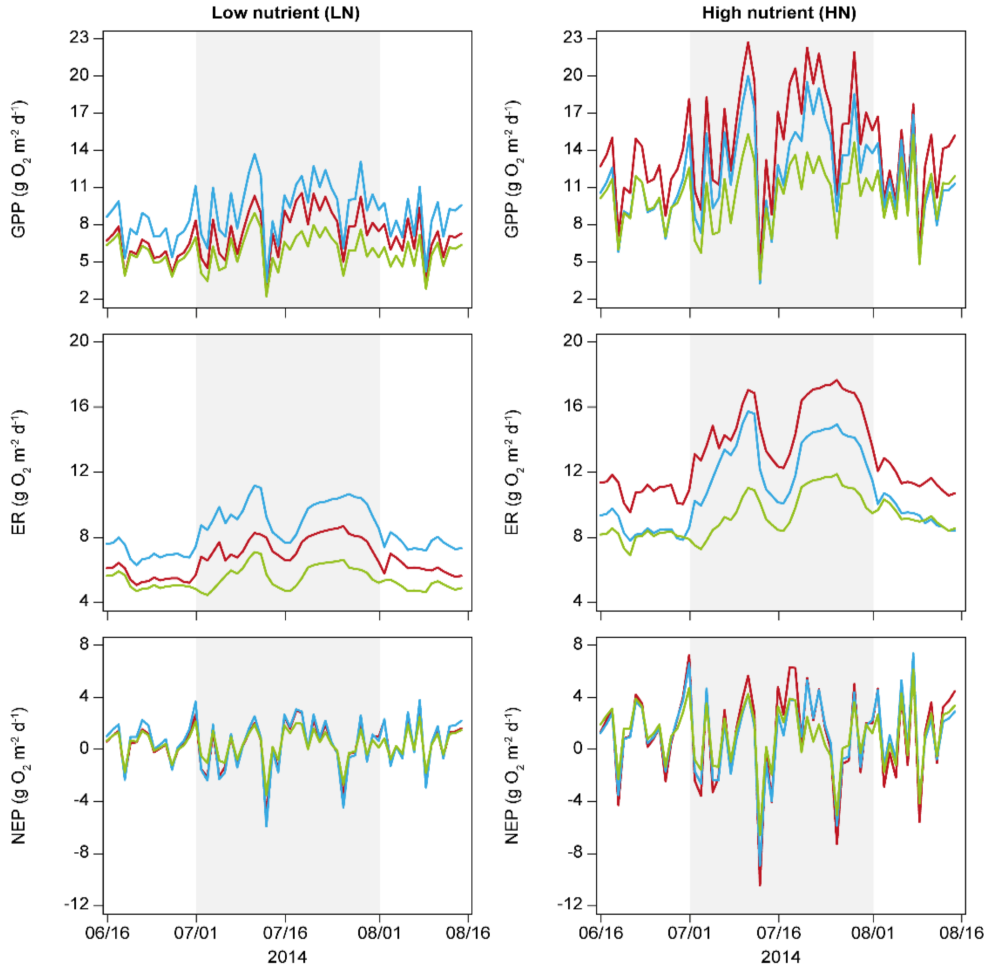

Figure 4. Changes in 24 h gross primary production (GPP), ecosystem respiration (ER)and net ecosystem production (NEP) in the low and high nutrient mesocosms before, during and after the heat wave. The three temperature scenarios are: A0 (green), A2 (blue) and A2+ (red). Values are means of all four replicates. Variation—see Figure 5. Grey indicates the experimental heat wave period.

Table 3. Results from the linear mixed effect models used to test the effect of nutrient, temperature and heat wave treatments on gross ecosystem primary production (GPP), ecosystem respiration (ER), net ecosystem production (NEP) and bacterioplankton production (BACPR)—ns, not significant, * $p < 0.5$; ** $p < 0.01$; *** $p < 0.001$. Further explanation is given in the legend of Table 2.

Data Set	Treatment	GPP	ER	NEP	BACPR
Full	Nutrient	*** (+)	*** (+)	* (+)	*** (+)
	Temperature	ns	* (+)	ns	** (+)
	Heat wave	*** (+)1	*** (+)1	*** (−)0	*** (+)0
LN	Temperature	* (+)	*** (+)	ns	** (+)
	Heat wave	*** (+)1	*** (+)1	ns	*** (+)0
HN	Temperature	* (+)	* (+)	ns	* (+)
	Heat wave	*** (+)0	*** (+)0	ns	*** (+)0

We also ran the models excluding the cold period 9–18 July, as it may preclude disclosure of significant results of the HW. However, only minor changes appeared. A significant HW effect on GPP now emerged also when accounting for changes in A0, and the HW led to significantly lower O_2 and O_2% (all $p < 0.05$) in LN, but otherwise no effect of removing the cold period data was observed.

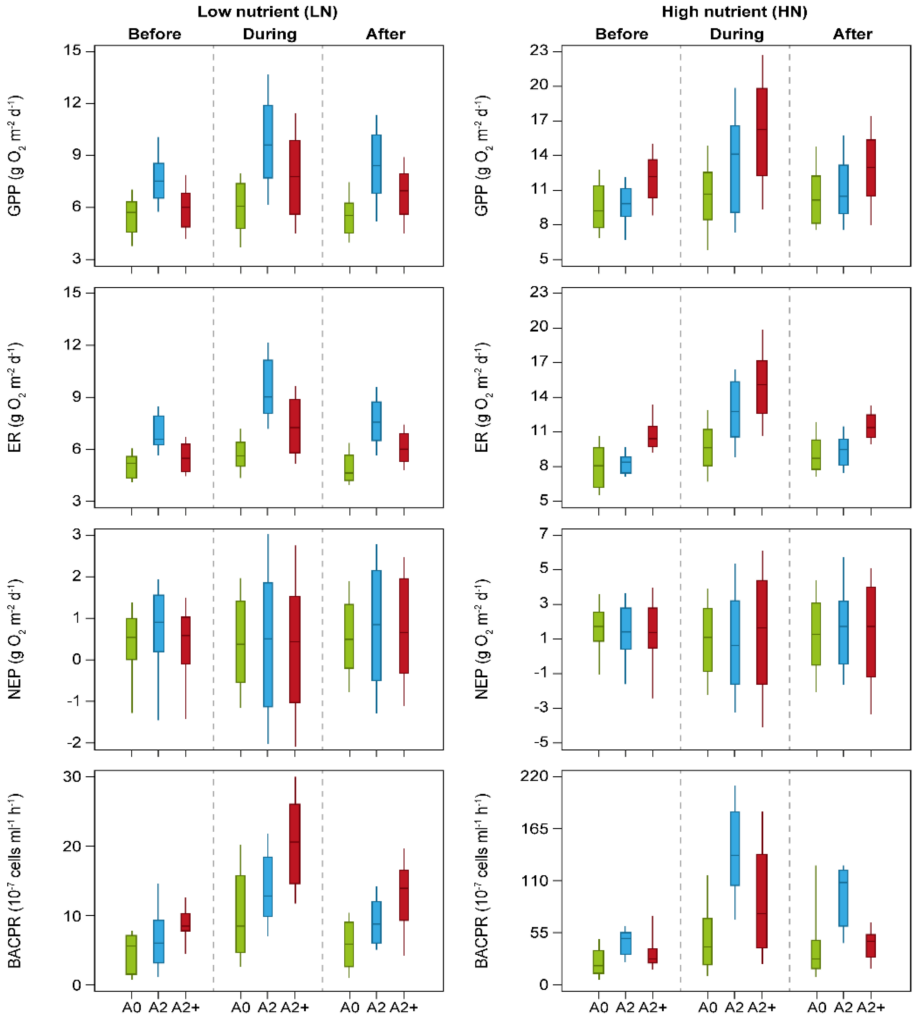

Figure 5. Boxplot (10%, 25%, 75%, 90% percentiles) of 24 h mean oxygen concentration (O_2), oxygen percentage (O_2%), gross primary production (GPP), ecosystem respiration (ER), net ecosystem production (NEP) and bacterioplankton production (BACPR) in the low and high nutrient mesocosms before, during and after the heat wave (the 4 replicates pooled). The three temperature scenarios are: A0 (green), A2 (blue) and A2+ (red). Note the different scale on the y-axes of the LN and HN treatments.

We further elucidated the relationship between GPP, ER and NEP standardized to 20 °C (GPP20, ER20 and NEP20) using Equation (1) and the key environmental variables. This was done to explore the importance of other environmental variables other than temperature which was already included in the calculation of the metabolism variables. As the dataset for environmental variables was modest, we combined all treatments and sampling dates. The results of the linear mixed effect model regression analyses are presented in Table 4. When using Chl-a, TP and TN as explanatory variables, TN and/or Chl-a were retained in the final models. When divided into LN and HN, TN and TP were retained in the models for GPP20 and NEP20 and TN for ER20 in LN, while Chl-a and TP were retained for ER20 in HN. None of the other variables were related to these three environmental variables. We also ran analyses with additional inclusion of the amount of submerged macrophytes in the mesocosms, PVI; however, this reduced the dataset further (Table 4). No significant effects were found on the full dataset or in HN, while GPP20 and ER20 in LN were related positively to PVI and, while ER20 was also related negatively to TN (Table 4). No other significant relationships were found.

Table 4. Multiple regression (backwards procedure) relating gross primary production standardized to 20 °C (Equation (1)), GPP20 and the same figures for ecosystem respiration (ER20) and NEP20 = GPP20-ER20 with chlorophyll a (Chl-a), total phosphorus (TP) and total nitrogen (TN) as explanatory variables and when also including the plant volume inhabited (PVI). Note that the dataset is smaller for the latter set (less PVI measurements). All p values are so low that even if they were corrected for multi-sampling, they would still be significant. Standard deviation is shown in brackets.

Data Set	With Chl-a TP and TN
Full	Log(GPP20) = 2.50(0.04) + 0.022(0.009) Log (Chl-a), $p < 0.0001$, $n = 337$ Log(ER20) = 2.40(0.04) + 0.019(0.005) Log (Chl-a) − 0.026(0.007) Log(TN), $p < 0.0001$, $n = 337$ Log(NEP20) = 2.43(0.01) + 0.031(0.009) Log(TN), $p < 0.02$, $n = 337$
LN	Log(GPP20) = 2.25(0.07) + 0.029(0.013) Log (TN) − 0.044(0.013) Log(TP), $p < 0.0001$, $n = 168$ Log(ER20) = 2.28(0.03) − 0.033(0.008) Log (TN), $p < 0.0001$, $n = 168$ Log(NEP20) = 2.27(0.07) + 0.055(0.013) Log(TN) − 0.041(0.013) Log(TP), $p < 0.0003$, $n = 168$
HN	Log(ER20) = 2.40(0.06) + 0.027(0.009) Log (Chl-a) − 0.058(0.016) Log (TP), $p < 0.0001$, $n = 169$ GPP20 and NEP20 were not significantly related to these variables.
	With PVI, Chl-a TP and TN, and PVI Must Be Included
Full and HN	GPP20, ER20 and NEP20 were not significantly related to PVI.
LN	Log(GPP20) = 2.23(0.03) + 0.011(0.02) Log (PVI + 1), $p < 0.02$, $n = 83$ Log(ER20) = 2.21(0.04) − 0.04(0.01) Log (TN) + 0.05(0.02) Log(PVI + 1), $p < 0.0001$, $n = 83$ NEP20 was not significantly related to PVI.

We conducted similar analyses for BACPR (Table 5), but here we also included water temperature as an explanatory variable, as temperature was not used in the calculation of BACPR. On the full dataset, Chl-a and water temperature contributed positively to BACPR, and when divided into LN and HN, TP also contributed to the relationship in both LN and TN.

Table 5. Multiple regression (backwards procedure) relating bacterioplankton production (BACPR) with chlorophyll a (Chl-a), total phosphorus (TP), total nitrogen (TN) and water temperature (TEMP) as explanatory variables without and with inclusion of plant volume inhabited (PVI). Note that the dataset is smaller for the latter set (less PVI measurements). All p values are so low that even if they were corrected for multi-sampling, they would still be significant. SD is shown in brackets.

Data Set	With Chl-a TP and TN
Full	Log(BACPR) = −2.62(0.35) + 0.14(0.01) Log (Chl-a) + 1.03(0.11) Log (TEMP), $p < 0.0001$, $n = 328$
LN	Log(BACPR) =−4.97(0.80) − 0.16(0.04) Log (Chl-a) − 0.15(0.06) Log (TP) + 1.51(0.22) Log (TEMP), $p < 0.0001$, $n = 160$
HN	Log(BACPR) = −0.66(0.19) − 0.024(0.011) Log(Chl-a) + 0.13(0.02) Log (TP) + 0.66(0.05) Log (TEMP), $p < 0.0001$, $n = 168$
	With PVI, Chl-a TP, TN, and PVI Must Be Included
	No significant relationships

4. Discussion

We studied nutrients, oxygen metabolism and bacterioplankton production before, during and after a simulated 1-month summer heat wave (HW) in shallow lake mesocosms run at contrasting nutrient concentrations and temperatures. As the mesocosms had already been in operation for 11 years prior to this experiment, we assumed that the different compartments (i.e., sediment, water and biota) have reached an equilibrium, allowing reliable comparison with natural systems. However, disentangling the effects of the HW from those of seasonality was not straightforward with our design lacking true controls. We, therefore, compared the data from the HW with those from the periods before

and after, considering the changes in A0 having no artificial HW but warmer temperatures in July arising from a natural HW (Figure 1).

We found a strong nutrient treatment effect on Chl-a, TP, TN and PO_4, as expected, given the major difference in nutrient loading of the LN and HN mesocosms. No significant difference for NO_3 is also reasonable, since NO_3 levels in shallow lakes often decrease in the summer due to uptake in organisms (higher TN in HN) or loss by denitrification [50]. No temperature treatment effects were recorded for the four variables; however, Chl-a and PO_4 significantly increased during the HW, but not when accounting for the changes in A0, which is more likely a reflection of the naturally higher temperatures (Figure 1) during the HW than before and after. This suggests that eutrophication was intensified during this overall natural warmer period occurring during the period that we applied the artificial HW (Figure 3), but the effect of the 5 °C extra temperature increase in A2 and A2+ could not be discerned. When dividing the data into the two nutrient levels (treatments), we found that PO_4 increased with temperature in HN but decreased in LN (Table 2). The higher PO_4 in HN likely reflects increased internal loading under eutrophic conditions, as has been observed for eutrophic lakes in both experimental studies [51] and when conducting lake mass balances [52]. We also found higher Chl-a in LN with increasing temperature, which is likely a result of the overall lower PVI of plants in the warmer mesocosms [35] and thus is a diminished constraining effect of plants on phytoplankton development [53]. During the HW, Chl-a increased relative to the pre/post-HW period in LN, but not when accounting for changes in A0, indicating that the extra heating during the HW was not of importance.

The mixed effect models revealed a strong significant positive effect of nutrient treatments on the metabolism variables, GPP, ER, NPP and BACPR (Figures 4 and 5, Table 3). That higher nutrient loading leads to increased metabolic rates and higher net productivity is well-known [13,14]. It is also well-established that BACPR increases with eutrophication (e.g., [17]). We found a significant positive effect of temperature on ER and BACPR, both when the dataset was analyzed in full and when it was divided into LN and HN, but no effect was found on GPP and NEP. While the positive effects on GPP, ER and BACPR were expected because these processes are temperature dependent ([7–10], see Equation (1), we anticipated a lower NEP with increasing temperature as ER, in theory, is more dependent on temperature than on GPP [9,10]. While the 24 h daily mean NEP varied between positive and negative values during the course of the experiment in all temperature scenarios (Figure 4), the average for the study period was positive and similar across the three temperature treatments (A0, A2 and A2+) for both LN and HN, indicating organic matter accumulation during summer irrespective of temperature and HW variances. However, NEP was about 2–3 times higher in HN than in LN, which suggests an overall higher net accumulation of organic matter at high nutrient levels.

We also related the temperature-corrected GPP, ER and NET (20 °C selected, GPP20, ER20 and NEP20, using Equation (1)) to explore the effect of other environmental variables (Table 4). We found that GPP20 and ER20 were related to nutrients in the full dataset and in HN. By contrast, PVI was the most important variable in LN, emphasizing the key role that macrophytes and associated periphyton may play for the metabolism in clear water shallow lakes with abundant macrophytes (e.g., [54]). BACPR was not affected by PVI but was affected by Chl-a and/or nutrients and temperature, suggesting that the bacterioplankton was mainly fuelled by pelagic resources, even at high PVI.

In both LN and HN, HW effects on GPP, ER and BACPR appeared, but only the effect on GPP and ER in LN remained significant when accounting for the changes in the A0 mesocosms during this period (Table 3). A substantial drop in air temperature and PAR in the middle of the HW, however, led to a major short-term reduction of GPP, followed by a reduction of ER, as well as a major reduction of NEP to large negative values, all changes being more pronounced in HN than in LN (Figure 4). Accordingly, O_2 and O_2% declined markedly, not least in the heated mesocosms (Figure 2). In some of the heated HN mesocosms, O_2 values were as low as 0.6–0.9 mg L^{-1}, indicating that eutrophic systems

in particular are highly sensitive to short-term changes in weather conditions under HW conditions. Concomitant with the decline in O_2, an increase in the emission of methane was observed during the HW [47]. During low-O_2 period, fish kills occurred in HN-A2 and HN-A2+ as well, with cascading effects on the zooplankton [35] and the phytoplankton communities [55]. However, the metabolism was rapidly restored when the temperature increased again and seemed to be only insignificantly affected by the fish kill.

5. Conclusions

To conclude, we found strong effects of nutrients on oxygen processes and bacterioplankton production as well as temperature effects on ecosystem respiration and bacterioplankton production. Overall, the HW effect was minor. We did, however, find major short-term changes in metabolism during a sudden cooling period with reduced irradiance; the responses were strongest in the heated nutrient-rich mesocosms and manifested the low oxygen concentrations and fish kills in the high nutrient and high temperature treatments. These results demonstrate the higher risk of dramatic shifts in shallow lake ecosystems in HW periods in a future warmer world and that the risk increases with eutrophication. Rapid responses to changes in temperature, including the cooling period in the middle of the HW, were also found for the biomasses of bacterioplankton, heterotrophic flagellates, ciliates [56] and for the methane emission [47]. By contrast, a modest effect of the HW was found for phytoplankton [55] and zooplankton [35], suggesting that the microbial community and process rates are particularly sensitive to heat waves in already warm lakes, at least in the short-term.

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