

Effects of artificial thermocline deepening on sedimentation rates and microbial processes in the sediment

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Abstract Global warming affects the thermal stratification of freshwater lakes, and thus can lead to thermocline deepening. To follow the direct and indirect effects of thermocline shifts on processes at the sediment–water interface, the thermoclines in six of twelve lake enclosures were deepened by pumping warm surface water below the thermocline. Hypolimnetic temperatures increased and oxygen concentrations decreased due to the treatment. Path modeling suggests a correlation of the treatment to epilimnetic

chlorophytes and hypolimnetic diatoms, while the dominant cryptophytes and cyanobacteria remained unaffected by the treatment. Sedimentation rates, ranging between 0.1 and 1.9 g m⁻² day⁻¹ per enclosure, correlated strongly with the selected phytoplankton groups, but were not affected by the treatment. Concentrations of elements (Ba, Mn, S, Sr), nutrients (NH₄⁺, PO₄³⁻, Si), and greenhouse gases (CO₂, CH₄, N₂O) in the upper centimeter of the sediment were not affected by the hypolimnetic changes. Methane and carbon dioxide emission from the enclosures to the atmosphere were negligible. Bacterial and viral abundance in sediments were not affected by the treatment. Results suggest that the benthic ecosystem remains unaffected by slight changes in the pelagial. Further,

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we conclude that short-term warming does not increase greenhouse gas emissions from temperate, deep, oligo-mesotrophic lakes.

Keywords Climate change · Lake metabolism · Phytoplankton biomass · Sediment microbes · Greenhouse gases · Mesocosms

Introduction

Thermocline formation is the result of heat fluxes within lakes (Schindler, 1997) and stratifies the water column of dimictic lakes into nutrient-depleted surface waters (epilimnion) and nutrient-rich deep waters (hypolimnion) in summer. Moreover, the thermocline forms a density boundary that limits the passage of fresh organic matter from the epilimnion to profundal sediments and of recycled nutrients from the sediment to the uptaking organisms in the epilimnion. Thereby, the thermocline determines the growth and loss of phytoplankton (Sommer et al., 1986; Diehl, 2002) and zooplankton (Sastri et al., 2014) populations and the sedimentation of organic matter. Onset, duration, and depth of the thermocline are influenced by water transparency (Fee et al., 1996; Snucins & Gunn, 2000; Houser, 2006), lake morphometry, climate, and salinity (Sterner, 1990; Archer, 1995; Imboden & Wuest, 1995), and global warming is supposed to intensify the stratification and the suppression of nutrient fluxes from the hypolimnion to the epilimnion by increasing density gradients (Verburg et al., 2003; Behrenfeld et al., 2006; Schindler, 2009). However, the predicted consequences vary among lakes, depending mainly on the morphometry. Lake thermoclines can either deepen, due to wind stress and mixing (France, 1997; Schindler, 2009), or due to lower precipitation frequencies (IPCC, 2007) resulting in clearer waters (Schindler, 2001), or become shallower, due to higher precipitation frequencies resulting in increased influx of colored, terrestrial DOC (Snucins & Gunn, 2000).

Thermal stratification fundamentally affects the hydrophysical and chemical conditions in the lake, and thus also plankton community compositions and their sedimentation to the sediment. In a mesocosm experiment, a deeper thermocline resulted in decreased spring phytoplankton biomass and *Daphnia* density peaks (Berger et al., 2014), while whole lake

experiments demonstrated greater phytoplankton productivity in the epilimnion during the stratified season (Cantin et al., 2011) and an increase of total zooplankton biomass along with a shift to smaller-bodied zooplankton species (Gauthier et al., 2014; Sastri et al., 2014) due to a deepened thermocline. Such changes in the plankton community alter sedimentation rates, and thus connect the pelagic with the benthic ecosystem. Organic matter input into the sediment as well as its microbial turnover and release of nutrients into the water column are vital to supply both environments with nutrient and energy sources.

Lake sediments are affected indirectly by a deepened thermocline, via the sedimentation of organic matter and changing physical and chemical conditions in the hypolimnion. Published information about the consequences of a deepened thermocline on microbial processes at the sediment–water interface (SWI) is scarce. Thermocline deepening is correlated with hypolimnion warming (Sastri et al., 2014), thus hypolimnetic respiration rates and microbial productivity could increase and reduce the oxygen concentrations at the SWI. Additionally, enhanced release of nutrients to the water column would be channeled into the microbial loop (Azam et al., 1983) and may stimulate zooplankton productivity (Cantin et al., 2011; Gauthier et al., 2014). Further, SWI anoxia could increase P-mobilization and increase anaerobic decomposition processes such as methanogenesis. Methane is one of the main products of anaerobic carbon mineralization during organic matter decomposition (Conrad et al., 2010), with 20–60% of the sedimenting carbon being converted to CH₄ (Rudd & Hamilton, 1978; Fallon et al., 1980; Wetzel, 2001). In addition, methanogenesis is positively correlated to temperature (Zeikus & Winfrey, 1976; Kelly & Chynoweth, 1981; Schulz et al., 1997; Glissmann et al., 2004; Duc et al., 2010). In mesocosm experiments, Flury et al. (2010) did not find increased methane emission rates at higher temperatures, while Yvon-Durocher et al. (2014) observed that warming of 4°C increased the emission of CH₄ from carbon remineralization by 20%.

A number of studies have focused on the effects of global warming on plankton and fish dynamics in lakes, but to our knowledge there has not been any targeted study to assess the impacts on sediments. It is not clear so far if and how sediment microbes are affected by stratification patterns in the water column,

as the effects will mainly be indirect. Thermocline shifts increase microbial respiration and/or extend the duration of summer stratification, resulting in an increase of anoxic conditions at the SWI, which might be followed by severe consequences affecting the whole lake ecosystem. The objectives of this study were to investigate direct and indirect effects of global warming, in the form of thermocline deepening, on lake sediments. We simulated thermocline deepening in the so-called LakeLab (huge enclosures in Lake Stechlin; Bauchrowitz, 2012) and measured the effects on sedimentation rates and microbial responses in sediments two months after the treatment. The following chain of hypotheses was tested (see Fig. 1):

H1 A deepened thermocline leads to an increase of hypolimnetic temperature, and thus of microbial activity, and to a decrease of the oxygen concentrations above the sediment.

H2 A deepened thermocline and increased phytoplankton biomass will increase sedimentation rates to the sediment.

H3 An increase of temperature and sedimentation rates at the SWI will enhance microbial processes, thereby increasing the abundance of sediment bacteria as well as the products of organic matter decomposition, such as NO_3^- , NO_2^- , NH_4^+ , and H_2S , PO_4^{3-} . An increase of bacterial abundance will in turn increase viral abundance.

H4 Increasing temperatures and decreasing oxygen concentrations above the sediment will increase the release of porewater elements.

H5 An increase of temperature, sedimentation rates, and a decrease of oxygen concentrations will increase the concentrations of the greenhouse gases CO_2 and

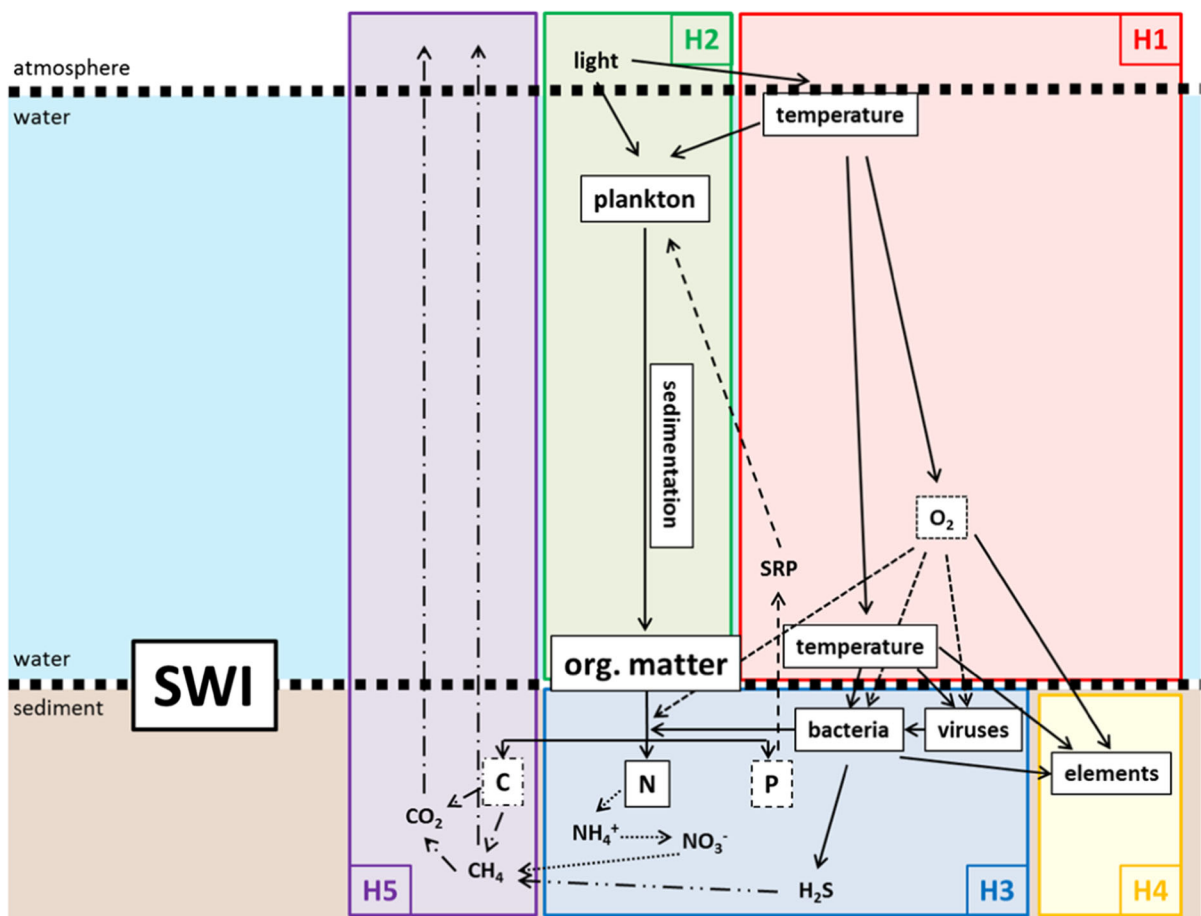


Fig. 1 Hypotheses interaction scheme

CH₄ at the sediment surface. Simultaneously, NO₃[−] and SO₄^{2−} can inhibit the production of CH₄.

To test these hypotheses we applied path analysis. A path model takes into account the combination of direct and indirect effects that would not be sufficiently covered by multivariate statistics or linear models. In this path model, different blocks of variables, each of them representing one step within the carbon cycle, were defined by latent variables that significantly influenced the linear relations among the blocks (Sanchez, 2013), independent of their abundance or significance for the ecosystem in general.

Materials and methods

Enclosures facility and treatment

The study was performed in 12 enclosures (diameter: 9 m, depth ca. 20 m, Table 1) which are part of a 24-enclosures LakeLab facility (Kwok, 2013; Giling et al., 2016; Fig. 2) in Lake Stechlin (Brandenburg, Germany). Lake Stechlin is an oligo-mesotrophic, temperate lake with a mean depth of 23.3 m, maximum depth of 69.5 m, and a surface area of 4.23 km² (Koschel & Adams, 2003). The lakes' shoreline is covered to ~96% by trees (Casper, 1985). Parameters influencing the thermal stratification of the lake during the last 55 years in the form of artificial or global temperature increases were reported by Kirillin et al. (2013). The enclosures consist of floating aluminum rings with curtains (0–11 m depth: 5 mm thickness,

STAMOID 4800, 12 m-sediment: 1 mm thickness, STAMOID 1250) reaching down until 1 m into the sediment (Fig. 2). Enclosure pairs were E1 with E13, E3 with E15, E4 with E16, E5 with E17, E7 with E20, and E8 with E19, based on similar depths and ecological conditions. Pairs of enclosures were chosen randomly for treatment or control. Thus, E1, E4, E8, E15, E17, and E20 were chosen for treatment (Table 1). Prior to the manipulation, water was exchanged between the enclosures and the lake by pumping for two weeks, and between paired enclosures for another two weeks, to equalize the starting conditions among enclosures.

In each enclosure, underwater pumps (SUPS 4-12-5, SPECK Pumpen Verkaufsgesellschaft GmbH, Neunkirchen am Sand, Germany) transported 5.5 m³ h^{−1} of warm epilimnetic water via aluminum release rings into deeper water layers (Fig. 2). The release rings (ring diameter: 7 m, tube diameter: 44.3 mm), containing holes (diameter: 3 mm) every 50 cm, were placed into the depth of the lake thermocline in control enclosures, and 2 m lower in the treated enclosures; thus all enclosures were equally affected by pumping activities. As the thermocline in the lake moved due to temperature changes, the release rings in the enclosures were moved 1 m down each on 24 July, 25 July, and 04 September 2013. During the first month, only a weak pre-treatment (8 h of pumping per day) was applied, to enable organisms to adapt to the pumping gently, and the pumping rhythm was then increased (12 h of pumping per day) in the second month to force the thermocline into a

Table 1 List of enclosures used in this study, showing treatments, water depths, and the depth of the release rings, with (E) = epilimnion; (H) = hypolimnion

Each treated enclosure was paired with a control enclosure (E3–E15, E13–E1, E5–E17, E16–E4, E7–E20, E19–E8)

Treatment	Enclosure	Depth (m)	Release ring depth (m)	Volume (E)	Volume (H)
Control	E3	20.5	5–8	318/509	986/795
Control	E13	20.8	5–8	318/509	1,005/814
Control	E5	19.2	5–8	318/509	903/713
Control	E16	19.9	5–8	318/509	948/757
Control	E7	17.9	5–8	318/509	821/630
Control	E19	17.7	5–8	318/509	808/617
Treated	E15	20.0	7–10	445/636	827/636
Treated	E1	19.9	7–10	445/636	821/630
Treated	E17	18.9	7–10	445/636	757/566
Treated	E4	19.5	7–10	445/636	795/604
Treated	E20	17.1	7–10	445/636	643/452
Treated	E8	17.2	7–10	445/636	649/458

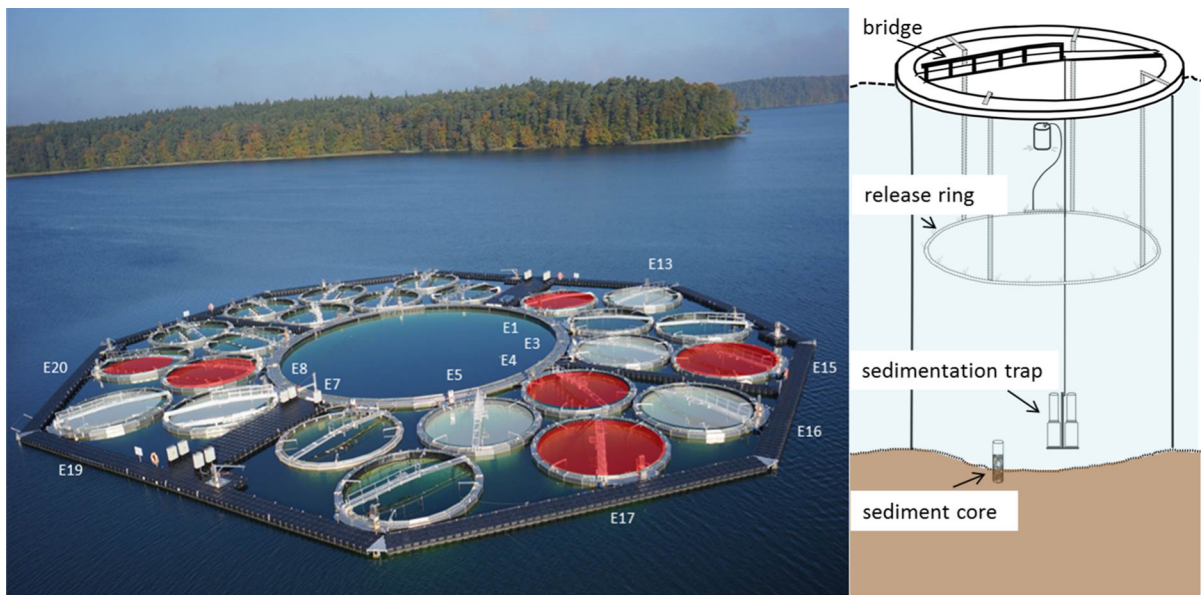


Fig. 2 LakeLab facility in Lake Stechlin with control (gray) and treated (red) enclosures and a scheme of a single enclosure including sampling devices (photo: Martin Oczipka, IGB/HTW Dresden)

deepened position. Pumping was only applied during daytime to allow natural cooling over night.

Water column

Physical and chemical parameters

The water column was sampled every 2 weeks, on 25 June, 10 July, 23 July, 06 August, 20 August, and 11 September 2013. Temperatures and oxygen concentrations in the water column were measured in half-meter increments (from the surface to a minimum of 0.5 m above the sediment) and at 1 h intervals by automated multiprobes (YSI 6600, YSI Inc., Yellow Springs Instrument, OH, USA) installed in each enclosure. Average temperatures and oxygen concentrations in the epilimnion and hypolimnion were calculated from the average temperature and oxygen concentration per volume of each enclosure on each sampling day, considering the varying volumes of each depth horizon over time due to thermocline deepening. Due to the depth differences among enclosures (Table 1), we calculated the averages in the hypolimnion only from values until 16 m depth in each of them. In addition, we calculated the total amount of oxygen and temperatures within each

horizon of each enclosure. Values at the SWI were measured in the last meter above the sediment.

Phytoplankton biomass

Integrated water samples (volume: 5 l each, IWS 2, Hydro-Bios, Kiel, Germany) were taken from the epilimnion and hypolimnion and preserved in Lugol's solution. At minimum 400 settling units (cells, filaments, or colonies) were counted in each sample using an inverted microscope (Zeiss Axiovert 35, Oberkochen, Germany). Phytoplankton numbers were determined with the methodology by Lund et al. (1958) and biomass was calculated volumetrically assisted by the OptiCount software (SequentiX, Klein Raden, Germany).

Sedimentation rates

Sedimentation traps (Uwitec®, Mondsee, Austria) were deployed in the hypolimnion 1 m above ground for three months, tied with a rope centrally to the bridge leading across the enclosures, and a weight at the bottom to keep the trap in position. Traps consisted of two tubes (diameter: 9 cm, height: 100 cm) pushed into two wide-neck PVC collection bottles (2 l) filled

prior to deployment with hypolimnetic water. Just one collection bottle per enclosure was used to determine sedimentation rates. As a fixative, 100 ml formaldehyde (final concentration 1.85%) was added to each bottle prior to deployment to inhibit further decomposition and zooplankton feeding (Fuchs et al., 2016a). Traps were deployed from 27 June to 19 September and were exchanged in monthly intervals. Samples were stored at 4°C until processing.

Trapped material was allowed to settle and the total volume was decanted to 500 ml. The sample was then homogenized and sieved through 280 µm gauze to remove zooplankton that tended to actively swim into traps instead of sinking in. Sieved material was filtrated over big (diameter: 45 mm, 50 ml of homogenized sample filtrated) and small (diameter: 25 mm, 10–15 ml of homogenized sample filtrated) glass fiber filters (GFF) in three replicates, each. GFF were combusted at 500°C for 4 h before filtration and dried at 105°C over night after filtration. Big GFF were used for dry weight analyses, while two of the three small GFF were analyzed for carbon and nitrogen (Vario El analyzer, Elementar Analysensysteme, Hanau). The third small GFFs were used for analyses of total phosphorus (TP) and total nitrogen (TN) after extraction with potassium peroxodisulfate (Ebina et al., 1983) and subsequent photometric analysis using flow injection analysis.

Sediments

Sediment cores

Two sediment cores (diameter: 6 cm) per enclosure were collected before the start of the experiment (26–28 June) and after two months of thermocline deepening (28–30 August), using a gravity corer (Uwitec®, Mondsee, Austria). Cores were taken in the center of each enclosure, carefully choosing a new spot for every core. Sediments were pushed to the top of the core and sliced into the layers 0–1, 1–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–14, 14–16, 16–18, and 18–20 cm. We retrieved only 0–8 cm of sediment from cores of E8, E19, and E20 in June, and of E19 and E20 in August due to low water content in deeper layers. While sediments of one core were analyzed for sediment characteristics and porewater chemistry in each layer, sediments of the second core were analyzed for greenhouse gas concentrations and the abundance of bacteria and viruses at the SWI.

Porewater chemistry and sediment characteristics

Rhizons (length: 5 cm, diameter: 2.5 mm, pore size: 0.1 µm, Rhizosphere, Wageningen, Netherlands) were used to extract a maximum of 10 ml porewater per depth, while remaining sediments were dried over night at 105°C and weighed before and after, to analyze the dry weight content. Of each porewater sample, 2 ml was distributed in Eppendorf tubes for the analyses of nutrients (phosphate, nitrate, nitrite, ammonium), sulfide, silicate (SiO_4^{2-}), and dissolved metals, respectively. Tubes for nutrients and silicate were washed with Milli-Q water in advance, and samples were measured using an autoanalyzer (CFA QuAAtro, SEAL, Norderstedt, Germany). Tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) were treated with 65% v/v suprapure HNO_3 prior to sampling, and porewater samples were acidified with 1% v/v suprapure HNO_3 . Samples were stored at 4°C until the measurement by ICP-OES (iCAP 6300 Duo, Thermo Fisher Scientific, Waltham, MA, USA). H_2S was measured spectrophotometrically (SPEKOL 1100, Analytik Jena, Jena, Germany) according to the method of Cline (1969).

Bacterial and viral abundance

From each sediment core, 0.5 ml of wet sediment was mixed with 0.5 ml of sodium pyrophosphate (final concentration 5 mM) and 4 ml of virus-free sterile H_2O . After an incubation of 10 min at room temperature, samples were gently sonicated in a water bath sonicator (Transsonic Digital, Elma, Singen, Germany) three times for 20 s interrupting for 10 s every time during which the sample was shaken manually. Then a centrifugation step at $800\times g$ at 15°C was applied for 1 min, and 1 ml of the supernatant was withdrawn in duplicate and mixed with virus-free distilled water in order to achieve the desired dilution. The extracted sample (1 ml) was first treated with DNase (final concentration 1 U ml^{-1}) and then filtered through 0.02 µm ultra-fine pore size filter (Anodisc, Whatman, Little Chalfont, UK). As the filter dried, a drop (10 µl) of SYBR Green I ($2.5\times$ final concentration diluted in Milli-Q water, Invitrogen, Waltham, USA) was laid on the sample and kept in the dark for 15 min. Filters were then washed with virus-free lake water and put on a slide. Slides were stored at -20°C until counted using an epifluorescence

microscope (magnification 1.000×, blue light excitation filter cube L5, Leica DM5500B, Leitz, Wetzlar, Germany). 10 to 20 fields were viewed until a total of 200–300 virus-like particles (VLP) or bacteria were counted, respectively.

Greenhouse gases

Concentrations of carbon dioxide, methane, and nitrous oxide were measured from 2 ml of wet sediment per layer added to 4 ml distilled water in 10 ml glass vials. Vials were sealed gas-tightly by butyl caps. HgCl_2 (final concentration 200 mg l⁻¹) was added for fixation to avoid microbial production or consumption of gases. Vials were stored upside down at 4°C in the dark until measurement. Gas measurements were performed using a gas chromatograph (GC-14B, Shimadzu Europa GmbH, Duisburg, Germany) equipped with TCD (CO_2 analysis), FID (CH_4 analysis), and ECD (N_2O analysis) and an autosampler AOC 5000 (CTC Analytics AG, Zwingen, Switzerland). CO_2 and CH_4 concentrations were calculated per dry weight (DW) sediment based on Henry's Law solubility constants (k_{H,CH_4} : 0.00117192 mol kg⁻¹ bar⁻¹, k_{H,CO_2} : 0.034 mol kg⁻¹ bar⁻¹) retrieved from NIST.

Greenhouse gas fluxes at the air–water boundary were measured by an aluminum flux chamber (1 m² area, 240 l volume), shaped as a truncated pyramid and floating on an inflated rubber rectangle, in the center of each enclosure. Each flux chamber possessed a connection valve with a gas-tight septum. Chambers were placed on the enclosures at the end of the experiment, one day after the pumping stopped, and gas samples were taken immediately (t0), and after 6 h (t1), 24 h (t2), and 96 h (t3). 20 ml vials were capped gas-tightly and pre-evacuated by a vacuum pump for 90 s prior to sampling and connected to the flux chamber valves via cannulas for 90 s. Gas samples were measured immediately after sampling.

Statistics

All statistical analyses and graphs were performed using RStudio, Version 3.1.2 (R Studio, Inc. 2013, Boston, MA, USA). For several parameters, the variability between replicate enclosures was high (raw data given in Supplement Table 1). In addition, the natural development of summer stratification

resulted in changing concentrations of many parameters over time. Thus, we calculated for each parameter in each enclosure the delta values, as $T1 - T0$. Boxplots and statistical analyses were performed using these delta values, excluding values from enclosures 5 (control) and 8 (treated) due to missing data.

The direct and indirect effects of the treatment on sedimentation rates and sediments were tested by Partial Least Squares Path Modeling using the package “plsrm” (Sanchez, 2013). PLS path modeling has rarely been used for ecological approaches (Fu et al., 2015; Wang et al., 2016); however, it is advantageous to other models when analyzing complex networks of variables. In a path model, the correlation between latent variables is analyzed, taking into account direct as well as indirect effects. Each latent variable is defined by various measured manifest variables. In this study, the inner model of latent variables was constructed based on our hypotheses. We defined the treatment by the changes of temperatures and oxygen concentrations in the epilimnion and hypolimnion, assuming that water column parameters (such as plankton biomass and sedimentation rates) would be affected only by the epilimnetic treatment, while the sediment parameters (such as microbial abundances, porewater elements, and greenhouse gas concentrations) would be affected by the hypolimnetic treatment and sedimentation. The PLS path model was considered acceptable when the goodness-of-fit (GoF) estimate reached >0.7.

Additionally, all variables were tested blockwise on significant differences between control and treated enclosures using multivariate statistics (vegan-package, Oksanen et al., 2016). For this, all measured parameters were included, independent of their use in the PLS path model.

Results

The path model reached a goodness-of-fit estimate of $\text{GoF} = 0.701$. The inner model summary shows the relevance of the latent variables to the path model (Table 2), with the elements having the highest R^2 (0.837) and nutrients having the lowest R^2 (0.145). The hypolimnion treatment, porewater elements, and greenhouse gases were highly important for the inner model, reaching R^2 values >0.7, while phytoplankton

Table 2 Summary of the inner path model, with Treat = treatment, Phyto = phytoplankton, Traps = sedimentation traps, (E) = epilimnion, and (H) = hypolimnion

R^2 values above 0.7 (in bold) indicate significant response variable blocks within the model

Block	Type	R^2	Block communality	Mean redundancy
Treat (E)	Exogenous	0.00	0.75	0.00
Treat (H)	Endogenous	0.72	0.70	0.50
Phyto	Endogenous	0.49	0.71	0.35
Traps	Endogenous	0.69	0.95	0.66
Nutrients	Endogenous	0.14	0.96	0.14
Elements	Endogenous	0.84	0.84	0.70
Gases	Endogenous	0.70	0.77	0.54

and nutrients had a $R^2 < 0.5$, showing no significant relevance to the path analysis.

Treatment

During the first month of pre-treatment, the thermocline was not deepened in order to allow organisms to establish a community under the new treatment-like conditions. From 26 July onwards pumping intensity was increased and the thermocline was deepened by 2 m (Fig. 3). However, we did not achieve a complete mixing of the epilimnion between 25 July and 14 August. During this time, a 3–5 m thick density gradient formed above the thermocline with 21–22°C in the control and 15–18°C in the treated enclosures (Fig. S1—Supplementary Material). After this period,

decreasing irradiation cooled the surface water and facilitated a homogeneously mixed epilimnion.

Temperatures and oxygen concentrations (H1)

At the SWI, mean temperatures were 5.5°C in the control and treated enclosures, both in June (control: $\pm 0.2^\circ\text{C}$ SD, treated: $\pm 0.3^\circ\text{C}$ SD) and August (control: $\pm 0.1^\circ\text{C}$ SD, treated: $\pm 0.2^\circ\text{C}$ SD), while mean oxygen concentrations decreased from $10.2 \pm 1.4 \text{ mg l}^{-1}$ (control) and $10.5 \pm 1.1 \text{ mg l}^{-1}$ (treated) in June to $7.4 \pm 1.6 \text{ mg l}^{-1}$ (control) and $6.1 \pm 1.1 \text{ mg l}^{-1}$ (treated) in August. However, considering the delta values per enclosure in the hypolimnion, we observed an increase of the temperature (Fig. 3) as well as a decrease of oxygen concentrations (Fig. 4). Path

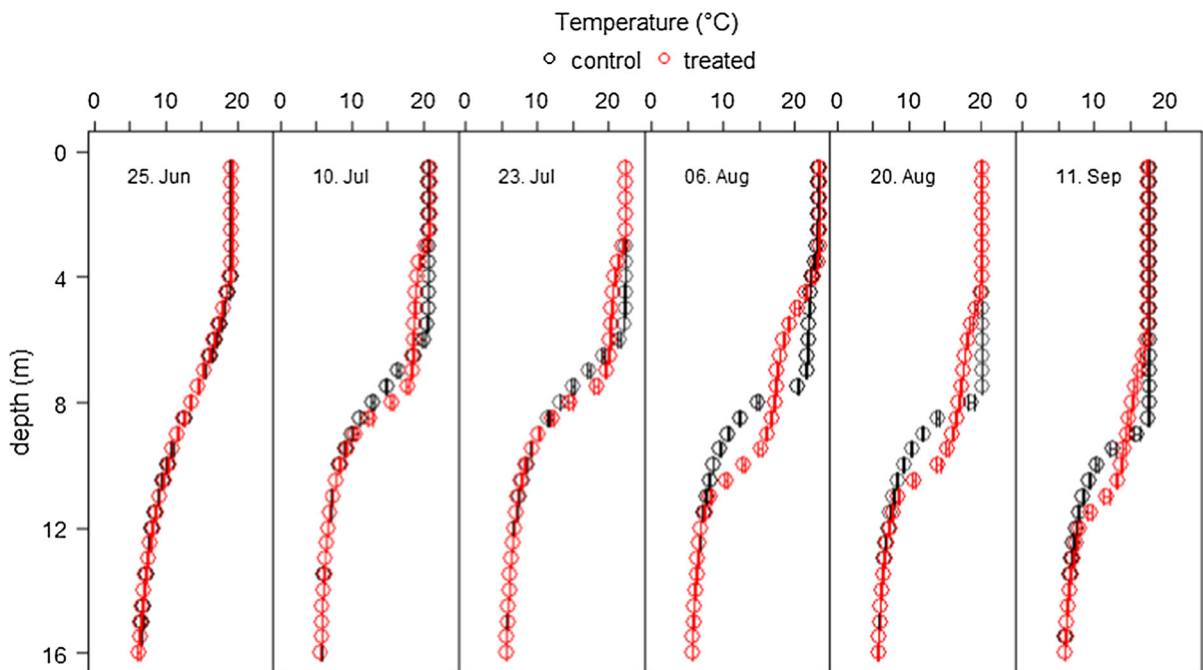


Fig. 3 Temperature profiles (mean \pm SE) in the water column during the experiment in the control (gray) and treated (red) enclosures

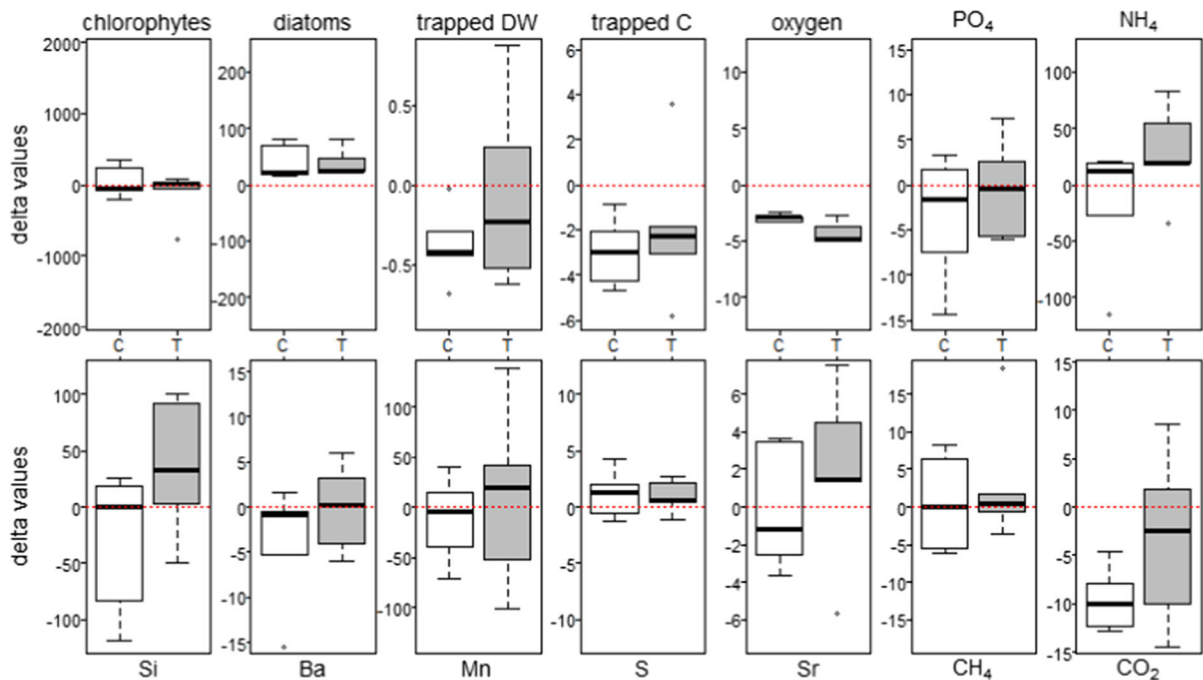


Fig. 4 Delta values of the parameters at the WSI that were used in the path analysis, showing an increase (*positive values*) or decrease (*negative values*) of the concentrations between June

and August in control (*white*) and treated (*gray*) enclosures. Boxes include the median (*solid lines*), minimum and maximum values (*whiskers*), and outliers (*circles*)

analysis confirmed a positive correlation between epilimnetic and hypolimnetic treatment effects. Multivariate statistics (Table 3) confirmed that the treatment affected average temperatures and oxygen concentration in both the epilimnion (PERMANOVA, $R^2 = 0.405$, $P = 0.018$) and the hypolimnion (PERMANOVA, $R^2 = 0.552$, $P = 0.01$), as well as total temperatures and oxygen concentrations in the epilimnion (PERMANOVA, $R^2 = 0.634$, $P = 0.011$) and the hypolimnion (PERMANOVA, $R^2 = 0.319$, $P = 0.027$), but not at the SWI (PERMANOVA, $R^2 = 0.261$, $P = 0.079$). The permanent profiling did not reveal anoxic conditions in any of the enclosures.

Phytoplankton biomass and sedimentation rates (H2)

Total phytoplankton biomass was not affected by the treatment and showed contrasting developments in epilimnion and hypolimnion: between July and August, the mean overall phytoplankton biomass in the epilimnion remained similar (525–564 $\mu\text{g l}^{-1}$), but increased in the hypolimnion (517–668 $\mu\text{g l}^{-1}$). The phytoplankton community was initially dominated by epilimnetic cryptophytes and shifted to an overall

Table 3 Results of PERMANOVA analysis (method = Euclidean), with Treat = treatment, Phyto = phytoplankton, Traps = sedimentation traps, (E) = epilimnion, (H) = hypolimnion, and (SWI) = sediment-water interface

	Df	SS	F	R^2	P value
Treat (E)	1	7.292	5.448	0.405	0.020
Treat (H)	1	9.936	9.857	0.552	0.013
Treat (SWI)	1	4.693	2.821	0.261	0.052
Phyto (E)	1	5.577	0.777	0.088	0.726
Phyto (H)	1	4.593	0.629	0.073	0.775
Traps	1	3.039	1.015	0.112	0.382
Nutrients	1	6	1.000	0.111	0.424
Elements	1	11.331	0.938	0.105	0.448
Gases	1	2.108	1.061	0.117	0.427

Df degrees of freedom, SS sums of squares, F F-model, P values based on 999 permutations

Statistical significance at $P < 0.05$ is indicated in bold

dominance of cyanobacteria (main species: *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek) in August, forming a deep chlorophyll maximum in the hypolimnion (see data in Selmečzy et al., 2016). These changes in phytoplankton community composition were not reflected in

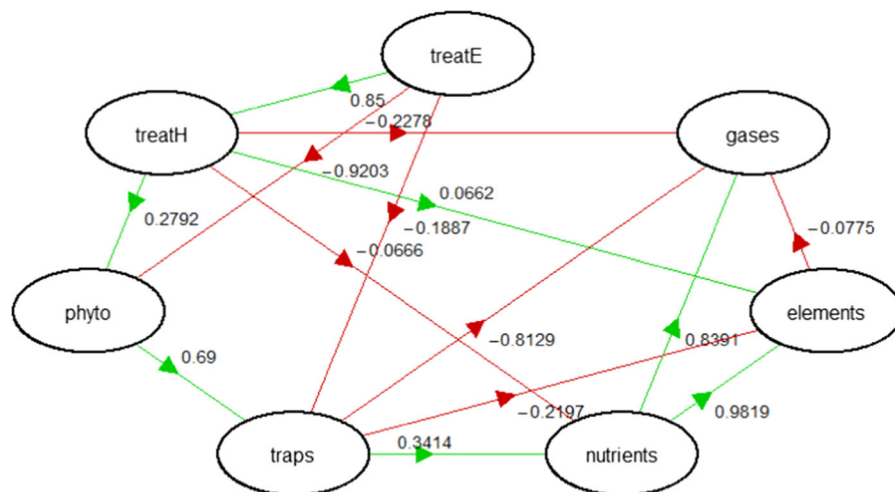


Fig. 5 Path analysis plot indicating positive (green) and negative (red) correlations of the inner model. The inner model consists of seven latent variables representing epilimnetic treatment (*treatE*), hypolimnetic treatment (*treatH*),

phytoplankton biomass (*phyto*), sedimentation rates (*traps*), sediment nutrients (*nutrients*), porewater chemistry (*elements*), and greenhouse gases (*gases*)

sedimentation rates, which decreased from an average of $686.6 \pm 197.8 \text{ mg m}^{-2} \text{ day}^{-1}$ in July to an average $495.7 \pm 498.1 \text{ mg m}^{-2} \text{ day}^{-1}$ in August. In August, sedimentation rates showed very high variation between enclosures, with dry weights ranging from $129.2 \text{ mg m}^{-2} \text{ day}^{-1}$ in E17 (treated) to $1,890.5 \text{ mg m}^{-2} \text{ day}^{-1}$ in E20 (treated). However, the change in the phytoplankton community composition was reflected by the composition of the sedimented material. The average C:N ratio of the sedimented material decreased from 10.6 ± 0.9 in July to 8.2 ± 1.8 in August, due to increases in the percentage of nitrogen. While E20 showed the highest sedimentation rate and total phytoplankton biomass in July and moderately high values in August, the C:N ratio of the sedimented material reached the lowest rate in July and the highest in August. Although cyanobacteria and cryptophytes dominated the phytoplankton community, they did not show any relevance for the path model and were thus removed from it. This is justified by their known marginal contribution to sedimentation. Instead, epilimnetic chlorophytes and hypolimnetic diatoms (Fig. 4) defined the parameter “*phyto*,” despite their little contribution to the phytoplankton biomass. Path analysis indicates a strong negative correlation between the treatment parameters in the epilimnion and a weak positive correlation of the hypolimnetic treatment parameters on these phytoplankton groups (Fig. 5).

The parameter “*traps*” was defined by dry weight of sedimented matter and its carbon content. Path analysis shows that the sedimentation rate was strongly influenced by the selected phytoplankton groups and not affected by the treatment. However, despite a small increase of the diatom biomass, sedimentation rates and their carbon content decreased over time. Phytoplankton biomass and sedimentation rates were not significantly different between control and treated enclosures (PERMANOVA, Table 3).

Abundances of bacteria and viruses (H3)

Mean viral abundance doubled between June ($2.4 \times 10^{10} \pm 1.4 \times 10^{10} \text{ VLP g}^{-1}$) and August ($5.5 \times 10^{10} \pm 2.5 \times 10^{10} \text{ VLP g}^{-1}$) and was one order of magnitude higher than mean bacterial abundance ($3.5 \times 10^9 \pm 3.2 \times 10^9 \text{ cells g}^{-1}$ in June, $2.4 \times 10^9 \pm 2.3 \times 10^9 \text{ cells g}^{-1}$ in August) at the SWI. Neither bacterial nor viral abundance were affected by thermocline deepening, and additionally did not contribute with any impact to the path model.

Nutrients (NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-}), H_2S , and Si (H3)

Nutrients-, H_2S -, and dissolved Si concentrations in the porewater showed no significant difference between control and treated enclosures (Table 3).

Nitrate, nitrite, and sulfide showed no relevance to the path model variable block “nutrients.” In contrast, mean concentrations of NH_4^+ ($60.4 \pm 40.7 \mu\text{mol l}^{-1}$ in June, $65.4 \pm 46.3 \mu\text{mol l}^{-1}$ in August), PO_4^{3-} ($7.1 \pm 5.0 \mu\text{mol l}^{-1}$ in June, $5.0 \pm 4.9 \mu\text{mol l}^{-1}$ in August), and also Si ($95.0 \pm 52.0 \mu\text{mol l}^{-1}$ in June, $97.0 \pm 65.2 \mu\text{mol l}^{-1}$ in August) contributed to the path model and were strongly negatively correlated to the hypolimnion treatment parameters (Fig. 5). Mean values (Fig. 4) of all three variables were higher in the treated than in the control enclosures (ΔNH_4^+ : $91.9 \pm 47.5 \mu\text{mol l}^{-1}$ in control, $98.1 \pm 61.6 \mu\text{mol l}^{-1}$ in treated; ΔPO_4^{3-} : $-3.7 \pm 7.3 \mu\text{mol l}^{-1}$ in control, $-0.5 \pm 5.7 \mu\text{mol l}^{-1}$ in treated, ΔSi : $-31.7 \pm 65.1 \mu\text{mol l}^{-1}$ in control, $35.7 \pm 62.5 \mu\text{mol l}^{-1}$ in treated). Amount and carbon content of sedimented materials were weakly correlated to the nutrient parameters.

Porewater elements: Ba, Ca, K, Li, Mg, Mn, Na, S, and Sr (H4)

Porewater element concentrations did not differ between control and treated enclosures (Table 3). We tested barium, calcium, potassium, lithium, magnesium, manganese, sodium, sulfur, and strontium to fit into the path analysis, but only barium, manganese, sulfur, and strontium correlated with the model. Furthermore, path analysis (Fig. 5) showed a strong correlation between porewater nutrients and elements, but only weak and negative influences of the treatment and the sedimented material on element concentrations. Concentrations ranged between $26.1 \pm 5.7 \mu\text{g l}^{-1}$ (June) and $24.0 \pm 3.6 \mu\text{g l}^{-1}$ (August) for barium, $122.1 \pm 52.1 \mu\text{g l}^{-1}$ (June) and $120.7 \pm 48.7 \mu\text{g l}^{-1}$ (August) for manganese, $9.1 \pm 1.4 \text{ mg l}^{-1}$ (June) and $10.1 \pm 1.5 \text{ mg l}^{-1}$ (August) for sulfur, and $76.4 \pm 1.4 \mu\text{g l}^{-1}$ (June) and $77.3 \pm 3.8 \mu\text{g l}^{-1}$ (August) for strontium (delta values given in Fig. 4).

Greenhouse gases: CO_2 , CH_4 , and N_2O (H5)

Average greenhouse gas concentrations at the SWI were between $19.2 \pm 5.6 \mu\text{mol g}^{-1} \text{ DW}$ (June) and $12.8 \pm 6.2 \mu\text{mol g}^{-1} \text{ DW}$ (August) for carbon dioxide, and between $5.5 \pm 4.0 \mu\text{mol g}^{-1} \text{ DW}$ (June) and $7.4 \pm 9.8 \mu\text{mol g}^{-1} \text{ DW}$ (August) for methane. Concentrations of nitrous oxide were below detection limit. Statistically, CO_2 and CH_4 concentrations did

not differ between control and treated enclosures (Table 3). In contrast to CH_4 , mean CO_2 concentrations decreased over time, but less in the treated than in the control enclosures (Fig. 4). Path analysis (Fig. 5) revealed a strong positive correlation of nutrients and a strong negative correlation of sedimentation rates to the greenhouse gases, while the porewater chemistry as well as hypolimnetic temperatures and oxygen concentration showed no influence.

Greenhouse gas emissions at the air–water boundary, as measured by flux chambers, were below the detection limit during the first 24 h. After 96 h, methane fluxes were detected in seven enclosures in amounts below $50 \mu\text{mol m}^{-2} \text{ h}^{-1}$, only exceeded by emissions in E16 ($157.2 \mu\text{mol m}^{-2} \text{ h}^{-1}$) and E15 ($444.9 \mu\text{mol m}^{-2} \text{ h}^{-1}$), but did not differ between control and treated enclosures. Fluxes in E1, E8, and E13 had slopes with an $R^2 < 0.6$ and were not considered for further calculations. Fluxes of carbon dioxide and nitrous oxide could not be detected at any time.

Discussion

Artificial thermocline deepening in lake enclosures led to increased temperatures and decreased oxygen concentrations in the hypolimnion, but did not result in any direct or indirect effect on sedimentation rates or on the microbial abundance, greenhouse gas concentrations, and porewater chemistry at the SWI. The formation and deepening of the thermocline in a lake during summer stratification is supposed to have dramatic effects on all physical, chemical, and biological processes; therefore, the lack of responses to such a treatment emphasizes the complexity of the processes as well as the autonomy of the sediment community, as already indicated by distinct microbial communities in waters and sediments in various environments (Lozupone & Knight, 2007; Cole et al., 2012). In contrast to extreme events like storms and droughts, the impact of thermocline deepening might be visible only in the long term, as slight changes in the SWI temperature and oxygen regime at the end of summer might be carried over to the following winter and spring season and add up to more severe changes after several years. However, our experiments shed more light on short-term effects on the chemical and biological interactions at the SWI.

Hypothesis 1 A deepened thermocline leads to an increase in hypolimnetic temperature and thus in microbial activity, leading to a decrease of the oxygen concentrations above the sediment.

The statistical approaches used (path analysis model and multivariate statistics) confirmed that a deepening of the thermocline in ~20 m deep lake enclosures results in an increase of temperature and a decrease of oxygen concentrations in the hypolimnion. This was observed for average as well as total values in relation to the water volume, although not at the SWI directly. The differences in temperature in the hypolimnion were mainly found in the water layers below the thermocline, and decreased in the deeper layers. In contrast, differences in oxygen concentrations increased with depth. However, oxygen concentrations at the SWI varied by up to 4.3 mg l^{-1} among replicate enclosures, mainly due to differences in depth. The results imply that changes in the stratification depth can affect pelagic organisms, but have no immediate direct effect on the biogeochemistry and microbial abundance at the sediment surface. Nevertheless, the trend towards higher temperatures and lower oxygen concentrations in the hypolimnion indicates that more severe consequences could occur in shallow or eutrophic lakes in the long term. Moreover, the results suggest that sediments at the depth level below the thermocline will experience temperature increases, but probably no changes regarding the oxygen regime. However, due to seiches, sediments at the thermocline depth level experience changing epilimnetic and hypolimnetic conditions regularly (Kirillin et al., 2009), without dramatic effects on the biogeochemistry or microbial activity (Frindte et al., 2013).

Hypothesis 2 A deepened thermocline and increased phytoplankton biomass will increase sedimentation rates of the sediment.

Phytoplankton biomass and sedimentation rates were not directly affected by thermocline deepening. The path model indicated a strong negative correlation of the epilimnetic treatment with the biomass of epilimnetic chlorophytes and hypolimnetic diatoms, as well as a strong positive correlation of these phytoplankton groups with sedimentation rates. Chlorophytes also contributed to increased phytoplankton production in the epilimnion of lake basins

where the thermocline was deepened, as reported by Cantin et al. (2011). In our study, however, at the beginning of the experiment the phytoplankton community was dominated by cryptophytes, a motile group that dominates in Lake Stechlin during the non-stratified period and prevails in the upper hypolimnion during summer (Padisák et al., 1998). Thus, their initial dominance in the enclosures' epilimnia was probably related to the previous homogenized mixing (Phillips & Fawley, 2002; Teubner et al., 2003; Ongun Sevindik et al., 2015), followed by strong biomass losses in the epilimnion when the mixing ended and the thermal stratification was established, but without increasing sedimentation rates. Presumably, the algae were consumed by grazers or microbially decomposed within the water column, although it may be possible that they do not contribute enough weight to significantly alter sedimentation rates. Simultaneously, phytoplankton biomass in the hypolimnion, dominated by the cyanobacterium *Planktothrix rubescens*, increased during the experimental period, and resulted in an increase of the total phytoplankton biomass between June and August, despite the steady total biomass in the epilimnion. *P. rubescens* becomes abundant in Lake Stechlin after very cold winters (Padisák et al., 2010), and was involved in the formation of a deep chlorophyll maximum (DCM) in Lake Stechlin in the same year (Selmeczy et al., 2016). Another cyanobacterium species, *Dolichospermum flos-aquae* (Brébisson ex Bornet & Flahault) Wacklin, Hoffmann & Komárek, increased biomass 2.5 weeks after artificial strong mixing in a different LakeLab experiment (Giling et al., 2016). As reported previously, such events can lead to massive calcite precipitation (Kasprzak et al., 2017) followed by massive sedimentation (Fuchs et al., 2016a), but such consequences were not observed in our study since thermocline deepening showed no effect on the mass of sedimenting particles reaching the sediment. In enclosures, sedimentation rates are biased by the lack of currents and by an altered impact of wind force, but sedimentation rates measured close to the LakeLab facility in the previous year (Fuchs et al., 2016a) exhibited similar values in July ($568.3 \text{ mg m}^{-2} \text{ day}^{-1}$) and 1.8 times higher rates in August ($942.8 \text{ mg m}^{-2} \text{ day}^{-1}$). The increasing percentage of nitrogen in the sedimented material between July and August could occur as a result of N_2 -fixation by cyanobacteria (Dokulil & Teubner, 2000) or fecal

pellets deposition, as in parallel the phytoplankton community shifted to a dominance of cyanobacteria in August, but N_2 -fixing species did not reach prominent biomasses within this group. Neither the nitrogen content of the sedimented material nor the cyanobacteria biomass were correlated to the treatment or showed any relevance to the path model.

Hypothesis 3 An increase of temperature and sedimentation rates at the SWI will enhance microbial processes, thereby increasing the abundance of bacteria as well as the products of organic matter decomposition, such as NO_3^- , NO_2^- , NH_4^+ , H_2S , or PO_4^{3-} . An increase of bacterial abundance will in turn increase viral abundance.

Bacterial abundance was affected neither by the treatment nor by the temporally decreasing sedimentation rate and its changing C:N composition. Similarly, Goedkoop et al. (1997) demonstrated that a difference in organic matter supply, in the form of diatom addition, to incubated Lake Erken sediments did not affect bacterial abundance, although it increased bacterial production and growth rates. Despite previous indications that all these parameters (bacterial production, abundance, and growth rates) are more strongly affected by temperature than substrates (Boström et al., 1989; Shiah & Ducklow, 1994), bacterial abundances correlated neither to the treatment nor to the sedimentation rate in the path model.

While the deepened thermocline did not result in an increase of viral abundance at the SWI, we found an increase of viral abundance over time. It has been shown that viral abundance is highly correlated to oxygen (Ricciardi-Rigault et al., 2000), organic matter (Maranger & Bird, 1996), and bacterial abundance (Proctor et al., 1993). In contrast, we observed an increase of viral abundance towards the end of summer stratification, where oxygen concentrations and sedimentation rates were the lowest, while the bacterial abundance remained unaffected. As Pinto et al. (2013) discussed, it is still not clear which are the drivers of viral abundance variability. Local conditions at the end of the summer (low oxygen concentration) might have induced the release of lysogenic viruses, and thus resulted in a higher viral abundance.

When oxygen is depleted in the upper few centimeters of the sediment, nitrate and sulfate are the main electron acceptors transformed by microbes

into nitrite, nitrous oxide, or molecular nitrogen, and sulfide. As mentioned above, the treatment had no direct effect on the biogeochemistry at the SWI, thus also the concentrations of nitrate, nitrite, and sulfide remained unaffected. Interestingly, the shift of phytoplankton composition and sedimenting C:N ratio also did not correlate to the nitrate concentrations at the SWI. Instead, ammonium, phosphate, and silicate contributed to the “nutrients” part of the path model and were strongly correlated to the ion and greenhouse gas concentrations in the sediment. Ammonium and phosphate are products of microbially mediated organic matter decomposition and have been shown to increase under anoxic condition (Frindte et al., 2013), indicating an increase of microbial activity and a shift to anaerobic processes towards the end of summer. Additionally, phosphate can be released from the sediment by altering redox conditions (Hupfer & Lewandowski, 2008) and compete with Si, which is derived from sedimented diatoms, for sorption sites (Gonsiorczyk et al., 2003). Nevertheless, the path model showed no significant correlation of these nutrients to hypolimnetic temperatures and oxygen concentrations.

Hypothesis 4 Increasing temperatures and decreasing oxygen concentrations above the sediment will increase the release of porewater elements.

Porewater elements are part of the sediment geochemistry, being dissolved into the porewater by rock weathering or introduced by rainfall or anthropogenic activities. Temperature increase and oxygen decrease can affect the binding complexes of these elements, due to shifts in pH values and shifts in the availability of electron acceptors and carbonates as binding partners. We did not observe a significant correlation of the treatment or the sedimented material on the concentrations of manganese, strontium, sulfur, and barium; however, the nutrients phosphate and ammonium, and silicate, were very highly correlated to these elements, indicating simultaneous mobilization.

Hypothesis 5 An increase of temperature, sedimentation rates, and a decrease of oxygen concentrations will increase the concentrations of the greenhouse gases CO_2 and CH_4 at the sediment surface. Simultaneously, NO_3^- and SO_4^{2-} can outcompete the production of CH_4 .

Anoxia is one of the most severe environmental impacts, restraining the life of benthic eukaryotes and larval stages and shifting the composition and activities of microbial sediment communities towards anaerobic processes. The final step of organic matter decomposition is the production of the greenhouse gas methane. Methanogenesis occurs anaerobically, nonetheless, methanogens are present in all layers down to 25 cm depth in Lake Stechlin sediments (Fuchs et al., 2016b) and have been shown to produce methane even at the sediment surface when exposed to favorable conditions (Dos Santos Furtado & Casper, 2000; Conrad et al., 2007). Previous incubation experiments with sediments of Lake Stechlin have shown that elevated temperatures decreased methane concentrations (Fuchs et al., 2016b), but shifts from oxic to anoxic conditions weakly enhanced the functional gene expression of methanogens and methanotrophs (Frindte et al., 2015). Studies in other lake and river sediments have also shown that methane production as well as methane oxidation are both elevated at higher temperatures and can outbalance each other (Kelly & Chynoweth, 1981; Shelley et al., 2015). In this study, we did not observe any impact of the treatment on methane concentrations. However, the treatment effects were not as strong as in the previous sediment incubation experiments by Frindte et al. (2015) and Fuchs et al. (2016b). Additionally, methanogenesis could have been outcompeted by energetically more favorable sulfate reducers, but H_2S concentrations at the SWI did not indicate an increase of sulfate reduction rates in response to decreasing oxygen concentrations or changes in the composition of sedimenting organic material.

Recent research indicates that global warming will substantially impact methane emission rates, mainly due to methane release from permafrost areas (Anisimov, 2007), thawing lakes (Walter et al., 2006), and from ice-bubble storage after melting in spring (Sepulveda-Jauregi et al., 2015), but also due to increased gas transport through emergent plants related to sediment temperature (Kankaala et al., 2004). In contrast, global warming does not seem to increase methane emission from deep temperate lakes. We did not observe an increase or decrease of methane emission due to a lowered thermocline. Likewise, Flury et al. (2010) did not find any effect of warming on greenhouse gas emission from mesocosms in Lake Hallwil (Switzerland). Previous studies in Lake

Stechlin have shown high methane production rates in deeper sediment layers (>20 cm, Casper, 1992; Casper et al., 2005; Fuchs et al., 2016b) as well as methane production in the water column (Grossart et al., 2011; Tang et al., 2014), but fluxes of methane from the enclosures to the atmosphere were very low. Thermoclines act as a physical barrier for the passage of gases, thereby additionally enabling methane oxidation at the barrier. Up to 99% of the methane released from sediments can be oxidized before reaching the atmosphere, with highest methane oxidation rates at the oxic/anoxic interface (Bastviken et al., 2002).

Conclusions

The impact of global warming-induced thermocline deepening on a cascade of variables affecting the sediment biogeochemistry was analyzed in a deep stratified lake. We found that a 2 m deepened thermocline in lake enclosures affected neither sedimentation rates, nor microbial and viral abundances in the sediment, nor greenhouse gas concentrations at the SWI or emissions to the atmosphere. Thus, we reject our Hypotheses 2–5. Nevertheless, our results confirmed Hypothesis 1, indicating a high potential of reaching or increasing anoxia due to a deepened thermocline. Stronger effects could be expected especially in lakes that already experience low oxygen concentrations at the end of summer stratification. Results showed that the deepened thermocline was strongly correlated to specific phytoplankton groups, and has the potential to indirectly affect sedimentation rates and concentrations of nutrients in sediment porewater, which were strongly correlated to greenhouse gas concentrations in the sediments. However, our results do not indicate a biomass increase of phytoplankton groups leading to mass sedimentation events. Moreover, the path model demonstrates that the dominant phytoplankton groups, as well as elements related to the N-cycle and microbial abundances at the SWI, did not explain the correlations between the blocks of variables considering the impact of the treatment on the C-cycle. Furthermore, nutrients, elements, and greenhouse gas concentrations at the SWI seemed to be more affected by the interactions among each other and the organic matter input than by temperatures and the availability of electron

acceptors. For the future, more work is needed focusing on the impacts of global warming on the sediment. Certainly the repetition of such experiments over several years, and the inclusion of different plankton communities, would allow us to see remarkable trends in the response of the ecosystem.

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