



Impacts of hypoxia on boreal lake biogeochemistry and productivity: a 4-year whole-ecosystem BACI experiment

I. C. Puts¹ · S. Koizumi² · J. M. Sarneel³ · A. Jonsson⁴ ·
H. A. Verheijen⁵ · J. Karlsson⁶ · D. Seekell⁷ · P. Byström ·
A.-K. Bergström⁸

Received: 27 February 2025 / Accepted: 6 August 2025
© The Author(s) 2025

Abstract Climate warming is increasing thermal stratification depth, strength, and duration in lakes, leading to more frequent hypolimnetic oxygen depletion. Most research has focused on eutrophic temperate lakes, which differ significantly from boreal lakes that dominate Earth's landscape. However, assessing the impact of hypoxia, confounded by browning, warming, and altered stratification, on biogeochemistry and ecological processes in boreal lakes is particularly challenging. Here, we test how oxygenating a hypoxic hypolimnion affects water chemistry, bacterial and primary production (BP and PP), and detritus degradation in a shallow humic boreal lake divided into two basins in an experimental four-year

Before-After Control-Impact (BACI) design. After two control years, we oxygenated the hypolimnion of one basin during two stratified periods without disturbing the seasonal development of the thermocline. Hypolimnetic oxygen concentrations moderately impacted lake biogeochemistry. Reoxygenation altered nitrification pathways (increased NO_3^-) of the hypolimnion, and slightly decreased epilimnion and lake BP (− 6.1% of annual average) and green tea degradation (− 6.0%), whereas Rooibos degradation slightly increased (7.3%). Other water chemistry parameters remained within natural variation. We compared our BACI approach, which separates natural variation, to the simpler Before vs After approach, which does not. We find that studies not accounting for seasonal and among-year variability may overestimate the effects of oxygenation on hypolimnion biogeochemistry, as much of the observed impact is due to natural climate variation. Climate warming

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10533-025-01262-3>.

Responsible editor: Stephen D. Sebestyen

I. C. Puts (✉) · S. Koizumi · J. M. Sarneel · A. Jonsson ·
H. A. Verheijen · J. Karlsson · D. Seekell · P. Byström ·
A.-K. Bergström
Department of Ecology, Environment & Geoscience,
Umeå University, Umeå, Sweden
e-mail: isolde.puts@umu.se

I. C. Puts
Department of Ecoscience, Aarhus University, Aarhus,
Denmark

I. C. Puts
Department of Biosciences, Oslo University, Oslo, Norway

S. Koizumi
LFI Laboratory for Freshwater Ecology and Inland
Fisheries, NORCE Norwegian Research Centre, Bergen,
Norway

H. A. Verheijen
Swedish Meteorological and Hydrological Institute,
Norrköping, Sweden

D. Seekell
Current Address: Atle Fund Management, Stockholm,
Sweden

and altered stratification patterns are therefore likely to impact boreal lake algal and bacterial production and degradation more than hypolimnion hypoxia during the stratified period.

Keywords Oxygenation · Stratification · Global change · Bacterial production · Degradation · Hypolimnetic oxygen depletion

Introduction

Climate warming is intensifying thermal stratification in lakes, leading to more frequent hypolimnetic oxygen depletion (Jansen et al. 2024; Klanten et al. 2023; Klaus et al. 2021). While most of Earth's lakes are in the boreal zone, far from human development, research on hypoxia's biogeochemical impacts has predominantly focused on hyper-eutrophic lakes in the temperate zone (Jane et al. 2021), which exhibit significantly different patterns and processes compared to their northern counterparts. Global warming has led to widespread increases in surface water (epilimnion) temperatures and extended the duration of summer thermal stratification in northern latitudes lakes over the last decades (Jansen et al. 2024; Jenny et al. 2016; Klanten et al. 2023; Woolway & Merchant 2019), although in north-eastern Canada only the thermal stability and not its phenology has changed (Hadley et al. 2014). In addition, these boreal lakes are impacted by increased inputs of terrigenous colored dissolved organic matter (cDOM; "i.e., lake browning") caused by warming and altered precipitation patterns (De Wit et al. 2016), recovery from acid deposition (Garmon et al. 2014; Monteith et al. 2007), intensified agriculture and forestry (Kritzberg 2017), and catchment greening (Berner & Goetz 2022; Finstad et al. 2016; Guay et al. 2014). Lake browning enhances thermal stratification and intensifies the warming of the epilimnion due to the high light absorption capacity of cDOM, which is converted to heat in the process (Couture et al. 2015; Jones 1992; Puts et al. 2023). Boreal lakes tend to be fairly shallow and organic rich (Cael & Seekell 2016; Toming et al. 2020), leading to rapid warming with strong thermal stratification in summer, which together with high microbial activity and oxygen consumption (Staehr et al. 2012; Strock et al. 2017) frequently causes anoxic or hypoxic conditions (Klanten

et al. 2023; Klaus et al. 2021). Combined impacts of global changes therefore promote warmer and shallower epilimnetic waters and larger volumes of cold oxygen-poor hypolimnetic (bottom) waters due to changes in thermal stratification (Bartosiewicz et al. 2019; Pilla et al. 2018), which in turn may impact biogeochemical cycles and the productivity and general ecology of lakes.

Low concentrations, or even absence of dissolved oxygen (hypoxia < 3 mg/L and anoxia; 0.001 mg/L, respectively) impact lake chemistry in several ways. The nitrogen cycle is complex but may shift to decreased nitrification and increased denitrification under anoxic conditions, resulting in higher ammonium (NH_4^+) and variable, but often lower nitrate (NO_3^-) concentrations (Gautreau et al. 2020; Romilac 2019). In addition, periodic anoxic conditions in the hypolimnion induces sediment release of phosphate (PO_4^{3-}) (Mortimer 1971; North et al. 2014), cDOM (Brothers et al. 2014; Peter et al. 2017; Peter & Sobek 2018), nitrogen, and iron (Dimberg & Bryhn 2015; Von Wachenfeldt & Tranvik 2008) from carbon rich sediments or waters. Reversing these anoxic processes by reoxygenating the hypolimnion would thus likely result in decreased PO_4^{3-} and NH_4^+ , but increased NO_3^- concentrations especially in the hypolimnion (Carey et al. 2022).

Algal and bacterial production are crucial components for lake food webs that respond closely to changes in biogeochemistry. Primary production (PP) by phytoplankton follows spatial-temporal gradients in nutrient, light and temperature conditions over the water column and is mainly confined to the epilimnion in stratified brown boreal lakes (Jones 1992; Puts et al. 2023), unlike temperate or boreal clear-water lakes that often have a deep chlorophyll maximum (Leach et al. 2018; Fee 1976; Tonin et al. 2022). During the stratification period, the epilimnion of boreal lakes is mainly influenced by external inputs of elements from catchments and by surface water heating, capturing the seasonal climate variability in summer (Bergström & Jansson 2000). In contrast, the hypolimnion's biogeochemical processes are sealed off from these external influences due to stable thermoclines and are largely affected by variations in oxygen concentration. In the absence of extreme storm events, nutrients released from sediments due to summer hypoxia are therefore unlikely to reach the epilimnion, and for that reason likely to have minor

impacts on primary production (PP). However, algae may use nutrients from the hypolimnion during vertical diel-migration, which is mostly recorded in temperate lakes with high abundances of blue-green algae (Ganf and Oliver 1982; Molot et al. 2014). Blue-green algae are uncommon in northern Boreal brown lakes (Deininger et al. 2017; Puts et al., 2023), and hypolimnion-nutrient utilization would thus more likely happen by migrating mixotrophic and autotrophic flagellates that are highly abundant in high cDOM lakes (Hansson et al. 2019).

Heterotrophic bacterial communities, on the other hand, adapt to the available electron donors, following the electron tower hierarchy (in falling hierarchy; O₂, nitrate, iron, sulfate, CO₂), resulting in distinct bacterial producer patterns across depth and temporal-spatial gradients (Berggren et al. 2010; Martínez-García et al. 2013; Shade et al. 2008). Moreover, redox conditions (specifically O₂ availability) determine the rates of CO₂ and CH₄ production, and thus the emission potential of greenhouse gasses (Carlsen et al. 2025; Pajala et al. 2023). Induced anoxic conditions likely shift the microbial community from using O₂ to using more energy demanding nitrate or sulfate as electron acceptors, potentially reducing bacterial production (BP) and degradation of organic material (Burgin et al. 2011; Bush et al. 2017). However, since organic material degradation is complex and involves various factors (like photochemical oxidation, nutrient and mineral availability) and organisms (heterotrophic and autotrophic bacteria, fungi), it can be complemented with measurements of organic matter degradation. Research has shown that reversing oxic-anoxic conditions experimentally often results in similar degradation rates (Bastviken et al. 2004), indicating that the complexity in breakdown is accurately constrained this way. Therefore, degradation of standardized OM can be used as a complement for BP measurements.

Overall, the effects of altered stratification patterns on biogeochemistry, BP, PP and degradation and resulting changes in oxygen levels on whole ecosystems are understudied. Changes in stratification and induced hypoxia are linked to warming and browning (De Wit et al. 2016; Leech et al. 2018) and are projected to continue to increase with global change with likely significant impacts on boreal lake ecosystems. Despite the increasing occurrence of hypolimnion hypoxia and extended stratification periods and

strengths, studies isolating its impacts from climate variability on whole lake ecosystems are rare. Existing studies often use a Before vs. After (BvA) design, which does not control for other factors including natural seasonal and interannual variability, or a Control vs. Impact (CvI) design, which does not account for variation due to differences between the sites. To address these limitations, a Before-After Control-Impact (BACI) design is proposed, involving measurements at both an impact site and a control site before and after the intervention (Fisher et al. 2019; Underwood 1994). This design requires double the effort but helps attribute changes specifically and minimizes the limitations of seasonality (BvA) and site differences (CvI). Given hypoxia's long-term effects occurring alongside natural and anthropogenic climatic changes, assessing its impact without the confounding influences of seasonality and/or site characteristics requires comprehensive BACI measurements. The few previous lake reoxygenation experiments (using BvA or CvI) confirm complex ecosystems impacts, with both reduced (Carey et al. 2022) and increased (Inoue et al. 2017; Riccardi and Mangoni 1996) carbon and nutrient concentrations, accompanied by altered electron acceptor pathways (McClure et al. 2021). However, impacts on PP and BP were not considered in previous studies in brown boreal lakes, and neither were the effects of hypoxia separated from weather variability. Here, we first address this knowledge gap, and test how hypoxia affects biogeochemistry, PP and BP, and the degradation of organic material. For this we use a four-year BACI setup where we reoxygenated the hypolimnion in one of two well-separated basins of a small boreal lake. Secondly, we compared conclusions of our BACI design to the outcome of the BvA design.

We predict that oxygenation of the hypolimnion results in the following observations:

1. Hypoxia and climate will have distinct independent effects on lake biogeochemistry, as well as bacterial and primary production.
2. During stratification, epilimnion biogeochemistry remains unaffected by oxygenation of the hypolimnion.
3. Increased NO₃⁻ and reduced TN, TP, DOC, PO₄³⁻, NH₄⁺ in the hypolimnion.

4. The results using BACI approach and analysis will differ from the outcome using an analysis based on a BvA design.

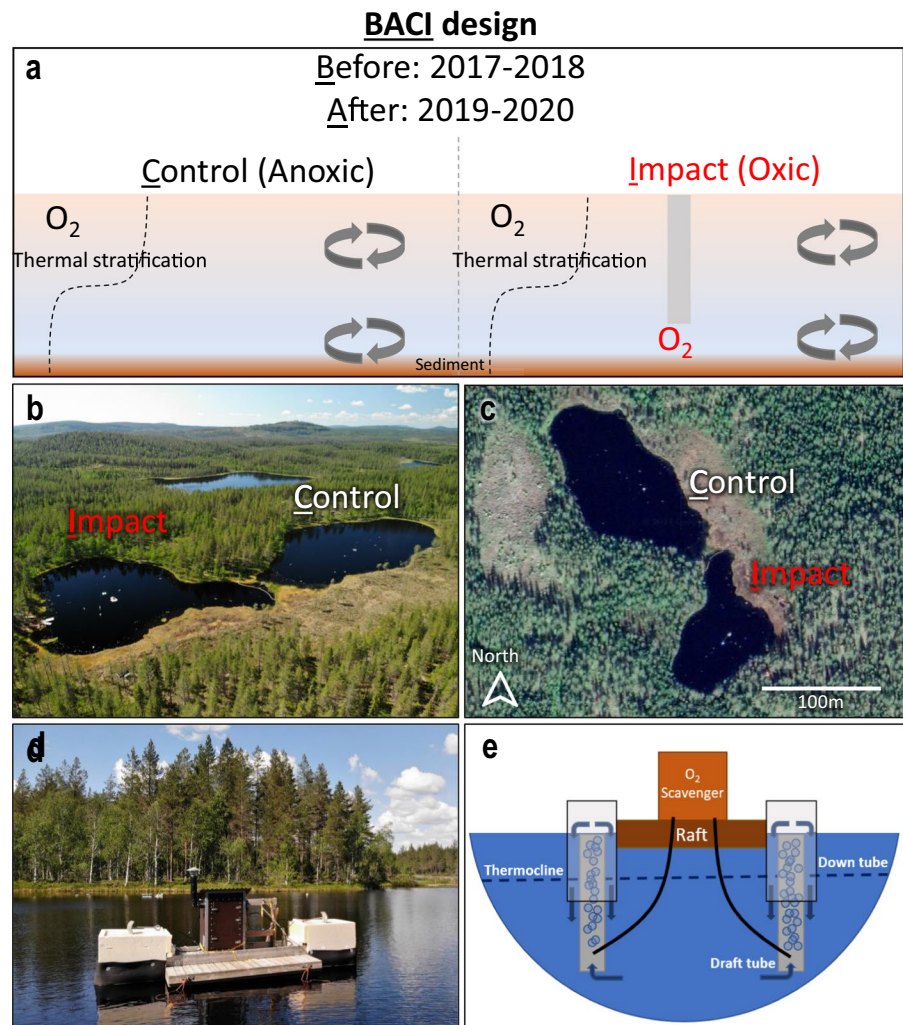
Methodology & approach

Experimental setup and approach

To test how hypoxia affects the biogeochemistry and ecological functioning in boreal shallow lakes, we adopted a backwards-experimental approach. We use this approach because adding oxygen to the hypolimnion is easier than removing it, and we can leverage a long history of hypolimnetic oxygenation technologies to maximize success. We oxygenated a naturally

hypoxic hypolimnion in a typical boreal shallow lake with two well-separated basins with brown water (the “Ljusvattentjärn” lakes; coordinates in decimal degrees: 39.311°, – 102.292°, seasonal average K_d for the basins are 2.0 ± 0.3 and 1.9 ± 0.2) in northern Sweden, and manipulated the hypolimnion of the southern (“Impact”) basin while keeping the northern adjacent basin as (untreated) reference (“Control”; Fig. 1a–c). We monitored changes in water chemistry, basal (primary + bacterial) productivity, and degradation of standardized detritus material. To differentiate the measured impacts from climatic variations, we implemented a Before-After Control-Impact (BACI) design. The basins have a maximum depth of 9.1 m/9.5 m and a surface area of 0.63/1.09 ha for the Impact and Control basin, respectively.

Fig. 1 the Before-After Control-Impact setup with (a) a schematic visualization indicating the hypolimnion oxygenation treatment in the Impact lake, and continuous thermal and chemical stratification indicated by different color and mixing arrows in the epi- and hypolimnion for the two Before (2017, 2018, and 1st sampling 2019) and two After (2019 minus 1st sampling, and 2020) lakes. (b) a drone image of the Control and Impact basin, and (c) a scaled satellite image with North-indication. (d) a picture and (e) a schematic overview of the Impact-lake with the oxygenation installation in the middle of the lake



Oxygenation of the hypolimnion

The Impact basin was oxygenated between 19 June and 26 August 2019, and turned on again on 25 November 2019, until the last sampling in 2020, and thus commenced after sampling occasion “1” in 2019. Oxygenation of the hypolimnion was reached using a full-lift aeration system with double tubes hung at 6 m depth (Fig. 1d–e). The full-lift aeration system was placed on a raft (Fig. 1d) at the deepest part of the Impact basin, and oxygenation occurred throughout the warm summer season. Compressed O₂ was injected into hypolimnetic water through a micro-diffuser at the bottom of a 0.6 m diameter tube mounted vertically to a raft floating over the center of the basin. The oxygen bubbles dissolved into the hypolimnetic water as they draw it up to the surface, where the cold oxygenated water spills over into the space between the draft tube and a 1.2 m return tube circumscribing it. The return tube extends to just below the thermocline, where the oxygenated water then spreads horizontally in the hypolimnion (Pajala et al. 2023; Fig. 1d–e). The water moves through the system fast enough so that there is no significant change in water temperature, and there was no disruption of stratification because the moving water is contained within tubes (Fig. 2). Water drains from the reference basin to the experimental basin, ensuring the treatment doesn’t impact the reference basin. This setup is like other paired basin studies using two basins of the same lake (Carpenter and Kitchell, 1993).

Field sampling and water chemistry

Water chemistry was analyzed in water sampled using a Ruttner sampler at the deepest point in the basin at 1 m, 3 m and 6 m every third week during the ice-free season for 4 consecutive years (May–October, years 2018–2020). The water chemistry during the first year (2017) was measured on a mixture of 1 m, 3 m and 6 m, and the other years we analyzed the water chemistry for each specific depth. For each year, sampling frequency and dates are identical for both basins, and exact sample dates for each sampling per year can be found in the openly available data. Dissolved oxygen (DO), water temperature, and PAR were measured at additional depths, specifically at 0 m, 0.25 m, 0.5 m, 1 m, and at each consecutive meter, until reaching the

basin bottom using a handheld probe (YSI ProODO and LI-193 Spherical Quantum Sensor). Water chemistry was analyzed for total and inorganic nitrogen and phosphorus (TN, TP, NO₃[−], NH₄⁺, and PO₄^{3−}), dissolved organic and inorganic carbon (DOC and DIC), in the epi- and hypolimnion (1 m and 6 m depth). After sampling, water was frozen and stored dark and cool until analysis. TN and TP (unfiltered) samples were stored frozen until analysis. Dissolved nutrients and DOC were filtered through a 0.45 µm filter (Sarstedt Filtropur) and stored in a refrigerator and acidified (with HCl) to an end concentration of 12 mM before being analyzed within a few weeks after sampling. DOC samples were acidified to an end concentration of 12 mM using HCl and analyzed through high-temperature combustion using a Formacs HT-I analyzer (Skalar). DIC samples, used to calculate primary production, were prepared by injecting 4 mL (air-free) water into a tightly sealed 18 mL glass vial (pre-flushed with N₂) containing 0.1 mL 1.2 M HCl and analyzed as soon as possible on a Clarus 500 gas chromatograph (Perkin Elmer) using N₂ as carrier gas. Nutrients concentrations were detected using a segmented flow analyzer (QuAAtro, SEAL Analytical GmbH, Norderstedt, Germany).

Basal production and decomposition

Bacterial- and primary production

Pelagic primary- and bacterial production (PP and BP) measurements were performed on water taken at different depths over the water column using isotopic tracers. The BP was measured from water at similar depths as water chemistry (1 m, 3 m (additional), and 6 m), by incubating the water in triplicates with an isotopically labeled tracer (³H, leucine) in the laboratory as soon as possible after the water sample was acquired. The water was incubated for approximately 1 h at in situ temperature in the dark, according to Smith and Azam (1992) with slight modifications (Karlsson et al. 2001). To estimate average areal BP (“lake BP”), the BP at specific depths were integrated over the water column using trapezoid integration, up to 9 m depth. The PP was measured in situ using a ¹⁴C isotopic tracer as described by (Schindler et al. 1972) at 0 m, 0.25 m, 0.5 m, 1 m, and at every following meter, up to the depth where no light was available for photosynthesis (at 4 m

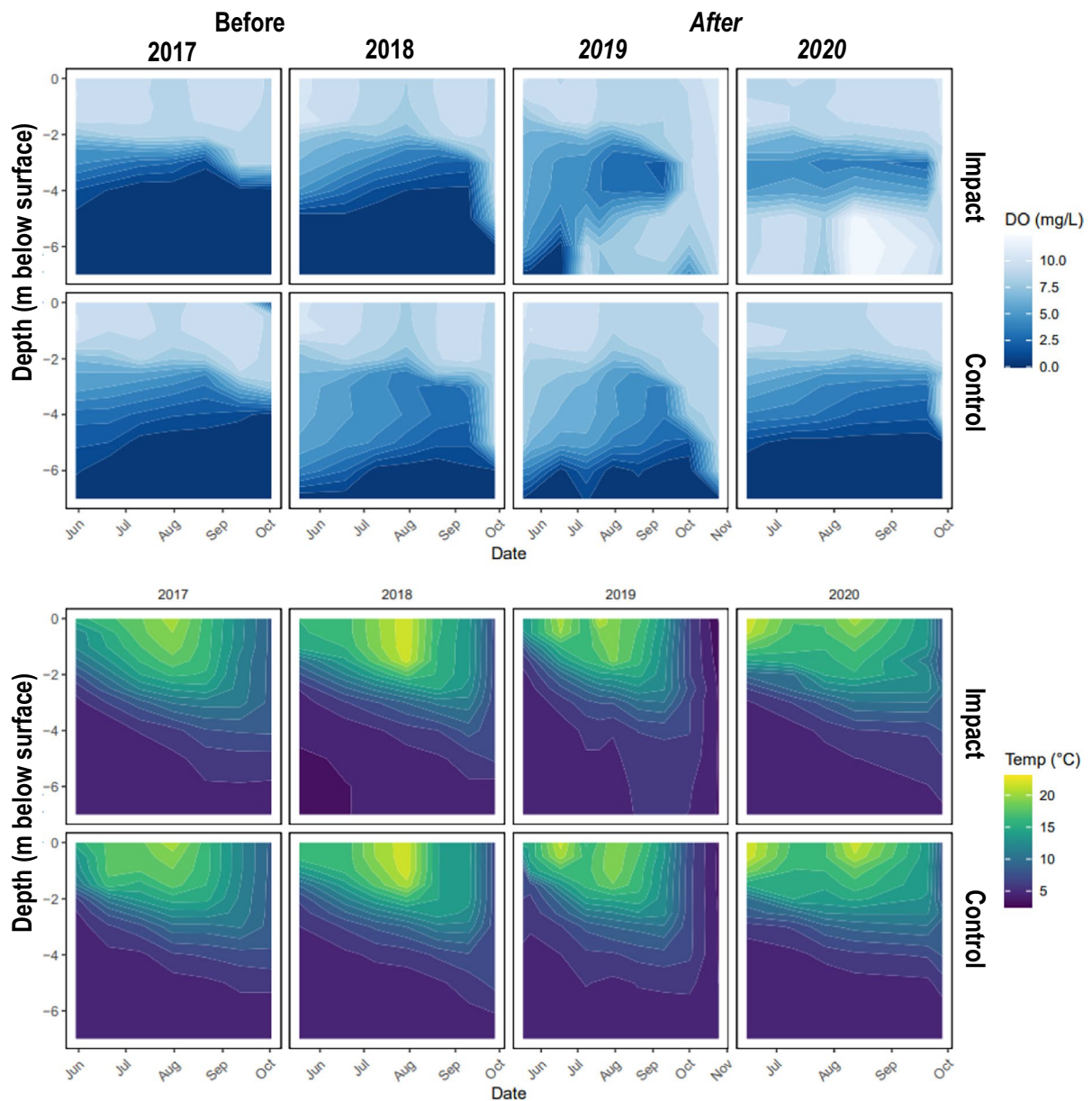


Fig. 2 Depth profiles during monitoring season of (a) dissolved oxygen (DO) and (b) temperature profiles of the impact (top panels) and control (bottom panels) lakes for the Before

(2017 and 2018, 1st sampling 2019 and After (2019 minus 1st sampling and 2020) years

depth). We filled (single or triplicate, see openly available data) transparent glass bottles with water from the sampling depth, and dark bottles at shallowest and deepest measurements only, and subtracted the dark bottle values at deep (respectively shallow) depths from deep (respectively shallow) measurements below (respectively above) 2 m depth.

Bottles were incubated in-situ at sampling depth for about 4 h during midday. Average areal PP ("lake PP") was estimated by integrating the rates over the water column, also by trapezoid integration.

Degradation of organic material

Decomposition rates were measured by mass-loss rates of rooibos and green tea, that represent two types of standardized plant material enclosed in plastic mesh bags (Keuskamp et al. 2013). The tea bags were weighted, and pairs of one rooibos and one green tea bag (non-woven mesh material) were enclosed in cases of coarse plastic mesh (1 cm). The cages were deployed at four depths (0, 2, 4, 6 m depth) in the deepest point of the basin in summer 2018, 2019, and 2020, and retrieved after ca 90 days. Bags were dried at least 48 h at 60 °C and the mass loss of the tea in the bag was calculated ($\% \text{ day}^{-1}$). To account for differences in incubation duration in before and after years, we calculated the mass loss rate assuming an exponential decay: $(\ln(M_1) - \ln(M_2))/T$, where M_1 is the mass at the start, and M_2 is the mass after incubation and T is the incubation duration in days. Since the tea bags were not dried at the start, we first determined the moisture from the air in the tea from eight additional bags per tea type ($4.25\% \pm 0.18$ S.E. for green tea and $5.74\% \pm 0.04$ S.E. for rooibos) and then corrected all starting masses by subtracting this fraction from the starting mass of the tea inside the bags. Values were represented by the average loss rate of bags hung at 0 m and 2 m (epilimnion) and 4 m and 6 m (hypolimnion) depth, resulting in duplicates in the epi- and hypolimnion for each year, and only assessed hypolimnion values in the pairwise comparison.

Data interpretation

We monitored the response of seasonal average water chemistry, production, and decomposition in the epi- and hypolimnion for all four years (three years for decomposition, and first year water chemistry with mixed water column values as described below). We removed the 1st sampling of all years from statistical data interpretation, as the oxygen treatment had only started at the 2nd sampling in 2019. We compared the natural variation measured in six sampling episodes for two years (“Before”, of which the 1st year mixed water column samples were used) with the variation after experimental oxygenation of the hypolimnion for two years (“After”), resulting in 12 “Before” and 12 “After” datapoints (after removal of sampling 1 from each year). Firstly, we used

ANOVAs to evaluate our experimental setup and the similarity of the two lakes (CvI) and natural variation (BvA), where we excluded the 1st year of mixed-water chemistry. Secondly, we assessed significant changes post-impact (oxygenation) in our BACI setup using the ePower package (referred to as “BACI” and described in detail below) using water chemistry of all four years. Thirdly, we compared the statistical results of the BACI impact to the outcome of the BvA (here: “BvA” term, sensu Carey et al. 2022; McClure et al. 2021). Additionally, to quantify the magnitude of impacts, we assessed the average paired difference (per sampling) between the Impact and the Control basin in the Before years to the After years, indicating direction of changes in % of annual averages for the biological parameters (PP, BP, PP:BP, green tea and rooibos mass loss). Additionally, we investigated how DO correlates with different water chemistry variables (Supplementary). We further tested what water chemistry variables best predicted lake PP, lake BP, and BP rates in the epilimnion and hypolimnion in a multiple linear regression (MLR) with forward selection, where Akaike values are included to assess the model performance.

We separated (excluded) the effect of natural variability among years from experimentally induced variation by the oxygen treatment in the “epower” package designed for BACI interpretations (Fisher et al. 2019). In the “epower” package, two Bayesian Generalized Linear Models are compared—one with, and one without interaction terms. The significance of the impact is determined by comparing the probabilities of these models, e.g. when the probability is higher with the interaction effect, it means the observed effect is due to the oxygen treatment and not due to seasonal climate variability (BvA) or to differences between sites (CvI) variation. This is true under certain assumptions, most notably the parallel trends assumption (eg. see Wauchope et al. 2021; Roth et al. 2023). In the epower package, a probability ratio greater than 0.5 indicates stronger support for the BACI interaction model and an effect of the oxygen treatment. Following the parameters of essential elements for a BACI design as presented in Fisher et al. 2019, in our BACI design “BA” terms were years, “Time” was 6 samplings per year (sampling events 2–7), CI were the two basins (control vs oxygenated). “Location” replicates are represented by double years per location (2017–2018, and 2019–2020, resp.). The

package could not be run for tea degradation because measurements were missing from 2017. The package was run independently for measurements from the epi- and hypolimnion. All models were run using default integrated nested Laplace approximation (INLA) priors, and probability integration transform (PIT) plots were generated to assess each model. We chose gamma or gaussian distribution depending on the datatype, as suggested by the package. Analyses were carried out using the package ‘epower’ V1.3 (BMT 2019) as described in Fisher et al (2019) and based on the statistical programming platform R (R-Core Team 2025).

The water chemistry measurements run on water combined from 1 and 6 m depth (in 2017) provide a Control vs. Impact comparison and are included in the environmental monitoring data (Table 1; presented in the epilimnion values). However, as a central question of the experimental set-up regards distinguishing processes in epi- and hypolimnion, we excluded these measurements from statistical analyses that specifically investigate differences in these habitats (Table 1 and related figures and analyses). PO_4^{3-} values that were negative, indicating they are at the detection limit, were set to 0. Analyses and graphics were performed in R and SPSS. All data, including replicates, can be found in the openly available data (<https://doi.org/10.5281/zenodo.14930455> and <https://zenodo.org/records/15517814>).

Results

Experimental conditions

Pre-oxygenation and oxygenation conditions of biogeochemistry and production

The oxygenation of the hypolimnion in the Impact-basin was successful (annual average $\pm$ SD DO values in mg/L Before: 2.2 ± 0.8 , After: 6.1 ± 1.7) although less impactful in the first Impact-year (2019) compared to the second Impact-year (2020) (Fig. 2). The experimental oxygenation did not affect the thermal stratification nor basin water temperature (Fig. 2, 4a–b; Table 1: (i.e., DO in the hypolimnion was reduced according to the BACI and BvA p-value, but temperature did not change). To assess the similarity of the two basins, we compared them in the Before

years (CvI in the Before years; Table S1). The Control basin had slightly higher annual average hypolimnion DO concentrations (Control: 4.0 ± 1.5 and Impact: 2.2 ± 0.8) and did also stratify somewhat later and at deeper depths compared to the Impact basin (Fig. 2). Differences in water chemistry between the basins occurred mostly in the hypolimnion, whereas epilimnion values were similar (Fig. 3, Table S1). Higher annual average NO_3^- (11.9 ± 6.6 vs 5.0 ± 1.4), and lower TN (309 ± 25 vs 552 ± 88), TP (13.5 ± 4.2 vs 40.1 ± 8.8), NH_4^+ (93.5 ± 24.6 vs 311.2 ± 61.6) and PO_4^{3-} (6.8 ± 4.3 vs 36.3 ± 8.1) occurred in the hypolimnion of the Control compared to the Impact basins, respectively (Table S1). The basins were on average similarly dark as indicated by Kd values (Kd; 2.0 ± 0.3 vs 1.7 ± 0.4 for Control and Impact basin, respectively; Table S1). There were no differences in lake PP, lake BP, lake PP:BP, and degradation and BP in either epilimnion or hypolimnion between the Control and the Impact basin during the before years (Table S1; Fig. 4). Hence, the basins were similar in the epilimnion at the onset of oxygenation although slight differences in the hypolimnion chemistry occurred in the Before years, while no differences in the measured biological responses were observed.

Weather and environmental (natural) variability in water chemistry among Before and After years

The temperature variation between the years was substantial; while 2018 (second “Before” Year) was an exceptionally warm year, 2019 (first “After” year) was a more normal year/summer with a later warm period (sampling 6 and 7; Figs. 2b; 4b). Seasonal onsets of warm and cold periods also varied: both “Before” years (2017 and 2018) had a warm peak around the 4th sampling (between 28 and 30 July). In “After” year 2019 such a warm peak was absent and in 2020 two more dampened warm peaks occurred, early and later in the year (2nd and 5th sampling; 16–18 June and 16–21 August resp.; Figs. 2b, 4b). The variability in seasonality (the onset of warm and cold periods) and weather (the average and peak temperatures) between years resulted in a slightly colder seasonal average epilimnion and hypolimnion during the “After” period compared to the “Before” period (Table 1; Figs. 2b, 4b). The basins were thermally stratified during the sampling seasons, except for the first and last sampling occasions in some years

Table 1 Annual average $\pm$ SD of water physico-chemistry and biology measured as lake estimates, and in the epi- and hypolimnion for the BACI design, along with the p-value for the Before vs After (BvA) assessment in an ANOVA, BA*CI probability ratio and effect direction calculated using the epower package (significant effects in bold)

	Before		After		BvA (p-value)		BACI (ePower)	BvA vs BACI design	
	Control*	Impact	Control	Impact	Control	Impact	BA*CI		Direction
<i>Lake estimates</i>									
Lake PP (mgC m ⁻² day ⁻¹)	61.0±62.9	60.0±53.0	43.3±28.1	37.2±21.2	0.385	0.182	0.011		Decrease
Lake BP (mgC m ⁻² day ⁻¹)	24.0±16.0	25.2±16.3	36.21±12.3	35.2±19.3	0.048	0.183	0.900		Decrease
Lake PP:BP	3.5±4.1	3.5±4.5	1.2±0.8	1.3±1.0	0.077	0.120	0.015		Decrease
Kd (m ⁻¹)	2.0±0.3	1.7±0.4	1.9±0.2	1.8±0.2	0.119	0.665	0.009		Increase
<i>Epilimnion</i>									
NO ₃ ⁻ (µg L ⁻¹)	6.2±3.3	4.4±2.2	9.8±11.6	4.9±3.7	0.556	0.788	0.015		
NH ₄ ⁺ (µg L ⁻¹)	11.1±3.3	9.7±4.1	10.0±4.0	8.6±5.0	0.589	0.443	0.030		
PO ₄ ³⁻ (µg L ⁻¹)	3.5±3.3	5.7±6.6	1.0±1.0	0.8±0.9	0.084	0.037	NA	False	
DOC (mg L ⁻¹)	10.3±1.3	11.9±4.2	10.9±4.0	10.0±3.7	0.583	0.396	0.007		
TP (µg L ⁻¹)	6.7±1.8	5.6±1.2	7.3±1.3	7.5±1.9	0.462	0.057	0.013	False	
TN (µg L ⁻¹)	233±12	259±37	242±13	239±20	0.192	0.169	0.013		
Twater (°C)	14.7±4.6	15±4.6	13.6±3.1	13.3±3.2	0.516	0.336	0.004		
DO (mg L ⁻¹)	8.8±0.7	8.7±0.6	8.8±0.6	8.8±0.6	0.483	0.423	0.001		
BP (µg·L ⁻¹ ·day ⁻¹)	3.9±2.9	4.0±2.9	7.7±3.6	6.9±3.9	0.009	0.054	0.535		Decrease
Green tea mass loss (% day ⁻¹)	0.024±0.003	0.025±0.006	0.015±0.003	0.015±0.002	0.027	0.024	—	—	
Rooibos mass loss (% day ⁻¹)	0.005±0.001	0.005±0.001	0.003±0.001	0.003±0.000	0.035	0.053	—	—	
<i>Hypolimnion</i>									
NO ₃ ⁻ (µg L ⁻¹)	11.9±6.6	5.0±1.4	23.3±23.1	63.0±37.5	0.370	0.004	0.721		Increase
NH ₄ ⁺ (µg L ⁻¹)	93.5±24.6	311.2±61.6	30±16.5	37.8±39.9	<.001	<.001	0.069	False	
PO ₄ ³⁻ (µg L ⁻¹)	6.8±4.3	36.3±8.1	1.7±1.4	5.3±3.5	<.001	<.001	0.047	False	
DOC (mg L ⁻¹)	12.9±4.6	17.8±7.9	10.3±1.4	11.5±6.2	0.095	0.249	0.012		
TP (µg L ⁻¹)	13.5±4.2	40.1±8.8	9.8±3.2	14±2.8	0.062	<.001	0.158	False	
TN (µg L ⁻¹)	309±25	552±88	270±34	337±47	0.033	<.001	0.389	False	
Twater (°C)	7.1±1.3	6.6±1.8	5.6±1.0	5.7±1.2	0.058	0.552	0.008		
DO (mg L ⁻¹)	4±1.5	2.2±0.8	3.9±1.6	6.1±1.7	0.765	<.001	0.591		Increase
BP (µg·L ⁻¹ ·day ⁻¹)	2.8±2.2	3.0±2.8	3.2±1.2	3.4±2.2	0.603	0.733	0.100		
Green tea mass loss (% day ⁻¹)	0.014±0.001	0.013±0.001	0.011±0.002	0.009±0.001	0.061	0.005	—	—	Decrease
Rooibos mass loss (% day ⁻¹)	0.004±0.000	0.003±0.000	0.002±0.001	0.002±0.000	0.096	0.007	—	—	Increase

A BA*CI probability ratio of >0.5 indicates a stronger support for the BACI interaction model, and an effect of the treatment. In the “BvA vs BACI design” column is indicated if the significant effect found in the BvA effect in the Impact basin is “FALSE” compared to the effect found with the epower package. Epilimnion values are seasonal average values of the water column at 1 m, and hypolimnion 6 m below water surface. Data from 2017 and 1st sampling of each year are excluded. The rooibos and green tea mass loss rates presented are calculated assuming an exponential decay

(Fig. 2b), and there was a distinct thermocline and nutricline (Figs. 2, 3). In summary, the experiment was carried out over a 4-year period with substantial

seasonal and climate variations, where Before years were somewhat warmer than the After years, with different timing of the warm peak.

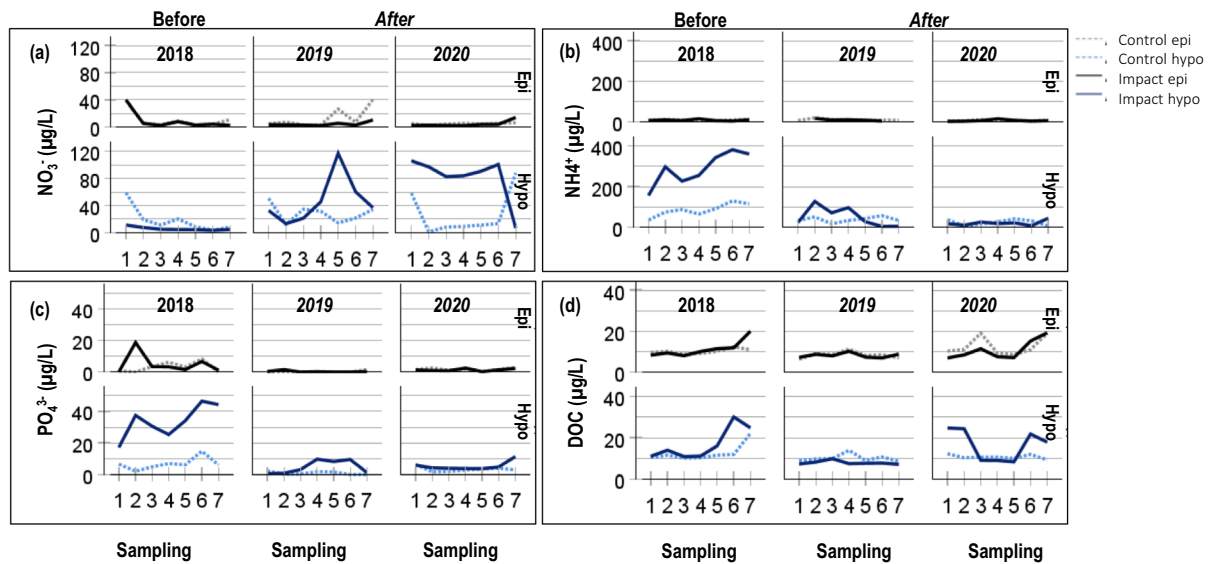


Fig. 3 Water chemistry concentrations of (a) NO_3^- , (b) NH_4^+ , (c) PO_4^{3-} , and (d) DOC in the epilimnion (black and grey) and hypolimnion (light and dark blue) for the Impact (solid lines)

and Control (dotted lines) lakes over seven sampling occasions each during the Before ((2017, 2018)) and After (2019 and 2020) years, with years depicted in panels

Substantial variability (measured over 3 years in the Control basin) was observed for the nutrient concentrations, specifically in the hypolimnion (Fig. 3). Annual average NO_3^- concentrations were higher and much more variable in the hypolimnion during the Before years compared to the After years, but not so in the epilimnion (Table 1; Fig. 3). In the hypolimnion of the control basin, lower annual averages were observed in the After period compared to the Before period for NH_4^+ , PO_4^{3-} and TN (Table 1).

Natural biological variability in Before and After years

The lake PP measurements were confined to the epilimnion and were influenced by variations in water temperature (Fig. 4b–c; average $\pm$ SD PP rates ($\mu\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$); 27.2 ± 33.0 vs 1.8 ± 5.0 for all measurements between 0–2 m depth and 2–4 m depth, respectively (see open data)). Annual average lake PP and temperature were slightly lower in both basins in the after years when comparing Before and After years (Table 1; Fig. 4). In contrast, lake BP was higher in both basins in the slightly cooler After years (Before: 24.0 ± 16.0 vs After: 36.2 ± 12.3 ; $p < 0.05$ for Control basin) and therefore did not strictly follow temperature variations (Fig. 2; Table 1). The

lake PP:BP ratios were not different when comparing the After and Before years in both basins, (BvA, Table 1). Green tea mass loss decreased in the epilimnion and hypolimnion in the Control basin, while Rooibos mass loss decreased only in the epilimnion in the Control basin (Table 1, BvA; Fig. 4).

Oxygenation impacts

Effects of hypolimnic oxygenation on water chemistry in epi- and hypolimnion

Oxygenation of the hypolimnion increased hypolimnion NO_3^- but statistically significant effects on other variables were not detected (TN, TP, PO_4^{3-} , NH_4^+ , and DOC; Table 1: BACI). Furthermore, the water chemistry in the epilimnion was unaffected by the oxygen treatment (Fig. 3; Table 1). The BACI assessment indicates that only the increase in DO values, and the increase in NO_3^- values in the hypolimnion are due to the experimental oxygenation ($\text{BA}^*\text{CI} = 0.72$ and 0.59 , resp.; Table 1). Still, (insignificant) patterns in altered water chemistry occurred. Essentially, these patterns are similar to a traditional BvA setup as described below and in Table 1. These patterns include that water in the hypolimnion shifted from domination of NH_4^+ ($\text{BA}^*\text{CI} = 0.07$)

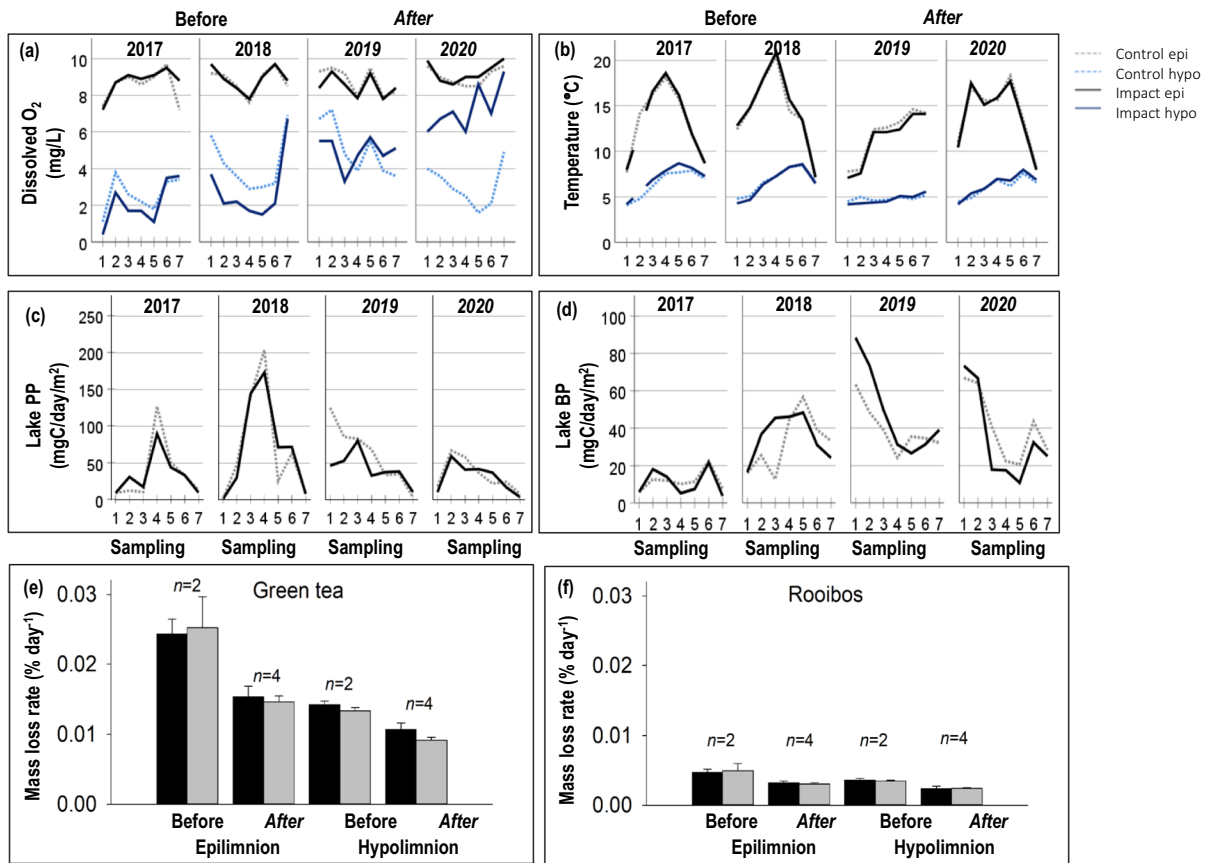


Fig. 4 Responses of (a) dissolved oxygen, (b) temperature, (c) lake primary production, and (d) lake bacterial production (e) green tea and (f) rooibos mass loss rates to the Before-After Controlled-Impact (BACI) treatments. In (a–d), seasonal changes in epilimnion (black and grey: Impact and Control, respectively) and hypolimnion (light and dark blue: Impact and Control, respectively) are shown for the Impact (solid lines) and Control (dotted lines) lakes over seven sampling occasions each during the Before (2017, 2018, and 1st sampling 2019)

and After (2019 minus 1st sampling and 2020) years. Dissolved oxygen and temperature are taken at 1 m and 6 m depth in the epilimnion and hypolimnion, respectively. In e,f, means (black=Impact, grey=Control) are shown with standard errors and the number above the bar indicates the replication on which each mean is based. Hypolimnion primary production in (c) is negligible in both Control and Impact lake and left out of this Figure. *PP:BP is log 10 transformed in the figure

to domination of NO_3^- ($\text{BA} \cdot \text{CI} = 0.72$) after oxygenation, and that PO_4^{3-} ($\text{BA} \cdot \text{CI} = 0.047$) values decreased after hypolimnion oxygenation (Fig. 4; Table 1). However, for all other water chemistry variables besides NO_3^- the $\text{BA} \cdot \text{CI}$ term was lower than 0.5, indicating the probability is higher that these patterns are most likely due to climate and environmental variability. Scatterplots of DO against other water chemistry variables for both basins per year, in both the epilimnion and hypolimnion, show that PO_4^{3-} (and TP) concentrations are elevated in low oxygen conditions in the hypolimnion during 2018 (Before year), but not in the After years

(Figure S1a–b). Moreover, hypolimnetic PO_4^{3-} concentrations appear to be influenced by dissolved oxygen (DO) levels; consistently low PO_4^{3-} under high DO conditions suggests that hypoxia may trigger sedimentary release of phosphate (Figure S1b). NO_3^- concentrations increase with rising oxygen concentrations in the hypolimnion, but not in the epilimnion (Figure S1c–d). The pattern for DOC is different in the epilimnion and hypolimnion; values below 20 mg/L exhibit a slight positive correlation with oxygen levels in the epilimnion, while showing a slight negative correlation in the hypolimnion (Figure S1e–f).

Oxygenation impacts on production and decomposition and the magnitude of change

The lake BP decreased due to the hypolimnion oxygenation in the Impact basin, and increased in both basins, but less so in the Impact basin (Table 1; $BA*CI=0.900$, Fig. 4d). The effect was modest, as the annual average lake BP only decreased by 6.1%, calculated using the average paired difference (per sampling) between the basins, and annual average lake BP in the Control basin over four years (Table 2). Annual average lake PP, lake PP:BP, and rooibos and green tea mass loss in the hypolimnion only slightly changed (-11.7% , 4.2% , -6.0% and 7.3% , respectively) and changes were not detected with the BACI assessment (Table 2). Thus, lake BP was the only biological parameter that significantly decreased with the reoxygenation of the hypolimnion according to the BACI design (Table 1; BACI epower).

However, further analysis comparing the effects at different depths (1 and 6m depth) show that the changes in lake BP are primarily attributed to high epilimnetic values in the 'After' years in both the Control and Impact basins, but mostly in the Control basin (Table 1). To understand better what drove changes in lake BP, BP in the epilimnion and hypolimnion, and in lake PP at 1m depth, we tested which water chemistry variables (DO, NO_3^- , NH_4^+ , PO_4^{3-} , DOC, TP, water temperature at depth ($T_{water, 1m}$), Year) correlated best with these variables in MLR analyses with forward selection. These results show that lake PP correlates positively with $T_{water, 1m}$

and negatively to NO_3^- (Table 3; Fig S2). Lake BP and BP in the epilimnion correlates positively with DO and negatively with NH_4^+ and NO_3^- . The BP in the hypolimnion correlated positively with TN (Table 3). Therefore, the epilimnetic BP changes in the After years overshadowed the response in BP in the hypolimnion, and there was no significant effect of reoxygenation on BP in the hypolimnion based on the seasonal average (Tables 1–3). In addition, PP is primality correlated to water temperature (Table 2; Fig S2).

Comparing the BACI design with a traditional BvA setup

We compared the outcome of the BACI design to the simplified BvA design that does not control for other variables, such as natural seasonal and inter-annual variations. The BvA setup (BvA in Impact basin) identified changes due to oxygenation for several parameters compared to the BACI (Table 1, "BvA vs BACI"). In the BvA analyses for the Impact basin, oxygenation affected PO_4^{3-} , TP, and green tea mass loss in both the epilimnion and hypolimnion, and NO_3^- . NH_4^+ , PO_4^{3-} , TP, TN, and rooibos mass loss in hypolimnion only (Table 1). None of the other productivity measurements (lake PP, lake BP, lake PP:BP) were affected in the BvA setup for the Impact basin. Since these effects were not significant when accounting for natural variability using a BACI design (not available for tea degradation), the effects

Table 2 Quantifying the impacts of oxygenation by tracking the average paired differences (Impact-Control) per sampling event for the Before (dBefore) and After (dAfter) samplings

Proxy	dBefore (Impact-Control)	dAfter (Impact-Control)	dAfter-dBefore (Impact effect)	Seasonal 2y avg (Control basin, After)	% change of seasonal avg	Sig- nificant BACI?
Lake PP ($mg\ C\ m^{-2}\ day^{-1}$)	- 1.02	- 6.08	5.06	43.3 ± 28.1	- 11.69%	No
Lake BP ($mg\ C\ m^{-2}\ day^{-1}$)	1.19	- 1.00	- 2.19	36.21 ± 12.3	- 6.05%	Yes
PP:BP	- 2.27	- 2.22	0.05	1.2 ± 0.8	4.17%	No
Green tea mass loss ($\%\ day^{-1}$)*	- 9.13E-04	- 1.55E-03	- 6.38E-04	1.07E-02	- 5.96%	-
Rooibos mass loss ($\%\ day^{-1}$)*	- 1.29E-04	4.66E-05	1.75E-04	2.40E-03	7.32%	-

Changes in average paired differences (% change of seasonal avg) are expressed as percentage of the annual average of all years and samplings of the Control basin (annual avg) compared to the outcome of the BACI analyses presented in Table 1

*Tea decomposition is represented for hypolimnion values

Table 3 Multiple linear regression results with forward selection of variables for predicting lake PP, and BP in the epilimnion and hypolimnion

Coefficients of explanatory variables	R ² (adj)	DF	AIC	F	Deselected variables
<i>Lake PP</i>					
3.656·T _{water, 1m}	0.209	51	387.034	80.795	O ₂ , NO ₃ ⁻ , NH ₄ ⁺ , PO ₄ ³⁻ , DOC, TP, TN, Year
4.482·T _{water, 1m} - 1.288 NO ₃ ⁻	0.645	50	382.539	48.200	O ₂ , NH ₄ ⁺ , PO ₄ ³⁻ , DOC, TP, TN, Year
<i>Lake BP</i>					
3.534 O ₂	0.735	51	305.219	145.511	NO ₃ ⁻ , NH ₄ ⁺ , PO ₄ ³⁻ , DOC, TP, TN, Year, T _{water, 1m}
4.142·O ₂ - 0.228·NH ₄ ⁺	0.787	50	294.842	97.214	NO ₃ ⁻ , PO ₄ ³⁻ , DOC, TP, TN, Year, T _{water, 1m}
4.811·O ₂ - 0.227·NH ₄ ⁺ - 0.622·NO ₃ ⁻	0.816	49	288.214	77.925	PO ₄ ³⁻ , DOC, TP, TN, Year, T _{water, 1m}
<i>BP epilimnion (1m)</i>					
0.687 O ₂	0.637	51		92.169	NO ₃ ⁻ , NH ₄ ⁺ , PO ₄ ³⁻ , DOC, TP, TN, T _{water, 1m} , Year
0.802·O ₂ - 0.045·NH ₄ ⁺	0.680	50		56.350	NO ₃ ⁻ , PO ₄ ³⁻ , DOC, TP, TN, T _{water, 1m} , Year
0.950·O ₂ - 0.045·NH ₄ ⁺ - 0.133·NO ₃ ⁻	0.709	49		43.208	PO ₄ ³⁻ , DOC, TP, TN, T _{water, 1m} , Year
<i>BP hypolimnion (6m)</i>					
0.011·TN	0.726	39		107.017	O ₂ , NO ₃ ⁻ , NH ₄ ⁺ , PO ₄ ³⁻ , DOC, TP, T _{water, 6m} , Year

DF degrees of freedom, AIC Akaike Information Criterion

identified by the traditional BvA setup are concluded to be within the range of natural variability (Table 1).

Discussion

Our four-year BACI experiment demonstrates that hypolimnetic oxygen concentrations have a moderate impact on lake biogeochemistry and basal production (PP, BP). Re-oxygenation altered nitrification pathways (increased NO₃⁻) of the hypoxic hypolimnion, and only modestly decreased lake BP (- 6.1%

of annual average). The decrease in lake BP was driven by changes in the epilimnion, and therefore the decrease was more likely due to natural variations. Litter degradation (in the hypolimnion) of rooibos increased, while green tea degradation decreased, but could not be assessed in a BACI setup due to missing the first year. No statistically detectable changes in other water chemistry parameters (TP, PO₄³⁻, NO₃⁻, NH₄⁺, DOC) and PP were observed when considering the impacts by oxygenation alone, while accounting for natural variability among years. Moreover, we found that across sampling dates PP was primarily

positively correlated to water temperature, and not to water chemistry. We compared two experimental designs—one that accounts for natural variation (BACI) and one that does not (BvA)—and found that the BvA approach showed more significant effects from oxygenation than the BACI design. Our results therefore suggest that changes induced by oxygenation are mostly within the range of natural variation, and that only NO_3^- and lake BP and epilimnion BP are significantly, but modestly, affected. Natural variation in climate as well as variability in stratification among years is therefore likely to have a larger impact on humic boreal lake functioning than hypolimnion hypoxia itself, at least during the stratification period.

Climate effects rather than hypoxia drive lake biogeochemical functioning

In the hypolimnion, reoxygenation increased NO_3^- concentrations as expected in the impact basin (prediction 3). However, only significant reductions in PO_4^{3-} , NH_4^+ , TN, and TP were observed when considering the BvA setup in the Impact basin but not in the BACI analysis. Decreased TN, NH_4^+ and PO_4^{3-} were also present in the Control basin when interpreting the BvA term, confirming strong effects of natural variations in the hypolimnion (Table 1). These findings are further supported by a large-scale study including 64 lakes in Sweden, that found no relationship between hypolimnetic hypoxia and TP (Dimberg & Bryhn 2015). Nonetheless, the experimentally induced increase in NO_3^- , and reductions due to natural variation in PO_4^{3-} , NH_4^+ , TN, and TP in the hypolimnion of the oxygenated Impact basin are likely to be due to increased nitrification (Gautreau et al. 2020; Romillac 2019) and inhibited sediment release of PO_4^{3-} (Mortimer 1971; North et al. 2014) during the sampling period. In the epilimnion we found no effects of oxygenation on the water chemistry within the BACI approach. However, very weak decreases of annual average TP and PO_4^{3-} in the Impact basin and nearly significant changes in NH_4^+ and TN in the Control basin were identified in the BvA analyses, which suggest mixed effects of natural variation and oxygenation. Altogether, our results indicate that hypolimnion oxygenation significantly increases nitrification (increase in NO_3^-), but without further affecting the nutrient pools or other water chemistry. This contrasts with potential effects

on (type and magnitude of) greenhouse gas emissions, as redox conditions (specifically O_2 availability) determine the rates of CO_2 and CH_4 production (Carlsen et al. 2025; Pajala et al. 2023). Furthermore, variations in nutrient (PO_4^{3-} , TP, TN, DOC) concentrations in both the epilimnion and hypolimnion are more influenced by seasonal changes than by hypoxia.

Indeed, the seasonal patterns of water chemistry and temperature fluctuated throughout the year and varied substantially across the four years of monitoring with notably warmer temperatures in the Before compared to the After years. In contrast, the temperatures in the hypolimnion were lower and considerably more stable due to the separation of these water masses caused by the thermoclines (Bartosiewicz et al. 2019; Jones 1992; Pilla et al. 2018). In both basins during the Before-years, and in the Control basin in the After-years, the concentrations of DOC were significantly higher in the hypolimnion compared to the epilimnion. This suggests a degree of seasonal fluctuation also in the hypolimnion water chemistry, which is likely due to the limited and somewhat variable mixing of the basins during spring and/or autumn circulation (Dimberg & Bryhn 2015). Moreover, DOC-DO patterns differed between the epilimnion and hypolimnion; values below 20 mg/L showed a slight positive trend with oxygen in the epilimnion and a slight negative trend in the hypolimnion (Figure S1e–f), reflecting sediment release of DOC under low to anoxic conditions, and terrestrial runoff of DOC entering the epilimnion. High seasonal variability in climate among years has also been observed in other settings with experimental oxygenation in a BvA approach without accounting for seasonal impacts (Carey et al. 2018, 2022; McClure et al. 2021; Riccardi & Mangoni 1996). The results of these studies indicate that observed significant changes in hypolimnion biogeochemistry due to altered oxygen regimes in the hypolimnion may be overestimated, as seasonal variability is not accounted for.

Our results suggest that the water chemistry in the epilimnion is strongly driven by weather fluctuations and the hydrological connectivity of the lakes and their catchments (Einola et al. 2011; Puts et al. 2022; Rantakari & Kortelainen 2008). On the other hand, the chemistry in the hypolimnion is more linked to physical–chemical drivers, such as sediment–water interactions, oxygen availability, and water mixing events (North et al. 2014), illustrating that these water

masses are differentially impacted by climate change and increasing hypoxia. Natural (including seasonal) fluctuations have a significant impact on nutrient cycling, even in the hypolimnion with a constant mean temperature. This suggests that climate effects and their impact on temperature and stratification patterns, rather than just variations in oxygen concentrations and anoxia alone, are more important drivers for biogeochemical functioning in shallow, brown, boreal lakes, at least during the stratification period.

Oxygenation affects bacterial production and detritus degradation with minimal effects on primary production

Oxygenation of the hypolimnion decreased the annual average lake BP and the BP in the epilimnion but had no effects on lake PP and lake PP:BP (BACI in Table 1). However, the oxygenation effect on lake BP was not significant in the BvA setup for the Impact basin, which illustrates that the BvA approach underestimates the effect of oxygenation on lake BP (Table 1). Tea degradation rates, a key measure of bacterial functioning, could not be assessed in the BACI design because of the absence of measurements from the first control year. However, in the BvA term, tea degradation rates were differently affected by the oxygenation treatment, with decreased green tea degradation and increased rooibos degradation in the hypolimnion. These differences -though small in absolute terms- may be driven by different responses of decomposer communities to various litter types. Alternatively, we observed more algal growth on green tea compared Rooibos tea, suggesting that hypolimnion oxygenation may have stimulated algae growth on green tea. Since the algae couldn't be separated from the final mass, this could have decreased the mass loss rates of green tea more than Rooibos.

The observed effects in decreased lake BP and potentially in degradation rates are likely because of several processes connected to reoxygenation. The overall impact of hypoxia on lake BP is a balance of several opposing processes: i.e. increased PO_4^{3-} and DOC release from sediments (positive effect) and losses of O_2 as the most efficient electron donor (negative effect; (Burgin et al. 2011; Garcia et al. 2013; Shadeet al. 2008)). Nonetheless, lake BP was thus higher in years with slightly colder water temperatures in After years especially in the epilimnion

in both basins with somewhat higher epilimnion DOC concentrations, a difference most likely caused by seasonal variability in catchment input of bio-available colored DOC influencing the BP (Table 1, Figure S1b–c); Berggren et al. 2010; Bergström and Jansson 2000). While increased oxygen availability was associated to reduced lake BP -due to a reduction in the epilimnion-, the Rooibos degradation increased (Table 2). This might be due to simultaneous decreases in available PO_4^{3-} and temperature (Jansson et al. 1999; Rulli et al. 2022). Consequently, yearly climatic variations were able to override the expected impact of oxygen treatment on a whole-ecosystem scale when accounting for effects induced by natural variability. This highlights the importance of whole-ecosystem BACI studies; laboratory and BvA or CvI studies might simply conclude that oxygenation leads to altered BP and degradation rates with increased oxygen, but this isn't always the case in natural systems.

We further observed weak trends of decreasing lake PP (− 11.7%) and increasing lake PP:BP (4.1%: Seasonal average paired differences; Table 2). The patterns of slightly decreased lake PP and increased lake BP resulted in increased lake PP:BP ratios in the pairwise comparison, although PP:BP decreased from around 3 in the Before years, to ratios around 1 in the After years for both lakes. The largest part of this “shift” is attributed to PP primarily related to water temperatures, and lake and epilimnion BP related to DO, NH_4^+ and NO_3^- (Table 3). This suggests that the impact of natural climate variations on the conditions in the epilimnion of stratified humic boreal lakes during summer overrides the effects of changes in hypoxia on the net ecosystem balance and ecosystem functioning. In addition, to some extent the lake PP:BP ratio could further have been modified by changes in the food web structure (fish, zooplankton and phytoplankton community composition) impacting the response in lake PP and lake BP across seasons. In our system only one fish species (Eurasian Perch, *Perca fluviatilis*) existed within the two basins, and there was no observable effect of the oxygenation treatment on the fish population dynamics, or their stomach content, during the period which this experiment was conducted. Thus, our findings imply that considerable variations in lake PP:BP are likely to occur in humic boreal, shallow lakes in the future. The magnitude and occurrence of these fluctuations

depend on the oscillations of natural climate variability related to the ongoing (long-term) changes in climate, that impact lake temperatures, precipitation and catchment loadings of colored DOC and nutrient to lakes.

In all, our results suggest a modest direct impact of local O₂ conditions and hypoxia on lake BP and litter degradation. These findings partly support prediction 1 and 2; the hypothesis that reoxygenation of the hypolimnion does not impact PP as it is confined to the epilimnion and show that the impact of climate is relatively stronger than the impact of reoxygenation as lake BP and lake PP responded most to environmental changes in the epilimnion.

Conclusion

As global change continues to drive significant alterations in the stratification and surface temperature of boreal lakes, understanding these dynamics and how they are linked to natural variation in climate is crucial for predicting and managing the impacts on lake food webs and freshwater ecosystems. Our results indicate that hypoxia had minor influence on nutrient cycling and the lower food web in a seasonally stratified and small humic boreal lake. Our study highlights the complex interplay between hypolimnetic hypoxia and natural climate variation embedded in ongoing global changes. In contrast to the potential magnitude and type of greenhouse gas emissions, that follow redox conditions, we find biogeochemistry is minimally affected. Still, as our study of 2 years of oxygenation had limited effects on water chemistry and basal productivity it remains to be assessed if long-term effects of oxygen are delayed past the scope of the study, possibly impacting biogeochemistry and ecology of the system during autumn turnover and during winter. We predicted that hypoxia and weather variations would have distinct effects on lake biogeochemistry and BP and PP (prediction 1). Our results contradict our hypothesis and underline that climate variations affecting the conditions of stratified boreal lakes during summer override the effects of changes in hypoxia on ecosystem functioning. Moreover, the results imply that results of studies that do not account for seasonal variability and find significant changes of hypolimnion biogeochemistry

may lead to flawed conclusions, as a large part of effects can be due to natural weather variations (prediction 4). The magnitude and occurrence of these fluctuations depend on natural climate variability and ongoing long-term climate changes, impacting lake temperatures, stratification depth, strength, and duration, precipitation, and catchment loadings of colored DOC and nutrients, and lake DO concentrations.

Acknowledgements We thank Erik Gebrink for help maintaining the experimental treatment, and all students and curious colleagues that helped out in the field.

Author contributions ICP wrote the first draft of the manuscript. AKB, DS, ICP, JK, PB and SK initiated research questions and designed the study approach. DS, PB and SK maintained the technical parts of experimental setup. AJ, HAV, ICP, JMS and SK performed fieldwork and laboratory analyses. ICP and SK conducted statistical analyses and graphical illustrations. AKB, ICP, JK, and PB acquired funding. All coauthors read and commented on the manuscript; author mentions in this statement are in alphabetical order.

Funding Open access funding provided by Umea University. This work was supported by the Knut and Alice Wallenberg Foundation (d.nr. 2016.0083). ICP has received research support from Swedish research council FORMAS (d.nr. 2022-02830) and from an Inge Lehman grant.

Data availability The datasets generated during and/or analyzed during the current study are available via zenodo: <https://zenodo.org/records/14930455> (temperature and O₂ profiles, BP and PP in 1st version, water chemistry in 2nd version), and <https://zenodo.org/records/15517814> (tea bags).

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Bartosiewicz M, Przytulska A, Lapierre JF, Laurion I, Lehmann MF, Maranger R (2019) Hot tops, cold bottoms: Synergistic climate warming and shielding effects increase carbon burial in lakes. *Limnol Oceanogr Lett* 4(5):132–144. <https://doi.org/10.1002/lo2.10117>
- Bastviken D, Persson L, Odham G, Tranvik L (2004). Bastviken, David, Linn Persson, Göran Odham, and Lars Tranvik. Degradation of dissolved organic matter in oxic and anoxic lake water. *Limnol. Oceanogr.* 49(1):109–116. <http://www.ihss.gatech.edu/>
- Berggren M, Ström L, Laudon H, Karlsson J, Jonsson A, Giesler R, Bergström AK, Jansson M (2010) Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. *Ecol Lett* 13(7):870–880. <https://doi.org/10.1111/j.1461-0248.2010.01483.x>
- Bergström AK, Jansson M (2000) Bacterioplankton production in humic lake Ortrasket in relation to input of bacterial cells and input of allochthonous organic carbon. *Microb Ecol* 39(2):101–115. <https://doi.org/10.1007/s002480000007>
- Berner LT, Goetz SJ (2022) Satellite observations document trends consistent with a boreal forest biome shift. *Glob Change Biol* 28(10):3275–3292. <https://doi.org/10.1111/gcb.16121>
- Brothers S, Köhler J, Attermeyer K, Grossart HP, Mehner T, Meyer N, Scharnweber K, Hilt S (2014) A feedback loop links brownification and anoxia in a temperate, shallow lake. *Limnol Oceanogr* 59(4):1388–1398. <https://doi.org/10.4319/lo.2014.59.4.1388>
- Burgin AJ, Yang WH, Hamilton SK, Silver WL (2011) Beyond carbon and nitrogen: how the microbial energy economy couples elemental cycles in diverse ecosystems. *Front Ecol Environ* 9(1):44–52. <https://doi.org/10.1890/090227>
- Bush T, Diao M, Allen RJ, Sinnige R, Muyzer G, Huisman J (2017) Oxidic-anoxic regime shifts mediated by feedbacks between biogeochemical processes and microbial community dynamics. *Nat Commun.* <https://doi.org/10.1038/s41467-017-00912-x>
- Cael BB, Seekell DA (2016) The size-distribution of Earth's lakes. *Sci Rep.* <https://doi.org/10.1038/srep29633>
- Carey CC, Doubek JP, McClure RP, Hanson PC, Stanley E, Del Giorgio P (2018) Special issue-letter oxygen dynamics control the burial of organic carbon in a eutrophic reservoir. *Limnol Oceanogr Lett* 3:293–301. <https://doi.org/10.6073/pasta/10545e44a05b1a688c1561d602ca8a62>
- Carey CC, Hanson PC, Thomas RQ, Gerling AB, Hounshell AG, Lewis ASL, Lofton ME, McClure RP, Wander HL, Woelmer WM, Niederlehner BR, Schreiber ME (2022) Anoxia decreases the magnitude of the carbon, nitrogen, and phosphorus sink in freshwaters. *Glob Change Biol* 28(16):4861–4881. <https://doi.org/10.1111/gcb.16228>
- Carlsen ECL, Wei J, Lejzerowicz F, Trier Kjær S, Westermann S, Hessen DO, Dörsch P, Eiler A (2025) Redox determines greenhouse gas production kinetics and metabolic traits in water-saturated thawing permafrost peat. *ISME Commun.* <https://doi.org/10.1093/ismeco/ycaf009>
- Carpenter SR, Kitchell JF eds. (1993) *The Trophic Cascade in Lakes*. Cambridge University Press
- Couture RM, De Wit HA, Tominaga K, Kiuru P, Markelov I (2015) Oxygen dynamics in a boreal lake responds to long-term changes in climate, ice phenology, and DOC inputs. *J Geophys Res Biogeosci* 120(11):2441–2456. <https://doi.org/10.1002/2015JG003065>
- De Wit HA, Valinia S, Weyhenmeyer GA, Futter MN, Kortelainen P, Austnes K, Hessen DO, Räske A, Laudon H, Vuorenmaa J (2016) Current browning of surface waters will be further promoted by wetter climate. *Environ Sci Technol Lett* 3(12):430–435. <https://doi.org/10.1021/acs.estlett.6b00396>
- Deininger A, Faithfull CL, Bergström AK (2017) Phytoplankton response to whole lake inorganic N fertilization along a gradient in dissolved organic carbon. *Ecology* 98(4):982–994. <https://doi.org/10.1002/ecy.1758>
- Dimberg PH, Bryhn AC (2015) Predicting total nitrogen, total phosphorus, total organic carbon, dissolved oxygen and iron in deep waters of Swedish lakes. *Environ Model Assess* 20(5):411–423. <https://doi.org/10.1007/s10666-015-9456-4>
- Einola E, Rantakari M, Kankaala P, Kortelainen P, Ojala A, Pajunen H, Mäkelä S, Arvola L (2011) Carbon pools and fluxes in a chain of five boreal lakes: a dry and wet year comparison. *J Geophys Res.* <https://doi.org/10.1029/2010JG001636>
- Fee EJ (1976) The vertical and seasonal distribution of chlorophyll in lakes of the Experimental Lakes Area, north-western Ontario: implications for primary production estimates. *Limnol Oceanogr* 21(6):767–783
- Finstad AG, Andersen T, Larsen S, Tominaga K, Blumentrath S, De Wit HA, Tømmervik H, Hessen DO (2016) From greening to browning: Catchment vegetation development and reduced S-deposition promote organic carbon load on decadal time scales in Nordic lakes. *Sci Rep.* <https://doi.org/10.1038/srep31944>
- Fisher R, Shiell GR, Sadler RJ, Inostroza K, Shedrawi G, Holmes TH, McGree JM (2019) Epower: an r package for power analysis of Before-After-Control-Impact (BACI) designs. *Methods Ecol Evol* 10(11):1843–1853. <https://doi.org/10.1111/2041-210X.13287>
- Ganf GG, Oliver RL (1982) Vertical separation of light and available nutrients as a factor causing replacement of green algae by blue-green algae in the plankton of a stratified. *Journal of Ecology* 70(3) <https://about.jstor.org/terms>
- Garcia SL, Salka I, Grossart H, Warnecke F (2013) Depth-discrete profiles of bacterial communities reveal pronounced spatio-temporal dynamics related to lake stratification. *Environ Microbiol Rep* 5(4):549–555. <https://doi.org/10.1111/1758-2229.12044>
- Garmo ØA, Skjelkvåle BL, De Wit HA, Colombo L, Curtis C, Fölster J, Hoffmann A, Hruška J, Høgåsen T, Jeffries DS, Keller WB, Krám P, Majer V, Monteith DT, Paterson AM, Rogora M, Rzychon D, Steingruber S, Stoddard JL, Vuorenmaa J, Worsztynowicz A (2014) Trends in surface water chemistry in acidified areas in Europe and North America from 1990 to 2008. *Water Air Soil Pollut.* <https://doi.org/10.1007/s11270-014-1880-6>
- Gautreau E, Volatier L, Nogaro G, Gouze E, Mermillod-Blondin F (2020) The influence of bioturbation and water

- column oxygenation on nutrient recycling in reservoir sediments. *Hydrobiologia* 847(4):1027–1040
- Guay KC, Beck PSA, Berner LT, Goetz SJ, Baccini A, Buermann W (2014) Vegetation productivity patterns at high northern latitudes: a multi-sensor satellite data assessment. *Glob Change Biol* 20(10):3147–3158. <https://doi.org/10.1111/gcb.12647>
- Hadley KR, Paterson AM, Stainsby EA, Michelutti N, Yao H, Rusak JA, Ingram R, McConnell C, Smol JP (2014) Climate warming alters thermal stability but not stratification phenology in a small north-temperate lake. *Hydrol Process* 28(26):6309–6319. <https://doi.org/10.1002/hyp.10120>
- Hansson TH, Grossart HP, del Giorgio PA, St-Gelais NF, Beisner BE (2019) Environmental drivers of mixotrophs in boreal lakes. *Limnol Oceanogr* 64(4):1688–1705. <https://doi.org/10.1002/lno.11144>
- Inoue T, Sugahara S, Seike Y, Kamiya H, Nakamura Y (2017) Short-term variation in benthic phosphorus transfer due to discontinuous aeration/oxygenation operation. *Limnol* 18(2):195–207. <https://doi.org/10.1007/s10201-016-0501-z>
- Jane SF, Hansen GJA, Kraemer BM, Leavitt PR, Mincer JL, North RL, Pilla RM, Stetler JT, Williamson CE, Woolway RI, Arvola L, Chandra S, DeGasperis CL, Diemer L, Dunalska J, Erina O, Flaim G, Grossart HP, Hambright KD, Hein C, Hejzlar J, Janus LL, Jenny JP, Jones JR, Knoll LB, Leoni B, Mackay E, Matsuzaki SS, McBride C, Müller-Navarra DC, Paterson AM, Pierson D, Rogora M, Rusak JA, Sadro S, Saulnier-Talbot E, Schmid M, Sommaruga R, Thiery W, Verburg P, Weathers KC, Weyhenmeyer GA, Yokota K, Rose KC (2021) Widespread deoxygenation of temperate lakes. *Nature* 594(7861):66–70. <https://doi.org/10.1038/s41586-021-03550-y>
- Jansen J, Simpson GL, Weyhenmeyer GA, Härkönen LH, Paterson AM, del Giorgio PA, Prairie YT (2024) Climate-driven deoxygenation of northern lakes. *Nat Clim Chang* 14(8):832–838. <https://doi.org/10.1038/s41558-024-02058-3>
- Jansson M, Bergström AK, Blomqvist P, Isaksson A, Jansson A (1999) Impact of allochthonous organic carbon on microbial food web carbon dynamics and structure in Lake Ortrasket. *Arch Hydrobiol* 144(4):409–428. <https://doi.org/10.1127/archiv-hydrobiol/144/1999/409>
- Jenny JP, Francus P, Normandeau A, Lapointe F, Perga ME, Ojala A, Schimmelmänn A, Zolitschka B (2016) Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. *Glob Change Biol* 22(4):1481–1489. <https://doi.org/10.1111/gcb.13193>
- Jones RI (1992) Dissolved organic matter in lacustrine ecosystems: energy source and system regulator. *Hydrobiologia* 229
- Karlsson J, Jonsson A, Jansson M (2001) Bacterioplankton production in lakes along an altitude gradient in the subarctic north of Sweden. *Microb Ecol* 42:372–382. <https://doi.org/10.1007/s00248-001-0009-9>
- Keuskamp JA, Dingemans BJJ, Lehtinen T, Sarneel JM, Hefting MM (2013) Tea bag index: a novel approach to collect uniform decomposition data across ecosystems. *Methods Ecol Evol* 4(11):1070–1075. <https://doi.org/10.1111/2041-210X.12097>
- Klanten Y, Couture RM, Christoffersen KS, Vincent WF, Antoniadis D (2023) Oxygen depletion in Arctic Lakes: circumpolar trends, biogeochemical processes, and implications of climate change. *Global Biogeochem Cycles*. <https://doi.org/10.1029/2022GB007616>
- Klaus M, Karlsson J, Seekell D (2021) Tree line advance reduces mixing and oxygen concentrations in arctic-alpine lakes through wind sheltering and organic carbon supply. *Glob Change Biol* 27(18):4238–4253. <https://doi.org/10.1111/gcb.15660>
- Kritzborg ES (2017) Centennial-long trends of lake browning show major effect of afforestation. *Limnol Oceanogr Lett* 2(4):105–112. <https://doi.org/10.1002/lol2.10041>
- Leach TH, Beisner BE, Carey CC, Pernica P, Rose KC, Huot Y, Brentrup JA, Domaizon I, Grossart HP, Ibelings BW, Jacquet S, Kelly PT, Rusak JA, Stockwell JD, Straile D, Verburg P (2018) Patterns and drivers of deep chlorophyll maxima structure in 100 lakes: the relative importance of light and thermal stratification. *Limnol Oceanogr* 63(2):628–646. <https://doi.org/10.1002/lno.10656>
- Leech DM, Pollard AI, Labou SG, Hampton SE (2018) Fewer blue lakes and more murky lakes across the continental U.S.: implications for planktonic food webs. *Limnol Oceanogr* 63(6):2661–2680. <https://doi.org/10.1002/lno.10967>
- Martínez-García S, Fernández E, Calvo-Díaz A, Cermeño P, Marañón E, Morán XAG, Teira E (2013) Differential response of microbial plankton to nutrient inputs in oligotrophic versus mesotrophic waters of the North Atlantic. *Mar Biol Res* 9(4):358–370. <https://doi.org/10.1080/17451000.2012.745002>
- McClure RP, Schreiber ME, Lofton ME, Chen S, Krueger KM, Carey CC (2021) Ecosystem-scale oxygen manipulations alter terminal electron acceptor pathways in a eutrophic reservoir. *Ecosystems* 24(6):1281–1298. <https://doi.org/10.1007/s10021-020-00582-9>
- Molot LA, Watson SB, Creed IF, Trick CG, McCabe SK, Verschoor MJ, Sorichetti RJ, Powe C, Venkiteswaran JJ, Schiff SL (2014) A novel model for cyanobacteria bloom formation: the critical role of anoxia and ferrous iron. *Freshw Biol* 59(6):1323–1340. <https://doi.org/10.1111/fwb.12334>
- Monteith DT, Stoddard JL, Evans CD, De Wit HA, Forsius M, Høgåsen T, Wilander A, Skjelkvåle BL, Jeffries DS, Vuorenmaa J, Keller B, Kopéček J, Vesely J (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450(7169):537–540. <https://doi.org/10.1038/nature06316>
- Mortimer CH (1971) Chemical exchanges between sediments and water in the Great Lakes-speculations on probable regulatory mechanisms. *Limnol Oceanogr* 16(2):387–404. <https://doi.org/10.4319/lno.1971.16.2.0387>
- North RP, North RL, Livingstone DM, Köster O, Kipfer R (2014) Long-term changes in hypoxia and soluble reactive phosphorus in the hypolimnion of a large temperate lake: consequences of a climate regime shift. *Glob Change Biol* 20(3):811–823. <https://doi.org/10.1111/gcb.12371>

- Pajala G, Sawakuchi HO, Rudberg D, Schenk J, Sieczko A, Gålfalk M, Seekell D, Sundgren I, Thanh Duc N, Karlsson J, Bastviken D (2023) The effects of water column dissolved oxygen concentrations on Lake Methane Emissions—Results from a whole-lake oxygenation experiment. *J Geophys Res: Biogeosci*. <https://doi.org/10.1029/2022JG007185>
- Peter S, Sobek S (2018) High variability in iron-bound organic carbon among five boreal lake sediments. *Biogeochemistry* 139(1):19–29. <https://doi.org/10.1007/s10533-018-0456-8>
- Peter S, Agstam O, Sobek S (2017) Widespread release of dissolved organic carbon from anoxic boreal lake sediments. *Inland Waters* 7(2):151–163. <https://doi.org/10.1080/20442041.2017.1300226>
- Pilla RM, Williamson CE, Zhang J, Smyth RL, Lenters JD, Brentrup JA, Knoll LB, Fisher TJ (2018) Browning-related decreases in water transparency lead to long-term increases in surface water temperature and thermal stratification in two small lakes. *J Geophys Res Biogeosci* 123(5):1651–1665. <https://doi.org/10.1029/2017JG004321>
- Puts IC, Ask J, Siewert MB, Sponseller RA, Hessen DO, Bergström AK (2022) Landscape determinants of pelagic and benthic primary production in northern lakes. *Glob Change Biol* 28(23):7063–7077. <https://doi.org/10.1111/gcb.16409>
- Puts IC, Ask J, Deininger A, Jonsson A, Karlsson J, Bergström AK (2023) Browning affects pelagic productivity in northern lakes by surface water warming and carbon fertilization. *Glob Change Biol* 29(2):375–390. <https://doi.org/10.1111/gcb.16469>
- R Core Team (2025) R: A language and environment for statistical computing. R foundation for statistical computing. <https://www.r-project.org/>
- Rantakari M, Kortelainen P (2008) Controls of organic and inorganic carbon in randomly selected boreal lakes in varied catchments. *Biogeochemistry* 91(2–3):151–162. <https://doi.org/10.1007/s10533-008-9266-8>
- Riccardi N, Mangoni M (1996) Chemical consequences of oxygenation in a shallow eutrophic lake studied with mesocosms. *J Aquat Ecosyst Health*. <https://doi.org/10.1007/BF00691730>
- Romillac N (2019) Ammonification. In *Encyclopedia of Ecology: Volume 1–4, Second Edition* (Vol. 2, pp. 256–263). Elsevier. <https://doi.org/10.1016/B978-0-12-409548-9.10889-9>
- Roth J et al (2023) What's trending in difference-in-differences? A synthesis of the recent econometrics literature. *J Econom* 235(2):2218–2244
- Rulli MPD, Bergström AK, Sponseller RA, Berggren M (2022) Seasonal patterns in nutrient bioavailability in boreal headwater streams. *Limnol Oceanogr* 67(5):1169–1183. <https://doi.org/10.1002/lno.12064>
- Schindler DW, Schmidt RV, Reid RA (1972) Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. *J Fish Res Board Can* 29(11):1627–1631. <https://doi.org/10.1139/f72-250>
- Shade A, Jones SE, McMahon KD (2008) The influence of habitat heterogeneity on freshwater bacterial community composition and dynamics. *Environ Microbiol* 10(4):1057–1067. <https://doi.org/10.1111/j.1462-2920.2007.01527.x>
- Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using tritiated leucine. *Mar Microb Food Webs* 6:107–114
- Staehr PA, Baastrup-Spohr L, Sand-Jensen K, Stedmon C (2012) Lake metabolism scales with lake morphometry and catchment conditions. *Aquat Sci* 74(1):155–169. <https://doi.org/10.1007/s00027-011-0207-6>
- Strock KE, Theodore N, Gawley WG, Ellsworth AC, Saros JE (2017) Increasing dissolved organic carbon concentrations in northern boreal lakes: implications for lake water transparency and thermal structure. *J Geophys Res Biogeosci* 122(5):1022–1035. <https://doi.org/10.1002/2017JG003767>
- Toming K, Kotta J, Uuemaa E, Sobek S, Kutser T, Tranvik LJ (2020) Predicting lake dissolved organic carbon at a global scale. *Sci Rep*. <https://doi.org/10.1038/s41598-020-65010-3>
- Tonin J et al (2022) Dissolved organic carbon affects the occurrence of deep chlorophyll peaks and zooplankton resource use and biomass. *Freshw Biol* 67(8):1357–1369
- Underwood AJ (1994) On beyond BACI: sampling designs that might reliably detect environmental disturbances on beyond baci: sampling designs that might reliably detect environmental disturbances. *Ecological Applications* 4(1)
- Von Wachenfeldt E, Tranvik LJ (2008) Sedimentation in boreal lakes - the role of flocculation of allochthonous dissolved organic matter in the water column. *Ecosystems* 11(5):803–814. <https://doi.org/10.1007/s10021-008-9162-z>
- Wauchope HS et al (2021) Evaluating impact using time-series data. *Trends Ecol Evol* 36(3):196–205
- Woolway RI, Merchant CJ (2019) Worldwide alteration of lake mixing regimes in response to climate change. *Nat Geosci* 12(4):271–276. <https://doi.org/10.1038/s41561-019-0322-x>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.