Water Air Soil Pollut (2026) 237:385
https://doi.org/10.1007/s11270-025-08991-z

Impacts of an Oxygen Slow-Releasing Calcium Peroxide Formulation on Oxygen Availability and Microbiota in Sediments of a Shallow Eutrophic Lake

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Received: 6 June 2025 / Accepted: 6 December 2025 / Published online: 13 January 2026

Abstract Small lakes and ponds are often of critical ecological and economic importance. At the same time, they are increasingly impacted by anthropogenic activities, leading to eutrophic or hypertrophic conditions. These are typically accompanied by increasing primary production, nutrient, and detritus accumulation. Owners and stakeholders are often challenged to decide on feasible and appropriate measures for lake restoration. Addressing one of these measures, this study explores passive sediment aeration through oxygen slow-release from a calcium peroxide (CaO_2) formulation amended at the sediment surface. Compared to other peroxides, CaO_2 has a slow dissociation rate, which may enhance organic matter degradation and microbial activities over months. This study investigates the application of a CaO_2-based lake restoration agent (CLRA) by a fine-scale dissection of its application to an eutrophic lake sediment. Using laboratory sediment columns, a dose–response approach, fine-scale microsensor analytics, and 16S rRNA gene amplicon sequencing, we provide evidence for an increased oxygen availability within sediments while other biogeochemical parameters remained unaffected. Significant impacts on sediment height were not detected, and microbial diversity remained stable across depths and CLRA dosages. However, characteristic shifts in microbial communities in upper sediments, specifically within aerobic heterotrophs and carbohydrate-degrading taxa within the *Gammaproteobacteria, Bacterioidota* and others were clearly detected. The findings provide first detailed insights into the sedimentary and microbiological impacts of a CaO_2 formulation applied in lake restoration. Better understanding the mechanisms and ecosystem impacts of CLRA applications will be vital for the implementation of effective and ecologically sound lake restoration strategies.

Keywords Ecological lake restoration · Sedimentary microbial communities · Microsensor analytics · Sediment aeriation · Elevated dissolved oxygen (eDO)

Thomas Kaupper and Felix Pfaff contributed equally.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11270-025-08991-z.

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1 Introduction

Small lakes and ponds often play a crucial role in local economic and ecologic context. The ecosystem services provided by small lakes include cultural services, e.g., for tourism and recreational activities, but chiefly involve provisioning, regulatory and buffering capacities (e.g., water retention, habitats, biodiversity or microclimatic regulation; Jakubiak & Chmielowski, 2020). While larger lakes (> 50 ha) are actively monitored under the European Water Framework Directive (Directive 2000/60/EC of the European Parliament, Council of the European Union (2000); Ruecker et al., 2015; Ruecker et al., 2019), the qualitative status of smaller waterbodies is often disregarded. Despite their small size and not always being included in routine monitoring efforts, small lakes actually make up for the largest part of global lake surface area, and are of crucial importance for global freshwater ecosystem services (Downing, 2010). Recently, interest in research addressing small water bodies and wetlands has increased markedly (e.g., Hill et al., 2021; Meerhoff and González-Sagrario, 2021; 2019 and Shen et al., 2025). Small waterbodies (here defined as ponds, small lakes, small streams, ditches or springs) increase the heterogeneity of landscapes and of microclimatic zones at regional scales; thus providing important environmental niches for aquatic and terrestrial flora and fauna (Biggs et al., 2017; E.P.C.N., 2008 and references therein). Moreover, climate, as well as local water and nutrient cycles are closely linked to small waterbodies (Downing, 2010; Nixdorf et al., 2016).

Albeit being highly important for local biodiversity and socio-ecology, small waterbodies are strongly impacted by land use and other direct anthropogenic influences. In combination with effects of global warming and climate change, small and shallow waterbodies are increasingly threatened by eutrophication (Zhou et al., 2022). Nutrient-rich runoff can stimulate cyanobacterial blooms, boosting biomass growth and organic detritus accumulation. In turn, microbial degradation of detritus can induce seasonal oxygen depletion and release of nutrients (i.e., P, N and trace metals), spurring further eutrophication cycles (Søndergaard et al., 2003, 2007; Yao et al., 2022).

Cyanobacterial blooms can release bioactive compounds that are toxic to many invertebrates, animals, and even humans (Metcalf & Codd, 2012), which can lead to the closure of a lake or pond for leisure activities or water usage. Lakes with marked anthropogenic eutrophication, high nutrient loading and severe algal blooms will develop an altered sediment biogeochemistry as well as microbial (Han et al., 2020; Yao et al., 2022) and zoobenthic communities (Heip, 1995). Algal blooms and consequent oxygen depletion in deeper waters and sediment can also boost greenhouse gas emissions (Beaulieu et al., 2019; Casper et al., 2000).

To address these challenges, lake restoration measures are increasingly sought and implemented by local stakeholders. Lake restoration involves both catchment-wide measures, primarily reducing external nutrient inputs, and lake-internal measures that act directly within the system. While catchment management is often considered of primary importance for sustainable success, internal measures are often more readily applied, especially for smaller lakes, where larger catchment-wide measures may be much more difficult to implement. Common internal measures include active aeration, nutrient binding or removal, or sediment dredging, which removes the accumulated nutrient rich and often highly organic sediments. Especially the latter can be connected to high costs. Thus, several alternative and less labour-intensive lake restoration strategies have been introduced, e.g. via the application or manipulation of limnic (micro-)organisms (Shan et al., 2009), the selective elimination of nutrients from the water phase (e.g., Meis et al., 2012; Zamparas & Zacharias, 2014), or the increased aeriation and provisioning of oxygen to limit P-release from anoxic sediments (e.g., Waite et al., 1999; Zhang et al., 2020). In addition, an active sparging with H_2O_2 or other aqueous ROS agents is usually intended to have a direct biocidal effect on algal blooms (i.e. *Cyanobacteria*; Lusty & Gobler, 2020; Matthijs et al., 2012).

Also a passive aeration via oxygen slow-releasing peroxides can increase concentrations of O_2 and reactive oxygen species (ROS) at the sediment/water interphase (Ma et al., 2007). While O_2 is assumed to directly stimulate microbial organic matter degradation, ROS may chemically oxidize organic molecules that may otherwise be hard to degrade for microbes (e.g., Gholami et al., 2019; Kim et al., 2021; Watts & Teel, 2005). Compared to other metal peroxides, CaO_2 dissociation is slow, releasing oxygen over a course of approx. 8 weeks (Ulrich et al., 2020). The overall reaction is:

$$CaO_2 + H_2O \rightarrow Ca(OH)_2 + \frac{1}{2}O_2 \quad (1)$$

Some commercially available CaO_2-based lake restoration agents (CLRA) also contain buffers, P-binding agents, or even lyophilized bacteria, that are thought to further increase organic matter degradation. Even though CLRA formulations have been employed as chemical oxidants and microbial stimulants in organic matter and pollutant degradation over decades (e.g., Arienzo, 2000; Cassidy & Irvine, 1999; Northup & Cassidy, 2008; Nykänen et al., 2012; Wang et al., 2019b), their activation mechanisms and impacts on a complex lake sediment microbiota are still not understood. In this manuscript, we use microcosm incubations treated with different dosages of CLRA to investigate effects on biogeochemical parameters and prokaryotic community composition. We hypothesize that CaO_2 (I) increases the oxygen availability in the upper zone of the treated sediment and (II) stimulates the activity and proliferation of a more aerobic, typically heterotrophic prokaryotic community. Our work contributes to a better understanding and a sustainable application of CLRA in the management and restoration of small eutrophicated waterbodies.

2 Materials and Methods

2.1 Sediment Acquisition and Site Description

Sediment was sampled at Lake "Melangsee" in the Scharmützelsee region in Eastern Brandenburg, Germany (52°9′42.28 "N, 13°59′20.14 "E). The lake is surrounded by spruce forests and situated between various settlements. The Melangsee surface area is 11 ha, with a mean depth of 1.6 m and a maximum depth of up to 3 m. The lake has been hypertrophic and polymictic for over 15 years, with frequently occurring algal blooms. Further lake characteristics are described elsewhere (Nixdorf et al., 2003; Ruecker et al., 1997). Profundal sediment was acquired using a corer (USC 06000, UWITEC, Mondsee, Austria) at the lake's center. At sampling, the air temperature was 12 °C, and the surface water temperature was 10 °C. Lake sediment was visually dominated by algal detritus and autochthonous organic matter (blackish color) with a gravimetric water content of up to 90%. The sediment was transported to the lab and stored at 15 °C in the dark for 5 days until microcosm set-up. Gravimetric water content was determined after drying the sediment at 60 °C for 1 week.

2.2 Microcosm Set-Up and Sampling

Lake sediment was sieved via three subsequent fractions (stainless steel mesh aperture with 2, 1, and 0.64 mm; Haver Boecker, Oelde, Germany) to remove large plant material or mineral particles. Afterward, the sediment was homogenized by manual stirring. Sterilized glass cylinder columns (inner Ø: 35 mm; height: 125 mm) were sealed at the bottom using parafilm. Approx. 60 g of homogenized sediment was used per column. Columns were submerged in a freshwater aquarium (~ 50 L total volume) filled with non-sterile tap water, and incubated as done before (Sachs et al., 2022). The water was aerated continuously using diffusers and an aquatic pump (Tetra APS 400, Tetra, Melle, Germany). Columns were incubated at room temperature (18–23 ºC) on the benchtop with natural day-night cycles. During incubation, columns were randomly redistributed in the aquarium to minimize spatial patterns and increase homogeneous exposure. Aeriation efficiency at sediment–water interphases within glass columns was routinely checked via microsensors.

After a settling period of 7 days, 2 columns were sacrificed and sampled (see below), as T_0 starting conditions. Afterward, a commercial CLRA (SchlixX®, Soell GmbH, Hof, Germany; ≈ 20% CaO_2; grain size: 100% < 2000 µm and 90% < 500 µm; density: 1.1–1.3 kg/dm^3; pH of a 1% aqueous solution: 8.2; the remaining 80% of the CLRA consisting of pH-buffering insoluble and soluble salts) was added onto the sediment surfaces. To investigate dosage effects, columns were incubated with either 100 g/m^2, 200 g/m^2, or 400 g/m^2 CLRA (n = 4 for each treatment). Additional controls without addition of CLRA were also exposed (n = 4). During incubation, evaporated water was regularly re-filled with fresh tap water to maintain a consistent water overlay. After 63 and 77 days, columns were sacrificed and sampled. Before sampling, the glass cylinders were removed from the aquarium, and microsensor measurements were conducted as detailed below. Subsequently, sediment cores (n = 2 per treatment) were sliced into subsections (0–0.5 cm; 0.5–1 cm; 1–2 cm; 2—3 cm; 3—4 cm) as described in Sachs et al. (2022). Briefly, cores were pushed out of the cylinders from the

bottom using a plunger of a syringe that perfectly sealed the glass cylinder. After carefully moving the sediment to the top of the cylinder to not disturb sediment layers, fractions of given depth intervals were pushed out of the cylinder and transferred into individual sterile petri dishes. Afterwards, each fraction was homogenized and stored at −20 ºC for further analysis.

2.3 Oxygen, Redox, and pH Depth Profiles

Column depth profiles were measured using redox, pH, and O_2 microsensors (500 µm tip size; Redox: RD500C, pH: PH500C, and O_2: OX500; UniSense, Aarhus, Denmark) as described before (Sachs et al., 2022). Depth profiling was performed using an automated Micro-Profiling System (UniSense, Aarhus, Denmark) with a motorized z-axis. For data acquisition, the manufacturers' software (SensorTrace Profiling; UniSense, Aarhus, Denmark) was used. Measurements were started in water at 5 mm above the sediment surface. After equilibration, the depth profile was analysed (step size 200 µm, depth 50.000 µm, singular measurement, 3 s. of equilibrations before data acquisition, sensor progression rate: 50 µm/s). For replication of profiles, sediment columns were rotated by approx. 30 degrees and measurements were repeated. For an adequate comparison of oxygen profiles, despite slightly variable sediment surface depths, the slopes of decreasing oxygen profiles were inferred. Between highest and lowest O_2 concentrations, linear regression models ($R^2 > 0.99$) were inferred for oxygen decline over at least $n > 22$ incremental data points, thus spanning a depth interval of > 4.4 mm. For these, the change in oxygen concentrations over depth was compared between columns and treatments. The slope was used as a proxy for oxygen penetration into and availability within sediments, since absolute concentrations and profiles were difficult to compare for identical depths between columns.

2.4 Determination of Sediment Physico-Chemical Parameters

For total phosphorus (P_{tot}) and carbon to nitrogen (C:N) ratio determination, sediment was dried at 60 °C for 7 days and ground using a mortar. The C:N ratio was determined using an element analyzer (Thermo Quest, Flash EA, 1112; Thermo Fisher Scientific, Waltham, USA). For total phosphorus (P_{tot}) quantification a complete HF-ashing was conducted according to Schramel et al. (1987), using a mixture of HNO_3/HF. Subsequently, sediment extracts were analysed in an elemental analyser (Agilent 5800 ICP-OES; Agilent Technologies, Santa Clara, USA). Depth profiles of soluble ammonium, nitrate and nitrite (NO_x), and sulfate concentrations were generated from sediment fractions extracted in deionised H_2O (1:2 w/v) after filtration (0.22 µm) using standard colorimetric assays (NH_4^+ and NO_x^- according to Gadkari (1984); SO_4^{2-} after Wolfson (1980)). All colorimetric assays were conducted using a BioTEK µQuant plate reader (BioTek Instruments Inc., Winooski, VT, USA) in technical duplicates.

2.5 Sediment DNA Extraction

DNA was extracted based on a modified protocol after Lueders et al. (2004). For each extraction, approx. 200 mg fresh weight of sediment was used. In detail, sediment was mixed with sterile Zirconium/Silica beads (0.1 and 0.7 mm, respectively), PCI (Phenole:Chlorophorm:Isoamylalcohol 25:24:1; Carl Roth GmbH + Co.KG, Karlsruhe, Germany) and extraction buffer (10% w/v Sodium-Dodecyl-Sulphate in 1 × phosphate buffer) before bead beating (TissueLyzer II; Qiagen, Venlo, Netherlands) at 30 Hz for 1 min. The aqueous supernatant was re-extracted with PCI using Phase lock tubes (5PRIME Phase Lock Gel—Heavy; Quantabio LCC, Beverly, USA). Aqueous supernatant was washed with CI (Chlorophorm:Isoamylalcohol 24:1; Carl Roth GmbH + Co.KG, Karlsruhe, Germany). DNA was precipitated on ice for 2 h after the addition of two parts PEG buffer (30% w/w Poly-Ethylene-Glycole-6000, Carl Roth GmbH + Co.KG, Karlsruhe, Germany; in 1.6 M NaCl). The pellet was washed in 80% Ethanol. The DNA was solubilized in elution buffer (Qiagen, Venlo, Netherlands). Subsequent quantification was done using a spectrophotometer (NanoDrop One; Thermo Fisher Scientific, Waltham, USA), then the DNA was stored at −20 °C. DNA yield was 3.3 ± 1.9 µg g_{dw}^{-1} and DNA was of good quality ($A_{260nm}/A_{230nm} > 1.86 \pm 0.12$; $A_{260nm}/A_{280nm} > 1.73 \pm 0.16$).

2.6 Amplicon Preparation for Illumina Sequencing

To identify enriched members of the microbial community, 16S rRNA genes were amplified via PCR and sequenced via Illumina amplicon sequencing, as done before in Rauscher et al., 2023. In a first PCR, the 16S rRNA gene was targeted using primers Ba-515f und Ba-806R (Parada et al., 2016 and Apprill et al., 2015, respectively) tagged with Illumina adapters. Here, each PCR reaction (total volume: 30 µL), comprised of 15 µL NEBNext Ultra II Q5 Master Mix (New England BioLabs, Ipswich, USA), 0.18 µL of each primer (50 µM; Biomers.net GmbH, Ulm, Germany), 0.3 µL bovine serum albumin (20 ng/µL; Roche GmbH, Basel, Switzerland), 12.84 µL nuclease-free water, and 1.5 µL of DNA template. The thermal profile of the PCR consisted of an initial denaturation step at 94 °C for 5 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and elongation at 72 °C for 60 s. PCR reactions were performed in technical duplicates. Amplification specificity was verified via agarose gel electrophoresis. For downstream analysis, duplicate reactions were pooled before clean-up.

PCR clean-up was performed using the NucleoMag DNA/RNA purification protocol (Macherey–Nagel, Düren, Germany) on a magnetic particle separator (KingFisher; Thermo Fisher Scientific, Waltham, USA). The PCR product length distribution was verified on a fragment analyzer (5200 Fragment Analyzer System; Agilent, Santa Clara, USA), and DNA was quantified using Qubit dsDNA HS assay according to the manufacturer's protocol (Thermo Fisher Scientific, Waltham, USA) for subsequent indexing. For indexing, the Nextera XT index kit v2 (Set A and B; Illumina, San Diego, USA) and KAPA HiFi HotStart ready mix (Roche GmbH, Basel, Switzerland) were used according to the manufacturer's protocol for 8 cycles. Again, fragment length composition was investigated using a fragment analyzer. Indexed samples were pooled at 4 nM and again cleaned using magnetic beads. Additionally, the pooled library was size selected for 200 to 400 bp long fragments by gel-electrophoresis (Pippin Prep; Sage Science, Newcastle, UK). Subsequently, the library was loaded on the flow cell at a 80 pM dilution with 10% PhiX as a control library and sequenced on an Illumina iSeq100 sequencer using iSeq 100 i1 Reagen v2 sequencing chemistry (Illumina, San Diego, USA) and 300 cycles. Only forward reads with a length of approximately 301 bp were generated. Afterward, indexing primers were trimmed off and sequences were demultiplexed. Raw sequence files are deposited in the NCBI Sequence Read Archive under Project number PRJNA1227283.

2.7 16S rRNA Gene-Targeted Amplicon Sequencing Analysis

16S rRNA gene sequences were processed in R (R version 4.2.2; R Core Team, 2021). After primer removal via cutadapt (Martin, 2011), quality control, demultiplexing, chimera removal and taxonomy assignment were done with 'DADA2' package (Callahan et al., 2016). After low quality reads were removed and error rate was learned, the dada command was run in self-consist mode to reach convergence between sample inference and error rate estimation applying the error model to all samples ('pooled mode'); then, chimera removal was run in consensus mode. The classification was performed at ASV level against the SILVA SSU database version 138 (Quast et al., 2013). After singleton and doubleton removal, ASVs classified as mitochondrial or chloroplasts and ASVs unclassified at the phylum level were removed (approx. 9.1% of total sequences). Approx. 1700000 sequence reads with 18391 reads (±8237) per sample were obtained over 90 samples in total. Then, samples were rarefied to 7833 sequences per sample, according to the sample with the lowest read count (Schloss, 2024). Sequencing data was analyzed in R using 'phyloseq' (Version: 1.42.0), 'vegan' (Version: 2.6–4), 'ggplot2', and 'DESeq2' (Version: 1.38.3) packages. Ordination of samples by non-linear multidimensional scaling (NMDS) was calculated from Bray–Curtis distance from square root transformed relative abundance data ('Hellinger transformation'). The applicability of PERMANOVA (Anderson, 2001) with Bray–Curtis distance was tested by analyzing beta dispersion of square root transformed relative abundance data. Then, PERMANOVA was performed, comparing the control to each treatment individually for each depth fraction. Furthermore, DESeq2 analysis was performed on differentially abundant taxa in top layer (0—0.5 cm). For the DESeq2 analysis, non-rarefied data was filtered for paired comparison of individual treatments to the control; then, taxa were agglomerated at genus level.

Additionally, a filtration for agglomerated genera that had more than 10 reads summarized across analysed samples was performed. Results only include significant log2-fold changes ($p < 0.05$) comparing respective treatments to the control.

2.8 Statistical Analysis

Statistical analysis was performed in the PAST4 software (Hammer et al., 2001). Data from abiotic parameters were tested for normality using Shapiro–Wilk test. If data was not normally distributed, the data was log-transformed. Equality of variance was tested via Levene's test. Normally distributed data with equal variances was tested via ANOVA with Tukey post-hoc test for differences between treatments and the control incubation and over depth. Where criteria for ANOVA were not met, a Kruskal–Wallis-ANOVA with a pairwise Wilcoxon post hoc test was performed. To generate a bigger sample size for statistical analysis and a more rigorous comparison of treatments, samples from day 63 and 77 were combined as one data set (T_{end}). As at both days biogeochemical profiles were not distinguishable, we assume a steady state (in microbial communities and geochemical parameters) had been reached by then.

3 Results

3.1 Changes in Biogeochemical Parameters Due to CLRA Application

Microsensors for oxygen saturation, redox potential, and pH were used to track CaO_2-induced depth-dependent biogeochemical changes in sediment columns. As microsensor measurements are not easy to start at exactly the same depth over sediment between columns, we refrained from using absolute data, but compared slopes of oxygen decline. The CLRA application significantly changed the slopes of oxygen concentration ($p < 0.001$; Fig. 1) towards a shallower course. Consequently, an increase in O_2 penetration depth was revealed. The absolute values of the microsensor measurements (Fig. 1B and S1) suggested an increase of oxygen penetration depth by approx. 2—5 mm.

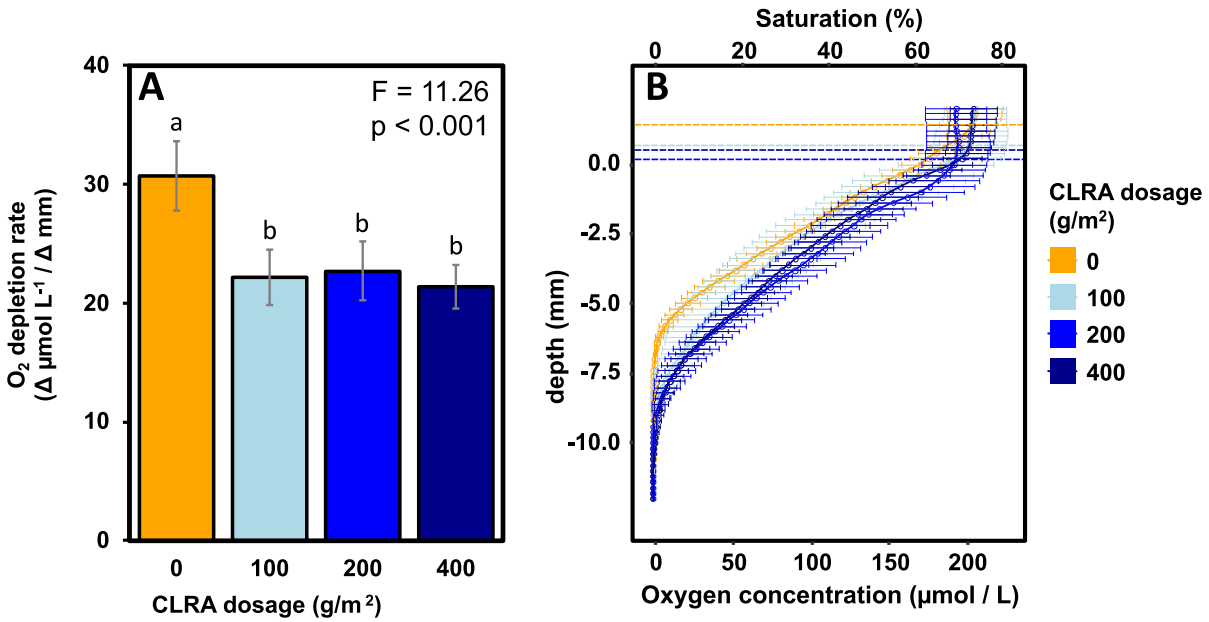


Fig. 1 Change in oxygen concentration over depth inside the sediment columns comparing the different CLRA dosages (orange: 0 g/m^2; light-blue: 100 g/m^2; blue: 200 g/m^2 and dark blue: 400 g/m^2). Slope of oxygen concentration change (panel **A**) was calculated from linear oxygen decrease in the sediment ($n > 22$ consecutive data points; $R^2 > 0.99$) over depth (panel **B**). A detailed description on the calculation is given in the Materials and Methods section (Section 2.3). Lowercase letters indicate significant differences ($p < 0.001$). Error bars depict the standard deviation. Panel B: Horizontal dashed lines indicate sediment surface

The redox potential remained high throughout the upper ~ 20 mm in depth, dropping to ~ 0 mV below (Fig S1) in all treatments independent of the CaO_2 dosage. pH profiles over depth started at pH 8.9 (± 0.1) in the control and 8.9 (± 0.18) in the highest treatment in water, dropping to pH ~ 7.9 in all incubations in the sediment below (Fig S1). The depth profiles were comparable, independent of CLRA dosage, and profound impacts on sediment or water pH were not observed.

Besides microsensor measurements, the concentration of solutes (here: NH_4^+, NO_x^-, and SO_4^{2-}) was also analysed over dept, to identify potential changes in redox cycling dependent on the CaO_2 amendment. NH_4^+ concentrations in the columns increased from the upper layers (approx. at 1 cm; Tab S1 and Fig S2A) towards deeper zones (2–6 cm). Compared to the control, CLRA application did not significantly alter NH_4^+ concentrations in all fractions in the sediment columns; nevertheless, NH_4^+ levels at the sediment surface (0–0.5 cm) seemed to be lower in the treatments compared to the control. Contrary to increasing NH_4^+ concentrations inside the columns, nitrate concentrations decreased with depth (Fig S2B). Again, significant differences were not identified between treatments and controls, same as also observed for sulfate (Tab S1 and Fig S2C).

The total carbon and nitrogen content of sediments was analysed to identify potentially increased mineralization under elevated dissolved oxygen. Even though not significant, a trend towards a decrease in total C was detected in the top sediments (0–0.5 cm), comparing the 200 and 400 g/m^2 CLRA treatments to the control (Fig. 2 and Table S2). Here, the total C content decreased to 16.3% (± 1.2%) and 17.0% (± 0.7%) compared to 18.0% (± 1.3%), respectively. A decrease in total N was also detected. Here, the total N decreased from 1.4% (± 0.2%) in the control incubation to 1.07% (± 0.2%) and 1.2% (± 0.2%) in the two highest CLRA dosages. Again, these decreases were not significant. The lower sediment layers (0.5 cm to 6 cm) remained unchanged; thus, total C and total N

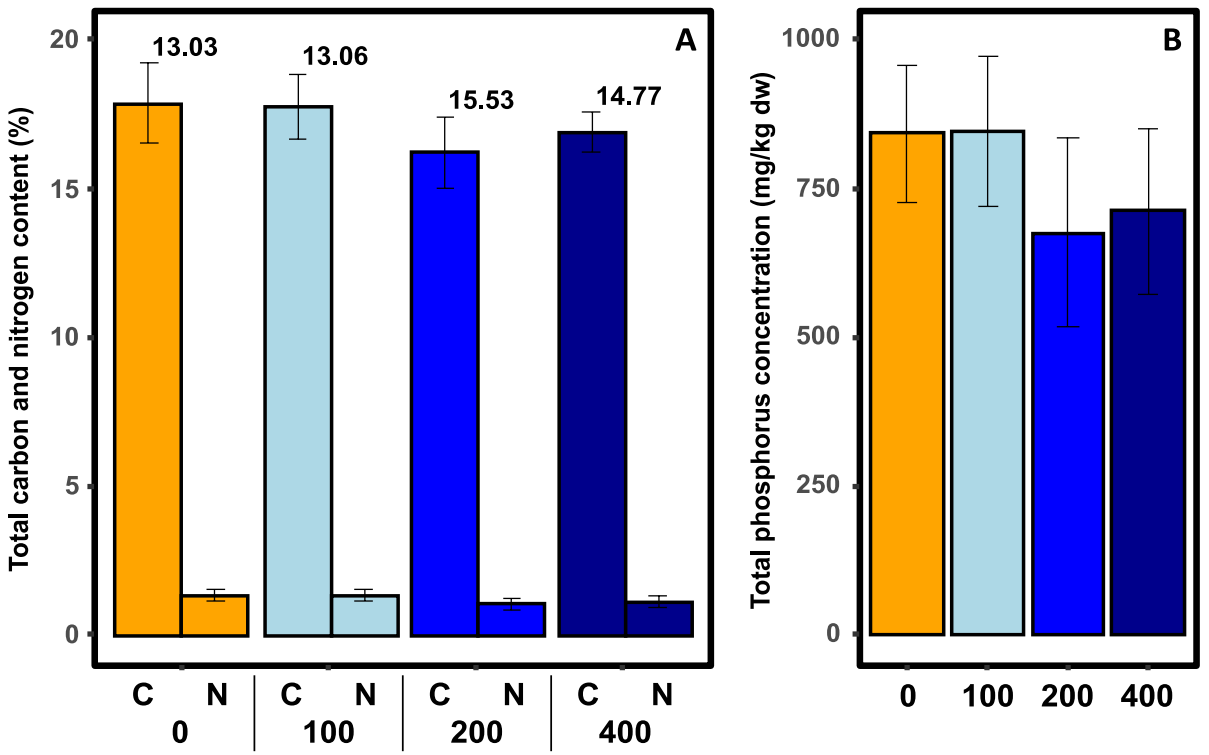


Fig. 2 Change in total carbon and nitrogen **A** and total phosphorus **B** concentration in the sediment treated with the different CLRA dosage (orange: 0 g/m^2; light blue: 100 g/m^2; blue: 200 g/m^2 and dark blue: 400 g/m^2). The values above the bars give the C:N ratio in the sediment. **A** Corresponding data is given in Table S2. Error bars depict the standard deviation

Table 1 Pairwise comparison of the beta diversity (see Fig. 3) comparing the different CLRA treatments to the control incubation over depth via PERMANOVA analysis. Asterisks indicate significant differences ($p < 0.05$)

	Depth (cmbsf)				
	0–0.5	0.5–1	1–2	2–3	3–6
0 vs. 100 g/m^2	F = 0.972 p = 0.64	F = 0.876 p = 1	F = 1.007 p = 0.436	F = 0.978 p = 0.732	F = 1.027 p = 0.228
0 vs. 200 g/m^2	F = 1.164 p = 0.061	F = 0.9771 p = 0.435	F = 1.031 p = 0.19	F = 1.015 p = 0.34	F = 0.961 p = 0.893
0 vs. 400 g/m^2	F = 1.537 p = 0.026 *	F = 1.020 p = 0.267	F = 1.021 p = 0.314	F = 1.048 p = 0.185	F = 1.011 p = 0.386

remained similar. Together with nitrogen, phosphorus is the main driver of eutrophication. In the top sediment layer (0–0.5 cm), total P concentration tended to decrease at higher CLRA dosage, but did again not change significantly (Fig. 2B and Tab S2).

3.2 CLRA-Induced Changes in the Microbial Community Composition

The effects of different doses of CLRA on the prokaryotic community were assessed by sequencing of prokaryotic 16S rRNA genes. Alpha diversity measures indicated a decrease in community diversity in top sediment layers (0–0.5 cm) during all incubations, independent of the CaO_2 treatment (Fig S3). Shannon and Simpson diversity indices were lower compared to the deeper sediment layers. As CLRA was applied in different dosages, dose-dependent changes in sediment microbiota were queried. In fact, significant shifts in community composition were detected only for the upper sediment layers (Fig. 3A; 0–0.5 cm; $p = 0.01$). However, PERMANOVA revealed no significant community changes when comparing the lowest dose of CLRA to controls ($p > 0.05$). However, significant changes were observed with increasing dosage ($p = 0.061$ at 200 g/m^2 and $p = 0.026$ at 400 g/m^2) compared to the controls. For deeper sediment samples within the columns, significant impacts of CLRA amendment were not detected (Fig. 3B and C; $p > 0.05$). The NMDS plot comparing all depths within columns showed a high similarity of communities below ~ 1 cm depth (Fig. 3D).

When comparing the 200 g/m^2 CLRA treatment to the control incubations, only minor changes in differentially abundant taxa were detected (p = 0.061). Only five genus-level taxa were found to significantly change in abundance ($p < 0.05$). Nevertheless, ASVs assigned to the *Sphingobacteriales* and *Pseudomonas spp.* increased by approx. sixfold and fourfold in the 200 g/m^2 treatment, respectively, while members of the *Nannocystaceae* family, the *Thermoanaerobaculaceae* family, and a *Methylobacter* spp. decreased approx. twofold compared to controls.

Much more pronounced changes were detected for the 400 g/m^2 CLRA treatment. Here, 40 taxa were identified to change significantly ($p < 0.05$) compared to the controls (Fig. 4, S4 and S5). Members of the phyla *Bacterioidota* (i.e., members of the family ST-12K33, Blvii28 wastewater sludge group, *Lacihabitans* and *Emticicia spp.*), *Pseudomonadota* (e.g., *Brachymonas, Pseudomonas*, and *Aeromonas spp.*) and *Campylobacteriota (*i.e., *Sulfurimonas sp.)* had a higher log2-fold change, with increased abundance compared to the controls. Opposingly, members of the phyla *Myxococcota (i.e., members of the Family Sandaracinaceae and Blrii41), Spirochaeta* (i.e., *Turneriella sp.*), *Actinobacteria* (i.e., CL500-29 marine group), and *Cyanobacteria* (members of the deep-branching non-photosynthetic class *Sericytochromatia*) had a negative log2-fold change, indicating a decrease in the treated samples. Overall, the taxa identified to be differentially abundant accounted for approx. 8% of the reads per sample (Fig S5B). Thus, while the remaining ~ 93% remained unaffected, changes observed for ~ 8% of the sediment microbiota were significant under CLRA amendment.

4 Discussion

4.1 CLRA Increased Oxygen Availability in Upper Sediments

In this study, we used a CaO_2 containing CLRA to test dose-dependent effects in biogeochemistry,

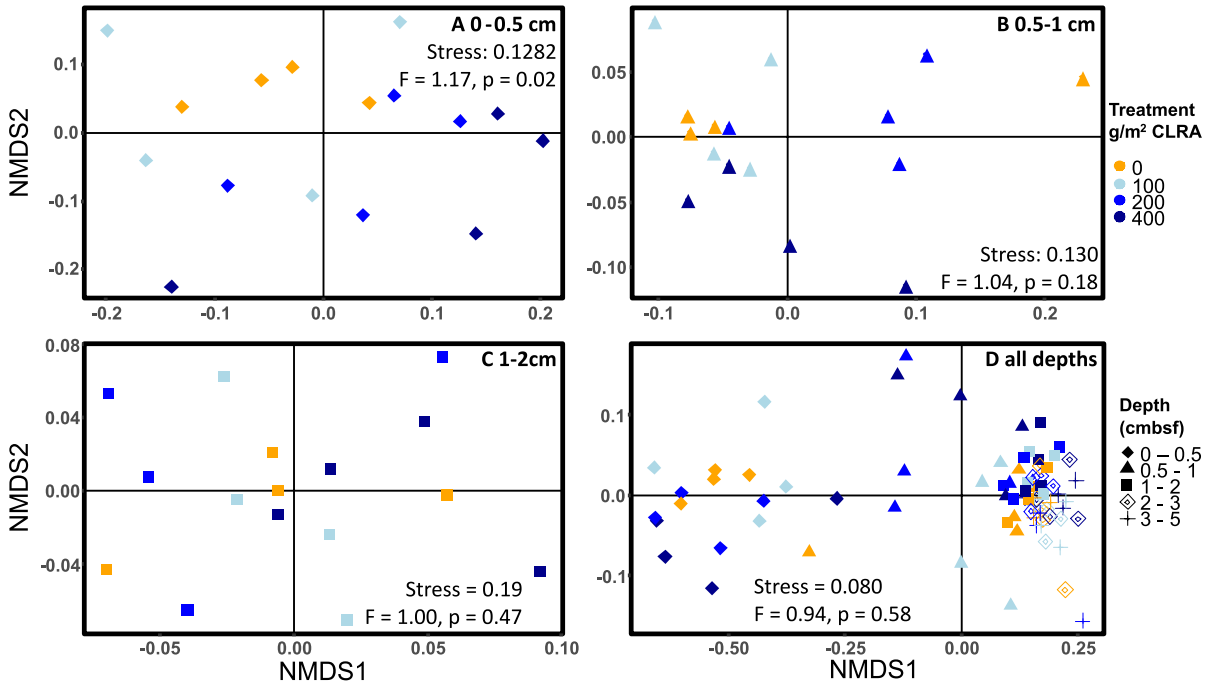
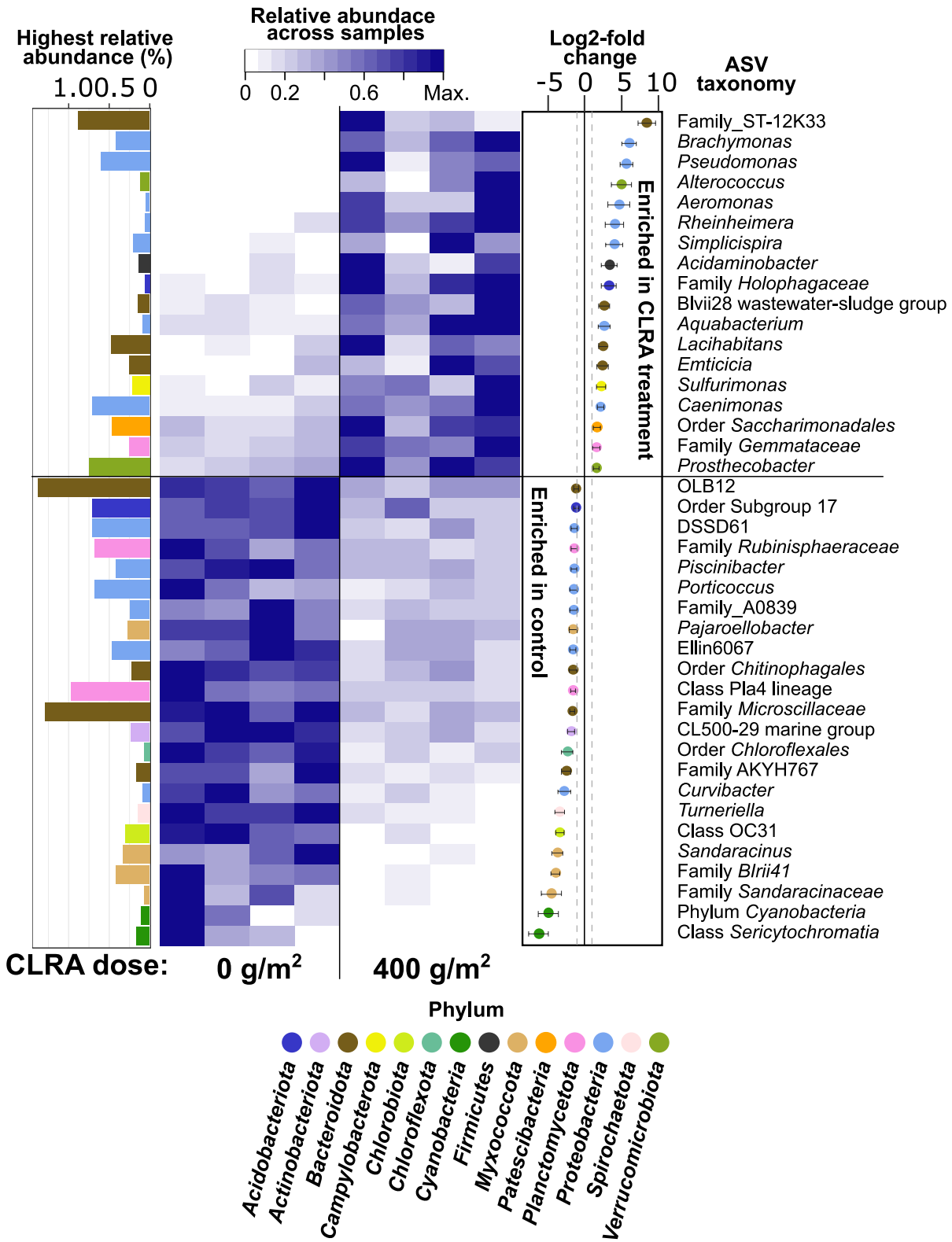


Fig. 3 Non-metric multidimensional scaling (NMDS) plot using Bray–Curtis dissimilarity distances comparing the prokaryotic community composition in the sediment over depth (at 0–0.5 cm, **A**; 0.5–1 cm, B; and 1–2 cm, **C**) and over all depths **D** treated with different dosages of CLRA (Orange: 0 g/m^2; light blue: 100 g/m^2; blue: 200 g/m^2 and dark blue: 400 g/m^2). Statistical differences in the community composition were tested using PERMANOVA and are given in the respective plot. Statistical analysis of pairwise comparison of the treatments to the control was performed to identify changes due to the treatment (see Table 1). Abbreviations: cmbsf: cm below surface

physico-chemical parameters and prokaryotic community composition of a eutrophicated lake sediment. CaO_2 was applied as oxygen slow-release agent, releasing ROS and molecular oxygen when in contact with water (Eq. 1 and Ma et al., 2007). Indeed, oxygen availability increased in our microsensor depth profiles, depicted by a lower slope and increased penetration depth of O_2 diffusion gradients (approx. 2–5 mm; see Fig S1). In a previous experiment with comparable settings (hypertrophic lake sediments, incubated in microcosms using "pure" CaO_2 of up to 75 g/m^2; equalling up to 400 g/m^2 CLRA) Nykänen et al. (2012) also observed an increase in oxygen concentration in top sediment layers after 8 weeks, paralleled by an increase in bacteria counted via colony forming units (CFUs). Consequently, one can postulate a proliferation of aerobic microorganisms in the treated sediments due to an increase of available oxygen (discussed below). Furthermore, an increase in oxygen concentrations in the upper mm of sediment can reduce the potential for reductive nutrient and metal release from sediments to an overlying anoxic water column (Bormans et al., 2016; Hindersmann & Mansfeldt, 2014). However, a definite answer to our first hypothesis is not straightforward, as a detected increase in oxygen penetration depth could have been either a result of increased oxygen release by the CaO_2, or of an inhibition of aerobic respiration in certain depths. Still, given the minor impacts of CLRA amendment on overall sediment microbiomes, and also an observed stimulation of selected typically aerobic and heterotrophic taxa, our results substantiate the first scenario.

An increase in oxygen availability in the first few mm of sediment may also cause other redox cycling reactions to shift deeper into the sediment. Unfortunately, the spatial resolution of our depth-resolved solute analyses (NH_4^+, NO_x^-, SO_4^{2-}) was not fine enough to detect potential effects below the ~cm-scale. Thus, overall trends in redox species appeared similar between treatments and control incubations, opposing substantial changes in redox chemistry


Highest relative abundance (%)
1.0 0.5 0
Relative abundace across samples
0 0.2 0.6 Max.
Log2-fold change
-5 0 5 10
ASV taxonomy
Enriched in CLRA treatment
Enriched in control
Family_ST-12K33
Brachymonas
Pseudomonas
Alterococcus
Aeromonas
Rheinheimera
Simplicispira
Acidaminobacter
Family Holophagaceae
Blvii28 wastewater-sludge group
Aquabacterium
Lacihabitans
Emticicia
Sulfurimonas
Caenimonas
Order Saccharimonadales
Family Gemmataceae
Prosthecobacter
OLB12
Order Subgroup 17
DSSD61
Family Rubinisphaeraceae
Piscinibacter
Porticoccus
Family_A0839
Pajaroellobacter
Ellin6067
Order Chitinophagales
Class Pla4 lineage
Family Microscillaceae
CL500-29 marine group
Order Chloroflexales
Family AKYH767
Curvibacter
Turneriella
Class OC31
Sandaracinus
Family Blrii41
Family Sandaracinaceae
Phylum Cyanobacteria
Class Sericytochromatia
CLRA dose:
0 g/m2
400 g/m2
Phylum
Acidobacteriota
Actinobacteriota
Bacteroidota
Campylobacterota
Chlorobiota
Chloroflexota
Cyanobacteria
Firmicutes
Myxococcota
Patescibacteria
Planctomycetota
Proteobacteria
Spirochaetota
Verrucomicrobiota

◂**Fig. 4** Distribution of significantly differentially abundant taxa ($p < 0.05$ and log2-fold change of > 0.75 or < -0.75 according to DeSeq2 analysis) across samples treated with the highest CLRA dosage (400 g/m.^{2}) and control samples. The bar chart on the left shows the highest relative abundance of an ASV in any sample. The heatmap shows the change in relative abundance across samples, relative to the maximum value shown on the left. The log2-fold change as calculated by DeSeq2 is indicated to the right of the heatmap, with vertical dashed lines indicating a log2-fold change of 1. Taxonomic categories are given at the Genus level or lowest taxonomic resolution possible. Colors of the bars indicate the attribution to a broader taxonomic category (phylum level). Full taxonomic community composition of all samples is shown in Fig. S4. A direct visualization of relative abundances of differentially abundant taxa is shown in Fig S5

in sediments after treatment. P concentrations also remained similar in sediment fractions, independent of the applied CLRA dosage. Unfortunately, our experimental setup prevented an analysis of P dissolved in overlying water per column. At any rate, a more oxidized sediment surface after CLRA application is expected to limit P release to the water, contrary to P released under reducing conditions or upon organic matter degradation.

As CaO_2 dissociation may release $Ca(OH)_2$ (Eq. 1; Ma et al., 2007), a slight increase in pH in upper sediment was expected. However, our microsensor measurements clearly showed that pH did not increase over the sediment columns. The overlying water was always somewhat more alkaline (pH 8.9) than the sediment (pH 7.8). Under CLRA amendment, the pH gradient in transition between both zones reached somewhat deeper into the sediment. This could potentially be explained via active bioturbation observed over our ~ 10-weeks of column incubation, where CLRA particles could have been translocated over several mm of sediment depth. In line with this, Wang et al. (2019a), using polluted urban waters and sediment, only observed minor pH changes even at higher CaO_2 doses (0.06 to 0.18 kg/m^2; corresponding to 300 to 900 g/m^2 CLRA). In contrast, Nykänen et al. (2012) reported an increased pH after adding 75 g/m^2 CaO_2 (equals 400 g/m^2 CLRA) to sediment from a eutrophic lake.

Significant differences in C or N content between sediment columns were not observed. A minor, dosage-dependent difference for uppermost sediment layers (0–0.5 cm) likely was attributed to sediment dilution by the CLRA agent. However, our analyses prevented a direct assessment of changes in organic carbon fractions. The direct reactivity of the CLRA is assumed to involve Fenton reactions. As CaO_2 is an oxygen slow-release agent, the release mechanism involves ROS intermediates (Ma et al., 2007). Fenton reactions refer to the catalytic oxidation of organic substrates via H_2O_2/ROS and iron salts under acidic pH (Northup & Cassidy, 2008; Watts & Teel, 2005). Still, as Fenton reactions are known to slow down under neutral or slightly alkaline pH (Northup & Cassidy, 2008), respective contributions to total C oxidation should have been minor in our columns. Thus, as the amount of O_2 released from CaO_2 increases with pH (Arienzo, 2000; Northup & Cassidy, 2008), this should again stimulate microbial oxidation rather than abiotic oxidation of organic carbon in sediments.

4.2 CLRA-Induced Decline in Prokaryotic Taxon Abundances

Clear changes in microbiomes were observed for upper sediments upon CLRA amendment, especially for the highest used dose. Potentially, mild oxidative stress could have been imposed on communities, as ROS and O_2 was slowly released from CaO_2. We posit that especially ROS-sensitive community members should have been affected, e.g., microaerophiles or strict anaerobes. Amongst lake phototrophs, *Cyanobacteria* have been reported to be more ROS-sensitive than eukaryotic algae. Matthijs et al. (2012) have shown that an *in situ* application of 2 mg/L H_2O_2 drastically reduced cyanobacterial abundance without major effects on eukaryotic phytoplankton. However, in our experiment, all cyanobacterial taxa remained unaffected by CLRA treatment, with one exeption: Members of the class *Sericytochromatia* significantly decrease in abundance. However, these are known as deep-branching, non-photosynthetic *Cyanobacteria* (Garcia-Pichel et al., 2020). Since chloroplast ASVs were excluded from data analysis after sequencing, possible effects on eukaryotic algae cannot be reported. Interestingly, a minor negative log2-fold change was also detected for two different genera of the *Bacteroidota* family *Microscillaceae* (−1.13 and −1.64 log2-fold change), which are tightly associated to *Cyanobacteria*, potentially as parasites (Chun et al., 2020; van Le et al., 2023).

Moreover, ASVs of the phylum *Myxococcota* (family *Sandaracinaceae* and the Blrii41 lineage)

were also negatively affected by the CLRA treatment at 400 g/m^2. Little is known about the function of the *Sandaracinaceae* in freshwater sediments. In general, members of this family are strictly aerobic degraders of complex organic carbon (Mohr et al., 2012). For this lineage, the observed depletion was thus in contrast to an intended stimulation by the increased O_2 availability in the CLRA treatments.

All of these community changes observed in the uppermost sediments were minor and the vast majority of sediment microbiota remained unaffected. Along with the very minor effects on sediment pH and presumably also ROS, this clearly excludes any profound negative or ecotoxicity impacts of the CLRA amendment on sediment microbiomes. According to the German Environment Agency (UBA), CaO_2 is classified as mildly hazardous to water upon improper handling, although generally accepted for drinking water and surface water treatment (German Environment Agency 2025). It's application as CLRA should thus not come with detrimental effects on treated sediment microbiomes, as we confirm in our study.

4.3 CLRA-Induced Shifts in Organic Matter Oxidizing Sediment Microbiota

Other microbial taxa were significantly enriched in abundance in the 400 g/m^2 CLRA treatment. Interestingly, a "exchange of taxa" was partially observed within the phyla *Bacteroidota* and *Pseudomonadota* (specifically *Gammaproteobacteria*) (Fig. S5), with some taxa within these phyla decreasing while others increase upon CLRA application. Since most of the affected taxa were typical heterotrophs, this change could indicate a change in organic matter composition and degradation (which e.g. many *Bacteroidota* typically specialize on). Another change following this pattern was the decrease of *Planctomycetota* and increase of *Verrucomicrobiota* taxa, members of both phyla typically associated with complex carbohydrate degradation (e.g. López-Mondéjar et al., 2022).

The highest increase in differential abundance was detected for an organism belonging to the order of *Sphingobacteriales* (uncultured family-level taxon ST-12K33, 8.5 log2-fold change in in highest CLRA treatment). This taxon was first associated with organic material breakdown in sewage sludge (Li et al., 2015; Liu et al., 2016). As many members of the *Sphingobacteriales* are typical heterotrophic aerobes, or facultative anaerobes, the observed enrichment of such lineages seems credible. Still, further metagenomic or cultivation-based analyses will be necessary for more rigorous functional assignment of such uncultivated taxa.

Also other aerobic taxa with diverse physiologies were clearly enriched in the 400 g/m^2 treatment, such as *Pseudomonas, Brachymonas*, *Caenimonas, Aqubacterium, or Aeromonas spp.* within the *Gammaproteobacteria (*e.g., Edwards et al., 2001; Staley et al., 1976; van Le et al., 2022; Zhu et al., 2019*).* All of these are typical aerobic, heterotrophic or mixotrophic bacteria typically found in freshwater environments. Additionally, a potential autotrophic *Sulfurimonas sp.* was also enriched. Members of the genus *Sulfurimonas* are known to have a multifaceted chemolithoautotrophic metabolism, including oxidation of different reduced sulfur compounds coupled to oxygen respiration or denitrification (Han & Perner, 2015).

In summary, a fair number of typically aerobic and mostly heterotrophic freshwater microorganisms, especially within the *Gammaproteobacteria*, appeared to be stimulated by the CLRA amendment. In addition, the exchange of specific typically carbohydrate-degrading taxa seemed to indicate a shift in organic carbon utilization during sediment incubation. Both lines of evidence corroborate the second hypothesis of this study, a community shift towards a more active sediment microbiota involved in the oxidation of organic matter, triggered by the CLRA.

4.4 Implications for CLRA Field Application

In our study, only the highest dosage of CLRA applied caused notable changes in sediment microbiota, while the biogeochemical change in O_2 availability was also apparent at lower doses. However, the change in organic carbon degrading aerobic microorganisms was not reflected in a visually detectable reduction of sediment height. Still, the application of CLRA at a dosage 4×higher than what is usually applied in the field (recommended 75–100 g/m^2) seemed to not come with detrimental effects on the investigated sediment microbiome. CaO_2 as a pure chemical is listed as slightly hazardous (Water hazard class 1) by the German Environment Agency. Albeit this classification, CaO_2 is widely applied for drinking water purification, lake restoration and sewage

sludge treatment in Germany (German Environment Agency, 2025). Furthermore, this peroxide is also used in products of daily use and in food (food additive E930; Health Canada, 2024). Together with our own observations, concerns about potential negative impacts of its application in the field thus seem unwarranted.

To further expand our understanding of CLRA applications, the agent is currently also applied to a selection of small lakes *in situ*, to identify key parameters of application success. However, the quantification of organic sediment decrease *in situ* is not straightforward, as established methods to identify variations in sediment strength are not robust at the cm-scale (Ruecker et al., 2024). Even though the heterogeneity of lakes typically complicates data interpretation *in situ*, field results will hopefully shed more light on the mechanisms of CLRA application under natural conditions. In summary, our results show that the applied CLRA resulted in a stimulatory effect on biogeochemistry and prokaryotic communities in the selected eutrophic lake sediment. As our findings are based on a single sediment only, their broader implications and transferrability remain uncertain and may be different for other sediment types. Nevertheless, our results point towards interesting mechanisms of action by the investigated lake restoration agent that warrant further in-depth elucidation across different lake and sediments in the field.

5 Conclusions

- The CaO_2-based lake restoration agent enhanced sedimentary oxygen availability at a mm-scale
- Other biogeochemical parameters were unaffected by the treatment
- The restoration agent promoted the activity of an aerobic, heterotrophic sediment microbiota
- Adverse effects of CaO_2 dosage on sediment physicochemistry or microorganisms were not observed

Acknowledgements This work was funded by the German Federal Ministry of Economic Affairs and Energy (BMWE) via the Central Innovation Programme for Small and Medium-Sized Enterprises (ZIM) within the Water4All Innovation Network under the project number 16KN091023 “Schlamm-TEC” to TL. We are grateful to Brigitte Nixdorf and Jaqueline Rücker (Aquatic Ecology, Brandenburg University of Technology (BTU) Cottbus-Senftenberg, Germany) and Thomas Willuweit and Dominique Gampe (Söll GmbH, Hof, Germany) for their feedback on the manuscript. We also thank Tobias Neumann and Franziska Horn (WERTEC GmbH, Chemnitz, Germany) for their support during field sampling. Additionally, we thank the Keylab Experimental Biogeochemistry (Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth (UBT), Germany) and the Genomics and Bioinformatics KeyLab (BayCEER, UBT) for their support in data acquisition.

Authors Contribution Thomas Kaupper: Writing – original draft, Investigation, Formal analysis, Data curation. Felix Pfaff: Writing – review & editing, Investigation, Formal analysis, Data curation. Dimitri Meier: Writing – review & editing, Formal analysis. Tillmann Lueders: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data Availability Generated 16S rRNA gene sequences were deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the project number PRJNA1227283.

Declarations

Competing Interest The funder of this project mandates direct collaboration between university and industry partners. As such, the SMEs WERTEC GmbH (Chemnitz, Germany) and Söll GmbH (Hof, Germany) were involved in the project consortium and have provided support in field work and materials. Their contribution was made without expectation of compensation, favourable representation, or influence on project outcomes. In addition, the authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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