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The effect of a short oxygen exposure period on algal biomass degradation and methane release from eutrophic and oligotrophic lake sediments

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Abstract

Algal blooms in lakes result in large amounts of labile carbon being transported down the water column towards the sediments, often resulting in temporary water column hypoxia. The algal biomass is deposited at the surface sediments, where it is degraded by the microbial community. Negative effects of algal blooms and biomass depositions are sometimes mitigated by pumping air or oxygen into the bottom waters of lakes. The fate of the algal biomass, in terms of greenhouse gas release, is however often unknown. We investigated methane emissions from sediments originating from both a eutrophic and oligotrophic lake and tested the effect of additional algal C inputs. Additionally, we investigated the effect of a pulse supply of oxygen, a mediating measure that is currently being used in the investigated eutrophic lake. Our results show a difference in the control experiments based on the state of eutrophication, but the methane release from new algal biomass additions was the same, although the process proceeded more rapidly in the eutrophic sediments. A 3-week pulse of oxygen lowered the emitted methane from both types of sediments by 50%, not only reducing the emissions of algal biomass additions, but also reducing methane emissions from the experiments without fresh organic matter inputs. This effect was relatively long-lasting: its effects were visible for several weeks after anoxic conditions were re-established, making it a potentially interesting measure to lower methane emissions over a longer period.

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Introduction

Lakes are known to be significant contributors to global methane emissions, despite their relatively small surface area(Bastviken et al. 2004). Methane emissions are the result of the net outcome of two processes: the production of methane, called methanogenesis, and the consumption of methane, methanotrophy. Methane emissions from aquatic environments originate mostly from sediments. Organic matter is delivered from either internal lacustrine (autochthonous) or external, e.g. riverine and terrestrial (allochthonous) sources. Autochthonous primary production, e.g. by planktonic microalgae in the water column, captures CO_2 from the atmosphere to produce biomass. After cell death, this biomass sinks down the water column, and becomes part of the sediment. The decomposition of this biomass lowers dissolved oxygen concentrations in bottom water and surface sediments and frequently enhances sedimentary methane production (Fiskal et al. 2019; van Grinsven et al. 2022). Eutrophication, the increase in (mainly algal) primary production due to increased nutrient concentrations in lakes, has thus been shown to increase methane emissions from lakes (Beaulieu, DelSontro, and Downing 2019).

Most sublittoral lake sediments are anoxic from a depth of a few mm to cm below the sediment surface. This is due to the mainly diffusive transport of oxygen into sediment and the high rates of aerobic decomposition processes at the sediment surface. In the underlying anoxic sediment, organic matter breakdown is performed by a community of hydrolytic, fermentative, and respiring microorganisms. Generally, methanogenesis is expected to occur only after the depletion of other, more energy-rich anaerobic oxidants, such as nitrate, nitrite, metal-oxides and sulfate (Bastviken et al. 2004),

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though strong overlaps in the distribution of methanogenesis with other anaerobic respiration reactions have also been observed (Fiskal et al. 2019).

Generally, the anaerobic breakdown of organic matter follows three steps: first, the large, complex organic compounds (e.g. macromolecules, polymers) are broken down to their building blocks (e.g. monomers, oligomers, fatty acids) by extracellular reactions (e.g. hydrolysis). Subsequently, these building blocks are taken up by microbial cells and fermented to smaller chemical compounds, such as H_2, alcohols (e.g. methanol, ethanol) and volatile fatty acids (VFAs; e.g., acetate, propionate, butyrate, isovalerate, formate, and pyruvate). These smaller compounds can then undergo a secondary fermentation step by syntrophic microorganisms, prior to respiration, or be directly respired to CO_2 or methane using nitrate, nitrite, metal-oxides, sulfate, or CO_2 as electron acceptors. Methanogens, which are respiring organisms that gain energy through the production of methane, are mostly obligate anaerobes, although their tolerance to oxygen is debated and may differ between clades (Kato, Field, and Lettinga 1993; Zinder 1993; Kiener and Leisinger 1983). Methanogenesis in sediments is believed to proceed mainly via three different pathways: CO_2 reduction using H2 or formate as electron sources, acetoclastic involving the disproportionation of acetate into CO_2 and methane, or methylotrophic methanogenesis, which involves the conversion of methylated compounds, such as methanol, methylamines and methyl sulfides to methane. With few exceptions, these pathways are performed by distinct taxa of methanogens.

A significant fraction of methane is consumed by methanotrophy, the process of methane consumption. Methanotrophy can occur both aerobically or anaerobically via

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the reduction of various anaerobic electron acceptors. Methanotrophs are found both within the archaeal and bacterial domain and include strict aerobes, facultative anaerobes, and obligate anaerobes. Methanotrophic activity often peaks at the oxic-anoxic interface in either the sediment or water column, presumably due to the high energy yields of aerobic methanotrophy.

Past research indicates that high organic matter inputs to lake sediments following algal blooms result in increases in methane concentrations in the sediment (Schulz and Conrad 1994). Both eutrophication and reduced water column mixing due to increased thermal stratification as a result of global warming will likely increase the frequency of algal blooms and contribute to more widespread bottom water anoxia in the future (Hou et al. 2022). While this promotes the deposition and burial of organic carbon in lake sediments, it will also increase methane emissions by increasing methanogenesis rates and lowering rates of aerobic methanotrophy. In addition to future algal blooms, legacy effects, e.g. continued high rates of methane production sustained by the decomposition of older organic carbon from past periods of eutrophication may contribute to these elevated methane emissions. These increases in methane emissions may be a lesser concern for oligotrophic sediments, which are generally lower in organic carbon content and hence methane production rates.

In order to mediate the effects of current and past eutrophication, artificial aeration is applied to lakes in Switzerland. This aeration reduces the detrimental ecological and socioeconomic consequences of seasonal anoxia. In addition, artificial aeration may lower methane emissions from lakes by promoting aerobic methanotrophy and reducing methane production in deep water columns and surface sediments, though

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the efficacy of artificial aeration in achieving lower methane emissions is not known. Here, we experimentally test the impact of artificial aeration on methane emissions under different trophic regimes by short, pulse-wise, oxygen supply to sediments from oligotrophic Lake Lucerne and eutrophic Lake Baldegg (both Switzerland). Based on slurry and whole-core incubations, we study the impact of oxygen pulses on methane emissions from sediments with and without an initial spike of algal biomass to mimic the situation shortly after an algal bloom. Samples were extracted for gas concentration and isotope analysis, as well as VFA and microbial community analyses. Our results show that oxygen exposure had effects lasting past the oxic period such as that methane emission rates remained lower for up to 10 weeks. The methanogenic and methanotrophic communities did not seem affected by the oxygen exposure.

Methods

Study sites

Two lakes with a different trophic state were sampled for various experiments. A map showing the location of both lakes is shown in Fig. S1.

Lake Lucerne is located at the northern alpine front in Central Switzerland (47°N, 8°E, 434 m a.s.l). It has a surface area of 116 km^2, and is fed by four alpine rivers that provide ±80% of the lakes total water supply (Schnellmann et al. 2002). It is oligotrophic, with a maximum P-concentration within the past century of 1.7 μM (Bürgi and Stadelmann 2002). Further details on the trophic history of both lakes can be found in (Fiskal et al. 2019).

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Lake Baldegg is in an area with intensive cattle and pig farming within Central Switzerland. It has a surface area of 5.22 km^2. Eutrophication has been ongoing, with a peak in the 1970s, reaching P concentrations of 15.4 μM before remediation measures were put in place. It has had an anoxic hypolimnion for almost 100 years until artificial aeration was started in 1982 (Gächter and Wehrli 1998).

Field sampling

Lake Lucerne sediment cores were taken in November 2020 and February 2021 at a location near the village of Kastanienbaum (47.00085N, 8.33697E). Lake Baldegg sediment cores were taken in June 2021 from the center of the lake (47.193071N, 8.265238E). Both lakes were sampled with a multicorer device containing 10 cm diameter, transparent butyrate plastic core liners of 65 cm, which were never filled more than 3/4rd. The average core length was 40 cm. All sediment cores were brought into a climate room of 10°C within 2 hours after core collection and stored until further processing. Bottom water temperatures are between 5 and 9°C, according to (Fiskal et al. 2019).

Slurry experiments in bottles

Oxygen regime	Biomass addition*	Lake	Headspace gas analysis	VFA	Microbial community
N_2 flushed at start	0.1 g	Lucerne	+	+	+
N_2 flushed after 1 week	0.1 g	Lucerne	+	+	+
N_2 flushed after 3 weeks	0.1 g	Lucerne	+	+	+
N_2 flushed at start	-	Lucerne	+	+	+
N_2 flushed after 1 week	-	Lucerne	+	+	+
N_2 flushed after 3 weeks	-	Lucerne	+	+	+
N_2 flushed at start	0.1 g	Baldegg	+	+	+
N_2 flushed after 3 weeks	0.1 g	Baldegg	+	+	+

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N_2 flushed at start	-	Baldegg	+	+	+
N_2 flushed after 3 weeks	-	Baldegg	+	+	+

Whole sediment core experiments

Oxygen regime	Biomass addition*	Lake	Headspace gas analysis	VFA	Microbial community
N_2 flushed at start	0.3 g	Lucerne	+	-	-
No N_2 flushing, air headspace	0.3 g	Lucerne	+	-	-
N_2 flushed at start	-	Lucerne	+	-	-
No N_2 flushing, air headspace	-	Lucerne	+	-	-

* freeze-dried Spirulina algae (slurries) or 1:1 mixture of freeze-dried Spirulina + Chlorella algae (whole cores)

Table 1. Overview of experiments. Further details are provided in Table 2 and the Methods section.

Experimental setups

The experimental setups were designed to mimic oxygen intrusion from overlying oxic water. Two different experimental setups were used: slurry experiments (Lakes Baldegg and Lucerne) and whole core experiments (Lake Lucerne only). An overview of the experiments and analyses is presented in Table 1. Oxygen concentrations were followed and are shown in Fig. S2 and S3.

Slurry incubation experiments

The oxygen penetration depth of aquatic sediments generally does not exceed 1 cm (Fiskal et al. 2019; Horppila et al. 2015), with outliers in very organic material poor (marine) sediments up to 6 cm(Cai and Sayles 1996). It is highly unlikely that sediments deeper than 5 cm will experience oxygen intrusion. Therefore, we choose

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a setup which does not expose the sediments deeper than 5 cm to oxygen, as this would inhibit methanogenesis in a way that is highly unlikely to appear in lake sediments.

To ensure this, sediment cores were separated in a surface part (0 – 5 cm depth) and a deep part (5 – 15 cm depth). Lake bottom water was used to dilute sediment material 1:1, to create slurries. For the experiments that started completely anoxically directly from the start, both the surface sediment and deeper sediment were added at the same time and flushed with N_2. For the experiments that started under oxic conditions (see Table 1), only surface sediments were placed into the incubation vials. The deeper sediments were added upon O_2 removal, which was after either 1 or 3 weeks. An overview is provided in Table 2. The oxygen concentrations inside a subset of incubation bottles was followed to ensure the oxic and anoxic periods were indeed established as aimed for (Fig. S2).

Treatment	Sediment provided at start	Sediment added after 1 week	Sediment added after 3 weeks	Final contents
N2 flushed at start	0 – 15 cm	-	-	0 – 15 cm
N2 flushed after 1 week	0 – 5 cm	5 – 15 cm	-	0 – 15 cm
N2 flushed after 3 weeks	0 – 5 cm	-	5 – 15 cm	0 – 15 cm

Table 2. Overview of sediment additions within different experimental treatments of the slurry experiment.

Slurry experiments were performed in triplicate in 0.5L Schott bottles, filled with a final volume of 258 ml sediment slurry. Each bottle was closed with an adapted stopper, containing a three-way-stopcock that could be opened to allow throughflow of sediment slurry and gas without exchange with the air. Freeze-dried algal biomass (0.1 g of freeze-dried Chlorella; DietFoods CH) was added to a subset of the bottles.

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Incubations proceeded at 10°C in the dark. The oxygen concentration was measured daily to bi-weekly in 8 out of the 12 oxic bottles, using optical oxygen sensor spots (Pyroscience, UK).

After 1 week or 3 weeks, the oxic bottles were flushed with N_2 for 15 minutes to remove oxygen, after which they received additional anoxic sediments, according to Table 2. The resulting oxygen trends are shown in Fig. S2. Sediments were added via the sampling ports in the stoppers.

At each gas sampling time point, headspace gas was sampled via the sampling port in the bottle caps. Prior to gas extraction, 10 ml of N_2 or air was pushed into the bottle. Immediately after, 10 ml of headspace gas was sampled and stored in N_2 flushed 70 ml serum bottles. At each VFA (volatile fatty acids) and DNA sampling time point, 1.5 ml overlying water and 1.5 ml mixed slurry were sampled as described below. For the VFA samples, samples without particulates were required. To achieve this, the bottles were carefully tipped over, so the natural layering of sediment at the bottom and water at the top remained. The water was then sampled via the sampling port. For the DNA sample, the bottle was briefly shaken and then held upside down to take a mixed water + sediment DNA sample. Both VFA and DNA samples were put directly on ice, and after the sampling series was finished, moved to -20°C (VFA) or -80°C (DNA) freezers.

Whole core incubation experiments

In contrast to the slurry experiments, the cores for the whole core incubation experiments were not disturbed or opened. Whole core experiments were only performed with Lake Lucerne sediments for practical reasons, as shown in Table 1.

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The core retrieval resulted in cores that were filled with on average 40 cm of sediment and 25 cm of overlying water. To allow for headspace gas extraction over the experiment duration, 12 cm of overlying water was carefully removed without disturbing the sediment surface, leaving on average 13 cm of overlying water above the sediment-water interface of each core. Whole core experiments were performed in quadruplicate, an overview of the treatments is provided in Table 1.

To set up the treatments, freeze-dried algal biomass was added to selected cores ca. 18 hours after sampling (0.3 g per core, 1:1 mixture of freeze-dried Chlorella and Spirulina; DietFoods, CH), corresponding to the same amount of algal biomass per sediment mass as in the slurry incubations, and following earlier studies(Hiltunen, Nykänen, and Syväranta 2021; Dai et al. 2005). The algal biomass was carefully deposited on the sediment surface using a pipet, without disturbing the sediment-water interface. The headspace and overlying water of the cores for the anoxic incubations were flushed for at least 10 minutes with N_2.

Adjusted rubber stoppers with sampling ports were used to seal the cores on the top, the bottoms were sealed off with rubber stoppers plus plastic caps. All cores were then placed at 10°C in the dark. One core of each oxic treatment contained an oxygen sensor spot (Pyroscience, UK), glued to the inner wall of the core liner.

Oxygen concentrations were measured at the start of the experiment and at irregular intervals over the course of the experiment (shown in Fig. S3) and showed oxic conditions were indeed retained over the full course of the oxic experiment, as was aimed for.

Over the course of the experiment, gas samples for CH_4, CO_2 and N_2O analysis were taken via the sampling ports. 10 ml of the gas headspace was extracted and placed

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into 70 ml N_2-flushed serum bottles with butyl stoppers. After the gas sampling, 10-15 ml of N_2 gas (anoxic cores) or N_2 or air (oxic cores) was added via the sampling ports, to equilibrate the internal and external pressure and limit the risk of leakage or contamination. The gas pressure prior to sampling was determined with a pressure meter and noted for each timepoint (not shown). One core was discarded due to water leakage during the experiment.

Gas concentration and stable isotope analysis

Gas samples were analyzed for the concentration of CH_4 and CO_2 by gas chromatography (GC; Agilent 6890N, Agilent Technologies) using a Carboxen 1010 column (30 m x 0.53 mm, Supelco), a flame ionization detector and an auto-sampler (Valco Instruments Co. Inc.) for both the slurry and whole core experiments. Isotopic ratios of methane 13C/12C (presented in the standard δ13C-notation relative to the Vienna Pee Dee Belemnite (VPDB) reference) were measured in selected headspace samples by isotope ratio mass spectrometry (IRMS; GV Instruments, Isoprime). To purify, concentrate and combust the CH_4 to CO_2, injected samples were passed through a trace gas unit (T/GAS PRECON, Micromass UK Ldt).

VFA analysis

Volatile fatty acids (VFA) analysis was only performed on samples from the slurry experiment. Samples were filtered through pre-cleaned syringe filters Acrodisc™, 0.2 µm PES membrane, Supor™) and analyzed by two dimensional ion chromatography (2D IC) at ETHZ according to the method described in (Glombitza et al. 2014) with some modifications. The instrument used was a Dinonex™ ICS6000 (Thermo Fisher Scientific) equipped with two 2.5-mm columns (AS24 for the first dimension and

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AC11HC for the second dimension). The Retention time window on the first IC dimension to collect the bulk VFAs for injection onto the second IC column was set to 3 min – 6.5 min to account for the low salinity of the freshwater samples compared to the original method, as described in (Schaedler et al. 2018; Vuillemin et al. 2023). Likewise, the VFA standards for quantification (mixed standards of formate, acetate, propionate, butyrate, valerate, isovalerate and pyruvate at 1, 5, 10, 50 and 100 µmol L^{-1}) were prepared in Milli-Q® water instead of IAPSO seawater as described in the original method. Quantification was done using the conductivity detector signal of the second IC dimension.

Microbial community analysis

Microbial community analysis was only performed on samples from the slurry experiment. Each DNA sampled was stored at -80°C until processing. DNA was extracted using the Qiagen Powersoil DNeasy kit without adaptations. The DNA concentration of all extracts was measured on a Nanodrop device (Thermo Scientific). When the concentration was below 2 ng/µl, an additional extraction was performed, and samples were pooled. DNA extracts from all experiments were combined in two lanes, including extraction blanks, and send for 16S rRNA NovaSeq PE250 sequencing (30K tags per sample) to Novogene UK, using the general 16S rRNA archaeal and bacteria primer pair 515F and 806R, targeting the V4 region (Caporaso et al. 2012). Quality control and species annotation were performed using the standard Novogene pipelines (https://www.novogene.com/eu-en/services/research-services/metagenome-sequencing/16s-18s-its-amplicon-metagenomic-sequencing/). Raw sequencing data is deposited in the public repository [available upon publication, or on reviewers' request].

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Results

Slurry experiments oligotrophic Lake Lucerne

All oligotrophic slurry experiments that received algal biomass emitted significant quantities of methane. The control experiment, without additional carbon source, only emitted methane in the permanently anoxic setup (ca. 9 μmol per week). The control slurries in which the top 5 cm was initially exposed to oxygen did not emit methane, also not after anoxic conditions were established and the deeper anoxic sediments were added (Fig. 1; Fig. 2). All given concentrations and concentration increases are given in μM per liter headspace volume.

The addition of algal biomass to the oligotrophic slurries increased the methane emission almost 25-fold under permanently anoxic conditions (to 161 μM per week), but only 17-fold and 14-fold under the 1-week and 3-week oxic start conditions (118 μM and 93 μM per week), respectively (Fig. 1, Fig. 2).

Methane emissions started directly in the first week after the start of the anoxic experiments (Fig. 2). After oxygen removal from the oxic start slurries, thus re-establishing anoxic conditions and adding deeper sediments, methane emission also started immediately. Net methane emission continued until week 13 in the anoxic oligotrophic slurries, and two weeks longer in both types of the oxic slurries, despite the 2 weeks difference between the start of methane emission in these two oxic incubations (Fig. 2). The methane concentration in the oligotrophic slurries plateaued and remained constant between weeks 13 or 15 to week 28, at a concentration of 1900 (anoxic), 1500 (1-week oxic start) or 1200 (3-weeks oxic start) μmol per L headspace.

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Slurry experiments eutrophic Lake Baldegg

Methane emission was observed in all eutrophic slurry setups, both the control and algal biomass addition setups, after establishment of anoxic conditions and the addition of deeper sediments. Under oxic conditions, and with only the top 5 cm, no methane emission was observed. However, similar to the oligotrophic setup, the onset of methane emission happened directly after establishment of anoxic conditions and the addition of the deeper sediments.

The anoxic background methane emission in the eutrophic control slurries exceeded those of the oligotrophic control slurries over 12-fold (85 versus 6.9 μM per week, respectively, Fig. 1; Table S1).

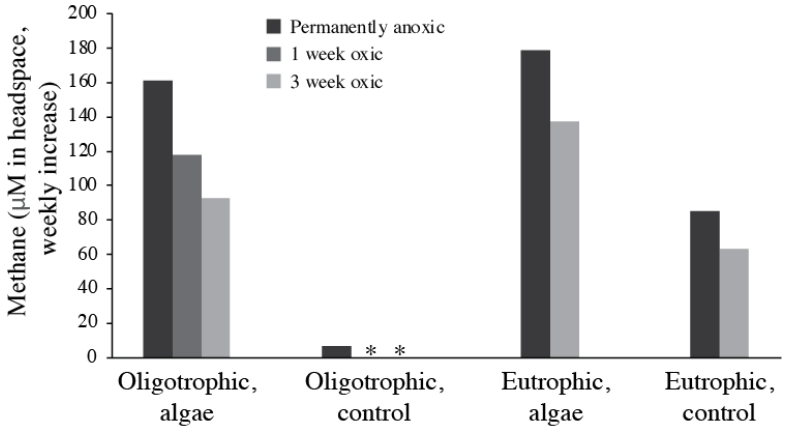

Fig. 1. Weekly increase in headspace methane concentration in the oligotrophic and eutrophic slurry experiments, respectively, as derived from the linear phase of the methane concentration plots (Fig. 2). * no increase in concentration detected.

The methane concentration in the eutrophic slurries did not plateau, although two phases could be identified in the algae-fed slurries: A phase of rapid increase in

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338 headspace CH_4 was observed from t_0 until week 7 in the anoxic algae-fed slurries, and
339 from week 3 to week 9 in the 3-week oxic start algae-fed slurries (Fig. 2). After this
340 initial phase, the methane emission rate stabilized at a similar rate as was observed
341 in the control experiments, as can be observed by the parallel lines in the graph of Fig.
342 2.
343

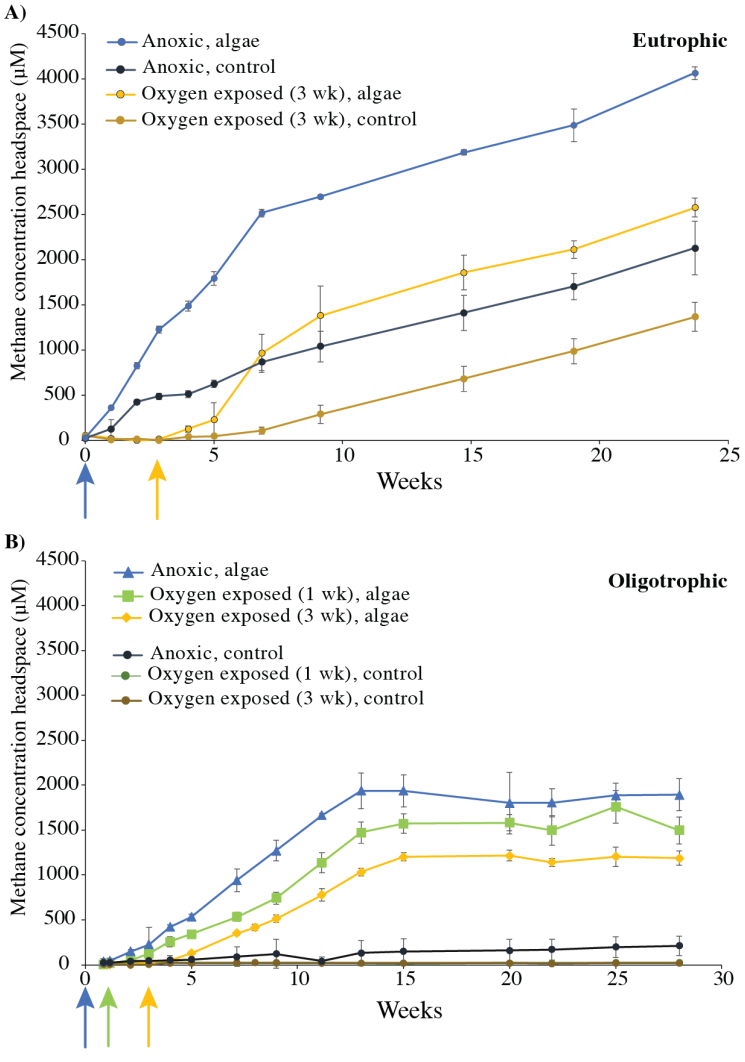

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Fig. 2. Methane concentration in the headspace of the slurry experiments with A) eutrophic and B) oligotrophic sediments. Arrows indicate the moment of oxygen removal, by flushing with N_2, and the addition of anoxic sediments (see methods).

Even though the addition of algal biomass did increase the methane emission from the eutrophic slurries, it was partly diminished when oxygen was present at the start of the experiment. The background methane emission under anoxic control conditions was similar to that of the oxic conditions with additional algal biomass, resulting in a total amount of methane emitted of 2600 and 2100 μM for the oxic with algae and anoxic control, respectively, corresponding to an increase of only 20% in emitted methane (Table S1; Fig. 2). The methane emission rate was initially higher in the oxic incubations with algae, but because the phase of high emission was of a short duration (6 weeks, from week 3 to week 9), the total emission did not strongly exceed the total anoxic control emission. The high emission phase in the anoxic algae experiment was also short (t_0 until week 7), but due to the high weekly emission rate of 180 μmol, the total amount of methane produced after 24 weeks was twice as high as the methane emission in the anoxic control experiment, and 1.5 times higher than in the oxic algae experiment (Fig. 2; Table S1).

Both the oligotrophic and eutrophic incubation experiments received equal amounts of algal biomass. The methane emission rate in the oligotrophic anoxic experiments increased from 6.9 to 161 μM per week due to the algae addition, whereas the eutrophic anoxic rate increased from 85 to 179 μM per week, showing a much larger increase in the oligotrophic experiments. The same holds for the 3-weeks oxic experiments, which increased in weekly rate from 0 (control) to 93 (with algae) in the

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oligotrophic experiments and from 63 to 137 in the eutrophic experiments, respectively. Due to the shorter duration of this high-rate methane emission the total methane produced as a result of the algal biomass was, however, similar between the oligotrophic and eutrophic sediments (Fig. 2).

Intact core experiments oligotrophic Lake Lucerne

Experiments with whole sediment cores, rather than sediment slurries, showed a similar effect of oxygen exposure and algal biomass additions as the oligotrophic slurry experiments. Although the variation within the experiments with whole cores was much larger than in the more controlled slurry experiments, still a significant effect of oxygen exposure, and of algal biomass addition, was observed (Fig. 3).

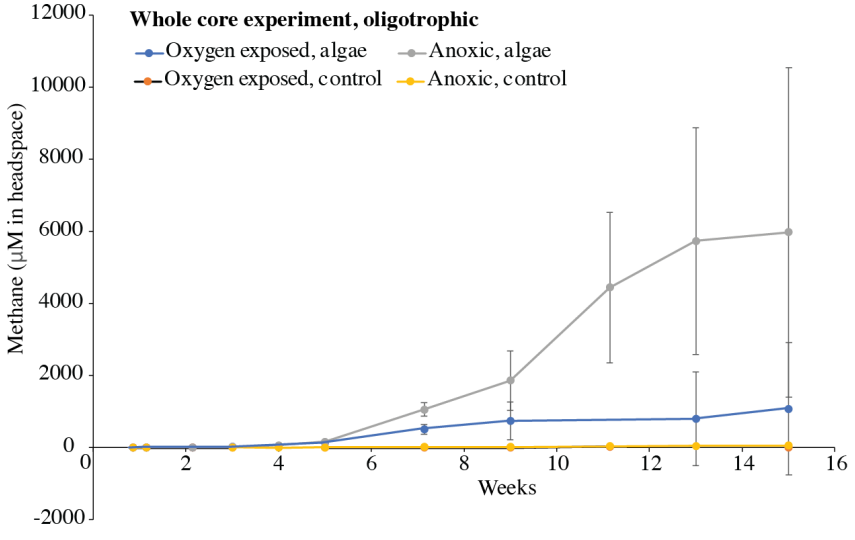

Fig. 3. Methane concentration in the headspace of the whole core experiment under oxygen exposed (air headspace above overlying water) or anoxic (N_2 headspace) conditions, with and without the addition of algal biomass, respectively. A cut-out of

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the lower values that highlights the methane concentrations at the start of the experiments is available in Fig. S4. The oxygen exposed control line is hidden from view behind the anoxic control line in this graph.

Microbial community in slurry experiments

Effect of algal biomass on microbial community

The phyla of the Proteobacteria and the Bacteriodota were abundant in both the eutrophic and oligotrophic sediment incubations. Other phyla that were found among the 10 most abundant phyla in both setups were the Verrucomicrobiota, Chloroflexi, Acidobacteriota, Desulfobacterota and Planctomycetota.

The microbial community was similar in the permanently anoxic and initially oxic treatments, in both the oligotrophic and eutrophic sediment incubations. The addition of the algal biomass influenced the microbial community composition. In the oligotrophic incubations, the relative abundance of the Proteobacteria was higher in all incubations with algae, both with and without oxygen exposure. In the eutrophic experiments, the Proteobacteria abundance was actually lower in incubations with algal biomass than without, both oxygen exposed and permanently anoxic. The abundance of the Bacteroidota was higher in incubations with algal biomass than the control, in all treatments in both setups. The Nitrospirota had a high abundance in the oligotrophic sediments (11 – 14% in control setups), that was lowered under conditions with algal biomass (8 – 10%, Fig. 4). In the eutrophic sediments, the relative abundance of Nitrospirota was <1%. The abundance of the Acidobacteriota was the same in oligotrophic sediments with and without algal biomass (5 – 7%). In

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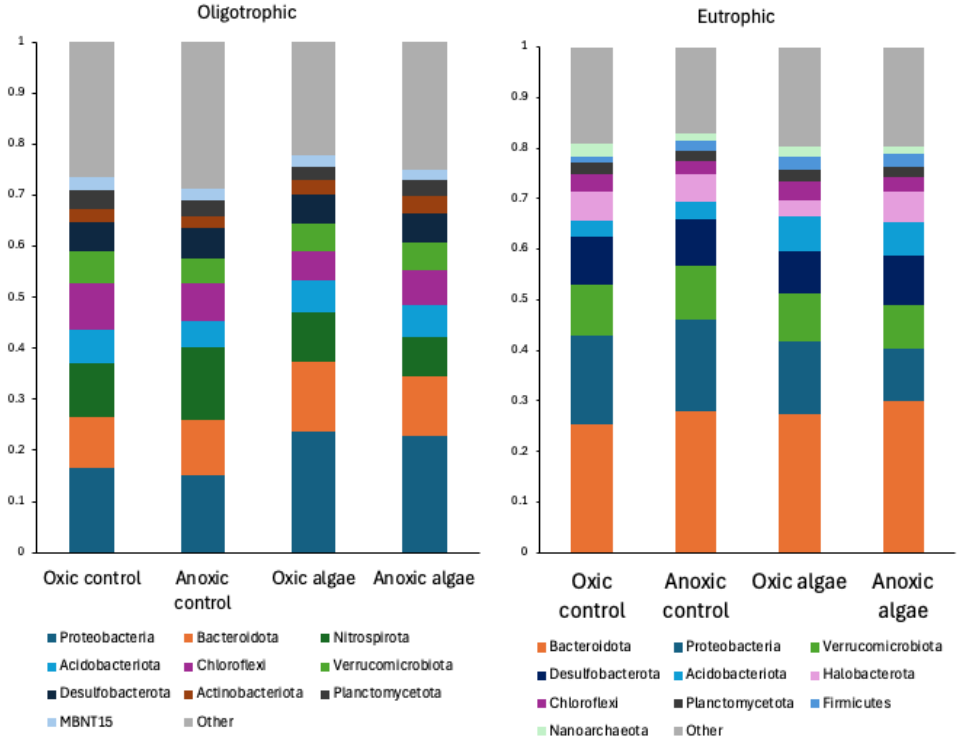
412 the eutrophic sediments, the relative abundance in the control incubations was lower
413 (3%) than in the algal addition incubations (7%).



414

415 **Fig. 4.** Microbial phyla in the oligotrophic (A) and eutrophic (B) sediment incubations,
416 after 15 weeks and 9 weeks, respectively. The 10 most abundant phyla, as detected
417 by 16S rRNA sequencing, are shown. The timepoints correspond to the start of the
418 stationary methane release phase in both setups, as shown in Fig. 2.

419

420 *Methanogenic/methanotrophic clades*

421 Surprisingly, nor the oxygen exposure or the algal biomass additions had a significant
422 effect on the relative abundance of methanogenic and methanotrophic clades in the
423 microbial community (Fig. S5). There was a profound difference between the
424 oligotrophic and eutrophic sediments, but only for the methanogen relative abundance.

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In the oligotrophic incubations, the relative abundance of operational taxonomic units (OTUs) assigned to methanogenic orders was between 0.5 and 3% of the total 16S rRNA detected sequences (Fig. S5) at all tested timepoints between 0 and 27 weeks. In the eutrophic experiments, the relative abundance of OTUs assigned to methanogenic orders was mostly between 4 and 7% of the total 16S rRNA detected sequences in all selected timepoints between 0 and 15 weeks (Fig. S5).

The methanogenic community was dominated by OTUs assigned to the order Methanomicrobiales (Fig. S6). The contribution of Methanomicrobiales to the total 16S rRNA sequences assigned to methanogenic orders was, however, higher in the eutrophic (average of 75%) than in the oligotrophic experiments (average of 65%). Methanomassiliicoccales made up a larger fraction in the oligotrophic experiments (average of 29%, versus 12% in eutrophic experiments). Methanosarcinales were relatively more abundant in the eutrophic experiments (Fig. S6). In the oligotrophic experiments, no patterns were observed over time, nor differences between treatments. In the eutrophic experiments, Methanomicrobiales dominated both the oxic and anoxic experiments. However, in the permanently anoxic incubations, more OTUs are assigned to Methanosarcinales, whereas in the temporarily oxic incubations, more OTUs are assigned to the order Methanomassiliicoccales.

In both the oligotrophic and eutrophic incubations, the relative abundance of methanotrophs belonging to the order Methylococcales was around 1 – 3% for all treatments (Fig. 5). The majority of OTUs within the Methylococcales order were assigned to the genus *Crenothrix* in all eutrophic treatments (20 – 99% of Methylococcales reads), followed by the genus *Methylobacter* (0.9 – 30.6% of

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Methylococcales reads, Fig. 5). However, recent research on the SILVA annotation within the Methylococcales order has shown that a differentiation between *Crenothrix* and certain groups of *Methylococcaceae* cannot be supported, and the weight of the differentiation between these two groups is thus limited, currently (van Grinsven et al. 2022). The methanotrophic community in the oligotrophic experiments was less dominated by sequences assigned to "*Crenothrix*", and rather than a higher abundance of *Methylobacter* assigned OTUs (Fig. 5) and a higher abundance of genera that were marginal in the eutrophic incubations, such as *Methyloparacoccus*. At specific time points, the genus *Methylomonas* showed particularly high peaks in its relative abundance (up to 74% of Methylococcales reads) in both the eutrophic and oligotrophic incubations (Fig. 5). Overall, the methanotrophic communities looked relatively similar and no trends could be established over time, nor differences between the oxygen or algae treatments (Fig. 5, Fig. S7).

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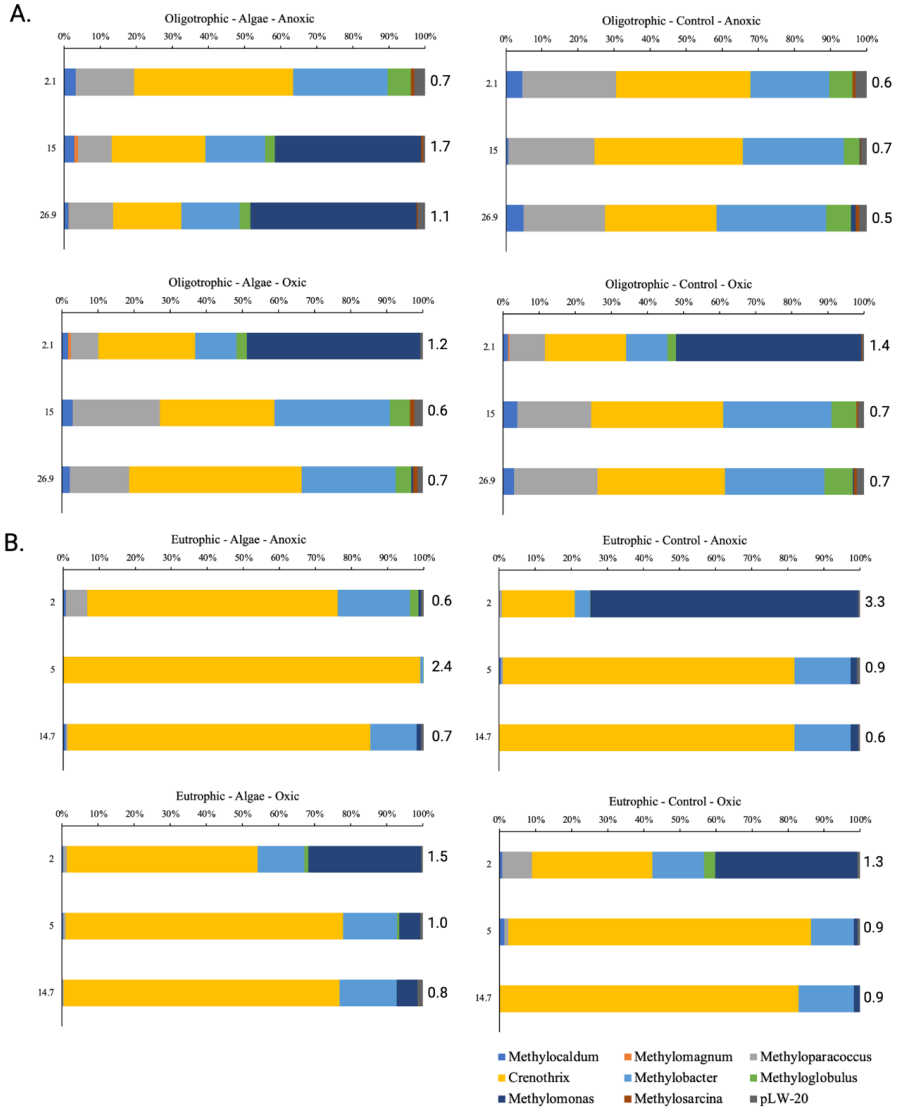

Fig. 5. Relative abundance of OTUs assigned to methanotrophic genera in the oligotrophic (A) and eutrophic (B) incubation experiments. The bar plots show the abundance of specific genera relative to the total methanotroph abundance, whereas the number behind each bar indicates the abundance of methanotrophs relative to

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the total microbial community (in % of 16S rRNA reads of each sample). The y-axis shows the time in weeks since the start of the experiment.

Volatile fatty acids in oligotrophic incubation experiments

To compare the release of different volatile fatty acids (VFAs) after the algal biomass additions, VFA concentrations were traced in the oligotrophic slurry experiments during the first 8 weeks, as shown in Fig. 6. VFA concentrations were highest in anoxic incubations, and were significantly lowered by temporary oxygen exposure. The addition of algal biomass led to a strong increase in VFA concentrations (5 - 500 fold increase) compared to control incubations. One of the anoxic control incubations had 20-fold higher VFA concentrations than the other two bottles of this treatment, resulting in the large error bars (Fig. 6A).

Acetate was the dominant VFA, with concentrations 10-100x higher than the other VFAs (Fig. S8), and the key VFA to differ between oxic and anoxic treatments. The concentrations of formate and pyruvate were not significantly affected by the oxygen exposure.

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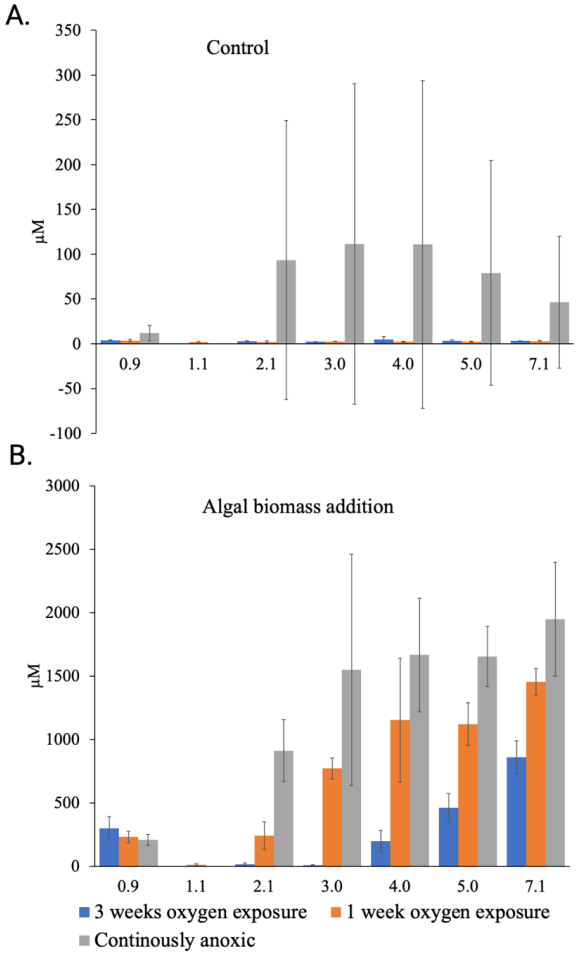

485

486 **Fig. 6.** Total volatile fatty acid concentrations during the first 8 weeks of the oligotrophic
487 control (A) and algal biomass addition (B) experiment. The x-axis indicates weeks
488 since the start of the experiment. Note the different y-axis for A and B. Each bar
489 represents the average of triplicate samples at each timepoint. No samples were taken
490 at 1.1 weeks (8 days) of the anoxic and 3 weeks-exposure experiments.

491

492 **Carbon isotopes of methane in slurry incubations**

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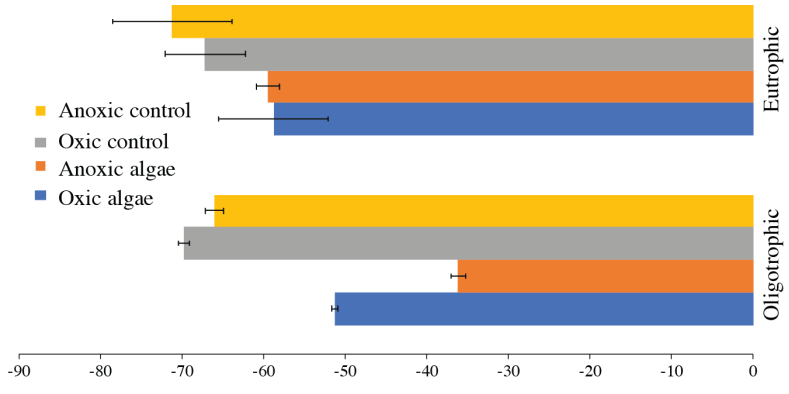
493 The stable isotope profile of the headspace methane was determined only in the
494 stable, post-algal biomass degradation phase in the slurry experiments, when
495 methane emission rates no longer increased over time. This was at 17 weeks in the
496 oligotrophic, and at 11 weeks in the eutrophic experiments. The stable isotope ratio is
497 given in the standard δ13C-notation, relative to the Vienna Pee Dee Belemnite (VPDB)
498 reference. When comparing the treatments, we see more negative $\delta^{13}CH_4$ values in
499 the algal biomass experiments in both the eutrophic and oligotrophic slurry
500 experiments, compared to the controls. A significant difference between the oxygen
501 exposed and anoxic treatments was only visible in the oligotrophic sediments.
502
503



504

505 *Fig. 7. $\delta^{13}CH_4$ values of headspace methane, after 11 weeks (eutrophic sediments)*
506 *or 17 weeks (oligotrophic) of slurry incubations.*
507
508

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Discussion

Trophic state and legacy effects on methane emissions and methane-cycling communities

The two investigated lakes differ in their current and historical trophic status. Lake Lucerne is currently oligotrophic and has a history of low phosphate inputs. Lake Baldegg is eutrophic and has been receiving high phosphate loading in the past, resulting in water column anoxia and algal blooms in the period of 1910 – 1985(Fiskal et al. 2019). Our results show that the eutrophication state of the lake affects the methane emissions throughout the entire incubation period, both with and without fresh organic matter inputs. The importance of legacy effects on biogeochemical processes and communities in these lakes has been shown in earlier studies as well (Fiskal et al. 2019; Han et al. 2020) and was also shown for other lakes along a trophic gradient (Zhou et al. 2024). The methane emission was 12 times higher in control experiments with eutrophic sediments than oligotrophic sediments, though no fresh material input was delivered over 160 days (Fig. 1, Table S1). Contrastingly to their historic and current carbon inputs, the TOC concentrations in sediments of both lakes are comparable, according to a recent study at the same sampling locations as used in this study (Fiskal et al. 2019).

Although these legacy effects are clearly visible in the methane emissions of the control setups, the response to the input of new algal material was similar in magnitude in both oligotrophic and eutrophic sediments (Fig. 1; Fig. 2). The addition of easily degradable carbon compounds to environmental samples can spark a priming effect, in which carbon stocks in the original sample are degraded more rapidly upon addition

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of additional fresh material (Y. Wang et al. 2021; Guenet et al. 2010). Although we cannot separate the contribution of algal carbon and sedimentary carbon to the emitted CH_4, the equal emission responses from both lakes to the addition of fresh organic matter suggest that the OM degradation response was also similar, and likely primarily driven by degradation of the algal biomass rather than by older sedimentary organic matter. A study with a similar setup regarding the addition of algal biomass also showed a direct response in methane production rates during the first 60 days, after which methane production rates stabilized (T. Wang et al. 2023).

Previous research has shown higher abundances and diversities of methanogens and methanotrophs in eutrophic than in oligotrophic sediments(Yang et al. 2019), although other studies indicate that the effect of sediment depth is of stronger effect(Yang et al. 2017) than the trophic state of the lake. The continuous high methane emission in our eutrophic sediments (weekly increase of 85 μM in control experiments), in contrast to the low methane emission in the oligotrophic sediments (weekly increase of 7 μM in control experiments), also suggests that a more abundant and more active methanogenic microbial community may exist in the eutrophic sediments. The methanogenic community was indeed higher in its relative abundance (3 – 7%) in the eutrophic experiments than in the oligotrophic sediments (0.5 – 4%).

Effect of algal biomass and oxygen exposure on substrate availability to the microbial community

The conversion of algal biomass to gaseous methane emissions requires an initial step of carbon degradation by fermenters, and a second step in which the reaction products are converted into methane. The reaction products can consist of various

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organic molecules, of which part can be used by methanogens directly, but others need to be degraded further to become a suitable substrate for methane production. Here, we found that the concentration of organic compounds indeed increased strongly after the addition of algal biomass to the slurries, up to 500-fold (Fig. 6). This was similar to a study by Schwarz et al. (Schwarz, Eckert, and Conrad 2008), who found increased acetate and propionate concentrations in lake sediment incubations with algal additions. A study by (Zhou et al. 2024) showed algal deposition on top of the surface sediments led to a distinct increase in TOC in the top 8 cm of the sediment cores, also without active mixing. The same two compounds as found in the Schwarz et al. 2008 study, acetate and propionate, were also the major compounds detected in our experiments. Surprisingly, these compounds were also produced (and build up) under oxic conditions (200 μM acetate and 13 μM propionate under oxic conditions at day 6, Fig. S8). However, the production of both acetate and propionate did not reach the same values as the concentrations reached in the continuously anoxic incubations, and a clear difference in the VFA buildup was also visible between 1 or 3 weeks oxygen exposure treatments. The short exposure to oxic condition did lower the acetate and propionate buildup, but did not diminish it. A recent study by (Kallistova et al. 2023) showed that acetate additions strongly enhanced methane production from surface sediments, showing it had an active function as methane precursor and higher concentrations of acetate are likely directly correlated to higher methane emissions from the sediments into the water column. In our experiments, substrates for acetate-consuming methanogens were present in both the oxygen-exposed and permanently anoxic experiments, but the concentrations were significantly lowered by oxygen exposure at the start of the experiment. This corresponds to the methane production in each of these treatments (Fig. 2). The methanogenic community did not show

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similar patterns, suggesting that the substrate concentrations rather than the microbial presence determines and predicts the methane emission rates in lake sediments.

Microbial community

A recent study by (Yang et al. 2021) followed the succession of a sedimentary microbial community during an algal bloom, and found that both the archaeal and bacterial community transitioned, taking part in biomass degradation steps that changed over the time since the start of the algal bloom. (Schwarz, Eckert, and Conrad 2008) noted that in their experiments with algal biomass additions, the Deltaproteobacteria and Clostridiales increased immediately, and the Bacteroidetes after 6 days. Methanogens, specifically acetate-using methanogens of the type Methanosaetaceae increased in abundance after 6 days(Schwarz, Eckert, and Conrad 2008). In our experiments, we noticed a similar increase in the Proteobacterial relative abundance in the oligotrophic incubations due to algal biomass additions, but a decrease in the eutrophic sediments (Fig. 4). The Bacteroidota also increased in abundance in the oligotrophic incubations only. We did not see an increase in the relative abundance in methanogenic clades in response to the algal biomass addition, despite the much higher emission rates. A similar effect was observed in experiments with algal biomass additions by (T. Wang et al. 2023), who also saw large effects on the methane emission, but no increase in methanogen copy numbers.

The effect of the oxygen exposure on the microbial community composition was limited, both on the total prokaryotic community and on the specific methane-cycling community. The methanogenic archaea are predominantly present in deeper sediment layers (> 5 cm depth, as shown for these lakes by (Meier et al. 2024). They

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will therefore likely not be affected by oxygen supply to lake bottom waters, which was the process that was mimicked here. In our experimental setup, only sediments of 0 – 5 cm depth were oxygen exposed, and 5 – 15 cm depth sediments were added after oxygen removal. Therefore, the methanogenic community was predominantly affected via the availability of substrates, and not due to direct oxygen toxicity. Methanotrophs were presented throughout the sediment in these lakes (van Grinsven et al. 2022). The methanotrophic bacteria found in our experiments are all know as aerobic methanotrophs. However, these methanotrophs have been found in anoxic environments, in these lakes and others, more often (van Grinsven et al. 2022). Although the methanotrophic community was diverse, especially in the oligotrophic sediments, and showed changes in structure over time, these did not seem related to oxygen exposure (Fig. 5).

Oxygen exposure decreases methane emissions

The effect of oxygen penetration depth on methane emission from lake sediments is well established. However, these studies generally address long term stable oxygen conditions ((Sobek et al. 2009; Huttunen et al. 2006). Here, we look at short oxygen pulses, as a potential mediative measure for lakes with anoxic bottom water. The presence of oxygen for a short, 3-week period at the start of the incubation had major implications for methane emissions over the course of the entire experiments. The total release of methane was significantly lower in the treatments that had experienced an oxic period (Fig. 1; Fig. 2, Table S1). Most likely, part of the algal biomass was converted to CO_2 and/or biomass during the oxic period and was therefore not directly available for methanogenesis anymore. This is supported by the peak in CO_2 emissions that was observed during the oxic period of the experiments (Fig. S9; S10).

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However, due to difficulties in translating headspace CO_2 concentrations to dissolved CO_2, it is not possible to make a carbon mass balance, to see how much is indeed released as CO_2. Part of the produced CO_2 will again be converted prior to release to the headspace, leading to underestimates that cannot be sufficiently quantified. The bubbling with N_2 to remove oxygen, that occurred at different timepoints in the different experiments, removed CO_2 and may therefore has changed the pH in the system. pH was not measured. Given the immediate production of CO_2 after bubbling (Fig. S9, S10), we however assume that a [CO_2] close to natural conditions was rapidly established following N_2 bubbling.

As CO_2 has a much lower warming potential per mole than methane (approximately 28 times lower on a hundred year basis, (Forster et al. 2021)) the release of CO_2 is strongly preferred over that of methane in light of global warming. Besides CO_2, part of the carbon may have been converted to microbial biomass during the oxic period, and is stored as such in the sediments. (Sobek et al. 2009) published a weak linear relationship between the diffusive methane flux from lake sediments, and the oxygen penetration depth at those locations. A direct comparison with this study is, however, difficult to make, as there are likely other factors involved that affect both the oxygen penetration depth and the methane production, such as carbon content of the sediments.

Directly after oxygen was removed from the incubation bottles and sediments from 5 – 15 cm depth were added, methane started to build up (Fig. 2). Algal biomass was directly available for methane production, or the fresh organic matter enabled the production of methane from previously present organic compounds (priming) or CO_2. Methanotrophy may have been electron acceptor limited under the anoxic conditions,

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and could not consume all methane produced. Even though the sediments recovered directly after the establishment of anoxic conditions, and emitted methane, oxygen pulse additions did decrease the methane release from the algal inputs.

A similar effect of an oxic-anoxic switch was observed by (Frenzel, Thebrath, and Conrad 1990), who observed an abrupt increase in sedimentary methane emissions when the oxygen concentration in the water overlying their core experiments dropped below 18 μM. They assigned the difference between oxic and anoxic methane emissions solely to an increased activity of methanotrophs under oxic bottom water conditions.

Stable isotope analysis of the headspace methane in the stable, post-algal biomass degradation phase (17 weeks of oligotrophic, and 11 weeks of eutrophic experiments, Fig. 7), showed more negative $\delta^{13}CH_4$ values in the algal biomass experiments. The $\delta^{13}C$ signal of the algal biomass likely decreased the $\delta^{13}CH_4$ values in the algal addition experiments, with a larger effect in the oligotrophic lake, where the relative contribution of algal biomass was largest, compared to the organic matter already present in the sediments. Another potential factor is the shift in methanogenesis pathway due to the algal biomass availability. (Zhou et al. 2022) showed that cyanobacteria accumulation in lake sediments shifted the availability of organic compounds for methane production and increased the potential for methylotrophic methane production. Methylotrophic methanogenesis results in more depleted $\delta^{13}CH_4$ values compared to hydrogenotrophic methanogenesis (Summons, Franzmann, and Nichols 1998). When comparing the oxic and anoxic experiments, only the oligotrophic experiment showed significant differences: the $\delta^{13}CH_4$ values were lower (more

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682 depleted) in the anoxic than in the oxic incubations, both with and without algal
683 biomass additions. This could also be caused by differences in methanogenesis
684 pathways, as hydrogenotrophic methane production (from CO_2) yields more ^{13}C-
685 depleted methane than acetoclastic methanogenesis (Conrad 2005). As no changes
686 in the methanogenic community were observed between the oxic and anoxic
687 oligotrophic treatments, it is unlikely that a change in the community caused the
688 dominant methanogenesis pathway to swap and to cause the differences in the δ
689 $^{13}CH_4$ values. A further explanation is that differences in rates of methanotrophy
690 caused the observed changes in ^{13}C-compositions of methane. Indeed, increased
691 rates of methanotrophy under oxic conditions would be expected to contribute to a
692 less depleted isotopic composition of the remaining methane (Barker and Fritz 1981).
693
694 *Methane emissions and implications*
695 Sedimentation of (algal) biomass is a key factor in the magnitude and seasonal
696 variation in lake methane emission rates(Gruca-Rokosz and Cieśla 2021). Our
697 experiments with intact sediment cores, rather than slurries, showed a significant
698 decrease in methane emissions under oxic bottom water conditions compared to
699 anoxic bottom waters, similar to our slurry experiments. Algal biomass led to a strong
700 increase in methane emissions, which was dampened by oxygen exposure. Oxygen
701 was not actively mixed into the sediments: only the overlying water and headspace
702 were made oxic, oxygen penetration into the sediments was due to natural occurring
703 diffusion. Algal biomass was deposited on top of the sediments, and not mixed in
704 either, to mimic natural algal deposition. Both in the oxic and anoxic algal-addition
705 experiments, methane emission started almost immediately after algal biomass
706 addition (Fig. 3; Fig. S4). The weekly methane release was however lower under oxic

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conditions and resulted in lower concentrations at the end of the 15-week experiment, despite the methanogenic zone of the sediments (> 5 cm depth) not being in direct contact with either the oxygen or algal biomass.

Generally, only the sediment surface is affected by the oxygen conditions in the bottom water; deeper sediments are anoxic, due to the low diffusion coefficient through sediments. (Maerki et al. 2009) investigated the oxygen, carbon and nitrogen dynamics of lake sediments, and stated that short term (weeks to months) oxygen exposure is insufficient to change the reactivity spectrum of eutrophic Lake Zug sediments, that the exposure times are too short for that. Our whole core experiment shows, however, that despite the fact that the methanogenic layer is deeper in the sediments than the bottom water affected layer, the conditions in the bottom water are still of key importance for the methane emissions from the sediments following the deposition of algal material, for example after an algae bloom in the surface waters. (Maerki et al. 2009) also state that over 95% of the anaerobic mineralization in Lake Zug sediments was due to methanogenesis, and that methane oxidation was responsible for over half of the oxygen consumption at the sediment surface. If a similar situation is the case in our eutrophic Lake Baldegg, changes in the methane cycling are likely to have substantial effects on the carbon and oxygen cycling in the shallow sediments.

Our experiments show that the effects of a short (1-3 week) oxygen exposure can last for several months, i.e. decreasing methane emissions without changing the methane-related microbial community (Fig. 5, Fig. S6, S5, S10). We believe these findings should be further explored in environmental settings. In certain Swiss lakes,

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artificial aeration is already applied to combat bottom water anoxia. If brief pulses of oxygen, like the 1- and 3-week oxygen exposure periods tested here, have the capacity to reduce longer-term methane emissions, we believe this could be promising, especially if applied directly after an algal bloom, as tested here. Given the expectations of ongoing eutrophication in the upcoming decades, plus the global warming of lakes that further draws down oxygen levels, we believe this should be a topic for further research.

Data availability statement

Raw reads of the 16S rRNA sequencing data is deposited and made publicly available in the online repository NCBI SRA, under accession number XXX (in progress).

Author contribution statement

Conceptualization by SvG, MAL and CJS. Original draft preparation by SvG, review and editing by SvG, NM, CG, MAL and CJS. Investigation and Methodology by SvG, NM and CG.

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Competing interests

At least one of the authors is a member of the editorial board of BG. The authors have no further conflicts of interest to declare.

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