Warming and eutrophication interactively drive changes in the methane-oxidizing community of shallow lakes

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Freshwater ecosystems are globally the largest source of natural emissions of the greenhouse gas methane (CH4). They account for 43% (159 Tg CH4 year⁻¹) of total natural CH4 emissions, of which about 70% originates from lakes, especially shallow lakes. Currently, many freshwater ecosystems are changing due to a combination of climate warming and increased phosphorus and nitrogen loading, which is expected to increase shallow lake CH4 emissions. In shallow lakes, methanogenic archaea produce CH4 predominantly in anoxic sediments. Subsequently, up to 90% of this CH4 is oxidized by methanotrophic microorganisms, mostly methane-oxidizing bacteria (MOB), which are abundant in the oxic top layer of the sediment. Therefore, MOB are key in mitigating methane-oxidizing community of shallow lakes.

While it is known that eutrophication and warming lead to increased CH4 emissions,4,5 we lack a mechanistic understanding of the response of MOB communities to these stressors. Limitation and excess of nitrogen (N) and phosphorus (P) may significantly affect MOB activity depending on species-specific traits, while rising temperatures have been found to enhance methane oxidation potential. However, we do not yet know their combined effect on methane oxidation, and we particularly lack insight into the changes in MOB community composition as a result of these disturbances. Since MOB communities comprise a diverse set of microorganisms possessing different functional traits and roles, understanding the changes in the MOB community composition will help to understand the dynamics of methane oxidation in lake sediments.

The two largest groups of aerobic MOB are type I (Gammaproteobacteria) and type II (Alphaproteobacteria) MOB. Several studies have shown that type I and II MOB occupy different niches. While type I MOB are generally favoured by high methane and nutrient availability, type II have been found to be more resilient to disturbance and thrive under oligotrophic conditions. However, these characteristics were not explicitly linked to functioning of the community, most importantly methane oxidation potential. How the relative abundance of type I and II MOB affects methane oxidation potential will shed new light on the mitigation capacity of MOB communities. Here, we aim to identify how MOB communities change in response to eutrophication and warming and how that impacts the methane oxidation potential of freshwater sediments.

We sampled the sediment of a long-running lake mesocosm experiment to test the combined and separate effect of temperature and nutrients on methane oxidation potential, MOB...
abundance and MOB community composition. The setup consisted of two nutrient treatments (N+P added or not added) and three temperature treatments (ambient, +2–3 °C and +4–5 °C). To test for specific effects of N-availability, the year before sampling no N was added, while weekly P-addition in the high nutrient treatments continued. We sampled the top 4 cm of the sediment four days before N-addition was resumed (week -1, June), two months after (week 8, August) and one year after (week 52, June). We measured methane oxidation potential in bottle incubations and estimated MOB abundance by qPCR targeting the pmoA gene using the A189F and mmb661R primers. While these primers omit some MOB, in particular Verrucomicrobia and NC10, they target the majority of proteobacterial MOB. MOB community composition was determined by 16S rRNA gene amplicon sequencing and analysed in the dada2 pipeline using the Silva v138 database to assign taxonomy. In addition, we calculated the type I:type II MOB ratio and measured diffusive and ebullitive CH4 fluxes and a range of physicochemical properties of the sediment and water column (Supplementary methods, Figs. S1–11).

MOB abundance was significantly affected by nutrients (p = 0.018), N-addition (p = 0.044) and temperature (p < 0.001, Fig. 1A, Table S1A, three-way mixed ANOVA). MOB abundance increased two months after resuming N-addition (p = 0.012), and was higher in warmed mesocosms than at ambient temperature (p < 0.0001 for both +2–3 °C and +4–5 °C, Table S1B). Methane oxidation potential was also affected by nutrients (p = 0.002) and N-availability (p < 0.0001) but not by temperature (p = 0.292, Fig. 1B, Table S2), and therefore did not follow the temperature-response of MOB abundance, leading to a lower apparent cell-specific activity with increased temperature (p < 0.0001, Fig. 2A, Table S3).

NMDS analysis showed that MOB communities clustered based on temperature and nutrients (Fig. 2B). The fact that the community clustered more based on temperature and nutrients than sole N-addition, indicates that in this experiment community composition is likely mostly influenced by long-term processes. Temperature (p < 0.0001), nutrients (p = 0.001) and N-addition (p < 0.0001) significantly affected community composition, with temperature having the strongest effect, based on PERMANOVA analysis (Table S4A). Warmed treatments had a significantly different community composition from the ambient treatment in both nutrient treatments (Table S4B).

Eutrophication increased relative abundance of Methylopara-
coccus (Fig. 2C, Table S5). Warming shifted MOB communities from...
type I dominance, in particular because of high relative abundance of type IA Methylomonadaceae, to type I and II co-dominance, mostly due to an increase in Methylocystis, decreasing the type I:type II ratio ($p < 0.0001$, Fig. S1, Tables S6, S7). This coincided with a decrease in apparent cell-specific activity (Fig. 2A), indicating a potential mechanistic effect.

A recent study showed that slow-growing microorganisms are more competitive at higher temperatures, and are expected to be favoured by global warming. Indeed, type II MOB generally exhibit a more oligotrophic, slow-growing life strategy than type I MOB, and are resistant to high temperatures, potentially explaining their increased relative abundance at higher temperature. Also, slower-growing oligotrophs have a higher yield per molecule of substrate and take up substrate at a lower rate than fast-growers. Thus, in a community dominated by slow-growing organisms, the total amount of substrate used per day per organism is lower than in a community dominated by fast-growers. This may explain why the shift to type II MOB coincided with lower apparent cell-specific activity, and why the increased MOB abundance in warmed mesocosms, which was mostly due to an increase in type II MOB, did not increase methane oxidation potential.

In contrast, N-addition increased methane oxidation potential. The effects of N have been found to be dose-dependent, substrate dependent, and species-dependent. N additions can either relieve MOB from N-limitation, or inhibit methane oxidation potential. Here, N-dependent stimulation of methane production, as shown by increased methane emissions (Fig. S2), likely fuelled the higher methane oxidation potential, in concert with relieved N-limitation of the MOB.

In conclusion, our results show important differential effects of eutrophication and warming on MOB communities. While warming shifts lake sediments from type I MOB dominance to type I and II MOB co-dominance without affecting overall
community activity, methane oxidation potential is enhanced by eutrophication. To accurately predict effects of these MOB community shifts on CH4 emissions, future studies should also address interactive warming and eutrophication effects on methane producing communities.

DATA AVAILABILITY
Experimental data are available at the Dryad Digital Repository (https://doi.org/10.5061/dryad.j9w0w0q01). Sequencing data are deposited at the European Nucleotide Archive under accession number PRJEB43466.

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COMPETING INTERESTS
The authors declare no competing interests.

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