frontiers | Frontiers in Environmental Science

BRIEF RESEARCH REPORT
published: 01 April 2022
doi: 10.3389/fenvs.2022.804512

Relief of Phosphate Limitation Stimulates Methane Oxidation

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Aquatic ecosystems such as shallow lakes and wetlands are important emitters of the greenhouse gas methane (CH_4). Increased phosphorus (P) loading is expected to increase CH_4 production in these ecosystems. This increased CH_4 production can potentially be mitigated by increased CH_4 oxidation, but how P availability affects methane-oxidizing bacterial (MOB) community composition and potential CH_4 oxidation remains to be tested. Here, we incubated MOB from sediments of four subtropical lakes of different trophic states for 7 days at different phosphate (PO_4^{3-}) concentrations to determine the effects of P on MOB community composition and potential CH_4 oxidation. We measured CH_4 consumption daily and compared CH_4 oxidation during the exponential growth phase. Furthermore, we determined MOB community composition at the end of the incubations using qPCR of the *pmoA* gene. To test for differences in N and P uptake, we determined bacterial biomass N and P content. We found that increases in PO_4^{3-} concentrations until 10 µM significantly increased CH_4 oxidation. PO_4^{3-} also increased bacterial biomass P content, while N content was not affected. MOB community composition was not affected by PO_4^{3-} but more strongly correlated to lake of origin, likely due to the short duration of the incubations. Our results show that PO_4^{3-} can not only stimulate CH_4 oxidation indirectly through increased CH_4 production, but also directly by increasing MOB growth. Importantly, these effects only occur at low PO_4^{3-} concentrations, indicating that at high nutrient loads the increased CH_4 oxidation will likely not mitigate the increased CH_4 production.

Keywords: methane oxidation, phosphate, eutrophication, methane, aquatic sediment, freshwater, methanotroph, microbial community composition

OPEN ACCESS

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Specialty section:
This article was submitted to Biogeochemical Dynamics, a section of the journal Frontiers in Environmental Science

Received: *29 October 2021*
Accepted: *17 February 2022*
Published: *01 April 2022*

Citation:
Nijman TPA, Amado AM, Bodelier PLE and Veraart AJ (2022) Relief of Phosphate Limitation Stimulates Methane Oxidation. Front. Environ. Sci. 10:804512. doi: 10.3389/fenvs.2022.804512

INTRODUCTION

Methane (CH_4) is the second most important greenhouse gas, contributing to 16–25% of global warming (IPCC, 2014). Microbial processes in aquatic ecosystems, such as wetlands and shallow lakes, are the main source of this greenhouse gas, accounting for over 50% of global CH_4 emissions (Rosentreter et al., 2021). Currently, the nutrient status of many shallow lake ecosystems is changing because of eutrophication, which is expected to increase shallow lake CH_4 emissions (Aben et al., 2017; Davidson et al., 2018), mostly driven by phosphorus (P) and nitrogen (N) enrichment in water and sediment (Correll, 1998). While interactions between N cycling and CH_4 emissions have been well studied (Bodelier and Steenbergh, 2014), we still lack understanding about the effects of P on CH_4 emissions.

CH_4 cycling in shallow lakes is largely driven by the sediment microbiome. In the anoxic zone of the sediment, methanogens produce CH_4 (Conrad, 2007). Subsequently, a large part of the produced CH_4 is mitigated by methane-oxidizing bacteria (MOB), which consume CH_4 mostly aerobically, converting CH_4 into CO_2. In freshwater ecosystems with high concentrations of alternative electron acceptors, such as nitrate and sulfate, CH_4 can also be converted anaerobically (Deutzmann, 2020). This study focuses on aerobic MOB, which belong to the Gammaproteobacteria (type I), Alphaproteobacteria (type II), and Verrucomicrobia, of which type I and type II MOB are dominant in most freshwater lakes. These MOB differ in their phylogeny, physiology, biochemistry, and morphology (Semrau et al., 2010). While both perform the same process—CH_4 oxidation—they prefer different environments. Type I MOB are most abundant in stable environments with high substrate availability while type II are most abundant in environments with disturbances such as heat waves and desiccation and low substrate availability (Ho et al., 2013). CH_4 oxidation by MOB is the sole biological CH_4 sink (Adamsen and King, 1993), and it is therefore important to know how MOB react to disturbances such as eutrophication.

The accumulation of phosphate (PO_4^{3-}) in aquatic ecosystems can affect CH_4 oxidation. In a field study, higher potential CH_4 oxidation rates were found in sediments that had higher PO_4^{3-} concentrations (Veraart et al., 2015). Increased [PO_4^{3-}] is also hypothesized to indirectly affect CH_4 oxidation rates by changing the MOB community. It has been suggested that type I MOB thrive in eutrophic environments with high [PO_4^{3-}] while type II MOB compete better in more oligotrophic environments with low [PO_4^{3-}] (Ho et al., 2013). One of the reasons for this is that type II MOB generally harbor more enzymes which liberate P from organic molecules and have membranes with lower P content (Veraart et al., 2015). Changes in the dominant type of MOB can potentially also lead to changes in CH_4 oxidation rates (Reis et al., 2020; Nijman et al., 2021), thereby altering the mitigation capacity of the MOB community.

Only one study has addressed the direct relationship between [PO_4^{3-}] and MOB functioning (Sawakuchi et al., 2021). In this study, surface water MOB from ice-covered lakes oxidized more CH_4 at 500 μg/L (~5 μM) PO_4^{3-} than without PO_4^{3-} in long-term incubations at 4 °C. However, the specific conditions of this experiment make it difficult to apply to MOB from other ecosystems. Most important, the dominant MOB in the ice-covered lake experiment are very rare outside of arctic regions (50.3% *Methylobacter tundripaludum* and 44.6% *Methylovulum psychrotolerans*). Second, the CH_4 oxidation rates were very low (2.5–29.4 μmol CH_4 L^{-1} d^{-1}), compared to many other MOB communities, such as those in lake sediments (e.g. Shelley et al., 2015). Finally, the question arises whether higher PO_4^{3-} concentrations would further increase potential CH_4 oxidation rates, and up to which concentration PO_4^{3-} could still be the limiting growth factor for MOB.

Therefore, we still lack a good understanding of the direct relationship between [PO_4^{3-}] and CH_4 oxidation. Other studies of MOB, which showed effects of PO_4^{3-} on CH_4 oxidation rates (Veraart et al., 2015) and on the type of MOB (Ho et al., 2013), were based on correlative field observations, and thus only showed an indirect relationship between [PO_4^{3-}] and MOB functioning. Since many biogeochemical variables are correlated, the relationship between [PO_4^{3-}] and CH_4 oxidation found in these studies could also have been due to other variables, such as organic matter content (Czepiel et al., 1995) or N availability (Bodelier and Laanbroek, 2004). Furthermore, since increased [PO_4^{3-}] stimulates lake CH_4 emissions, likely through enhanced CH_4 production (Aben et al., 2017; Davidson et al., 2018), oxidation may also be indirectly stimulated by PO_4^{3-} due to higher CH_4 availability. However, considering that bacteria need P for growth, and especially type I MOB since they contain phospholipid-rich stacked membranes (Bodelier et al., 2009), it is likely that PO_4^{3-} also directly affects MOB, especially at low PO_4^{3-} concentrations.

Here, we investigated the effect of PO_4^{3-} concentration on potential CH_4 oxidation and MOB community composition. We expected increases in PO_4^{3-} concentration would increase potential CH_4 oxidation. Furthermore, we expected that increases in PO_4^{3-} concentration would favor type I MOB over type II MOB.

METHODS

To test the effects of [PO_4^{3-}] on potential CH_4 oxidation and MOB community composition, we incubated MOB from four different subtropical lake sediments in 48 microcosms at different [PO_4^{3-}], analyzing CH_4 oxidation rates during exponential growth and final MOB community composition (**Figure 1**).

Sediment Sampling and Inoculum Preparation

Four subtropical lake sediments in south-eastern Brazil were sampled in april 2019 to obtain different MOB communities for the microcosms (**Supplementary Table S1**). We sampled Funil lake (latitude, longitude: 2231′31″S, 44°33′1″W), Manacás lake (2146′43″S, 4322′8″W), Botanical Garden lake of Universidade Federal de Juiz de Fora (UFJF; 2143′55″S, 4322′14″W), and Chapéu D'Uvas lake (CDU; 2134′55″S, 4333′16″W). The lakes were chosen because of their different trophic state. Funil lake (Pacheco et al., 2015) and Manacás lake (Lawall et al., 2005) are eutrophic, UFJF botanical garden lake is mesotrophic (Nunes et al., 2021) and CDU lake is oligotrophic (Almeida et al., 2019). We took one sediment core from each lake with a Uwitech corer (ø 60 mm), of which we sliced off the top 2 cm (~56.5 ml), and used those for the microcosms. While one core is not enough for a representative picture of the community of a single lake sediment, the use of single cores from four different lakes allowed us to investigate the relationship between [PO_4^{3-}] and CH_4 oxidation for MOB communities with different community composition.

The sediments were used to prepare inocula for the microcosms. The sliced sediment was first diluted with lake water from the corresponding lake to obtain a total volume of

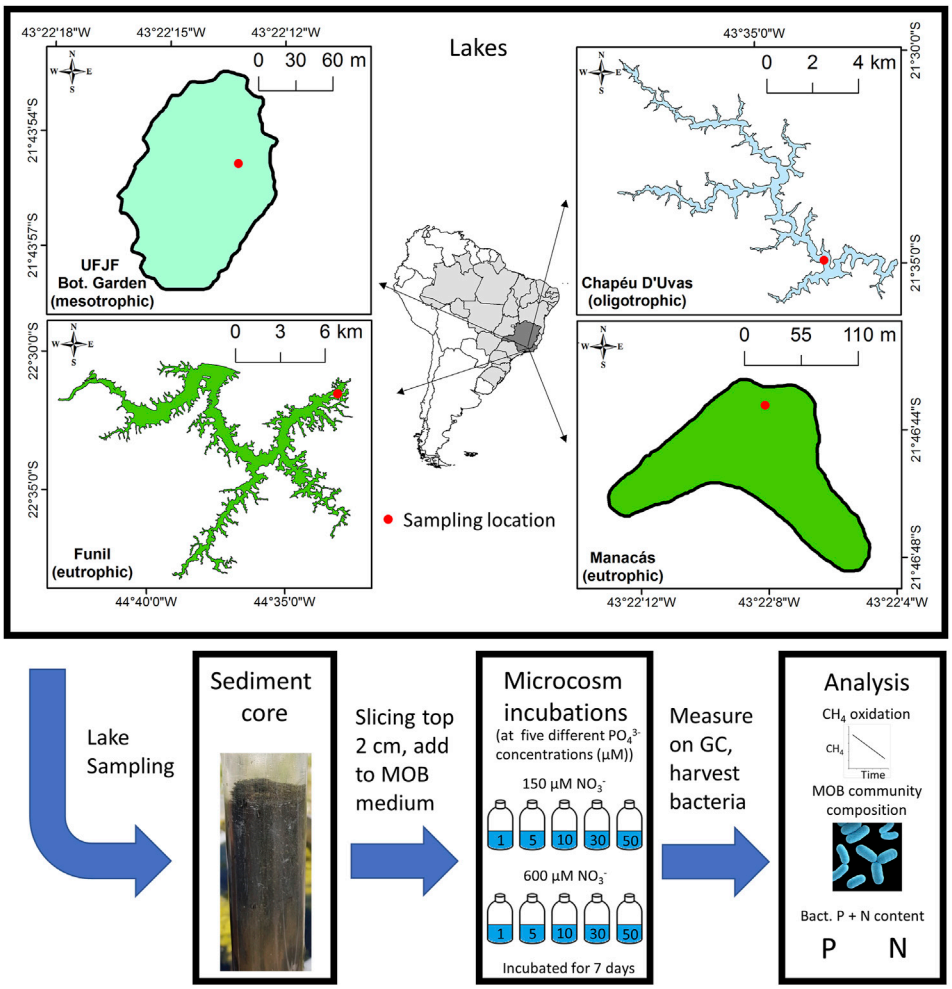

FIGURE 1 | Sampling sites and experimental design.

150 ml, and homogenized using a blender. While blending could have lysed some cells, the low speed of the blender was likely not enough to significantly affect the microbial community. After mixing, the suspended sediment was pipetted into 15 ml falcon tubes and centrifuged at 4,000 rpm for 5 min. Then, the supernatant, containing a large number of bacteria, was taken. The supernatant was filtered through 1.2 μm glass fiber filters to remove most protozoa and remaining sediment particles, and the filtrate was used as inoculum for the microcosms.

Microcosms

We used microcosms to test how [PO_4^{3-}] affected MOB growth and community composition. We prepared Nitrate Mineral Salts (NMS) medium specific for MOB (Whittenbury et al., 1970, ATCC 1306) with 10 μM copper and five different PO_4^{3-} concentrations (1, 5, 10, 30, and 50 μM, added as KH_2PO_4 and $NaHPO_4$ in a 1:2.5 ratio). We also used two different NO_3^- concentrations (150 and 600 μM, added as KNO_3) to control for effects of N and N:P ratio, and included a negative control (0 μM PO_4^{3-} and 0 μM NO_3^-, no inoculum added) and positive control (1 mM PO_4^{3-} and 1 mM NO_3^-), resulting in twelve microcosms per lake. The PO_4^{3-} and NO_3^- concentrations were chosen based on comparable concentrations in mesotrophic to eutrophic freshwater sediments (Geurts et al., 2008; Veraart et al., 2015). We ran one series of microcosm experiments per lake, yielding four replicates per combination of [PO_4^{3-}] and [NO_3^-]. Microcosms were prepared by adding 57 ml NMS medium and 3 ml inoculum of the respective lake to 120 ml serum bottles. Bottles were closed with butyl stoppers and capped, after which 10% of the total headspace was replaced with CH_4 (purity grade 4.5). This left an oxygen (O_2) concentration of 18.9%. We incubated the microcosms in the dark for 7 days at 20°C, while stirring continuously. Because MOB use O_2 in a 2:1 ratio to oxidize CH_4, there was not enough O_2 in the microcosms to completely consume all CH_4. However, we found that in all microcosms, the [CH_4] still decreased after the time of exponential growth, indicating that O_2 was not yet depleted. It was also unlikely CH_4 decreased because of anaerobic oxidation of methane (AOM), as described below.

Since the microcosms had a much higher O_2 concentration than is present in sediments (Wang et al., 2014), we did not aim to show how sediment MOB would respond to increased [PO_4^{3-}]. Rather, the aim was to show how model MOB communities would respond to different PO_4^{3-} concentrations. Using an initially high [O_2] had two advantages. First, it allowed the MOB to reach exponential growth because they could consume the majority of CH_4 before O_2 would become limiting. Second, it prevented AOM and methanogenesis. For AOM, 2% O_2 decreased CH_4 oxidation of '*Candidatus* Methylomirabilis oxyfera' by 60% within 22 h without recovery (Kampman et al., 2018), while 5% O_2 caused '*Candidatus* Methanoperedens nitroreducens' to be outcompeted by *Methylobacter*, an aerobic MOB (Guerrero-Cruz et al., 2018). Also, while some methanogens can survive O_2 exposure (Peters and Conrad, 1995), these only showed activity after 15 days without O_2. Therefore, it is unlikely there was AOM or methanogenesis in the microcosms.

To determine CH_4 consumption rates, we measured CH_4 concentration in the headspace directly after CH_4 addition and once per day afterwards. The CH_4 concentration was measured by taking 1 ml of the headspace and injecting into a Bruker 450-GC (Bruker Technologies, Australia) equipped with a flame ionization detector. The concentration was calculated by comparing to a standard curve. Furthermore, 1 ml of medium was taken daily for MOB analysis, although these samples were not used because the number of cells was too low for DNA extractions.

CH_4 Oxidation During Exponential Growth

To estimate CH_4 oxidation rates, we calculated the rate at which CH_4 decreased per day per ml of medium, accounting for removal of water and air from the microcosms (supplementary methods). Surprisingly, there was no CH_4 oxidation in the microcosms of mesotrophic UFJF botanical garden lake. Little data on this lake are available thus far, but potential MOB inhibitors such as low pH, high NH_4^+ and humic substance concentration have not been observed. However, one possibility is the lakes' high oxygen concentration (11 mg/L, Nunes et al., 2021) may have prevented most CH_4 production, exemplified by very low CH_4 emissions (3.39 mg CH_4 m^{-2} day^{-1} September 2021, personal communication Nasário and Barros), and thereby the development of an active MOB community, which remains to be experimentally confirmed. Because of the lack of CH_4 oxidation, we removed those microcosms from our analysis. For the remaining microcosms, we used the time of exponential growth to compare CH_4 oxidation at different PO_4^{3-} concentrations, based on the day with the largest decrease. All incubations had >0.75% CH_4 (12 μM CH_4 in the water) left after this day, which is not yet limiting (Shelley et al., 2015). Exponential growth occurred 1 day earlier for the eutrophic sediments than for the oligotrophic sediment (**Supplementary Figure S4**).

MOB Community Composition

To evaluate MOB community composition, we harvested the bacteria from the microcosms after 7 days. Thirty ml of the final medium was filtered through 0.2 μm filters, using half for DNA extractions. Filters of the positive control were used to test the DNA extraction method, while the negative control was not filtered as there was no bacterial growth. Four filters were accidently lost during transportation and the remaining 26 filters were used for qPCR. Bacteria were scraped off the filters using sterile cell scrapers (Greiner Bio-One 541070) and DNA was extracted with the DNeasy PowerSoil kit (Qiagen 12888-100) according to manufacturer's instructions with one modification: bead-beating was performed using a Powerlyzer for 45 s at 2,500 rpm. We then determined copy number of type Ia (*Methylobacter*, *Methylomonas*), type Ib (*Methylocaldum*, *Methylococcus*) and type II (*Methylosinus*, *Methylocella*, *Methylocystis*) MOB by qPCR using different primers for the *pmoA* gene, according to Kolb et al. (2003) with some small modifications (**Supplementary Table S2**), using iQ SYBR® Green Supermix as mastermix. All samples and standards were measured in triplicate on a BioRad iQ5 Multicolour Real-Time PCR detection system (Vers. 2.0, BioRad, Gothenburg Sweden), on which we checked the melt curves.

Bacterial Biomass N and P Content

To investigate whether the PO_4^{3-} concentrations changed the N and P content of the MOB, we filtered 10 ml of the final medium through two 0.7 μm glass fiber filters per microcosm. Filters were weighted before and after filtering, and bacterial N and P concentration was determined from the bacterial biomass retained on the filters after potassium persulfate digestion (Carmouze, 1994). N concentration in bacterial biomass was measured on a total organic carbon analyzer, equipped with a TNM-1 total nitrogen module (TOC-V CPN, Shimadzu), whereas P in bacterial biomass was measured spectrophotometrically (Lambda 365 UV/VIS, Perkin Elmer) by the ascorbic acid method (Mackereth et al., 1979). Filters were pre-combusted (550°C for 4 h) before filtration and oven dried before analysis (60°C for >24 h). The filtration method for microbial biomass nutrient contents using 0.7 μm pore size filters may not account for all the microbial community - smaller cells may pass through filters - but a previous study showed an average 80% retention of bacterial community using this pore size (They et al., 2017). Because the main available carbon substrate for microbial growth was CH_4, and because we used NMS medium specific for MOB, we assumed that MOB were the predominant bacteria, and hence bacterial biomass N and P content was closely related to MOB N and P content. Also, because most CH_4 was consumed in all incubations, similar total growth of MOB had taken place, indicating that N and P content per cell were related to total N and P content of the bacterial community. However, these results should be interpreted with caution, because P could also have formed suspended particles, increasing P content of the filters.

Statistical Analysis

Statistical analyses were performed in R 3.6.3 (R Core Team, 2019). To analyze how [PO_4^{3-}] affected CH_4 oxidation during exponential growth, we calculated potential CH_4 oxidation rates at different [PO_4^{3-}] (supplementary methods). Next, we

determined which model fit the data best to compare effects of $[PO_4^{3-}]$ on CH_4 consumption for the different lakes. We tested a linear regression, a nonlinear least-squares regression using the *nls* function, and a segmented regression using the *segmented* function (Muggeo, 2003). Based on a comparison of AIC values (**Supplementary Table S3**), the best-fitting model was the nonlinear least-squares regression, which we further analyzed. We also compared the nonlinear least-squares regression for 150 and 600 µM NO_3^- separately (**Supplementary Figure S1**), but since the differences between these models were small, we finally used one model combining all data.

To determine at which PO_4^{3-} concentrations CH_4 oxidation rates during exponential growth significantly differed from each other, we determined the confidence intervals (CIs) of CH_4 oxidation at different PO_4^{3-} concentrations and calculated the *p*-value at which CIs of different concentrations overlapped.

Relative abundance of different MOB types was calculated by dividing the copy number per MOB type by the copy number of all MOB. To achieve normality, the proportions of MOB type Ia and II were logit transformed while the type Ib proportion was log transformed. Linear models were used to test the effects of $[PO_4^{3-}]$ $[NO_3^-]$, and lake of origin on relative abundance of different MOB types, and the *emmeans* function (Lenth et al., 2020) was used for post-hoc between-lake comparisons. We also inspected the relationship between N:P ratio and proportions of MOB (**Supplementary Figure S2**), but found no clear patterns.

Bacterial P content was log-transformed to achieve normality and linear models were used to test the effects of NO_3^- and PO_4^{3-} on bacterial N content and log-transformed bacterial P content. A similar model comparison to CH_4 oxidation showed the best fitting model was a linear regression rather than a nonlinear least-squares regression or segmented regression (**Supplementary Table S4**). Similar to CH_4 oxidation, we compared the models for 150 and 600 µM NO_3^- separately (**Supplementary Figure S3**), but finally decided to combine all data since the differences between the models were very small.

RESULTS

To investigate the effects of $[PO_4^{3-}]$ on model MOB communities, 30 microcosms from three different lakes (Manacás, Funil, CDU) were incubated at five different PO_4^{3-} concentrations (1–50 µM), while a fourth lake (UFJF botanical garden) yielded no MOB growth and was therefore removed from the analysis.

CH_4 oxidation increased with $[PO_4^{3-}]$ (**Figure 2**). Interestingly, PO_4^{3-} stimulated CH_4 oxidation during exponential growth mostly at low $[PO_4^{3-}]$ up to 10 µM, as shown by the good fit of the nonlinear least-squares regression to the CH_4 oxidation data (**Supplementary Table S3**). Analysis of confidence intervals showed that CH_4 oxidation at 1 µM PO_4^{3-} was significantly lower than at all higher concentrations (5 µM: $p = 0.0036$; 10, 30, and 50 µM: $p < 0.0001$) while CH_4 oxidation at 5 µM PO_4^{3-} was significantly lower than at 30 µM ($p = 0.0051$) and 50 µM PO_4^{3-} ($p = 0.0039$), and slightly lower at 10 µM PO_4^{3-} than at 50 µM PO_4^{3-} ($p = 0.048$). There was no effect of $[NO_3^-]$ or lake of origin on CH_4 oxidation.

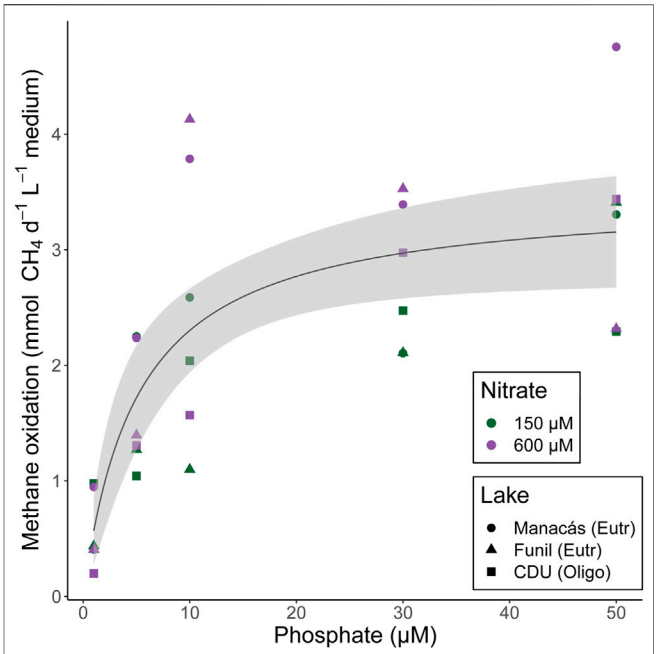

FIGURE 2 | CH_4 oxidation per day per ml of medium at different $[PO_4^{3-}]$ and $[NO_3^-]$ during exponential growth phase of MOB from Manacás lake, Funil lake, and CDU lake. Fitted line according to nonlinear least-squares regression, shading represents 95% confidence interval. Individual measurements are shown, colours represent $[NO_3^-]$ treatments, shapes represent lake of origin, $n = 30$.

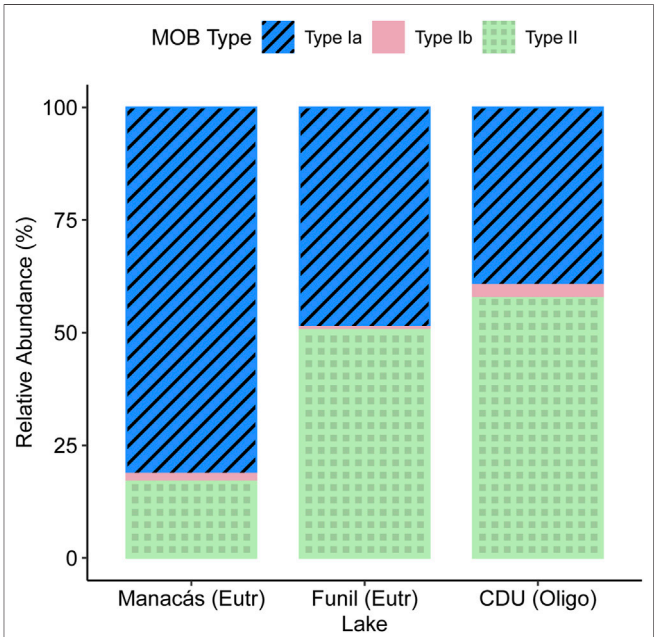

FIGURE 3 | proportional abundance of different types of MOB per lake based on qPCR copy number of the *pmoA* gene at the end of microcosm incubations. Different colours represent different types of MOB, $n = 26$.

The change in CH_4 oxidation did not coincide with changes in relative abundance of different MOB types, which were mostly influenced by the lake of origin (**Supplementary Table S5**;

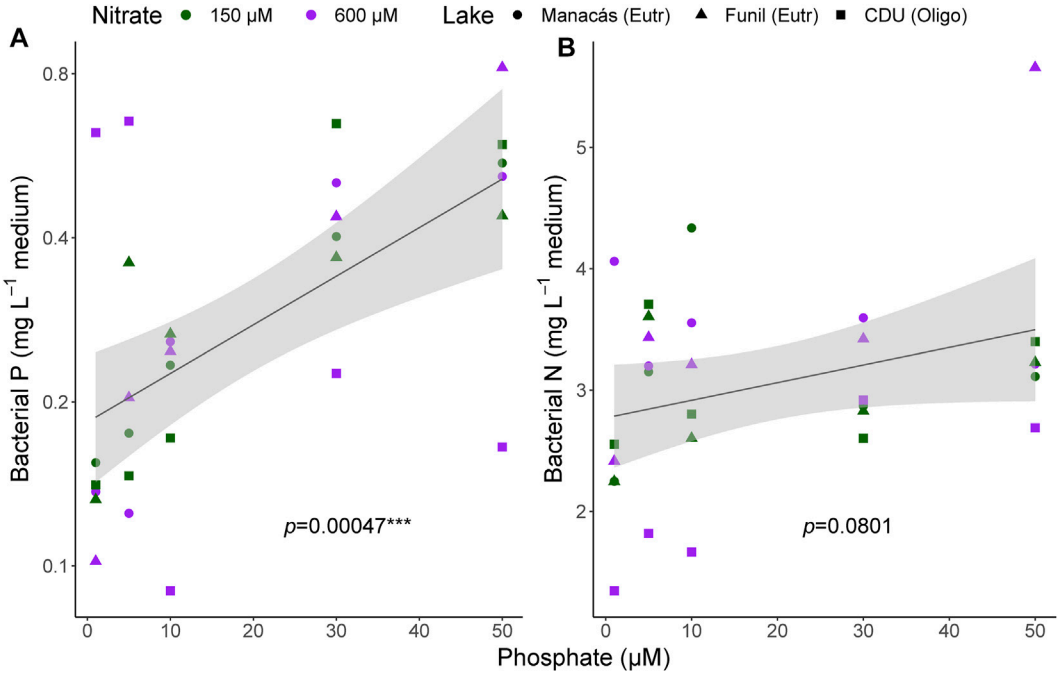

FIGURE 4 | Bacterial P (log-transformed, **(A)** and bacterial N **(B)** content per litre of medium at the end of the microcosm incubations. Fitted line according to linear regression, shading represents 95% confidence interval. Individual measurements are shown, colours represent [NO_3^-] treatments, shapes represent lake of origin, n = 30.

Figure 3). Most MOB were either type Ia (58.8%) or type II (39.6%), while only a small percentage was type Ib (1.7%). Type Ia MOB were more abundant in the Manacás microcosms than in the CDU microcosms ($p < 0.05$) but not influenced by [NO_3^-] or [PO_4^{3-}] concentrations. Type II MOB showed the opposite trend and were more abundant in the CDU lake microcosms than in the Manacás microcosms ($p < 0.05$), while also not being affected by [NO_3^-] or [PO_4^{3-}]. Type Ib were more abundant in the Manacás and CDU microcosms than in the Funil microcosms ($p < 0.05$ for both, **Supplementary Table S5**). Interestingly, the number of type Ib MOB significantly decreased at higher [NO_3^-] ($p = 0.05$, **Supplementary Table S5**) but since the abundance of type Ib was relatively low, this had little impact on overall community composition.

Bacterial P content showed a strong relationship with [PO_4^{3-}] ($p = 0.00047$, **Figure 4A**), while bacterial N content (**Figure 4B**) was not influenced by either [NO_3^-] or [PO_4^{3-}]. In contrast to CH_4 oxidation rates, the increase in bacterial P content was also observed at high [PO_4^{3-}] and not only at low concentrations, as there was a linear relationship between the log transformed bacterial P content and [PO_4^{3-}].

DISCUSSION

In our microcosm experiment, we found that increased [PO_4^{3-}] led to an increase in potential CH_4 oxidation rates, in agreement with our hypothesis. This was likely because MOB incorporated more P to increase their growth rates, as shown by the increase in bacterial P biomass content at higher [PO_4^{3-}]. However, the [PO_4^{3-}] did not affect the relative abundance of different types of MOB.

PO_4^{3-} increased CH_4 oxidation during exponential growth in a nonlinear way, indicating that PO_4^{3-} was likely the limiting growth factor for the MOB communities at low concentrations. Increasing PO_4^{3-} to concentrations up to 10 µM relieved MOB from PO_4^{3-} limitation and thereby increased their growth. This is in line with previous research showing higher rates of CH_4 oxidation in systems with higher PO_4^{3-} availability (Veraart et al., 2015; Nijman et al., 2021). Increased P content suggested that bacteria used more P at higher PO_4^{3-}, possibly to invest in rRNA to increase their growth rate - as suggested in the growth rate hypothesis (Elser et al., 2000) - thereby increasing CH_4 oxidation rates. At PO_4^{3-} concentrations above 10 µM, P on the filters still increased but this did not coincide with increased CH_4 oxidation, possibly because bacteria stored the excess PO_4^{3-} in organic and inorganic molecules inside the cytoplasm (Kulakovskaya, 2014) rather than investing in new cells. Likely, another limiting factor was preventing faster growth of MOB, such as copper (Semrau et al., 2010) or maximum growth rate (Ho et al., 2013). Future studies could investigate if PO_4^{3-} concentrations lower than 1 µM, which are common in surface waters (Geurts et al., 2008), limit CH_4 oxidation even more.

It is not clear whether the response of the model MOB communities in our experiment would be comparable to the response of sediment MOB communities, as those generally experience much lower O_2 concentrations (Wang et al., 2014). Interestingly, MOB communities in the water column have the highest growth rates at low [O_2], and the question remains whether the effects of PO_4^{3-} that we found in this experiment would also be found at low [O_2]. A future experiment at lower initial [O_2], in which [O_2] is closely monitored and added when necessary, could give further insight into these dynamics.

Since the increased CH_4 oxidation in the microcosms was only apparent at low PO_4^{3-} concentrations, the direct effect of PO_4^{3-} on CH_4 oxidation might contribute little to the CH_4 cycle in highly eutrophic lakes. Rather, these lakes might be more strongly influenced by an increase in methanogenesis because of higher algal carbon decomposition (West et al., 2012) and decreased oxygen (Bastviken et al., 2004). If MOB in sediments are stimulated by PO_4^{3-} in a comparable way to those in the microcosms, that may be one of the reasons why CH_4 emissions are higher in eutrophic lakes (Aben et al., 2017; Davidson et al., 2018), while MOB are a more effective CH_4 filter in mesotrophic lakes where they still benefit from increased PO_4^{3-} concentrations (Veraart et al., 2015).

Our results are in line with the increased CH_4 oxidation rates found in P-amended incubations of surface water from ice-covered lakes (Sawakuchi et al., 2021). In both studies, adding 5 μM PO_4^{3-} increased CH_4 oxidation rates. In contrast, the rates in our experiment were on average about 100-fold higher (0.19–4.75 mmol CH_4 d^{-1} L^{-1} vs. 0.0025–0.029 mmol CH_4 d^{-1} L^{-1}), and type II MOB were far more abundant (40% vs. <5%). That is likely because our model communities originated from freshwater sediments, which often harbour highly active MOB communities (Oremland and Culbertson, 1992).

Increased [PO_4^{3-}] had little effect on MOB community composition, which was more related to the lake of origin. The eutrophic Manacás lake had a higher proportion of type Ia MOB, while the oligotrophic CDU lake had more type II MOB. This is in line with predictions that type I MOB are better competitors and thrive in high nutrient environments, while type II are better stress-tolerators that can deal with low nutrient conditions (Ho et al., 2013). However, the fact that MOB community composition did not change in response to [PO_4^{3-}] was in contrast with these predictions. Most likely, the short duration of 1 week for our experiment explains this result, which was too short to change MOB community composition. Future research could simulate MOB growth at different [PO_4^{3-}] for a longer period to see possible shifts in community composition.

The microcosms did not show relationships between N:P ratio and CH_4 oxidation, bacterial N and P content, and MOB community composition (**Supplementary Figures S1–3**). Likely, this was because [PO_4^{3-}] was limiting and therefore driving most changes in the microcosms, while changes in MOB community composition would require more time. A future experiment using concentrations of NO_3^- and PO_4^{3-} that are not limiting, e.g., 100–600 μM NO_3^- and 10–100 μM PO_4^{3-}, could provide further insight into the effects of N:P ratio on CH_4 oxidation.

To conclude, we show that MOB originating from lake sediments can be PO_4^{3-} limited and show a nonlinear response to increased PO_4^{3-} concentrations, indicating that they can potentially mitigate increased methanogenesis in mesotrophic lakes that become more eutrophic.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: (10.17026/dans-x59-warf) (DANS EASY).

AUTHOR CONTRIBUTIONS

TN, AA, and AV designed the experiment. TN and AA carried out the practical work, with support from PB for the qPCR analyses. All authors discussed the results and contributed to the final manuscript.

FUNDING

AV was funded by Aquafarm 2.0. This work was partly funded by an Erasmus + Grant awarded to TN, by Brazil's National Council for Scientific and Technological Development (CNPq), for providing Productivity grants to AA (PQ 310033/2017-9 and PQ 312772/2020-2), and by the PROPP-UFJF Reverse Sandwich Program.

ACKNOWLEDGMENTS

We thank Icaro Barbosa for sampling Chapéu D'Uvas and helping with slicing, Layla Fonseca, Alisa Shubina, and Annemiek Tiekstra for help with molecular analyses, José Paranaiba for help plotting the sampling locations, Jonas Nasário and Nathan Barros for providing CH_4 emission data of the UFJF botanical garden lake, and Fons Smolders for advising on porewater PO_4^{3-} concentrations.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.804512/full#supplementary-material

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