



## INORGANIC NITROGEN STIMULATES METHANE OXIDATION IN COASTAL LAGOON SEDIMENTS

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**Abstract:** Methane (CH<sub>4</sub>) oxidation is a critical process to reduce CH<sub>4</sub> emissions from aquatic environments to the atmosphere. Considering the continuous increase in nitrogen in rivers, lakes, and lagoons from human sources, we re-evaluated the still controversial potential effect of inorganic nitrogen on CH<sub>4</sub> oxidation. Here, we approached three shallow coastal lagoons that represent great environmental heterogeneity and

used slurry sediments as a model system. The addition of ammonium chloride ( $\text{NH}_4\text{Cl}$ ) and potassium nitrate ( $\text{KNO}_3$ ) significantly stimulated  $\text{CH}_4$  oxidation in the sediments of all studied lagoons, indicating the potential limitation of nitrogen for the growth of  $\text{CH}_4$  oxidizing bacteria. Our findings contrast to some previous reports, where ammonium and nitrate inhibited  $\text{CH}_4$  oxidation in sediments. Indeed, our experiment was performed in a more realistic range in relation to natural concentrations of inorganic nitrogen in aquatic systems (0.5 to 1 mM) and was opposed to extreme concentrations previously used (2 to 50 mM). Our results point to the need to further assess the connection between nitrogen inputs and  $\text{CH}_4$  budgets in aquatic sediments, considering the potential fuel for  $\text{CH}_4$  oxidation that may affect the global greenhouse gas balance.

**Keywords:**  $\text{CH}_4$ ; greenhouse gas;  $\text{NH}_4^+$ ;  $\text{NO}_3^-$ ; methanotrophy.

## INTRODUCTION

Next to carbon dioxide, methane ( $\text{CH}_4$ ) is the most important greenhouse gas, contributing significantly to radiative forcing (Myhre *et al.* 2013). Aquatic ecosystems, including those natural and human-altered, are considered to contribute more than 50% to the global  $\text{CH}_4$  emission (Saunio *et al.* 2016, Carlson *et al.* 2017). The role of lakes in the global  $\text{CH}_4$  budget has recently been reevaluated by estimating their  $\text{CH}_4$  emissions as 16% of all non-anthropogenic sources (Bastviken *et al.* 2004). This percentage may be even higher, as the area of small lakes and lagoons used in previous global budgets has been seriously underestimated (Downing *et al.* 2006, Parker *et al.* 2018, Saunio *et al.* 2020). Atmospheric  $\text{CH}_4$  would be much higher if part of the produced  $\text{CH}_4$  had not been oxidized by  $\text{CH}_4$  oxidizing bacteria (MOB) before escaping to the atmosphere (Conrad & Rothfuss 1991, Banger *et al.* 2012, Sawakuchi *et al.* 2016). The emission of  $\text{CH}_4$  from aquatic systems to the atmosphere results from a balance between the amount of methane produced in the anoxic sediments (methanogenesis) and  $\text{CH}_4$  consumption by oxidation at oxic-anoxic interfaces (Frenzel *et al.* 1990, Conrad 2007). In addition, there is increasing evidence that  $\text{CH}_4$  production in the water column contributes significantly to lakes  $\text{CH}_4$  emissions (Tang *et al.* 2014, DelSontro *et al.* 2018, Bižić *et al.* 2019). Despite the large methanogenesis in naturally flooded areas, high rates of  $\text{CH}_4$  oxidation might keep concentration and fluxes low (Sawakuchi *et al.* 2021).

Despite the importance of MOB in controlling the  $\text{CH}_4$  emission in aquatic environments, studies on the factors that regulate its activity in sediments are scarce. All MOB known so far share

the unique feature of using  $\text{CH}_4$  not only as an energy source, but also for carbon assimilation. A few species may assimilate additional substrates with C-C bounds (Dedysch *et al.* 2005, Theisen *et al.* 2005). Many  $\text{CH}_4$  oxidizing bacteria can fix  $\text{N}_2$  (Haroon *et al.* 2013, Arshad *et al.* 2015), but they can also assimilate mineral nitrogen, *e.g.*,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . For upland soils, it has been established for many years that the consumption of  $\text{CH}_4$  by MOB can be inhibited by mineral nitrogen (Sabrekov *et al.* 2016), while other studies have shown the opposite (*e.g.*, Bodelier & Laanbroek 2004). In freshwater sediments, some reports have confirmed the inhibitory effect of  $\text{NH}_4^+$  on  $\text{CH}_4$  oxidation (Bosse *et al.* 1993, Nold *et al.* 1999, Hu *et al.* 2018). However,  $\text{NH}_4^+$  may stimulate  $\text{CH}_4$  oxidation in cultivated paddy fields (Bodelier *et al.* 2000, Krüger *et al.* 2002, Krüger & Frenzel 2003, Noll *et al.* 2008) and in other ecosystems (Bodelier & Laanbroek 2004, Bodelier & Steenbergh 2014, Liu & Greaver 2009). In extensive coastal aquatic ecosystems with variable inputs from terrestrial and marine sources, the effects of N enrichment on  $\text{CH}_4$  oxidation are still poorly understood.

The ever-growing demand for food will expose all aquatic ecosystems to an increasing nitrogen load (Galloway *et al.* 2008, Rockström *et al.* 2009). Cultivation of nitrogen-fixing crop species (*e.g.*, soybean) in Latin America is promoting a large increase of N inputs in agricultural systems through anthropogenic and natural biological fixation. Nitrogen inputs have significantly increased in Brazilian ecosystems due to human activities (Martinelli *et al.* 2006). For instance, the urbanization on the south coast of Brazil was already associated with untreated wastewater discharges (Fistarol *et al.* 2015), affecting numerous coastal lagoons (Esteves *et al.* 2008).

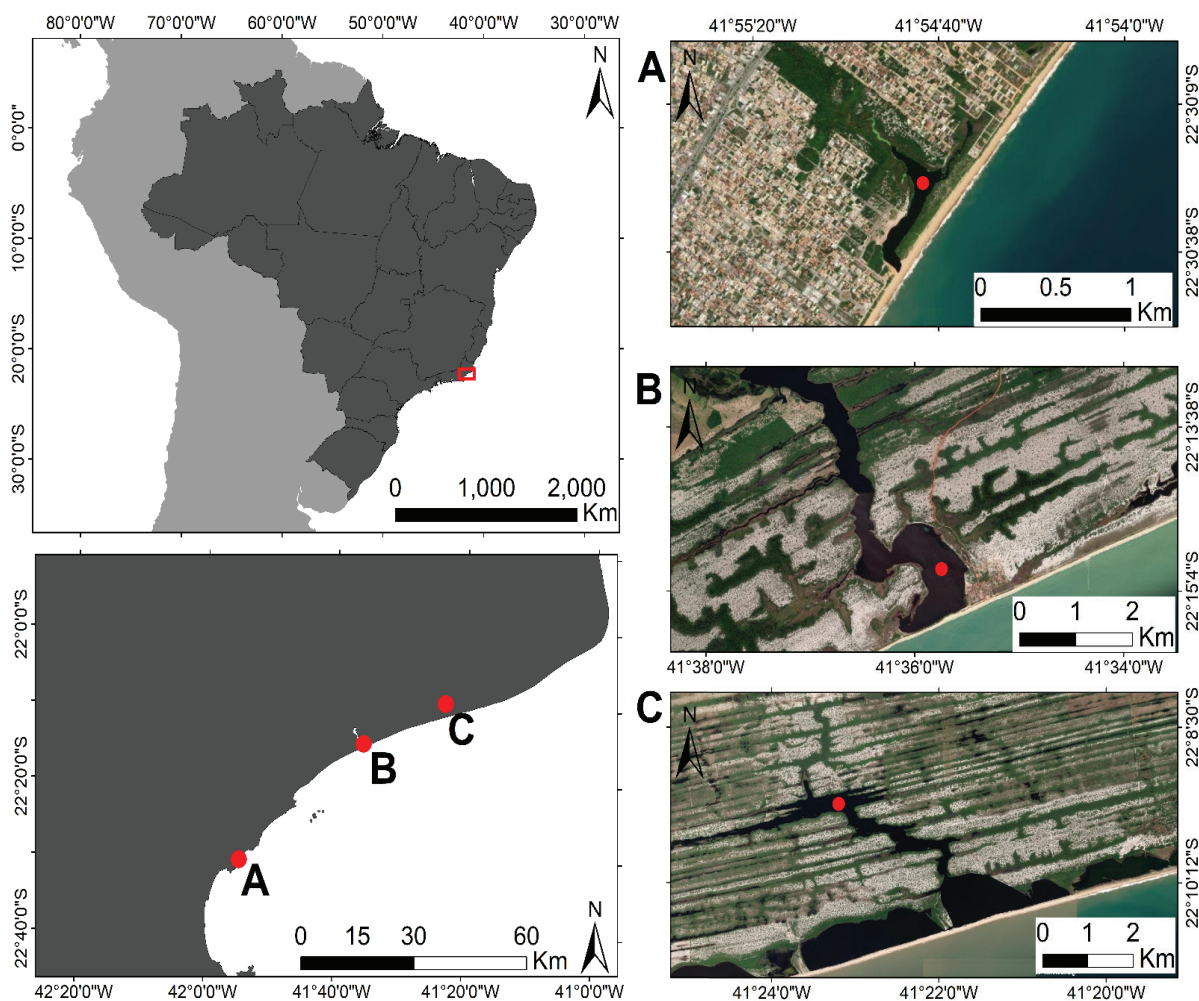
Considering these changes, it is essential to know how  $\text{CH}_4$  oxidation will be affected by nitrogen input in aquatic systems due to its contribution to the greenhouse effect. Hence, in this study, we aimed to evaluate the effect of mineral nitrogen on  $\text{CH}_4$  oxidation in coastal aquatic sediments. This knowledge is essential for predicting the future role of aquatic environments in the global  $\text{CH}_4$  cycle.

## MATERIAL AND METHODS

### Study area

The study was conducted with sediments collected from three shallow tropical coastal lagoons, Iodada ( $22^\circ 30' \text{ S}$ ;  $41^\circ 54' \text{ W}$ ), Comprida ( $22^\circ 16' \text{ S}$ ;  $41^\circ 39' \text{ W}$ ), and Carapebus ( $22^\circ 15' \text{ S}$ ;  $41^\circ 35' \text{ W}$ ), located along the North coast of Rio de Janeiro State, Brazil (Figure 1). These paralic depositional environments are subjected to the

mixing of fresh and salt waters at the landward side of coastal barriers (Woszczyk *et al.* 2014), receiving large inputs of humic substances from the coastal sandy plain. Iodada and Comprida lagoons are permanently humic, while the Carapebus lagoon shows periods of humic and clear waters over the year (Marotta *et al.* 2010). Both permanently humic lagoons are also smaller than the Carapebus lagoon, reaching  $\sim 0.12$ ,  $\sim 0.11$ , and  $\sim 6.50 \text{ km}^2$ , respectively, and only the Iodada lagoon is situated in a denser urban area, compared with both Comprida and Carapebus lagoons at a conservation unit (Jurubatiba National Park). Despite such differences, all studied sites showed similar oligotrophic brackish waters at the sampling moment (Table 1). The ecosystems have been chosen for being small and shallow, representing the majority of continental aquatic ecosystems in South America (Downing *et al.* 2006), and by presenting



**Figure 1.** Geographical location of the studied coastal lagoons: (A) Iodada, (B) Carapebus, and (C) Comprida.

similar chlorophyll-*a* (Lorenzen *et al.* 1967), total nitrogen and total phosphorus (Golterman *et al.* 1978) concentrations, but different measured concentrations of dissolved organic carbon (TOC 5000, Shimadzu Scientific instruments), salinity (Jenway 4200) and pH (Digimed 3000) values (Table 1). The regional climate is characterized as tropical sub-humid/humid (Henriques *et al.* 1988), the annual mean temperature is 26.6°C, and the mean annual precipitation is 1000 mm (INMET 2005).

Water and sediments were sampled in the Iodada lagoon in January 2003, Carapebus lagoon in February 2004, and Comprida lagoon in July 2005. In each lagoon, we collected sediment samples from the central area using tubes (Perspex, diameter 8 cm). We subsampled the top centimeter from 10 different cores and transferred it into 10 sterile 500 mL polycarbonate bottles. The sediment was transported to the laboratory in Germany within 7 to 10 days after sampling. On arrival, the sediment was shaken at 10-12 rpm for 3 days to maintain oxic conditions. Afterward, we transferred 1 mL of sediment to 25 mL pre-autoclaved glass tubes and added 4 mL of autoclaved lagoon water forming 5 mL slurry.

### Experimental methods

Different experimental setups were used to evaluate (1) the effect of NH<sub>4</sub>Cl and KNO<sub>3</sub> (0.5 mM of each substrate) in sediments from Iodada lagoon, and (2) the effect of KNO<sub>3</sub> (1.0 mM) in sediments from Comprida and Carapebus lagoons on CH<sub>4</sub> oxidation rates. In the first setup made in Iodada sediments, NH<sub>4</sub>Cl was added without

the precaution of avoiding possible NH<sub>3</sub> toxicity. Because of that, in the second setup made in Comprida and Carapebus sediments, the addition of just KNO<sub>3</sub> was decided to avoid the potential toxic effect of NH<sub>3</sub>. Each tube was sealed with a butyl stopper. The slurries were supplemented with CH<sub>4</sub> (99.995%, Messer Griesheim, Germany) to a final mixing ratio of 1-3% in the headspace and incubated at 25°C on a gyratory shaker (100-120 rpm) to maintain the aerobic conditions required to CH<sub>4</sub> oxidation process, which was also provided by the movement during slurry preparation.

Depending on the activity, CH<sub>4</sub> concentrations were measured 1-3 times per day for 5 to 13 days. In some tubes with very high activity, the initial CH<sub>4</sub> concentration was re-established before it could be completely consumed. CH<sub>4</sub> was analyzed with a Shimadzu GC-8A gas chromatograph equipped with a HayesepD column. Calibration was done using standard gas mixtures with 1,000 and 50,000 ppm<sub>v</sub> CH<sub>4</sub> in N<sub>2</sub> (Messer Griesheim, Germany). CH<sub>4</sub> concentrations in the slurries were calculated from Henry's law and the Bunsen solubility coefficient for CH<sub>4</sub> in freshwater at a temperature of 25°C.

In Carapebus and Comprida sediments, which did not receive NH<sub>4</sub>Cl (explanation above), two setups of experiment were done. In one experimental setup, KNO<sub>3</sub> was added again to the slurries from Carapebus and Comprida after 142 and 165 hours of incubation, respectively, to check the response of the methane oxidizers in a continuous high KNO<sub>3</sub> concentration, to compare with the other setup with no extra addition. In another setup, KNO<sub>3</sub> was added at the beginning of the incubation.

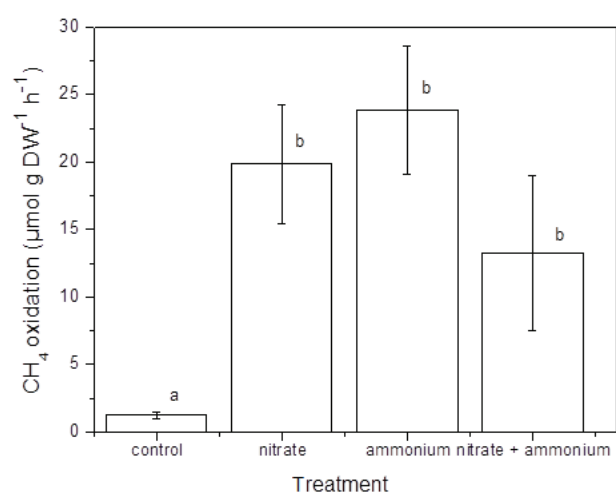
**Table 1.** Some features of the water column of the studied sites at the sampling time.

Variables	Coastal Lagoons		
	Iodada	Comprida	Carapebus
Depth (m)	2.9	2.0	1.7
Salinity (‰)	1.1	0.4	5.0
Conductivity (mS cm <sup>-1</sup> )	1.3	0.8	6.0
Secchi disc (m)	1.8	0.7	1.3
pH	7.3	5.5	7.7
Total N (µM)	56.0	65.0	67.0
Total P (µM)	0.36	0.53	0.62
Total Organic Carbon (mM)	25.0	35.0	10.0
Color (at 430 nm)	0.15	0.23	0.02
Chlorophyll- <i>a</i> (µg l <sup>-1</sup> )	5.0	2.2	3.70



**Table 2.** Synopsis of the experiments performed during this study; nd = not determined.

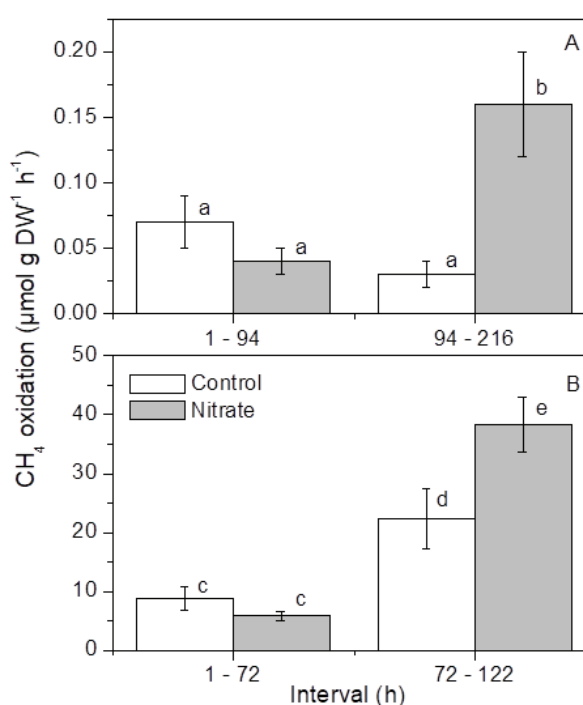
Lagoon	Sampling date	N addition (mM)	Incubation time (h)	Interval measurements of methane oxidation rates (h)	Moment of N addition (h)	Lag phase (h)	Figure
Iodada	Jan/2003	0.5 KNO <sub>3</sub> or NH <sub>4</sub> Cl	150	100 - 150	0	100	1
Carapebus	Feb/2004	0.5 KNO <sub>3</sub>	122	1 - 72 and 72 - 122	0	20 - 75	2B, 6
	Dec/2005	0.5 KNO <sub>3</sub>	330	nd	142	20 - 75	4
Comprida	Feb/2004	0.5 KNO <sub>3</sub>	216	1 - 94 and 94 - 216	0	94	2A, 5
	Dec/2005	0.5 KNO <sub>3</sub>	330	nd	165	70	3

**Figure 2.** Potential CH<sub>4</sub> oxidation rates from Iodada Lagoon after addition of potassium NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> chloride and a combination of both. Mean ± SE (N = 3). Different letters indicate significant differences (Anova, p < 0.01). Rates calculated between 100 and 150 hours of incubation.

Replicate sediment slurries from the three lagoons were incubated in 150 ml glass bottles in similar conditions, for daily determinations of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> concentrations and pH values (see Tables 1 and 2). We used 2 mL of the slurry for pH determination (Schott pH meter) and later centrifuged at 10.000 rpm (Eppendorf 5417-R). After centrifugation, the supernatant was carefully removed and NH<sub>4</sub><sup>+</sup> concentration was determined with a colorimetric assay (Bower & Holm-Hansen 1980) and NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations with UV detection by ion chromatography.

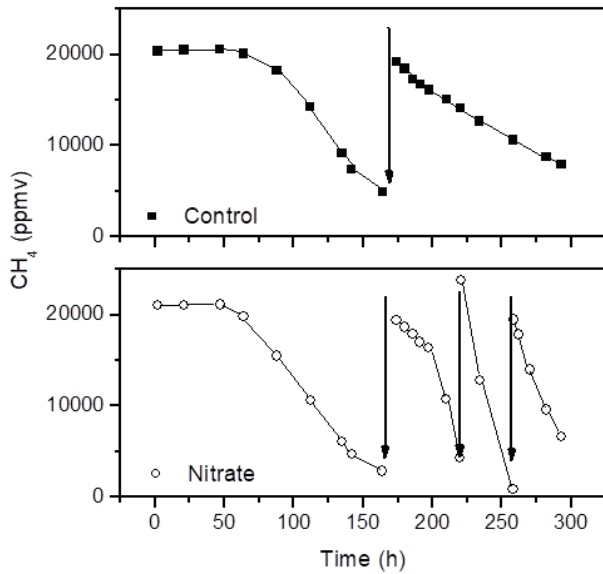
### Statistical analyses

We used the analysis of variance (ANOVA One-Way) followed by Tukey post hoc test to investigate whether there are differences among controls and treatments of inorganic nitrogen additions. In

**Figure 3.** Potential CH<sub>4</sub> oxidation rates from control and KNO<sub>3</sub> treatments in sediments from Comprida (A) and Carapebus (B) lagoons at different incubation intervals. Mean ± SE (N = 4). Different letters indicate significant differences (Anova, p < 0.01). Note differences in scales.

addition, we performed paired t-test to investigate significant differences in pH and NH<sub>4</sub><sup>+</sup> between lagoons. We assessed all tests using the significant level of 95%.

Changes in pH values and NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations were followed during the incubation of the slurries from Comprida and Carapebus (Figures 6 and 7). pH and NH<sub>4</sub><sup>+</sup> concentrations were higher in Carapebus than Comprida sediments, respectively (Paired t-test, p < 0.05). However, NH<sub>4</sub><sup>+</sup> concentrations in Carapebus sediments (up to 500 µM) are two

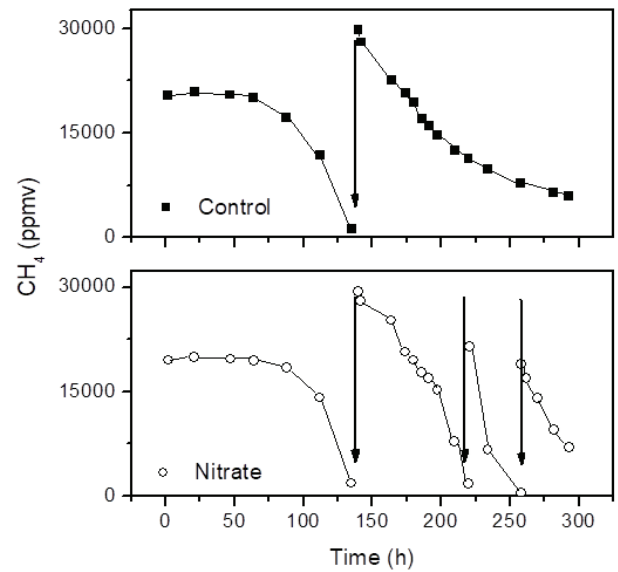


**Figure 4.** Changes in CH<sub>4</sub> concentrations with and without KNO<sub>3</sub> amendment in slurry from Comprida lagoon. KNO<sub>3</sub> was added 165 hours after beginning of incubation. Arrows indicate extra CH<sub>4</sub> addition.

orders of magnitude higher than in Comprida. NH<sub>4</sub><sup>+</sup> concentrations in Carapebus slurries remained higher than 200 μM during the first 94 hours of incubation but declined from 94 to 122 hours of incubation dropping to < 5 μM. NH<sub>4</sub><sup>+</sup> concentrations in Comprida slurries were < 7 μM in all measurements. NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> concentrations were < 1 μM and < 10 μM, in slurries from Comprida and Carapebus lagoons. Water salinity, which could influence CH<sub>4</sub> oxidation rates when is high (above 5; Osudar *et al.* 2017), were measured in all three lagoons (Table 1). Carapebus lagoon has showed the highest salinity (5; Table 1); however, the CH<sub>4</sub> oxidation rates seems not to be influenced, showing high rates (Table 3).

## DISCUSSION

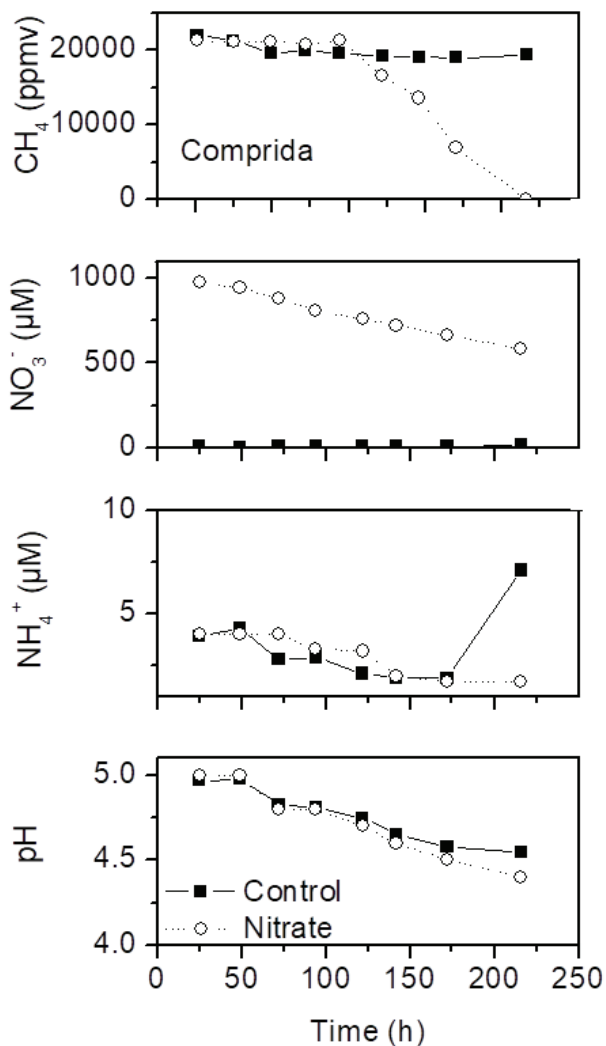
The addition of both forms of inorganic nitrogen, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, stimulated CH<sub>4</sub> oxidation after a lag phase, suggesting that nitrogen was limiting the growth of MOB. Methanotrophs are slow-growing bacteria (Mancinelli, 1995, Nold *et al.* 1999); however, they have relatively high growth with genetic manipulation (Kim *et al.* 2018), as well as a requirement for nitrogen, principally ammonium (Tays *et al.* 2018), with an ideal C:N growth ratio of 4:1 (Anthony 1978). Bodelier & Laanbroek (2004) summarized some results



**Figure 5.** Changes in CH<sub>4</sub> concentrations with and without KNO<sub>3</sub> amendment in slurry from Carapebus lagoon. KNO<sub>3</sub> was added 142 hours after beginning of incubation. Arrows indicate extra CH<sub>4</sub> addition.

on the effect of nitrogen in soils and aquatic sediments and established that nitrogen can be considered a limiting factor for the growth of MOB, suggesting that this could be also valid for natural aquatic sediments. For instance, a syntrophic consortium of archaea can perform anaerobic CH<sub>4</sub> oxidation by using nitrate as electron acceptor, while bacteria might use nitrite in inland waters (Bodelier & Steenbergh 2014).

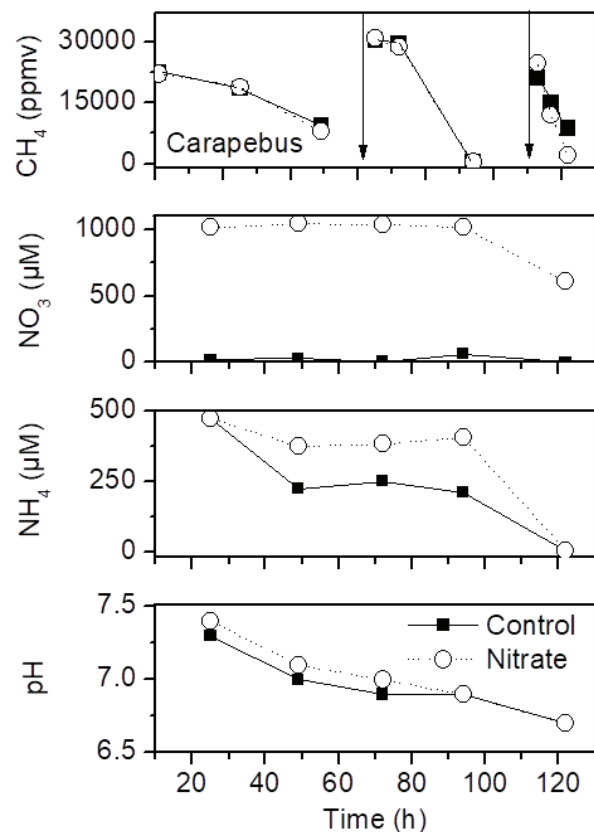
Our results contrast previous evidence on inorganic nitrogen inhibiting the CH<sub>4</sub> oxidation in aquatic bottom sediments (Conrad & Rothfuss 1991, Boon & Lee 1997, Nold *et al.* 1999, Hu *et al.* 2011), confirming that its response may be variable (Table 3). Besides the concentrations of inorganic nitrogen applied here were considered high (from 0.5 to 1 mM; Table 1), they remain one order of magnitude lower than previous studies that found inhibiting effect on CH<sub>4</sub> oxidation (Table 3). Although nitrogen is an important nutrient for oxidation this chemosynthetic process as in many habitats (Liikanen *et al.* 2002, Shen *et al.* 2020, Rudd *et al.* 1976), extremely higher concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup> may constrain its rates by mechanisms that are not yet clear (Bodelier 2011, Bodelier & Steenbergh 2014). For instance, concentrations of up to 50 mM NH<sub>4</sub>Cl have been related to reduced CH<sub>4</sub> oxidation in aquatic sediments (Table 3),



**Figure 6.** Changes in  $\text{CH}_4$ ,  $\text{KNO}_3$ ,  $\text{NH}_4^+$  concentrations and pH values with and without  $\text{KNO}_3$  amendment in slurries from Comprida lagoon.  $\text{KNO}_3$  was added at the beginning of the incubation.

including deleterious osmotic effects on MOB (Shrestha *et al.* 2010).

Also, mechanisms of nitrogen inhibition in the methane oxidation may be controversial (Bodelier 2011). Some authors point out that the inhibition mechanism of  $\text{NH}_4^+$  on  $\text{CH}_4$  oxidation seems to be rather complex, including not only competitive inhibition of the methane monooxygenase by  $\text{NH}_4^+$ , but also the toxic inhibition by hydroxylamine and  $\text{NO}_2^-$  produced by the oxidation of  $\text{NH}_4^+$  (Dunfield & Knowles 1995, O'Neill & Wilkinson 1977). In high concentrations,  $\text{NH}_4^+$  could inhibit  $\text{CH}_4$  oxidation in freshwater sediments (Nold *et al.* 1999) by the competition of the key-enzyme (methane monooxygenase) by  $\text{NH}_4^+$  (Bodelier & Steenbergh 2014). Likewise,



**Figure 7.** Changes in  $\text{CH}_4$ ,  $\text{KNO}_3$ ,  $\text{NH}_4^+$  concentrations and pH values with and without  $\text{KNO}_3$  amendment in slurries from Carapebus lagoon.  $\text{KNO}_3$  was added at the beginning of the incubation.

$\text{NO}_3^-$  in concentrations higher than 10 mM would inhibit  $\text{CH}_4$  oxidation (O'Neill & Wilkinson 1977, Dunfield & Knowles 1995). However, this inhibition depends on the  $\text{CH}_4$  concentration (Duan *et al.* 2013) that has been attributed to osmotic effects (Bodelier & Laanbroek 2004).

A combination of both competitive and toxic mechanisms may also explain the increase in inhibition of  $\text{CH}_4$  oxidation observed with increasing amounts of  $\text{NH}_4^+$ .  $\text{NH}_4^+$  must exceed  $\text{CH}_4$  concentrations 30 to 100-fold to become effective for inhibition (Van der Nat *et al.* 1997, Yang *et al.* 2020). Depending on pH, high  $\text{NH}_4^+$  concentrations can correspond to relatively high concentrations of ammonia ( $\text{NH}_3$ ) that can be toxic to methanotrophs (He *et al.* 2017, Mohammadi *et al.* 2017). High levels of  $\text{NO}_2^-$ , a toxic compound for MOB, can also occur in environments with high oxidation rates of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  (Mohammadi *et al.* 2017).

Indeed, there was a clear variation in  $\text{CH}_4$  oxidation rates in the sediment of the three lagoons

**Table 3.** Nitrogen effects on methane oxidation in sediment and water from different aquatic environments. Stimulation (+); inhibition (-); stimulation or inhibition (+ / -) depending on the concentration added, and no effect (0). Methods: A = difference of CH<sub>4</sub> emission from sediment cores under oxic and anoxic conditions; B = decrease of CH<sub>4</sub> concentrations over time; C = incorporation of <sup>13</sup>CCH<sub>4</sub>; nd = not determined or not described.

CH <sub>4</sub> μM	NH <sub>4</sub> <sup>+</sup> μM	Addition of N mM	Effect	Method	Lake	Reference
<i>Sediment</i>						
100	nd	2 – 50 NH <sub>4</sub> Cl	-	A	Rice paddies	(Conrad & Rothfuss 1991)
0.2 – 20	nd	0 – 25 NH <sub>4</sub> Cl	+ / -	A	Lake Constance (Germany)	(Bosse & others 1993)
1 – 70	nd	2 – 20 NH <sub>4</sub> Cl	+ / -	B	Ryan I Billabong (Australia)	(Boon & Lee 1997)
1 – 70	nd	2 – 20 NaNO <sub>3</sub>	-	B	Ryan I Billabong (Australia)	(Boon & Lee 1997)
1400	1000	10 NH <sub>4</sub> Cl	-	C	Lake Loosdrecht (The Netherlands)	(Nold <i>et al.</i> 1999)
0.02 – 10	160	0.3 – 1.5 NH <sub>4</sub> Cl	-	B	Schelde Estuary (Belgium)	(van der Nat <i>et al.</i> 1997)
nd	0 – 360	0.005 – 15 NH <sub>4</sub> Cl	0	A	Lake Kevätön (Finland)	(Liikanen <i>et al.</i> 2003)
nd	nd	0.03 – 2 KNO <sub>3</sub> / NaNO <sub>3</sub>	+	A	Lake Kevätön (Finland)	(Liikanen <i>et al.</i> 2002)
0.15	nd	1.5 x 10 <sup>6</sup> NaNO <sub>2</sub>	0	C	Lake Constance (Germany)	(Deutzmann & Schink 2011)
0.15	nd	2 x 10 <sup>6</sup> NaNO <sub>3</sub>	0	C	Lake Constance (Germany)	(Deutzmann & Schink 2011)
10000	800	2 x 10 <sup>6</sup> KNO <sub>3</sub>	+ / -	A	Shanyutan estuary wetland (China)	(Hu <i>et al.</i> 2018)
10000	800	2 x 10 <sup>6</sup> NH <sub>4</sub> Cl	-	A	Shanyutan estuary wetland (China)	(Hu <i>et al.</i> 2018)
nd	480	5 x 10 <sup>5</sup> NO <sub>2</sub> <sup>-</sup>	+	B	Jiulonghu Reservoir (China)	(Shen <i>et al.</i> 2020)
nd	480	2 x 10 <sup>6</sup> NO <sub>3</sub> <sup>-</sup>	+	B	Jiulonghu Reservoir (China)	(Shen <i>et al.</i> 2020)
nd	nd	5 x 10 <sup>5</sup> NaNO <sub>3</sub>	+ / -	C	Brunsummerheide peatland	(Zhu <i>et al.</i> 2012)
175.6	nd	0 – 5 x 10 <sup>6</sup> KNO <sub>3</sub>	0	C	Lake Pääjärvi (Finland)	(Rissani <i>et al.</i> 2021)
nd	nd	0 – 3000 NH <sub>4</sub> Cl	-	A	Paddy soils - Rice fields (China)	(Cai & Mosier, 2000)
12000 - 20000	nd	6 x 10 <sup>5</sup> NO <sub>2</sub> <sup>-</sup>	-	B	Fresh Water Lake sediments	(Hu <i>et al.</i> 2011)
14	5	0.5 NH <sub>4</sub> Cl / 0,5 KNO <sub>3</sub>	+	B	Iodada Lagoon (Brazil)	This study
14 – 45	5	1.0 KNO <sub>3</sub>	+	B	Comprida Lagoon (Brazil)	This study
14 – 45	500	1.0 KNO <sub>3</sub>	+	B	Carapebus Lagoon (Brazil)	This study
<i>Water column</i>						
80	3	7 NH <sub>4</sub> Cl	+	B	Lake 227 ELA (Canada)	(Rudd <i>et al.</i> 1976)
0.1 – 0.6	12-30	0.06 KNO <sub>3</sub>	0	B	Hudson River (USA)	(de Angelis & Scranton 1993)
0.1 – 0.6	12-30	0.04 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	-	B	Hudson River (USA)	(de Angelis & Scranton 1993)
0.05 – 0.25	<1	0.2 NH <sub>4</sub> NO <sub>3</sub>	-	B	Lake Biwa (Japan)	(Murase & Sugimoto 2005)



studied. The CH<sub>4</sub> oxidation rates were one order of magnitude higher in Carapebus than those of the Comprida and Iodada sediments at the beginning of the incubation, indicating that methanotrophs were more active in the Carapebus sediment. The methanotrophic physiological stages will depend on the availability of CH<sub>4</sub> and oxygen in the sediment oxic zone. In such way, Bender & Conrad (1992) suggested that the sediment methanotrophs can present three different physiological stages: (1) fully active cells, that are oxidizing CH<sub>4</sub> in the oxic part of the sediment; (2) cells with low physiological activity in the upper anoxic zone, capable of spontaneously induced to oxidize CH<sub>4</sub> in the presence of oxygen, and (3) as resting stages as cysts or exospores and able to be activated only by prolonged incubation under high oxygen and CH<sub>4</sub> ratios. According to this classification, the methanotrophs from Iodada and Comprida lagoons may be in stage 3, while from Carapebus lagoon in stage 2, suggesting that a longer experiment approach might result in change from stage 3 to 2, in Iodada and Comprida lagoons

The results of the influence of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentration in tropical coastal lagoons showed here should also be considered in the regulation of CH<sub>4</sub> oxidation in lacustrine ecosystems worldwide. The ongoing increase of inorganic nitrogen inputs in the watershed must play a critical role in improving CH<sub>4</sub> oxidation in aquatic sediments, in addition to previously reported increases in greenhouse gas production and loss of water quality. Our findings contrast with other studies where nitrogen has been observed to inhibit CH<sub>4</sub> oxidation and we argue that our experiment was performed in a more realistic range of nitrogen concentrations regarding the natural concentration of aquatic sediments. However, it is important to highlight that CH<sub>4</sub> oxidation might be sensitive to environmental change, since it varies spatially and temporally in natural and human-altered ecosystems, which was not assessed here. Also, the influence of fertilizers on the CH<sub>4</sub> budget in aquatic ecosystems needs to be evaluated considering the net effects of increased inorganic N on both methanogenesis and methanotrophy. This study brings forward the insufficient knowledge about the processes that control CH<sub>4</sub> dynamics in

coastal aquatic sediments, which is essential to determine the impact of nitrogen loads on CH<sub>4</sub> budgets on a regional and global scale.

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