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# The effects of temperature and resource availability on denitrification and relative N<sub>2</sub>O production in boreal lake sediments

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## ARTICLE INFO

### Article history:

Received 9 November 2015

Revised 3 March 2016

Accepted 7 March 2016

Available online 22 March 2016

### Keywords:

Acetylene

NO<sub>3</sub>

Carbon

DOC

Nitrous oxide ratio

Sediment

## ABSTRACT

Anthropogenic environmental stressors (like atmospheric deposition, land use change, and climate warming) are predicted to increase inorganic nitrogen and organic carbon loading to northern boreal lakes, with potential consequences for denitrification in lakes. However, our ability to predict effects of these changes is currently limited as northern boreal lakes have been largely neglected in denitrification studies. The aim of this study was therefore to assess how maximum potential denitrification and N<sub>2</sub>O production rates, and the relationship between the two (relative N<sub>2</sub>O production), is controlled by availability of nitrate (NO<sub>3</sub><sup>-</sup>), carbon (C), phosphorus (P), and temperature. Experiments were performed using the acetylene inhibition technique on sediments from a small, nutrient poor boreal lake in northern Sweden in 2014. Maximum potential denitrification and N<sub>2</sub>O production rates at 4°C were reached already at NO<sub>3</sub><sup>-</sup> additions of 106–120 μg NO<sub>3</sub><sup>-</sup>-N/L, and remained unchanged with higher NO<sub>3</sub> amendments. Higher incubation temperatures increased maximum potential denitrification and N<sub>2</sub>O production rates, and Q<sub>10</sub> was somewhat higher for N<sub>2</sub>O production (1.77) than for denitrification (1.69). The relative N<sub>2</sub>O production ranged between 13% and 64%, and was not related to NO<sub>3</sub><sup>-</sup> concentration, but the ratio increased when incubations were amended with C and P (from a median of 16% to 27%). Combined, our results suggests that unproductive northern boreal lakes currently have low potential for denitrification but are susceptible to small changes in NO<sub>3</sub> loading especially if these are accompanied by enhanced C and P availability, likely promoting higher N<sub>2</sub>O production relative to N<sub>2</sub>.

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## Introduction

It is well known that humans have greatly altered the nitrogen (N) cycle of our planet. Atmospheric N deposition has increased dramatically since the industrial age (Galloway, 1998; IPCC, 2007) and predictions are that by 2050, we will see a 2-fold increase relative to the 1990s (Galloway et al., 2004). This drastic increase in atmospheric inputs of bioavailable

inorganic N compounds affects the global N cycle, modifies a variety of aquatic processes such as primary production, and can lead to both eutrophication and acidification (Bergström and Jansson, 2006; Camargo and Alonso, 2006; Seitzinger et al., 2006; Vitousek et al., 1997). Other human activities enhancing inorganic N leakage to boreal lakes includes logging (Kreutzweiser et al., 2008; Löfgren et al., 2009). Global change is also predicted to enhance mobilization of N and carbon (C)

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from organic pools in soils (de Wit et al., 2007) and increase terrestrially dissolved organic matter input to lakes (Forsberg, 1992; Roulet and Moore, 2006). An important ecosystem process that mitigates excess bioavailable N is denitrification, a process where heterotrophic bacteria reduce nitrate ( $\text{NO}_3^-$ ) to nitrogen gas ( $\text{N}_2$ ) through oxidation of organic matter to gain energy. However, in case of incomplete denitrification, dinitrogen gas ( $\text{N}_2\text{O}$ ) is also produced, a potent greenhouse gas which also depletes atmospheric ozone (Ravishankara et al., 2009).

Denitrification occurs across a variety of ecosystems (water-saturated soils, wetlands, streams, estuaries, oceans and lakes) at low oxygen conditions where production rates vary depending on nutrient and carbon availability, and physical and chemical conditions, including pH and temperature. How these potential regulating factors interact and counteract have been studied since the late 1800 (Payne, 1981), but it was not until mid-1900s that denitrification was actually measured in a lake ecosystem (Goering and Dugdale, 1966). Since then, important factors found to constrain denitrification and  $\text{N}_2\text{O}$  production rates in lakes include  $\text{NO}_3^-$  availability (McCrackin and Elser, 2010, 2012), temperature (Liikanen et al., 2002; Saunders and Kalff, 2001) and redox potential — the latter by constraining nitrification and thus  $\text{NO}_3^-$  supply, and by regulating inhibiting elements like sulfide (Small et al., 2014). Organic C concentration in sediments have further been found to correlate with denitrification rates in lakes (Saunders and Kalff, 2001) and streams (Fork and Heffernan, 2014). In streams, the quality of organic matter has also been shown to be an important regulating factor for denitrification (Fork and Heffernan, 2014; Barnes et al., 2012).

Given that the abovementioned environmental factors affect denitrification and  $\text{N}_2\text{O}$  production, trophic status and climatic settings will also influence these bacterially mediated processes in lakes. However, a large part of aquatic research on denitrification and  $\text{N}_2\text{O}$  production have so far been conducted in large, eutrophic lake ecosystems with  $\text{NO}_3^-$  concentrations  $>100 \mu\text{g N/L}$  (c.f. Seitzinger et al., 2006). Hence, little is known about these processes in small ( $<0.1 \text{ km}^2$ ), boreal and unproductive lakes, which globally (by number) represent one of the most common lake types (Downing et al., 2006; Veerporter et al., 2014). In many of these lake systems concentrations of inorganic N are low (in areas with low N deposition) and phytoplankton are N-limited (Bergström and Jansson, 2006; Elser et al., 2009), thus the competition for available pools of inorganic N between denitrifying bacteria and phytoplankton should be severe (cf. Seitzinger et al., 2006). Increased inorganic N availability could therefore potentially affect denitrification in these lake types differently when compared with more nutrient rich and productive systems (i.e., eutrophic lakes). Denitrification studies in small and shallow lakes are additionally clearly lacking (cf. Seitzinger et al., 2006). In a climate change perspective this could be important especially because sediments of small, shallow lakes may represent important hot spots for denitrification and  $\text{N}_2\text{O}$  production in the landscape which will become heated and proportionally warmer than sediments of deeper, larger lakes as air temperatures increase globally.

The extent to which denitrification affects atmospheric greenhouse gas content is in part regulated by the relative  $\text{N}_2\text{O}$

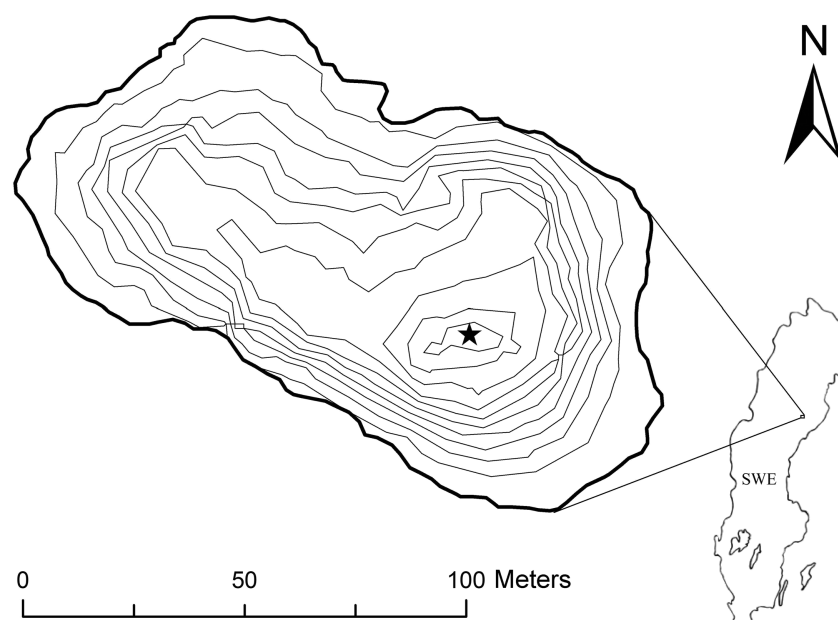
production, i.e., the ratio of  $\text{N}_2\text{O}$  production to total denitrification. Because  $\text{N}_2\text{O}$  production does not necessarily correlate with denitrification (Firestone et al., 1980; Heuvel et al., 2009), it is critical to study these processes in tandem. So far, relative  $\text{N}_2\text{O}$  production have mainly been investigated in soils, coastal areas and estuaries (Firestone et al., 1980; Seitzinger, 1988), where acidity and greater  $\text{NO}_3^-$  availability have been found to promote  $\text{N}_2\text{O}$  over  $\text{N}_2$  production. Furthermore, temperature has been suggested to increase the  $\text{N}_2\text{O}:\text{N}_2$  ratios in soils because of possible influence of thermophilic genus of denitrifying bacteria that are associated with incomplete  $\text{NO}_3^-$  reduction (Knowles, 1982). In eutrophic lakes, moderate oxygen concentration has shown to favor  $\text{N}_2\text{O}$  relative to  $\text{N}_2$  production, due to coupled nitrification and denitrification (Beaulieu et al., 2014; Deemer et al., 2011). We only know of one study performed in unproductive clear water lakes where relative  $\text{N}_2\text{O}$  production was evaluated, in which ambient  $\text{NO}_3^-$  concentrations were  $<100 \mu\text{g N/L}$  (McCrackin and Elser, 2010). Here,  $\text{NO}_3^-$  availability was important in regulating the relative  $\text{N}_2\text{O}$  production in lakes with high atmospheric N deposition ( $10\text{--}20 \text{ kg N/ha/year}$ ) and higher surface water  $\text{NO}_3^-$  concentrations (ranging between  $7.8$  and  $45.3 \mu\text{mol NO}_3^-/\text{N/L}$ ). However, in lakes with low atmospheric N deposition ( $<4 \text{ kg N/ha/year}$ ) and lower surface water  $\text{NO}_3^-$  concentrations (between  $<0.04$  and  $0.13 \mu\text{mol NO}_3^-/\text{N/L}$ ) the  $\text{N}_2\text{O}$  to  $\text{N}_2$  relationship was not evaluated because the  $\text{N}_2\text{O}$  production was below detection (McCrackin and Elser, 2010). Consequently, it remains to be tested how relative  $\text{N}_2\text{O}$  production is affected by different environmental factors in small, unproductive and boreal lakes.

The aim of this study was to assess possible constraining factors on maximum potential denitrification and  $\text{N}_2\text{O}$  production rates, and the relationship between the two processes (i.e., relative  $\text{N}_2\text{O}$  production) in a small, nutrient poor, boreal lake in northern Sweden with low N deposition. This was done by using the acetylene inhibition technique on sediment slurries. We performed dose-response experiments using different amendments of  $\text{NO}_3^-$  alone ( $10$  to  $1250 \mu\text{g NO}_3^-/\text{N/L}$ ),  $\text{NO}_3^-$  combined with C and P, and an additional experiment that evaluated the effect of temperature (i.e., warming) performed at non-limiting concentrations of C, N, and phosphorus (P). We hypothesized that (1) higher  $\text{NO}_3^-$  concentrations and temperatures would increase both maximum potential denitrification and  $\text{N}_2\text{O}$  production rates, that (2) the relative  $\text{N}_2\text{O}$  production would increase with  $\text{NO}_3^-$  availability, and (3) also be affected by changes in sediment temperatures.

## 1. Material and methods

### 1.1. Site description

Experiments were carried out on sediments collected from Lake Nästjärn, situated some  $100 \text{ km}$  north west of Umeå, northern Sweden (Fig. 1). The catchment is dominated by granite bedrock, overlain by glacial till and coniferous forests with shrub and lichen understory. The lake is marginally affected by forestry and the mean ( $\pm\text{SD}$ ) wet deposition of  $\text{NO}_3^-$ -N between 1990 and 2013 (measured at the closest weather station, Rickleå station,  $100 \text{ km}$  from the lake) was  $1.6$



**Fig. 1 – The location and bathymetric map of Lake Nätjärn. The star indicates the deepest area of the lake (10 m).**

( $\pm 0.33$ ) kg N/ha/year (IVL, 2014). The catchment area of the lake ( $0.034 \text{ km}^2$ ) is relatively small compared to the lake surface area ( $0.01 \text{ km}^2$ ), and the theoretical water retention time is fairly long (1144 days) (cf. Lindström et al., 2006). The lake has a mean depth of 4.2 m and a max depth of 10.4 m. The lake is unproductive, and had a mean ( $\pm$ SD) chlorophyll a concentration of  $1.6 (\pm 0.5) \mu\text{g/L}$  ( $n = 7$ ) in the epilimnion during summer 2013 (May–August; Bergström unpublished data).

## 1.2. Field sampling and experimental analyses

Sampling for the different denitrification experiments was carried out three times in February to March in 2014 (sampling dates in detail under Section 1.3.3), during ice cover and base-flow conditions. Lake water temperature and oxygen were measured *in situ* using oxygen probe YSI ProODO. Water for analysis of pH, dissolved organic carbon (DOC) and nutrients (dissolved  $\text{NO}_3^-$ -N, ammonium:  $\text{NH}_4^+$ -N and phosphate:  $\text{PO}_4^{3-}$ -P, total dissolved nitrogen: TDN) and water for the incubations was sampled at the deepest point of the lake, in the hypolimnion, just above the sediment surface where sediment samples were cored. The top 7 cm of the sediment was collected using a gravity corer (Renberg and Hansson, 2008), bulked into one sample, brought to the lab and stored at  $6^\circ\text{C}$ . On each sampling occasion, 8–11 cores were collected and bulked, to minimize errors introduced via sampling variability and heterogeneity of the sediment. All denitrification experiments were started within 48 hr of sampling.

## 1.3. Experimental set ups and sediment incubations

The hypotheses were tested by performing two different denitrification experiments. Experiment (1) was performed with different  $\text{NO}_3^-$  amendments alone, or combined with C and P ( $\text{NO}_3^- + \text{C} + \text{P}$ ). Experiment (2) was performed with different incubation temperatures under non-limiting C, N

and P conditions. Every amendment was made with and without acetylene to be able to analyze total potential denitrification (with acetylene) and  $\text{N}_2\text{O}$  production (without acetylene). The experimental set-ups and sediment incubations are explained in detail below.

### 1.3.1. Experiment (1) the $\text{NO}_3^-$ addition experiment

Here two different incubation set-ups were made, which were all incubated at  $4^\circ\text{C}$ . Set-up one consisted of 6 different  $\text{NO}_3^-$  amendments alone (0, 50, 100, 400, 625, 1250  $\mu\text{g NO}_3^-$ -N/L), and set-up two consisted of 6 different  $\text{NO}_3^-$  amendments (10, 20, 40, 100, 400, 800  $\mu\text{g NO}_3^-$ -N/L) combined with  $\text{PO}_4^{3-}$  (P) and glucose (C) amendments to a final C:N:P molar ratio of 50:10:1 (based on bacterial C:N:P stoichiometry found by Fagerbakke et al., 1996). Three bottles were incubated for each amendment, to account for variance in the method. Each bottle was also replicated with acetylene (i.e., in total each  $\text{NO}_3^-$  or  $\text{NO}_3^- + \text{C} + \text{P}$  amendment was incubated with three bottles without acetylene and three bottles with acetylene). The rationale for not using the same  $\text{NO}_3^-$  amendments in the different set-ups was that the denitrification reached its maximum potential already at the lowest amendment in the first set-up, so we added lower amendment levels to the second set-up. The median CV between the triplicate incubation bottles was 12% (CV ranged between 0.1% and 86%), with the highest variance in the ambient amendments, where denitrification was lowest.

### 1.3.2. Experiment (2) — the temperature experiment

Here three different temperatures (10, 15 and  $25^\circ\text{C}$ ) were used and all incubations were amended with 800  $\mu\text{g NO}_3^-$ -N/L, combined with C and P, to the same C:N:P molar ratio (50:10:1) as in the  $\text{NO}_3^-$  addition experiment (c.f. above). Data on denitrification and  $\text{N}_2\text{O}$  production rates at  $4^\circ\text{C}$  were used from the  $\text{NO}_3^-$  experiment (amendment with 800  $\mu\text{g NO}_3^-$ -N/L combined with C and P; cf. above). Nutrient amendments

were performed in order to assess the temperature effect *per se*, without the confounding effect of potential nutrient and/or C limitation. Three bottles were incubated at each temperature to account for the variance in the method. Each bottle was also replicated with acetylene, as in the  $\text{NO}_3^-$  addition experiment (cf. above). The median CV between the triplicate incubation bottles was 7% (CV ranged between 1% and 15%).

### 1.3.3. Sediment incubations

All sediment incubations were carried out following McCrackin and Elser (2010) with some minor adjustments. The sediment was homogenized before subsamples of 50 g wet sediment were added to 80 mL of hypolimnion lake water in 320 mL serum bottles. Bottles were closed with rubber stoppers and two plastic tubes with gas valves for sampling. To deplete bottles of oxygen, they were bubbled with  $\text{N}_2$  for 10 min before incubation. Each treatment was also replicated with acetylene amendments to a final saturation of 0.7% (Sørensen, 1978), which yield estimates of potential denitrification (capturing both  $\text{N}_2\text{O}$  and  $\text{N}_2$  production). Before sampling headspace, bottles were vigorously shaken for 1 min to attain gas–water equilibrium, after which 10 mL of gas was sampled from the headspace at the same time as 10 mL of  $\text{N}_2$  gas was injected, to keep constant pressure in the bottles. The headspace of the incubations was sampled at 2, 4, 8, 12 and 24 hr. Sampled gas was injected into pre-flushed (with  $\text{N}_2$ ) 22 mL crimped sealed glass vials, which were depressurized before analysis.

Sediment and lake water for the different experiments were sampled at different times. Amendments with 0, 625 and 1250  $\mu\text{g NO}_3^-$ -N/L (set-up one) were sampled on February 10th, sediment for the whole  $\text{NO}_3^- + \text{C} + \text{P}$  amendments (set-up two) on February 24th and sediment for the 50, 100, 400  $\mu\text{g NO}_3^-$ -N/L amendments (set-up one again) and sediment for the temperature experiment was sampled on March 24th. The sampling for the  $\text{NO}_3^-$  only experiments (set-up one) was done at two occasions due to technical problems. The final  $\text{NO}_3^-$  concentrations in the incubations presented in the Results and Discussion sections, represent the sum of the ambient lake water concentration at each sampling occasion plus the amended amount of  $\text{NO}_3^-$ .

### 1.4. Analyses

Water samples for DOC and TDN were filtered (0.7  $\mu\text{m}$ , acid washed and ignited GF/F filters) and acidified (0.5 mL 1.2 mol/L HCl 50 mL<sup>-1</sup> sample) before analysis in a 550 TOC-TN analyzer, Hach IL, Germany. Dissolved nutrients in hypolimnion lake water were filtered (0.45  $\mu\text{m}$ ) and analyzed using an automated flow injection analyzer (FIA star 5000, FOSS, Denmark). Extractable  $\text{NO}_3^-$ -N +  $\text{NO}_2^-$ -N (hereafter presented as  $\text{NO}_3^-$ -N),  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$ -P in the sediments were analyzed with the same method, after extraction of 1 g of dry sediment with 30 mL of 0.5 mol/L potassium sulfate ( $\text{K}_2\text{SO}_4$ ) (Stark et al., 2012). Loss on ignition (LOI) was analyzed as a proxy for organic matter in the sediment. The sediment was dried in 105°C for 12 hr for sediment water content and burned in 550°C for 4 hr for loss on ignition analyses.

$\text{N}_2\text{O}$  was analyzed on a gas chromatograph (Perkin Elmer Clarus 500, USA) equipped with a capillary column (Q-PLLOT)

and an electron capture detector, using  $\text{N}_2$  as carrier gas and having a make-up gas flow of 30 mL of argon with 5% methane mixture. The  $\text{N}_2\text{O}$  concentration in the headspace was recalculated to water concentrations based on Henry's law. All gas samples were analyzed within a week of sampling. All the above analyses were performed at the Department of Ecology and Environmental Science, Umeå University.

Maximum potential denitrification and  $\text{N}_2\text{O}$  production rates are presented as the per hour and per area ( $\text{m}^2$ ) production, calculated based on the area of the sediment corer (58  $\text{cm}^2$ ) and the amount of sediment used for each incubation (50 g, about 1/5th of a core). Production rates of denitrification and  $\text{N}_2\text{O}$  were calculated from the accumulation of gas per time unit during the incubation. At all times, and in all experiments, maximum potential production rate (i.e., both for  $\text{N}_2\text{O}$ - and  $\text{N}_2$  production) was calculated from the steepest slope of a linear regression between two adjacent sampling points during incubation. Relative  $\text{N}_2\text{O}$  production was then calculated as the ratio between the maximum potential rates of  $\text{N}_2\text{O}$  production and total denitrification (i.e.,  $\text{N}_2 + \text{N}_2\text{O}$ ) and presented as  $\text{N}_2\text{O}$  production in percent of total denitrification.  $\text{NO}_3^-$  consumption was calculated as the amount of N (in moles) used over the course of the experiment (24 hr); which is the produced  $\text{N}_2\text{O}$ , divided by the amount of added N.

### 1.5. Statistics

One-way ANOVA (analysis of variance) was used to analyze differences between the average denitrification and/or  $\text{N}_2\text{O}$  production rates across different levels of  $\text{NO}_3^-$  alone-, and  $\text{NO}_3^- + \text{C} + \text{P}$  amendments, and temperature treatments. If significant, ANOVA was followed by Tukey (HSD) honest significant difference test for pairwise differences. Two tailed t-test was used to analyze differences in maximum denitrification rates between incubations amended with  $\text{NO}_3^-$  versus  $\text{NO}_3^- + \text{C} + \text{P}$ . Two tailed Wilcoxon's rank-sum test (or Mann–Whitney U test) was used to test if median relative  $\text{N}_2\text{O}$  production differed between incubations amended with  $\text{NO}_3^-$  versus  $\text{NO}_3^- + \text{C} + \text{P}$ . All statistical analyses were performed in R and the threshold for statistical significance was set at  $\alpha = 0.05$ .

Q10 values were calculated from the relationship between temperature and production rate using the following equation:

$$Q_{10} = e^{(R \cdot t)} \quad (1)$$

where,  $e$  is the base of the natural logarithm and  $R$  is the change in denitrification or  $\text{N}_2\text{O}$  production per 1°C (i.e., the slope) and  $t$  (°C) is the temperature, and  $t$  is set to 10°C.

## 2. Results

### 2.1. Lake water and sediment chemistry

Water at the deep spot of the lake was anoxic and had a temperature of 4°C during all sampling occasions. DOC concentrations in the deep hypolimnion water ranged between 8.9 and 13.4 mg/L (Table 1). One pronounced change to



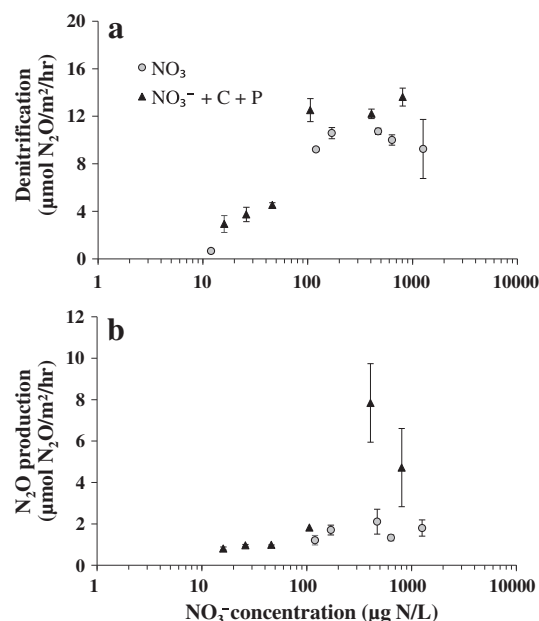
be noted is the  $\text{NO}_3^-$ -N concentrations in the hypolimnion water increased ten-fold (from 6 to 70  $\mu\text{g NO}_3^-$ -N/L), whilst  $\text{PO}_4^{3-}$ -P and  $\text{NH}_4^+$ -N concentrations decreased drastically, between February 24th and March 24th. TDN was halved during the same period and pH increased from 6.1 to 6.40. The sediment had a water content of 96% and LOI ranged between 71% and 75%.  $\text{NH}_4^+$ -N was the dominant nutrient in the sediment (0.91–1.06 mg/g) while  $\text{NO}_3^-$ -N concentration was low (5.0–5.7  $\mu\text{g/g}$ ) and  $\text{PO}_4^{3-}$ -P was below detection (1  $\mu\text{g/g}$ ).

## 2.2. Nitrate addition experiment

At ambient conditions, the maximum potential denitrification rate was 0.66  $\mu\text{mol N}_2\text{O}/\text{m}^2/\text{hr}$  whereas no  $\text{N}_2\text{O}$  production was observed.  $\text{NO}_3^-$  amended slurries had many times higher production rates (Fig. 2a–b) and they were all significantly higher compared to the ambient amendments (ANOVA, and Tukey HSD, all  $p < 0.05$ ). This was true both for amendments where  $\text{NO}_3^-$  was added alone and in amendments where  $\text{NO}_3^-$  was added together with C and P. The consumption of added  $\text{NO}_3^-$ -N ranged between 1% and 30% during the 24 hr incubation, counting all  $\text{NO}_3^-$  amendments, meaning that there was always inorganic N left at the end of incubations.

Amendments with  $\text{NO}_3^-$  alone at levels of 120, 170, 406, 637 and 1269  $\mu\text{g NO}_3^-$ -N/L had similar denitrification rates around 10  $\mu\text{mol N}_2\text{O}/\text{m}^2/\text{hr}$  (one-way ANOVA  $p < 0.05$ ; Fig. 2a), indicating that the bacteria reached their maximum potential denitrification rate already at concentrations of 120  $\mu\text{g NO}_3^-$ -N/L. Maximum potential  $\text{N}_2\text{O}$  production rate showed the same pattern and the maximum rate reached was 2.1  $\mu\text{mol N}_2\text{O}/\text{m}^2/\text{hr}$ .

When  $\text{NO}_3^-$  was added together with C and P, the denitrification rate increased to a maximum of 14  $\mu\text{mol N}_2\text{O}/\text{m}^2/\text{hr}$  which was a significant increase compared to maximum rates when  $\text{NO}_3^-$  was added alone (two tailed t-test,  $p = 0.001$ ). The threshold for maximum potential denitrification rate was found at a similar nitrate level; 106  $\mu\text{g NO}_3^-$ -N/L. Subsequently, amendments with 406 and 806  $\mu\text{g NO}_3^-$ -N/L did not increase the rates any further. The average rate in the lower amendments (of 16, 26 and 46  $\mu\text{g NO}_3^-$ -N/L) was 3.7  $\mu\text{mol N}_2\text{O}$ . Maximum

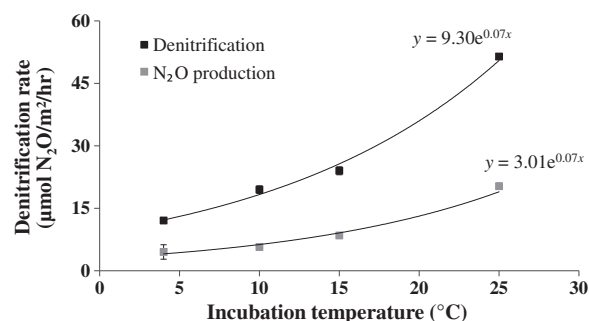


**Fig. 2 – (a) Maximum potential denitrification (with acetylene) and (b)  $\text{N}_2\text{O}$  production rates (without acetylene) in experiments incubated at 4°C with different  $\text{NO}_3^-$  amendments. Error bars represent standard error.**

potential  $\text{N}_2\text{O}$  production rate was  $< 2 \mu\text{mol N}_2\text{O}/\text{m}^2/\text{hr}$  at 16 to 46  $\text{N}_2\text{O}/\text{m}^2/\text{hr}$  amendments. There was then a marked increase in  $\text{N}_2\text{O}$  production rates in the 406 and 806  $\mu\text{g NO}_3^-$ -N/L amendments, where  $\text{N}_2\text{O}$  production rate reached its maximum of 7.8 ( $\pm 1.8$ , SE)  $\mu\text{mol N}_2\text{O}/\text{m}^2/\text{hr}$ .

## 2.3. Temperature experiment

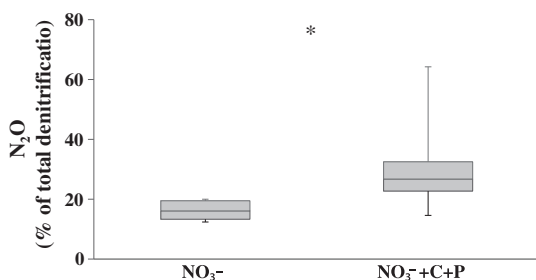
Temperature had a significant, positive effect on the maximum potential denitrification rate (one-way ANOVA, Tukey HSD, all  $p < 0.05$ ; Fig. 3), such that all temperature treatments (from 4 to 25°C) differed significantly from each other. The maximum potential  $\text{N}_2\text{O}$  production rate only showed a significant increase compared to ambient temperature treatments (4°C), when temperature reached 25°C (ANOVA, Tukey



**Fig. 3 – Maximum potential denitrification (with acetylene) and  $\text{N}_2\text{O}$  production rates (without acetylene) (at non-limiting nutrient and carbon conditions) incubated at different temperatures from 4 to 25°C. Error bars represent standard error.**

**Table 1 – Water and sediment chemistry. Note that all water samples are hypolimnion water from the sediment–water interface. Sediment  $\text{PO}_4^{3-}$ -P,  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N represent extractable (with  $\text{K}_2\text{SO}_4$ ) concentrations.**

		Feb 10	Feb 24	March 24
Water chemistry	Temperature (°C)	4.1	4.2	4.2
	$\text{O}_2$ ( $\mu\text{g/L}$ )	0.1	0	0
	pH	6.1	6.4	6.4
	DOC (mg/L)	11.8	13.4	8.9
	$\text{PO}_4^{3-}$ -N ( $\mu\text{g/L}$ )	18	35	7
	$\text{NO}_3^-$ -N ( $\mu\text{g/L}$ )	12	6	70
	$\text{NH}_4^+$ -N ( $\mu\text{g/L}$ )	189	249	40
	TDN ( $\mu\text{g/L}$ )	607	666	367
	LOI (%)		75	71
Sediment chemistry	Water content (%)		96	96
	$\text{PO}_4^{3-}$ -N ( $\mu\text{g/g}$ )	$< 1$		$< 1$
	$\text{NO}_3^-$ -N ( $\mu\text{g/g}$ )	5.0		5.7
	$\text{NH}_4^+$ -N (mg/g)	0.91		1.06



**Fig. 4 – Relative N<sub>2</sub>O production with amendments of NO<sub>3</sub><sup>-</sup> alone, and with NO<sub>3</sub><sup>-</sup>+C+P. The relative N<sub>2</sub>O production was calculated as the N<sub>2</sub>O production rate divided by the total denitrification rate. \* denotes significant difference between the groups.**

HSD  $p = 0.003$ ). The maximum potential denitrification and N<sub>2</sub>O production rate increased from 14 to 43  $\mu\text{mol}/\text{m}^2/\text{hr}$ , and 4.5 to 22  $\mu\text{mol}/\text{m}^2/\text{hr}$ , respectively, when temperatures were increased from 4 to 25°C. This increase corresponded to a Q10 value of 1.69 for denitrification, and 1.77 for N<sub>2</sub>O production. This means that maximum potential N<sub>2</sub>O production rate showed a marginally higher response to increased temperature (over the whole temperature range) than did maximum potential denitrification rate (Fig. 3). During the 24 hr incubation, in the 25°C incubation with acetylene (where production was the highest), denitrifying bacteria only consumed 28% of added inorganic N.

#### 2.4. Relative N<sub>2</sub>O production

The relative N<sub>2</sub>O production ranged from 13% to 64% (of total denitrification). The relative N<sub>2</sub>O production was not affected by different NO<sub>3</sub><sup>-</sup> amendments, or by temperature. However, the relative N<sub>2</sub>O production in amendments with NO<sub>3</sub><sup>-</sup> + C + P was significantly higher (median: 27%) than the corresponding ratio in amendments with NO<sub>3</sub><sup>-</sup> alone (median: 16%, Fig. 4; Two way Wilcoxon rank-sum test,  $p = 0.03$ ). In ambient treatments, the relative N<sub>2</sub>O production could not be calculated because the N<sub>2</sub>O production was below detection limit.

### 3. Discussion

Results from this study showed that maximum potential denitrification and N<sub>2</sub>O production rates in the sediments of this small, unproductive, boreal lake were primarily limited by NO<sub>3</sub><sup>-</sup> but clearly also limited by C and/or P availability, and constrained by temperature. We also found that when nutrients were added with C (at non-limiting concentrations) the N<sub>2</sub>O production rate responded stronger than the denitrification rate which resulted in higher relative N<sub>2</sub>O production. It can also be concluded that relative N<sub>2</sub>O production was higher than previously reported from lakes.

The maximum potential denitrification rate (0.66  $\mu\text{mol}$  N<sub>2</sub>O/ $\text{m}^2/\text{hr}$  at ambient conditions; 45.2  $\mu\text{mol}$  N<sub>2</sub>O/ $\text{m}^2/\text{hr}$  with 806  $\mu\text{g}$  NO<sub>3</sub><sup>-</sup>-N/L with C and P at 25°C) and maximum potential N<sub>2</sub>O production rate (no production at ambient conditions; 22  $\mu\text{mol}$  N<sub>2</sub>O/ $\text{m}^2/\text{hr}$  with 806  $\mu\text{g}$  NO<sub>3</sub><sup>-</sup>-N/L with C and P at 25°C) reached in this study are within the same range reported from

other sediment studies originating from different experimental approaches. Hence, this includes lake sediment experiments performed at ambient temperature and nutrient conditions from unproductive clear water lakes (McCrackin and Elser, 2010, measured at 15°C), and studies on maximum potential denitrification rates in a eutrophic lake using different nutrient amendments (Ahlgren et al., 1994, measured at 20°C; Rissanen et al., 2011, using in situ temperatures from 0.5 to 21°C). However, our results on maximum potential denitrification without nutrient amendments (incubated at 4°C) are in the lower range of rates presented in the review by Seitzinger (1988), which mostly included sediment studies from more nutrient rich and productive lakes. Thus, we want to stress the difficulties of comparing denitrification results between studies due to the discrepancies in the experimental designs used as well as the different methods used for measuring and presenting data. We have tried to highlight some of these difficulties in the following discussion.

In line with our first hypothesis, NO<sub>3</sub><sup>-</sup> was found to have a significant role in limiting denitrification and N<sub>2</sub>O production rate, i.e., both maximum potential denitrification and N<sub>2</sub>O production rates increased with NO<sub>3</sub><sup>-</sup> additions. Surprisingly, NO<sub>3</sub><sup>-</sup> limitation was alleviated at amendment levels of only 106–120  $\mu\text{g}$  NO<sub>3</sub><sup>-</sup>-N/L. Previous studies made on NO<sub>3</sub> limitation have shown highly variable results. A similar dose-response experiment performed by McCrackin and Elser (2012) conducted in sediments from unproductive, clear water mountain lakes in Colorado, USA, showed that denitrification was relieved from nitrate limitation in amendments somewhere between 50 and 500  $\mu\text{mol}$  NO<sub>3</sub><sup>-</sup>-N/L (700–7000  $\mu\text{g}$  NO<sub>3</sub><sup>-</sup>-N/L). Further, studies by García-Ruiz et al. (1998) on sediments from the Swale–Ouse river system (UK) found that nitrate limitation was alleviated at concentrations between 20 to 2000  $\mu\text{mol}$  NO<sub>3</sub><sup>-</sup>-N/L and that nitrate limitation was closely linked to stream total N concentration. Combined these studies subsequently illustrate large variations in half saturation constant ( $K_m$ ) between systems; i.e.,  $K_m$  in our experiment was 50  $\mu\text{g}$  NO<sub>3</sub><sup>-</sup>-N/L vs.  $K_m$  between 3 and 1100  $\mu\text{mol}$  NO<sub>3</sub><sup>-</sup>-N/L in the McCrackin and Elser (2012) study. Notable is that our sediments were incubated at 4°C, the García-Ruiz et al. (1998) study at 15°C, and the McCrackin and Elser (2012) study at 17–20°C. However, these differences in incubation temperature cannot explain the lower  $K_m$  values found in our study as lower temperatures have been shown to reduce the affinity for nitrate in bacteria, meaning they need higher NO<sub>3</sub> concentrations to reach steady state growth at low temperatures (Reay et al., 1999). Hence, something else besides temperature was most likely limiting the denitrifying bacterial population in our system compared to the other mentioned studies above, and we suggest that this is likely attributed to differences in water and sediment character (i.e., different chemical composition, such as C and P quality and quantity) between systems.

The sediments of Lake Nätjärn are composed of large shares of allochthonous C, likely of poor quality (cf. Fork and Heffernan, 2014), and with low concentrations of extractable (loosely sorbed) P in the sediments compared to many other lakes (cf. Table 1 and Ostrofsky, 1987). The denitrifying bacterial population in our experiments was likely adapted to these sediment conditions (cf. O'Connor et al., 2006). Thus,

because our experiments with C and P amendments were made only during a few hours, the denitrifying bacterial population may have been unable to adapt to these new environmental incubation conditions during the incubation time. McCrackin and Elser (2012) also found that the abundance of denitrifiers co-varied with both the abundance and the quality of organic matter and that potential denitrification co-varied with the C:P ratio. Combined, our results indicate that denitrification in Lake Nätstjärn, a typical boreal forest lake in northern Sweden, likely was constrained by sediment characteristics and low  $\text{NO}_3^-$  concentrations that resulted in low maximum potential denitrification and  $\text{N}_2\text{O}$  production rates. Currently, this system is also relieved from nitrate limitation already at low additions of nitrate ( $106\text{--}120\text{ }\mu\text{g NO}_3^-\text{--N/L}$ ), but this threshold could potentially increase with changed sediment characteristics.

Few studies have been published on the effect of temperature on denitrification in lake sediments. Andersen (1977) saw no effect from temperature in incubations of sediments from productive Danish lakes. This study was however not comparable to ours because the measurements were made in oxygenated incubations. But in line with our first hypothesis we found that increased temperatures significantly increased maximum potential denitrification and  $\text{N}_2\text{O}$  production rates. Both maximum potential denitrification rate and  $\text{N}_2\text{O}$  production rate increased exponentially with increasing temperature and were relatively similar ( $Q_{10}$  was 1.69 for denitrification and 1.77 for  $\text{N}_2\text{O}$  production). Even though not measured directly, both McCrackin and Elser (2012) and Liikanen et al. (2002) speculated that temperature was an important constraining factor for denitrification in their studies. Large temperature effects on denitrification in the sediment was also found in the nutrient rich Lake Taihu in China (Zhong et al., 2010), even though the  $Q_{10}$  value was not calculated. A positive response of the denitrification process to increased temperature can thus be expected, even in nutrient poor, unproductive lakes. The  $Q_{10}$  value for the studied lake is in the lower range of reported values of pure cultured aquatic bacteria (2–3) (Pomeroy and Wiebe, 2001). It could be speculated that this is related to the lake's nutrient status, with low  $Q_{10}$  values in unproductive boreal lakes and higher values in nutrient rich lakes. We can conclude that differences in temperature response in different systems raises the problem of interpreting and comparing data from denitrification studies where different incubation temperatures have been used.

The results used for testing the second and third hypotheses, concerning relative  $\text{N}_2\text{O}$  production, are more ambiguous. Our relative  $\text{N}_2\text{O}$  production (ranging from 13% to 64%) was within the range reported by García-Ruiz et al. (1998), similar to the ones presented by Beaulieu et al. (2011, 2014) but high in comparison to most ranges previously reported (Deemer et al., 2011; Firestone et al., 1980; McCrackin and Elser, 2010; Seitzinger, 1988). However, none of the ratios in the abovementioned studies originates from unproductive boreal lakes. Potentially it could be problematic to evaluate relative  $\text{N}_2\text{O}$  production from experiments using the acetylene inhibition technique because the acetylene may inhibit nitrification (Groffman et al., 2006), but as our incubations were made with  $\text{NO}_3^-$  additions the ratio between  $\text{N}_2\text{O}$  and  $\text{N}_2$  should not have been affected by a deficiency of  $\text{NO}_3^-$ . The

acetylene technique was evaluated by Bernot et al. (2003) who found comparable results using the acetylene and Membrane Inlet Mass Spectrometry (MIMS) techniques when measuring denitrification rates in sediments from a Texas (USA) estuary. Bernot et al. (2003), used chloramphenicol to inhibit new enzyme production from the denitrifying bacteria during acetylene incubations, a process which could enhance denitrification. It has not been evaluated whether chloramphenicol affects the relationship between  $\text{N}_2$  and  $\text{N}_2\text{O}$  production and thus we did not use it. Combined we feel that it is adequate to use the acetylene inhibition technique, in experiments with excess nitrate amendments, to evaluate factors governing  $\text{N}_2\text{O}:\text{N}_2$  ratios. Hence, we do not think that the high relative  $\text{N}_2\text{O}$  production in this study is an artifact of the method, but rather suggest that it reflects the conditions in unproductive boreal lakes with low atmospheric N deposition which, at least to our knowledge, has been poorly studied so far.

Our second hypothesis was not supported, as the relative  $\text{N}_2\text{O}$  production was not affected by the  $\text{NO}_3^-$  amendments. This hypothesis was based on the fact that if the bacteria are not limited by  $\text{NO}_3^-$ , they prefer reduction of  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$ , before  $\text{N}_2\text{O}$  to  $\text{N}_2$ , because the  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$  reduction is more thermodynamically favorable (Schlesinger and Bernhardt, 2013), creating more energy per N molecule reduced for the bacteria. However, when this theory has been tested, contrasting results have been found. Earlier lake sediment (McCrackin and Elser, 2010) and soil studies (Firestone et al., 1980) have found that  $\text{N}_2\text{O}:\text{N}_2$  ratios increased when N availability increased. In a denitrification study by Beaulieu et al. (2011) conducted in 72 stream sediments from different land-use types across US,  $\text{N}_2\text{O}:\text{N}_2$  ratios remained stable even though  $\text{NO}_3^-$  concentrations spanned more than five orders of magnitude (from 0.1 to  $10,000\text{ }\mu\text{g NO}_3^-\text{--N/L}$ ); i.e., in line with our results. In our study, increased  $\text{NO}_3^-$  concentration did not affect relative  $\text{N}_2\text{O}$  production, but it was increased when  $\text{NO}_3^-$  was added combined with C and P. This outcome in our experiments is further supported by Heuvel et al. (2009), who found that glucose amendments favored  $\text{N}_2\text{O}$  production over  $\text{N}_2$  production in experiments performed in riparian soils. Overall, the underlying mechanism of the discrepancy between studies, and between different environmental conditions (such as temperature), concerning how  $\text{NO}_3^-$  availability alone, and/or in combination with higher C and P availability, affects the relative  $\text{N}_2\text{O}$  production remains to be further investigated.

Given the fact that primary production is N-limited in northern boreal lakes (Bergström and Jansson, 2006) we expect that primary producer responses to inorganic N loading will be mediated through the effects of DOM (dissolved organic matter) on light (Jones, 1992; Seekell et al., 2015). How this response will affect rates of denitrification is far less clear. Since there is plenty of available C across boreal lakes to support denitrification, rates should primarily be governed by the availability of nitrate, which was also supported by our results. However, denitrification rates may also increase with the bulk DOM content, reflecting larger supplies of C (i.e., quantities), and higher rates of bacterial production and respiration that drive down oxygen levels (Hessen, 1985; Bergström and Jansson, 2000) and induces P mobilization from anoxic sediments (Boström et al., 1988). But if C quality is more important than quantity, denitrification rates may on the other hand decline with higher DOM content



as the terrestrial DOC increasingly dilutes labile DOC exudates from phytoplankton and suppress algal production (Jones, 1992; Karlsson et al., 2009). There are consequently both direct and indirect mechanisms that may affect denitrification rates in boreal lakes, since the N processing is so tightly linked to the C-cycle. Our results point in the direction that unproductive northern boreal lakes may respond to even slight increases in  $\text{NO}_3^-$  loading, with increased denitrification and  $\text{N}_2\text{O}$  production, and that these processes will be especially enhanced if  $\text{NO}_3^-$  loadings are accompanied by environmental drivers which enhance both C and P availability in lakes, and by climate warming.

#### 4. Conclusions

We found that both maximum potential denitrification and  $\text{N}_2\text{O}$  production rates in the sediments of the unproductive boreal Lake Nästjärn were low compared to more nutrient loaded systems. The production rates increased with  $\text{NO}_3^-$  amendments but they were relieved from nutrient limitation at fairly low  $\text{NO}_3^-$  concentrations (already at 106–120  $\mu\text{g NO}_3^-/\text{N/L}$ ). Adding unlimited resources of C and P significantly increased the maximum potential denitrification and  $\text{N}_2\text{O}$  production rates and both of these processes were highly temperature dependent. Also, the relative  $\text{N}_2\text{O}$  production found in the various experiments was higher than many studies have previously reported and it increased when C and P availability was increased. The high throughput of organic matter in boreal lakes potentially make denitrification in these systems sensitive to environmental changes such as warming (Veraart et al., 2011) and the results show that even slight increases in enhanced inorganic nutrient and C availability will likely promote increased denitrification and  $\text{N}_2\text{O}$  production, and proportionally higher  $\text{N}_2\text{O}$  production relative to  $\text{N}_2$ . The studied system, an unproductive northern boreal lake, with low N deposition and naturally low catchment inputs of inorganic N, represents a lake type that, to our knowledge, has been overlooked in denitrification studies. In order to make accurate up-scalings of  $\text{N}_2\text{O}$  production, and its linkage to climate and land-use changes, we emphasize the need of future comparable studies on denitrification in unproductive boreal lakes.

#### Acknowledgments

Many thanks to Ryan Sponseller and two anonymous reviewers for providing valuable comments to improve the manuscript. Financial support was granted from The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas). (No. 215-2010-992).

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