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# NO<sub>x</sub> reduction is the main pathway for benthic N<sub>2</sub>O production in a eutrophic, monomictic south-alpine lake

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## Abstract

Nitrous oxide ( $\text{N}_2\text{O}$ ) is a potent greenhouse gas, generated through microbial nitrogen (N) turnover processes, such as nitrification, nitrifier denitrification, and denitrification. Previous studies quantifying natural sources have mainly focused on soils and the ocean, but the potential role of terrestrial water bodies in the global  $\text{N}_2\text{O}$  budget has been widely neglected. Furthermore, the biogeochemical controls on the production rates and the microbial pathways that produce benthic  $\text{N}_2\text{O}$  in lakes are essentially unknown. In this study, benthic  $\text{N}_2\text{O}$  fluxes and the contributions of the microbial pathways that produce  $\text{N}_2\text{O}$  were assessed using  $^{15}\text{N}$  label flow-through sediment incubations in the eutrophic, monomictic south basin of Lake Lugano in Switzerland. The sediments were a significant source of  $\text{N}_2\text{O}$  throughout the year, with production rates ranging between 140 and 2605  $\text{nmol N}_2\text{O h}^{-1} \text{m}^{-2}$ , and the highest observed rates coinciding with periods of water column stratification and stably anoxic conditions in the overlying bottom water. Nitrate ( $\text{NO}_3^-$ ) reduction via denitrification was found to be the major  $\text{N}_2\text{O}$  production pathway in the sediments under both oxygen-depleted and oxygen-replete conditions in the overlying water, while ammonium oxidation did not significantly contribute to the benthic  $\text{N}_2\text{O}$  flux. A significant portion (up to 15%) of the total  $\text{NO}_3^-$  consumed by denitrification was reduced only to  $\text{N}_2\text{O}$ , without complete denitrification to  $\text{N}_2$ . These fluxes were highest when the bottom water had completely stabilized to a low-oxygen state, in contrast with the notion that stable anoxia is particularly conducive to complete denitrification without accumulation of  $\text{N}_2\text{O}$ . This study provides evidence that lake sediments are a significant source of  $\text{N}_2\text{O}$  to the overlying water and may produce large  $\text{N}_2\text{O}$  fluxes to the atmosphere during seasonal mixing events.

## 1 Introduction

Nitrous oxide ( $\text{N}_2\text{O}$ ) is a potent greenhouse gas with a global warming potential that is  $\sim 300$  times higher than that of  $\text{CO}_2$  over a 100-yr time horizon (Forster et al., 2007).

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Furthermore, N<sub>2</sub>O is the most important stratospheric ozone-depleting substance currently being emitted to the atmosphere (Ravishankara et al., 2009). The atmospheric concentration has increased from 270 ppb in 1750 (Forster et al., 2007) to 323 ppb in 2011 (AGAGE, 2012), but there are still large uncertainties with regard to the relative contributions of the major sources and sinks (Forster et al., 2007). Microbiological processes in soils and the ocean are the most important natural N<sub>2</sub>O sources (Forster et al., 2007). However, the recent increase in atmospheric N<sub>2</sub>O concentration is largely due to human intervention in the nitrogen cycle, in particular through the agricultural use of synthetic nitrogen-based fertilizers (Codispoti et al., 2001; Bouwman et al., 2002; Mosier et al., 1998). In aquatic systems, anthropogenic fixed nitrogen (N) loading can have multiple detrimental environmental effects, such as eutrophication, acidification, and the reduction of biodiversity (Galloway et al., 2003). In these environments, microbial processes taking place in redox transition zones play an important role in removing fixed N. Denitrification, for example, can be an important mechanism for removing fixed N along the land-ocean continuum by reducing it back to N<sub>2</sub>. N<sub>2</sub>O is a free intermediate in this process that may be released to the environment under certain conditions. N<sub>2</sub>O is also produced during other N transformation reactions (Galloway et al., 2003) such as nitrification (specifically, ammonia oxidation) and nitrifier denitrification.

Distinguishing the relative contributions of each of these major N<sub>2</sub>O production and consumption pathways to the total N<sub>2</sub>O flux is often challenging in aquatic systems because different types of microorganisms perform these pathways under overlapping environmental conditions. Furthermore, certain microbes carry out more than one pathway in response to changes in biogeochemical conditions. N<sub>2</sub>O is produced during the aerobic oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>) when hydroxylamine (NH<sub>2</sub>OH), an intermediate in the reaction, decomposes (Stein, 2011). Rates of ammonia oxidation depend primarily on substrate (NH<sub>4</sub><sup>+</sup>) and oxygen (O<sub>2</sub>) availability (Ward, 2008). However, in sediments, aerobic ammonia oxidation and nitrite oxidation to nitrate can be closely coupled to anaerobic NO<sub>3</sub><sup>-</sup> reduction in the redox transition zone (coupled nitrification-denitrification, Ward, 2008). Ammonia oxidizers also produce N<sub>2</sub>O through

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a second mechanism known as nitrifier denitrification, an enzymatic pathway that sequentially reduces NO<sub>2</sub><sup>-</sup> to nitric oxide (NO), and then N<sub>2</sub>O (Wrage et al., 2001). The importance of nitrifier denitrification as a N<sub>2</sub>O source appears to be higher under low-O<sub>2</sub> conditions (Ritchie and Nicholas, 1972; Poth and Focht, 1985). However, unlike denitrification, O<sub>2</sub> does not seem to inhibit nitrifier denitrification to the same extent or through the same mechanisms (Kool et al., 2011). Denitrification is the reduction of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub> via the gaseous intermediates NO and N<sub>2</sub>O (Knowles, 1982) under anoxic or suboxic conditions (i.e. [O<sub>2</sub>] < 2–5 μmol L<sup>-1</sup>, Devol, 2008; Codispoti et al., 2001). Denitrification can both produce and consume dissolved N<sub>2</sub>O, releasing N<sub>2</sub>O under conditions that suppress the activity of the N<sub>2</sub>O reductase enzyme, e.g. under low O<sub>2</sub> concentrations (Firestone et al., 1979; Otte et al., 1996). Furthermore, rapid transitions between oxic and suboxic conditions may cause “stop and go” denitrification, which causes N<sub>2</sub>O accumulation in aquatic environments (Naqvi et al., 2000; Codispoti et al., 2001).

In lacustrine sediments, microbial activity consumes O<sub>2</sub> rapidly in the topmost millimeters, leading to suboxic or anoxic conditions in deeper sediment horizons, where denitrification becomes an important redox process (Hunting and van der Geest, 2011). The O<sub>2</sub> penetration depth is closely related to the O<sub>2</sub> concentration in the overlying water and the sediment reactivity (Lehmann et al., 2009; Thibodeau et al., 2010). A decrease in bottom water O<sub>2</sub> concentration is reflected in a narrower oxygenated zone in the sediment (Rasmussen and Jorgensen, 1992). Narrow redox zonation leads thus to an equally narrow succession of microbial processes (Stockdale et al., 2009). Changes in the redox zonation may have profound consequences on N<sub>2</sub>O production (Otte et al., 1996). Seasonal cycles of water column mixing and stagnation can influence the oxidation state of surface sediments and modulate the penetration of redox boundaries into the sediments, potentially changing the redox environments of nitrifiers and denitrifiers (Rasmussen and Jorgensen, 1992). The few studies that have quantified N<sub>2</sub>O fluxes from freshwater sediments, indicate that lake sediments can be a significant source of N<sub>2</sub>O. They also highlight that factors influencing N<sub>2</sub>O production pathways in the

benthic environment are still not clearly identified, particularly with regard to the relative importance of nitrification, nitrifier denitrification, and denitrification (Mengis et al., 1996; Liikanen and Martikainen, 2003; Liikanen et al., 2003b; McCrackin and Elser, 2010).

5 In this study, N<sub>2</sub>O production pathways in lacustrine sediments were studied using ex situ steady state flow-through incubations with intact sediment cores (Lavrentyev et al., 2000; McCarthy et al., 2007; Liikanen and Martikainen, 2003; Liikanen et al., 2002a,b,c, 2003a,b) in combination with substrate <sup>15</sup>N labeling to assess benthic N<sub>2</sub>O production rates and pathways. The experiments were conducted with sediments from  
10 a eutrophic, monomictic lake in southern Switzerland, the south basin (SB) of Lake Lugano. Monomixis and the resulting intermittent anoxia and suboxia of the bottom waters makes the SB an ideal study site for testing the effects of variable bottom water oxygenation on the benthic N<sub>2</sub>O production in a lake. Furthermore, previous measurements (Wenk, 2013) indicate high bottom water N<sub>2</sub>O accumulation in the deep  
15 hypolimnion during thermal stratification in summer and fall, begging the question as to what causes N<sub>2</sub>O accumulation in near bottom waters. Upon water column overturn in winter, N<sub>2</sub>O-laden bottom waters may be advected to the surface, enhancing N<sub>2</sub>O fluxes into the atmosphere.

20 The study's objectives were (1) to estimate N<sub>2</sub>O fluxes from the sediments to the overlying water column and to assess seasonal variations in these fluxes, (2) to identify the dominant benthic N<sub>2</sub>O producing processes in the lake, and (3) to study the possible impact of variable redox conditions of bottom waters during the seasonal cycle on N<sub>2</sub>O production rates and pathways.

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## 2 Sampling and methods

### 2.1 Site description

Lake Lugano is located in southern Switzerland/northern Italy at an altitude of 271 m above sea level (Fig. 1). It is divided into two main basins, the northern and southern basin. In addition, a smaller sub-basin of the southern basin is located in front of the only outlet, the Tresa River (Barbieri and Polli, 1992; Lehmann et al., 2004a). Due to the limited water exchange, the basins are characterized by a distinct limnology, so that the northern and southern basins can be regarded as two separate lake systems that are connected by a narrow opening at Melide. A detailed overview of the lake's limnology can be found in Barbieri and Polli (1992) and Barbieri and Simona (2001).

The SB mixes completely from January/February to April. It has an area of 20.3 km<sup>2</sup>, a volume of 1.14 km<sup>3</sup> and a maximum depth of 95 m (Barbieri and Polli, 1992).

Lazzaretti and Hanselmann (1992) and Lehmann et al. (2004a,b) described in detail the changes in seasonal redox conditions in the southern basin. During the mixing period, the whole water column becomes oxygenated and oxic conditions are found at the sediment/water interface until late spring. With the onset of thermo-stratification, generally in April, together with the increased phytoplankton production in surface waters and organic matter export to the hypolimnion, oxygen concentrations in the deep hypolimnion decrease, and by June/July, the redox transition zone has migrated from within the sediments by several meters into the water column. Complete anoxia prevails in the bottom waters until the water column turns over again in winter.

### 2.2 Sampling

Six ~ 50 cm sediment cores with 20 cm overlying bottom water were taken with a 5.7 cm diameter mini gravity corer at a site (45°57' N, 8°54' E) west of the village of Figino, close to the point of maximum water depth of this basin (Fig. 1) in 2010 (April, August, October) and 2011 (January, May). The cores were stored upright and in the dark

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during transport to the home laboratory on the day of sampling. In addition, 3 × 20 L of bottom water were sampled using 10 L Niskin bottles. Bottom waters were stored in open 20 L plastic containers with headspace for samples taken during oxic conditions (April 2010, May 2011). During thermal stratification and anoxia in the deep hypolimnion, bottom waters were filled into gastight 25 L bags (Gas Sampling Tedlar Bags, CEL Scientific Corporation, ITP-25) without headspace. In order to minimize the risk of O<sub>2</sub> contamination, only the lower three quarters of the Niskin bottle contents were used, and in October 2010 and January 2011, the bags were additionally stored underwater. Within approximately 6 h from sampling and after transportation to the lab, the flow-through experiments were set up in a cold room at a near-in situ temperature (6.5 °C).

### 2.3 Steady state flow-through experiments

Steady state flow-through experiments were set up according to Gardner et al. (1991) and Lavretyev et al. (2000) (Fig. 2). The top caps on the liners were removed and replaced with gastight, O-ring sealed PVC plungers containing two holes. The plungers were lowered into the liners until all headspace air was released through the holes. Subsequently, the inlet water reservoir was connected to the core with gastight tubes (FEP, 0.8 mm inner diameter). A second tube connected the core with the sampling vial. A constant flow of ~ 1 mL min<sup>-1</sup> was established with a peristaltic pump. For each sampling campaign, three duplicate flow-through experiments were set up, where two core incubations were supplied with water from one inlet water reservoir, respectively. One of the three inlet water reservoirs was amended with <sup>15</sup>N-NH<sub>4</sub><sup>+</sup> (Ammonium Chloride, > 99 % <sup>15</sup>N atom, Spectra Stable Isotopes), one with <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> (Potassium Nitrate, > 99 % <sup>15</sup>N atom, Spectra Stable Isotopes) and one was left unamended as a control. The labeled substrates were added so that the in situ concentrations were doubled (final <sup>15</sup>N content ~ 50 %), except in the aerobic ambient O<sub>2</sub> incubations, where the

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background  $\text{NH}_4^+$  concentration was  $< 1 \mu\text{mol L}^{-1}$  and  $10 \mu\text{mol L}^{-1}$  of  $^{15}\text{N-NH}_4^+$  label was added (final  $^{15}\text{N}$  content  $> 90\%$ ).

After a conditioning period of  $> 24$  h (Gardner and McCarthy, 2009), the in- and out-flows were sampled daily. The presented results are the average of 2 (August 2010) or 3 incubation days (October 2010, January 2011, May 2011). In April 2010, samples for  $\text{N}_2\text{O}$  analysis were only taken on the last day of the experiment.

For  $\text{N}_2\text{O}$  analyses, glass vials (21 mL) were filled from bottom to top, and allowed to overflow for at least two bottle volumes to minimize  $\text{N}_2\text{O}$  exchange with air. The vials were capped with aluminium crimp caps with silicone septa (CS chromatographie service GmbH, Art. Nr. 300227). Subsequently, a 10 mL He headspace was added in exchange with water. The samples were sterilized with 0.2 mL of  $10 \text{ mol L}^{-1}$  NaOH to prevent further microbial activity (Sigman et al., 2001). Sample treatment was done within one hour after sampling. After adding NaOH, the samples were analyzed within 3 days.

## 2.4 Determination of $\text{N}_2\text{O}$ concentrations and benthic fluxes

$\text{N}_2\text{O}$  concentrations and stable isotope ratios were determined using an isotope ratio mass spectrometer (IRMS, Thermo Finnigan Deltaplus XP), coupled to an automated purge and trap system (Thermo Finnigan GasBench II).  $\text{N}_2\text{O}$  concentration standards were produced using the denitrifier method (Sigman et al., 2001) to reduce  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$ . Six  $\text{KNO}_3$  solutions were produced to yield the following  $\text{N}_2\text{O}$  concentration standards: 0.03, 0.1, 0.2, 0.5, 1.0, 1.5  $\text{nmol N}_2\text{O mL}^{-1}$ . Detector-sensitivity corrected IRMS peak areas of  $\text{N}_2\text{O}$  standards were compared to the respective concentrations in a regression analysis, and the resulting transfer function was used to calculate the  $\text{N}_2\text{O}$  concentrations (in  $\text{nmol L}^{-1}$ ) based on the M/z 44, 45 and 46 detector signals. These correspond to the  $[^{14}\text{N}^{14}\text{N}^{16}\text{O}]$ ,  $[^{14}\text{N}^{15}\text{N}^{16}\text{O} + ^{15}\text{N}^{14}\text{N}^{16}\text{O} + ^{14}\text{N}^{14}\text{N}^{17}\text{O}]$ , and  $[^{15}\text{N}^{15}\text{N}^{16}\text{O} + ^{14}\text{N}^{14}\text{N}^{18}\text{O} + ^{14}\text{N}^{15}\text{N}^{17}\text{O} + ^{15}\text{N}^{14}\text{N}^{17}\text{O}]$  isotopologues, respectively. For the  $^{15}\text{N}$  amended incubations,  $[^{14}\text{N}^{15}\text{N}^{16}\text{O} + ^{15}\text{N}^{14}\text{N}^{16}\text{O}] \gg [^{14}\text{N}^{14}\text{N}^{17}\text{O}]$ , and

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$[^{15}\text{N}^{15}\text{N}^{16}\text{O}] \gg [^{14}\text{N}^{14}\text{N}^{18}\text{O} + ^{14}\text{N}^{15}\text{N}^{17}\text{O} + ^{15}\text{N}^{14}\text{N}^{17}\text{O}]$ , and we therefore assume that any changes in the contributions of the natural abundance  $^{18}\text{O}$  and  $^{17}\text{O}$  isotopologues were negligible.

Fluxes of  $\text{N}_2\text{O}$  of masses 44, 45 and 46 [ $\text{nmol h}^{-1} \text{m}^{-2}$ ] from the sediment to the water column were then calculated from the concentration changes in the in- and out-flowing water:

$$\text{Flux}_{\text{N}_2\text{O}} = ([\text{N}_2\text{O}_{\text{out}}] - [\text{N}_2\text{O}_{\text{in}}]) \times \frac{Q}{A} \quad (1)$$

where  $[\text{N}_2\text{O}_{\text{out/in}}]$  are the measured  $\text{N}_2\text{O}$  concentrations ( $\text{nmol L}^{-1}$ ),  $Q$  is the average flow rate ( $\text{L h}^{-1}$ ) and  $A$  is the sediment core surface area ( $\text{m}^2$ ). Positive fluxes indicate a net increase in  $\text{N}_2\text{O}$  concentration between the in- and out-flowing water, and thus fluxes out of the sediments.

## 3 Results

### 3.1 Water column characteristics

The physical and chemical bottom water parameters at the five sampling dates are presented in Table 1. In March and April 2010, the water column was well mixed so that bottom waters were fully oxygenated, the  $\text{NH}_4^+$  concentration was low ( $0.3 \mu\text{mol L}^{-1}$ ) and the  $\text{NO}_3^-$  concentration was comparatively high ( $83.7 \mu\text{mol L}^{-1}$ ). By August 2010, the lake was stratified, an anoxic near-bottom layer of about 2 m width had developed, and the  $\text{NH}_4^+$  concentration in the bottom water had risen ( $7.1 \mu\text{mol L}^{-1}$ ). In October 2010, with ongoing stratification and organic matter decomposition, the anoxic bottom layer expanded by another 6 to 7 m into the water column.  $\text{NO}_2^-$  ( $2.4 \mu\text{mol L}^{-1}$ ) as well as  $\text{NH}_4^+$  ( $44.5 \mu\text{mol L}^{-1}$ ) accumulated in the bottom water, but  $\text{NO}_3^-$  concentrations ( $50.3 \mu\text{mol L}^{-1}$ ) decreased. In January 2011, samples were collected right at the beginning of the winter overturn. The  $\text{O}_2$  gradient started to collapse, and suboxic to hypoxic

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concentrations of  $O_2$  ( $11 \mu\text{mol L}^{-1}$ ) were measured in bottom waters. In May 2011, the water column was fully oxygenated again.

### 3.2 Benthic $N_2O$ fluxes

Net benthic  $N_2O$  fluxes were calculated for the total  $N_2O$  as well as the unlabeled ( $^{14}\text{N}^{14}\text{N}^{16}\text{O}$ ), the singly- ( $^{14}\text{N}^{15}\text{N}^{16}\text{O}$ ,  $^{15}\text{N}^{14}\text{N}^{16}\text{O}$ ), and the doubly-labeled  $N_2O$  ( $^{15}\text{N}^{15}\text{N}^{16}\text{O}$ ) according to Eq. (1) (Fig. 3).

In almost all incubations, except in January 2011, average  $N_2O$  fluxes were positive, indicating that sediments released  $N_2O$  to the overlying water column.  $N_2O$  flux measurements in unamended cores (core 1 and core 2), which represent  $N_2O$  fluxes under in situ  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentration conditions, increased from 831 and 140  $\text{nmol N}_2\text{O h}^{-1} \text{m}^{-2}$  in April 2010 to 2426 and 2605  $\text{nmol N}_2\text{O h}^{-1} \text{m}^{-2}$  in October 2010. In January and May 2011, average  $N_2O$  fluxes were again relatively low (202 and 195  $\text{nmol N}_2\text{O h}^{-1} \text{m}^{-2}$  in January 2011 and 178 and 189  $\text{nmol N}_2\text{O h}^{-1} \text{m}^{-2}$  in May 2011 for core 1 and core 2, respectively).

Overall, total  $N_2O$  fluxes calculated from unamended cores showed strong variation between different months and between duplicate cores. In April and August 2010 the difference between the  $N_2O$  fluxes of the replicate cores was most pronounced (difference  $\sim 700$  and  $\sim 850 \text{ nmol N}_2\text{O h}^{-1} \text{m}^{-2}$ , respectively) probably reflecting the heterogeneity of the sediments (Fig. 3).

### 3.3 $^{15}\text{N}$ - $N_2O$ recovery from the $^{15}\text{N}$ labeling experiments

$^{15}\text{N}$ -label was detected as  $^{15}\text{N}$ - $N_2O$  for all  $^{15}\text{N}$ - $\text{NO}_3^-$  labeled sediment cores (Fig. 3). Generally,  $^{15}\text{N}$ - $N_2O$  fluxes in the  $^{15}\text{N}$ - $\text{NH}_4^+$  labeled cores did not exceed natural abundance levels. Total  $N_2O$  fluxes in cores with  $^{15}\text{N}$ - $\text{NH}_4^+$  and  $^{15}\text{N}$ - $\text{NO}_3^-$  additions were comparable to  $N_2O$  fluxes from the unamended cores, despite the two-fold increase

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in  $\text{NH}_4^+$  or  $\text{NO}_3^-$  concentrations. Enhanced  $\text{N}_2\text{O}$  fluxes in  $^{15}\text{N}\text{-NO}_3^-$  labeled cores with respect to control cores were only observed in April 2010.

## 4 Discussion

### 4.1 The sediments are a net source of $\text{N}_2\text{O}$ throughout the year

Lacustrine sediments are a known source of  $\text{N}_2\text{O}$  (McCrackin and Elser, 2010; Liikanen and Martikainen, 2003; Mengis et al., 1996). The  $\text{N}_2\text{O}$  fluxes reported here ranged between  $140 \text{ nmol N}_2\text{O h}^{-1} \text{ m}^{-2}$  (April 2010) and  $1115 \text{ nmol N}_2\text{O h}^{-1} \text{ m}^{-2}$  (August 2010), and were comparable to measurements reported previously for other eutrophic lakes. However, the October 2010 sampling stands out, with an exceptionally high production rate of  $2605 \text{ nmol N}_2\text{O h}^{-1} \text{ m}^{-2}$ . Mengis et al. (1996), for example, measured net benthic  $\text{N}_2\text{O}$  fluxes of  $458\text{--}542 \text{ nmol N}_2\text{O h}^{-1} \text{ m}^{-2}$  at the bottom of the artificially oxygenated Lake Baldegg in static chamber experiments. Liikanen and Martikainen (2003) used microcosm incubation experiments with intact sediment cores to measure  $\text{N}_2\text{O}$  fluxes of up to  $317 \text{ nmol N}_2\text{O h}^{-1} \text{ m}^{-2}$  in a eutrophic lake in Finland. McCrackin and Elser (2010) reported an average  $\text{N}_2\text{O}$  flux of  $550 \text{ nmol N}_2\text{O h}^{-1} \text{ m}^{-2}$  in Norwegian lakes.

Comparison of the total  $\text{N}_2\text{O}$  fluxes from unamended cores (Fig. 3) to the  $\text{NH}_4^+$  or  $\text{NO}_3^-$  amended cores shows no obvious stimulation of  $\text{N}_2\text{O}$  production pathways. This suggests that the in situ microbial processes were not substrate-limited so that the rates presented here are real rather than potential rates. The expectation that net  $\text{N}_2\text{O}$  fluxes vary seasonally, with potential links to water column stratification and oxygenation, was partially supported. Total  $\text{N}_2\text{O}$  fluxes increased during the stagnation period with increasing anoxia from August to October 2010, when maximal values were reached. Such high sedimentary  $\text{N}_2\text{O}$  production during stratification can lead to accumulation and oversaturation in the bottom water, as measured in 2009 (Wenk, 2013). At the

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beginning of water column overturn in January 2011, together with the breakdown of the  $O_2$  gradient, small amounts of  $O_2$  ( $11 \mu\text{mol L}^{-1} O_2$ ) were measured in bottom waters, and only low benthic  $N_2O$  fluxes were observed. The fact that more reduced conditions during stratification foster  $N_2O$  production, and that  $O_2$  seems to hinder benthic  $N_2O$  production, implies denitrification as the dominant  $N_2O$  production pathway (see below). It has been suggested that the rapid injection of small quantities of  $O_2$  into  $O_2$ -deficient environments may be conducive to  $N_2O$  release by denitrifiers (Codispoti et al., 2001). However, our results indicate that  $N_2O$  production rates were in fact very low during the initial phase of the destratification period (January 2011), the period when pulses of  $O_2$ -laden bottom waters would have been expected to cause this type of perturbation of the denitrifiers' redox environment. Rather, the reduced  $N_2O$  production in January 2011 suggests that the introduction of low concentrations of  $O_2$  may, in fact, inhibit  $N_2O$  release.

We cannot rule out the possibility that other factors besides  $O_2$  also impacted the activity of denitrifying bacteria. The likelihood of such impacts is raised by the fact that  $N_2O$  fluxes during the fully oxic April 2010 incubation were relatively high and comparable to those observed in the anoxic August 2010 incubation. Furthermore, fluxes were low in May 2011, when the water was still well-oxygenated. Still, although we cannot identify a direct relationship between  $N_2O$  production and bottom water oxygenation, additional measurements at a higher temporal and spatial resolution could shed light on the impact of  $O_2$  concentration fluctuations on a much smaller scale, e.g. daily fluctuations (Bartoli et al., 2003), or spatial differences leading to sediment core heterogeneity (Principi et al., 1994; Svensson and Leonardson, 1996). In addition, the impact of the availability of reducing substrates like organic carbon or sulfide (Wenk et al., 2013) that may also influence the activity of denitrifiers in the sediments remains an open question. Finally, the process of nitrifier denitrification may also produce  $N_2O$  in the presence of  $NO_2^-$  and small amounts of  $O_2$  (as low as  $5 \mu\text{mol L}^{-1}$ , Frame and Casciotti, 2010). While we did not observe the incorporation of  $^{15}\text{N}$  from  $^{15}\text{NH}_4^+$  into  $N_2O$ , the relative yield of  $N_2O$  produced by ammonia oxidizers increases substantially

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under conditions that stimulate their nitrifier denitrification pathway. As a result, as long as there is a source of  $\text{NO}_2^-$  (e.g. denitrification) nitrifier denitrification could be an important source of  $\text{N}_2\text{O}$  even if  $\text{O}_2$ -limitation has slowed the overall rate of ammonia oxidation (and therefore the rate of  $\text{N}_2\text{O}$  production via  $\text{NH}_2\text{OH}$  decomposition).

## 4.2 Denitrification is the main $\text{N}_2\text{O}$ producing process in the sediment

Significant  $^{15}\text{N}$ - $\text{N}_2\text{O}$  recovery was only observed in the overlying water of the  $^{15}\text{N}$ - $\text{NO}_3^-$  labeled cores, but not in the  $^{15}\text{N}$ - $\text{NH}_4^+$  labeled cores (Fig. 3), suggesting that denitrification is the predominant  $\text{N}_2\text{O}$  production pathway in the Lake Lugano SB sediments.

Even in April 2010 and May 2011, when the sediment/water interface was fully oxygenated and nitrification was likely to occur (Ward, 2008),  $\text{N}_2\text{O}$  production during ammonia oxidation was not observed. Therefore, it is very likely that canonical anoxic denitrification is the main source of N incorporated into the  $\text{N}_2\text{O}$  produced during the incubations. Rapid oxygen consumption supported by high rates of organic matter input to the sediments can reduce the oxygen penetration depth into the sediments, producing conditions favorable to denitrification close to the sediment/water interface (Li et al., 2007). It is not clear whether canonical denitrifiers carry out all of the steps involved in reducing  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$ . Although  $\text{NO}_3^-$  cannot serve in the place of  $\text{NO}_2^-$  as an electron acceptor during nitrifier denitrification, we cannot rule out the possibility that proximate denitrification supplies  $\text{NO}_2^-$  to ammonia oxidizers that then convert it to  $\text{N}_2\text{O}$  during nitrifier denitrification. Distinguishing between  $\text{N}_2\text{O}$  produced solely by denitrification and  $\text{N}_2\text{O}$  produced by nitrifier denitrification coupled to denitrification is not possible using the  $^{15}\text{N}$  tracer approach adopted here and is therefore beyond the scope of this study.

Denitrification rates were relatively high under the truly anoxic conditions observed in October 2010 ( $28.2 \mu\text{mol N h}^{-1} \text{m}^{-2}$ , Wenk, 2013), once redox gradients and, presumably, the microbial community had stabilized. At that time, up to 15% of total  $\text{NO}_3^-$  that was reduced, was released as  $\text{N}_2\text{O}$  without being completely reduced to  $\text{N}_2$ . However, variability in denitrification rates between the two cores in October 2010 was very

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high ( $10.2 \pm 5.0$  (SD) and  $46.2 \pm 20.3$  (SD)  $\mu\text{mol N h}^{-1} \text{m}^{-2}$ , respectively, Wenk, 2013). Furthermore, the October 2010 experiment was the only one in which steady-state conditions may not have been reached before the experiments were started.  $\text{N}_2$  as well as  $\text{N}_2\text{O}$  production rates increased over the experimental period (data not shown). Nevertheless, the overall high net  $\text{N}_2\text{O}$  fluxes measured during these incubations indicate that  $\text{N}_2\text{O}$  production was much more efficient than  $\text{N}_2\text{O}$  consumption.  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$  during the last step of denitrification is thought to be less tightly regulated than the preceding step that reduces  $\text{NO}$  to  $\text{N}_2\text{O}$  (Otte et al., 1996). As a result,  $\text{N}_2\text{O}$  produced by denitrifiers can accumulate when denitrification rates are high.

$\text{N}_2\text{O}$  production through  $\text{NH}_4^+$  oxidation was not observed at the oxygenated sediment/water interface in April 2010 and May 2011 (Fig. 3), even though conditions were seemingly conducive to benthic nitrification. Only in January 2011 did  $\text{NH}_4^+$  oxidation produce  $\text{N}_2\text{O}$ .  $\text{O}_2$  was low but not absent during this incubation, which agrees with previous findings that  $\text{N}_2\text{O}$  production during nitrification is enhanced under low  $\text{O}_2$  conditions (Stein, 2011; Bange et al., 2010). It is possible that dilution of the  $^{15}\text{N-NH}_4^+$  tracer by rapid  $\text{NH}_4^+$  regeneration from organic N in the sediments reduced the degree of  $^{15}\text{N}$ -labeling of the  $\text{N}_2\text{O}$  that was produced during  $\text{NH}_4^+$  oxidation, and thus our ability to detect  $\text{N}_2\text{O}$  production by this mechanism. In the same line, Norton and Stark (2011) suggest that immediate shunting of unlabeled  $\text{NH}_4^+$  from  $\text{NH}_4^+$ -remineralizing microorganisms to ammonia-oxidizing organisms could reduce the degree to which added  $^{15}\text{NH}_4^+$  tracer is incorporated into the pool of  $\text{NH}_4^+$  available for oxidation during soil incubations. It is feasible that a similar effect occurs in lake sediments.

Although methodological limitations prevent us from ruling out  $\text{N}_2\text{O}$  production during ammonia oxidation, it is clear that  $\text{N}_2\text{O}$  exported from the sediment is mainly produced through nitrate (and possibly nitrite) reduction and, therefore, that denitrification is required to produce the  $\text{N}_2\text{O}$  fluxes observed throughout the year.

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## 5 Conclusions

This study demonstrated that the sediments of the Lake Lugano SB are a net source of  $N_2O$  to the water column.  $NO_3^-$  reduction by denitrification was found to be the primary source of this  $N_2O$ , while  $N_2O$  production during ammonia oxidation was minimal or not observed at all, even when oxic conditions prevailed at the sediment/water interface. Net  $N_2O$  fluxes varied seasonally, and the proportion of  $NO_3^-$  reduced to  $N_2O$  versus  $N_2$  appeared to be enhanced by ongoing anoxia. Hence, 1–15% of the N reduced from  $NO_3^-$  accumulated as  $N_2O$  in the water column. Surprisingly, the highest percentage of  $NO_3^-$  reduced to  $N_2O$  was observed after thermal stratification occurred and stable anoxia developed, conditions that are generally thought to favor complete denitrification.

If we extrapolate the average of total  $N_2O$  fluxes presented in this study ( $879 \text{ nmol } N_2O \text{ h}^{-1} \text{ m}^{-2}$ ) over the area of the Lake Lugano SB ( $20.3 \text{ km}^2$ ) and the time span of one year, the sediments of the Lake Lugano SB release  $6.9 \text{ t } N_2O \text{ yr}^{-1}$  to the overlying water. The spatial resolution of our experiments is small compared to the lake area, and benthic conditions are no doubt highly variable in time and space, so that this extrapolation is accompanied by a large uncertainty. Nevertheless, it demonstrates that potentially large amounts of  $N_2O$  are produced in the sediments of the Lake Lugano SB. It is worth noting that the lacustrine  $N_2O$  fluxes measured in this study, which ranged between  $140$  and  $2605 \text{ nmol } N_2O \text{ h}^{-1} \text{ m}^{-2}$ , as well as benthic fluxes from several other studies (e.g. McCrackin and Elser, 2010; Liikanen and Martikainen, 2003; Mengis et al., 1996) are of the same order as global average fluxes estimated for soils (Bouwman et al., 2002).

$N_2O$  fluxes from the surface waters to the atmosphere were not addressed in this study and remain unknown, and the capacity of microbial processes in the water column of Lake Lugano SB to remove sedimentary  $N_2O$  before it escapes to the atmosphere has yet to be determined. Nevertheless, we argue that significant, year-round benthic  $N_2O$  production can lead to the accumulation of  $N_2O$  in the bottom waters of

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stratified lakes, and possibly to rapid evasion to the atmosphere during periods of de-stratification. The relevance of such lacustrine N<sub>2</sub>O degassing events for regional or global N<sub>2</sub>O budgets also awaits further investigation.

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## N<sub>2</sub>O production in a monomictic south-alpine lake

C. V. Freymond et al.

**Table 1.** In situ temperature and concentrations of dissolved O<sub>2</sub>, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> in bottom waters. DIN concentrations were measured in the inlet water reservoirs of the control experiments (Wenk, 2013).

	Temp. [°C]	O <sub>2</sub> [μmol L <sup>-1</sup> ]	NH <sub>4</sub> <sup>+</sup> [μmol L <sup>-1</sup> ]	NO <sub>3</sub> <sup>-</sup> [μmol L <sup>-1</sup> ]	NO <sub>2</sub> <sup>-</sup> [μmol L <sup>-1</sup> ]
April 2010	5.5	224	0.3	83.7	0.1
August 2010	5.7	0	7.1	75.7	1.7
October 2010	5.7	0	44.5	50.3	2.4
January 2011	5.9	11	13.2	69.6	2.3
May 2011	5.5	190	0.7	83.1	0

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**Fig. 1.** Location and map of Lake Lugano. The sampling station (red triangle) is located west of the village of Figino (SB), close to the point of maximum depth (modified from Barbieri and Polli, 1992).

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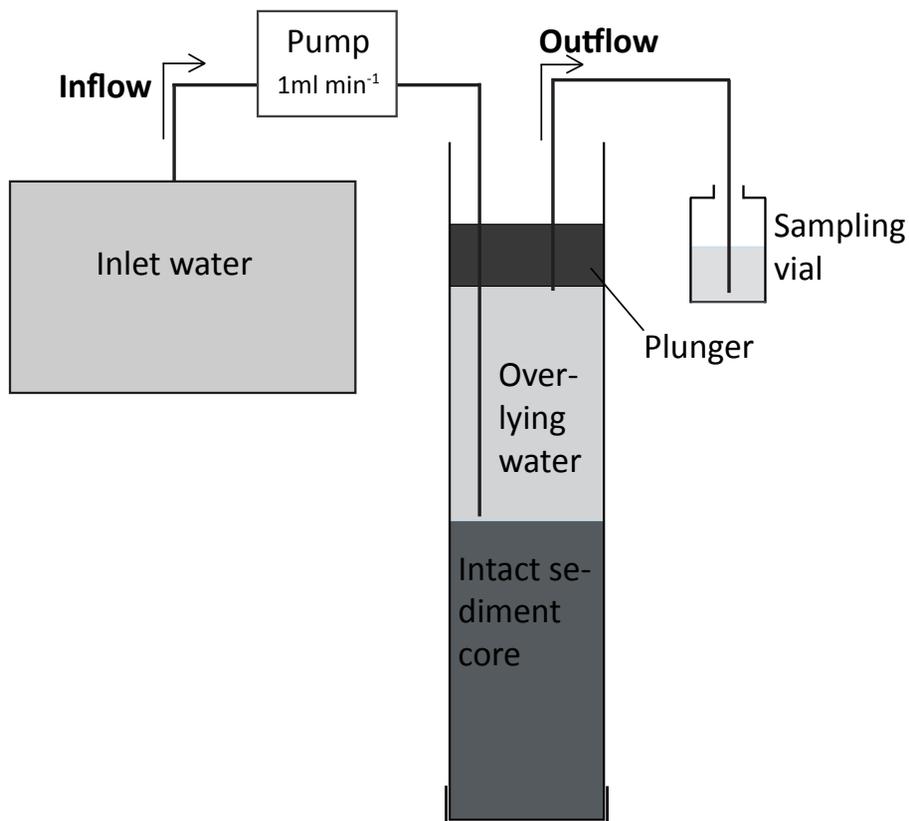
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**Fig. 2.** Steady state flow-through set-up.

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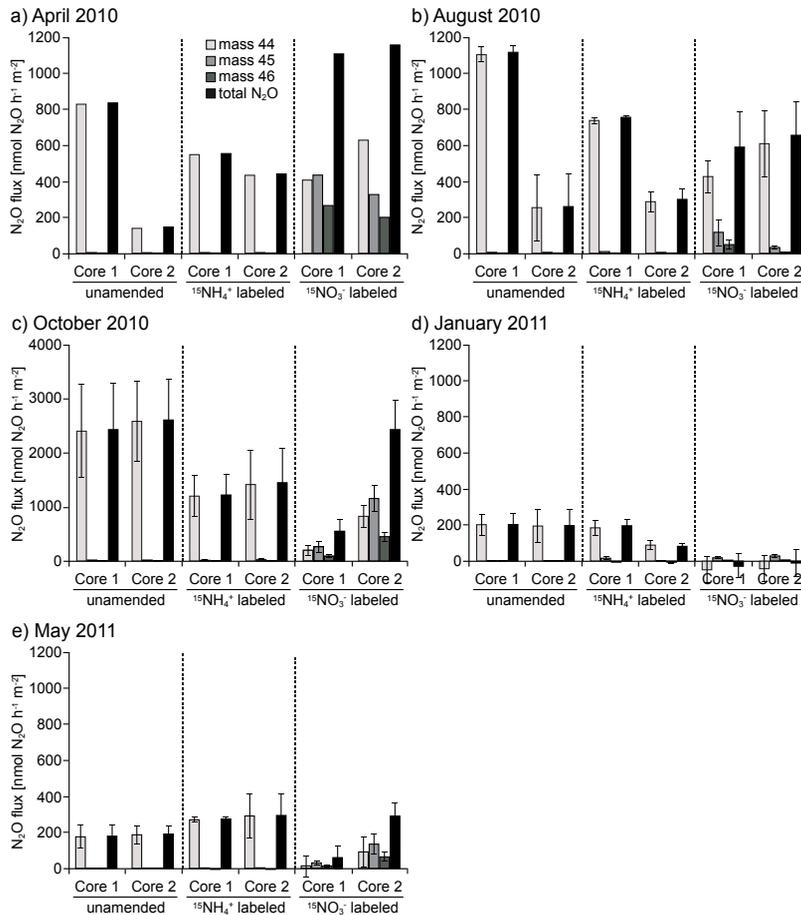
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**Fig. 3.** N<sub>2</sub>O fluxes from the sediment to the overlying water. Each column represents the average value of duplicate experiments. Error bars show the standard error of the fluctuations over incubation time. White: <sup>14</sup>N<sup>14</sup>NO (mass 44). Light grey: <sup>14</sup>N<sup>15</sup>NO and <sup>15</sup>N<sup>14</sup>NO (mass 45). Dark grey: <sup>15</sup>N<sup>15</sup>NO (mass 46). Black: Total N<sub>2</sub>O flux. Note the different scale in (c).