



## Direct O<sub>2</sub> control on the partitioning between denitrification and dissimilatory nitrate reduction to ammonium in lake sediments

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**Abstract.** Lacustrine sediments are important sites of fixed nitrogen (N) elimination through the reduction of nitrate to N<sub>2</sub> by denitrifying bacteria, and are thus critical for the mitigation of anthropogenic loading of fixed N in lakes. In contrast, dissimilatory nitrate reduction to ammonium (DNRA) retains bioavailable N within the system, promoting internal eutrophication. Both processes are thought to occur under oxygen-depleted conditions, but the exact O<sub>2</sub> thresholds particularly of DNRA inhibition are uncertain. In O<sub>2</sub>-manipulation laboratory experiments with dilute sediment slurries and <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions at low- to sub-micromolar O<sub>2</sub> levels, we investigated how, and to what extent, oxygen controls the balance between DNRA and denitrification in lake sediments. In all O<sub>2</sub>-amended treatments, oxygen significantly inhibited both denitrification and DNRA compared to anoxic controls, but even at relatively high O<sub>2</sub> concentrations (≥ 70 μmol L<sup>-1</sup>), nitrate reduction by both denitrification and DNRA was observed, suggesting a relatively high O<sub>2</sub> tolerance. Nevertheless, differential O<sub>2</sub> control and inhibition effects were observed for denitrification versus DNRA in the sediment slurries. Below 1 μmol L<sup>-1</sup> O<sub>2</sub>, denitrification was favored over DNRA, while DNRA was systematically more important than denitrification at higher O<sub>2</sub> levels. Our results thus demonstrate that O<sub>2</sub> is an important regulator of the partitioning between N-loss and N-recycling in sediments. In natural environments, where O<sub>2</sub> concentrations change in near bottom waters on an annual scale (e.g., overturning lakes with seasonal anoxia), a marked seasonality with regards to internal N eutrophication versus efficient benthic fixed N elimination can be expected.

### 1 Introduction

25 Over the last decades, intensive human activities have dramatically affected the nitrogen (N) cycle in aquatic systems through elevated inputs of reactive (biologically available) N. In some lakes, external N loading can lead to excessive algal blooms in the upper water column, and the subsequent decomposition of the sinking algal biomass is often associated with O<sub>2</sub> depletion in the deeper water column, and possibly, seasonal or permanent anoxia (e.g. Blees et al., 2014; Lehmann et al., 2004, 2015). Depending on the O<sub>2</sub>-concentrations in the water column, and the reactivity of the sediment organic matter, the oxygen penetration depth within lacustrine sediments can vary (e.g. Lehmann et al., 2009), and so will



the transition zone that separates aerobic from anaerobic biogeochemical reactions. Under oxygen-depleted conditions, both in the water column and in sediments, anaerobic N-transformation processes such as denitrification, anammox and/or dissimilatory nitrate reduction to ammonium (DNRA) become important. While denitrification and anammox can mitigate excessive N loading (eutrophication) by converting reactive nitrogen ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ) to  $\text{N}_2$  which subsequently returns to the atmosphere, DNRA retains a bioavailable form of nitrogen within the ecosystem, fostering internal eutrophication of lakes (Tiedje, 1988).

The biogeochemical conditions that regulate the partitioning between these different N-transforming processes (and others) in benthic environments remain uncertain, but links to other biogeochemical cycles are likely an important factor. It is commonly accepted, that when organic matter and nitrate concentrations are relatively high, nitrate is mostly reduced to  $\text{N}_2$  by denitrifying bacteria (Gruber, 2008; Seitzinger et al., 2006; Seitzinger, 1988). In contrast, DNRA seems to be favored in sediments with an excess of electron donors (total organic carbon (TOC),  $\text{H}_2\text{S}$ ,  $\text{Fe}^{2+}$ ) relative to nitrate (Brunet and Garcia-Gil, 1996; Roberts et al., 2014). In organic matter-rich lake sediments, the contribution of anammox to the total fixed-N transformation fluxes across sediment-water interfaces is most likely minor relative to both denitrification and DNRA, since anammox seems to occur primarily in sediments with low organic matter content (Babbín et al., 2014; Thamdrup and Dalsgaard, 2002).

Changes in the redox zonation may have profound impact on the benthic N cycle (Otte et al., 1996). Seasonal cycles of water column mixing and stagnation can modulate the penetration of redox boundaries into the sediments, potentially changing the redox environments of, for example, nitrifiers, denitrifiers, anammox, and DNRA bacteria. Oxygen can hence be considered a key regulator of benthic N exchange (Glud, 2008; Tiedje, 1988), and its effects are multifold. On one side, increasing  $\text{O}_2$  concentrations may expand the oxic/suboxic zone where nitrification can supply  $\text{NO}_3^-$  and  $\text{NO}_2^-$  for denitrification and anammox, enhancing the overall fixed-N loss (Lehmann et al. 2015). On the other hand,  $\text{O}_2$  can inhibit nitrate/nitrite reduction. When surface sediments are oxygenated, the facultative anaerobic microbes will preferably use oxygen, as the heterotrophic respiration with  $\text{O}_2$  yields more energy to cells for growth than with other oxidants (i.e.,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ; Froelich et al., 1979; Payne et al., 2009; Thauer et al., 1977). Moreover, under oxygenated conditions, the synthesis and/or the activity of the key enzymes involved in nitrate/nitrite reduction may be suppressed (Körner and Zumft 1989, Baumann et al. 1996, Dalsgaard et al., 2014). Existing reports on  $\text{O}_2$  tolerance and inhibition of denitrification and anammox in environments differ quite significantly. Inhibition may occur already at very low (nanomolar) levels of  $\text{O}_2$  (Dalsgaard et al., 2014), but experimental studies also revealed that relatively high  $\text{O}_2$  levels may be required (up 16% saturation levels) to induce a 50% inhibition of anammox (Jensen et al., 2008; Kalvelage et al., 2011). The apparent persistence of denitrification at relatively high  $\text{O}_2$  concentration levels led to a revision of the classical paradigm regarding the absolute  $\text{O}_2$  inhibition of nitrate reduction in nature (Tiedje et al., 1988), with important implications regarding the total volume of hypoxic zones in the ocean or in lakes that hosts microbial  $\text{N}_2$  production (Paulmier and Ruiz-Pino, 2009).



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While oxygen inhibition/tolerance of denitrification and anammox has been studied previously in the ocean water column (Jensen et al. 2008, Kalvelage et al. 2011, Babbín et al. 2014, Dalsgaard et al. 2014), investigations into the O<sub>2</sub> control on benthic N-reduction are rather rare, and limited to sandy and low organic matter marine sediments (Gao et al., 2010; Jántti and Hietanen, 2012; Rao et al., 2007). Despite intensified research, the exact O<sub>2</sub> thresholds with regards to the direct inhibition of benthic N reduction are still poorly constrained. This is particularly true for DNRA. Recent work has highlighted the significance of DNRA even in the presence of relatively high O<sub>2</sub> concentrations (i.e., at hypoxic levels (i.e., 10-62 mmol/L), or concentrations even >62 mmol/L) in estuarine sediments (Roberts et al., 2012, 2014) and marine sediments (Jántti and Hietanen, 2012), but a systematic investigation of how DNRA is impacted at low micromolar O<sub>2</sub> levels in aquatic sediments (and how in turn the balance between denitrification and DNRA is affected), does, to our knowledge, not exist.

In this study, we provide first experimental evidence for direct O<sub>2</sub> control on the fate of reactive N in lacustrine sediments with high organic matter content. Through slurry incubation experiments with sediment from a eutrophic lake in Switzerland (Lake Lugano), <sup>15</sup>N-labelled substrates and manipulated O<sub>2</sub> concentrations, we investigated the functional response of benthic N-reducing processes to changing O<sub>2</sub> levels. We demonstrate that denitrification and DNRA are differentially sensitive towards O<sub>2</sub>, which has important implications for fixed N removal in environments that undergo short- and longer-term O<sub>2</sub> changes, such as seasonally stratified (anoxic) lakes or other aquatic environments with expanding volumes of hypoxia/anoxia.

## 2 Sampling site, materials and methods

### 2.1 Sampling location

Sediment sampling took place in the south basin of Lake Lugano, a natural alpine lake situated at the border between Switzerland and Italy. Between April and January, the water column of the basin is stratified, with bottom-water suboxia/anoxia starting in late spring/early summer (e.g., Lehmann et al. 2004; Lehmann et al. 2015). During winter (February/March) the lake turns over and bottom waters are ventilated until the water column re-stratifies in spring, and bottom-water O<sub>2</sub> concentrations decrease again (Fig. 1). Water column O<sub>2</sub> and N-compound (NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) concentrations were measured as part of a long-term monitoring campaign promoted by the International commission for the protection of Italian-Swiss waters (CIPAIS; Commissione Internazionale per la Protezione delle Acque Italiano-Svizzere) and run by SUPSI (University of Applied Sciences and Arts of Southern Switzerland) on behalf of the Administration of Canton Ticino. Sediment cores were taken at two locations, Figino (8°53'37"E, 45°57'31"N, 94 m depth) and Melide (8°57'29"E, 45°56'22"N, 85 m depth) in October 2017, using a small gravity corer (inner diameter 6.2 cm). Figure 1 displays representative seasonal trends in the deep south basin. During oxygenation of the bottom waters, nitrate



concentrations in the water 2 m above the sediments reach about  $75 \mu\text{mol L}^{-1}$  and even during water column anoxia, near-sediment nitrate concentrations rarely dropped below 15 and  $5 \mu\text{mol L}^{-1}$  at Figino and Melide, respectively (Fig. 1; SUPSI data), so that the sediments are constantly exposed to nitrate-containing bottom waters. Ammonium concentrations in bottom water were relatively high ( $\sim 30\text{--}140 \mu\text{mol L}^{-1}$ ) during anoxia and close to the detection limit during months when the water column was mixed.

## 2.2 Porewater sampling

Porewater oxygen microprofiles were generated using an  $\text{O}_2$  microsensor (Unisense) with a tip diameter of  $100 \mu\text{m}$  in duplicate cores from both sites. The overlying water was gently stirred (without disturbing the sediment water interface) and aerated to determine the  $\text{O}_2$  penetration depth under oxygenated conditions. Porewater samples for the analysis of dissolved inorganic nitrogen concentrations were obtained by sectioning of a separate set of cores from the same sites at 1 cm-interval, and centrifuging of the samples.

## 2.3 N-transformation incubation experiments

In a first step, incubations to measure potential denitrification and DNRA rates under control (i.e., anoxic) conditions were performed. At each site, fresh surface sediments (upper 2 cm) from duplicate sediment cores were homogenized to prepare dilute sediment slurries. Aliquots of 1 g sediment and 70 mL anoxic artificial lake water ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ -free) were transferred into 120 mL serum bottles. The use of dissolved- $\text{NO}_x$ -free artificial water is important to avoid any potential underestimation of N-transformation process rates due to  $^{28}\text{N}_2$  production from ambient  $\text{NO}_3^-$  or  $\text{NO}_2^-$  present in bottom waters. Serum bottles were sealed and crimped using blue butyl rubber stoppers and aluminum caps. The sediment slurries (generally in triplicates, Table 1) were He-flushed for 10 min to lower the atmospheric  $\text{N}_2$  and  $\text{O}_2$  backgrounds, and placed overnight on a shaker (80 rpm) at  $8^\circ\text{C}$  in the dark to remove any residual  $\text{O}_2$ . Labeled  $^{15}\text{N}$  substrate (i.e.,  $\text{Na}^{15}\text{NO}_3^-$ , 99%  $^{15}\text{N}$ , Cambridge Isotopes Laboratories) was added in order to quantify potential rates of denitrification and DNRA. During the incubation period (ca. 10 hours), anoxic sediment slurries were kept in an incubator on an orbital shaker (80 rpm;  $8^\circ\text{C}$ ). For subsampling of gas and liquid, the incubation vials were transferred to an anaerobic chamber with  $\text{N}_2$ -atmosphere. There, two-milliliter gas samples were taken at four successive time points ( $T_0$ ,  $T_1$ ,  $T_2$ ,  $T_3$ ) for  $\text{N}_2$  isotope measurement, in exchange with 2 mL He ( $T_0$ ) or anoxic Milli-Q water ( $T_1$  to  $T_3$ ) in order to compensate for any pressure decrease inside the vials. Gas samples were then transferred into 3 mL exetainers (Labco), prefilled with anoxic water, and stored upside down until isotope analysis. Liquid samples (6 mL) were taken at  $T_0$  and  $T_3$  for the quantification of DNRA rates through N- $\text{NH}_4^+$  isotope analysis (see below) and for the assessment of nutrient ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ) concentrations.

## 2.4 $\text{O}_2$ manipulation experiments

For the  $\text{O}_2$  manipulation experiments, serum bottles were equipped with TRACE Oxygen Sensor Spots (TROXSP5, detection limit =  $6 \text{ nmol L}^{-1} \text{ O}_2$ , Pyroscience, Germany), allowing non-invasive, contactless monitoring of dissolved  $\text{O}_2$



concentrations in the dilute slurry. The sensor spots were fixed at the inner side of the glass wall with silicone glue and the sensor signal was read out from outside using a Piccolo2 fiber-optic oxygen meter (PyroScience). Different volumes of pure  
130 O<sub>2</sub> (99,995%) were injected into the headspace of pre-conditioned and <sup>15</sup>NO<sub>3</sub><sup>-</sup>-amended slurries using a glass syringe (Hamilton). For each treatment, the gas volume required to reach the targeted O<sub>2</sub> equilibrium concentration (0.8, 1.2, 2, ..., 78.6 μmol L<sup>-1</sup>) was calculated based on the headspace versus slurry volumes, salinity, and temperature (Gordon and Garcia, 1992). Measured O<sub>2</sub> concentrations in slurries after injection of the respective O<sub>2</sub> gas volumes were always close to the ones calculated (the first measurement was performed 30 minutes after injection to ensure gas equilibration between the gas and  
135 the liquid phase). Oxygen concentrations in the slurry incubations were monitored with the fiber-optic oxygen meter every 30 minutes and, in case of a marked decline in dissolved O<sub>2</sub> due to microbial consumption, O<sub>2</sub> was added in order to return to the initial target O<sub>2</sub> concentrations. In addition to continuous agitation on the shaking table, the dilute slurries were vigorously shaken by hand every 30 minutes to avoid the formation of anoxic microniches, which may act to increase rates of anaerobic N-transformation processes (Kalvelage et al., 2011).

#### 140 2.5 Hydrochemical analyses

Nitrite concentrations were determined colorimetrically using sulfanilamide and Griess-reagent, according to (Hansen and Koroleff 1999). Total NO<sub>x</sub> (i.e., NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>) concentrations were measured using a NOx-analyzer (Antek Model 745), by reduction to nitric oxide (NO) in an acidic V<sup>3+</sup> solution, and quantification of the generated NO by chemiluminescence detection (Braman and Hendrix, 1989). Nitrate concentrations were then calculated from the difference  
145 between NO<sub>x</sub> and NO<sub>2</sub><sup>-</sup> concentrations. Ammonium was measured by suppression-ion chromatography with conductivity detection (940 Professional IC Vario, Metrohm, Switzerland).

#### 2.6 <sup>15</sup>N-based rate measurements

For the determination of denitrification rates, gas samples from the <sup>15</sup>N-isotope enrichment experiments were analyzed for <sup>15</sup>N/<sup>14</sup>N isotope ratios in the N<sub>2</sub> using a Delta V Advantage isotope ratio mass spectrometer (IRMS; Thermo  
150 Fisher Scientific) with a ConFlo IV interface (Thermo Fisher Scientific). Denitrification (and negligible anammox) rates were calculated based on the quantification of <sup>15</sup>N in the N<sub>2</sub> gas in excess of the natural abundance, i.e. by assessing the linear increase in the concentrations of <sup>30</sup>N<sub>2</sub> (and to a minor extent <sup>29</sup>N<sub>2</sub>) with incubation time (Nielsen, 1992; Thamdrup and Dalsgaard, 2002). DNRA rates were quantified using the isotope-pairing method described by Risgaard-Petersen et al. (1995). Briefly, 2 mL liquid samples were transferred into 6 mL exetainers (Labco) and closed with plastic screw septum  
155 caps. The headspace was flushed with He for 2 min to reduce the <sup>28</sup>N<sub>2</sub> background, and 25 μL mL<sup>-1</sup> of alkaline (16 mol NaOH) hypobromite iodine solution (3.3 mol L<sup>-1</sup>) were added through the septum to convert NH<sub>4</sub><sup>+</sup> to N<sub>2</sub> (Robertson et al., 2016). Exetainers were stored upside down and placed on a shaker (100 rpm) for 24 h at room temperature. The produced N<sub>2</sub> was then analyzed by IRMS as described above. DNRA rates were determined based on the <sup>15</sup>NH<sub>4</sub><sup>+</sup> production with time, as



calculated from the total  $^{15}\text{N-N}_2$  in the hypobromite-treated samples (i.e., calculated from the excess  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  signals).  
160 The recovery of  $^{15}\text{N}$ -label from identically treated standards was >95%.

## 2.7 Statistics

Results are presented as the mean and standard error of n replicate experiments (Table 1). Significance differences between results were verified using a Student's *t*-test ( $P < 0.05$ ) in Microsoft Excel software.

## 3 Results

### 165 3.1 Porewater hydrochemistry

The  $\text{O}_2$  microsensor profiles revealed that the  $\text{O}_2$  penetration at the two sites under aerated conditions ranged between 2.4 mm (Melide) and 3.7 mm (Figino, Fig. 2). The relatively low oxygen penetration depth is consistent with a high organic carbon content (~8%, data not shown). According to the observed  $\text{O}_2$  concentration gradients at the two stations, the potential  $\text{O}_2$  flux into sediments was greater at Melide suggesting a higher reactivity of the sedimentary organic matter. In  
170 contrast to the microsensor profiling, the sectioning-centrifuging technique was not sufficient to resolve the exact porewater nitrate concentration gradient, yet the observed nitrate concentration data across the sediment-water interface (Fig. 2) clearly indicate that the sediments at both sites represent a sink for water-column nitrate, and that nitrate is consumed to completion already within the top centimeter of the sediments. In contrast, ammonium concentrations just below the sediment-water interface at Figino and Melide increased steeply from 830 and 600  $\mu\text{mol L}^{-1}\text{NH}_4^+$  to 1.7 and 1.2  $\text{mmol L}^{-1}$ , respectively.

### 175 3.2 N-transformations in control experiments

Potential rates of denitrification and DNRA under true anoxic conditions were quantified at both sampling sites in October 2017. Anammox rates were measured in a previous study at different times of the year, and its contribution to the total fixed-N removal was always less than 1%, thus negligible with respect to other processes (Cojean, in prep.). Indeed, in all experiments, denitrification and DNRA were the main benthic N-transformation processes with an essentially equal  
180 contribution to the total nitrate reduction ( $\approx 0.1 \mu\text{mol N g}^{-1}\text{ wet sed. d}^{-1}$ ; Table 1 caption). We ensured that measured DNRA rates were not underestimated due to  $^{15}\text{NH}_4^+$  loss through adsorption on mineral surfaces. Previous results (Cojean, in prep.) demonstrate that adsorption of ambient or tracer ammonium does not occur at detectable levels in the dilute sediment slurries. Ammonium consumption by nitrifiers in presence of  $\text{O}_2$ , however, might affect quantification of DNRA rates. Indeed, oxic slurry incubation experiments ( $\geq 73 \mu\text{mol L}^{-1}\text{O}_2$ ) revealed that at least at high  $\text{O}_2$  concentrations net  $\text{NO}_3^-$   
185 production occurs ( $\leq 1 \mu\text{mol N g}^{-1}\text{ wet sed. d}^{-1}$ ). Thanks to the comparatively large background pool of ammonium ( $\sim 42 \mu\text{mol L}^{-1}$  derived from the porewater of the wet sediment and from organic matter N remineralization), the little  $^{15}\text{NH}_4^+$



generated by DNRA during the incubation ( $< 0.1 \mu\text{mol L}^{-1}$ ) is strongly diluted, preventing that a significant fraction of the DNRA-derived  $^{15}\text{NH}_4^+$  is consumed by nitrifying microorganisms.

### 3.3 Impact of $\text{O}_2$ on $\text{NO}_3^-$ reduction in sediments

190 The  $\text{O}_2$  sensitivity of denitrification and DNRA and inhibition kinetics were investigated through slurry incubation  
experiments under  $\text{O}_2$ -controlled conditions. At both sites, potential denitrification and DNRA rates consistently decreased  
with increasing  $\text{O}_2$  concentration (Fig. 3). While the general pattern was systematic for both processes (i.e., an exponential  
attenuation of both denitrification and DNRA rates with increasing  $\text{O}_2$ ), the response of denitrifiers versus nitrate  
ammonifiers towards manipulated  $\text{O}_2$  differed across sites and treatments. We compared  $\text{O}_2$ -addition experiments to the  
195 anoxic controls to estimate the inhibition of nitrate reduction by  $\text{O}_2$ . At the lowest  $\text{O}_2$  concentration ( $\sim 1 \mu\text{mol L}^{-1} \text{O}_2$ ),  
denitrification was less inhibited than DNRA at Figino (29% and 51% inhibition, respectively) while the suppression was  
almost equivalent at Melide (43% and 37% inhibition of denitrification and DNRA respectively, Table 1). At  $\text{O}_2$   
concentrations around  $2 \pm 0.2 \mu\text{mol L}^{-1}$ , both denitrification and DNRA rates were more than 50% inhibited compared to the  
anoxic control (Table 1, Fig. 3). At  $\text{O}_2$  concentration  $\geq 2 \mu\text{mol L}^{-1}$ , DNRA rates were generally higher than those of  
200 denitrification (with one exception, i.e.,  $16 \mu\text{mol L}^{-1} \text{O}_2$  at Figino; Fig. 3), indicating that denitrification was more sensitive  
than DNRA to elevated  $\text{O}_2$  levels. Oxygen concentrations higher than  $73 \mu\text{mol L}^{-1}$  resulted in almost complete inhibition of  
denitrification at both sites (96% and 93% at Figino and Melide, respectively, Table 1). Oxygen inhibition thresholds for  
DNRA were even higher, as DNRA rates were significantly less impaired than denitrification at these elevated  $\text{O}_2$  levels  
(79% and 75% inhibition compared to the anoxic controls at Figino and Melide, respectively; Table 1). Hence, the relative  
205 contribution of the two processes to total nitrate reduction was significantly affected by changing  $\text{O}_2$  concentrations. At  
anoxic and submicromolar levels of  $\text{O}_2$  ( $\leq 1 \pm 0.2 \mu\text{mol L}^{-1} \text{O}_2$ ), denitrification rates were higher than those of DNRA, while  
at higher  $\text{O}_2$  concentration the ratio between denitrification and DNRA was shifted in favor of nitrate ammonifiers (Fig. 4).

Consistent with the observed decline in denitrification and DNRA rates based on the  $^{15}\text{N}_2$  and  $^{15}\text{NH}_4^+$  measurements  
210 in the  $^{15}\text{N}$ -label incubations, nitrate consumption in slurries decreased with increasing  $\text{O}_2$  concentration at both stations  
(Table 1). Similarly, maximum ammonium accumulation was observed in the anoxic controls, whereas at higher  $\text{O}_2$  levels  
ammonium underwent net consumption, indicating the concomitant decrease of DNRA and the increasing importance of  
nitrification under more oxic conditions, particularly at Melide. In incubations where nitrate concentrations decreased, the  
ratio of  $(\text{NO}_3^-)_{\text{reduced}} : (\text{N-N}_2 + ^{15}\text{NH}_4^+)_{\text{produced}}$  was always significantly higher ( $>5:1$ ) than expected (1:1). This observation is  
215 consistent with previous work in the Lake Lugano South Basin (Wenk et al., 2014). Here, whole-core flow-through  
incubations also revealed that  $\text{NO}_3^-$  fluxes into the sediments significantly exceeded benthic  $\text{N}_2$  production, an imbalance,  
which could neither be explained by nitrate reduction to ammonium nor incomplete reduction to  $\text{N}_2\text{O}$ . As it is not the scope  
of this study, we will not discuss this puzzling discrepancy further, but we speculate that excess  $\text{NO}_3^-$  consumption may be  
linked to bacterial and algal uptake (Bowles et al., 2012). Biotic immobilization of  $\text{NO}_3^-$  in marine sediments has been



220 attributed previously to the intracellular storage of nitrate by filamentous bacteria (Prokopenko et al., 2013; Zopfi et al.,  
2001) and/or diatoms (Kamp et al., 2011), but we do not know whether such nitrate sinks are important also in Lake Lugano  
sediments.

#### 4 Discussion

##### 4.1 Anaerobic N-cycling in the South Basin of Lake Lugano

225 Benthic denitrification and DNRA were the predominant anaerobic N-transformation processes at the two studied  
stations. Interestingly, the contribution of DNRA was systematically higher than observed in flow-through whole-core  
incubations performed with sediment from the same basin. Wenk et al. (2014) reported a maximum DNRA contribution to  
NO<sub>3</sub><sup>-</sup> reduction of not more than 12%, but also argued that their DNRA rate measurements must be considered conservative,  
because they did not account for the production of <sup>14</sup>NH<sub>4</sub><sup>+</sup> from ambient natural-abundance nitrate. The reason for such a  
230 discrepancy is unclear, but there seems to be a tendency for slurry incubations to yield higher DNRA rates compared to  
denitrification (Kaspar, 1983), implying biasing methodological effects. The observed discrepancies may also be related to  
natural sediment heterogeneity and/or seasonal/interannual fluctuations in benthic N transformation rates. As for the latter, in  
2016, the annual water overturn and bottom-water ventilation was exceptionally suspended and sediments remained anoxic  
for more than a year. In contrast, in 2017, the water column mixed in January and surface sediments were oxygenated  
235 throughout June. Our O<sub>2</sub> manipulation experiments revealed that redox conditions have a marked impact on the partitioning  
between the two nitrate reduction pathways, and consistent with the slurry incubation data, the extended O<sub>2</sub> exposure of  
microbes at the sediment-water interface in 2017 compared to the preceding year may have favored nitrate ammonifiers over  
denitrifiers. Independent of any possible spatio-temporal variability, in this study, DNRA rates were equal, or even higher,  
than denitrification. Such a partitioning of the two nitrate reducing processes is not implausible and was similarly observed  
240 in a wide range of environments, particularly in more reduced sediments with high organic matter content and comparatively  
low nitrate levels (Brunet and Garcia-Gil, 1996; Dong et al., 2011; Papaspyrou et al., 2014). More generally, substrate-  
availability changes induced by O<sub>2</sub> fluctuations may be important drivers of the partitioning between denitrification and  
DNRA (Cojean in prep.), and environmental conditions that favor DNRA over denitrification may be quite common.  
However, to our knowledge, experimental evidence for the direct O<sub>2</sub> control on the balance between these two nitrate-  
245 reducing processes is still lacking.

##### 4.2 O<sub>2</sub> inhibition thresholds of benthic nitrate reduction

Our study shows that submicromolar O<sub>2</sub> levels significantly lowered both, denitrification and DNRA rates.  
Denitrification and DNRA were inhibited by about 30-50% at 1 μmol L<sup>-1</sup> O<sub>2</sub>, while in previous studies that investigated O<sub>2</sub>  
effects on fixed-N elimination in the water column, denitrification was almost completely suppressed at this O<sub>2</sub> level already.  
250 For example, by conducting incubation experiments using samples from oxygen minimum zones in the Eastern Tropical





Pacific, a 50% inhibition of denitrification was noticed already at  $0.2 \mu\text{mol L}^{-1} \text{O}_2$ , and complete suppression at  $1.5\text{-}3 \mu\text{mol L}^{-1} \text{O}_2$  (Dalsgaard et al., 2014, Babbin et al., 2014). Similarly, incubation experiments with samples from a Danish fjord exhibited full inhibition of denitrification at  $8\text{-}15 \mu\text{mol L}^{-1} \text{O}_2$  (Jensen et al., 2009). In marine sediments, in contrast, denitrification was occurring even at  $\text{O}_2$  concentrations greater than  $60 \mu\text{mol L}^{-1}$  (Gao et al., 2010, Rao et al., 2007). This is  
255 in agreement with our results showing that at higher  $\text{O}_2$  levels ( $\geq 73 \mu\text{mol L}^{-1}$ ) denitrification was still active although at very low rates compared to the anoxic control ( $\geq 93\%$  inhibition). Similarly, DNRA was still occurring, and was less impaired by the elevated  $\text{O}_2$  concentration compared to denitrification ( $\geq 75\%$  inhibition relative to the anoxic control). An increase of DNRA relative to denitrification rates under oxic conditions ( $> 100 \mu\text{mol L}^{-1} \text{O}_2$ ) was also observed in estuarine sediments, though N-removal remained predominant (Roberts et al., 2012, 2014). In brackish sediments in the Gulf of Finland in the  
260 Baltic Sea, at elevated  $\text{O}_2$  concentrations (from  $50$  to  $110 \mu\text{mol L}^{-1}$  in bottom waters), benthic DNRA rates were generally higher than denitrification rates (Jäntti and Hietanen, 2012), further supporting our findings. Yet, in contrast to our study, their observations suggest a higher  $\text{O}_2$  sensitivity (i.e., greater inhibition) of DNRA compared to denitrification in sediments with higher bottom water  $\text{O}_2$  concentrations ( $> 110 \mu\text{mol L}^{-1}$ ). Given the paucity and discrepancy of existing data in this context, it is premature to conclude that DNRA microbes are generally less or more oxygen-tolerant than denitrifiers. A  
265 direct comparison of DNRA  $\text{O}_2$  inhibition thresholds in this study and in the study of Jäntti and Hietanen (2012) is difficult because of the differing methodological approaches. There, nitrate reduction rates were determined in whole-core incubations, without manipulating (and measuring) the  $\text{O}_2$  concentrations at the sediment depth where nitrate is actually reduced. And although the  $\text{O}_2$  penetration depth and porewater  $\text{O}_2$  concentrations will respond to a certain degree to the  $\text{O}_2$  content in the bottom water, deducing the actual  $\text{O}_2$  concentrations for the active nitrate reduction zone within the sediment  
270 from  $\text{O}_2$  concentrations in the overlying water is problematic. Here, we tested the oxygen sensitivity of a microbial community in suspension, directly exposed to defined  $\text{O}_2$  conditions. These incubation data indicate that DNRA is less inhibited than denitrification at  $\text{O}_2$  concentrations  $\geq 73 \mu\text{mol L}^{-1}$  and, at the same time, imply that anoxia per se is not a strict requirement for DNRA, as previous ecosystem-scale work has also suggested (Burgin and Hamilton, 2007). Our results also are consistent with observations made in soil microcosms showing that DNRA is less sensitive to increasing  $\text{O}_2$  partial  
275 pressures than denitrification within the range of  $0\text{-}2\% \text{O}_2$  v/v (Fazzolari et al., 1998; Morley and Baggs, 2010).

The observed  $\text{O}_2$  inhibition thresholds for nitrate reduction are significantly higher than reported from most incubation studies with water column samples (Dalsgaard et al., 2014, Babbin et al., 2014, Jensen et al., 2008). Elevated  $\text{O}_2$  tolerance in prior studies was often attributed to the formation of anoxic microniches that may foster anaerobic N-reduction  
280 (Kalvelage et al., 2011). It is unlikely that such microniches formed during our incubation experiments since slurries were heavily diluted ( $1 \text{ g}$  sediment in  $70 \text{ mL}$  water) and vigorously shaken by hand every  $30 \text{ min}$ , in addition to the continuous agitation on a shaking table during the incubation. Also, experiments were replicated  $2\text{-}3$  times for some  $\text{O}_2$ -amended treatments, and measured rates were very similar between replicates. If anoxic microniches had formed, we would have



285 expected that their formation is more variable, resulting in a lower reproducibility of the determined rates. One might speculate that benthic nitrate-reducing microbial communities are more tolerant towards higher  $O_2$  levels than pelagic ones.

The existence of aerobic denitrifiers (e.g. microbes that reduce  $NO_3^-/NO_2^-$  to  $N_2$  in presence of  $O_2$ ) in soils and sediments has been confirmed through isolation of bacterial strains (e.g. Robertson et al., 1995), and it was suggested that they contribute to the total fixed N loss in marine sediments (Carter et al., 1995; Patureau et al., 2000; Zehr and Ward, 2002).  
290 Recent studies of permeable marine sediments (Gao et al., 2010) and soils (Bateman and Baggs, 2005; Morley et al., 2008) also observed significant  $N_2$  production in the presence of  $O_2$  and attributed it to aerobic denitrification

#### 4.3 DNRA favored under less reducing conditions

It is generally assumed that strongly reducing conditions favor DNRA over denitrification, yet in our study, particularly at elevated  $O_2$  concentrations, DNRA rates were higher than those of denitrification. That DNRA often seems to  
295 be more important under true anoxic conditions may therefore not be linked directly to the absence of  $O_2$  and differential  $O_2$  inhibition levels of the two nitrate-reducing processes. Indirect mechanisms are likely to be important. For instance,  $H_2S$  accumulation, which often accompanies prolonged anoxia, can inhibit denitrification and simultaneously enhance DNRA (An and Gardner, 2002; Rysgaard et al., 1996). Another indirect, redox-dependent factor may be the availability of nitrate. Higher DNRA rates were observed under more  $NO_3^-$ -limiting conditions induced by prolonged anoxia, probably because  
300 nitrate ammonifiers are able to gain more energy per  $NO_3^-$  reduced than denitrifiers (Dong et al., 2011). As nitrate concentrations are generally much lower under oxygen-free conditions, it appears plausible that anoxia-associated nitrate and nitrite depletion is conducive to higher DNRA/denitrification rates. While these examples seem to support that DNRA is favored under true anoxic conditions, results of other studies are more consistent with our observation of higher DNRA than denitrification rates at elevated  $O_2$  concentrations. For example, in estuarine sediments, DNRA was stimulated relative to  
305 denitrification under more oxidizing conditions (Roberts et al., 2014, 2012). The authors argued that DNRA is enhanced by increasing  $Fe^{2+}$  availability at the oxic-anoxic sediment layer during more oxygenated conditions. These studies highlight the importance of redox conditions in regulating the balance between denitrification and DNRA, however, to what extent  $O_2$  directly controls the partitioning between the two nitrate-reducing processes at the enzyme levels remains, to our knowledge, still unknown. Apparent contradictions with regards to how changing  $O_2$  levels may impact nitrate reduction may simply be  
310 due to the counteracting and variable influence of direct versus indirect effects of the variable  $O_2$  concentrations.

We cannot fully exclude that through  $O_2$  manipulation in this study, we partly affected nitrate-reduction indirectly through its control of  $H_2S$  or  $Fe^{2+}$ . Yet, we set up the experiments in a way that indirect effects should be minimized (e.g., no free sulfide in any of the incubations, same organic matter content, same excess  $NO_3^-$  concentrations). Hence, this study can  
315 be considered the first experimental investigation into the direct  $O_2$  effect on the partitioning between N-loss by denitrification and N-recycling by DNRA in aquatic sediments. The fact that in our experiments we can essentially exclude



the effects of redox-related parameter changes (i.e.,  $H_2S$ ,  $NO_3^-$ , and  $Fe^{2+}$ ), leads us to the conclusion that in the studied sediments from Lake Lugano,  $O_2$  controls the balance between denitrification and DNRA at the organism-level, and that denitrification is in fact more sensitive towards increasing  $O_2$  concentrations than DNRA.

#### 320 4.4 Direct $O_2$ control on benthic $NO_3^-$ reduction

It has been previously reported that  $O_2$  can either suppress the synthesis of enzymes involved (Baumann et al., 1996) or the enzyme activity itself (Dalsgaard et al., 2014). The observed DIN concentration trends (i.e. decreasing nitrate consumption) with increasing  $O_2$  concentrations suggest that the overall activity is modulated mainly at the nitrate reduction step. The differential response of denitrifiers and nitrate ammonifiers further suggests a distinct  $O_2$  sensitivity of the nitrate reductase enzymes involved. Denitrifiers and nitrate ammonifiers utilize the same nitrate reductase enzymes (Nar, Nap), and while a differential  $O_2$  sensitivity of the same type of enzyme is difficult to explain, it is certainly possible for different enzymes. Indeed, the membrane-bound (Nar) and the periplasmic (Nap) nitrate reductases have distinct affinities towards  $NO_3^-$  and  $O_2$  tolerance (Mohan and Cole, 2007). Periplasmic nitrate reduction is almost exclusively found in the Proteobacteria and many of the organisms possess both Nar and Nap systems, whose production is regulated in response to ambient  $NO_3^-$  and  $O_2$  concentrations (Simon and Klotz, 2013). When  $NO_3^-$  is scarce, Nap provides a high-affinity (for  $NO_3^-$ ) but low-activity pathway that does not require  $NO_3^-$  transport into the cell cytoplasm (Mohan and Cole, 2007). In presence of oxygen, nitrate transport across the cell membrane is repressed, preventing nitrate reduction by the membrane-bound enzyme Nar with its cytoplasm-facing active site (Moir and Wood, 2001). In contrast, nitrate reduction in the periplasm is less  $O_2$  sensitive, so that microbes possessing and relying on Nap are likely to have an ecological advantage in environments that are subject to  $O_2$  fluctuation (Carter et al., 1995). In nature, nitrate reduction by denitrifiers is assumed to be catalyzed primarily by Nar (Richardson et al., 2007), while most nitrate ammonifiers seem to use Nap (Mohan and Cole, 2007).

Clearly, more fundamental research is required in environmentally relevant non-model microorganisms or mixed communities, to understand better the combined effects of  $O_2$  on the nitrogen-transforming metabolic pathways and their regulation. Additional  $O_2$  inhibitory effects at one of the down-cascade enzyme levels (Nir, Nrf, Nor, Nos) are likely to exhibit variable  $O_2$  sensitivities (Baumann et al., 1996, 1997; Körner H. and Zumft, 1989; Poock et al., 2002). Our observations of higher DNRA/denitrification ratios at higher  $O_2$ , however, provides at least putative evidence that microorganisms performing DNRA using Nap may be more  $O_2$ -tolerant than denitrifiers using Nar.

#### 4.5 Implication for N-elimination versus N-recycling in lakes with fluctuating $O_2$ conditions

The redox-sensitive partitioning of nitrate elimination (through  $N_2$  production by denitrification) versus fixed-N recycling (by nitrate ammonification) has likely important ecosystem-scale consequences. The annual water column turnover of holomictic lake basins such as the south basin of Lake Lugano plays an important role in regulating the contribution of N-removal and N-recycling in the water column (Lehmann et al. 2004; Wenk et al, 2014). To which extent  $O_2$  fluctuations



affect N transformation reactions within the sediments remains uncertain. Winter water column turnover ventilates the  
350 bottom waters and re-oxygenates surface sediments that were anoxic for several months. Hence, at least in the top  
millimeters of the sediment column we can expect changes in the benthic N cycling. Based on our incubation experiments,  
the O<sub>2</sub> inhibition threshold was lower for denitrification than for DNRA, possibly reflecting differential adaption of the in  
situ microbial community of denitrifiers and nitrate ammonifiers to fluctuating O<sub>2</sub> conditions of bottom waters. Indeed, many  
nitrate ammonifiers possess both nitrate reductase enzymes (Nap and Nar) and can switch between the two respiratory  
355 systems providing them with an ecological advantage over denitrifiers when substrates become limiting (i.e., with regards to  
the primary reductant used in energy metabolism; Mohan and Cole, 2007). During oxygenated bottom-water conditions,  
within the benthic redox transition zone, nitrate-reducing microbes at the sediment-water interface will be exposed to  
elevated O<sub>2</sub> concentrations, similar to the ones tested here. Our experimental data imply that then, at least in the uppermost  
sediments, DNRA is favored over denitrification. We may even expect an O<sub>2</sub>-regulated zonation of DNRA and  
360 denitrification. As a consequence, when denitrification-driven nitrate-reduction is pushed down, it is possible that NO<sub>3</sub><sup>-</sup> will  
be partially consumed through DNRA before it gets to the “denitrification layer”, as nitrate ammonifiers are less O<sub>2</sub> sensitive  
than denitrifiers. In contrast, denitrification is likely to be a more important nitrate-reducing process compared to DNRA  
during water column stratification (suboxia/anoxia of bottom waters), when the sediments are fully anoxic.

365 In the discussion thus far, we implicitly assume that the main control O<sub>2</sub> exerts on the absolute and relative rates of  
denitrification and DNRA is due to its inhibitory effects at the organism-level, yet the effect of O<sub>2</sub> on the coupling of  
nitrification and nitrate reduction by either denitrification or DNRA remained unaddressed. Oxygen fluctuations in the  
natural environment will affect nitrate regeneration by nitrification, and hence determine how much nitrate is available for  
microbial reduction. It has been shown previously that through oxygenation events (e.g., the increase in bottom water O<sub>2</sub>  
370 concentrations during episodic mixing/ventilation), the overall benthic N elimination in lakes may be enhanced through  
coupled nitrification-denitrification, at least transiently (Hietanen and Lukkari, 2007; Lehmann et al., 2015). So, while the  
direct effect of elevated O<sub>2</sub> would be to hamper fixed N elimination by denitrification at the organism-level, the oxygenation  
of previously ammonium-laden but nitrate free (pore-) waters would help to better exploit the benthic nitrate-reduction  
potential by increasing the nitrate availability for nitrate-reducing microbes within the sediments, so that the overall nitrate  
375 reduction may be stimulated (Lehmann et al. 2015). Yet, as shown in the present study, oxygenation of the water column and  
the upper surface sediments may also act to shift the balance between denitrification and DNRA towards DNRA, thus  
promoting N-recycling rather than fixed-N elimination through denitrification. Total nitrification rates were not measured in  
this study, but nitrate concentration changes in sediment slurries suggest that at elevated O<sub>2</sub> levels there is at least some  
production of nitrate. There is no obvious reason to assume that O<sub>2</sub>-stimulation of the coupling of nitrification and  
380 denitrification on the one hand, and of nitrification and DNRA on the other would per se be different. Yet, as demonstrated  
here, DNRA appears to be less O<sub>2</sub> sensitive compared to denitrification. It is thus reasonable to expect a higher coupling of  
nitrification with DNRA than with denitrification during oxygenated bottom-water conditions. Indeed, there is putative



evidence for such an indirect link between  $O_2$  and elevated coupled nitrification-DNRA. In a recent study with estuarine sediments, stronger stimulation of DNRA compared to denitrification was observed during oxygenation of bottom waters, in parts attributed to the coupling to nitrification (Roberts et al., 2012). Additional experimental work is required to better understand the role of nitrification in regulating the balance between benthic denitrification and DNRA during oxygenation of bottom waters.

It is important to understand that in the natural environment,  $O_2$  will not be the only regulator of the balance between denitrification and DNRA. As previously mentioned, the partitioning of the two nitrate-reducing processes can also be modulated by the substrate (e.g.,  $NO_3^-$ ,  $NO_2^-$ , TOC,  $H_2S$ ,  $Fe^{2+}$ ) availability. The latter may be redox controlled or not. Such regulation may be linked to the differential substrate affinity of the two processes when competing for the same electron acceptor (e.g., nitrate/nitrite) providing selective pressure that can drive communities either towards denitrification or DNRA (Kraft et al. 2014), or simply due to differing substrate requirements in the case of chemolithotrophic versus organotrophic nitrate reduction.

For example, nitrate concentrations in the water column of the lake sampled in this study (Lake Lugano) varied significantly over the year, with very low  $NO_3^-$  concentrations during the stagnation period (during anoxia) (Fig. 1). As a consequence, it is reasonable to assume that the relative partitioning between denitrification and DNRA in a natural environment is affected by the fluctuating nitrate concentrations (e.g., Tiedje et al., 1988, Dong et al., 2011). Similarly,  $Fe^{2+}$  levels in near-bottom waters and sediment porewaters in Lake Lugano are greater during the anoxia/stratification period (Lazzaretti et al., 1992). At least in environments where chemolithotrophic processes contribute to the overall nitrate reduction, such redox-dependent  $Fe^{2+}$  concentration changes (or changes of other electron donors such as  $HS^-$ ) may affect the balance between DNRA and denitrification (e.g., Robertson et al. 2015). Hence, in addition to the direct regulating effects of  $O_2$  on the partitioning between denitrification and DNRA, which we have demonstrated here experimentally,  $O_2$  can act as indirect regulator of fixed N elimination versus regeneration. The ultimate ecosystem-scale DNRA/denitrification ratio in environments that are subject to  $O_2$  fluctuating conditions is difficult to predict, because direct and indirect  $O_2$  regulation may act concomitantly and in opposite ways.

## 5 Conclusion

The presented results broaden the range of  $O_2$  inhibition thresholds of benthic denitrification at micromolar  $O_2$  levels, demonstrating that benthic denitrification may resist full inhibition up to almost  $80 \mu M O_2$ . Similarly, sedimentary DNRA does not necessarily require true anoxia, and was even less sensitive than denitrification to higher  $O_2$  levels. Our data suggest that the balance between DNRA and denitrification is modulated by  $O_2$  at the nitrate-reducing enzyme level (Nar versus Nap). However, more in-depth investigations on the exact role of oxygen in regulating other denitrification and/or



415 nitrate-ammonification enzymes in microbial pure culture experiments are needed. The differential tolerance of denitrifiers  
versus nitrate ammonifiers towards  $O_2$  has important implications for natural environments with fluctuating  $O_2$  conditions.  
Based on our results, one might argue that DNRA may be more important during phases of bottom-water oxygenation, while  
anoxic conditions during the stratification period may favor full denitrification to dinitrogen. Whether and when fixed  
nitrogen is preserved in a lake or eliminated by denitrification is, however, difficult to predict, as this will depend also on  
420 multiple indirect effects of changing  $O_2$  levels. For example, nitrification and the redox-dependent modulation of substrates  
that may be relevant for denitrification or DNRA (such as nitrite, the substrate at the branching point between the two  
processes, and/or sulfide as potential inhibitor of denitrification and stimulator of chemolithotrophic DNRA) will play an  
important role both with regards to the overall nitrate reduction rate, as well as the balance between different nitrate reducing  
processes. Internal eutrophication from N in high-productivity lakes is generally less of a concern than from P. Nevertheless,  
425 it needs to be considered that oxygenation may reduce the overall fixed N-elimination capacity of the bottom sediments by  
impairing denitrification more than DNRA, partially counteracting the generally positive effects of hypolimnetic ventilation  
in the context of benthic nutrient retention/elimination, and with implications on the nutrient status in the water column.

#### Author contribution

JZ and MFL initiated the project. ANYC performed all sample collection and conducted the experimental work  
430 with help from AG. FL provided the water column chemistry profiles. ANYC, JZ and MFL performed data analysis and  
interpretation. ANYC and MFL prepared the manuscript with input from all co-authors.

#### Competing interest

The authors declare that they have no conflict of interest.

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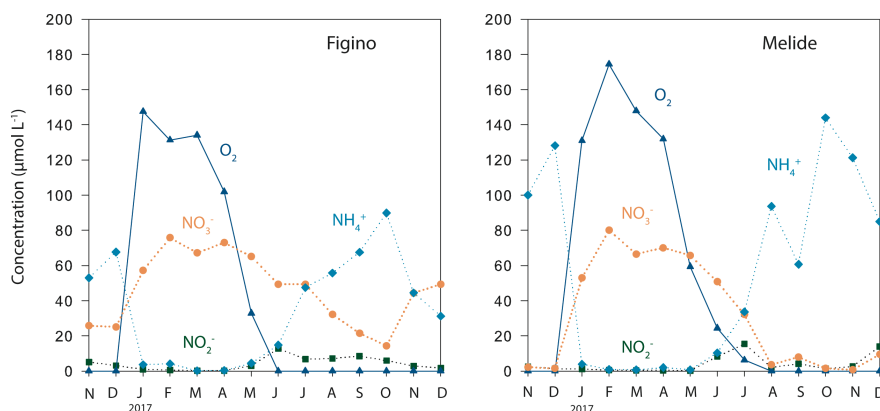
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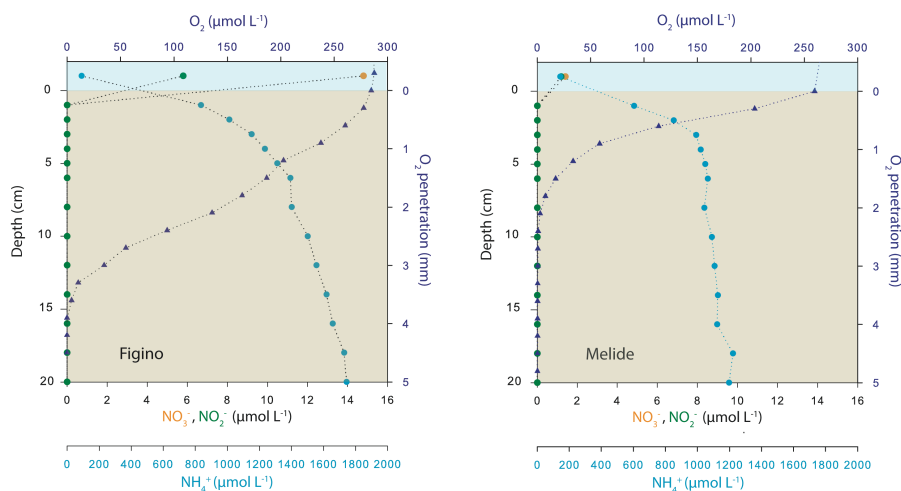


**Table 1: Overview of N transformation rates in O<sub>2</sub>-controlled slurry incubation experiments. Negative and positive values correspond to net NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> consumption and production rates over incubation time, respectively. Standard errors are indicated in bracket for *n* replicates. Average denitrification and DNRA rates (μmol N g<sup>-1</sup> wet sed. d<sup>-1</sup>) in anoxic control experiments were: 0.11 ± 0.01 and 0.12 ± 0.04, respectively, at Figino; 0.12 ± 0.01 and 0.11 ± 0.01, respectively, at Melide.**

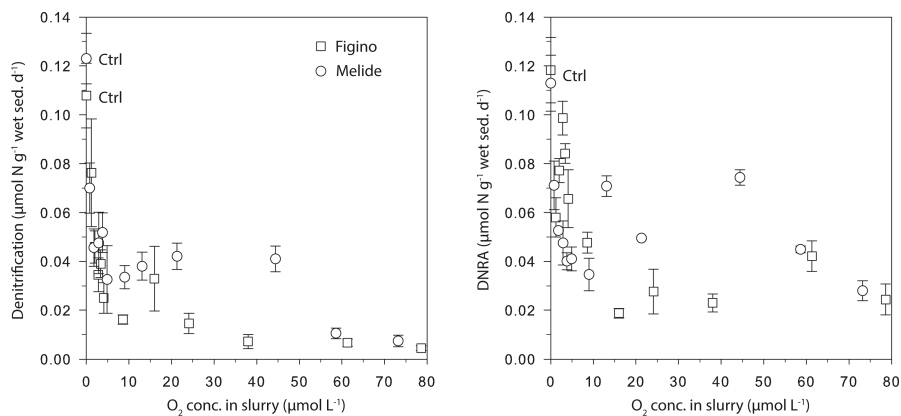
Sampling site	O <sub>2</sub> conc. in slurry μmol L <sup>-1</sup>	<i>n</i>	Inhibition compared to control (%)		NO <sub>3</sub> <sup>-</sup> μmol N g <sup>-1</sup> wet sed. d <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> μmol N g <sup>-1</sup> wet sed. d <sup>-1</sup>
			Denitrification	DNRA		
Figino	0 (control)	12			-1.4 (0.1)	0.7 (0.03)
	1.2	3	29	51	-0.8 (0.2)	0.8 (0.4)
	2	3	57	35	-0.4 (0.1)	0.8 (0.2)
	2.8	3	68	17	-0.4 (0.3)	0.7 (0.1)
	3.4	2	64	29	-0.6 (0.2)	0.6 (0.1)
	4.1	3	77	45	-0.9 (1.3)	0.5 (0.2)
	8.6	3	85	60	-1.1 (0.3)	0.4 (0.0)
	16	4	70	84	-0.2 (0.5)	0.1 (0.2)
	24.1	3	86	77	-0.2 (0.6)	0.2 (0.1)
	38	3	93	39	0.2 (2.1)	0.0 (0.2)
	61.3	3	94	64	-0.3 (0.3)	-0.2 (0.1)
78.6	6	96	79	1.1 (1.4)	-0.1 (0.0)	
Melide	0 (control)	9			-1.0 (0.4)	0.2 (0.0)
	0.8	2	43	37	-0.7 (0.2)	-0.1 (0.1)
	1.8	2	63	53	-0.6 (0.1)	0.2 (0.2)
	2.9	3	61	58	-0.5 (0.3)	-0.1 (0.2)
	3.8	4	58	65	-0.2 (0.1)	-0.1 (0.3)
	4.9	3	74	64	-0.3 (0.2)	-0.1 (0.3)
	9	7	73	69	0.0 (0.1)	-0.1 (0.2)
	13.1	2	69	37	-0.6 (0.1)	-0.1 (0.0)
	21.3	2	66	56	-0.4 (0.1)	-0.1 (0.1)
	44.4	2	67	34	-0.3 (0.2)	-0.1 (0.2)
	58.6	3	91	60	-0.1 (0.1)	-0.1 (0.2)
73.2	4	93	75	0.2 (0.2)	-0.4 (0.1)	



600 **Figure 1: Concentrations of dissolved O<sub>2</sub> and reactive nitrogen in the bottom waters (2 m above the sediments) of the Lake Lugano South Basin in 2017.**

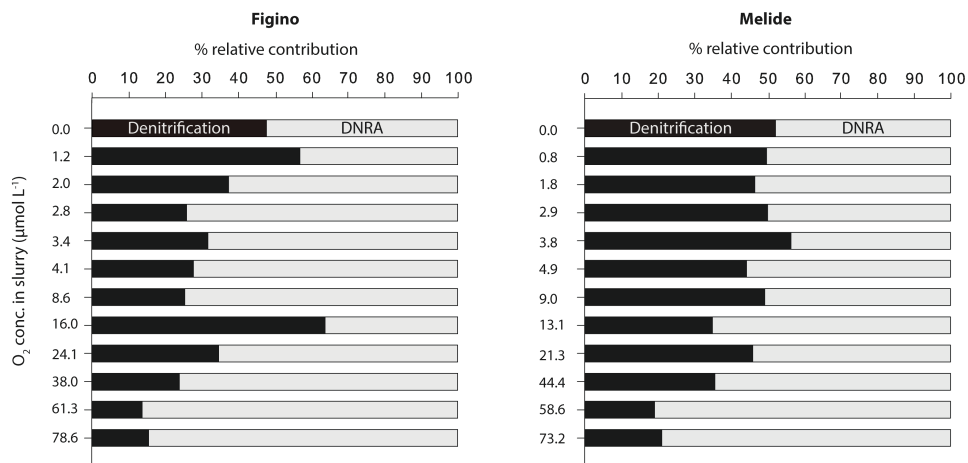


605 **Figure 2: Ex-situ sediment porewater profiles (O<sub>2</sub> and dissolved inorganic nitrogen) at the two sampling stations of the Lake Lugano South Basin in a sediment core collected in October 2017. Oxygen concentration profiles (note different depth units) were determined in aerated cores, and thus are representative of the O<sub>2</sub> penetration during aerated conditions in the water column, as seen between January and April (see Fig. 1).**



**Figure 3: Denitrification and DNRA rates as a function of dissolved O<sub>2</sub> concentration in dilute sediment slurry from Figino and Melide. Error bars represent the standard error of n replicate experiments and measurements (Table 1).**

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**Figure 4: Relative contribution (%) of denitrification and DNRA to total nitrate reduction under variable O<sub>2</sub> conditions.**

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