

Response to comments by Anonymous Referee #1

The authors have done a good job incorporating new information and clarifying aspects of the paper.

I only have a couple of corrections/suggestions.

We thank the reviewer for her/his valuable inputs. We will make changes in the revised manuscript accordingly.

L109. If procedures from Line 106-109 were made in anaerobic glove box as you mention in the reply, this should be included in the text.

Reply: This will be included in the revised manuscript.

L115. I did not argue tha purging will affect the DOC pool. Purging can potentially affect the balance of gases (CO₂, H₂, CH₄) and anaerobic processes in the sediment. Pre-incubation may affect the availability of labile DOC (even without purging).

Reply: We thank the reviewer for clarifying her/his point.

L121. Please mention that you filtered the samples in the glove box before freezing.

Reply: This will be included in the revised manuscript.

L173. In my previous comment "The profiles do indeed indicate that the surface sediment of the Figino is less at the surface than further below. Any reason why? However, the profiles of nitrate do not indicate directly that the sediment acts as a sink given that nitrate and nitrite microprofiles occur same as for oxygen at a mm scale." I did not express myself correctly.

Your profiles of NO₃ are in cm, not mm as you mention in your answer.

With a resolution of 1 cm slices, the authors would not be able to notice the nitrification/denitrification zone which can be as little as 1-2mm to 1 cm. So it may be that by integrating porewater profiles at such coarse resolution can indicate the wrong conclusion. What would happen if I make a porewater profile of oxygen in a highly productive sediment at a 1 cm resolution? I would see a decrease from 200 to 0 and I would assume that it is consuming oxygen whereas in reality it will be releasing O₂ across the sediment water interface. Same here. Without core incubations, you cannot really know if the sediment is a net sink or source.

My other question was, why do the authors think it might be that the sediment at Figino seems less reactive near the surface than further below? Changes in porosity? changes in OC?

Reply: We thank the reviewer for clarifying her/his point, and indeed we made a mistake in our previous answer, where we meant to write cm, and not mm. We agree that the depth resolution of the NO₃⁻ profiles is not sufficient to resolve the exact porewater nitrate concentration gradient at the sediment-water interface (SWI). This is why we mentioned in the text that the sediments represent a sink for NO₃⁻ within the top centimeter of the sediments and did not draw any conclusion about the NO₃⁻ turnover within the first millimeters. Given the multi-fold core-incubation evidence (e.g., Wenk et al. 2014) for the sediments taking up nitrate, it would be brave to assume that there might be a hidden NO₃⁻ concentration peak just below the SWI, which we were not able to resolve. Nevertheless, we do not question that NO₃⁻ is produced via nitrification in the 1-2 mm of the sediments, yet it was entirely consumed within the top centimeter, yielding a net concentration gradient that points to a flux of nitrate into the sediments.

This may be a misunderstanding. We do not think that the sediments at Figino are less reactive near the surface than further below. But there is biogeochemical evidence (e.g., oxygen penetration depth) that the sediments at Figino are less reactive than those at Melide, which may indeed be due to a higher reactivity of the sedimentary organic matter at Melide.

Wenk, C. B., Zopfi, J., Gardner, W. S., McCarthy, M. J., Niemann, H., Veronesi, M. and Lehmann, M. F.: Partitioning between benthic and pelagic nitrate reduction in the Lake Lugano south basin, *Limnol. Oceanogr.*, 59(4), 1421–1433, doi:10.4319/lo.2014.59.4.1421, 2014.

List of relevant changes

Adjustments according to our responses to comments by anonymous referee #1

Line 107: Additional information mentioning that incubations were performed into an anaerobic chamber was included in the text (Ref.1, R1)

Line 126: Details about the processing (e.g. filtering and freezing) of the samples were included in the text (Ref.1, R2)

Direct O₂ control on the partitioning between denitrification and dissimilatory nitrate reduction to ammonium in lake sediments

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10 **Abstract.** Lacustrine sediments are important sites of fixed nitrogen (N) elimination through the reduction of nitrate to N₂ 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 O₂ thresholds particularly
of DNRA inhibition are uncertain. In O₂-manipulation laboratory experiments with dilute sediment slurries and ¹⁵NO₃⁻
15 additions at low- to sub-micromolar O₂ levels, we investigated how, and to what extent, oxygen controls the balance between
DNRA and denitrification in lake sediments. In all O₂-amended treatments, oxygen significantly inhibited both denitrification
and DNRA compared to anoxic controls, but even at relatively high O₂ concentrations (≥ 70 μmol L⁻¹), nitrate reduction by
both denitrification and DNRA was observed, suggesting a relatively high O₂ tolerance. Nevertheless, differential O₂ control
and inhibition effects were observed for denitrification versus DNRA in the sediment slurries. Below 1 μmol L⁻¹ O₂,
20 denitrification was favored over DNRA, while DNRA was systematically more important than denitrification at higher O₂
levels. Our results thus demonstrate that O₂ is an important regulator of the partitioning between N-loss and N-recycling in
sediments. In natural environments, where O₂ 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.

25 1 Introduction

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 O₂ depletion
in the deeper water column, and possibly, seasonal or permanent anoxia (e.g. Blees et al., 2014; Lehmann et al., 2004, 2015).

30 Depending on the O₂-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 (NO_3^- , NO_2^- , NH_4^+) to 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 N_2 by denitrifying bacteria (Gruber, 2008; Seitzinger et al., 2006; Seitzinger, 1988). In contrast, DNRA seems to be favoured in sediments with an excess of electron donors (total organic carbon (TOC), H_2S , 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 the sediment-water interface is most likely minor relative to both denitrification and DNRA, since anammox seems to occur primarily in sediments with low organic matter content (Babbin 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 O_2 concentrations may expand the oxic/suboxic zone where nitrification can supply NO_3^- and NO_2^- for denitrification and anammox, enhancing the overall fixed-N loss (Lehmann et al. 2015). On the other hand, 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 O_2 yields more energy to cells for growth than with other oxidants (i.e., NO_3^- , 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 O_2 tolerance and inhibition of denitrification and anammox in environments differ quite significantly. Inhibition may occur already at very low (nanomolar) O_2 concentrations (Dalsgaard et al., 2014), but experimental studies also revealed that relatively high O_2 levels may be required (up to 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 O_2 concentration levels led to a revision of the classical paradigm regarding the absolute 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 N_2 production (Paulmier and Ruiz-Pino, 2009).

65 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, Babbin et al. 2014, Dalsgaard et al. 2014), investigations into the O₂ control on
benthic N-reduction are rather rare, and limited to sandy and low organic matter marine sediments (Gao et al., 2010; Jäntti and
Hietanen, 2012; Rao et al., 2007). Despite intensified research, the exact O₂ 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
70 significance of DNRA even in the presence of relatively high O₂ concentrations (i.e., at hypoxic levels (i.e., 10-62 μmol L⁻¹),
or concentrations even greater than 62 μmol L⁻¹) in estuarine sediments (Roberts et al., 2012, 2014) and marine sediments
(Jäntti and Hietanen, 2012), but a systematic investigation of how DNRA is impacted at low micromolar O₂ levels in aquatic
sediments (and how in turn the balance between denitrification and DNRA is affected), does to our knowledge not exist.

75 In this study, we provide first experimental evidence for direct O₂ 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), ¹⁵N-labelled substrates and manipulated O₂ concentrations, we investigated the functional response of benthic N-
reducing processes to changing O₂ levels. We demonstrate that denitrification and DNRA are differentially sensitive towards
O₂, which has important implications for fixed N removal in environments that undergo short- and longer-term O₂ changes,
80 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
85 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 oxygenated until the water column re-stratifies in spring, and
bottom-water O₂ concentrations decrease again (Fig. 1). Water column O₂ and N-compound (NO₃⁻, NO₂⁻, NH₄⁺) concentrations
were measured as part of a long-term monitoring campaign promoted by the International commission for the protection of
Italian-Swiss waters (CIP AIS; Commissione Internazionale per la Protezione delle Acque Italiano-Svizzere) and run by SUPSI
90 (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 μmol L⁻¹ and even during water column anoxia, near-sediment nitrate concentrations rarely dropped below 15
95 and 5 μmol 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 (~ 30-140 $\mu\text{mol L}^{-1}$) 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 O_2 microsensor (Unisense) with a tip diameter of 100 μm in duplicate cores from both sites. The overlying water was gently stirred (without disturbing the sediment-water interface) and aerated to determine the O_2 penetration depth under oxygenated conditions at room temperature (~20 °C). 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 under normal atmosphere and at room temperature (~20 °C), and centrifuging of the samples (4700 rpm, 10 min).

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 in an anaerobic chamber (N_2 atmosphere). 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 (NO_3^- , NO_2^- , NH_4^+ -free; Smith et al., 2002) were transferred into 120 mL serum bottles. The use of dissolved- 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 NO_3^- or 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 N_2 and O_2 backgrounds, and placed overnight on a shaker (80 rpm) at 8 °C in the dark to remove any residual O_2 . It needs to be noted that this He-flushing step, although crucial in our experimental set-up, may have interfered with in situ conditions by altering microbe-particle interactions through disruption of larger aggregates in the sediments or by slightly changing the pH in the sediment slurries. Labeled ^{15}N substrate (i.e., $\text{Na}^{15}\text{NO}_3^-$, 99% ^{15}N , Cambridge Isotopes Laboratories, $120 \pm 2 \mu\text{mol L}^{-1}$ final conc.) 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 °C). Preliminary tests were performed in order to assess the minimal incubation time required to obtain clear and robust ^{15}N - N_2 production trends, and during which it was feasible to maintain a more or less constant O_2 concentration in parallel slurry experiments. For subsampling of gas and liquid samples, the incubation vials were transferred to an anaerobic chamber with N_2 -atmosphere. There, 2-mL gas samples were taken at four successive time points (T_0 , T_1 , T_2 , T_3) for 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- NH_4^+ isotope analysis (see below) and for the assessment of nutrient (NO_3^- , NO_2^- , NH_4^+) concentrations. Samples were filtered (0.2 μm) inside the anaerobic chamber prior to freezing.

Commenté [MOU1]: Ref.1, R1

Commenté [MOU2]: Ref.1, R2

2.4 O₂ manipulation experiments

For the O₂ manipulation experiments, serum bottles were equipped with TRACE Oxygen Sensor Spots (TROXSP5, detection limit = 6 nmol L⁻¹ O₂, Pyroscience, Germany), allowing non-invasive, contactless monitoring of dissolved O₂ 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 O₂ (99,995%) were injected into the headspace of pre-conditioned and ¹⁵NO₃⁻-amended slurries using a glass syringe (Hamilton). For each treatment, the gas volume required to reach the targeted O₂ equilibrium concentration (0.8, 1.2, 2, ..., 78.6 μmol L⁻¹) was calculated based on the headspace versus slurry volumes, salinity, and temperature (Garcia and Gordon, 1992). Measured O₂ concentrations in slurries after injection of the respective O₂ 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 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 decline in dissolved O₂ due to microbial consumption, O₂ was added in order to return to the initial target O₂ concentrations (Supp. Table SI.1). 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).

2.5 Hydrochemical analyses

Nitrite concentrations were determined colorimetrically using sulfanilamide and Griess-reagent, according to Hansen and Koroleff (1999). Total NO_x (i.e., NO₃⁻ + NO₂⁻) concentrations were measured using a NO_x-analyser (Antek Model 745, detection limit = 0.02 μmol L⁻¹), by reduction to nitric oxide (NO) in an acidic V³⁺ solution, and quantification of the generated NO by chemiluminescence detection (Braman and Hendrix, 1989). Nitrate concentrations were then calculated from the difference between NO_x and NO₂⁻ concentrations. Ammonium was measured by suppression-ion chromatography with conductivity detection (940 Professional IC Vario, Metrohm, Switzerland).

2.6 ¹⁵N-based rate measurements

For the determination of denitrification rates, gas samples from the ¹⁵N-isotope enrichment experiments were analysed for ¹⁵N/¹⁴N isotope ratios in the N₂ using a Delta V Advantage isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific) with a ConFlo IV interface (Thermo Fisher Scientific). Denitrification (and negligible anammox) rates were calculated based on the quantification of ¹⁵N in the N₂ gas in excess of the natural abundance, i.e. by calculating the linear regression of ³⁰N₂ concentrations (and to a minor extent ²⁹N₂) over incubation time (Nielsen, 1992; Thamdrup and Dalsgaard, 2002; Supp. Fig. SI.1). 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 caps. The headspace was flushed with He for 2 min to reduce the ²⁸N₂ background, and 25 μL mL⁻¹ of alkaline (16 mol NaOH) hypobromite iodine

160 solution (3.3 mol L⁻¹) were added through the septum to convert NH₄⁺ to N₂ (Robertson et al., 2016). Exetainers were stored upside down and placed on a shaker (100 rpm) for 24 h at room temperature. The produced N₂ was then analysed by IRMS as described above. DNRA rates were determined based on the ¹⁵NH₄⁺ production with time, as calculated from the total ¹⁵N-N₂ in the hypobromite-treated samples (i.e., calculated from the excess ²⁹N₂ and ³⁰N₂ signals). The recovery of ¹⁵N-label from identically treated standards was >95%.

2.7 Statistics

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

3 Results

3.1 Porewater hydrochemistry

170 The O₂ microsensor profiles revealed that the O₂ 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 O₂ concentration gradients at the two stations, the potential O₂ flux into sediments was greater at Melide suggesting a higher reactivity of the sedimentary organic matter. In 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 175 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 μmol L⁻¹ NH₄⁺ to 1.7 and 1.2 mmol L⁻¹, respectively.

3.2 N-transformations in control experiments

180 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 et al., in prep.). Indeed, in all experiments, denitrification and DNRA were the main benthic N-transformation processes with an essentially equal contribution to the total nitrate reduction (≈0.1 μmol N g⁻¹ wet sed. d⁻¹; Table 1 caption). We ensured that measured DNRA rates were not underestimated due to ¹⁵NH₄⁺ loss through adsorption on mineral surfaces. Previous results (Cojean et al., in 185 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 O₂, however, might slightly 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 O_2 concentrations net NO_3^- production occurs ($\leq 1 \mu\text{mol N g}^{-1} \text{wet sed. d}^{-1}$).

3.3 Impact of O_2 on NO_3^- reduction in sediments

190 The O_2 sensitivity of denitrification and DNRA and inhibition kinetics were investigated through slurry incubation experiments under O_2 -controlled conditions. At both sites, potential denitrification and DNRA rates consistently decreased with increasing 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 O_2), the response of denitrifiers versus nitrate ammonifiers towards manipulated O_2 differed across sites and treatments. We compared O_2 -addition experiments to the anoxic controls to estimate
195 the inhibition of nitrate reduction by O_2 . At the lowest O_2 concentration ($\sim 1 \mu\text{mol L}^{-1} \text{O}_2$), denitrification was less inhibited than DNRA at Figino ($29 \pm 20\%$ and $51 \pm 7\%$ inhibition, respectively) while the suppression was almost equivalent at Melide ($43 \pm 8\%$ and $37 \pm 9\%$ inhibition of denitrification and DNRA respectively, Table 1). At 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 O_2 concentration $\geq 2 \mu\text{mol L}^{-1}$, DNRA rates were generally higher than those of denitrification (with one exception, i.e.,
200 $16 \mu\text{mol L}^{-1} \text{O}_2$ at Figino; Fig. 3), indicating that denitrification was more sensitive than DNRA to elevated O_2 levels. Oxygen concentrations higher than $73 \mu\text{mol L}^{-1}$ resulted in almost complete inhibition of denitrification at both sites ($96 \pm 1\%$ and $93 \pm 2\%$ at Figino and Melide, respectively, Table 1). Oxygen inhibition thresholds for DNRA were even higher, as DNRA rates were significantly less impaired compared to denitrification at these elevated O_2 levels ($79 \pm 5\%$ and $75 \pm 4\%$ inhibition compared to the anoxic controls at Figino and Melide, respectively; Table 1). A correlation analysis between the relative
205 contribution of DNRA to the total NO_3^- reduction (%) and the increase of O_2 concentration displayed a positive correlation coefficient of 0.57 and 0.91 for Figino and Melide, respectively (Supp. Fig. SI.2). Hence, the relative contribution of the two processes to total nitrate reduction was significantly affected by changing O_2 concentrations. At anoxic and submicromolar levels of 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 O_2 concentration the ratio between denitrification and DNRA was shifted in favour of the nitrate ammonifiers (Fig. 4).

210 Consistent with the observed decline in denitrification and DNRA rates based on the $^{15}\text{N}\text{-N}_2$ and $^{15}\text{NH}_4^+$ measurements in the ^{15}N -label incubations, nitrate consumption in slurries decreased with increasing O_2 concentration at both stations (Table 1). Similarly, maximum ammonium accumulation was observed in the anoxic controls, whereas at higher O_2 levels ammonium underwent net consumption, indicating the concomitant decrease of DNRA and the increasing importance of nitrification under
215 more oxic conditions, particularly at Melide. In incubations where nitrate concentrations decreased, the ratio of $(\text{NO}_3^-)_{\text{consumed}} : (\text{N}\text{-N}_2 + ^{15}\text{NH}_4^+)_{\text{produced}}$ was always significantly higher ($>5:1$) than expected (1:1). This observation is consistent with previous work in the Lake Lugano South Basin (Wenk et al., 2014). Here, whole-core flow-through incubations also revealed that NO_3^- fluxes into the sediments significantly exceeded benthic N_2 production, an imbalance, which could neither be explained by nitrate reduction to ammonium nor incomplete reduction to N_2O . As it is not the scope of this study, we will not

220 discuss this puzzling discrepancy further, but we speculate that excess NO_3^- consumption may be linked to bacterial and algal
uptake (Bowles et al., 2012). Biotic immobilization of NO_3^- in marine sediments has been 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 yet whether such nitrate sinks are important also in Lake Lugano sediments.

4 Discussion

225 4.1 Anaerobic N-cycling in the South Basin of Lake Lugano

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_3^- reduction
of not more than 12%, but also argued that their DNRA rate measurements must be considered conservative, because they did
230 not account for the production of $^{14}\text{NH}_4^+$ from ambient natural-abundance nitrate. The reason for such a 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,
235 in 2017, the water column mixed in January and surface sediments were oxygenated throughout June. Our O_2 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_2 exposure of microbes at the sediment-water interface
in 2017 compared to the preceding year may have favoured 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
240 the two nitrate reducing processes is not implausible and was similarly observed 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; Pappaspyrou et al., 2014). More generally, substrate-availability changes induced by O_2 fluctuations
may be important drivers of the partitioning between denitrification and DNRA (Cojean et al., in prep.), and environmental
conditions that favour DNRA over denitrification may be quite common. However, to our knowledge, experimental evidence
245 for the direct O_2 control on the balance between these two nitrate-reducing processes is still lacking.

4.2 O_2 inhibition thresholds of benthic nitrate reduction

Our study shows that submicromolar O_2 levels significantly lowered both, denitrification and DNRA rates. Denitrification and
DNRA were inhibited by about 30-50% at $1 \mu\text{mol L}^{-1} \text{O}_2$, while in previous studies that investigated O_2 effects on fixed-N
elimination in the water column, denitrification was almost completely suppressed at this O_2 level already. For example, by
250 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 $\mu\text{mol L}^{-1}$ O_2 , and complete suppression at 1.5-3 $\mu\text{mol L}^{-1}$ 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 8-15 $\mu\text{mol L}^{-1}$ O_2 (Jensen et al., 2009). In marine sediments, in contrast, denitrification was occurring even at O_2 concentrations greater than 60 $\mu\text{mol L}^{-1}$ (Gao et al., 2010, Rao et al., 2007). This is in agreement with our results showing that at higher 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 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 ($> 100 \mu\text{mol L}^{-1}$ 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 Baltic Sea, at elevated 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 O_2 sensitivity (i.e., greater inhibition) of DNRA compared to denitrification in sediments with higher bottom water 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 direct comparison of DNRA 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 O_2 concentrations at the sediment depth where nitrate is actually reduced. And although the O_2 penetration depth and porewater O_2 concentrations will respond to a certain degree to the O_2 content in the bottom water, deducing the actual O_2 concentrations for the active nitrate reduction zone within the sediment from 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 O_2 conditions. These incubation data indicate that DNRA is less inhibited than denitrification at 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 O_2 partial pressures than denitrification within the range of 0-2% O_2 v/v (Fazzolari et al., 1998; Morley and Baggs, 2010).

The observed 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 O_2 tolerance in prior studies was often attributed to the formation of anoxic microniches that may foster anaerobic N-reduction (Kalvelage et al., 2011). It is unlikely that such microniches formed during our incubation experiments since slurries were heavily diluted (1 g sediment in 70 mL water) and vigorously shaken by hand every 30 min, in addition to the continuous agitation on a shaking table during the incubation. Also, experiments were replicated 2-3 times for some O_2 -amended treatments, and measured rates were very similar between replicates. If anoxic microniches had formed, we would have expected that their formation is more variable, resulting in a lower reproducibility of the determined rates.

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The existence of aerobic denitrifiers (e.g. microbes that reduce $\text{NO}_3^-/\text{NO}_2^-$ 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). 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

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4.3 DNRA favoured under less reducing conditions

It is generally assumed that strongly reducing conditions favour 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 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 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 favoured 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 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 due to the counteracting and variable influence of direct versus indirect effects of the variable O_2 concentrations.

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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 measured in any of the incubations, same organic matter content, same excess NO_3^- concentrations), and this study can thus be considered an 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

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likely controls the balance between denitrification and DNRA at the organism-level, and that denitrification is in fact more sensitive towards increasing O₂ concentrations than DNRA.

4.4 Direct O₂ control on benthic NO₃⁻ reduction

320 It has been previously reported that O₂ 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 O₂ concentrations suggest that the overall activity is modulated mainly at the nitrate reduction step. Without
conclusive information on enzyme activities in hand, we can only speculate at this point about any real difference in O₂-
dependent response of the enzymes involved in denitrification versus DNRA. The differential response of denitrifiers and
325 nitrate ammonifiers may, however, suggest a distinct O₂ sensitivity of the nitrate reductase enzymes involved. Denitrifiers and
nitrate ammonifiers utilize the same nitrate reductase enzymes (Nar, Nap), and while a differential O₂ 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 NO₃⁻ and O₂ 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 NO₃⁻ and O₂ concentrations (Simon and Klotz, 2013).
330 When NO₃⁻ is scarce, Nap provides a high-affinity (for NO₃⁻) but low-activity pathway that does not require NO₃⁻ 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 O₂ sensitive, so that microbes possessing and relying on Nap are likely to
335 have an ecological advantage in environments that are subject to O₂ fluctuation (Carter et al., 1995). In nature, nitrate reduction
by denitrifiers is assumed to be catalysed 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,
340 to understand better the combined effects of O₂ on the nitrogen-transforming metabolic pathways and their regulation.
Additional O₂ inhibitory effects at one of the down-cascade enzyme levels (Nir, Nrf, Nor, Nos) are likely to exhibit variable
O₂ sensitivities (Baumann et al., 1996, 1997; Körner H. and Zumft, 1989; Poock et al., 2002). While we are aware that the
treatment above is speculative, we argue that our observations of higher DNRA/denitrification ratios at higher O₂ provides at
least putative evidence that microorganisms performing DNRA using Nap may be more O₂-tolerant than denitrifiers using
345 Nar, a hypothesis that requires further testing.

4.5 Implication for N-elimination versus N-recycling in lakes with fluctuating O₂ conditions

The redox-sensitive partitioning of nitrate elimination (through N₂ 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 O₂ fluctuations affect N transformation reactions within the sediments remains uncertain. Winter water column turnover ventilates the 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 O₂ inhibition threshold was lower for denitrification than for DNRA, possibly reflecting differential adaptation of the in situ microbial community of denitrifiers and nitrate ammonifiers to fluctuating O₂ conditions of bottom waters. Indeed, many nitrate ammonifiers possess both nitrate reductase enzymes (Nap and Nar) and can switch between the two respiratory 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 O₂ concentrations, similar to the ones tested here. Our experimental data imply that then, at least in the uppermost sediments, DNRA is favoured over denitrification. We may even expect an O₂-regulated zonation of DNRA and denitrification. As a consequence, when denitrification-driven nitrate-reduction is pushed down, it is possible that NO₃⁻ will be partially consumed through DNRA before it gets to the “denitrification layer”, as nitrate ammonifiers are less O₂ 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.

In the discussion thus far, we implicitly assume that the main control O₂ 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 O₂ 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 O₂ 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 O₂ 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 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 O₂ levels there is at least some production of nitrate. There is no obvious reason to assume that O₂-stimulation of the coupling of nitrification and 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 O₂ 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 O₂ 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, O₂ 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., NO₃⁻, NO₂⁻, TOC, H₂S, Fe²⁺) 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 NO₃⁻ 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, Fe²⁺ 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 Fe²⁺ 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 O₂ on the partitioning between denitrification and DNRA, which we have demonstrated here experimentally, O₂ can act as indirect regulator of fixed N elimination versus regeneration. The ultimate ecosystem-scale DNRA/denitrification ratio in environments that are subject to O₂ fluctuating conditions is difficult to predict, because direct and indirect O₂ regulation may act concomitantly and in opposite ways.

5 Conclusion

The presented results broaden the range of O₂ inhibition thresholds of benthic denitrification at micromolar O₂ levels, demonstrating that benthic denitrification may resist full inhibition up to almost 80 μM O₂. Similarly, sedimentary DNRA does not necessarily require true anoxia, and was even less sensitive than denitrification to higher O₂ levels. Our data suggest that the balance between DNRA and denitrification is modulated by O₂ at the nitrate-reducing enzyme level. However, more

415 in-depth investigations on the exact role of oxygen in regulating other denitrification and/or nitrate-ammonification enzymes
in microbial pure culture experiments are needed. The differential tolerance of denitrifiers versus nitrate ammonifiers towards
O₂ has important implications for natural environments with fluctuating O₂ 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 favour full denitrification to dinitrogen. Whether and when fixed nitrogen is preserved in a lake or
420 eliminated by denitrification is, however, difficult to predict, as this will depend also on multiple indirect effects of changing
O₂ 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
425 high-productivity lakes is generally less of a concern than from P. Nevertheless, 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.

Data availability

430 Data can be accessed upon request to the corresponding author.

Author contribution

JZ and MFL initiated the project. ANYC performed all sample collection and conducted the experimental work 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.

435 Competing interest

The authors declare that they have no conflict of interest.

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595 **Table 1: Overview of N transformation rates in O₂-controlled slurry incubation experiments. Negative and positive values correspond to net NO₃⁻ or NH₄⁺ 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⁻¹ wet sed. d⁻¹) 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 ₂ conc. in slurry μmol L ⁻¹	<i>n</i>	Inhibition compared to control (%)		NO ₃ ⁻ μmol N g ⁻¹ wet sed. d ⁻¹	NH ₄ ⁺
			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)

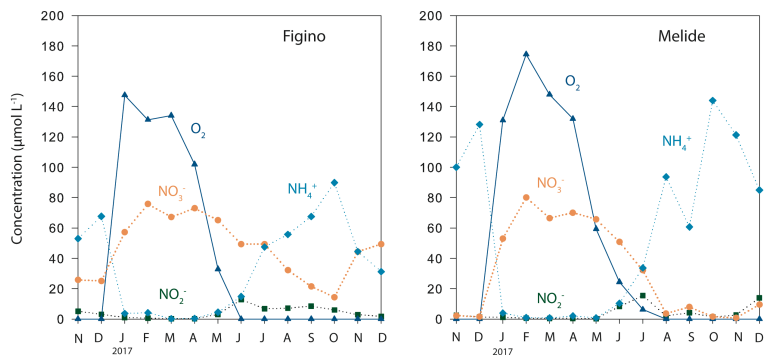
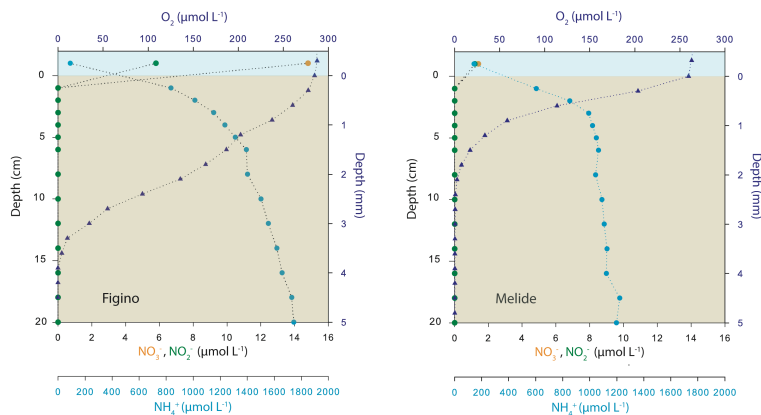
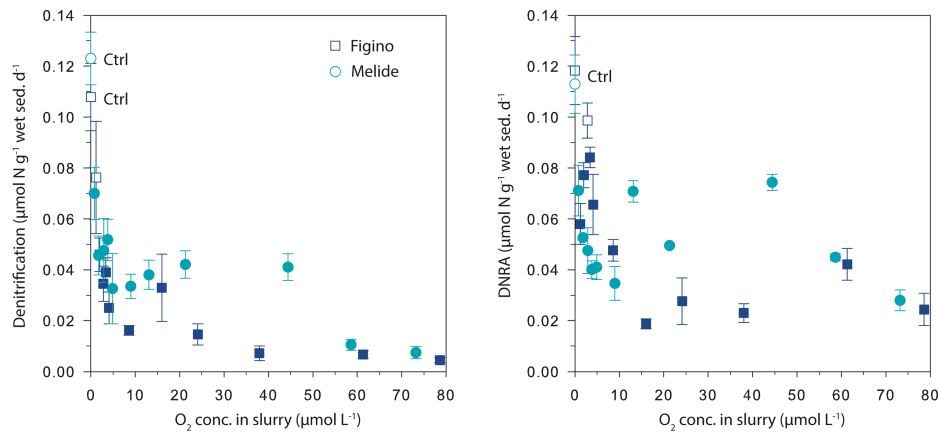


Figure 1: Concentrations of dissolved O₂ and reactive nitrogen in the bottom waters (2 m above the sediments) of the Lake Lugano South Basin in 2017.



605 Figure 2: Ex situ sediment porewater profiles (O₂ and dissolved inorganic nitrogen) at the two sampling stations of the Lake Lugano South Basin in sediment cores collected in October 2017. Symbols correspond to the mean value of O₂ and dissolved N species concentrations measured in triplicate and duplicate cores, respectively. Oxygen concentration profiles (note different depth units) were determined in aerated cores, and thus are representative of the O₂ penetration during aerated conditions in the water column, as seen between January and April (see Fig. 1).



615 **Figure 3: Denitrification and DNRA rates as a function of dissolved O₂ concentration in dilute sediment slurry from Figino and Melide. Statistically significant differences between denitrification/DNRA rates measured in the different O₂ treatments versus those in the respective control (Ctrl) experiments are shown by the filled symbols ($P < 0.05$, Supp. Table SI.2). Error bars represent the standard error of n replicate experiments and measurements (Table 1).**

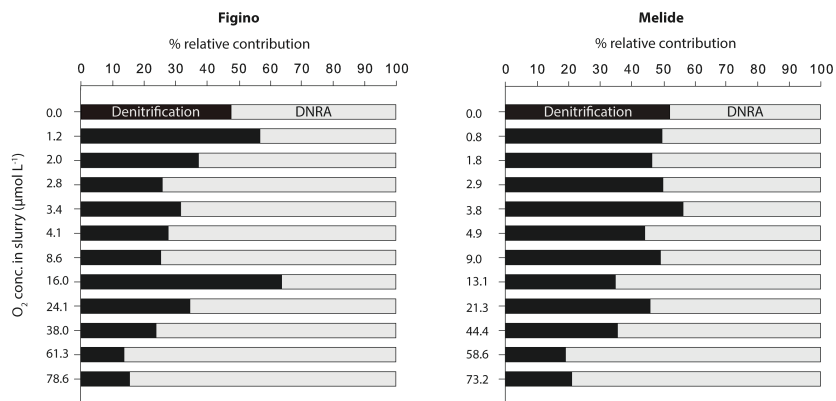


Figure 4: Relative contribution (%) of denitrification and DNRA to total nitrate reduction under variable O₂ conditions.