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## Isotopic signatures of biotic and abiotic N<sub>2</sub>O production and consumption in the water column of meromictic, ferruginous Lake La Cruz (Spain)

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### Abstract

Lakes can be important sources of the potent greenhouse gas nitrous oxide (N<sub>2</sub>O) to the atmosphere, but to what extent abiotic processes may contribute to lacustrine N<sub>2</sub>O production remains uncertain. We assessed pathways of N<sub>2</sub>O production and reduction in the water column of meromictic and iron-rich Lake La Cruz, Spain, including chemodenitrification-induced N<sub>2</sub>O formation via the reaction of reactive nitrogen (N) (e.g., NO<sub>2</sub><sup>-</sup>) with ferrous iron (Fe [II]). In the oxic waters (~8–10 m), N<sub>2</sub>O concentrations above atmospheric equilibrium were associated with comparatively low δ<sup>15</sup>N-N<sub>2</sub>O, high δ<sup>15</sup>N-NH<sub>4</sub><sup>+</sup>, and high N<sub>2</sub>O <sup>15</sup>N-site-preference (SP) values (up to ~29‰), suggesting N<sub>2</sub>O production by nitrification. N<sub>2</sub>O concentrations were highest (23–33 nM) near the depth of oxygen depletion (~11–14.5 m), likely due to production by nitrifier denitrification and/or denitrification, as indicated by decreasing SP values (as low as 12‰). Further below (~14.5–17 m), N<sub>2</sub>O consumption was indicated by increasing SP values and a δ<sup>18</sup>O-vs.-δ<sup>15</sup>N relationship (1.8–2.9) typical for stand-alone N<sub>2</sub>O reduction. The coupled N-vs.-O isotope signatures thus highlight the spatial, redox-dependent separation of incomplete and complete denitrification. In incubations with sterile-filtered lake water and <sup>15</sup>N-labeled or unlabeled substrate, NO<sub>2</sub><sup>-</sup> was reduced by Fe<sup>2+</sup> to N<sub>2</sub>O, even at low nitrite concentrations (5 μM NO<sub>2</sub><sup>-</sup>). In the water column, the spatial separation of NO<sub>2</sub><sup>-</sup> and Fe (II) during our samplings appears to preclude elevated rates of chemodenitrification, but during periods of overlapping NO<sub>2</sub><sup>-</sup> and Fe(II) in Lake La Cruz, and potentially in other lakes, its distinct N<sub>2</sub>O δ<sup>18</sup>O-vs.-δ<sup>15</sup>N relationship of ~1 : 1, as experimentally determined, could help to detect it.

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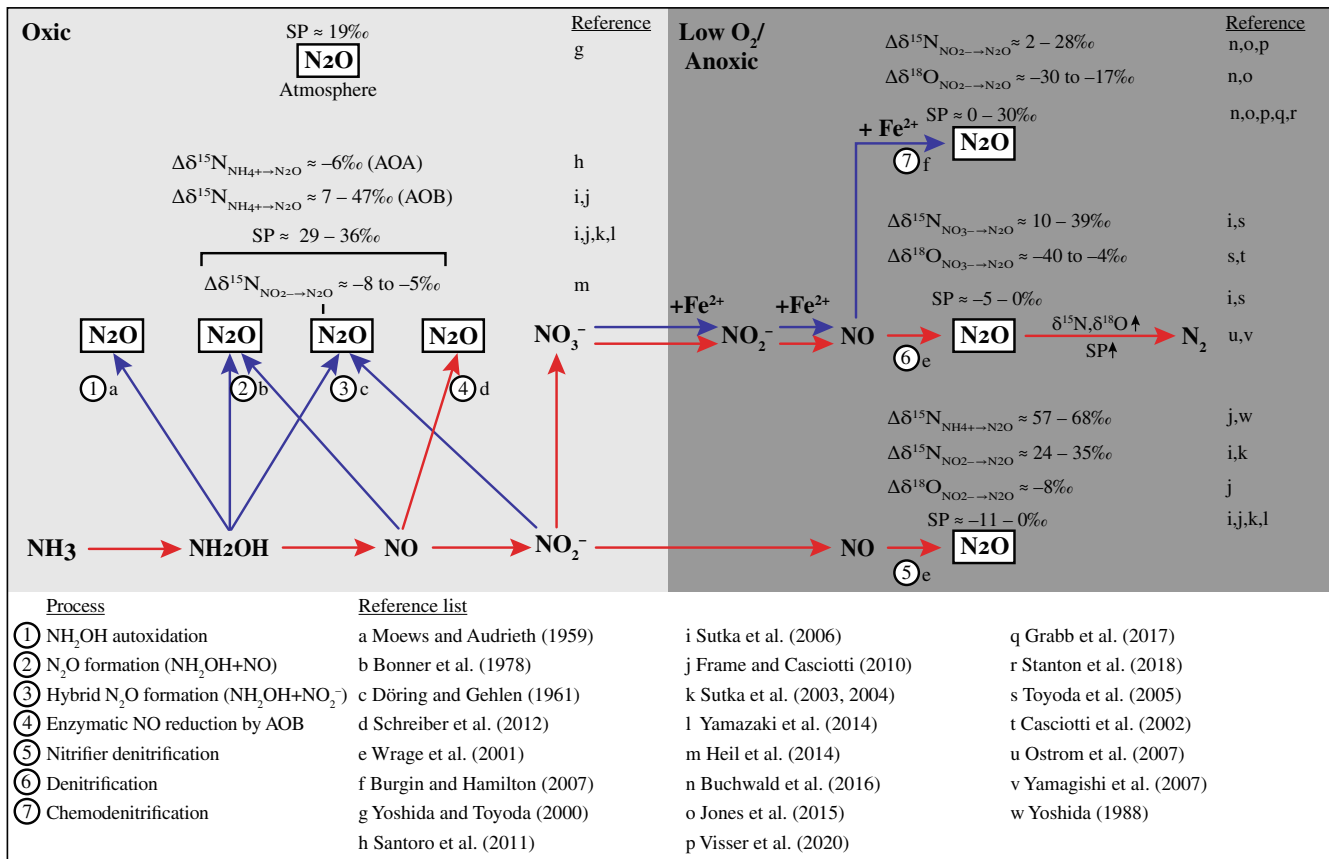
Nitrous oxide (N<sub>2</sub>O) is a potent greenhouse gas and an important ozone-layer-depleting substance in the atmosphere (Wang et al. 1976; Ravishankara et al. 2009). Terrestrial ecosystems including inland waters are relevant sources of both natural and anthropogenic N<sub>2</sub>O, but with a high uncertainty in their contribution to global N<sub>2</sub>O emissions (Maavara et al. 2019). N<sub>2</sub>O production in these ecosystems has been attributed primarily to microbially mediated nitrogen (N)-transforming processes (Tian et al. 2020). The relative contribution of abiotic N<sub>2</sub>O production mechanisms is still a matter of debate, particularly in lakes.

The main microbial N<sub>2</sub>O-producing processes are nitrification, nitrifier denitrification, and denitrification. During the first step of nitrification, where ammonia-oxidizing bacteria (AOB) and/or ammonia-oxidizing archaea (AOA) oxidize

ammonia (NH<sub>3</sub>) or ammonium (NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>) via hydroxylamine (NH<sub>2</sub>OH), and nitric oxide (NO) (Schreiber et al. 2012; Kozłowski et al. 2016), N<sub>2</sub>O can be produced as a side product via several pathways. Non-enzymatic N<sub>2</sub>O production may be attributed to the autoxidation of NH<sub>2</sub>OH (Fig. 1, pathway 1; Moews and Audrieth 1959), the reaction between NH<sub>2</sub>OH and NO both derived from NH<sub>3</sub> (Fig. 1, pathway 2; Bonner et al. 1978), or a hybrid mechanism, where one NH<sub>3</sub>-derived N atom (e.g., NH<sub>2</sub>OH) and one NO<sub>2</sub><sup>-</sup>-derived N atom form one N<sub>2</sub>O molecule (Fig. 1, pathway 3; Döring and Gehlen 1961). In AOB, N<sub>2</sub>O is thought to be produced primarily biologically through NO reduction by a yet unknown enzyme (Fig. 1, pathway 4; Schreiber et al. 2012). At relatively low oxygen (O<sub>2</sub>) levels, AOB respire NO<sub>2</sub><sup>-</sup> by reduction to NO and N<sub>2</sub>O (Fig. 1, pathway 5) via nitrifier denitrification (review in Wrage et al. 2001). A different subset of microorganisms carries out denitrification, the step-wise enzymatic reduction of nitrate (NO<sub>3</sub><sup>-</sup>) to NO<sub>2</sub><sup>-</sup>, NO, N<sub>2</sub>O, and eventually to dinitrogen gas (N<sub>2</sub>) (Fig. 1, pathway 6), under anaerobic and very low-O<sub>2</sub> conditions (Wrage et al. 2001). Abiotic N<sub>2</sub>O production may occur via

chemodenitrification, the reduction of NO<sub>2</sub><sup>-</sup> (and possibly NO<sub>3</sub><sup>-</sup>), with reduced iron (Fe(II); Fig. 1, pathway 7; Burgin and Hamilton 2007 and references therein). It can be accelerated by surface catalysts, such as mineral surfaces (Buchwald et al. 2016; Grabb et al. 2017), or dead bacterial detritus (Visser et al. 2020), and therefore indirectly connected to microbial activity, as microbes provide reactive N metabolites and catalyzing surfaces.

Process-specific isotope characteristics can help to trace N<sub>2</sub>O cycling processes that occur in close proximity in natural environments. One valuable diagnosing tool in this regard is the isotopic offset, here defined as the isotopic difference between the initial source and the final product during N<sub>2</sub>O production ( $\Delta\delta^{15}\text{N} = \delta^{15}\text{N}_{\text{source}} - \delta^{15}\text{N}_{\text{product}}$  and  $\Delta\delta^{18}\text{O} = \delta^{18}\text{O}_{\text{source}} - \delta^{18}\text{O}_{\text{product}}$ , respectively), where the N and O isotopic composition of N<sub>2</sub>O and its precursor compounds are reported in the conventional delta-notation of per mil (‰) units, that is,  $\delta^{15}\text{N}$  or  $\delta^{18}\text{O}$  values (Toyoda et al. 2015). Typical isotopic differences for N-transformation processes are presented in Fig. 1. For instance, they may help to identify AOB-associated N<sub>2</sub>O production from ammonium via nitrification



**Fig. 1.** Environmentally relevant enzymatic and non-enzymatic reactions that produce or consume N<sub>2</sub>O, including typical N<sub>2</sub>O SP values and characteristic isotope fractionation effects ( $\Delta\delta^{15}\text{N} \approx \delta^{15}\text{N}_{\text{substrate}} - \delta^{15}\text{N}_{\text{product}}$ ,  $\Delta\delta^{18}\text{O} \approx \delta^{18}\text{O}_{\text{substrate}} - \delta^{18}\text{O}_{\text{product}}$ ). Red arrows represent enzymatic reactions; blue arrows represent non-enzymatic reactions. Processes relevant in oxic environments are included in the light gray box; processes most relevant in low-O<sub>2</sub> and anoxic environments are in the dark gray box. See the text for more information.

( $\Delta\delta^{15}\text{N}_{\text{NH}_4^+ \rightarrow \text{N}_2\text{O}} \approx 7\text{‰}$ ) and nitrifier denitrification ( $\Delta\delta^{15}\text{N}_{\text{NH}_4^+ \rightarrow \text{N}_2\text{O}} \approx 57\text{‰}$ ) (Frame and Casciotti 2010). Contrarily, the N and O isotopic offsets with nitrite or nitrate as substrate can hardly distinguish between denitrification ( $\Delta\delta^{15}\text{N}_{\text{NO}_3^- \rightarrow \text{N}_2\text{O}} \approx 10\text{--}39\text{‰}$ ,  $\Delta\delta^{18}\text{O}_{\text{NO}_3^- \rightarrow \text{N}_2\text{O}} \approx -40$  to  $-4\text{‰}$ ; Casciotti et al. 2002; Toyoda et al. 2005; Sutka et al. 2006) and chemodenitrification ( $\Delta\delta^{15}\text{N}_{\text{NO}_2^- \rightarrow \text{N}_2\text{O}} \approx 2\text{--}30\text{‰}$ ,  $\Delta\delta^{18}\text{O}_{\text{NO}_2^- \rightarrow \text{N}_2\text{O}} \approx -30$  to  $-17\text{‰}$ ; Jones et al. 2015; Buchwald et al. 2016; Visser et al. 2020).

The  $\delta^{18}\text{O}$ -vs.- $\delta^{15}\text{N}$  relationship of N<sub>2</sub>O may be particularly helpful in constraining N<sub>2</sub>O sources and sinks in low O<sub>2</sub>/anoxic environments. While denitrification produces variable slopes in a  $\delta^{18}\text{O}$ -vs.- $\delta^{15}\text{N}$  space, for example,  $\Delta\delta^{18}\text{O} : \Delta\delta^{15}\text{N}$  values between  $\sim -1.5$  and  $2.5$  (Casciotti et al. 2002; Toyoda et al. 2005), N<sub>2</sub>O consumption via denitrification results in a robust  $\Delta\delta^{18}\text{O} : \Delta\delta^{15}\text{N}$  value of  $\sim 2.6$  (Yamagishi et al. 2007; Ostrom and Ostrom 2012). At the same time, chemodenitrification produces N<sub>2</sub>O with a relatively narrow range of  $\Delta\delta^{18}\text{O} : \Delta\delta^{15}\text{N}$  values between  $\sim 0.7$  and  $1$  (Jones et al. 2015; Visser et al. 2020).

The intramolecular <sup>15</sup>N distribution within N<sub>2</sub>O, or site preference (SP), defined as the difference in the relative abundance of <sup>15</sup>N in the central ( $\alpha$ ) and terminal ( $\beta$ ) N atoms in N<sub>2</sub>O ( $\text{SP} = \delta^{15}\text{N}^\alpha - \delta^{15}\text{N}^\beta$ ), provides information on the production pathway independent of the substrate N isotopic composition (Toyoda and Yoshida 1999). For example, the SP can distinguish between nitrification-associated N<sub>2</sub>O formation with high SP values ( $> 29\text{‰}$ ) and nitrifier denitrification and denitrification with relatively low values ( $< 0\text{--}10\text{‰}$ ) (see Fig. 1). N<sub>2</sub>O produced by abiotic reactions of NO<sub>2</sub><sup>-</sup> or NO and Fe(II) displays highly variable SP values between  $0$  and  $30\text{‰}$  (Grabb et al. 2017; Visser et al. 2020).

Iron-rich meromictic lakes are considered potential modern analogs for ferruginous ancient oceans and represent valuable model ecosystems to study biogeochemical processes involving iron (Walter et al. 2014; Camacho et al. 2017). Abiotic interactions between the iron and the N cycle have been demonstrated in laboratory experiments and studied in natural environments including soils, marine sediments, and Antarctic lakes and ponds (Venterea 2007; Peters et al. 2014; Wankel et al. 2017). Clear evidence for such abiotic interaction is lacking in lake water columns, despite the abundance and significance of boreal iron-rich shield lakes (Schiff et al. 2017). Chemodenitrification, for instance, may occur in ferruginous lakes like Lake Matano (Indonesia), Kabuno Bay (Democratic Republic of the Congo) or Lake La Cruz (Spain), where Fe(II)-oxidizing, nitrate-reducing bacteria, which are known to catalyze abiotic NO<sub>2</sub><sup>-</sup> reduction, have been identified (Walter 2011; Kopf et al. 2013; Michiels et al. 2017). In this study, we use a multifaceted isotope geochemical approach to investigate the N<sub>2</sub>O dynamics in meromictic, ferruginous Lake La Cruz. We combine (i) water column hydrochemical measurements, including the analysis of the stable isotope composition and SP of dissolved N<sub>2</sub>O; (ii) <sup>15</sup>N-isotope label incubation experiments to identify and quantify N<sub>2</sub>O production pathways; and (iii) experiments to

determine the isotope effects during the abiotic reaction between NO<sub>2</sub><sup>-</sup> and Fe<sup>2+</sup> to form N<sub>2</sub>O, as a prerequisite to identify this process potentially occurring in the lake. We assess the applied isotopic approaches in the context of their value in determining the relative importance and spatial partitioning of different N<sub>2</sub>O sources and sinks in the water column of Lake La Cruz. Furthermore, we aim at verifying whether under the ferruginous conditions in Lake La Cruz, chemodenitrification can be an important contributor to ecosystem-scale N<sub>2</sub>O production.

## Methods

### Field site

Lake La Cruz is located in the Iberian Ranges near the city of Cuenca, Spain, at an altitude of  $\sim 1000$  m above sea level. It is a circular carbonate karstic sinkhole with a mean diameter of  $132$  m and a maximum depth of  $\sim 20$  m (Vicente and Miracle 1988). The lake has been meromictic for more than  $300$  yr, due to a density gradient, which constitutes the chemocline, approximately  $5$  m above the lake bottom. The chemical stratification is mainly due to the high concentrations of calcium and magnesium bicarbonate ions in the permanent monimolimnion. High concentrations of other ions, including NH<sub>4</sub><sup>+</sup> and dissolved Fe<sup>2+</sup>, additionally contribute to the water column stability (Rodrigo et al. 2001; Walter et al. 2014). Lake La Cruz is a closed lake, and the total depth, as well as the position of the redox transition zone (RTZ, here defined as the layer between the depth, where the O<sub>2</sub> concentration reaches  $< 5$   $\mu\text{M}$  and the first depth of detectable sulfide (H<sub>2</sub>S)) vary depending on the precipitation regime (Rodrigo et al. 2001).

### In situ profiling and sample collection

We carried out two field campaigns, one in the first week of March 2015 and one in the third week of March 2017. Hydrocasts were performed at the deepest point in the center of the lake ( $39^\circ 59' 20''\text{N}$ ,  $01^\circ 52' 25''\text{W}$ ). As described in Oswald et al. (2016), a profiling in situ analyzer (PIA), equipped with a multi-parameter probe, as well as light (PAR; LI-193 Spherical Underwater Quantum Sensor, LI-COR) and chlorophyll *a* (Chl *a*; ECO-FL, Wetlands, EX/EM = 470/695) sensors was used to measure conductivity, turbidity, temperature, depth (pressure), pH, photosynthetically active radiation, Chl *a*, and H<sub>2</sub>S. Furthermore, two micro-optodes (PSt1 and TOS7, PreSens) attached to the PIA were used to detect dissolved O<sub>2</sub> concentrations down to  $125$  and  $20$  nM, respectively (Kirk et al. 2014). A battery-driven peristaltic pump was used for sample collection, whereby the outlet of an air-tight tubing (PVC Solaflex, Maagtechnic) was directly attached to the PIA, allowing sampling from precisely known depths. Unfiltered water was fixed with monobromobimane for H<sub>2</sub>S analysis (Fahey and Newton 1987). To avoid O<sub>2</sub> contamination, we filled a  $120\text{-mL}$  serum vial and let the water overflow one volume before pipetting water from the bottom of the vial

directly into a tube prepared with the fixing reagent. For total iron concentrations, we took unfiltered water samples by filling 60-mL plastic syringes with water and subsequently fixed the water in HNO<sub>3</sub> (~150 mM final concentration). For the analysis of dissolved Fe concentrations, we filtered water through 0.2- $\mu$ m syringe filters and fixed it in ~150 mM HNO<sub>3</sub>. For the analysis of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and sulfate (SO<sub>4</sub><sup>2-</sup>) concentrations, and for the isotope analysis of NO<sub>x</sub> (NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>)/nitrate, we filtered aliquots of water through a 0.45  $\mu$ m syringe filter [Correction added on 23 June 2022, after first online publication: “NO<sub>x</sub>” changed to read “NO<sub>x</sub> (NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>)”. Also corrected  $\mu$ M to  $\mu$ m.]. In the nitrate isotope samples, nitrite was removed using sulfamic acid (Granger and Sigman 2009). H<sub>2</sub>S, NO<sub>x</sub>/nitrate isotope, and ammonium isotope samples were kept frozen; all other samples were stored at 4°C prior to analysis. For N<sub>2</sub>O analyses, we collected samples in 160-mL serum bottles, and poisoned them immediately with 5 mL 10 M NaOH. Subsequently, a 1-mL headspace was introduced, and the vials were sealed with 20 mm gray butyl stoppers (VWR), shaken vigorously, and stored cold and dark until analysis. For incubation experiments in 2017, water from 8 and 14.5 m depth was pumped directly into sterile borosilicate glass bottles and sterile 10-L plastic canisters.

### Solute and metal concentrations

NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and N<sub>2</sub>O—Photometrical methods involving phenol and sulfanilamide and the Griess reagent (naphtal-ethylenediamine dihydrochloride) were used to measure NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup>, respectively (Hansen and Koroleff 1999). NO<sub>x</sub> was analyzed by reduction to NO in an acidic vanadium(III) (V<sup>3+</sup>) solution followed by chemiluminescence detection of the NO (Antek Model 745; Braman and Hendrix 1989). NO<sub>3</sub><sup>-</sup> concentrations were calculated as the difference of NO<sub>x</sub> and NO<sub>2</sub><sup>-</sup> concentrations. Dissolved N<sub>2</sub>O concentrations were determined by isotope-ratio mass spectrometry (IRMS) (see below).

Fe—In 2015, total and dissolved Fe concentrations were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Spectro Ciros Vision) and in 2017 by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent Technologies). Concentrations of particulate iron were calculated as the difference of total and dissolved iron.

H<sub>2</sub>S, SO<sub>4</sub><sup>2-</sup>—H<sub>2</sub>S was analyzed using high-performance liquid chromatography (HPLC, Dionex) with fluorescence detection (Zopfi et al. 2008). SO<sub>4</sub><sup>2-</sup> was quantified by ion chromatography (940 Professional IC Vario, Metrohm).

### Stable isotopes of nitrogen species

N<sub>2</sub>O—Using helium (He) as carrier gas, N<sub>2</sub>O was purged from the sample vials into a customized purge-and-trap system (McIlvin and Casciotti 2010), and analyzed by continuous-flow IRMS (GC-IRMS). CO<sub>2</sub> was trapped via Ascarite II and remaining traces additionally separated from N<sub>2</sub>O in the GC column (RT<sup>®</sup>-Q-BOND, 30 m, RESTEK, 25°C), resulting in baseline-separated peaks of CO<sub>2</sub> and

N<sub>2</sub>O. Measured N<sub>2</sub>O isotope ratios were calibrated against N<sub>2</sub>O injected from a reference N<sub>2</sub>O tank ( $\geq 99.9986\%$ , Messer) calibrated on the Tokyo Institute of Technology scale (Mohn et al. 2012) by J. Mohn (EMPA, Switzerland) for bulk and site-specific isotopic composition. Ratios of m/z 45/44, 46/44, and 31/30 signals were converted to  $\delta^{15}\text{N-N}_2\text{O}$  (referenced to AIR),  $\delta^{18}\text{O-N}_2\text{O}$  (referenced to Vienna Standard Mean Ocean Water, VSMOW), and site-specific  $\delta^{15}\text{N}^\alpha$  and  $\delta^{15}\text{N}^\beta$ -N<sub>2</sub>O according to Frame and Casciotti (2010), with an additional three-point correction (Mohn et al. 2014) using measurements of three isotopic mixtures of N<sub>2</sub>O in synthetic air (CA06261:  $\delta^{15}\text{N} = -35.74 \pm 0.07\%$ ,  $\delta^{15}\text{N}^\alpha = -22.21 \pm 0.39\%$ ,  $\delta^{15}\text{N}^\beta = -49.28 \pm 0.40\%$ ,  $\delta^{18}\text{O} = 26.94 \pm 0.23\%$ , Fl.53504:  $\delta^{15}\text{N} = 48.09\%$ ,  $\delta^{15}\text{N}^\alpha = 1.71\%$ ,  $\delta^{15}\text{N}^\beta = 94.44\%$ ,  $\delta^{18}\text{O} = 36.10\%$ , and CA08214:  $\delta^{15}\text{N} = 6.84\%$ ,  $\delta^{15}\text{N}^\alpha = 17.11\%$ ,  $\delta^{15}\text{N}^\beta = -3.43\%$ ,  $\delta^{18}\text{O} = 35.39\%$ ; kindly provided by J. Mohn). A final correction step was included to correct for the difference between gaseous and aquatic samples/standards by subtracting the values of measured atmospheric N<sub>2</sub>O equilibrated in water from the expected isotope values of atmospheric N<sub>2</sub>O using values reported in Yoshida and Toyoda (2000).

N<sub>2</sub>O concentrations were analyzed by calibrating the total peak areas in water column samples with N<sub>2</sub>O standards of known concentrations (2015: air and standards prepared with the denitrifier method, see next paragraph; 2017: isotope standard CA06261). The N<sub>2</sub>O concentrations in the water column at equilibrium with the atmosphere were calculated based on Weiss and Price (1980), taking into account water temperature and salinity. Atmospheric partial pressures of N<sub>2</sub>O were taken from the NOAA/ESRL halocarbons in situ program (<http://www.esrl.noaa.gov/gmd/>).

NO<sub>x</sub>, NO<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup>—NO<sub>x</sub> and NO<sub>3</sub><sup>-</sup> isotope ratios were analyzed using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002; McIlvin and Casciotti 2010). Briefly, after the bacterial conversion of NO<sub>x</sub> or NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>O, the N<sub>2</sub>O was analyzed using the customized purge-and-trap system and continuous-flow GC-IRMS described before. NO<sub>3</sub><sup>-</sup> isotopes were calibrated against the international NO<sub>3</sub><sup>-</sup> standards IAEA-N3 ( $\delta^{15}\text{N} = 4.7\%$ ,  $\delta^{18}\text{O} = 25.6$ ) and USGS34 ( $\delta^{15}\text{N} = -1.8\%$ ,  $\delta^{18}\text{O} = -27.9\%$ ), and an internal standard (UBN-1;  $\delta^{15}\text{N} = 14.15\%$ ,  $\delta^{18}\text{O} = 25.7\%$ ). The N and O isotopic composition of nitrite in incubation experiments was determined by the reduction of NO<sub>2</sub><sup>-</sup> to N<sub>2</sub>O with sodium azide, followed by GC-IRMS analysis (McIlvin and Altabet 2005). Isotopic calibration was performed by concurrent analysis of the international NO<sub>2</sub><sup>-</sup> standards N7373 ( $\delta^{15}\text{N} = -79.6\%$ ,  $\delta^{18}\text{O} = 4.5\%$ ) and N10219 ( $\delta^{15}\text{N} = 2.8\%$ ,  $\delta^{18}\text{O} = 88.5\%$ ). Finally, ammonium N isotope ratios were determined by the conversion of NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup> with hypobromite, followed by the transformation of the NO<sub>2</sub><sup>-</sup> to N<sub>2</sub>O with sodium azide (Zhang et al. 2007) and its isotopic analysis as described above. The NH<sub>4</sub><sup>+</sup>-derived N<sub>2</sub>O N isotope ratios were first referenced against the international NO<sub>2</sub><sup>-</sup> standards N7373 and N10219, and in a second

step against the international NH<sub>4</sub><sup>+</sup> standards IAEA-N1 ( $\delta^{15}\text{N} = 0.43\text{‰}$ ) and IAEA-N2 ( $\delta^{15}\text{N} = 20.41\text{‰}$ ), which underwent the same processing as the samples. All N and O isotopic data are reported on the per mil (‰) scale referenced to AIR and VSMOW, respectively:  $\delta^{15}\text{N} = ([^{15}\text{N}]/[^{14}\text{N}])_{\text{sample}}/[^{15}\text{N}]/[^{14}\text{N}]_{\text{AIR}} - 1) \times 1000\text{‰}$ , and  $\delta^{18}\text{O} = ([^{18}\text{O}]/[^{16}\text{O}])_{\text{sample}}/[^{18}\text{O}]/[^{16}\text{O}]_{\text{VSMOW}} - 1) \times 1000\text{‰}$ .

### <sup>15</sup>N-label incubation experiments

Potential N<sub>2</sub>O production rates in the presence or absence of dissolved Fe<sup>2+</sup> were determined in incubation experiments with <sup>15</sup>N-labeled substrates under different redox conditions (“Exp\_oxic <sup>15</sup>N<sub>2</sub>O” and “Exp\_anoxic <sup>15</sup>N<sub>2</sub>O”). Incubation conditions and added substrates are summarized in Supporting Information Table S1. For all abiotic treatments, lake water was filter-sterilized with 0.2- $\mu\text{m}$  cyclopore track-edged membrane filters (Whatman). For experiments with oxic water from 8 m depth, <sup>15</sup>N-tracers were added to a volume of 2 L in canisters (either 5  $\mu\text{M}$  <sup>15</sup>NO<sub>2</sub><sup>-</sup> at ambient NH<sub>4</sub><sup>+</sup> concentrations of  $\sim 40 \mu\text{M}$ , or 40  $\mu\text{M}$  <sup>15</sup>NH<sub>4</sub><sup>+</sup> + 5  $\mu\text{M}$  unlabeled NO<sub>2</sub><sup>-</sup>) from sterile 2–5 mM stock solutions (99% <sup>15</sup>N-NH<sub>4</sub>Cl or  $\geq 98\%$  <sup>15</sup>N-NaNO<sub>2</sub>; Cambridge Isotope Laboratories, Inc.). The water was distributed into 160-mL serum vials without a headspace and closed with butyl rubber stoppers and aluminum seals. For anoxic experiments with water from 14.5 m water depth, we purged the water in 2-L borosilicate bottles with He for 30 min before adding 5  $\mu\text{M}$  of the respective <sup>15</sup>N-tracer, and, in some of the treatments, 10  $\mu\text{M}$  dissolved Fe<sup>2+</sup>. At this sampling depth, no ambient Fe had been detected. We added <sup>15</sup>N-tracers from 2 mM stock solutions (99% <sup>15</sup>N-KNO<sub>3</sub>, or  $\geq 98\%$  <sup>15</sup>N-NaNO<sub>2</sub>; Cambridge Isotope Laboratories, Inc.), and unlabeled NO<sub>2</sub><sup>-</sup> and Fe<sup>2+</sup> as solutions of NaNO<sub>2</sub> and FeSO<sub>4</sub>, respectively. The water was anoxically transferred without leaving headspace into He-purged 160-mL serum vials already closed with butyl rubber stoppers and aluminum seals using an adjusted protocol from Holtappels et al. (2011). Both oxic and anoxic incubation bottles were incubated at  $\sim 16^\circ\text{C}$  in the dark. At each of three preassigned time points ( $\sim 12$ , 24, and 36 h), three bottles were sacrificed by introducing a 40-mL He headspace and terminating microbial activity, as well as potential chemodenitrification by adding 5 mL of 10 M NaOH, which precipitates ferrous iron and strongly decreases the reactivity of nitrite (Braidia and Ong 2000; Visser et al. 2020). One bottle was sacrificed directly after starting the experiment. The water samples were sterile-filtered and stored frozen. NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup> concentrations were measured as described above. The total N<sub>2</sub>O in each incubation bottle was analyzed for concentration and m/z ion ratios 45/44 and 46/44 as described for natural abundance stable isotope measurements of water column N<sub>2</sub>O samples. For highly <sup>15</sup>N-enriched N<sub>2</sub>O samples, we reduced the resistance on the m/z 46 detector from  $1 \times 10^{11} \Omega$  to  $1 \times 10^9 \Omega$ , to yield a 100-fold lower amplification of the <sup>15</sup>N<sup>15</sup>N<sup>16</sup>O signal. Some highly enriched <sup>15</sup>N samples were measured with a too high

resistance and therefore excluded from analysis. The raw data were corrected for linearity effects of the sample size/peak area on the 45/44 and 46/44 ratios through the analysis of N<sub>2</sub>O standards with different amounts of N<sub>2</sub>O. <sup>44</sup>N<sub>2</sub>O was determined as described above for the N<sub>2</sub>O concentration analysis of the in situ water samples. Masses <sup>45</sup>N<sub>2</sub>O and <sup>46</sup>N<sub>2</sub>O were then calculated based on the 46/44 and 45/44 ratios. Production rates of exogenous, endogenous, and total N<sub>2</sub>O were determined using equations described in Trimmer et al. (2016) (see Supporting Information Appendix S1). Exogenous N<sub>2</sub>O is produced entirely from the <sup>15</sup>N-labeled substrate added, considering the <sup>15</sup>N fraction in the substrate pool, while endogenous N<sub>2</sub>O mixes one N atom from the <sup>15</sup>N-labeled substrate and a second from another N-molecule in the incubation. Possible N isotope pairing outcomes for the oxic incubations are visualized in Supporting Information Fig. S1. The rates of N<sub>2</sub>O production were calculated based only on the linear increase in <sup>44</sup>N<sub>2</sub>O, <sup>45</sup>N<sub>2</sub>O, and <sup>46</sup>N<sub>2</sub>O, to exclude plateauing N species concentrations due to a terminated abiotic reaction and artifacts due to bottle effects mostly towards the end of the incubations. We estimated the detection limit as 1.5-times the standard error (SE) of the rates. The consumption of the <sup>15</sup>N-tracers was calculated based on the temporal change of the respective N substrate concentration, multiplied with the <sup>15</sup>N atom fraction, using the same timepoints as for the N<sub>2</sub>O rate determination. NO<sub>2</sub><sup>-</sup> production rates in the NO<sub>3</sub><sup>-</sup>-amended treatments were calculated analogously. A *t*-test was performed to test whether the rates were significant. N<sub>2</sub>O yields were calculated as (i) as the ratio of the <sup>15</sup>N-tracer consumption rate vs. the total N<sub>2</sub>O production rate and (ii) as the ratio of produced N<sub>2</sub>O vs. produced N<sub>2</sub> (see below) in the anoxic incubations. The production of <sup>15</sup>NH<sub>4</sub><sup>+</sup> was determined via chemical conversion to <sup>15</sup>N<sub>2</sub> using hypobromite (Risgaard-Petersen et al. 1995) and subsequent analysis of <sup>15</sup>N-N<sub>2</sub> by IRMS as described in Robertson et al. (2016). Parallel <sup>15</sup>N-label incubation experiments were conducted in 2017 to determine N<sub>2</sub> production rates (“Exp <sup>15</sup>N<sub>2</sub>”), using an adjusted protocol described in Wenk et al. (2016) (see Supporting Information Appendix S2; Table S2).

### Abiotic experiment with NO<sub>2</sub><sup>-</sup> and Fe<sup>2+</sup>

Concentration and isotope dynamics of chemodenitrification were determined experimentally (“Exp\_chemo”). Lake water from 14.5 m depth was filtered through 0.2- $\mu\text{m}$  membrane filters (Whatman), filled into sterile 20-mL serum vials, and closed airtight with rubber stoppers. The water was purged for 10 min, leaving a He headspace, before amending each vial with NO<sub>2</sub><sup>-</sup> and subsequently with Fe<sup>2+</sup> from sterile and anoxic stock solutions of NaNO<sub>2</sub> and FeSO<sub>4</sub>, respectively, in exchange with the headspace volume (i.e., leaving no headspace). The pH during the experiment was  $9.34 \pm 0.14$ . Target concentrations were 100  $\mu\text{M}$  NO<sub>2</sub><sup>-</sup> and 200  $\mu\text{M}$  Fe<sup>2+</sup>. At each of the seven time points between 0 and 48 h ( $t_0$ – $t_6$ ), three samples were sacrificed by taking 8 mL of the sample solution, replacing it

with He, and adding 0.5 mL 10 M NaOH. From the 8 mL sample, aliquots for NO<sub>2</sub><sup>-</sup> concentration measurements were fixed and analyzed as described above and samples for Fe<sup>2+</sup> were fixed in sulfamic acid (40 mM final concentration) and analyzed using the ferrozine assay (Stookey 1970; Klueglein and Kappler 2013). Aliquots for NO<sub>2</sub><sup>-</sup> isotopes were filtered through 0.2- $\mu$ m syringe filters, and frozen immediately at -80°C. After thawing, the samples were analyzed with the azide method (*see above*). Using the serum vials, we determined the N<sub>2</sub>O concentrations and isotope ratios as described above for the water column N<sub>2</sub>O isotope analyses.

## Results

### Geochemical conditions in Lake La Cruz

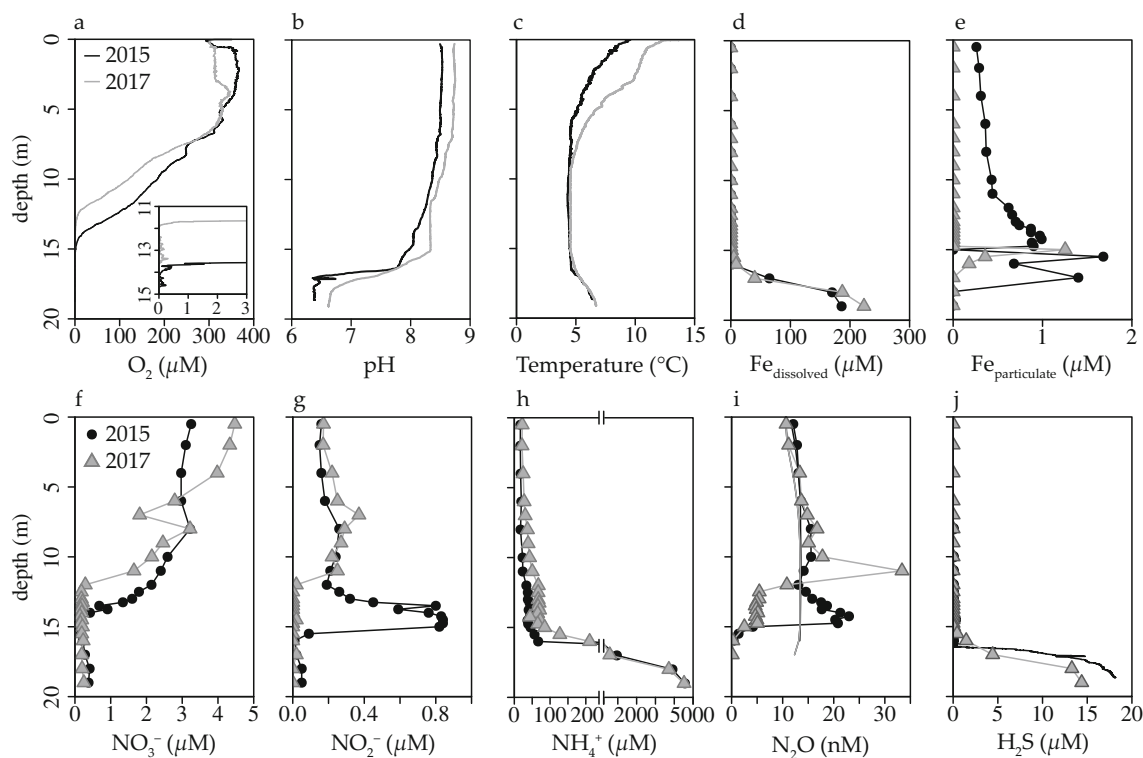
The water column of Lake La Cruz during our sampling campaigns can be divided into the oxic water column, the anoxic hypolimnion, and the deep permanently anoxic monimolimnion, where the RTZ consists of the lower oxic water column (<5  $\mu$ M O<sub>2</sub>) and the upper anoxic hypolimnion (Supporting Information Fig. S2). The oxic water column includes the photosynthetically most active zone, at 7–8 m, as indicated by a local Chl *a* maximum (Supporting Information Fig. S3b). O<sub>2</sub> was completely consumed at ~14 m in 2015 and at ~12 m in 2017 (Fig. 2a). Strong changes

in pH from ~8.5 (2015) and ~8.7 (2017) in the oxic water column, to ~6.3 (2015) and ~6.6 (2017) below ~15.5–17 m water depth mark the chemocline that separates the anoxic hypolimnion from the monimolimnion underneath (Fig. 2b). Paralleling the pH profile, conductivity increased from ~400  $\mu$ S cm<sup>-1</sup> in the chemocline to >1000  $\mu$ S cm<sup>-1</sup> in the bottom waters (Supporting Information Fig. S3c).

Below the RTZ, total Fe was dominated by dissolved Fe, with maximum concentrations of ~300  $\mu$ M in near-bottom waters (Fig. 2d). Particulate Fe concentrations were highest at the RTZ and in the anoxic water, but at comparatively low concentrations < 2  $\mu$ mol L<sup>-1</sup> (Fig. 2e; Supporting Information Fig. S2). H<sub>2</sub>S accumulated in the deep anoxic water column below 15 m with up to ~20  $\mu$ M (Fig. 2j), whereas SO<sub>4</sub><sup>2-</sup> decreased to < 10  $\mu$ M in the monimolimnion (Supporting Information Fig. S3f).

### N-species concentrations in the water column

The most abundant inorganic N compound throughout the water column was NH<sub>4</sub><sup>+</sup>, with concentrations increasing from ~20  $\mu$ M in the oxic epilimnion to almost 5 mM in the monimolimnion (Fig. 2h). NO<sub>3</sub><sup>-</sup> concentrations fluctuated around 3  $\mu$ M (2015) and 5  $\mu$ M (2017) in the oxic water, with a minor concentration peak at 8 m in both years, and decreased



**Fig. 2.** Physicochemical profiles from March 2015 and March 2017 in Lake La Cruz. (a) O<sub>2</sub> and trace O<sub>2</sub> (insert), (b) pH, (c) temperature, (d) dissolved Fe concentration, (e) particulate Fe concentration, (f) NO<sub>3</sub><sup>-</sup> concentration, (g) NO<sub>2</sub><sup>-</sup> concentration, (h) NH<sub>4</sub><sup>+</sup> concentration, (i) N<sub>2</sub>O concentration, and (j) H<sub>2</sub>S concentration. Water column profile data of O<sub>2</sub>, pH, temperature, Fe (particulate), NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup> from 2015 have been published previously in Oswald et al. (2016).

sharply at the RTZ to values below 0.5  $\mu\text{M}$  (Fig. 2f; Supporting Information Fig. S2). NO<sub>2</sub><sup>-</sup> was present in the epilimnion at low concentrations of  $\sim 0.2\text{--}0.4\ \mu\text{M}$ , with a subtle peak at 7–10 m in both years, but only in 2015 with a clear nitrite maximum (0.8  $\mu\text{M}$ ) in the RTZ (Fig. 2g; Supporting Information Fig. S2). The N<sub>2</sub>O concentration profiles revealed a subtle N<sub>2</sub>O oversaturation of  $\sim 2\ \text{nM}$  above equilibrium concentration in a discernible water layer at 8–10 m (up to 13.6 nM in 2015, and up to 16.7 nM in 2017, Fig. 2i). N<sub>2</sub>O concentrations were highest at 12–14.5 m (23.0 nM), near the depth of O<sub>2</sub> depletion, in 2015. In 2017, the N<sub>2</sub>O maximum (33.4 nM) was somewhat higher in the water column, at 11 m, where O<sub>2</sub> was still  $\sim 40\ \mu\text{M}$  (Fig. 2i; Supporting Information Fig. S2). Below 16 m, N<sub>2</sub>O concentrations were below the detection limit.

### Isotopic compositions of N-species in the water column

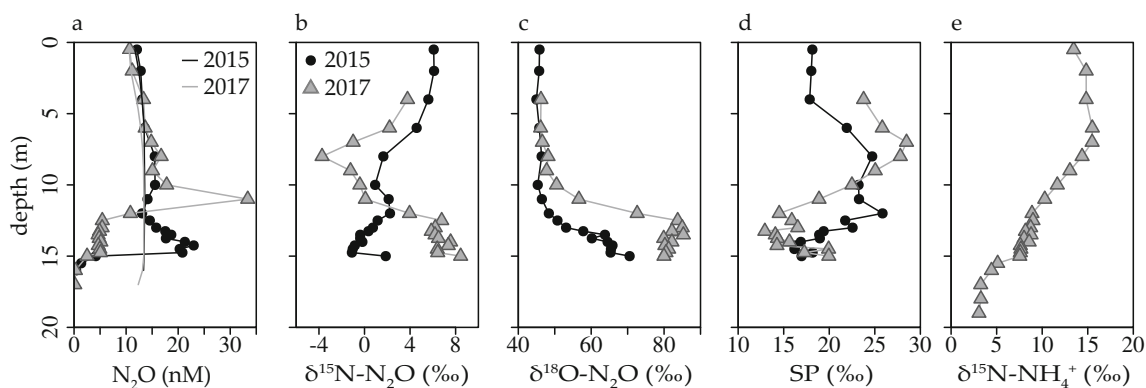
The  $\delta^{15}\text{N}$  values of N<sub>2</sub>O in the surface water were close to those of atmospheric N<sub>2</sub>O. With increasing N<sub>2</sub>O oversaturation, at  $\sim 8\text{--}10\ \text{m}$ , the  $\delta^{15}\text{N}\text{-N}_2\text{O}$  values decreased (Fig. 3b). In 2015, the increasing N<sub>2</sub>O concentrations between 12.0 and 15.5 m were accompanied by decreasing  $\delta^{15}\text{N}\text{-N}_2\text{O}$  values from 2.2 to  $-1.1\text{‰}$  and by increasing  $\delta^{15}\text{N}\text{-NO}_x$  values from  $\sim 8$  to  $\sim 22\text{‰}$  (Supporting Information Fig. S4a), resulting in a  $\Delta\delta^{15}\text{N}_{\text{NO}_x\rightarrow\text{N}_2\text{O}}$  value of  $\sim 23\text{‰}$  at the depth of maximum N<sub>2</sub>O concentration. In 2017, the  $\delta^{15}\text{N}\text{-N}_2\text{O}$  values increased slightly towards the concentration peak, from  $-3.2$  to  $-0.8\text{‰}$ . Further below, they continued to increase to final values of 6–8‰ (Fig. 3b).  $\delta^{15}\text{N}\text{-NH}_4^+$  values (measured only in 2017) were highest in the oxic water column at 6–7 m with 15–16‰ and decreased with increasing NH<sub>4</sub><sup>+</sup> concentrations through the RTZ to a robust value of 3‰ in the bottom waters (Fig. 3e).  $\delta^{18}\text{O}\text{-N}_2\text{O}$  values were more or less invariant at  $\sim 45\text{‰}$  within the oxic epilimnion, close to atmospheric N<sub>2</sub>O. They increased with increasing depth and plateaued at  $\sim 65\text{‰}$  (2015) and at  $\sim 85\text{‰}$  (2017) in the RTZ (Fig. 3c). The  $\delta^{18}\text{O}\text{-NO}_3^-$  values in the oxic water column ranged mostly between 16 and 20‰ (Supporting Information Fig. S4c) with

a  $\Delta\delta^{18}\text{O}_{\text{NO}_3\rightarrow\text{N}_2\text{O}}$  value of  $-37\text{‰}$  at the depth of maximum N<sub>2</sub>O concentration. N<sub>2</sub>O SP values increased from atmospheric values in the surface waters to 24.7‰ in 2015 and to 28.5‰ in 2017 at the layer of oxic N<sub>2</sub>O oversaturation (7–8 m; Fig. 3d). With the drop in N<sub>2</sub>O concentrations at  $\sim 15\ \text{m}$  depth in 2015, N<sub>2</sub>O SP values increased again. In 2017, the SP systematically decreased between the oxic N<sub>2</sub>O maximum and the second N<sub>2</sub>O maximum to a value of 13.0‰, and then increased again to 20.0‰ with decreasing N<sub>2</sub>O concentrations (Fig. 3d).

### N<sub>2</sub>O production and reduction potential

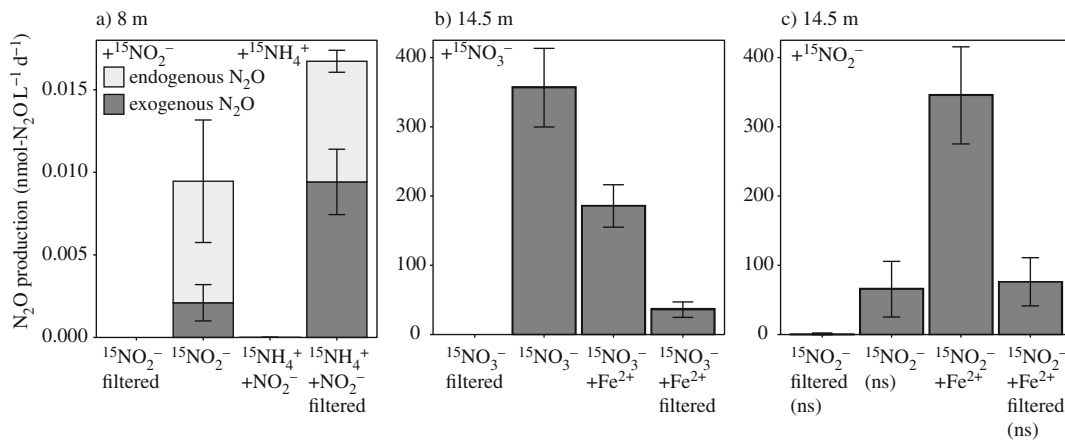
The results from the <sup>15</sup>N-N<sub>2</sub>O production experiments (Exp\_oxic <sup>15</sup>N<sub>2</sub>O and Exp\_anoxic <sup>15</sup>N<sub>2</sub>O) are shown in Fig. 4 and Supporting Information Table S3. In the oxic unfiltered, that is, microbially active, incubation experiments, N<sub>2</sub>O production rates were relatively low but significant. The addition of <sup>15</sup>NH<sub>4</sub><sup>+</sup> and non-labeled NO<sub>2</sub><sup>-</sup> led to higher total N<sub>2</sub>O production rates ( $16.7 \pm 2.1 \times 10^{-3}\ \text{nmol-N}_2\text{O L}^{-1}\ \text{d}^{-1}$ ,  $p < 0.001$ ,  $n = 10$ ) than the sole addition of <sup>15</sup>NO<sub>2</sub><sup>-</sup> ( $9.5 \pm 3.9 \times 10^{-3}\ \text{nmol-N}_2\text{O L}^{-1}\ \text{d}^{-1}$ ,  $p < 0.05$ ,  $n = 10$ ). The percentage of endogenous (above-expected mass 45) N<sub>2</sub>O production (Fig. 4a) was lower in the <sup>15</sup>NH<sub>4</sub><sup>+</sup> treatment (44%) than in the <sup>15</sup>NO<sub>2</sub><sup>-</sup> treatment (78%).

N<sub>2</sub>O production in the anoxic incubations was several orders of magnitude higher than the oxic N<sub>2</sub>O production rates. In the experiments with <sup>15</sup>NO<sub>3</sub><sup>-</sup> (Fig. 4b), the rates of <sup>15</sup>N transformation were highest in the <sup>15</sup>NO<sub>3</sub><sup>-</sup>-only treatment ( $356.5 \pm 56.8\ \text{nmol-N}_2\text{O L}^{-1}\ \text{d}^{-1}$ ,  $p < 0.01$ ,  $n = 4$ ). In the <sup>15</sup>NO<sub>3</sub><sup>-</sup> + Fe<sup>2+</sup> treatment, the rate was significantly lower ( $185.7 \pm 30.7\ \text{nmol-N}_2\text{O L}^{-1}\ \text{d}^{-1}$ ,  $p < 0.001$ ,  $n = 9$ ). In the filtered <sup>15</sup>NO<sub>3</sub><sup>-</sup> + Fe<sup>2+</sup> treatment, N<sub>2</sub>O production was significant ( $35.9 \pm 11.2\ \text{nmol-N}_2\text{O L}^{-1}\ \text{d}^{-1}$ ,  $p < 0.01$ ,  $n = 8$ ) compared to the control without Fe<sup>2+</sup> (rate below detection limit). In both unfiltered incubations with <sup>15</sup>NO<sub>3</sub><sup>-</sup>, some N<sub>2</sub>O was observed to be consumed toward the last timepoint ( $\sim 1.5\ \text{d}$ ), at least in some of the bottles (Supporting Information Fig. S5e). In the <sup>15</sup>NO<sub>2</sub><sup>-</sup>-amended experiments (Fig. 4c), N<sub>2</sub>O production was



**Fig. 3.** N<sub>2</sub>O concentration and N-species isotope profiles from March 2015 and March 2017 in Lake La Cruz. (a) N<sub>2</sub>O concentration, the lines indicate the N<sub>2</sub>O concentration expected for atmospheric equilibration, (b)  $\delta^{15}\text{N}\text{-N}_2\text{O}$ , (c)  $\delta^{18}\text{O}\text{-N}_2\text{O}$ , (d) N<sub>2</sub>O SP, and (e)  $\delta^{15}\text{N}\text{-NH}_4^+$ .

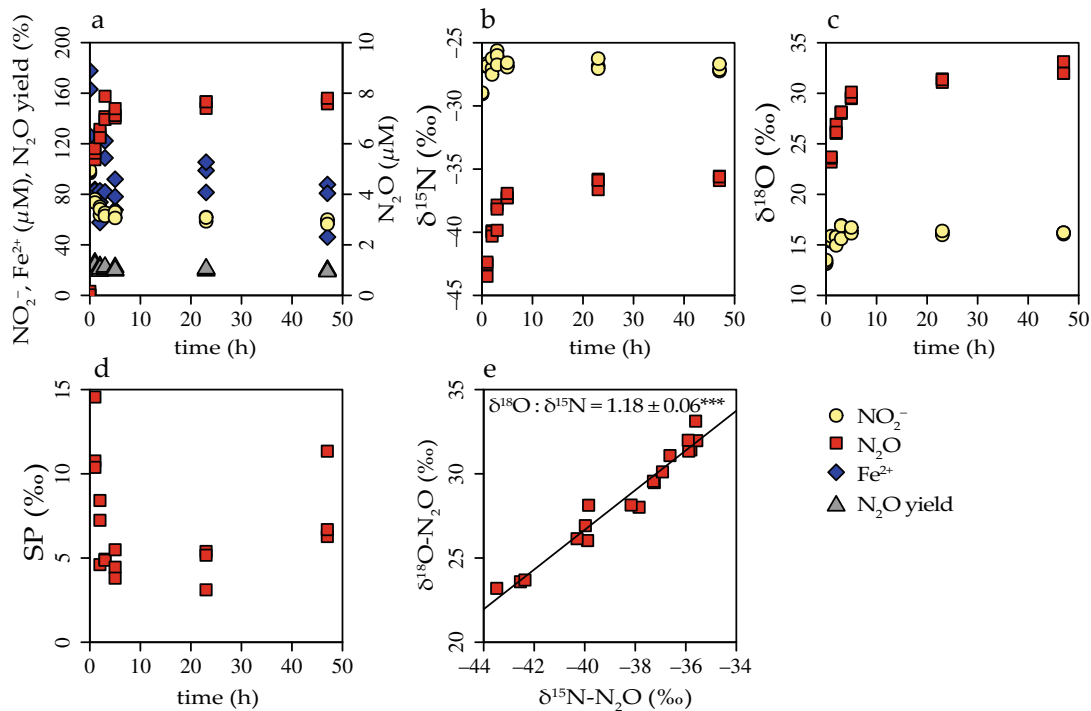




**Fig. 4.** N<sub>2</sub>O production rates in incubation experiments performed with <sup>15</sup>N-labeled substrates in March 2017. **(a)** Oxidic incubations in 8 m depth with <sup>15</sup>NO<sub>2</sub><sup>-</sup> and <sup>15</sup>NH<sub>4</sub><sup>+</sup> tracer (Exp<sub>oxic</sub> <sup>15</sup>N<sub>2</sub>O). **(b)** Anoxic incubations in 14.5 m depth with <sup>15</sup>NO<sub>3</sub><sup>-</sup> tracer, and **(c)** anoxic incubations in 14.5 m depth with <sup>15</sup>NO<sub>2</sub><sup>-</sup> tracer (Exp<sub>oxic</sub> <sup>15</sup>N<sub>2</sub>O). Non-significant production slopes above detection limit are indicated by “ns.” Error bars indicate standard error of replicate means of the total N<sub>2</sub>O production. Exogenous N<sub>2</sub>O is produced solely via the <sup>15</sup>N-labeled substrate, taking into account the <sup>15</sup>N-fraction in the substrate pool. Endogenous N<sub>2</sub>O originates from one <sup>15</sup>N-tracer molecule and one ambient <sup>14</sup>N-molecule of a different substrate, hence it can help to distinguish between N<sub>2</sub>O production pathways. For details, see “Methods” section and Supporting Information Fig. S1. Note the different scale in **(a)**.

highest when Fe<sup>2+</sup> was added to the unfiltered sample (345.4 ± 70.1 nmol-N<sub>2</sub>O L<sup>-1</sup> d<sup>-1</sup>, *p* < 0.01, *n* = 6). N<sub>2</sub>O production was similar in the unfiltered <sup>15</sup>NO<sub>2</sub><sup>-</sup>-only treatment (65.5 ± 40.2 nmol-N<sub>2</sub>O L<sup>-1</sup> d<sup>-1</sup>, *p* = 0.082, *n* = 6) and in the filtered treatment with <sup>15</sup>NO<sub>2</sub><sup>-</sup> + Fe<sup>2+</sup> (76.2 ± 34.8 nmol-N<sub>2</sub>O L<sup>-1</sup> d<sup>-1</sup>, *p* = 0.080, *n* = 3). In the abiotic incubations with

added Fe<sup>2+</sup>, we neither observed a production of <sup>15</sup>N-NH<sub>4</sub><sup>+</sup> (Exp<sub>anoxic</sub> <sup>15</sup>N<sub>2</sub>O; Supporting Information Table S3) nor of <sup>30</sup>N<sub>2</sub> and <sup>29</sup>N<sub>2</sub> (Exp <sup>15</sup>N<sub>2</sub>; Supporting Information Table S2). N<sub>2</sub>O vs. N<sub>2</sub> production ratios in the unfiltered incubations from Exp<sub>anoxic</sub> <sup>15</sup>N<sub>2</sub>O and Exp <sup>15</sup>N<sub>2</sub>, respectively, were up to ~250% (Supporting Information Table S2).



**Fig. 5.** Concentration and isotope dynamics during the abiotic reduction of NO<sub>2</sub><sup>-</sup> with Fe<sup>2+</sup> (Exp<sub>chemo</sub>). **(a)** Concentrations of NO<sub>2</sub><sup>-</sup> and Fe<sup>2+</sup>, **(b)** δ<sup>15</sup>N of NO<sub>2</sub><sup>-</sup> and product N<sub>2</sub>O, **(c)** δ<sup>18</sup>O of NO<sub>2</sub><sup>-</sup> and product N<sub>2</sub>O, **(d)** site preference (SP) of product N<sub>2</sub>O, and **(e)** δ<sup>18</sup>O vs. δ<sup>15</sup>N of N<sub>2</sub>O.

### NO<sub>2</sub><sup>-</sup> and N<sub>2</sub>O isotope dynamics of chemodenitrification in incubation experiments

The NO<sub>2</sub><sup>-</sup> and N<sub>2</sub>O concentration and isotope dynamics during the incubation with unlabeled NO<sub>2</sub><sup>-</sup> (Exp\_chemo) are summarized in Fig. 5 and Supporting Information Fig. S6. During the reduction of NO<sub>2</sub><sup>-</sup> coupled to the oxidation of Fe<sup>2+</sup>, NO<sub>2</sub><sup>-</sup> decreased from ~100 to ~60 μM, and Fe<sup>2+</sup> from ~155 to ~75 μM within the first 5 h after adding the substrates. Hence, NO<sub>2</sub><sup>-</sup> and Fe<sup>2+</sup> decreased with a stoichiometric ratio of ~1 : 2.2, which is close to the expected ratio of 1 : 2 for chemodenitrification reactions with N<sub>2</sub>O as only N-product, but somewhat higher than the ratio of 1 : 1.4 when assuming that 75% of the NO<sub>2</sub><sup>-</sup> is transformed into NO and only 25% into N<sub>2</sub>O (see equations in Supporting Information Table S4). N<sub>2</sub>O reached maximum concentrations of 7.7 μM (Fig. 5a). The molar N<sub>2</sub>O yields, that is, the N<sub>2</sub>O produced relative to the NO<sub>2</sub><sup>-</sup> consumed, were 20% to 25% throughout the experimental period. After ~3 h, the reaction slowed down and finally stalled, although none of the substrates were consumed to completion. The δ<sup>15</sup>N-N<sub>2</sub>O values increased from -42.8 initially to -35.7‰ at the end of the incubation (Fig. 5b), as expected for closed-system dynamics. The δ<sup>18</sup>O-N<sub>2</sub>O values increased in parallel with the δ<sup>15</sup>N-N<sub>2</sub>O from 23.5 to 32.4‰ (Fig. 5c). The O : N isotope enrichment ratio for N<sub>2</sub>O was 1.18 ± 0.06 SE (Fig. 5e). During the reactive part of the abiotic experiment, N<sub>2</sub>O SP values decreased, starting at t<sub>1</sub> (N<sub>2</sub>O concentrations at t<sub>0</sub> were too low for N<sub>2</sub>O isotope and SP analysis), from 11.9 to 4.5‰, before rising to an average endpoint value of 8.1‰. The overall average SP was 6.9 ± 3.1‰ (Fig. 5d). The δ<sup>15</sup>N and δ<sup>18</sup>O values of NO<sub>2</sub><sup>-</sup> increased within the first 5 h from -29.0 to -26.8‰ and from 13.3 to 16.2‰, respectively, and then stabilized at this value (Fig. 5b,c). Isotope effects of nitrite are presented and discussed in Supporting Information Appendix S4.

## Discussion

### Biogeochemical processes in the water column

In the oxic water column, at 7–8 m, the photosynthetically most active zone coincided with elevated NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations, indicating that photosynthesis as well as ammonium and nitrite oxidation occur in close vicinity. In the RTZ, decreasing nitrate concentrations, and accumulating nitrite in 2015, indicate active denitrification or dissimilatory nitrate reduction to ammonium.

The generally low sulfate load in Lake La Cruz explains the high concentrations of dissolved Fe in the monimolimnion, because only minor FeS precipitation takes place, exporting iron to the sediment (Rodrigo et al. 2001; Walter et al. 2014). Clear evidence for microbial Fe(II) oxidation coupled to nitrate reduction in the summer has been reported by Walter (2011). The results from our samplings in spring show dissolved Fe concentrations declining to zero-level still below the depth of maximum NO<sub>3</sub><sup>-</sup> consumption, suggesting that Fe is not the

prime reductant for NO<sub>3</sub><sup>-</sup>. Fe oxidation can also be coupled to biotic or abiotic nitrite reduction (i.e., nitrite chemodenitrification). At least in March 2015, the dissolved Fe gradient seems to be associated with the nitrite gradient at the bottom of the secondary nitrite maximum. Yet, the relative concentrations of dissolved Fe in the monimolimnion (several hundreds of μM) vs. those of nitrite within the nitrite maximum (< 1 μM), require that, if at all, chemodenitrification is fueled by a cryptic nitrite (and potentially nitrate) cycle, that is, the close coupling of the production of nitrite by nitrate-reducing bacteria and consumption by Fe-dependent nitrite reduction (see below), without great net accumulation of nitrite.

### N<sub>2</sub>O production in the oxic water column

A zone of active enzymatic and/or non-enzymatic N<sub>2</sub>O production associated to nitrification was identified at ~8–10 m depths, where N<sub>2</sub>O concentrations were ~2 nM above atmospheric equilibrium. The depletion in <sup>15</sup>N in N<sub>2</sub>O, the concomitant enrichment of <sup>15</sup>N in the substrate NH<sub>4</sub><sup>+</sup> pool, as well as the increase in N<sub>2</sub>O SP to 25–27‰ are strong indicators for nitrification. On the other hand, the isotopic offset between substrate NH<sub>4</sub><sup>+</sup> and product N<sub>2</sub>O (Δδ<sup>15</sup>N<sub>NH4+→N2O</sub> ≈ 18‰) did not allow us to identify N<sub>2</sub>O production pathways, since the observed value fell between the expected values for nitrification and nitrifier denitrification (Frame and Casciotti 2010).

The oxic <sup>15</sup>N-tracer incubation experiments provided some evidence for the activity of nitrifier denitrification, especially in the <sup>15</sup>NO<sub>2</sub><sup>-</sup> tracer incubations, where 22% of produced N<sub>2</sub>O was derived from two <sup>15</sup>N-tracer molecules. Furthermore, the production of endogenous N<sub>2</sub>O in the incubations with added <sup>15</sup>NH<sub>4</sub><sup>+</sup> indicates hybrid N<sub>2</sub>O formation and other non-enzymatic or enzymatic N<sub>2</sub>O production pathways. A detailed discussion of the isotopic constraints on N<sub>2</sub>O production in the oxic water column is available in Supporting Information Appendix S3.

### Identifying the dominant N<sub>2</sub>O production pathways in the RTZ

We argue that the N<sub>2</sub>O concentration peaks at the depths of O<sub>2</sub> depletion mainly had a biological origin, despite the potential for chemodenitrification demonstrated in the abiotic incubation experiments (Exp\_anoxic <sup>15</sup>N<sub>2</sub>O and Exp\_chemo, see discussion below). Denitrification appears as the most likely N<sub>2</sub>O source in 2015, as indicated by the consumption of NO<sub>3</sub><sup>-</sup> just below the depth of O<sub>2</sub> depletion, concurrent with the production of NO<sub>2</sub><sup>-</sup> and N<sub>2</sub>O, while, to some extent, nitrifier denitrification might contribute. The biological production of up to ~500 nmol <sup>30</sup>N<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup> in unfiltered incubations with amended <sup>15</sup>NO<sub>x</sub> (Exp <sup>15</sup>N<sub>2</sub>) confirms the activity of denitrifying microorganisms. In 2017, the highest N<sub>2</sub>O concentration was observed above the RTZ at ~110 μM O<sub>2</sub> (11 m), suggesting that at that time nitrifier denitrification was the

prime N<sub>2</sub>O source, as it tolerates higher O<sub>2</sub> concentrations than canonical denitrification (Zhu et al. 2013; Babbitt et al. 2014). Furthermore, a still relatively high NO<sub>3</sub><sup>-</sup> concentration of 1.7 μM at 11 m indicates that the depth of nitrate consumption via denitrification was below the N<sub>2</sub>O peak.

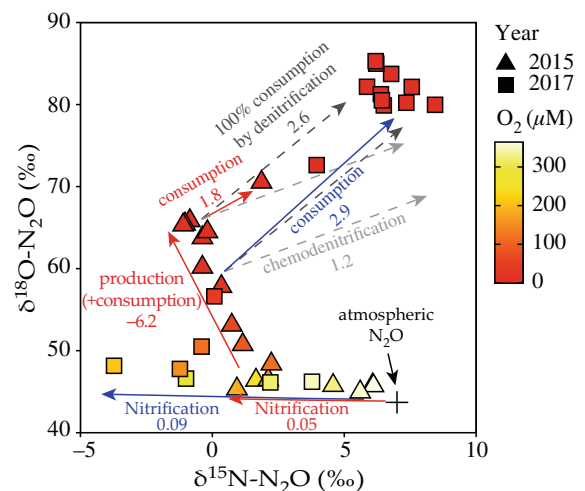
The N<sub>2</sub>O concentration peak in 2015 was accompanied by N isotope effects between the substrate NO<sub>x</sub> and the produced N<sub>2</sub>O (Δδ<sup>15</sup>N<sub>NO<sub>x</sub>→N<sub>2</sub>O</sub> of 23‰) in the range of previously reported isotope offsets for denitrification (Δδ<sup>15</sup>N<sub>NO<sub>3</sub>→N<sub>2</sub>O</sub> = 10–39‰, Fig. 1) and nitrifier denitrification (Δδ<sup>15</sup>N<sub>NO<sub>2</sub>→N<sub>2</sub>O</sub> = 24–35‰, Fig. 1). For δ<sup>18</sup>O, the observed Δδ<sup>18</sup>O<sub>NO<sub>3</sub>→N<sub>2</sub>O</sub> isotope effect of -37‰ in 2017 was at the lower end of the range of values reported for denitrification-derived N<sub>2</sub>O (Δδ<sup>18</sup>O = -40 to -4‰; Fig. 1). The negative isotopic offset for oxygen is due to O-isotope branching, that is, the selective release of <sup>16</sup>O during N<sub>2</sub>O formation and the preferential remaining of <sup>18</sup>O within the N<sub>2</sub>O bond. A contribution to N<sub>2</sub>O production by canonical denitrification and/or nitrifier denitrification is also indicated by the decreasing N<sub>2</sub>O SP values to ~16‰ (2015) and 13‰ (2017), respectively, at the depth of maximum N<sub>2</sub>O accumulation. Still, the intermediate SP values (i.e., between the endmember values of nitrification and denitrification) suggest that nitrification-associated N<sub>2</sub>O production still plays a role with regards to the RTZ SP signature. Increasing SP values in the deepest, anoxic N<sub>2</sub>O samples to 20‰ (2017) indicate partial N<sub>2</sub>O reduction. Chemodenitrification with an SP between 5 and 12‰ (Exp\_chemo) could have an impact on the observed intermediate SP values but, as we argue below mainly based on the dissolved Fe and NO<sub>2</sub><sup>-</sup> concentration profiles, its contribution in the RTZ is likely negligible.

Interestingly, in other O<sub>2</sub>-deficient environments, N<sub>2</sub>O concentration peaks were typically associated with high SP values, for example, in the Black Sea with values up to 46.5‰ (Westley et al. 2006), in Lake Lugano North Basin with up to 25.7‰, and in Lake Lugano South Basin with up to 42.3‰ (Wenk et al. 2016). There, denitrification was assumed to be important, and the high SP was seen as a result of overprinting isotope effects by nitrification or N<sub>2</sub>O reduction. The relatively low SP values in the RTZ of Lake La Cruz may result from a sluggish transfer of high-SP N<sub>2</sub>O from the oxic water column compared to other lakes, potentially due to high water column stability, and a lack of effective mixing between the oxic N<sub>2</sub>O maximum and the N<sub>2</sub>O peak at the oxic-anoxic interface.

A high N<sub>2</sub>O production potential by denitrification was confirmed in the <sup>15</sup>N-label incubation experiments at 14.5 m with <sup>15</sup>NO<sub>3</sub><sup>-</sup>, where 356.5 nmol-N<sub>2</sub>O L<sup>-1</sup> d<sup>-1</sup> was produced with a parallel accumulation of NO<sub>2</sub><sup>-</sup> (Exp\_anoxic <sup>15</sup>N<sub>2</sub>O). Yields of produced N<sub>2</sub>O vs. produced N<sub>2</sub> of 13% to 249% show that in some of the biologically active incubations, the accumulation of N<sub>2</sub>O can exceed the production of N<sub>2</sub>. We point out that high N<sub>2</sub>O : N<sub>2</sub> yields are uncommon in freshwater (e.g., in a reservoir, in situ N<sub>2</sub>O : N<sub>2</sub> ratios were much lower,

< 20%; Beaulieu et al. 2014). Yet, the accumulation of N<sub>2</sub>O from incomplete denitrification was likely only temporary, as indicated by the reduction of N<sub>2</sub>O at the last timepoint in the <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> treatment.

The slopes of δ<sup>18</sup>O-vs.-δ<sup>15</sup>N-N<sub>2</sub>O plots in the RTZ represent yet another useful tool to assign water layers in the RTZ, where either N<sub>2</sub>O production or N<sub>2</sub>O reduction is dominant, allowing us to further corroborate the different N<sub>2</sub>O production/consumption niches in the water column. We can pinpoint a zone of dominant N<sub>2</sub>O production via denitrification between 12 and 15 m in 2015, where we observed a negative N<sub>2</sub>O Δδ<sup>18</sup>O : Δδ<sup>15</sup>N slope of -6.2 (Fig. 6). Within the RTZ, the δ<sup>15</sup>N-N<sub>2</sub>O values decrease simultaneously with increasing N<sub>2</sub>O concentrations and decreasing NO<sub>x</sub> concentrations (i.e., complete consumption at ~15.5 m), and NO<sub>x</sub> becomes enriched in <sup>15</sup>N with continuous substrate consumption, as expected for denitrification. Contrarily, δ<sup>18</sup>O-N<sub>2</sub>O values increase as a consequence of Rayleigh distillation and the branching <sup>18</sup>O isotope effect during N<sub>2</sub>O production. As to what extent the observed δ<sup>18</sup>O : δ<sup>15</sup>N slope in N<sub>2</sub>O also reflects N<sub>2</sub>O reduction is difficult to say but the net decreasing δ<sup>15</sup>N values indicate the dominance of N<sub>2</sub>O production over N<sub>2</sub>O reduction. A stronger contribution from N<sub>2</sub>O reduction would be expected to drive the δ<sup>15</sup>N-N<sub>2</sub>O to higher values (Ostrom et al. 2007; Yamagishi et al. 2007). In 2017, the Δδ<sup>18</sup>O : Δδ<sup>15</sup>N slope between 10 and 12 m, where N<sub>2</sub>O concentrations reach a maximum, is less conclusive with regards to identifying a specific water layer where N<sub>2</sub>O production is dominating, as our



**Fig. 6.** Dual N- and O-isotope signatures of N<sub>2</sub>O in the water column of Lake La Cruz. The solid-line arrows represent measured Δδ<sup>18</sup>O : Δδ<sup>15</sup>N trends, and the interpretation of these trends in the context of specific N<sub>2</sub>O producing/consuming processes in 2015 (red) or 2017 (blue). The dashed-line arrows including numbers indicate experimentally determined δ<sup>18</sup>O : δ<sup>15</sup>N slopes of isolated processes: 100% consumption via denitrification (dark gray; Ostrom and Ostrom 2012) and chemodenitrification (light gray, this study).

sampling scheme did not cover these depths in detail. In both years, the  $\Delta\delta^{18}\text{O} : \Delta\delta^{15}\text{N}$  trends (1.8 in 2015 and 2.9 in 2017) associated with decreasing N<sub>2</sub>O concentrations (15–15.5 m in 2015 and 11–13 m in 2017) indicate that N<sub>2</sub>O reduction dominates the N<sub>2</sub>O isotopic signature, with little contribution from simultaneous gross N<sub>2</sub>O production. That is, these ratios are relatively close to the theoretical value of pure N<sub>2</sub>O reduction ( $\delta^{18}\text{O} : \delta^{15}\text{N} \approx 2.6$ ; Yamagishi et al. 2007; Ostrom and Ostrom 2012). The difference between the 2 years may possibly be attributed to the varying impact of chemodenitrification in these water layers (see discussion below). We highlight that the analysis of  $\delta^{18}\text{O}$ -vs.- $\delta^{15}\text{N}$ -N<sub>2</sub>O plots could be especially helpful in water columns, where N<sub>2</sub>O concentration peaks do not correspond to  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  extrema of N<sub>2</sub>O, and hence, the identification of N<sub>2</sub>O production and reduction processes is more difficult.

### Chemodenitrification and associated isotope effects

#### Evidence for potential chemodenitrification

Fe-amended <sup>15</sup>N-incubation experiments with water from 14.5 m water depth show a marked potential for chemodenitrification (Exp\_anoxic <sup>15</sup>N<sub>2</sub>O), suggesting that it could contribute to total N<sub>2</sub>O production in Lake La Cruz. For the first time, we provide evidence of chemodenitrification in experiments conducted with natural lake water, as opposed to previous studies investigating chemodenitrification in an artificial matrix (Jones et al. 2015; Buchwald et al. 2016). Thermodynamic calculations confirm that the reaction of Fe<sup>2+</sup> and NO<sub>2</sub><sup>-</sup> is not only favorable under the conditions in the incubation experiments but also under in situ conditions, even at nanomolar substrate concentrations (Supporting Information Tables S4, S5). In the incubations, N<sub>2</sub>O production stalled within 12 h, even though both NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> and Fe<sup>2+</sup> were still present. We speculate that the reaction terminated due to accumulation of the intermediate NO (Kampschreur et al. 2011), inhibiting the reduction of NO<sub>2</sub><sup>-</sup>. NO reduction to N<sub>2</sub>O, in turn, depends on the dynamics of gas equilibrium and can be impeded by the accumulation of N<sub>2</sub>O (Visser et al. 2020).

In the unfiltered <sup>15</sup>NO<sub>2</sub><sup>-</sup> incubation experiments with Fe<sup>2+</sup>, we observed a relatively high N<sub>2</sub>O production rate, whereas in both the filtered NO<sub>2</sub><sup>-</sup> + Fe<sup>2+</sup> (no cells and particles) experiment and in the unfiltered NO<sub>2</sub><sup>-</sup>-only (biological denitrification) experiment N<sub>2</sub>O production was just above detection limit, suggesting that reactive surfaces of bacterial or other cells and/or particulate iron catalyze the reaction of Fe<sup>2+</sup> with NO<sub>2</sub><sup>-</sup>. This effect has been previously demonstrated in various experiments under controlled lab conditions (Sutka et al. 2006; Kopf et al. 2013; Visser et al. 2020). In experiments with NO<sub>3</sub><sup>-</sup> and Fe<sup>2+</sup>, we did not observe catalyzing effects of reactive surfaces on N<sub>2</sub>O production as they were possibly masked by microbial N<sub>2</sub>O production through denitrification. Yet, although nitrite-driven chemodenitrification seemed to

be the most important abiotic N<sub>2</sub>O production process, N<sub>2</sub>O production in the filtered NO<sub>3</sub><sup>-</sup> + Fe<sup>2+</sup> treatment clearly occurred as well. Chemodenitrification of nitrate with Fe may be an underappreciated nitrate reduction process in natural environments, competing with the enzymatic nitrate reduction, at least in iron-rich settings such as Lake La Cruz.

#### Isotopic signatures during experimental chemodenitrification

The distinct in vitro isotope signature of Fe-coupled chemodenitrification, imparted on the N<sub>2</sub>O pool (Exp\_chemo), provides a basis for interpreting community N and O isotope effects in the water column (next subsection). The SP values in our experiment with an average of  $6.9 \pm 3.1\text{‰}$  are within the broad range of previously reported values for chemodenitrification between 0 and 30‰ (Buchwald et al. 2016; Visser et al. 2020). These highly variable SP values are likely due to the impact of intermediates such as NO or nitrosyl (Fe[NO]<sup>+</sup>), which are, in turn, influenced by the environmental conditions (Buchwald et al. 2016). A more detailed discussion on the SP dynamics during experimental chemodenitrification can be found in Supporting Information Appendix S5.

In contrast to the observed overall spread for SP, the coupled N and O isotope effect during N<sub>2</sub>O production by chemodenitrification yielded a constant  $\Delta\delta^{18}\text{O} : \Delta\delta^{15}\text{N}$  trend of  $1.18 \pm 0.06$ . This value is similar to, but somewhat higher than, values observed by Jones et al. (2015) and Visser et al. (2020), who reported  $\delta^{18}\text{O}$ -N<sub>2</sub>O vs.  $\delta^{15}\text{N}$ -N<sub>2</sub>O data from chemodenitrification experiments that correspond to slopes on  $\delta^{18}\text{O}$ -vs.- $\delta^{15}\text{N}$ -N<sub>2</sub>O plots between  $\sim 0.7$  and 1.0. Our experiments with natural lake water thus expand the existing evidence that N<sub>2</sub>O production by chemodenitrification displays coupled dual N<sub>2</sub>O N/O isotope signatures that are distinct from microbial processes such as N<sub>2</sub>O reduction, which displays a robust ratio of 2.6 (Yamagishi et al. 2007; Ostrom and Ostrom 2012).

#### Chemodenitrification in the water column

The concentration profiles of N- and Fe-compounds in Lake La Cruz revealed a relatively thin water layer (15 m depth in 2015, 12–15 m depth in 2017), where chemodenitrification is most likely to occur, with N<sub>2</sub>O accumulation and presence of traces of dissolved Fe and NO<sub>2</sub><sup>-</sup>. However, we point out that at least in spring 2017, based on the spatial separation of NO<sub>2</sub><sup>-</sup> and dissolved iron between 12 and 15 m depth, chemodenitrification does most likely not play a major role for N<sub>2</sub>O production in these depths. In contrast, below the N<sub>2</sub>O maximum in 2015, where N<sub>2</sub>O was reduced completely, chemodenitrification is more likely to have occurred, considering the nitrite and dissolved Fe concentration profiles. Here, particulate Fe accumulation indicates active Fe(II) oxidation to Fe(III). In our incubations (Exp\_anoxic <sup>15</sup>N<sub>2</sub>O, Exp\_chemo), the fractional N<sub>2</sub>O yield of chemodenitrification reached values of 20–30%, independent

of the initial substrate concentrations, and we thus expect similar yields in the water column of Lake La Cruz. When assuming concentrations of 0.5 μM for overlapping NO<sub>2</sub><sup>-</sup> and Fe(II), which may possibly apply to the situation in 2015, an N<sub>2</sub>O yield of 25% with respect to NO<sub>2</sub><sup>-</sup> reduction would result in 125 nM N<sub>2</sub>O produced. Yet, we observed rather low concentrations of N<sub>2</sub>O in the respective water layer (< 5 nM). In such a situation, chemodenitrification (i.e., its typical biogeochemical signature of high N<sub>2</sub>O production with respect to N<sub>2</sub>) could be hidden because active denitrification, as we have demonstrated, prevents N<sub>2</sub>O from accumulating.

Theoretically, N<sub>2</sub>O SP measurements allow identifying, or excluding, chemodenitrification, but we argue that two fundamental problems preclude the reliable use of SP for the detection of chemodenitrification in the RTZ of Lake La Cruz with SP values of 13–16‰. First, it is unclear whether the SP values around 5–12‰ determined in our incubation experiment (Exp\_chemo) are representative for the SP values of N<sub>2</sub>O produced in situ. Since NO concentrations only reach very low concentrations in natural water bodies (Tian et al. 2019 and references therein), as compared to the incubation experiment, where NO probably accumulated, reaction dynamics and SP effects could be different. Second, a single two-endmember SP approach cannot be applied, since contributions from canonical and/or nitrifier denitrification, simultaneous N<sub>2</sub>O reduction via denitrification, and remnant relict N<sub>2</sub>O from aerobic N<sub>2</sub>O production above the RTZ all shape the SP values within the RTZ. The SP minima of 16‰ (2015) and 13‰ (2017) are in fact similar to the SP values expected for chemodenitrification, but these values likely reflect a mixed signal of nitrification and denitrification rather than chemodenitrification. In the zone below the N<sub>2</sub>O maxima/SP minima, which is the most likely water depth for chemodenitrification to occur based on the geochemical profiles, SP values increase up to 20‰, which is higher than the expected SP of 5–12‰ for chemodenitrification. We suspect N<sub>2</sub>O reduction to be the driving process here (see above). Generally, the large uncertainties of the SP associated to N<sub>2</sub>O produced via chemodenitrification does not make it a suitable indicator to trace this process in the environment.

We turn to the dual N and O isotope trends of N<sub>2</sub>O to potentially disentangle chemodenitrification and microbial denitrification (Fig. 6). The Δδ<sup>18</sup>O : Δδ<sup>15</sup>N value of 1.8 for the deepest data points in 2015 falls in-between the theoretical expected trends for 100% N<sub>2</sub>O reduction via denitrification (2.6; Yamagishi et al. 2007; Ostrom and Ostrom 2012) and chemodenitrification (1.2, Exp\_Chemo), respectively, possibly representing a combined chemodenitrification/microbial N<sub>2</sub>O reduction signal. In 2017, decreasing N<sub>2</sub>O concentrations below the N<sub>2</sub>O peak at ~11–12.5 m were associated to a Δδ<sup>18</sup>O : Δδ<sup>15</sup>N ratio of 2.9 pointing toward a layer of N<sub>2</sub>O reduction without significant contribution of chemodenitrification, as this value is close to the theoretical value of 2.6 for microbial denitrification. This seems plausible, as during the sampling in 2017 the spatial separation

between NO<sub>x</sub>- and Fe(II)-containing waters was much more pronounced. In turn, this implies that chemodenitrification undergoes inter- and/or intra-annual fluctuations, probably modulated by seasonal/inter-annual changes in water column biogeochemistry. For example, higher Chl *a* concentrations in 2017 indicate a larger input of organic compounds to fuel canonical denitrification. Based on the apparent specificity of the dual N- and O-isotope ratio of N<sub>2</sub>O, the coupled N-vs.-O N<sub>2</sub>O isotope measurements were more useful than the SP analyses for identifying chemodenitrification.

Although the monimolimnion of Lake La Cruz displays high dissolved iron concentrations, Fe-coupled chemodenitrification was likely responsible for only a minor percentage of the total N<sub>2</sub>O observed in the RTZ. Incubation experiments revealed, however, that chemodenitrification has the potential to contribute to N<sub>2</sub>O production as much as canonical denitrification. For example, in winter, when the upper water column is well mixed and the depth of O<sub>2</sub> depletion is located at greater depth, NO<sub>x</sub> and dissolved Fe profiles are more likely to overlap, and with higher concentrations, chemodenitrification may play a more important role in the overall N<sub>2</sub>O production within the RTZ. Furthermore, the mixing of the upper water column could cause the transport of N<sub>2</sub>O from the RTZ to the upper water column and eventually to the atmosphere.

## Conclusions

We present a comprehensive tool set based on different stable isotopic approaches to investigate biotic and abiotic N<sub>2</sub>O production pathways in aquatic environments. We demonstrate that profiles of N<sub>2</sub>O concentration and the isotopic characteristics of N<sub>2</sub>O (δ<sup>15</sup>N, δ<sup>18</sup>O, and SP) are valuable indicators of nitrification-associated N<sub>2</sub>O production, N<sub>2</sub>O production via nitrifier denitrification and/or canonical denitrification, as well as N<sub>2</sub>O reduction via denitrification, in different compartments of Lake La Cruz. In particular, the coupled N<sub>2</sub>O N-vs.-O isotope signatures highlight the redox-dependent transition from incomplete and complete denitrification in the water column. Incubation experiments with lake water and amended <sup>15</sup>N-tracers proved useful for a more quantitative assessment of the above-mentioned processes. For example, <sup>15</sup>NO<sub>3</sub><sup>-</sup> incubations confirmed high rates of N<sub>2</sub>O production in the near bottom oxygen-deficient waters.

In addition to traditional microbial processes, we assessed the potential contribution of chemodenitrification to N<sub>2</sub>O production in Lake La Cruz. Lake water incubations with high N<sub>2</sub>O yields during the reaction between NO<sub>2</sub><sup>-</sup> and Fe<sup>2+</sup>, even at low substrate concentrations, suggest that chemodenitrification rates could be relevant under the observed in situ conditions in the water column. However, the identification of in situ gross N<sub>2</sub>O production by chemodenitrification is intricate, as it is likely overprinted by microbial N<sub>2</sub>O reduction. Most promising with regard to detecting chemodenitrification in the water column is the analysis of the N<sub>2</sub>O δ<sup>18</sup>O vs. δ<sup>15</sup>N, as the Δδ<sup>18</sup>O : Δδ<sup>15</sup>N ratio seems typical for chemodenitrification

(~1 : 1.2 in this study), that is, distinct from biological isotope effects. Periods of overlapping availability of NO<sub>x</sub> and iron in the anoxic hypolimnion of Lake La Cruz may transiently lead to a substantial increase in N<sub>2</sub>O production via chemodenitrification, and N<sub>2</sub>O  $\Delta\delta^{18}\text{O}$  :  $\Delta\delta^{15}\text{N}$  values could be deployed to detect this.

N<sub>2</sub>O produced via chemodenitrification may not only be relevant in Lake La Cruz or other meromictic, iron-rich lakes, but potentially also in seasonally stratified lakes, which accumulate dissolved iron in the temporarily anoxic water column. For example, numerous boreal shield lakes can develop an anoxic hypolimnion with high dissolved iron concentrations in summer (Schiff et al. 2017), possibly providing suitable conditions for chemodenitrification during stratification and N<sub>2</sub>O release to the atmosphere during mixing. Furthermore, in the context of ferruginous and anoxic ancient oceans, our findings imply that large amounts of the potent greenhouse gas N<sub>2</sub>O generated via chemodenitrification might have contributed to an ice-free Mesoproterozoic Ocean (Stanton et al. 2018).

#### Data availability statement

Data are available through the PANGAEA data repository at <https://www.pangaea.de> (Tischer et al. 2022, doi:10.1594/PANGAEA.943248).

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#### Conflict of Interest

None declared.

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