



Drivers of the apparent nitrate isotope effect during sedimentary net nitrate reduction: diffusion limitation and nitrate regeneration

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Abstract

10 Isotope effects associated with sedimentary nitrate (NO_3^-) reduction are often strongly suppressed at the ecosystem scale in the overlying water, resulting in low apparent isotope effects (ϵ_{app}) that are commonly attributed to physical transport limitation by diffusion. However, the extent to which environmental conditions and experimental manipulations modulate diffusive substrate transport and thereby regulate the expression of sedimentary isotope signals remains unclear. We quantified coupled nitrogen (N) and oxygen (O) isotope effects associated with net NO_3^- reduction in whole-core incubations with sediments from
15 two Swiss lakes. We isolated the potential controls on apparent isotope effects by manipulating sediment reactivity, macrofauna-mediated transport, NO_3^- regeneration via nitrification, and the extent/presence of the oxic diffusive barrier within the sediments. Under near-natural oxic conditions and across treatments with altered organic matter content and enhanced bioturbation, the apparent isotope effects remained strongly suppressed ($^{15,18}\epsilon_{\text{app}} \leq 3.5 \text{ ‰}$). In Lake Baldegg, inhibition of nitrification with N-allylthiourea increased apparent isotope effects ($^{15/18}\epsilon_{\text{app}} = 6.4/8.9 \text{ ‰}$), demonstrating that NO_3^-
20 regeneration contributes to suppressing isotope effects under oxic conditions within the surface sediment layer. In Lake Sarnen, however, inhibition of nitrification alone had little effect, indicating that transport limitation across the oxic diffusive barrier remained the dominant control. In anoxic experiments, when the oxic diffusive barrier was removed completely, apparent isotope effects increased further, reaching the highest values (8.3–10.5 ‰) in both lakes, reflecting alleviation of diffusion limitation and minimization or prevention of nitrification. Consistent with the stronger expression of denitrification isotope
25 effects under anoxic conditions, dual-isotope relationships most closely reflected the characteristic denitrification signature, with near-unity $\Delta\delta^{18}\text{O}-\text{NO}_3^-:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ coupling in Lake Baldegg (0.97) and a lower ratio still consistent with denitrification in Lake Sarnen (0.79). Together, these experiments show that apparent NO_3^- isotope effects in sediment-overlying waters are transport-filtered ecosystem-scale signals that are primarily controlled by diffusion limitation across the oxic surface sediment layer and further modulated by NO_3^- regeneration through nitrification.

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Keywords: nitrate, denitrification, apparent isotope effects, diffusion limitation



1 Introduction

Nitrate (NO_3^-) is one of the largest pools of bioavailable (or fixed) nitrogen (N) in lacustrine environments. It is produced primarily via nitrification, the aerobic oxidation of ammonium (NH_4^+). Denitrification - the dissimilatory reduction of NO_3^- to dinitrogen gas (N_2) - represents an important removal process for fixed N across both terrestrial and aquatic ecosystems. Denitrification occurs under suboxic ($< 6 \mu\text{mol L}^{-1}$) or anoxic conditions, when bacteria switch from oxygen (O_2) to NO_3^- as the terminal electron acceptor during the oxidation of electron donors such as organic matter (OM) or reduced sulphur compounds (Seitzinger et al., 2006; Burgin and Hamilton, 2007; Cojean et al., 2020). In addition to respiratory denitrification, further fixed-N-loss processes include anaerobic ammonia oxidation (anammox; Jetten et al., 2001; Burgin and Hamilton, 2007) and chemo-denitrification, an abiotic reaction where reduced metals (e.g., Fe^{+2} , FeS) react with NO_3^- or nitrite (NO_2^-) to produce gaseous N (Jones et al., 2015; Visser et al., 2020; Margalef-Marti et al., 2024).

The marine realm is by far the largest sink of bioavailable N, with benthic systems acting as hotspots of N-cycling activity where multiple processes coexist across steep and variable redox gradients (Lehmann et al., 2004). Marine sediments alone remove an estimated 100-300 Tg N yr^{-1} (Middelburg et al., 1996; Codispoti, 2007; DeVries et al., 2012). Seitzinger et al. (2006) estimated that continental and shelf sedimentary denitrification, water-column denitrification in marine O_2 minimum zones (OMZ), and denitrification in freshwater systems account for roughly 44 %, 14 %, and 20 % of global denitrification, respectively.

Denitrification in benthic habitats is sustained by and competes with co-occurring N transformation processes, particularly in redox transition zones, making it challenging to disentangle their individual contributions. N (^{14}N , ^{15}N) and O (^{16}O , ^{17}O , ^{18}O) each occur in multiple isotopic forms. Stable N and O isotope ratio measurements provide a powerful tool for tracing the origin and fate of NO_3^- . Isotopic abundances are expressed in delta (δ) notation (in ‰), relative to N_2 in air ($\delta^{15}\text{N}$) or VSMOW ($\delta^{18}\text{O}$) e.g.,

$$\delta^{15}\text{N} = [((^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{AIR}}) - 1] \times 1000 \quad (1)$$

Isotopic discrimination by processes is expressed as the isotope effect (ϵ). Biological transformations typically favour the lighter isotopes in what is termed normal isotopic fractionation. In a system following Rayleigh distillation dynamics, the magnitude of ϵ can be determined from changes in the isotopic composition of the substrate relative to its fractional consumption, as expressed in Eq. 2 (Mariotti et al., 1981; Sigman et al., 2009):

$$\epsilon = -[\delta^{15}\text{N}(c_t) - \delta^{15}\text{N}(c_0)] / \ln(f) \quad (2)$$

where c_0 and c_t are the initial and the remaining substrate concentrations, respectively, and $f = c_t / c_0$. Linking isotopic signals observed at the ecosystem (Lehmann et al., 2005) or even the global scale (Codispoti, 2007; Eugster and Gruber, 2012) to process-level fractionation provide key constraints on NO_3^- sources and sinks, but - critically - requires an understanding how process-specific isotope effects are expressed in the environment. In other words, N-isotope balance approaches used to quantify fixed-N sources and sinks require a solid accounting of the associated net isotope effects (Brandes and Devol, 2002).



Dissimilative NO_3^- reduction (e.g., via denitrification) at the organism-level is associated with large intrinsic isotope effects (ϵ_{cell} or ϵ_{bio}), ranging from 5 ‰ to 30 ‰ across different aquatic systems (Brandes et al., 1998; Voss et al., 2001; Brandes and Devol, 2002; Sigman et al., 2003, 2005; Lehmann et al., 2004, 2007; Kessler et al., 2014; Dähnke and Thamdrup, 2013, 2016; Paulus et al., 2026). Elevated isotope effects are typically observed in well-mixed environments where the substrate pool and the site of reduction are not spatially separated, as is often the case in the O_2 -deficient water columns of marine or lacustrine ecosystems. In contrast, in benthic environments, the NO_3^- pool (i.e., in the overlying water or in porewater near the sediment surface) is spatially separated from the active reduction zone in the deeper sediments (Lehmann et al., 2007), and the substrate supply to the denitrifying zone is often limited by molecular-diffusive transport. As a result of diffusion limitation (and in turn, quantitative consumption), the NO_3^- isotope effects of benthic denitrification can be strongly suppressed ($< 5 \text{ ‰}$), masking the intrinsic fractionation (Brandes and Devol, 1997; Lehmann et al., 2003, 2004, 2007; Sebilo et al., 2003; Kessler et al., 2014). The isotope effect then expressed in the overlying water is referred to as an apparent isotope effect (ϵ_{app}).

Lehmann et al. (2007) modelled both ϵ_{bio} and ϵ_{app} for denitrification based on porewater profiles from the Bering Sea under different biogeochemical conditions. They proposed that the extent to which ϵ_{bio} is attenuated in ϵ_{app} may depend on multiple factors, namely: i) the O_2 penetration depth, which is governed by OM supply, aerobic respiration, and O_2 consumption via nitrification; ii) denitrification rates, which depend on substrate availability and sediment OM reactivity; and, directly linked to the first two points, iii) the efficiency of diffusive transport supplying substrate to the denitrification zone. Additionally, bioturbation-enhanced solute exchange (especially NO_3^- and O_2) between the bottom water and sediments (Kristensen et al., 2012; Callbeck et al., 2026; Mazzoli et al., 2026a), may also influence ϵ_{app} . Furthermore, ϵ_{app} can be modified by concurrent NO_3^- regeneration via nitrification and/or anammox (Lehmann et al., 2004, 2007; Granger and Wankel, 2016).

Dual NO_3^- N and O isotope measurements provide a means to disentangle the imprints of these potentially overlapping pathways. Deviations in net denitrifying systems from the 1:1 relationship for $\Delta\delta^{18}\text{O}:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ expected for standalone denitrification involving the dissimilatory NO_3^- reductase NAR have been associated with the activity of distinct enzymatic NO_3^- reduction pathways (e.g., denitrification mediated by periplasmic NO_3^- reductase NAP) (Granger et al., 2008; Asamoto et al., 2021), and/or with NO_3^- regeneration via anammox (Brunner et al., 2013; Dähnke and Thamdrup, 2013; Granger and Wankel, 2016; Kobayashi et al., 2019, 2025; Magyar et al., 2021) or nitrification (Sigman et al., 2005; Buchwald and Casciotti, 2010; Boshers et al., 2019; Dale et al., 2022). Nitrification-derived NO_3^- is typically ^{15}N depleted, while its $\delta^{18}\text{O}$ is determined by the isotopic composition of the ambient water and O_2 , giving it a distinct O isotope signature often exceeding that of the ambient NO_3^- (Sigman et al., 2005; Granger and Wankel, 2016; Boshers et al., 2019). Nitrate regenerated via anammox is characterized by low or even negative $\Delta\delta^{18}\text{O}$ - to $\Delta\delta^{15}\text{N}-\text{NO}_3^-$ ratios (≤ 0.3) and non-linear trajectories (Kobayashi et al. 2025). Consequently, NO_3^- regeneration by nitrification tends to increase, and regeneration by anammox to decrease, the $\Delta\delta^{18}\text{O}$ - to $\Delta\delta^{15}\text{N}-\text{NO}_3^-$ ratio relative to that expected for pure denitrification, explaining characteristic deviations from the denitrification baseline.

While the NO_3^- isotopic fingerprint of water column N removal is well-characterized (Sigman et al., 2003, 2005), the isotopic signature of sedimentary denitrification at the ecosystem level and its modulating controls are poorly constrained. To date,



only a few studies have investigated, either through benthic chamber experiments (Brandes and Devol, 1997; Lehmann et al., 2004; Dale et al., 2022) or isotope-biogeochemical modelling (Lehmann et al., 2007), how signals from sedimentary
 100 denitrification and other co-occurring, partially coupled, N-cycling reactions propagate into oceanic or lacustrine water
 columns. The close links between ϵ_{app} and biogeochemical conditions affecting the depth of the benthic nitracline and N
 turnover rates suggested in model simulations by Lehmann et al. (2007) have thus far not been verified experimentally. Here,
 we present the first experimental study to directly examine the factors controlling the expression of ϵ_{app} . We conducted a suite
 of whole-core incubations with sediments from two Swiss lakes of contrasting trophic state (Lakes Sarnen and Baldegg) and
 105 compared the obtained values for ϵ_{app} to biological isotope effects for NO_3^- reduction (ϵ_{bio}), determined during the same
 campaigns (Paulus et al., 2026). The sediments from both lakes provide a tractable system to resolve the mechanistic influence
 of O_2 availability, NO_3^- regeneration via nitrification/anammox, bioturbation and sediment reactivity on the apparent dual
 isotope signature of NO_3^- . These are the first data of their kind from lakes, and their significance extends well beyond individual
 lake basins: they provide mechanistic insights into benthic isotope effects that are directly relevant to marine systems and
 110 critical for constraining global N budgets.

2 Material and methods

2.1 Sampling and site description

Sediment cores were collected from the deepest point of the Swiss lakes Baldegg (BAL; campaign August 2023, Canton of
 Lucerne, maximum depth 66 m, 5.2 km² surface area; Mengis et al., 1997) and Sarnen (SAR, campaign January 2024, Canton
 115 of Obwalden, maximum depth 49 m, 7.15 km² surface area; Müller et al. 2021) using two different-sized gravity corers fitted
 with PVC tubes (inner diameter 5.8 cm or 14 cm). Bottom water was sampled with Niskin bottles. Both sediments and water
 were kept on ice during transport to the onshore laboratory, where they were stored at 4 °C until further processing on the same
 day. Both lakes are monomictic, mixing between mid-November and March, and remaining thermally stratified from April
 onwards (Müller et al., 2021).

120 The pre-alpine oligotrophic Lake Sarnen (total phosphorus, TP: 5 mg m⁻³) receives substantial allochthonous material inputs
 from its mountainous catchment, which intensify during storm events (Müller et al., 2021). In contrast, the catchment of
 eutrophic Lake Baldegg (TP: 24 mg m⁻³) is dominated by animal farming, resulting in high phosphorus (P) and N inputs. Since
 1984, Lake Baldegg has been artificially aerated to counteract hypolimnetic anoxia. Pressurized air is applied in winter to
 enhance vertical circulation, while in summer, pure O_2 is supplied to the hypolimnion via fine-bubble diffusers to maintain
 125 oxygenation without disrupting thermal stratification. (Gächter and Wehrli, 1998)

The two lakes differ markedly in trophic state and catchment properties, resulting in distinct N-cycle dynamics. These
 differences are reflected in N transformation rates in sediments (Callbeck et al., 2026; Paulus et al., 2026), microbial functional
 gene repertoires (Baumann et al., 2022, 2024), and the biological isotope effects (ϵ_{bio}) associated with NO_3^- dissimilation
 (Paulus et al., 2026). Internal N recycling, including nitrification, dissimilatory nitrate reduction to ammonium (DNRA), and



130 anammox, is proportionally more important in Sarnen sediments than in Baldegg (Baumann et al., 2022). In contrast, the more
 biologically productive Lake Baldegg exhibits substantially higher N removal rates (NRR: $20 \pm 6.6 \text{ g N m}^{-2} \text{ year}^{-1}$) and N
 removal efficiency (NRE: 66 %) than the less productive Lake Sarnen (NRR: $3.2 \pm 4.2 \text{ g N m}^{-2} \text{ year}^{-1}$, NRE: 33 %) (Müller et
 al., 2021). These contrasting biogeochemical settings make the two lakes well suited to investigate how NO_3^- regeneration and
 transport limitation under different reactivity regimes influence the apparent isotope effects associated with sedimentary NO_3^-
 135 reduction.

2.2 Whole-core incubations

Whole-core incubation experiments comprised near-natural *Reference* treatments using intact sediments together with
 experimental manipulations targeting nitrification, bioturbation, O_2 availability, and sediment reactivity. An overview of all
 experimental treatments is given in Table 1.

140 2.2.1 General whole-core incubation set up

Sediment cores were shortened on the sampling day to a total length of 49 cm to fit the dimensions of the water bath. The core
 liner was comprised 30 cm of sediment and ~16 cm water, above the sediment-water interface. The overlying water volume
 was adjusted with sterile-filtered ($0.22 \mu\text{m}$) bottom water. Specifically, 265 mL of core water was supplemented with 155 mL
 of sterile bottom water, yielding a starting overlying-water volume of 420 mL per incubation. The amendments to the overlying
 145 water varied among treatments and included NO_3^- and, where applicable, additional amendments (e.g. ATU) as summarized
 in Table 1. For the *Anoxic* incubations, the overlying water was completely replaced with sterile-filtered bottom water to
 prevent "water-column" denitrification.

The cores were placed in a water bath maintained at 11.5°C (T Ecocoll 100, Grant Instruments). Each core was equipped with
 a stirring unit (see Supplementary Information Fig. S1), consisting of a microprocessor-controlled stroke engine with an
 150 attached stirrer, a microfilter candle (porosity grade 0) connected to a glass tube for purging the overlying water with either air
 (oxic incubations) or N_2 containing 400 ppm CO_2 (to maintain anoxia at constant pH; Dadi et al. 2015), a mount for an oxygen
 sensor, and a septum for sampling. The stirring speed (24 rpm) and gas flow (80 mL min^{-1}) ensured complete mixing of the
 overlying water without disturbing the sediment-water interface.

Sediment cores were pre-incubated overnight to minimize artifacts potentially associated with sampling, transport, and
 155 preparation. Sediment cores were subsequently incubated in the dark for 60 h (Lake Baldegg) or 72 h (Lake Sarnen). Full-
 range and trace oxygen robust probes (Pyroscience) were used to monitor O_2 saturation continuously and to confirm the
 absence of O_2 during anoxic incubations. At defined time points 15 mL water samples were withdrawn using a MilliQ prerinsed
 25 mL LuerLock syringe and needle, syringe-filtered ($0.22 \mu\text{m}$, MilliQ prerinsed and dried), and stored at -20°C in the dark
 until nutrient concentration and isotopic analyses.

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2.2.2 Experimental treatments using intact sediment cores

Reference: Duplicate, intact sediment cores from Lake Sarnen (amended with KNO_3 , Table 1) and Lake Baldegg (unamended) were incubated under oxic conditions following the general incubation protocol described in Sect. 2.2.1. These incubations served as the near-natural *Reference* treatment.

165 **Nitrification inhibition:** Whole-core incubations were performed according to the general incubation protocol, with nitrification inhibited by addition of N-allylthiourea (ATU) to the overlying water. ATU inhibits both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA, Martens-Habben et al. 2015). Final ATU concentrations are given in Table 1.

Enhanced bioturbation:

170 Bioturbation, the macrofauna-mediated mixing of sediment, including particle reworking and ventilation of burrows (bioirrigation), critically influences porewater and solid-phase sediment chemistry, redox state, and, consequently, the location and rates of biogeochemical processes (Kristensen et al., 2012). To increase macrofaunal biomass and thereby enhance bioturbation, the upper 8 cm of two additional 14-cm-diameter Lake Baldegg sediment cores were sieved through a 0.3 mm mesh, yielding 0.84 g of macrofauna each. One portion was added to each duplicate incubation (sediment core inner diameter
 175 5.8 cm), resulting in a 2.4-fold increase in macrofaunal biomass relative to the *Reference* treatment. Following macrofauna addition, the cores were left undisturbed for 5 h to allow the organisms to burrow before stirring commenced. Whole-core incubations were performed according to the general incubation protocol.

Anoxic incubations: Whole-core incubations with Lake Sarnen and Lake Baldegg sediments were performed in triplicate under anoxic conditions following the general incubation protocol, except that the overlying water was continuously purged
 180 with an N_2/CO_2 gas mixture to maintain anoxic conditions. One of the three incubations served as a proof-of-concept experiment to verify that denitrification in the sterile-filtered overlying water did not contribute substantially to the observed NO_3^- reduction. This core was sampled after 0, 24, and 48 h. At each time point, a 50 mL water sample was transferred into a 50 mL serum bottle, sealed with a septum and crimp cap, and purged with He for 1 h to remove O_2 introduced during sampling. O_2 concentrations were monitored using a trace-level O_2 sensor spot (Pyroscience) affixed to the serum bottle. The bottles
 185 were incubated at 11.5 °C in the dark and sampled for NO_3^- concentration analyses at three time points over the following 24 h. During sampling, the bottles were kept inverted while He was continuously supplied through one needle and samples withdrawn through a second needle to prevent oxygen contamination during sampling. Samples were immediately sterile-filtered with 0.22 μm pre-rinsed syringe filters and stored at -20 °C in the dark until analysis.

2.2.3 Whole-core incubations with modified sediment reactivity

190 Whole-core incubations with modified sediment reactivity were performed to investigate the influence of sediment organic matter reactivity on the apparent isotope effects associated with sedimentary NO_3^- reduction. A sediment slurry was prepared from Lake Baldegg sediments and pre-incubated at room temperature in a 5 L Schott bottle. During the 50-day pre-incubation, the slurry was continuously aerated and stirred to promote microbial degradation of labile organic matter and thereby reduce



sediment reactivity prior to packing the sediment into cores. Sediment reactivity was monitored throughout the conditioning period by repeated measurements of oxygen depletion rates (% h⁻¹), as a proxy for reactivity, using a full-range oxygen sensor spot (Pyroscience) (see Supplementary Information Fig. S2). Oxygen consumption rates progressively declined during pre-incubation, confirming successful reduction of sediment reactivity. Organic carbon content also decreased by approximately 25 % during the conditioning period. Pre-incubation was terminated once oxygen depletion rates approached a low and nearly stable level (approximately 8 % h⁻¹; Supplementary Information, Fig. S2).

Following pre-incubation, the sediments were separated from the slurry water using a Büchner funnel. Two cores were packed with the pre-aged sediments. Preliminary oxygen microprofiles showed that pre-aging alone resulted in only a modest increase in O₂ penetration depth. Therefore, two additional cores were prepared by mixing acid-cleaned, Milli-Q rinsed, and autoclaved silica sand with < 2.5 % (v/v) pre-aged sediment, to further reduce volumetric oxygen demand (i.e. overall reactivity), and increase O₂ penetration depth. All cores were filled with sterile-filtered bottom water and maintained under aerated, dark conditions at 4 °C for three months to establish stable geochemical gradients. O₂ penetration depth into the sediment was measured prior to the incubations (Table 2). Whole-core incubations were subsequently performed for 60 h at 11.5 °C in the dark according to the general incubation protocol described in Sect. 2.2.1. Experimental conditions are summarized in Table 1.

Table 1: Overview of whole-core incubation treatments and experimental amendments.

Incubation treatment	Purging	KNO ₃ addition	Further treatment
Lake Baldegg (intact sediment cores)			
Reference	Air	-	-
Nitrification inhibited	Air	-	1 mmol L ⁻¹ N-allylthiourea (ATU)
Enhanced bioturbation	Air	-	increased macrofauna biomass by a factor of 2.4
Anoxic	N ₂ (99.96 %):CO ₂ (0.04 %)	25 µmol L ⁻¹	overlying water replaced with sterile-filtered bottom water
Lake Sarnen (intact sediment cores)			
Reference	Air	20 µmol L ⁻¹	-
Nitrification inhibited	Air	20 µmol L ⁻¹	1 mmol L ⁻¹ N-allylthiourea (ATU)
Anoxic	N ₂ (99.96 %):CO ₂ (0.04 %)	20 µmol L ⁻¹	overlying water replaced with sterile-filtered bottom water
Modified sediment reactivity			
Reduced reactivity	Air	110 µmol L ⁻¹	pre-incubated sediments (100 %)
Low reactivity	Air	110 µmol L ⁻¹	pre-incubated sediments (< 2 %)



2.2.4 Flux calculations

Apparent net NO_3^- fluxes across the sediment-water interface were calculated for all whole-core incubations described above from changes in the NO_3^- inventory of the overlying water over the incubation period. The calculations follow a conservation-of-mass approach similar to that used in previous incubation studies (e.g. Du et al. 2022), but were adapted to account for repeated sampling without replenishment of the overlying water.

The NO_3^- inventory at time t_i was reconstructed from the NO_3^- remaining in the overlying water plus the cumulative NO_3^- removed during all previous sampling events:

$$Q_i = c_i V_i + \sum_{k=1}^{i-1} c_k V_{s,k} \quad (3)$$

where Q_i is the NO_3^- inventory at sampling time t_i , c_i is the NO_3^- concentration at sampling time t_i , and V_i is the remaining volume of the overlying water before sampling. The index i refers to the current sampling event. Within the summation term, c_k is the NO_3^- concentration measured in the water sample removed during sampling event k , and $V_{s,k}$ is the corresponding sample volume. The summation term runs over all previous sampling events ($k = 1, \dots, i-1$) and accounts for the cumulative NO_3^- mass removed by sampling.

Mean NO_3^- fluxes (F , Eq. 4) were calculated from the total change in NO_3^- inventory over the incubation period:

$$F = \frac{Q_{\text{end}} - Q_0}{A \Delta t} \quad (4)$$

Where A is the sediment surface area and Δt is the incubation time.

2.3 Oxygen measurements

O_2 concentrations in the sediments were determined by microsensor profiling, whereas O_2 concentrations in the overlying water during whole-core incubations were monitored continuously using optical O_2 sensors. Sediment O_2 concentration profiles were measured in intact cores during the BAL (August 2023) and SAR (March 2022) campaigns and in the cores with modified sediment reactivity prior to incubation using a Unisense microsensor system equipped with O_2 microsensors (tip diameter 50 μm). Profiles were recorded at 4 °C, while the overlying water was continuously aerated.

During whole-core incubations, oxygen saturation in oxic treatments and the absence of oxygen in *Anoxic* treatments was monitored continuously using Pyroscience full-range (LD: 0.01 mg L^{-1}) and trace (LD: 0.002 mg L^{-1}) oxygen robust probes (tip diameter 3 mm) or sensor spots (tip diameter 5 mm), respectively.

All oxygen sensors (Unisense microsensors and Pyroscience optical probes and sensor spots) were calibrated prior to use by two-point calibration using air-saturated water (100 %) and a sodium sulfite solution (0.24 mol L^{-1} Na_2SO_3 ; as 0 %).



2.4 Dissolved inorganic nitrogen analyses and nitrate isotope measurements

Concentrations of NO_3^- were determined by ion chromatography (IC; Metrohm 940 Professional IC Vario) equipped with an autosampler (Metrohm 889 IC Sampler Center), using filtered samples without additional pre-treatment (LOD: ~20-50 ppb). Concentrations of NO_2^- were determined using the Griess reaction, in which NO_2^- reacts with sulfanilamide (SAN) to form a diazonium intermediate that subsequently couples with N-(1-naphthyl)ethylenediamine (NED), producing an azo dye quantified photometrically at 540 nm (Pai et al., 1990).

For NO_3^- isotope measurements, where present, NO_2^- was removed by addition of sulfamic acid-saturated 20 % HCl (10 μL per mL sample), followed by neutralisation after 15 min with sodium hydroxide (Granger and Sigman, 2009). The N and O isotopic composition of NO_3^- ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$) was determined using the denitrifier method, involving the quantitative conversion of NO_3^- to N_2O by denitrifying bacteria (*P. chlororaphis*; *subsp. aureofaciens*) lacking the N_2O -reductase enzyme (Sigman et al. 2001; Casciotti et al. 2002; McIlvin and Casciotti 2011; Weigand et al. 2016). The isotopic composition of the resulting N_2O was subsequently determined using a continuous-flow isotope ratio mass spectrometer (IRMS; Delta V Plus, Thermo Fisher Scientific) coupled via a CONFLO IV interface to a custom-made cryogenic purge-and-trap system (McIlvin and Casciotti, 2010). The NO_3^- isotope measurements were calibrated against international (USGS34: $\delta^{15}\text{N} = -1.8 \text{ ‰}$, $\delta^{18}\text{O} = -27.9 \text{ ‰}$, IAEA-NO-3: $\delta^{15}\text{N} = 4.7 \text{ ‰}$, $\delta^{18}\text{O} = 25.6 \text{ ‰}$) and in-house (UBN-1: $\delta^{15}\text{N} = 14.15 \text{ ‰}$, $\delta^{18}\text{O} = 25.7 \text{ ‰}$) reference materials, with deep Pacific seawater serving as an internal long-term quality control.

2.5 Sediment elemental analysis

Total organic carbon (TOC) and total nitrogen (TN) were determined in sediment samples collected from the upper ~3 mm of the natural sediment cores and from the cores with modified sediment reactivity. Samples were freeze-dried, homogenized, weighed into silver capsules, decarbonized overnight under hydrochloric acid vapor, and analysed using a Flash EA 1112 elemental analyser. EDTA served as calibration reference (41.09 % C; 9.59 % N).



3 Results

3.1 NO₃⁻ consumption and partial NO₂⁻ accumulation during whole-core incubations

In all incubations, net NO₃⁻ reduction was observed (Fig. 1A-D). Sediment O₂ penetration depth and OM characteristics are summarized in Table 2. Net NO₃⁻ fluxes (Table 3) were generally higher in the more biologically productive Lake Baldegg (Fig. 1A) than in the less productive Lake Sarnen (Fig. 1B). In Lake Baldegg, NO₃⁻ reduction was accompanied by substantial NO₂⁻ accumulation in all treatments except those with *Nitrification inhibition*, where NO₂⁻ concentrations remained very low throughout the incubations. In contrast, Lake Sarnen sediments exhibited significant NO₂⁻ accumulation only under anoxic conditions, ultimately reaching concentrations exceeding those observed in Lake Baldegg. No significant concentration changes were detected during the anoxic serum-bottle incubations containing overlying water collected from an additional anoxic whole-core incubation (Fig. 1C). This confirmed that denitrification in the overlying water was negligible and that the observed NO₃⁻ decline during the whole-core incubations (and associated isotopic trends) exclusively reflected sedimentary NO₃⁻ reduction. Whole-core incubations with modified sediment reactivity also showed net NO₃⁻ reduction, accompanied by variable NO₂⁻ accumulation (Fig. 1D). In contrast to the intact sediment cores, cores with modified sediment reactivity, characterized by either reduced or low OM content, displayed divergent NO₃⁻ and NO₂⁻ dynamics and were therefore evaluated individually rather than as biological replicates. The two quartz-sand-diluted cores were prepared with different target OM contents but were both classified as *Low-reactivity* cores based on their measured OM contents (< 2.5 %).

Table 2: O₂ penetration depth and organic matter characteristics of the investigated sediments. O₂ penetration was measured under standardized conditions (4°C, aerated overlying water). For the cores with modified sediment reactivity, values represent the mean ± SD of repeated (n = 3) microsensor profiles at different locations within each core.

Sediment core	O ₂ penetration depth	TOC	TN	TOC:TN
	[mm]	[weight-%]	[weight-%]	
Lake Sarnen	5.1	1.693	0.187	10.6
Lake Baldegg	2.2	4.431	0.598	8.6
Reduced reactivity A	2.6 ± 0.3	3.131	0.357	10.2
Reduced reactivity B	2.8 ± 0	3.312	0.378	10.2
Low reactivity A	8.7 ± 1.5	0.073	0.009	9.9
Low reactivity B	9.8 ± 2.8	0.021	0.004	6.2

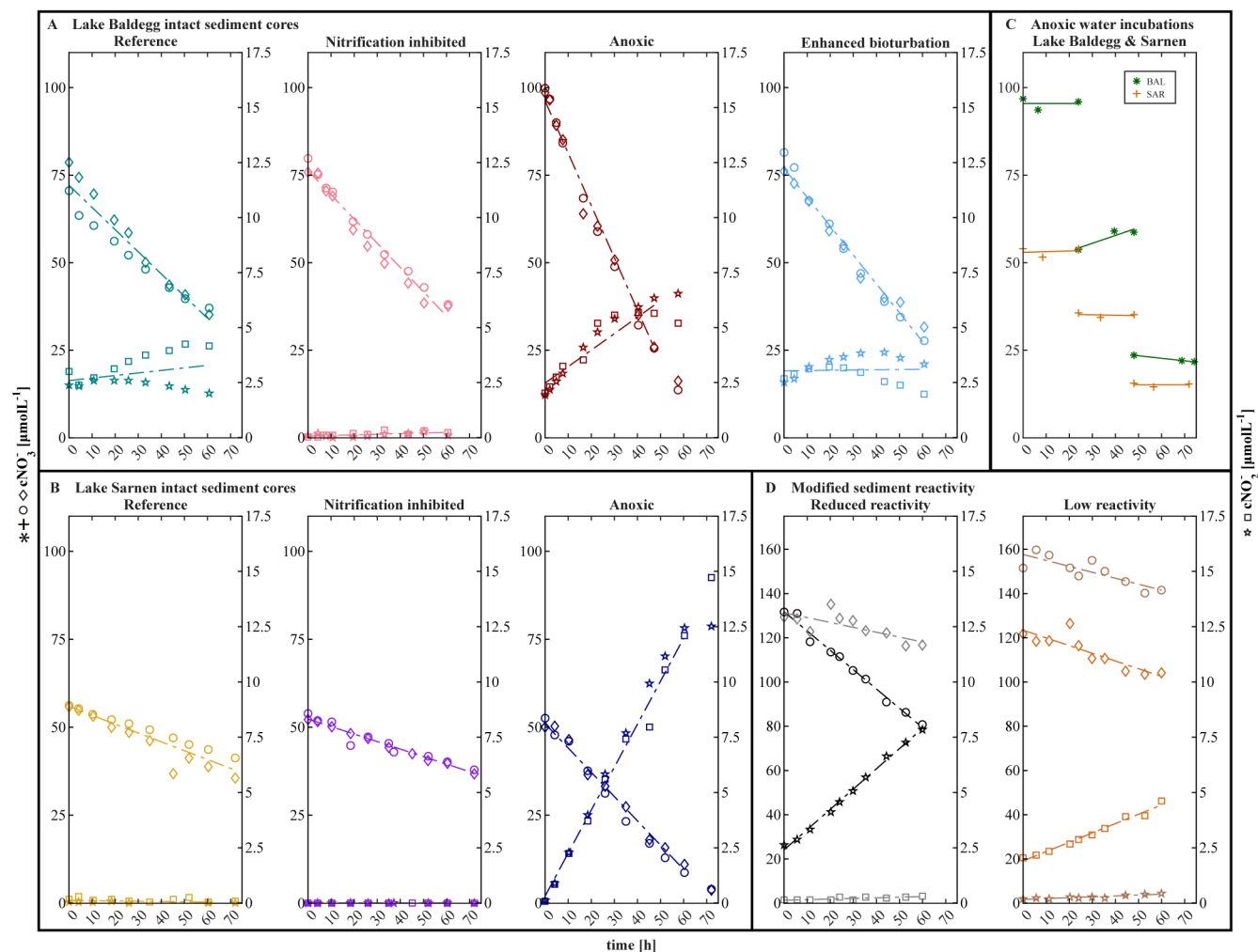


Figure 1: Concentrations of NO_3^- (circles, diamonds, pluses, asterisks) and NO_2^- (stars, squares) in the overlying water during whole-core sediment incubation experiments. Symbols are grouped by incubation, with circles and stars representing one incubation and diamonds and squares its biological replicate (A, B, D). Whole-core incubations with intact sediments are shown for Lake Baldegg (A) and Lake Sarnen (B). Whole-core incubations with experimentally manipulated sediments of modified reactivity (i.e., with pre-aged sediments from Lake Baldegg) are shown in panel D (see Sect. 2.2 for details). Panel C shows NO_3^- concentrations in anoxic serum bottle incubations containing overlying water collected from an additional anoxic whole-core incubation after 0, 24, and 48 h and subsequently incubated for 24 h. Lines represent linear regressions for the individual serum-bottle incubations and for the biological replicates of the whole-core incubations (A, B), while in panel D, the reduced- and low-reactivity cores exhibited divergent NO_3^- and NO_2^- dynamics and were therefore analysed individually. For the linear regressions, only data points with $\ln(f) \geq -1.5$ were considered, corresponding to ~78 % net consumption of the initial NO_3^- concentration. Note the different NO_3^- concentration scale in panel D.



3.2 Apparent nitrate isotope effects and dual-isotope relationships during net NO_3^- reduction

Apparent isotope effects ($^{15/18}\epsilon_{\text{app}}$) were calculated according to the Rayleigh approach (Eq. 2). During the whole-core incubations with intact sediment cores (Fig. 2A-D), the residual NO_3^- pool became generally enriched in the heavy isotopes, whereas some whole-core incubations with experimentally manipulated sediments exhibited a decline in $\delta^{15}\text{N}$ and especially $\delta^{18}\text{O}$ (Fig. 2E, F). Across both lakes, net NO_3^- reduction under treatments representing near-natural conditions was associated with low apparent isotope effects ranging from 0.2 ‰ to 3.1 ‰ ($^{15/18}\epsilon_{\text{app_ref}}$, Fig. 2A-D, Table 3). Apparent isotope effects did not differ significantly between the two lakes (Supplementary Information Table S1). In Lake Baldegg, *Enhanced bioturbation* yielded isotope effects similar to those of the *Reference* treatment (Fig. 2A, B, $^{15}\epsilon_{\text{app_ref}}$ vs. $^{15}\epsilon_{\text{app_bioirrig}}$: 0.9 ‰ vs. 1.1 ‰; $^{18}\epsilon_{\text{app_ref}}$ vs. $^{18}\epsilon_{\text{app_bioirrig}}$: 3.1 ‰ vs. 3 ‰; $^{15}p = 0.812$, $^{18}p = 0.948$). Following nitrification inhibition, both apparent N and O isotope effects increased to 6.4 ‰ ($^{15}\epsilon_{\text{app_nit_inh}}$) and 8.9 ‰ ($^{18}\epsilon_{\text{app_nit_inh}}$). Under anoxic conditions, apparent ^{15}N fractionation increased further, whereas $^{18}\epsilon$ remained at a similar level ($^{15}\epsilon_{\text{app_anoxic}} = 8.9$ ‰; $^{18}\epsilon_{\text{app_anoxic}} = 8.7$ ‰). Both treatments differed significantly from the *Reference* incubations ($p < 0.001$). In Lake Sarnen, nitrification inhibition resulted primarily in an increase in the apparent ^{18}O isotope effect (Fig. 2C, D, $^{15}\epsilon_{\text{app_nit_inh}} = 0.6$; $^{18}\epsilon_{\text{app_nit_inh}} = 4$ ‰). Relative to the *Reference* treatment the increase in the apparent ^{15}N isotope effect was small and not significant ($^{15}p = 0.910$), whereas the apparent ^{18}O isotope effect increased significantly ($^{18}p = 0.034$). Substantially higher apparent isotope effects were observed during the *Anoxic* incubations ($^{15}\epsilon_{\text{app_anoxic}} = 10.5$ ‰; $^{18}\epsilon_{\text{app_anoxic}} = 8.3$ ‰), with both isotope effects differing significantly from the *Reference* treatment ($p < 0.001$). Whole-core incubations with experimentally manipulated sediment reactivity and OM contents consistently exhibited low apparent isotope effects (Fig. 2E, F). At these overall low isotope-effect levels, however, cores with modified sediment reactivity showed considerable variability and were therefore analysed individually. Several incubations even exhibited apparent inverse isotope effects (Table 3).



315

Table 3. Overview of apparent nitrate N and O isotope effects, dual-isotope ratios ($^{18}\epsilon_{\text{app}}:^{15}\epsilon_{\text{app}}$), and NO_3^- fluxes. Errors reported for the isotope effects and dual-isotope ratios represent the standard errors of the regression coefficients, and R^2 denotes the coefficient of determination of the corresponding linear regressions. Asterisks indicate regressions with $R^2 < 0.5$. In these incubations, only limited changes in δ values and/or NO_3^- consumption occurred, resulting in poorly constrained Rayleigh regressions and correspondingly uncertain isotope effects and dual-isotope ratios. For these treatments, results are additionally reported for the individual replicates. For completeness, all values are reported but should be interpreted with appropriate caution. Errors associated with NO_3^- fluxes represent the standard deviation among biological replicate incubations.

Incubation treatment		$^{15}\epsilon_{\text{app}}$			$^{18}\epsilon_{\text{app}}$			$^{18}\epsilon_{\text{app}}:^{15}\epsilon_{\text{app}}$			Flux NO_3^-	
		‰	±	R^2	‰	±	R^2	ratio	±	R^2	mmol/m ² d	±
Lake Baldegg	Reference	0.9*	0.5	0.192	3.1	0.4	0.783	1.29	0.28	0.563	-2.2	0.4
	Reference A	-0.3*	0.4	0.055	2.2	0.2	0.930	-0.49*	0.72	0.062	-2.0	-
	Reference B	1.4	0.4	0.691	3.5	0.3	0.963	1.86	0.31	0.837	-2.5	-
	Enhanced bioturbation	1.1	0.2	0.713	3.0	0.2	0.931	2.09	0.26	0.804	-2.8	0.4
	Nitrification inhibited	6.4	0.2	0.978	8.9	0.4	0.962	1.39	0.06	0.968	-2.2	0.1
	Anoxic	8.9	0.3	0.977	8.7	0.3	0.983	0.97	0.02	0.994	-5.2	0.1
Lake Sarnen	Reference	0.7*	0.5	0.083	0.2*	0.8	0.003	-1.15	0.17	0.724	-0.9	0.2
	Reference A	2.8	0.3	0.910	-3.5	0.4	0.907	-1.13	0.19	0.812	-0.7	-
	Reference B	0.6*	0.3	0.391	0.6*	0.2	0.439	0.3*	0.3	0.096	-1.0	-
	Nitrification inhibited	0.6*	0.2	0.247	4.0	0.3	0.880	2.37*	0.70	0.391	-0.8	0.0
	Nitrification inhibited A	0.8	0.3	0.508	3.6	0.6	0.824	2.99	0.69	0.703	-0.8	-
	Nitrification inhibited B	0.4*	0.4	0.105	4.4	0.4	0.939	1.88*	1.25	0.220	-0.8	-
	Anoxic	10.5	0.3	0.985	8.3	0.3	0.982	0.79	0.02	0.987	-2.4	0.3
Mod. sed. reactivity	Reduced reactivity A	3.3	0.6	0.770	2.6	0.2	0.972	0.60	0.14	0.711	-3.0	-
	Reduced reactivity B	2.1*	1.5	0.196	-12.8	3.6	0.614	-2.20*	0.98	0.388	-0.9	-
	Low reactivity A	-2.5	0.6	0.665	-1.8	0.5	0.634	0.69	0.10	0.860	-0.8	-
	Low reactivity B	0.8	0.3	0.518	0.8*	0.3	0.383	0.87	0.24	0.620	-1.2	-

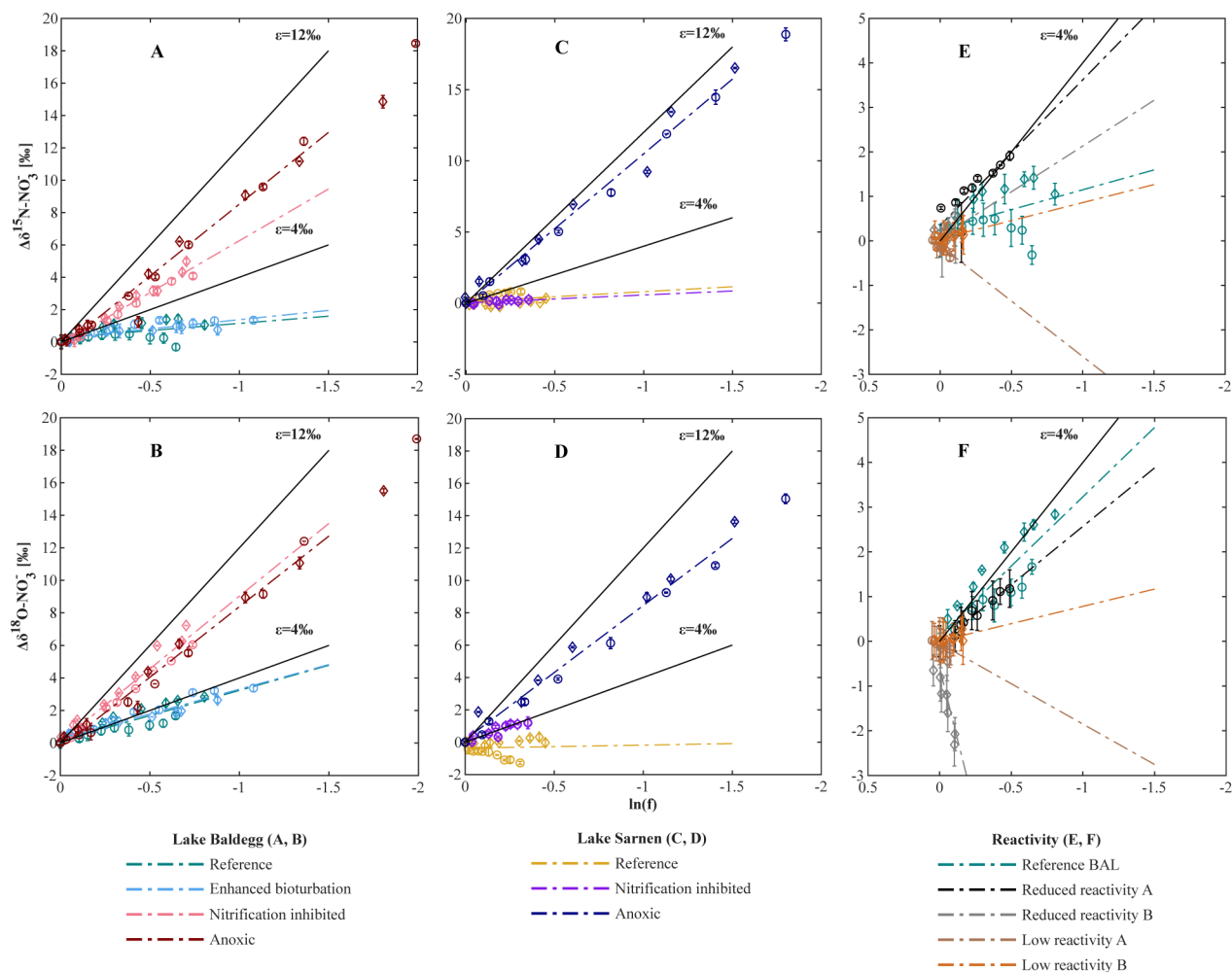


Figure 2. Rayleigh plots of $\Delta\delta^{15}\text{N-NO}_3^-$ (upper panels A, C, E) and $\Delta\delta^{18}\text{O-NO}_3^-$ (lower panels B, D, F). Nitrate isotopic compositions are expressed relative to the initial isotopic composition ($\Delta\delta$), plotted against $\ln(f)$, where f is the fraction of NO_3^- remaining (c_o/c_i). Whole-core incubations are grouped by lake (Lake Baldegg; A, B; Lake Sarnen: C, D), and sediment treatment (E, F), comparing intact Lake Baldegg sediments with experimentally manipulated (see Methods) Baldegg sediments of modified OM reactivity ("reduced" and "low"). Dashed lines indicate linear regressions fitted across duplicate incubations. In contrast, cores with reduced and low reactivity exhibited distinct isotope dynamics and were therefore analysed individually. Linear regressions include only data with $\ln(f) \geq -1.5$ (corresponding R^2 values are given in Table 1). Error bars represent the standard deviation of replicate sample preparation and isotope analysis. Note the different y-axis scales.

Across the different whole-core incubation treatments, the ratio of apparent oxygen-to-nitrogen isotope effects ($^{18}\epsilon_{\text{app}}/^{15}\epsilon_{\text{app}}$) ranged from -2.2 to 2.37 (Fig. 3A-D; Table 3). Under near-natural conditions, the ratio was negative in Lake Sarnen (-1.15) but positive in Lake Baldegg (1.29) and differed significantly between the two lakes ($p = 0.001$, Supplementary Information

Table S1). *Enhanced bioturbation* did not significantly affect the NO_3^- dual-isotope ratio in Lake Baldegg relative to the *Reference* treatment ($p = 0.328$). In Lake Sarnen, *Nitrification inhibition* significantly increased the $^{18}\epsilon_{\text{app}}:^{15}\epsilon_{\text{app}}$ ratio to 2.37 (Fig. 3A, B; to $p = 0.021$), whereas the corresponding increase in Lake Baldegg was not significant ($p = 0.871$). Under anoxic conditions, lower $^{18}\epsilon_{\text{app}}:^{15}\epsilon_{\text{app}}$ ratio were observed in both lakes (Fig. 3C), approaching the 1:1 ratio expected for standalone denitrification (Asamoto et al., 2021). Relative to the *Reference* incubations, this decrease was significant in Lake Sarnen (0.79; $p < 0.001$) but not in Lake Baldegg (0.97; $p = 0.604$). Relative to the *Nitrification-inhibited* treatment, the ratio remained statistically indistinguishable in Lake Sarnen ($p = 0.281$), despite the marked decrease in the mean ratio, reflecting the comparatively large uncertainty associated with the *Nitrification-inhibited* treatment (2.37 ± 0.7). In contrast, the decrease was significant in Lake Baldegg ($p = 0.002$). No consistent trend was observed across the whole-core incubations with experimentally manipulated sediment reactivity and organic matter contents. A complete overview of the apparent NO_3^- isotope effects, dual-isotope enrichment ratios, and NO_3^- fluxes is provided in Table 3, while the statistical comparisons are summarized in Supplementary Information Table S1.

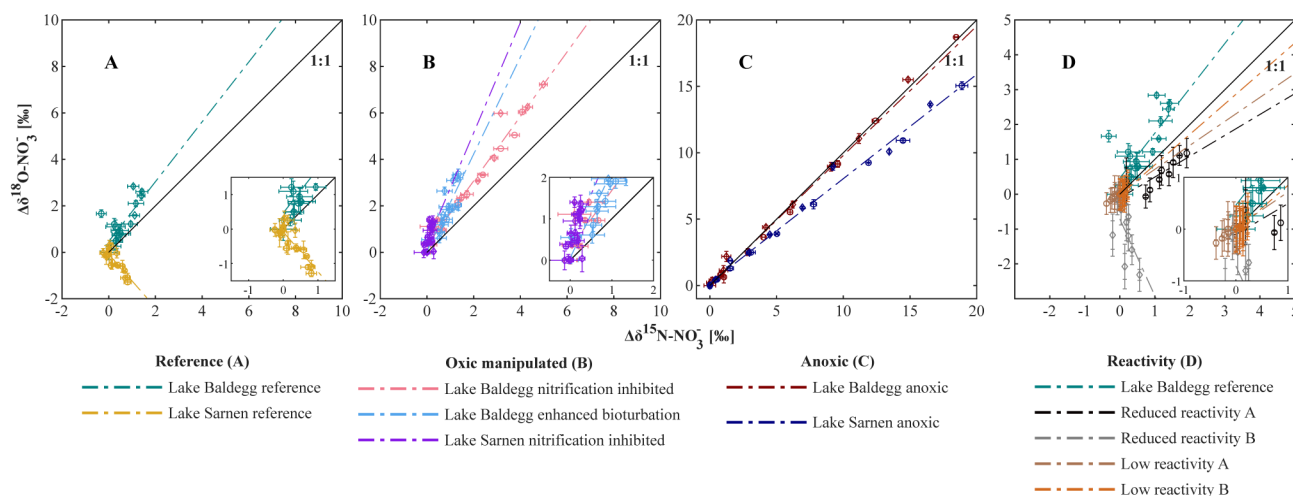


Figure 3. Nitrate dual-isotope plots showing $\Delta\delta^{18}\text{O-NO}_3^-$ versus $\Delta\delta^{15}\text{N-NO}_3^-$. Whole-core incubations are grouped as near-natural reference treatments (A), oxic experimental manipulations (B), anoxic incubations (C), and incubations with experimentally manipulated sediment OM reactivity/content (D). Panel D compares incubation results from intact Lake Baldegg sediment cores and cores with modified sediment reactivity (i.e., pre-oxidized and mixed with quartz sand). Dashed lines indicate linear regression across duplicate incubations. Cores with modified sediment reactivity were analysed separately because their isotope dynamics differed. Linear regressions include only values with $\ln(f) \geq -1.5$ (corresponding R^2 values are given in Table 1). Error bars represent the standard deviations of replicate sample preparation and isotope analysis. Note the different axis scales.



4 Discussion

355 We investigated how sediment reactivity, transport processes, and redox zonation shape the apparent isotope effects associated with benthic NO_3^- reduction. To this end, we combined whole-core incubations of near-natural, intact sediment cores from two lakes differing in trophic state, sediment reactivity, and oxygen penetration depths with a series of targeted experimental manipulations, designed to vary the depth and transport context of denitrification. These included macrofauna-enhanced bioturbation, inhibition of NO_3^- regeneration via nitrification, and experimentally manipulated (i.e. pre-oxidized) sediments
 360 with reduced reactivity and altered oxygen penetration depths. Together, these complementary approaches allowed us to disentangle the influence of sediment reactivity, transport processes/bioturbation, and the depth of denitrification on the apparent isotope effects of benthic NO_3^- reduction.

4.1 Transport-controlled suppression of NO_3^- isotope effect expression

Scale-dependent expression of NO_3^- isotope effects under transport limitation - Generally, under near-natural conditions,
 365 whole-core incubations without process inhibition (and with oxygen-saturated overlying water) consistently exhibited low apparent N- and O-isotope effects during net NO_3^- reduction in both study lakes (Lake Sarnen: $^{15}\epsilon_{\text{app_ref}} = 0.7\text{‰}$, $^{18}\epsilon_{\text{app_ref}} = 0.2\text{‰}$; Lake Baldegg: $^{15}\epsilon_{\text{app_ref}} = 0.9\text{‰}$, $^{18}\epsilon_{\text{app_ref}} = 3.1\text{‰}$). Compared to the intrinsic biological isotope effects (ϵ_{bio}) determined in homogenized sediment bag incubations (Lake Sarnen: $^{15}\epsilon_{\text{bio}} = 17\text{‰}$, $^{18}\epsilon_{\text{bio}} = 13.6\text{‰}$; Lake Baldegg: $^{15}\epsilon_{\text{bio}} = 25.3\text{‰}$, $^{18}\epsilon_{\text{bio}} = 20.7\text{‰}$; Paulus et al., 2026), these low apparent isotope effects ($^{15/18}\epsilon_{\text{app}}$) demonstrate a strong suppression of
 370 isotope-effect expression from the cellular to the ecosystem scale across the sediment-water interface.

Nitrate is produced via nitrification in the oxic compartment (here, the oxic surface sediments), and reduced by denitrification in the suboxic ($< 6\text{ }\mu\text{mol L}^{-1}$) or anoxic compartment (here, the deeper sediments) (Seitzinger et al., 2006). These compartments are coupled by diffusive NO_3^- transport across concentration gradients (Brandes and Devol, 1997), a kinetically slow process that can become rate limiting (Sebilo et al., 2003; Kessler et al., 2014). In particular, when ambient NO_3^- concentrations
 375 approach the half-saturation constant ($K_m = 2\text{--}18\text{ }\mu\text{M}$; Kritee et al., 2012; Wankel et al., 2015), diffusion into a NO_3^- -reducing cell can limit substrate supply. Under these conditions, NO_3^- substrate uptake rather than enzymatic N-O bond cleavage during the first irreversible step of denitrification governs the expressed isotope effect, resulting in substantially diminished isotope fractionation (Granger et al., 2008; Kritee et al., 2012; Wenk et al., 2014a). Accordingly, ϵ_{bio} and ϵ_{app} represent NO_3^- -reduction isotope effects at contrasting levels of spatial integration and transport limitation: ϵ_{bio} reflects the intrinsic cellular isotope
 380 effect, whereas ϵ_{app} incorporates ecosystem-scale transport constraints related to limited substrate supply across the sediment-water interface. The attenuation of ϵ_{app} relative to ϵ_{bio} therefore demonstrates that ecosystem-scale isotope effects are emergent properties of physical transport constraints and sediment redox structure rather than enzymatic kinetics alone, in agreement with model-based analyses of benthic N cycling (Lehmann et al., 2007; Rooze and Meile, 2016). Likewise, dampened isotope effects ($< 5\text{‰}$) have repeatedly been attributed to diffusion-limited transport in marine (Brandes and Devol, 1997; Lehmann



et al., 2004, 2007; Alkhatib et al., 2012; Kessler et al., 2014), lacustrine (Lehmann et al., 2003) and riverine (Sebilo et al., 2003) environments.

Diffusion limitation, however, is not the only process influencing the apparent NO_3^- isotope signal. Oxygen penetration depth determines the thickness of the diffusive transport barrier (Brandes and Devol, 1997), while sediment organic matter quantity and quality, together with fauna-mediated transport (bioturbation), regulate oxygen penetration and the position of the denitrification zone. In the following section, we discuss how these factors modify the transport-controlled expression of apparent isotope effects. The additional influence of coupled nitrification-denitrification on the apparent isotope effects and NO_3^- dual-isotope relationships is discussed subsequently in Sects. 4.2 and 4.3.

Influence of sediment reactivity - In sediments, O_2 is primarily consumed by aerobic OM mineralisation and by the reoxidation of reduced solutes produced in deeper, anoxic sediment layers (Steinsberger et al., 2020). These processes regulate O_2 penetration depth and thereby control the position of the denitrification zone, and in turn the diffusive distance to the overlying water. Model simulations predict that OM reactivity, O_2 penetration, and redox conditions strongly influence the benthic N-isotope signal of denitrification (Lehmann et al., 2007). Consistent with this interpretation, field observations indicate that OM reactivity and O_2 penetration modulate ϵ_{app} through their control on the depth of the denitrification zone and the associated transport limitation, with particularly strong suppression (i.e., a positive relationship between ϵ_{app} and OM reactivity) observed in weakly reactive, deeply oxygenated sediments (Alkhatib et al., 2012). Sedimentation rates, and thus the flux of labile OM to the sediments, are approximately twofold higher in eutrophic Lake Baldegg than in oligotrophic Lake Sarnen (Müller et al., 2021), contributing to enhanced benthic mineralisation, an approximately 3 mm shallower oxygen penetration depth (Table 2), and more than twofold higher NO_3^- consumption in Lake Baldegg. In theory, enhanced NO_3^- turnover under these conditions may reduce NO_3^- availability at the denitrification site, thereby favouring substrate limitation and dampening isotope effect expression (e.g., Brandes and Devol 1997; Lehmann et al. 2004, 2007; Alkhatib et al. 2012). Conversely, lower OM reactivity is associated with deeper O_2 penetration, shifting denitrification deeper into the sediment and strengthening the diffusive barrier to NO_3^- supply, likewise suppressing ϵ_{app} (Lehmann et al., 2007; Alkhatib et al., 2012).

Despite marked contrasts in sedimentation rates, NO_3^- fluxes, and O_2 penetration depths between the two lakes, both $^{15}\epsilon_{\text{app_ref}}$ and $^{18}\epsilon_{\text{app_ref}}$ remained indistinguishable, indicating that these contrasting sediment-biogeochemical conditions were not reflected in the NO_3^- isotope effect as expressed in the overlying water (i.e., at the ecosystem scale). This is consistent with the model-based prediction that O_2 penetration depth and denitrification rate are inherently anti-correlated, such that NO_3^- availability at the reaction site remains low and apparent isotope fractionation remains suppressed across a broad range of sediment reactivity regimes (Lehmann et al., 2007). Likewise, similar apparent NO_3^- isotope effects have been reported from the Santa Monica Basin despite substantial differences in denitrification rates, consistent with diffusion-limited, near-complete NO_3^- consumption within the sediments (Lehmann et al., 2004). Together, these observations suggest that ϵ_{app} depends less on sediment reactivity per se than on the degree of NO_3^- depletion at the denitrification site and on the efficiency with which this isotopic signal is transmitted across the sediment-water interface into the overlying water.



However, the natural contrast between the lake sediments may not sufficiently span the range of conditions required to fundamentally alter transport limitation. To extend the range of sediment reactivity beyond that observed under natural conditions, Lake Baldegg sediments were pre-aged to deplete labile organic matter fractions through pre-oxidation. Although pre-aging substantially reduced the sedimentary O_2 demand (Supplementary Information, Fig. S2) and lowered the organic carbon content by approximately 25 %, O_2 penetration increased only marginally (Table 2), indicating that O_2 consumption remained substrate-replete and largely controlled by O_2 availability, consistent with Michaelis-Menten-type kinetics under substrate-replete conditions (Arndt et al., 2013). Markedly deeper O_2 penetration was achieved only after further dilution of the pre-aged sediments with quartz sand, such that sediment material accounted for less than 2.5 % of the mixture (*Low reactivity A/B*: O_2 penetration depths of 8.7 and 9.8 mm).

While no statistically significant differences were observed between the near-natural *Reference* and *Reduced-reactivity* treatments for either $^{15}\epsilon_{app}$ or $^{18}\epsilon_{app}$, both *Low-reactivity* treatments differed significantly from the *Reference* in $^{18}\epsilon_{app}$, and *Low-reactivity* treatment A also differed significantly in $^{15}\epsilon_{app}$. Yet, overall, all OM treatments were characterized by similarly low apparent isotope effects (i.e., individual deviations occurred around a very low ϵ_{app} baseline), and no consistent directional trend emerged across the different OM scenarios (*Reference*, *Reduced* and *Low reactivity*). Nevertheless, individual incubations occasionally deviated from this general pattern. Most notably, the *Low-reactivity A* incubation (Table 3) exhibited an apparent inverse isotope effect ($^{15}\epsilon_{app} = -2.5$; $^{18}\epsilon_{app} = -1.8$ ‰), deviating markedly from the expected denitrification signal and coinciding with a progressive ^{15}N depletion of the NO_3^- pool. This behaviour may indicate substantial NO_3^- regeneration within the sediment (e.g. Casciotti et al. 2003; Casciotti 2009; Buchwald and Casciotti 2010; Buchwald et al. 2012) and is explored further in Sect. 4.3.

Influence of fauna-mediated transport - The quality and amount of organic matter governs the position of redox zonation and the denitrification layer, but through bioturbation across the sediment-water interface, macrofauna can mediate additional transport of O_2 , nutrients, and other solutes (Lehmann et al., 2004, 2007; Stief, 2013; Rooze and Meile, 2016; Callbeck et al., 2026). Oxygen intrusion associated with these processes is expected to stimulate aerobic pathways (e.g. respiration and nitrification) while locally suppressing anaerobic N-cycling processes (e.g. denitrification) within otherwise anoxic sediments (Stief, 2013; Rooze and Meile, 2016; Callbeck et al., 2026). However, comparison of the *Reference* and *Enhanced-bioturbation* treatments in Lake Baldegg suggests that, even if additional O_2 and/or nutrient intrusion/transport modified sedimentary N cycling, this signal was not transmitted across the sediment-water interface. Although enhanced bioturbation increases local transport of solutes, our results indicate that this does not necessarily translate into a measurable relaxation of substrate transport limitation at the ecosystem scale, as reflected by the isotope signals in the overlying water. Owing to the experimental design, we cannot exclude that bioturbation altered nitrogen cycling and the associated isotope effects/signatures within the sediment porewater. If so, however, the effects of such changes apparently remained confined to the sediments and were not expressed in the apparent isotope effect across the sediment-water interface. In other words, if bioturbation stimulated additional nitrification, the regenerated NO_3^- was apparently, in most cases, consumed within the sediments before its isotopic



signature could propagate across the sediment-water interface. This interpretation aligns with observations from Santa Monica Bay, where the N- and O-isotope composition of water-column NO_3^- likewise remained unaffected despite active sedimentary bioturbation (Lehmann et al., 2004).

The consistently low apparent isotope effects associated with net NO_3^- consumption in the sediments, together with the absence of substantial changes in the apparent isotope fractionation under various contrasting conditions - including oligotrophic and eutrophic lake sediments, varying OM contents and O_2 penetration depths, and *Enhanced bioturbation* - suggest that transmission of isotope signals across the sediment-water interface is governed less by sediment-internal redox zonation than by physical substrate transport. Diffusion limitation appears to be the overarching control, acting as an efficient filter that dampens sediment-scale variability in N-cycling before it becomes expressed in the overlying water, and ultimately irons out differences among sediments at the ecosystem scale. Nevertheless, while transport limitation establishes this generally low baseline expression of ϵ_{app} , NO_3^- regeneration may provide an additional level of modulation. Below, we consider the effects of further altering the experimental boundary conditions by inhibiting NO_3^- regeneration via nitrification, while the diffusive transport barrier is maintained or completely removed.

4.2 Influence of nitrate regeneration on apparent nitrate isotope effects

Nitrification inhibition under an intact oxic transport barrier - In the whole-core incubations amended with ATU to inhibit nitrification, the natural denitrification-zone geometry, including the unaltered O_2 penetration depth, was maintained. Consequently, the oxic/diffusive transport barrier separating the overlying water from the suboxic/anoxic denitrification zone remained unchanged. Assuming effective suppression of nitrification by the inhibitor (Martens-Habbena et al., 2015), differences between the inhibited and *Reference* incubations can therefore be attributed primarily to the removal of a nitrification-derived NO_3^- supply. This experimental comparison therefore allows us to evaluate the extent to which NO_3^- regeneration further modulates the apparent isotope effects established under transport-limited conditions (or contributes to the low apparent isotope effect expression). The two lake systems showed distinctly different responses. In oligotrophic Lake Sarnen, the $^{15}\epsilon_{\text{app_nit_inh}}$ remained unchanged compared to the *Reference* treatment, whereas $^{18}\epsilon_{\text{app}}$ increased significantly to 4 ‰ ($^{18}p = 0.034$), indicating a marked decoupling of the dual-isotope response. Nitrification produces isotopically light NO_3^- relative to the denitrification- ^{15}N -enriched NO_3^- pool and can dilute the apparent denitrification signal (Sigman et al., 2005; Granger and Wankel, 2016; Boshers et al., 2019; Dale et al., 2022; Mazzoli et al., 2026b). If this nitrification-derived NO_3^- contributes substantially to the NO_3^- pool, suppression of nitrification would be expected to result in a coupled enrichment of both ^{15}N - and ^{18}O - NO_3^- (i.e. stand-alone denitrification; Granger and Wankel 2016). However, because only $^{18}\epsilon_{\text{app}}$ increased, the elevated $^{18}\epsilon_{\text{app}}$ under nitrification inhibition cannot readily be explained by suppression of nitrification alone. This decoupling points to an additional process affecting the dual-isotope expression, which is further explored in Sect. 4.3. In contrast, Lake Baldegg sediments showed a substantial increase in both $^{15}\epsilon_{\text{app}}$ and $^{18}\epsilon_{\text{app}}$ upon nitrification inhibition, reaching



485 6.4 ‰ and 8.9 ‰, respectively. This coupled increase in the absence of NO_3^- regeneration indicates that, in addition to the generally low apparent isotope effects imposed by transport limitation, isotopically light NO_3^- produced by nitrification can further suppress the expression of denitrification isotope effects where NO_3^- regeneration contributes substantially to the NO_3^- pool, as is likely the case in eutrophic Lake Baldegg. This interpretation is consistent with isotope mass-balance estimates by Mazzoli et al. (2026a) that indicated nitrification maintained up to 82 % of the hypolimnetic NO_3^- pool during stratification in
 490 Lake Baldegg.

Nitrification suppression following removal of the oxic transport barrier - In the *Anoxic* treatment, aerobic nitrification was instead suppressed by O_2 exclusion rather than by chemical inhibition. As in the inhibitor treatment, this effectively eliminated NO_3^- regeneration. Unlike in the inhibitor treatment, however, O_2 exclusion also altered the vertical redox structure of the
 495 sediment (Cojean et al., 2019), shifting the denitrification zone upward toward the sediment surface. Consequently, the oxic diffusive transport barrier between the NO_3^- reduction zone and the overlying water was effectively removed, as transport was no longer constrained by an intervening oxic sediment layer (Brandes and Devol, 1997; Lehmann et al., 2007; Alkhatib et al., 2012). Thus, the *Anoxic* treatment simultaneously eliminated NO_3^- regeneration and removed the oxic transport barrier, allowing us to evaluate the additional influence of transport limitation on the expression of apparent isotope effects. Additional
 500 control experiments confirmed that denitrification remained restricted to the sediments, with no detectable denitrification in the water column under the experimental conditions (Fig. 1C).

Under anoxic conditions, the NO_3^- N and O isotope effects increased substantially in both lakes relative to the *Reference* treatments, reaching 8.3-10.5 ‰ (see Table 3, Fig. 2A-D), broadly similar to apparent isotope effects (~ 7 ‰) reported for marine sediments overlain by anoxic waters (Dale et al., 2019). Compared to the *Nitrification-inhibited* treatment, both N and
 505 O isotope effects increased significantly in Lake Sarnen ($^{15}\text{p} < 0.001$, $^{18}\text{p} < 0.001$), whereas only the N isotope effect increased significantly in Lake Baldegg ($^{15}\text{p} = 0.005$, $^{18}\text{p} = 0.808$). This stronger response in Lake Sarnen is consistent with its more than twofold greater O_2 penetration depth compared with Lake Baldegg (Table 2), implying a longer diffusive-transport pathway between the overlying water and the NO_3^- reduction zone under oxic conditions (Brandes and Devol, 1997). In addition, the finer-grained Lake Sarnen sediments, with a larger contribution of clay-sized to fine-silt-sized particles than Lake Baldegg
 510 sediments (Supplementary Information Fig. S3), may have further restricted effective solute exchange. In Lake Baldegg, by contrast, the shallower O_2 penetration depth and coarser-grained, less compact sediment texture may explain the closer agreement between the *Anoxic* and *Nitrification-inhibited* treatments. There, the shorter diffusive-transport pathway would have contributed comparatively little beyond removal of the nitrification overprint. Consequently, isotope effects under anoxic conditions did not differ significantly between the two lakes ($^{15}\text{p} = 0.104$, $^{18}\text{p} = 0.651$).

515 Nevertheless, even in the *Anoxic* whole-core experiments, where isotope effects were the highest, the intrinsic biological isotope effect was still only relatively weakly expressed in the overlying water. Apparent isotope effects remained strongly suppressed relative to the biological isotope effects determined for the same sediments (Lake Sarnen: $^{15}\epsilon_{\text{bio}} = 17$ ‰, $^{18}\epsilon_{\text{bio}} = 13.6$ ‰; Lake Baldegg: $^{15}\epsilon_{\text{bio}} = 25.3$ ‰, $^{18}\epsilon_{\text{bio}} = 20.7$ ‰; Paulus et al., 2026), in agreement with observations from



marine oxygen-minimum-zone sediments (Dale et al., 2019). This persistent suppression indicates that even after eliminating
520 nitrification-derived inputs of isotopically light NO_3^- and effectively removing the oxic transport barrier (i.e., reducing the
diffusion distance to the NO_3^- reduction zone), only part of the biological denitrification isotope signal generated within the
sediment was transmitted back across the sediment-water interface. Thus, although apparent isotope effects associated with
net NO_3^- consumption in the sediment increased substantially under anoxic conditions, diffusive exchange across the sediment-
water interface continued to limit their full expression. These results experimentally confirm the model predictions of Lehmann
525 et al. (2007) that the depth of the denitrification zone, through its control on transport limitation, exerts a major influence on
ecosystem-scale isotope-effect expression. Although apparent isotope effects remained considerably lower than the intrinsic
biological isotope effects of denitrification, they nevertheless reached values of up to $\sim 10\%$, demonstrating that under
conditions of a shallow sedimentary NO_3^- reduction zone, benthic denitrification can impart a substantial isotopic imprint on
the overlying NO_3^- pool. Such apparent isotope effects are sufficiently large to influence isotope-based estimates of benthic N
530 removal and should therefore be considered in quantitative NO_3^- isotope budgets.

4.3 Dual isotope dynamics of NO_3^-

Covariation of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ provides a framework for assessing contributions from the opposing processes of NO_3^- reduction
and regeneration. Relationships in $\Delta\delta^{18}\text{O}\text{-NO}_3^-:\Delta\delta^{15}\text{N}\text{-NO}_3^-$ near 1:1 indicate relatively coherent dissimilatory NO_3^- reduction,
whereas deviations from this pattern point to additional controls, including alternative enzymatic NO_3^- reduction mechanism
535 (Granger et al., 2008; Asamoto et al., 2021) and NO_3^- regeneration via nitrification (Sigman et al., 2005; Buchwald and
Casciotti, 2010; Boshers et al., 2019) or anammox (Brunner et al., 2013; Dähnke and Thamdrup, 2013; Granger and Wankel,
2016; Kobayashi et al., 2019, 2025; Magyar et al., 2021). The different incubation treatments applied here imparted a wide
range of dynamics in NO_3^- dual isotopes; here we evaluate whether this comparison yields any additional mechanistic
information beyond the apparent isotope effects discussed above (Fig. 3). Yet, because several treatments exhibited only small
540 apparent isotope effects and correspondingly limited changes in NO_3^- isotope composition, $\Delta\delta^{18}\text{O}\text{-NO}_3^-:\Delta\delta^{15}\text{N}\text{-NO}_3^-$
relationships are inherently sensitive to analytical and experimental uncertainty. We therefore focus on whether coherent
relationships emerge across treatments and on broad deviations from the near-unity relationship expected for dissimilatory
 NO_3^- reduction, rather than on precise slope values.

The clearest and most interpretable pattern was observed in the *Anoxic* Lake Baldegg incubations, where the
545 $\Delta\delta^{18}\text{O}\text{-NO}_3^-:\Delta\delta^{15}\text{N}\text{-NO}_3^-$ ratio of 0.97 matched the expectation for Nar-mediated dissimilatory NO_3^- reduction (Fig. 3C). This
outcome indicates that coupled N and O isotope enrichment was most clearly resolved when aerobic nitrification was excluded
and the diffusive-transport barrier was minimized. The ratio was even closer to unity than those obtained in previous batch
incubations with the same sediments (bag: 0.82; slurry: 0.85-0.95; Paulus et al., 2026). *Anoxic* Lake Sarnen incubations also
showed a clear linear relationship between $\Delta\delta^{18}\text{O}\text{-NO}_3^-$ and $\Delta\delta^{15}\text{N}\text{-NO}_3^-$ and a ratio (0.79) within the range expected for



enzymatic NO_3^- reduction (0.5-1, Asamoto et al. 2021) and comparable to those observed in Lake Sarnen batch incubations (0.82, Paulus et al., 2026) and in Lake Lugano (0.83-0.89; Wenk et al. 2014b, a). The lower ratio compared with Lake Baldegg sediments may reflect differences in the relative contribution of alternative NO_3^- reductases - Nar-mediated NO_3^- reduction is generally associated with ratios close to unity (Granger et al., 2008; Karsh et al., 2012; Wunderlich et al., 2012; Treibergs and Granger, 2017; Asamoto et al., 2021; Tischer et al., 2026; Kritee et al., 2012), whereas periplasmic NO_3^- reductase (Nap) has been linked to lower ratios (0.5-0.7; Granger et al. 2008; Frey et al. 2014b; Treibergs and Granger 2017; Asamoto et al. 2021). Alternatively, secondary NO_3^- regeneration processes, especially anammox, may have contributed in Lake Sarnen. While anammox activity was negligible in Lake Baldegg, it was more relevant in Lake Sarnen, reaching 30.9 % of the denitrification rate (Paulus et al., 2026). Anammox-related NO_3^- regeneration has been associated with very low $\Delta\delta^{18}\text{O}-\text{NO}_3^-:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ ratios (< 0.3 , Kobayashi et al. 2025).

These *Anoxic* experiments, matching expected NO_3^- isotopic trends during denitrification, provide a clear context for the *Reference* experiments from both Lakes Baldegg and Sarnen. Most data are clustered around the origin in $\Delta\delta^{15}\text{N}-\text{NO}_3^-$ and $\Delta\delta^{18}\text{O}-\text{NO}_3^-$, reflecting visually the low values of ϵ_{app} (Fig. 3A). In Lake Baldegg, a subset of data (*Reference B*, slope = 1.86) have a slope steeper than 1, possibly reflecting denitrification overprinted by nitrification. Likewise, in Lake Sarnen, results from *Reference A* (slope = -1.13) suggest a role for significant contributions from anammox or other NO_3^- processes. In both lakes, inhibition of nitrification using ATU induced some change in the observed results. The result in Lake Baldegg falls between the *Anoxic* and *Reference* experiments, with relatively elevated values of $^{15/18}\epsilon_{\text{app}}$ and a slope of 1.39, illustrating the role NO_3^- regeneration by nitrification plays in this setting. In the inhibitor treatment, nitrification was chemically suppressed but may not have been completely eliminated (Martens-Habbenha et al., 2015); therefore, small residual inputs of isotopically light, nitrification-derived NO_3^- could have dampened the ^{15}N enrichment and shifted the $\Delta\delta^{18}\text{O}-\text{NO}_3^-:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ ratio towards higher values. In Lake Sarnen the differences between *Reference* and inhibitor treatments are less straightforward to interpret. The *Reference* treatment yielded a negative $\Delta\delta^{18}\text{O}-\text{NO}_3^-:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ relationship, whereas the inhibitor treatment exhibited a comparatively high ratio, with the difference driven entirely by the evolution in $\delta^{18}\text{O}-\text{NO}_3^-$. Ratios exceeding unity are commonly interpreted as indicative of NO_3^- regeneration via nitrification (Granger and Wankel, 2016; Dale et al., 2022; Mazzoli et al., 2026b). The high ratio in the *Nitrification inhibition* treatment is therefore unlikely to reflect nitrification-driven NO_3^- regeneration, because this mechanism would predict higher ratios in the *Reference* treatment, regardless of whether the inhibition was complete. Although ATU is primarily used to suppress aerobic NH_4^+ oxidation, partial non-specific effects on anammox and denitrification cannot be fully excluded, as Jensen et al. (2007) reported an approximately 20 % lower rate for both processes under some incubation conditions. However, because ATU did not selectively inhibit anammox, this effect is unlikely to explain treatment-specific changes in the $\Delta\delta^{18}\text{O}-\text{NO}_3^-:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ ratio on its own. Which secondary processes were modulated during inhibition remains unclear; without additional controls, this observation cannot be constrained, and given the limited replication, it may also reflect replicate variability.



Finally, in whole-core incubations with modified sediment reactivity, NO_3^- dual isotopes show substantial variability not systematically controlled by organic matter quantity (Fig. 3D). Two cases - *Reduced reactivity A* and *Low reactivity B* - match the *Reference* experiments well with low $^{15}\epsilon_{\text{app}}$ and $^{18}\epsilon_{\text{app}}$, but with markedly lower $\Delta\delta^{18}\text{O}-\text{NO}_3^-:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ ratios (0.60 and 0.87, respectively), suggesting persistent suppression of ϵ_{bio} but still a significant role for expression of NO_3^- reduction isotope effects. In contrast, the inverse isotope effects apparent for $^{18}\epsilon_{\text{app}}$ in the experiments *Reduced reactivity B* and for both $^{15}\epsilon_{\text{app}}$ and $^{18}\epsilon_{\text{app}}$ in *Low reactivity A* demonstrate that the perturbation of microbial N cycling through sediment pre-oxidation facilitates the autotrophic processes such as nitrification or anammox, which impart inverse isotope effects that may then overprint those of dissimilatory NO_3^- reduction. Although only limited conclusions can be drawn from individual incubations, these cases provide phenomenological evidence for additional ways in which NO_3^- regeneration can, under specific conditions, markedly alter both the magnitude of the apparent NO_3^- isotope effect and the associated dual-isotope behaviour.

5 Conclusions

We quantified the apparent isotope effects of sedimentary net NO_3^- reduction expressed in the overlying water under natural and experimentally modulated conditions, using sediments from oligotrophic Lake Sarnen and eutrophic Lake Baldegg. Across oxic and anoxic intact whole-core incubations of both lakes, apparent N and O isotope effects in the overlying water were suppressed relative to biological isotope effects measured for the same sediments (Paulus et al., 2026). This suppression was strongest in oxic incubations, and persisted across contrasting sediment reactivity, NO_3^- fluxes, O_2 penetration depths, OM contents, and enhanced bioturbation. These observations identify transport limitation, specifically diffusive exchange between the sedimentary reaction zone and the sediment-overlying water, as the overarching control governing the expression of apparent isotope effects at the ecosystem scale.

Manipulating nitrification and redox zonation revealed superimposed processes/controls that modulate this transport-limited low ϵ_{app} baseline. First, NO_3^- regeneration via nitrification can further suppress the apparent isotope effects of denitrification by supplying newly produced, isotopically light NO_3^- , as most clearly indicated by the concurrent increase in N and O isotope effects following nitrification inhibition in Lake Baldegg. In Lake Sarnen, by contrast, nitrification inhibition alone was not sufficient to recover a clear coupled denitrification isotope signal, indicating that the oxic diffusive barrier remained sufficiently effective to limit isotope-signal transmission. Second, when the oxic diffusive barrier and nitrification-derived overprinting were removed by making the overlying water anoxic, apparent isotope effects increased relative to the near-natural *Reference* treatments in both lakes, reaching values up to $\sim 10\%$. This experimentally confirms the prediction of Lehmann et al. (2007) that the depth of the sedimentary NO_3^- -reduction zone exerts a major influence on ecosystem-scale isotope-effect expression through its control on transport limitation. These results demonstrate that benthic denitrification can impart a substantial isotopic imprint on the overlying NO_3^- pool when the NO_3^- -reduction zone is located close to the sediment-



water interface. Even under these conditions, however, ϵ_{app} remained below the intrinsic biological isotope effects, indicating that transport limitation continued to prevent full expression of the sedimentary isotope signal.

615 Dual-isotope relationships provided complementary mechanistic information on benthic N cycling. Suppressing NO_3^- regeneration and relaxing transport limitation revealed the clearest expression of coupled N- and O-isotope effects. Under these simplified conditions, the dual-isotope relationships approached the expected behaviour of dissimilatory NO_3^- reduction, revealing near-unity coupling in Lake Baldegg and a lower ratio that was nevertheless consistent with enzymatic NO_3^- reduction in Lake Sarnen. Under natural benthic conditions, by contrast, the generally small variation in $\Delta\delta^{18}\text{O}-\text{NO}_3^-$ and
 620 $\Delta\delta^{15}\text{N}-\text{NO}_3^-$ characteristic of transport-limited benthic systems limit the precision with which individual $\Delta\delta^{18}\text{O}-\text{NO}_3^-:\Delta\delta^{15}\text{N}-\text{NO}_3^-$ ratios can be resolved. Nevertheless, the overall NO_3^- dual-isotope patterns remained broadly consistent with the expected influence of NO_3^- regeneration, demonstrating that dual-isotope relationships retain considerable mechanistic value for describing the competing roles of NO_3^- reduction, regeneration, and transport limitation.

Together, these whole-core incubations provide direct experimental constraints on the scale-dependent transfer of NO_3^- isotope effects from sedimentary reaction zones to the overlying water. They demonstrate that ϵ_{app} is a transport-filtered ecosystem-scale signal shaped primarily by the efficiency of isotope-signal transmission across the sediment-water interface, which is strongly influenced by the vertical position of the denitrification zone, and secondarily modulated by concurrent processes, particularly NO_3^- regeneration via nitrification. Our results therefore experimentally substantiate and refine previous model- and field-based interpretations of diffusion-limited isotope-effect expression in benthic environments (e.g. Brandes and Devol
 630 1997; Lehmann et al. 2007; Rooze and Meile 2016). They demonstrate that diffusion limitation not only suppresses ϵ_{app} , but also largely masks the influence of sediment reactivity, redox zonation, and fauna-mediated transport on the isotope signal ultimately expressed in the overlying water. These findings provide a mechanistic framework for interpreting apparent NO_3^- isotope effects in benthic environments and improve the quantitative use of NO_3^- isotopes to constrain sedimentary nitrogen removal in natural aquatic systems.

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Data availability

The data supporting the findings of this study are publicly available in the on zenodo at <https://doi.org/10.5281/zenodo.22810348>.

Author contributions

640 Conceptualization: T.J.P. and M.F.L.; Data Curation: T.J.P.; Formal Analysis: T.J.P.; Funding Acquisition: M.F.L. and T.J.P.; Investigation: T.J.P., A.M. and T.K; Methodology: T.J.P., M.F.L., P.M.M., C.F., C.M.C., J.Z. and T.K.; Project Administration: M.F.L.; Resources: M.F.L; Supervision: M.F.L.; Visualization: T.J.P. and A.M.; Writing - original draft: T.J.P. and M.F.L.; Writing - review & editing: all co-authors.

Competing interests

645 The authors declare that they have no conflict of interest.

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