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Effects of denitrification and transport on the isotopic composition of nitrate ($\delta^{18}O$, $\delta^{15}N$) in freshwater systems

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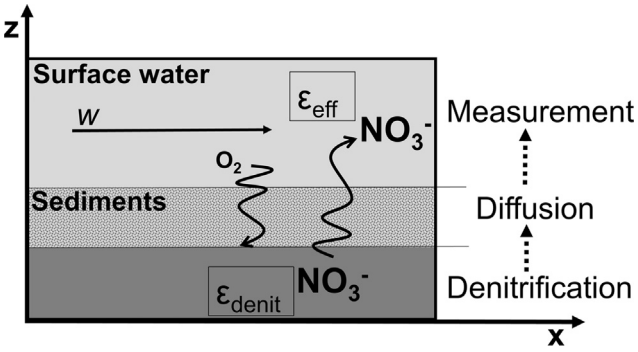
HIGHLIGHTS

- Isotope fractionation during benthic NO_3^- reduction can vary widely between sites.
- Diffusive transport homogenised local variations in NO_3^- isotope fractionation.
- Hydrology affects the use of NO_3^- isotopes to assess freshwater denitrification.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrate isotopes ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) are a potentially powerful tool for tracking the biological removal of reactive nitrogen (N) as it is transported from land to sea. However, uncertainties about, 1) the variability of the strength of biological isotopic fractionation during anaerobic benthic NO_3^- reduction (the kinetic enrichment factor: ε_{denit}), and, 2) how accurately these ε_{denit} values are expressed in overlying aerobic surface waters (the effective enrichment factor: ε_{eff}), currently limit their use in freshwater systems. Here we used a combination of incubation experiments and numerical modelling to construct a simple framework for defining freshwater ε_{denit} based on interactions between benthic denitrification and diffusive transport to surface waters. Under non-limited, anaerobic conditions the ε_{denit} values produced in submerged soils ($n = 3$) and sediments ($n = 4$) with denitrification rates between 10 and 600 mg N m^{-2} d^{-1} ranged from −3‰ to −28‰. Critically, model results indicated that diffusive transport would homogenise this to an effective fractionation range of -6 ± 4‰. Evidence for biological and hydrological variability of NO_3^- isotope fractionation means that values measured in aerobic surface water environments are most appropriately evaluated by a range of fractionation values, rather than commonly used single 'site specific' ε_{denit} values.

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1. Introduction

Nitrate dual isotopes ($\delta^{18}O$, $\delta^{15}N$) are now popular tools for distinguishing between freshwater nitrogen (N) contamination from atmospheric, agricultural, and sewage sources (Ji et al., 2017; Xue et al., 2009). These isotopes also contain information that can be used to generate integrative measures of denitrification (N attenuation via the step-wise reduction of NO_3^- to nitrous oxide (N_2O) and dinitrogen (N_2) using carbon (C) as an electron donor) over time or distance (Granger and Wankel, 2016; Mariotti et al., 1981; Søvik and Mørkved, 2008). This has enabled NO_3^- isotope data to provide critical constraints on marine denitrification (Bourbonnais et al., 2015; Sigman et al., 2005). Comparable use of isotope information could provide critical information for managing the 'cascade' of environmentally deleterious effects caused by land-to-sea N transport (Galloway et al., 2003). However, NO_3^- isotopes are largely overlooked as a denitrification metric in surface water environments due to uncertainties in the isotopic fractionation underpinning it.

Nitrate isotopes can be used to quantify denitrification because microbes preferentially use lighter isotopes, as described by Rayleigh kinetics (Eq. (1)):

$$\frac{R}{R_0} = \left(\frac{C}{C_0}\right)^{\alpha_{denit}} \quad (1)$$

where the measured ratio of heavy to light isotopes in the sample (R) relative to the ratio in the original pool (R_0) is directly related to the residual substrate concentration (C) relative to its initial concentration (C_0) by the strength of preference for light isotopes during denitrification (α). The fractionation factor for denitrification, α_{denit}, is subsequently referred to in the more common δ notation, which normalises the abundance of heavy:light isotopes to that of a standard material ($\delta‰ = (R/R_{standard} - 1) \times 1000$), as ε_{denit}, where $\varepsilon_{denit} = (\alpha_{denit} - 1) \times 1000$ (Kendall, 1998; Mariotti et al., 1981). Biological isotopic fractionation occurs as NO_3^- is transported across denitrifier cell walls (Kritee et al., 2012). The $^{15}\varepsilon_{denit}$ (ε_{denit} for $^{15/14}N$) of different microbial strains vary between −2‰ and −30‰, with ratio between $^{18}\varepsilon_{denit}$ (ε_{denit} for $^{18/16}O$) to $^{15}\varepsilon_{denit}$ of 1:1 (Granger et al., 2008; Kritee et al., 2012).

However, substantial uncertainties arise when applying this approach to aerobic surface waters, where denitrification occurs in the underlying benthos. First, ε_{denit} values for the microbially complex benthic sediments are poorly constrained. Increasing denitrification rates by increasing temperature (T) or adding labile C may decrease ε_{denit} values (Korom et al., 2012; Mariotti et al., 1981; Mariotti et al., 1982). Yet reported relationships between C, denitrification, and ε_{denit} are inconsistent: pure culture studies show that altering C quality can either alter denitrification rates independent of ε_{denit} (Knöller et al., 2011) or alter ε_{denit} independent of denitrification rates (Wunderlich et al., 2012). Second, hydrologic transport may cause these biologically-produced ε_{denit} values to be incompletely reflected in the isotope patterns measured in the surface water NO_3^- pool (effective fractionation: ε_{eff}). This is because transport of NO_3^- from the denitrifying zone (sediments) to the measurement zone (surface water) can cause mass-dependent fractionation as light isotopes diffuse faster than heavy isotopes (Richter et al., 2006). 'Masking' by a diffusive limitation is used to explain why fractionation during denitrification in marine sediments is negligible (Brandes and Devol, 1997; Lehmann et al., 2007), but its effect on sediment-surface water NO_3^- isotope fluxes remains poorly constrained. This makes ε_{eff} values, and thus the interpretation of NO_3^- isotope signatures, particularly uncertain in aerobic freshwater environments.

Here we aim to complement recent findings that the competing processes of NO_3^- production (nitrification) and reduction (denitrification) affect surface water $^{18}\varepsilon_{denit}$:$^{15}\varepsilon_{denit}$ ratios (Granger and Wankel, 2016) by quantifying: 1) the site-specificity of ε_{denit}, and, 2) the effect of diffusive transport from denitrifying zones to the water column on these values (Fig. 1). The mathematical framework is intended for understanding isotope effects resulting from denitrification along flowpaths with upwelling NO_3^- (e.g., gaining streams and agricultural drains), but potentially also includes soil macropores.

2. Materials and methods

2.1. Incubation experiments

To understand site variations in ε_{denit}, which we practically defined as fractionation within the anaerobic denitrifying zone, substrates were collected from two locations: A) Harts Creek, a shallow spring-fed stream in the Canterbury plains region of New Zealand (43°46′S, 172°16′E), and, B) submerged paddies at the International Rice Research Institute experimental farm (IRRI) in the Philippines (14°1′N; 121°15′E). Sampled locations within A ($n = 4$) and B ($n = 3$) were selected to span critical environmental gradients associated with denitrification and microbial community change. In (A) sediments were collected over a gradient of increasing discharge and water depth and decreasing organic matter between the stream's source spring (A1) and mouth (A4) (Findlay et al., 2011; Garcia-Ruiz et al., 1998; Wells et al., 2016). This design provided a snapshot of regional (cross-biome) versus local (m-km) scale variations in freshwater denitrifier communities, and thus ε_{denit} (Fig. 2).

In (B) soils were collected from fields with different clay contents and cultivation histories: B1 (61% clay) had two irrigated rice crops a year for >20 years, B2 (33% clay) had zero to two irrigated rice crops a year for ~10 years, and B3, located ~500 m from B1, was uncultivated (Bannert et al., 2011; Buresh et al., 2008). Twenty cores (10 cm depth × 5 cm diameter) were collected from each location using a pre-prepared randomised grid to capture a representative denitrifier community (Bissett et al., 2010). Subsamples (~50 g) were collected to establish antecedent nutrient concentrations.

After collection, samples were bulked and sieved (<0.5 mm) to remove roots, larger particles and soil fauna. To discern fundamental differences in ε_{denit} between sites and locations, anaerobic incubations were carried out under non-substrate limited, continuously mixed, and temperature controlled (20 °C) conditions (Kritee et al., 2012). Glucose-C (1 mM) and KNO_3 (25 mg N l^{-1}) were added after an initial pre-incubation period, and samples collected up to

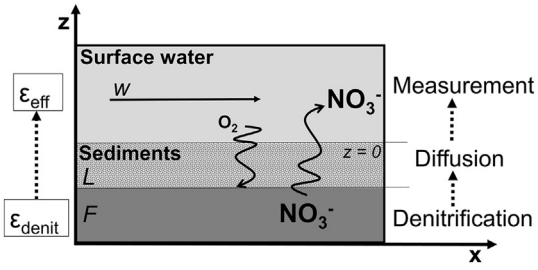

Fig. 1. Nitrate measured in aerobic freshwater environments is influenced by denitrification occurring in the anaerobic sediments over time and space (x). The effect of sediment denitrification on NO_3^- isotopic composition in the aerobic surface water (ε_{eff}) therefore depends on, 1) the site-specific ε_{denit} conferred during denitrification in the reactive layer located between the aerobic and anaerobic sediment zones (F, dark grey box), and, 2) physical modification to NO_3^- concentrations and isotopic composition during diffusive transport within the sediments between the denitrifying zone and the sediment-water interface ($z = 0$) in equilibrium with the surface water (measurement zone; light grey). The distance of diffusive transport is determined by the depth of O_2 penetration (L; textured area), while advective transport in the x direction (w) controls NO_3^- availability.