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Multi-species measurements of nitrogen isotopic composition reveal the spatial constraints and biological drivers of ammonium attenuation across a highly contaminated groundwater system

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ABSTRACT

Groundwater under industrial sites is characterised by heterogeneous chemical mixtures, making it difficult to assess the fate and transport of individual contaminants. Quantifying the *in-situ* biological removal (attenuation) of nitrogen (N) is particularly difficult due to its reactivity and ubiquity. Here a multi-isotope approach is developed to distinguish N sources and sinks within groundwater affected by complex industrial pollution. Samples were collected from 70 wells across the two aquifers underlying a historic industrial area in Belgium. Below the industrial site the groundwater contained up to 1000 mg N l^{-1} ammonium (NH_4^+) and 300 mg N l^{-1} nitrate (NO_3^-), while downgradient concentrations decreased to ~1 mg l^{-1} DIN ([DIN] = [NH_4^+–N] + [NO_3^-–N] + [NO_2^-–N]). Mean δ^{15}N-DIN increased from ~2‰ to +20‰ over this flow path, broadly confirming that biological N attenuation drove the measured concentration decrease. Multi-variate analysis of water chemistry identified two distinct NH_4^+ sources ($\delta^{15}N-NH_4^+$ from −14‰ and +5‰) within the contaminated zone of both aquifers. Nitrate dual isotopes co-varied (δ^{15}N: −3‰ – +60‰; δ^{18}O: 0‰ – +50‰) within the range expected for coupled nitrification and denitrification of the identified sources. The fact that $\delta^{15}N-NO_2^-$ values were 50‰–20‰ less than $\delta^{15}N-NH_4^+$ values in the majority of wells confirmed that nitrification controlled N turnover across the site. However, the fact that $\delta^{15}N-NO_2^-$ was greater than $\delta^{15}N-NH_4^+$ in wells with the highest [NH_4^+] shows that an autotrophic NO_2^- reduction pathway (anaerobic NH_4^+ oxidation or nitrifier-denitrification) drove N attenuation closest to the contaminant plume. This direct empirical evidence that both autotrophic and heterotrophic biogeochemical processes drive N attenuation in contaminated aquifers demonstrates the power of multiple N isotopes to untangle N cycling in highly complex systems.

1. Introduction

Global freshwater resources, 30% of which are held in subsurface aquifers, are under pressure due to the combination of increased human demand and decreasing natural supply (Griebler and Avramov, 2015; Klove et al., 2014). Effective means of remediating (removing) groundwater contaminants are therefore needed as on-going pollution simultaneously diminishes the supply of potable water. Groundwater management strategies are often limited by a poor understanding of the biogeochemical controls on contaminant cycling. Improving measurements of nitrogen's (N) fate and transport in groundwater is a priority due to both its ubiquity, and the 'cascade' of environmentally deleterious outcomes produced during transport due to its reactivity (Galloway et al., 2003). In natural systems, groundwater [N] is determined by residence time (Hinkle and Tesoriero, 2014). However, diffuse nitrate (NO_3^-) inputs (excess soil fertilisation, animal excreta) and point ammonium (NH_4^+) inputs (sewage, industrial effluent) overwhelm time-based constraints on N fate and transport. Turnover is complicated further in industrially contaminated sites, where multiple, asynchronous, contaminants (including salts, heavy metals, and hydrocarbons) can alter both the processes and rates of N transformations (Kleinsteuber et al., 2012; Ponsin et al., 2014).

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Attenuation of groundwater N (defined as the conversion of reactive N species to inert nitrogen gas (N_2)) is thought to be driven by denitrification, the step-wise reduction of NO_3^- to nitrous oxide (N_2O) and N_2. Biological denitrification occurs under anaerobic conditions, using carbon (C) or sulphide minerals, as electron donors (Burgin and Hamilton, 2008; Rivett et al., 2008). Abiotic denitrification (chemodenitrification) that uses iron as an electron donor an occur, although its prevalence remains uncertain (Jones et al., 2015). The attenuation of NH_4^+ in groundwater therefore depends on the coupling of NH_4^+ oxidation (nitrification: autotrophic conversion of ammonia (NH_3) to nitrite (NO_2^-) and then NO_3^- under aerobic conditions) with denitrification (Izbicki, 2014). This limits N attenuation to the plume fringe, as anaerobic conditions within the plume inhibit nitrification while oxygen (O_2) outside of the plume inhibits denitrification (Meckenstock et al., 2015). Yet evidence for the importance of processes such as anaerobic NH_4^+ oxidation (anammox: autotrophic conversion of NH_4^+ and NO_2^- to N_2 (Sonthiphand et al., 2014)), co-denitrification (conversion of NO_2^- and organic N to $N_2O + N_2$ (Selbie et al., 2015)), and nitrifier-denitrification (reduction of NO_2^- to $N_2O + N_2$ by autotrophic nitrifying bacteria (Kool et al., 2010)) challenge the assumption that NH_4^+ attenuation is controlled by coupled nitrification-denitrification. The different energetic controls on these attenuation pathways make identifying their role in N turnover fundamental to the development of effective remediation schemes.

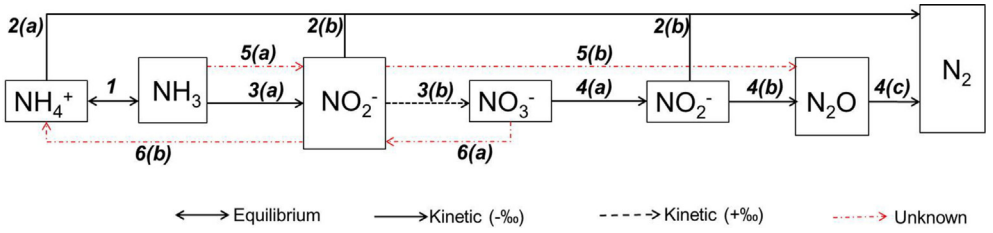
However, accurately measuring the importance of these pathways in contaminated systems is difficult. Modelling N losses from redox chemistry is complicated by the fact that N transformations occur in micro-scale 'hot spots' that are easily missed in such regional-scale sampling campaigns (Meckenstock et al., 2015; Rivett et al., 2008). Stoichiometric approaches can be used to estimate N attenuation rates and/or source mixing (Koh et al., 2010; Murgulet and Tick, 2013), but cannot be used in many contaminated groundwater sites when multiple sources of multiple chemical contaminants violate assumptions of mass conservation. Injecting ^{15}N labels, a typically robust tool for measuring N attenuation (Kellogg et al., 2005), is also not viable in many contaminated sites as it relies on the presence of a conservative tracer.

Advances in analysing the natural abundance composition of N species therefore create a potentially unique opportunity to assess N attenuation in contaminated groundwater (Hatzinger et al., 2013). This approach is based on the knowledge that the preferential use of heavy v. light isotopes during microbial reactions creates predictable Rayleigh-based patterns in the residual substrate pool: the ratio between the measured and initial substrate concentration (C/C_0) is related to the ratio between its measured and initial isotopic composition (R/R_0) by the reaction-specific fractionation factor (α) (Eq. (1)).

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

Isotope values are reported in δ‰, where the relative concentration is normalised to a standard; α values are reported as enrichment factors (ε; $\varepsilon = (\alpha - 1) \times 1000$). ε values are known for a growing number of N processes (Table 1): generally microbial preference for light isotopes causes the $\delta^{15}N$ of the residual substrate to increase as the reaction progresses ($\varepsilon = -$‰), although some reactions cause inverse fractionation ($\varepsilon = +$‰). As physical [N] changes (dilution or sorption) do not affect $\delta^{15}N$ composition, $\delta^{15}N$ patterns over time/distance can be used distinguish biological turnover from transport (Fenech et al., 2012).

Table 1
Overview of the microbial processes potentially affecting N fate in NH_4^+ contaminated aquifers. The N isotopic fractionation factors ($^{15}\varepsilon$) for each step of each pathway are listed in table. ***(1)*** pH determines the chemical equilibrium between NH_4^+ and NH_3, across which $^{15}\varepsilon_{eq}$ is constant. ***(2)*** Under anaerobic conditions, NH_4^+ ($^{15}\varepsilon_{amx,NH4}$) can be coupled with NO_2^- ($^{15}\varepsilon_{amx,NO2}$) to create N_2 by anammox bacteria and archaea. ***(3)*** Under aerobic conditions, NH_3 is oxidised to NO_2^- ($^{15}\varepsilon_{amo,NH3}$) and then NO_3^- ($^{15}\varepsilon_{amo,NO2}$). ***(4)*** Denitrification sequentially reduces NO_3^- to NO_2^- ($^{15}\varepsilon_{denit,NO3}$), N_2O ($^{15}\varepsilon_{denit,NO2}$), and N_2 ($^{15}\varepsilon_{denit,N2O}$) under anaerobic conditions by using C as an electron donor. Denitrification driven by mineral oxidation (chemodenitrification) is also possible. ***(5)*** NH_3 oxidation can progress to N_2O production under low O_2 conditions, bypassing production and reduction of NO_3^- ***(6)***, and DNRA can occur under electron donor rich, low O_2 conditions, both with unknown effects on the isotopic composition of NH_3, NO_3^- or NO_2^-.



ID	Process	Fractionation factor(s)	References
1	Chemical equilibrium	$^{15}\varepsilon_{eq} = 20$‰	Casciotti et al., 2003
2	Anammox	(a) $^{15}\varepsilon_{amx,NH4} = -27 \pm 3$‰ (b) $^{15}\varepsilon_{amx,NO2} = -16 \pm 5$‰	(a,b) Brunner et al., 2013
3	Ammonia oxidation	(a) $^{15}\varepsilon_{amo,NH3} = -14 \rightarrow -38$‰ (b) $^{15}\varepsilon_{amo,NO2} = +12.8$‰	(a) Casciotti et al., 2003 (b) Casciotti 2009
4	Denitrification/chemodenitrification[a]	(a) $^{15}\varepsilon_{denit,NO3} = -3 \rightarrow -30$‰ (b) $^{15}\varepsilon_{denit,NO2} = -5 \rightarrow -25$‰ (c) $^{15}\varepsilon_{denit,N2O} = -31 \rightarrow -25$‰	(a) Granger et al., 2008, Kritee et al., 2012, Sebilo et al., 2003, Jones et al., 2015 [a] (b) Bryan et al., 1983, Casciotti et al., 2002 (c) Sutka et al., 2003, 2004
5	Nitrifier-denitrification[b]	(a) $^{15}\varepsilon_{n\text{-}d,NH3} = ?$ (b) $^{15}\varepsilon_{n\text{-}d,NO2} = ?$	
6	DRNA[c]	(a) $^{15}\varepsilon_{DRNA,NO3} = ?$ (b) $^{15}\varepsilon_{DRNA,NO2} = ?$	

[a] Chemodenitrification causes comparable N isotope fractionation (Jones et al., 2015).
[b] Fractionation factors for nitrifier-denitrification have not been directly measured, but may reasonable be expected to be comparable to those for the NH_3 oxidation for step (a) as the same enzymes and microbial populations are involved (Kool et al., 2010; Colliver and Stephenson, 2000).
[c] There are no direct measurements of fractionation factors for DNRA, but anomalous relationships between $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ have been reported in regions where DNRA is known to occur (Dhondt et al., 2003).