

Scale-dependent linkages between nitrate isotopes and denitrification in surface soils: implications for isotope measurements and models

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Abstract Natural abundance nitrate (NO_3^-) isotopes represent a powerful tool for assessing denitrification, yet the scale and context dependence of relationships between isotopes and denitrification have received little attention, especially in surface soils. We measured the NO_3^- isotope compositions in soil extractions and lysimeter water from a semi-arid meadow and lawn during snowmelt, along with the denitrification potential, bulk O_2 , and a proxy for anaerobic microsites. Denitrification potential varied by three orders of magnitude and the slope of $\delta^{18}\text{O}/\delta^{15}\text{N}$ in soil-extracted NO_3^- from all samples measured 1.04 ± 0.12 ($R^2 = 0.64$, $p < 0.0001$), consistent with fractionation from denitrification. However, $\delta^{15}\text{N}$ of extracted NO_3^- was often lower than bulk soil $\delta^{15}\text{N}$ (by up to 24 ‰), indicative of fractionation during nitrification that was partially overprinted by denitrification. Mean NO_3^- isotopes in lysimeter water differed from soil extractions by up to 19 ‰ in $\delta^{18}\text{O}$ and 12 ‰ in $\delta^{15}\text{N}$, indicating distinct biogeochemical processing in relatively mobile water versus soil microsites. This implies that NO_3^- isotopes in streams, which are predominantly fed by mobile water, do not fully reflect

terrestrial soil N cycling. Relationships between potential denitrification and $\delta^{15}\text{N}$ of extracted NO_3^- showed a strong threshold effect culminating in a null relationship at high denitrification rates. Our observations of (1) competing fractionation from nitrification and denitrification in redox-heterogeneous surface soils, (2) large NO_3^- isotopic differences between relatively immobile and mobile water pools, (3) and the spatial dependence of $\delta^{18}\text{O}/\delta^{15}\text{N}$ relationships suggest caution in using NO_3^- isotopes to infer site or watershed-scale patterns in denitrification.

Keywords Isotope mass balance model · Mobile water · Nitrification · Redox · Snowmelt

Introduction

Assessing gaseous nitrogen (N) losses via denitrification from soils and watersheds remains a major challenge in our understanding of ecosystem N dynamics (Groffman 2012; Kulkarni et al. 2008; Yang et al. 2011). An improved understanding of the spatial and temporal controls on denitrification rates, and their importance in ecosystem N budgets, is critical for addressing problems linked to the cascading impacts of reactive nitrogen in our environment (Galloway et al. 2003). One important method for assessing spatial and temporal patterns in denitrification exploits the variation in the natural abundance isotopic composition ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) of nitrate (NO_3^-) and/or $\delta^{15}\text{N}$ of bulk soils to constrain denitrification losses using $\delta^{15}\text{N}$ mass balance (Bai and Houlton 2009; Houlton et al. 2006; Houlton and Bai 2009). Recent studies have expanded on this approach by combining $\delta^{15}\text{N}$ of NO_3^- with $\Delta^{17}\text{O}$, a sensitive tracer of atmospheric NO_3^- (Michalski et al. 2004), to derive gross NO_3^- production (Riha et al. 2014) and denitrification at

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the watershed scale (Fang et al. 2015). Natural abundance isotopes clearly represent a powerful and minimally invasive tool to assess patterns and controls on denitrification. However, several uncertainties remain with regards to the interpretation of natural abundance NO_3^- isotope measurements in the context of denitrification.

The degree to which NO_3^- stable isotopes can record patterns of denitrification in surface soil horizons (i.e., to a depth of tens of cm), as opposed to deeper subsurface or groundwater environments, has received little attention. It has long been known that significant hotspots of denitrification can occur in anaerobic microsites in otherwise aerobic soils (Parkin et al. 1987). Houlton et al. (2006) found strong evidence for denitrification in wet surface soils from humid tropical forests using natural abundance N isotopes. In contrast, Fang et al. (2015) used surface soil NO_3^- isotope compositions as inputs to models of watershed-scale denitrification, assuming that nitrification and denitrification were spatially segregated between surface and subsurface soils, respectively. It remains unclear how surface soil NO_3^- samples might best be collected and interpreted to inform ecosystem-scale isotope models and our broader understanding of denitrification.

Different types of samples, such as soil salt extractions or soil water samples, could differ substantially in their capacity to record NO_3^- isotope effects from denitrification due to differences in water residence time and spatial distribution in the soil—an important scale dependence that we highlight in this manuscript. Water has a continuum of mobility in soil due to physical and chemical interactions with soil constituents, which can be quantified according to water potential. Soil extractions capture most of the inorganic nitrogen (including N interacting electrostatically with soil exchange sites) in a sampled soil volume, whereas water collected in vadose zone lysimeters under minimal applied tension may reflect N in comparatively mobile water pools that have a shorter residence time in the soil matrix. Water pools associated with soil mineral and organic surfaces (i.e., less mobile pools) often differ greatly in H_2O isotope composition relative to highly mobile water in soil macropores, reflecting differences in water pool transit time and thus their capacity to record terrestrial biophysical processes (Evaristo et al. 2015; Good et al. 2015). We hypothesize that these water pools might also differ in their capacity to record N biogeochemical processing via stable isotopes. Both soil extractions and lysimeter water collected under various tensions have been used in ecosystem-scale studies involving NO_3^- isotopes, but they have seldom been directly compared. Perhaps even more importantly, differences in NO_3^- stable isotopes among samples have not typically been compared with canonical indices of denitrification, such as potential denitrification enzyme activity.

Comparisons of NO_3^- isotope composition with measured potential denitrification activities could help address possible ambiguities in isotope interpretation. The use of NO_3^- isotopes to infer the occurrence of denitrification relies on kinetic fractionation from denitrifying bacteria, which increases the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of residual NO_3^- (Mariotti et al. 1981). Linear relationships between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of NO_3^- with slopes between 0.5 and 1 are assumed to reflect kinetic fractionation by denitrifiers, a relationship that appears to hold across soils, groundwater, and aquatic ecosystems (Cohen et al. 2012; Granger et al. 2008; Houlton et al. 2006; Lehmann et al. 2003; Sigman et al. 2005). This assumption is plausible given that assimilatory N fractionation by plants and microbes, another explanation for NO_3^- isotope enrichment, is typically minor in N-limited ecosystems (Evans 2001; Granger et al. 2010). However, co-occurring fractionation from other N-cycling processes, such as nitrification (Mariotti et al. 1981), or N inputs with differing isotope compositions, could potentially obscure isotope enrichment from denitrification.

In surface soils with heterogeneous O_2 availability, nitrification (an aerobic process) and denitrification (an anaerobic process) can potentially co-occur. In open systems, nitrifying bacteria tend to decrease the $\delta^{15}\text{N}$ of soil NO_3^- relative to NH_4^+ by 14–38 ‰ (Casciotti et al. 2003; Mariotti et al. 1981). Open-system fractionation by denitrifying bacteria spans a similar range, increasing $\delta^{15}\text{N}$ of the residual NO_3^- pool by between 5 and 29 ‰ (Granger et al. 2008; Mariotti et al. 1981). In cases where NH_4^+ or NO_3^- are fully consumed in soil microsites (i.e., closed systems), it is also possible that fractionation is partially or not expressed during either nitrification or denitrification (Kendall et al. 2007). However, even in cases where fractionation from nitrification and denitrification *are* both expressed at the microsite (i.e., μm) scale, it is possible that their effects could be mutually obscured over larger spatial scales (i.e., cm) characteristic of soil samples due to their opposite effects on $\delta^{15}\text{N}$ of NO_3^- . This situation potentially creates a challenge to interpretation by obscuring the original isotope composition of NO_3^- inputs, a necessary parameter for quantitative isotope models (e.g., Fang et al. 2015), and diluting the pool of $\delta^{15}\text{N}$ -enriched NO_3^- that is typically used to indicate the presence of denitrification (Billy et al. 2010; Houlton et al. 2006; Wexler et al. 2014). Comparing traditional indices of denitrification and related biogeochemical parameters with NO_3^- isotopes could help to decipher if isotope overprinting from nitrification and denitrification can be important in surface soil environments.

Combining measurements of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- with $\delta^{15}\text{N}$ of N sources and $\delta^{18}\text{O}$ of soil water could also assist in identifying the occurrence of denitrification in surface soils. The $\delta^{15}\text{N}$ of nitrified NO_3^- depends on both the

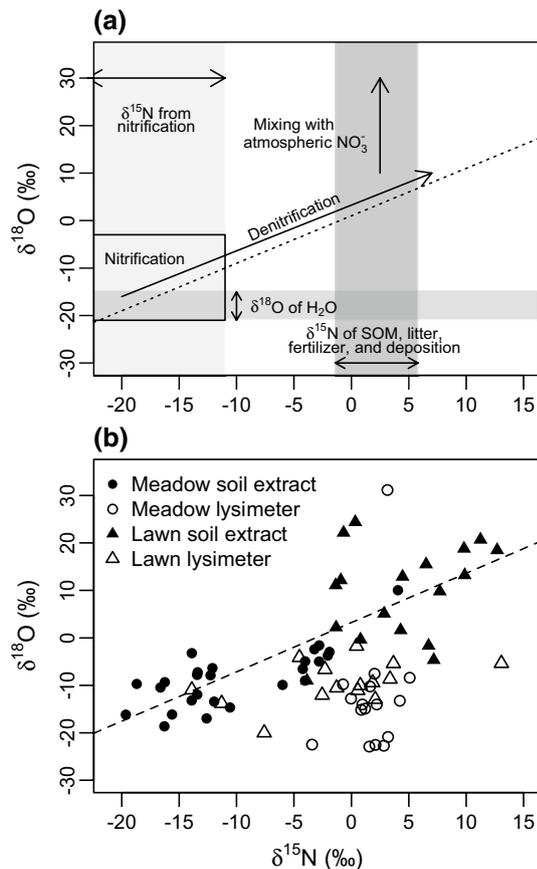


Fig. 1 **a** Conceptual dual isotope plot ($\delta^{18}\text{O}$ and $\delta^{15}\text{N}$) showing the measured variation in $\delta^{15}\text{N}$ of SOM, litter, and fertilizer as a vertical rectangle and the measured variation in precipitation $\delta^{18}\text{O}$ as a horizontal rectangle. The predicted isotope composition of nitrified NO_3^- with complete expression of kinetic isotope effects on $\delta^{15}\text{N}$ is bounded by a black rectangle. This incorporates potential N fractionation during nitrification of an NH_4^+ source with $\delta^{15}\text{N}$ within the observed range of SOM, litter, and fertilizer, and NO_3^- $\delta^{18}\text{O}$ ranging from complete incorporation of soil water to a 1/3 contribution from atmospheric O_2 with $\delta^{18}\text{O} = 23.5$ ‰ (Kool et al. 2011; Mayer et al. 2001). The dotted line represents an illustrative trend of kinetic denitrification isotope effects with a $\delta^{18}\text{O}/\delta^{15}\text{N}$ slope of 1, beginning at an arbitrary point in $\delta^{18}\text{O}/\delta^{15}\text{N}$ space. The vertical arrow indicates potential impacts of mixing with atmospheric NO_3^- enriched in $\delta^{18}\text{O}$. **b** Measurements of NO_3^- isotopes in soil KCl extractions (black symbols) and lysimeter water samples (open symbols). Triangles and circles represent the lawn and meadow sites, respectively. The dashed line represents a linear regression of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values in soil KCl extractions from both sites (slope = 1.04 ± 0.12 , $R^2 = 0.64$, $p < 0.0001$)

$\delta^{15}\text{N}$ of NH_4^+ sources and the extent of fractionation during nitrification. Ammonium typically reflects the $\delta^{15}\text{N}$ of the plant litter, soil organic matter (SOM), or fertilizer from which it is derived, with minimal (~ 1 ‰) fractionation (Kendall et al. 2007). Atmospheric deposition is another potentially important NH_4^+ source. A representative range of $\delta^{15}\text{N}$ in these NH_4^+ sources that is specifically applicable to the present study is indicated by the vertical shaded

rectangle in Fig. 1a. Nitrification of NH_4^+ fractionates $\delta^{15}\text{N}$ substantially in open systems (Casciotti et al. 2003; Mariotti et al. 1981), with potential values indicated by the “nitrification” vertical shaded rectangle in Fig. 1a. Nitrification also imparts a characteristic $\delta^{18}\text{O}$ composition to NO_3^- . Oxygen in nitrified NO_3^- is dominantly (i.e., $\geq 2/3$) derived from soil water, with a variable contribution from atmospheric O_2 (23.5 ‰) that depends on the degree of in situ O exchange with water (Kool et al. 2011; Mayer et al. 2001). This range is indicated by the nitrification box in Fig. 1a. Subsequent denitrification of this NO_3^- pool would presumably be reflected by $\sim 1:1$ increases in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrified NO_3^- (indicated by the denitrification arrow in Fig. 1a) which could ultimately return $\delta^{15}\text{N}$ of NO_3^- to values similar to or greater than the ecosystem N sources (Fig. 1a). However, $\delta^{18}\text{O}$ of NO_3^- would then remain substantially elevated relative to $\delta^{18}\text{O}$ of soil H_2O , which is inconsistent with NO_3^- derived from nitrification. Mixing of soil NO_3^- with atmospheric inputs is another important consideration. Atmospheric NO_3^- has very high $\delta^{18}\text{O}$ (i.e., 60–80 ‰) as a consequence of atmospheric oxidative processes (Kendall et al. 2007), which could also increase the $\delta^{18}\text{O}$ of soil NO_3^- relative to the products of nitrification, as indicated by the vertical mixing arrow in Fig. 1a.

To assess relationships between NO_3^- stable isotope composition and microsite-scale denitrification in surface soils, we designed a study involving two nearby sites (a riparian meadow and a managed urban lawn) in a semi-arid montane ecosystem where denitrification likely represents an important fate of N during spring snowmelt. In temperate ecosystems with a seasonal snowpack, snowmelt often represents a period of maximum soil moisture and potential N loss, when soil biogeochemical processes are especially important in attenuating N inputs to streams (Brooks and Williams 1999; Zak et al. 1990). Our sites provided a natural gradient in denitrification potential, allowing us to assess their relationship with NO_3^- isotopes while maintaining similar climate and soil physical/chemical characteristics. Importantly, these sites also had similar surface soil (0–15 cm) NH_4^+ pools, similar plant litter $\delta^{15}\text{N}$, and relatively similar bulk soil $\delta^{15}\text{N}$ (within 1.5 ‰), implying that the initial $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- pools produced by mineralization and nitrification were also likely to be similar between sites.

Methods

We sampled soils from two sites in the Red Butte Creek watershed in Salt Lake City, Utah, USA: a natural riparian meadow with herbaceous vegetation (Dawson and Ehleringer 1991) and a managed lawn on the University of

Utah campus. Both sites received $>4 \text{ kg N ha}^{-1} \text{ year}^{-1}$ in atmospheric deposition (Hall et al., *J Geophys Res Biogeosci*, in revision). The lawn received $\sim 100 \text{ kg ha}^{-1} \text{ year}^{-1}$ of fertilizer N as a urea blend (32–5–7 ratio of N, P, and K) applied during late spring, and ammonium sulfate (20–0–0) applied during October. Fertilizer was obtained from the Intermountain Farmers Association (Salt Lake City, UT, USA). Soils were sampled following spring snowmelt (February and March 2014 at the lawn and meadow, respectively), approximately 5 months after the last fertilizer application to the lawn. Snowmelt typically represents the period of maximum soil moisture in semi-arid montane ecosystems.

The meadow soil was a loamy Mollisol with a predominant rooting depth above 35 cm, and the lawn soil was a sandy loam Inceptisol derived from local alluvium added on top of rocks. Bulk density and pH were similar between sites (Table 1). We sampled the meadow at depths of 0–5, 5–15, and 15–35 cm with a 6-cm diameter auger ($n = 9$ for each depth); the lawn could only be sampled to 15 cm due to an impenetrable rock layer ($n = 12$ for each depth). Tension lysimeters (Prenart Super Quartz, Frederiksberg, Denmark) were installed at 15 and 35 cm in the lawn and meadow, respectively, at the bottom of the main rooting zone at each site. Lysimeters were installed approximately 5 months prior to sampling for this study. Lysimeter water was sampled by applying a vacuum of -28 kPa relative to ambient pressure (86 kPa). This sampling pressure was chosen because its absolute value is similar to but less than field capacity, typically defined as -30 kPa for loamy soils (Schaetzl and Anderson 2005). Field capacity is an estimate of the residual soil water remaining after macropores have drained. Thus, our lysimeters captured water at potentials above field capacity, or a comparatively “mobile” water pool. Others have used tension lysimeters to sample soil water under much more negative water potential (-70 kPa) than we used here, but they still judged these samples to reflect water capable of significant advection (Castellano et al. 2013). We stress that the concepts of “mobile” and “immobile” water do not represent a dichotomy but rather an operationally defined continuum. Because our lysimeters were sampled under tension, they contain some fraction of soil-associated water differing in composition from water that instantaneously drains through soils during melt or precipitation events (Landon et al. 1999). Our operational separation between more and less mobile water pools, respectively sampled in lysimeters and soil extractions, is thus conservative. Water samples were collected throughout the period of snowmelt (total $n = 186$; Jan–April 2014).

Oxygen (O_2) sensors (Apogee Instruments, Logan, UT, USA) were installed at depths of 10 cm in 4.8 cm diameter polyvinylchloride pipes with sealed caps (equilibration

Table 1 Soil biogeochemical characteristics by depth for the lawn and meadow sites. Standard errors are shown in parentheses

Site	Depth	pH	Bulk density (g cm^{-3})	Soil $\delta^{15}\text{N}$	Soil N (%)	Soil N stock (kg N ha^{-1})	Fe(II) ($\mu\text{g g}^{-1}$)	Fe(II)/Fe _{tot}	KCl- extractable NH_4^+ ($\mu\text{g g}^{-1}$)	KCl-extractable NO_3^- ($\mu\text{g g}^{-1}$)	Lysimeter NO_3^- -N (mg l^{-1})	Potential denitrification ($\mu\text{g N}_2\text{O-N g}^{-1}\text{h}^{-1}$ at 25 °C)	Potential denitrification ($\mu\text{g N}_2\text{O-N g}^{-1}\text{h}^{-1}$ at 4 °C)	Bulk soil O_2 (%)
Lawn	0–5	7.5 (0.1)	0.86 (0.02)	3.3 (0.3)	0.36 (0.02)	1500 (200)	53 (7)	0.46 (0.08)	8.8 (1.5)	4.4 (1.8)	–	2955 (194)	406 (55)	–
	5–15	7.8 (0.1)	–	5.8 (0.1)	0.12 (0.01)	1800 (300)	23 (1)	0.32 (0.08)	2.1 (0.3)	1.3 (0.5)	0.67 (0.23)	709 (40)	35 (3)	19.6 (1.1)
Meadow	0–5	7.7 (0.1)	0.87 (0.02)	3.8 (0.1)	0.41 (0.02)	1800 (200)	7 (1)	0.04 (0.01)	2.4 (0.2)	2.5 (0.2)	–	465 (62)	25 (3)	–
	5–15	7.7 (0.1)	–	4.3 (0.1)	0.36 (0.01)	3100 (300)	8 (1)	0.03 (0)	3.9 (0.3)	2.9 (0.2)	–	217 (20)	11 (3)	20.7 (0.1)
	15–35	7.8 (0.1)	–	5.5 (0.1)	0.23 (0.01)	4000 (400)	7 (1)	0.03 (0)	3.6 (0.3)	1.1 (0.1)	0.24 (0.04)	65 (15)	3 (0)	–

chambers), where the bottom lip of the chamber was pressed into undisturbed soil (Hall et al. 2013). Soil O₂ content was recorded on a datalogger at 15-min intervals. Here, we report O₂ during the period of NO₃⁻ sample collection. All of the above measurements were made in the context of a snow removal experiment at each site (Hall et al. 2016a), but given the absence of treatment effects on any biogeochemical response variable, we do not report treatment identities for ease of interpretation. Precipitation was collected on an event basis during the study period for analysis of NO₃⁻ and NH₄⁺ concentrations and isotope composition. Lysimeter and precipitation samples were filtered through pre-combusted and rinsed Whatman GF/F filters and frozen until analysis by ion chromatography.

We measured potential denitrification rates in shaken slurries of soil samples within 24 h of sampling using the acetylene inhibition method (Groffman et al. 1999), using an incubation temperature of 4 °C to approximate field conditions and 25 °C to facilitate comparisons with other studies. Potential denitrification rates are an appropriate metric to compare with the isotope composition of NO₃⁻ pools because they are tightly linked to the abundance of bacterial denitrification functional genes, which integrate recent process activity in the soil environment (Petersen et al. 2012). Soil subsamples were extracted in 2 M potassium chloride (KCl) solutions for analysis of NH₄⁺ and NO₃⁻ concentrations and isotope composition. We measured δ¹⁵N and δ¹⁸O of NO₃⁻ in soil extractions (*n* = 50), lysimeter water samples (*n* = 31), and precipitation samples (*n* = 8) using *Pseudomonas aureofaciens* and the denitrifier method (Bell and Sickman 2014; Casciotti et al. 2002) to generate N₂O for analysis by isotope ratio mass spectrometry at the Stable Isotope Ratio Facility for Environmental Research at the University of Utah. When analyzing the soil extractions using the denitrifier method, we corrected for the presence of trace NO₃⁻ in KCl and any physiological effects on the denitrifying bacteria by analyzing reference materials prepared in the same matrix (Bell and Sickman 2014). Samples were normalized to δ notation, relative to atmospheric N₂ for N and VSMOW for O, using a combination of the international NO₃⁻ reference materials USGS 34, 35, and 32. Precision and accuracy were 0.6 and 0.7 ‰ (respectively) for δ¹⁵N, and 0.6 and 0.3 ‰ (respectively) for δ¹⁸O, assessed by repeated analysis of IAEA-NO3 (for δ¹⁵N) and USGS 32 (for δ¹⁸O) treated as unknowns. Sample reproducibility was 0.3 ‰, based on duplicate analyses of 1/8 of the samples. To characterize the δ¹⁵N of fertilizer inputs to the lawn, three subsamples were analyzed from each of two production batches of each fertilizer type (ammonium sulfate and urea). Bulk soil δ¹⁵N from each site and sampled depth increment as well as surface litter (*n* = 9 plots per site) were analyzed on samples dried at 60 °C. Soil and fertilizer δ¹⁵N were measured via

combustion on an elemental analyzer coupled to an isotope ratio mass spectrometer (Thermo MAT 253, Waltham, MA, USA). A subset of lysimeter water samples from the meadow site (*n* = 17) was analyzed for δ¹⁸O of H₂O by isotope ratio infrared spectroscopy (Picarro L-2130i, Santa Clara, CA, USA).

A subset of precipitation samples (*n* = 15) was analyzed for δ¹⁵N of NH₄⁺ using a modified NH₃ diffusion method (Holmes et al. 1998). Briefly, 30 ml of the sample were added to a 60-ml HDPE bottle with 1.5 g sodium chloride. A glass-fiber filter acidified with 30 μl of 4 M phosphoric acid was pressed between Teflon tape and added to the bottle. Magnesium oxide (90 mg) was added to volatilize NH₃, and the bottle was immediately capped and incubated for 7 days on an orbital shaker/incubator at 40 °C to allow NH₃ to be completely trapped as NH₄⁺ on the acidified filter. Filters were analyzed for δ¹⁵N (precision < 0.2 ‰) by combustion as described above. Analysis of ammonium sulfate solutions with known δ¹⁵N values verified a lack of fractionation during diffusion.

Soil subsamples were also extracted in the field immediately after sampling with 0.5 M hydrochloric acid for analysis of reduced and oxidized iron [Fe(II) and Fe(III), respectively] as a redox indicator (Hall et al. 2013). The presence of Fe(II) implies the presence of anaerobic microsites where dissimilatory Fe reduction exceeds Fe(II) oxidation by O₂. The ratio of Fe(II)/Fe_{HCl} reflects the proportion of HCl-extractable Fe that has been reduced, facilitating comparisons among sites that may differ in total HCl-extractable Fe content (Hall and Silver 2015). Thermodynamic principles imply that NO₃⁻ should have been consumed via denitrification in microsites where Fe(II) is present (Chapelle et al. 1995).

We analyzed linear relationships between δ¹⁵N and δ¹⁸O of NO₃⁻ both within and among sites and for each separate sample type (soil extractions vs. lysimeter water) using ANCOVA. Mean differences in δ¹⁵N and δ¹⁸O composition among sites were assessed using MANOVA (i.e., ANOVA testing two response variables simultaneously). Differences in other response variables among sites were assessed with ANOVA. Relationships between δ¹⁵N of NO₃⁻ and potential denitrification and Fe(II)/Fe_{HCl} were highly nonlinear, so we fitted trends with a two-component piecewise linear regression using the SiZer package in R (Sonderegger 2012).

Results

While both sites typically had near-atmospheric O₂ concentrations in the bulk soil throughout the period of measurement, they also showed evidence of redox heterogeneity and the presence of reducing conditions in soil microsites,

Table 2 Nitrate isotope compositions in soil KCl extractions and lysimeter samples from the lawn and meadow

Site	Sample type	Sample size	NO ₃ ⁻ δ ¹⁵ N (‰)	NO ₃ ⁻ δ ¹⁸ O (‰)
Lawn	Lysimeter (NO ₃ ⁻)	15	-1.2 (1.7)	-9.5 (1.2)
Lawn	Soil extract (NO ₃ ⁻)	23	4.2 (1.2)	9.6 (2.4)
Meadow	Lysimeter (NO ₃ ⁻)	16	1.7 (0.5)	-12.5 (3.2)
Meadow	Soil extract (NO ₃ ⁻)	27	-9.9 (1.2)	-8.4 (1.2)
Meadow	Lysimeter (H ₂ O)	17	-	-17.7 (0.4)
Salt Lake City	Precipitation (NO ₃ ⁻)	8	2.3 (0.7)	81.9 (4.0)

Water δ¹⁸O values from meadow lysimeters are also presented; these were very similar to the precipitation δ¹⁸O during the period of sampling (Hall et al. 2016b). Values in parentheses represent standard errors

as reflected by Fe(II)/Fe_{HCl} ratios (Table 1). The lawn had much higher Fe(II)/Fe_{HCl} relative to the meadow ($p < 0.0001$), coinciding with increased potential denitrification rates. Denitrification potential was 16-fold greater in lawn soils at 4 °C ($p < 0.0001$) and sixfold greater at 25 °C ($p < 0.001$; Table 1) when considering all depth increments combined. Nitrate concentrations in soil extractions were generally similar between sites, although lysimeter NO₃⁻ was significantly greater in the lawn (Table 1).

Bulk soil δ¹⁵N was similar between sites in 0–5 cm soil, but was slightly (1.5 ‰) and significantly ($p < 0.0001$) greater in lawn 5–15 cm soil (Table 1). Despite the fact that NH₄⁺ concentrations significantly differed between sites at a given depth increment ($p < 0.001$), total KCl-extractable NH₄⁺ mass to 15 cm was similar between the meadow and lawn, measuring 0.056 (0.013) and 0.044 (0.003) kg NH₄⁺-N ha⁻¹, respectively. At the lawn, comparison of the KCl-extractable NH₄⁺ pool with annual NH₄⁺ fertilizer inputs indicated that >99.9 % of the previous year's fertilizer application was assimilated, or otherwise transformed or exported, prior to measurement. Surface litter δ¹⁵N was also similar between the meadow (-0.5 ± 0.1 ‰) and lawn (0.1 ± 0.4 ‰). Fertilizer δ¹⁵N was highly consistent within and between production batches and was relatively similar between the two fertilizer types applied here. Two separate batches of ammonium sulfate δ¹⁵N measured -0.9 ± 0.2 and -1.0 ± 0.3 ‰, and the urea blend δ¹⁵N was -1.7 ± 0.1 and -1.6 ± 0.3 ‰. Values of δ¹⁵N of NH₄⁺ in bulk atmospheric deposition measured -1.2 ± 1.5 ‰, similar to fertilizer.

Trends in δ¹⁸O and δ¹⁵N of NO₃⁻ in soil extractions from both sites considered together were consistent with linear isotopic effects from the denitrification of NO₃⁻ sources with similar initial isotope values, reflected by a slope of 1.04 ± 0.12 ($R^2 = 0.64$, $p < 0.0001$; Fig. 1b). The slope of this relationship did not differ significantly between sites. However, the relationship between δ¹⁸O and δ¹⁵N was insignificant when assessing samples from the lawn alone (slope = 0.62, $R^2 = 0.10$, $p = 0.21$), and was much stronger in the meadow (slope = 0.72 ± 0.13 , $p < 0.0001$, $R^2 = 0.55$). Nevertheless, the lawn extractions

had significantly greater (more enriched) NO₃⁻ δ¹⁸O and δ¹⁵N values than the meadow ($p < 0.0001$; Fig. 1b; Table 2). Nitrate δ¹⁵N in soil extractions also had a weakly significant site by depth interaction ($p = 0.02$), where values did not differ by depth in the lawn but were significantly greater in meadow 0–5 cm samples than the deeper samples.

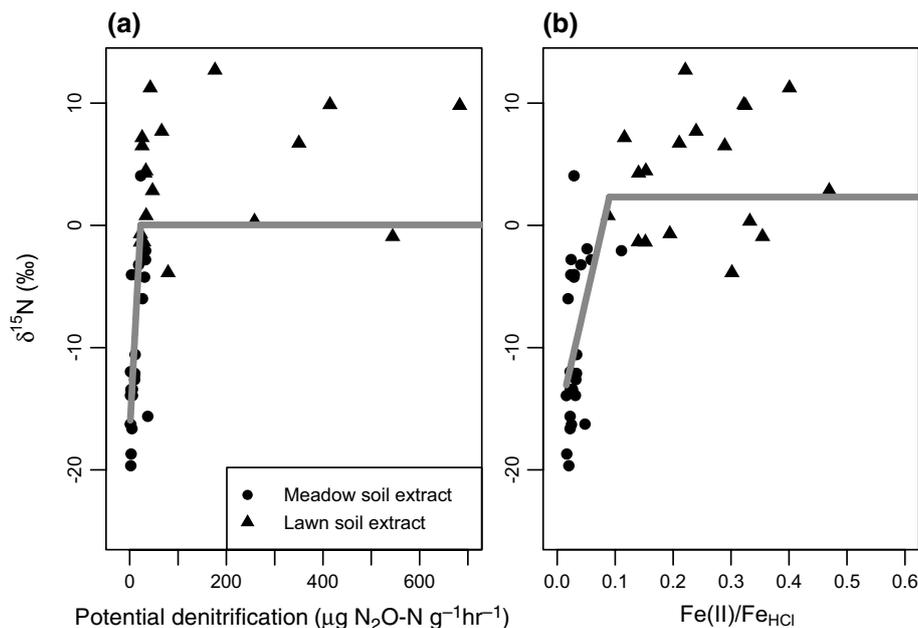
In contrast to the soil extractions, δ¹⁸O and δ¹⁵N of lysimeter NO₃⁻ were not significantly correlated, and lysimeter NO₃⁻ isotope composition did not differ between sites (Fig. 1b; Table 2). Furthermore, lysimeter samples significantly differed in NO₃⁻ isotope composition when compared with soil extractions from the same site (MANOVA, $p < 0.0001$; Fig. 1b). Values of δ¹⁸O in precipitation NO₃⁻ (81.9 ± 4.0 ‰) were substantially greater than any of the soil extractions or soil lysimeter samples, while δ¹⁵N of precipitation NO₃⁻ (2.3 ± 0.7 ‰; Table 2) fell within the bounds of soil extractions and lysimeter samples. Neither the soil extractions nor lysimeter samples displayed significant relationships between NO₃⁻ isotope composition and NO₃⁻ concentrations (data not shown).

Nitrate δ¹⁵N showed distinct trends with potential denitrification and Fe(II)/Fe_{HCl} at both sites: significant linear trends at the meadow ($p < 0.001$ and 0.03, respectively), but no relationship at the lawn. Combined, these data suggested significant threshold relationships between δ¹⁵N, potential denitrification, and Fe(II)/Fe_{HCl} as determined by piecewise linear regressions (Fig. 2). Nitrate δ¹⁵N initially increased sharply with potential denitrification (slope = 0.75, between 0.10 and 0.90 with 95 % confidence) and Fe(II)/Fe_{HCl} (slope = 170, between 60 and 550 with 95 % confidence), but subsequently plateaued to slopes indistinguishable from zero for both response variables (Fig. 2).

Discussion

We found evidence that denitrification was likely occurring during snowmelt in surface soil horizons at both sites, as reflected by substantial rates of potential denitrification activity combined with the presence of anaerobic

Fig. 2 **a** Relationship between $\delta^{15}\text{N}$ of NO_3^- in soil extractions and potential denitrification rate; the gray line represents a piecewise linear regression as described in the text. **b** Relationships between $\delta^{15}\text{N}$ of NO_3^- in soil extractions and the $\text{Fe(II)}/\text{Fe}_{\text{HCl}}$ ratio (a redox indicator, described in the text and in Hall and Silver 2015); the gray line represents a piecewise linear regression



microsites as reflected by $\text{Fe(II)}/\text{Fe}_{\text{HCl}}$. Although high potential denitrification rates indicate the presence of denitrifying organisms, they do not definitively indicate when denitrification may have occurred. In these ecosystems, increased soil moisture during snowmelt likely generated anaerobic microsites that promoted denitrification, given that very low soil moisture (<10 % by volume) prevailed for several months prior at each site until snowmelt began (Hall et al. 2016a). The importance of denitrification in cold soils during snowmelt has been well established in other studies (Brooks et al. 2011), and our measured potential denitrification activities at 25 °C were similar to rates from wetland and riparian soils in other studies (Roach and Grimm 2011). Yet, different NO_3^- samples (soil extractions vs. lysimeter water) and sites (the lawn and meadow) varied dramatically in their relationships between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ and proxies for denitrification.

Trends in NO_3^- isotopes in soil extractions and proxies for denitrification

Plotting $\delta^{18}\text{O}$ versus $\delta^{15}\text{N}$ of NO_3^- in soil extractions from both sites yielded a slope similar to 1, consistent with the upper bound of slopes invoked as evidence for denitrification in previous field studies in terrestrial, groundwater, and marine environments (Cohen et al. 2012; Houlton et al. 2006; Lehmann et al. 2003; Sigman et al. 2005), and similar to laboratory experiments with denitrifying bacteria (Granger et al. 2008). However, $\delta^{15}\text{N}$ of NO_3^- remained lower than bulk soil N in most samples, despite the fact that denitrification had most likely occurred. These trends are consistent with a large initial isotope fractionation from

nitrification (Casciotti et al. 2003) which was subsequently impacted by denitrification to a variable extent among samples. A large fractionation from nitrification is consistent with the observed presence of excess NH_4^+ at both sites relative to NO_3^- (Table 1), and comparatively slow enzymatic catalysis in cold soils following snowmelt. A large isotopic effect of nitrification was also recently observed in New Zealand pasture soils during winter (Wells et al. 2015).

Our interpretations of $\delta^{15}\text{N}$ of NO_3^- are enhanced by considering potential variation in $\delta^{15}\text{N}$ of the NH_4^+ from which NO_3^- is derived. We argue that there were not likely to be systematic differences in $\delta^{15}\text{N}$ of NH_4^+ between sites, according to the following reasoning. The major potential sources of NH_4^+ at these sites during the period of sampling include mineralization of litter and SOM, residual fertilizer at the lawn, and atmospheric deposition. Most importantly for our study, these potential NH_4^+ sources had relatively similar $\delta^{15}\text{N}$ compositions in comparison with the observed variation in $\delta^{15}\text{N}$ of NO_3^- , and tended to be similar between sites. First, mineralized NH_4^+ has similar $\delta^{15}\text{N}$ (± 1 ‰) relative to the organic matter (e.g., litter or SOM) from which it was derived (Kendall et al. 2007), and differences in litter and SOM $\delta^{15}\text{N}$ pools between the two sites were small (<0.5 and 1.5 ‰, respectively). Multiple litter and SOM pools mineralize NH_4^+ at different rates, so it is likely that the measured differences between litter and bulk soil $\delta^{15}\text{N}$ (−0.5 to 5.8 ‰) approximate the range of variation in $\delta^{15}\text{N}$ of mineralized NH_4^+ at these sites. Mean fertilizer (−0.9 to 0.1 ‰) and atmospheric deposition (2.3 ‰) $\delta^{15}\text{N}$ fell close to or within this range. We suggest that mineralization of litter and SOM (as opposed to fertilizer) were especially

important sources of NH_4^+ in both the meadow and the lawn during the period of sampling. This interpretation was supported by (1) the fact that total extractable NH_4^+ stocks were *equivalent* between lawn and meadow despite fertilization; (2) the very small extractable pool of NH_4^+ relative to annual fertilizer inputs, indicative of rapid cycling of mineral N where >99.9 % of fertilizer N inputs were assimilated or lost via gaseous or leaching pathways prior to sampling; (3) the fact that 6 months had elapsed since fertilizer application, providing ample opportunity for assimilation or other transformations. Even if residual fertilizer did represent a significant component of soil NH_4^+ , it is unlikely to lead to substantial differences in $\delta^{15}\text{N}$ of NH_4^+ between sites, given that fertilizer $\delta^{15}\text{N}$ was very similar to litter $\delta^{15}\text{N}$ at both sites.

Secondly, it is plausible to assume that initial $\delta^{15}\text{N}$ values of nitrified NO_3^- may also have been similar between sites and depths. This assumption is reasonable given overall similarities in NH_4^+ concentrations, bulk soil N concentration and $\delta^{15}\text{N}$, microbial biomass N, pH, bulk density, and climate between the lawn and meadow (Hall et al. 2016a). Under this assumption, the lawn showed greater enrichment of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ subsequent to nitrification, implying a greater fraction of NO_3^- loss to denitrification relative to the meadow, in agreement with the denitrification potential assays. Trends in $\delta^{15}\text{N}$ of NO_3^- with depth in the lawn also corresponded with the denitrification potential assays: $\delta^{15}\text{N}$ of NO_3^- was greater in 0–5 cm soils, which had greater potential denitrification rates. Previous studies have similarly demonstrated greater denitrification potential in surface than subsurface soil horizons (Groffman et al. 2002) and have reported high denitrification rates in lawns (Raciti et al. 2011). Both factors reflect the importance of plant C and N inputs (and potentially fertilizer additions) as controls on denitrification in surface soils, as opposed to O_2 diffusion limitation in the subsurface. However, if we had assessed relationships between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of NO_3^- at the lawn site alone without the context of the meadow samples with much lower denitrification rates, this conclusion would have been obscured. The meadow samples revealed the likely importance of fractionation during nitrification, and without accounting for this fractionation we would have had little isotopic evidence for denitrification at the lawn—even though denitrification potential assays and Fe(II) measurements strongly suggested it had occurred. This highlights a critical limitation on the use of NO_3^- isotopes to infer site-specific or watershed-scale patterns of denitrification, as recently implemented elsewhere.

Contrasting NO_3^- isotope compositions in soil extractions and lysimeter water

In contrast to the linear trend in the soil extractions, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values in soil lysimeter NO_3^- exhibited significant scatter, likely reflecting multiple fractionating processes

and mixing with atmospheric NO_3^- that was enriched in ^{18}O . The NO_3^- isotope disparities between lysimeters and soil extractions may also reflect the redox heterogeneity of terrestrial soils (Sexstone et al. 1985). Measurements of Fe(II)/Fe_{HCl} in soil extractions suggested the presence of reducing conditions in soil microsites, whereas the soil O_2 sensors indicated highly aerobic conditions at the scale of the bulk soil atmosphere in both sites (Table 1). Differences in dual isotope ($\delta^{18}\text{O}/\delta^{15}\text{N}$) slopes and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values between the soil extractions and the lysimeters support our hypothesis that soil extractions and lysimeter samples differ in their capacity to record ecosystem-scale N biogeochemical cycling via stable isotopes. A plausible explanation for this pattern is that soil extractions sampled the anaerobic microsites inside soil aggregates where denitrification can occur (Parkin et al. 1987), whereas lysimeters largely reflected the transport of N that was nitrified in the aerobic macropore environment or in surface litter.

This distinction may be critical in interpreting patterns of denitrification at the watershed scale, where the NO_3^- isotopic composition of stream water is often assumed to integrate gross nitrification or denitrification across entire watersheds (Fang et al. 2015; Riha et al. 2014). Our data suggest that this assumption does not necessarily hold, given that relatively mobile lysimeter water and soil extractions from the same sites differed substantially in their NO_3^- isotopic compositions. Rather, as supported by recent syntheses of H_2O isotope dynamics, these samples may reflect two “water worlds:” poorly mobile water accessible to plants and microbes, and comparatively mobile water that dominates groundwater recharge and stream discharge (Evaristo et al. 2015; Good et al. 2015). Thus, stream or groundwater NO_3^- isotope composition may not necessarily reflect denitrification in surface soil microsites, which are a dominant zone of potential denitrification activity at the ecosystem scale (Groffman et al. 2002). As a consequence, we suspect that model estimates based on stream NO_3^- isotopes might systematically underestimate watershed-scale denitrification in some ecosystems. However, even in studies aimed at estimating riparian denitrification alone, our data imply that characterizing the initial isotope composition of NO_3^- in relatively mobile soil water is a critical, if nontrivial, step in interpreting stream NO_3^- isotopes. Our data show that total soil NO_3^- and NO_3^- in relatively mobile water can differ substantially, and misspecification of the initial isotope composition of mobile soil NO_3^- could variably lead to under- or overestimation of denitrification.

Implications for interpreting $\delta^{15}\text{N}$ in models and environmental samples

Importantly, dual isotope trends in soil KCl-extractable NO_3^- from both sites combined were consistent with

denitrification isotope effects (Fig. 1) and were related to potential denitrification rates (Fig. 2) despite the fact that $\delta^{15}\text{N}$ values of NO_3^- were typically less than bulk soil $\delta^{15}\text{N}$. This finding has interesting implications for models that estimate denitrification at the ecosystem scale using isotopic measurements of NO_3^- (Billy et al. 2010; Fang et al. 2015). Our data suggest that substantial denitrification might occur even in situations where $\delta^{15}\text{N}$ values of NO_3^- do not display substantial enrichment relative to N inputs or other ecosystem pools, due to the potentially large initial N isotope fractionation associated with nitrification. Thus, isotope models which assume that $\delta^{15}\text{N}$ of surface soil NO_3^- predominantly represents the product of nitrification (Fang et al. 2015) would tend to underestimate the relative importance of denitrification in the case where large fractionation effects of nitrification were partially counteracted by denitrification. In the latter study (ibid.), correlations between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- were not significant in surface soil KCl extractions, leading the authors to conclude that NO_3^- dynamics were spatially separated in these sites, with nitrification dominating in near-surface soils and denitrification prevailing in deeper soils.

The very weak linear trends between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ we observed at the lawn site alone (as opposed to considering all samples together), combined with our process-level measurements, show that denitrification is not necessarily well recorded by site-specific trends in NO_3^- isotope composition in surface soils. Rather, $\delta^{15}\text{N}$ of NO_3^- showed a threshold relationship with potential denitrification rates and reducing conditions—strong trends at low rates that became a null relationship at higher rates. This may reflect closed-system kinetics where NO_3^- is mostly consumed via denitrification in soil microsites, leading to an under-expression of kinetic isotope effects, as noted previously in both soils and sediments (Houlton et al. 2006; Sebilo et al. 2003). Thus, pairwise trends between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- and their relationships with potential denitrification only became strongly significant when considering a very broad gradient in redox variability and potential denitrification activity (which spanned three orders of magnitude among samples and sites, Fig. 2). Moreover, our results are consistent with a recent global-scale analysis of soil $\delta^{15}\text{N}$, which found that much of the variability in bulk soil $\delta^{15}\text{N}$ previously attributed to denitrification might instead be explained by clay content and preferential retention of microbially processed organic matter (Craine et al. 2015). Differences in clay content between our sites (with loam and sandy loam texture, respectively) could contribute to the fact that bulk soil $\delta^{15}\text{N}$ did not reflect the large site differences in denitrification potential and $\delta^{15}\text{N}$ of soil extracted NO_3^- .

In summary, recent analytical and conceptual advances related to NO_3^- isotopes provide powerful opportunities

for exploring controls on N sources and biogeochemical processing over multiple spatial scales (Fang et al. 2015; Houlton et al. 2006; Kendall et al. 2007; Michalski et al. 2004; Riha et al. 2014). We showed that surface soils characterized by denitrification at the microsite scale in a heterogeneous redox environment can exhibit NO_3^- isotope trends consistent with denitrification when examining samples exhibiting a broad range of potential denitrification rates. However, these patterns are highly dependent on spatial scale. They vary between mobile water and total extractable soil NO_3^- , and depend on whether comparisons are conducted within or among sites. They also depend on assumptions about NH_4^+ isotope composition, nitrification isotope effects, and initial NO_3^- isotope composition prior to any denitrification. Thus, we suggest that NO_3^- isotope data should be interpreted with careful consideration of boundary conditions, sample types, and hydrologic connectivity between sampled pools before using them as parameter inputs or response variables in N cycling models.

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Author contribution statement S.J.H. designed the study, S.R.W. and D.R.B. contributed to sample analysis and interpretation, and S.J.H. wrote the paper with contributions from S.R.W. and D.R.B.

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