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The isotopic composition of nitrate produced from nitrification in a hardwood forest floor

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Abstract

Dual isotopic analysis of nitrate ($^{15}N/^{14}N$ and $^{18}O/^{16}O$) is increasingly used to investigate the environmental impacts of human-induced elevated atmospheric nitrate deposition. In forested ecosystems, the nitrate found in surface water and groundwater can originate from two sources: (1) atmospheric deposition, and (2) nitrate produced from nitrification in forest soils (microbial nitrate). Application of the dual nitrate isotope technique for determining the relative importance of nitrate sources in forested catchments requires knowledge of the isotopic composition of microbial nitrate. We excluded precipitation inputs to three zero-tension lysimeters installed below the F-horizon (Oe) at the Turkey Lakes Watershed (TLW) in order to measure the isotopic composition of microbial nitrate produced *in situ*. To our knowledge, this is the first *in situ* study of the isotopic composition of microbial nitrate in forest soils. Over a 2-week period, nitrate produced by nitrification was periodically flushed to the lysimeters by watering the area with a nitrogen-free solution. Nitrate produced in the forest floor had $\delta^{18}O$ values ranging from +3.1‰ to +10.1‰ with a mean of +5.2‰. These values were only slightly higher than from the expected value of +1.0‰ calculated for chemolithoautotrophic nitrification, which depends on the $\delta^{18}O$ of available O_2 and H_2O. In addition to nitrate, we also collected soil gas to determine if soil respiration and O_2 diffusion affected soil gas $\delta^{18}O$–O_2, which is typically assumed to be identical to atmospheric O_2 (+23.5‰) when calculating microbial nitrate $\delta^{18}O$ values. No significant difference in $\delta^{18}O$–O_2 from atmospheric O_2 was found in forest soils to a depth of 55 cm, and therefore ^{18}O-enrichment of soil gas O_2 could not explain the modest enrichment of nitrate ^{18}O. Evaporative ^{18}O-enrichment of soil water available to nitrifiers in the forest floor is a plausible mechanism for slightly elevated nitrate $\delta^{18}O$ values. However, the observed nitrate $\delta^{18}O$ values could also be explained by a minor contribution of nitrate from heterotrophic nitrifiers. The $\delta^{15}N$ of nitrate produced ranged from −10.4 to −7.3‰ and, as expected, was depleted in ^{15}N relative to soil organic nitrogen. Microbial nitrate produced in the forest floor was also significantly depleted in ^{15}N relative to microbial nitrate exported in groundwater and headwater streams at the TLW. We hypothesize that ^{15}N-depleted forest floor nitrate is not detected in groundwaters largely because of: (1) the immobilization of forest floor nitrate in the mineral soil and (2) the mixing of the remaining forest floor nitrate with nitrate generated in the mineral soil, which is expected to have higher $\delta^{15}N$ values. This study demonstrates that current methods of calculating *a priori* the $\delta^{18}O$ of microbial nitrate provide a reasonable value for nitrate produced by nitrification at the TLW.

1. INTRODUCTION

Human activity has resulted in a doubling of the global pool of reactive nitrogen since pre-industrial times (Galloway et al., 1995). As a result, many parts of the world are

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experiencing elevated levels of atmospheric nitrogen deposition (Galloway et al., 1995). Some forested ecosystems that have traditionally been thought of as nitrogen-limited, are becoming nitrogen-saturated (Agren and Bosatta, 1988; Aber et al., 1989). Nitrogen saturation can decrease forest health through water stress, reduced frost tolerance, acidification, leaching of nutrients, and decreased root biomass (Aber et al., 1989).

Nitrogen-saturated forested catchments become leaky with respect to inorganic nitrogen and often experience elevated levels of nitrate in surface and groundwaters (e.g., Dise and Wright, 1995; Peterjohn et al., 1996). This nitrate can be from two sources: (1) nitrate from atmospheric deposition and (2) nitrate produced from the nitrification of ammonium in forest soils (microbial nitrate). Microbial and atmospheric nitrate have distinctly different isotopic signatures, particularly with respect to $\delta^{18}O$ (e.g., Durka et al., 1994). Analysis of stable isotope ratios in nitrate ($^{18}O/^{16}O$ and $^{15}N/^{14}N$) have proven to be a powerful tool for investigating nitrate sources and cycling in forested watersheds (Durka et al., 1994; Kendall et al., 1996; Spoelstra et al., 2001; Williard et al., 2001; Burns and Kendall, 2002; Schiff et al., 2002; Sickman et al., 2003).

$\delta^{15}N$ and $\delta^{18}O$ values of both atmospheric and microbial nitrate are needed to calculate the fraction of nitrate from each source in streams and groundwater. The isotopic signature of atmospheric nitrate can be measured directly by collecting and analyzing precipitation ($\delta^{15}N = -6$ to $+12‰$, $\delta^{18}O = +14$ to $+76‰$; Kendall, 1998). However, the microbial end-member is not as easily determined from field samples, which usually contain a mixture of nitrate from both sources. Laboratory studies have shown that, during the conversion of ammonium to nitrate by chemolithoautotrophic bacteria, two oxygens come from water and one from O_2 (Aleem et al., 1965; Andersson and Hooper, 1983; Kumar et al., 1983; Hollocher, 1984). Therefore, Eq. (1) can be used to calculate microbial nitrate $\delta^{18}O$ values from the $\delta^{18}O$–H_2O of available water and the $\delta^{18}O$ of atmospheric O_2. Using representative values for $\delta^{18}O$–H_2O and $\delta^{18}O$–O_2, microbial nitrate $\delta^{18}O$ values should generally range from −10 to +10‰ (Kendall, 1998).

$$\delta^{18}O\text{—}NO_3^-(\text{microbial}) = 1/3(\delta^{18}O\text{—}O_2) + 2/3(\delta^{18}O\text{—}H_2O) \tag{1}$$

Nitrate $\delta^{18}O$ values in the range of the predicted microbial end-member have been measured in groundwaters in agricultural areas (Böttcher et al., 1990; Wassenaar, 1995; Cey et al., 1999; Mengis et al., 1999, 2001) and septic plumes (Aravena et al., 1993; Aravena and Robertson, 1998). Although some studies in forested watersheds have observed stream and groundwater nitrate $\delta^{18}O$ values low enough to be 100% microbial nitrate (Spoelstra et al., 2001; Schiff et al., 2002; Williard et al., 2001), others only report values well above the calculated microbial nitrate end-member (Durka et al., 1994; Kendall et al., 1996; Burns and Kendall, 2002; Campbell et al., 2002; Sickman et al., 2003). Based on the results of several laboratory and field-based studies, Kendall (1998) concluded that the $\delta^{18}O$ of microbial nitrate may be as high as 16‰. Therefore Eq. (1) may not be applicable to all forest soils and its use could lead to errors in the calculation of nitrate source contributions in some systems.

Several hypotheses have been suggested to explain microbial nitrate $\delta^{18}O$ values higher than predicted by Eq. (1) (summarized in Kendall, 1998). Water in shallow soils might be enriched in ^{18}O by evaporation. Therefore $\delta^{18}O$–H_2O values of precipitation or groundwater would not accurately reflect the $\delta^{18}O$ of water available to nitrifiers. By measuring the isotopic composition of twig water ($\delta^{18}O$ and δD), Tang and Feng (2001) found that the water taken up by a sugar maple tree had been enriched in ^{18}O by evaporation by as much as 7‰. However, the enrichment effects of evaporation decrease rapidly with depth in the soil profile (e.g., Allison, 1983). Since trees can take up water from both shallow and deep sources, the $\delta^{18}O$ value of shallow soil water taken up by the tree in the Tang and Feng (2001) study must have been greater than 7‰. Therefore it seems likely that the water available to nitrifiers would periodically be enriched in ^{18}O by evaporation, especially in the upper soil layers.

Heterotrophic nitrification incorporates a maximum of one oxygen from water and could also be a possible mechanism for producing microbial nitrate with higher $\delta^{18}O$ values (Mayer et al., 2001). Heterotrophic nitrifiers, which consist largely of fungi and some bacteria species, predominantly convert organic nitrogen to nitrite and nitrate through biochemical pathways that do not provide energy to the microorganisms (Killham, 1986; Bock et al., 1991). Relatively little is known about the conditions that favor heterotrophic nitrification in soils (Killham, 1986; Pedersen et al., 1999). Heterotrophic nitrifiers only produce small amounts of inorganic nitrogen and therefore chemolithoautotrophs are generally the dominant nitrifiers in environments with high nitrate production (Focht and Verstraete, 1977; Killham, 1986; Bock et al., 1991). Conversely, heterotrophic nitrification may be important in forest soils where nitrification rates are comparatively low.

Finally, the $\delta^{18}O$ value of oxygen gas, which is constant in the atmosphere (+23.5‰ relative to Vienna Standard Mean Ocean Water (VSMOW), Kroopnick and Craig, 1972), might be modified by processes in the subsurface, resulting in higher or lower $\delta^{18}O$–O_2 values. Laboratory experiments have shown that respiratory oxygen consumption by plant roots and microbes can cause ^{18}O-enrichment of soil O_2 (Lane and Dole, 1956; Forstel and Schleser, 1976; Schleser, 1979; Guy et al., 1989, 1993; Robinson et al., 1992; Angert and Luz, 2001a; Lee et al., 2003). In environments where diffusion controls soil gas oxygen isotope ratios, lower $\delta^{18}O$ values are expected with depth since $^{16}O^{16}O$ diffuses faster than $^{18}O^{16}O$ (e.g., Severinghaus et al., 1996). Factors such as respiration rate, microbial community, soil moisture, temperature, and soil structure, control the balance between the fractionation effects of respiration and those of diffusion and therefore determine the magnitude and direction of any isotopic shift in soil $\delta^{18}O$–O_2 values.

A limited number of field studies in non-forest systems have measured $\delta^{18}O$ values of soil O_2 and these studies have shown both directions of isotopic shift with depth. Wassenaar and Koehler (1999) and Lee et al. (2003) found