Soil Biology & Biochemistry 57 (2013) 731–738

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Nitrate–nitrogen and oxygen isotope ratios for identification of nitrate sources and dominant nitrogen cycle processes in a tile-drained dryland agricultural field

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ARTICLE INFO

Article history:
Received 20 February 2012
Received in revised form
11 October 2012
Accepted 14 October 2012
Available online 8 November 2012

Keywords:
Nitrification
Nitrate-–nitrogen and oxygen isotope ratios
Nitrogen cycle
Nitrate leaching
Nitrate fluxes

ABSTRACT

Agricultural systems are a leading source of reactive nitrogen to aquatic and atmospheric ecosystems. In this study environmental $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ are used to identify the dominant nitrogen cycle processes and sources of NO_3^- leached from a tile-drained, dryland agricultural field. Tile-drain water discharge $\delta^{18}O_{nitrate}$ values suggest nitrification is the dominant soil nitrogen cycle process throughout the 5-year study period, because the expected $\delta^{18}O_{nitrate}$ from nitrification is indistinguishable from the measured value of $-1.3 \pm 1.5‰$. Given this there is no evidence that denitrification was occurring at a large enough scale to influence $[NO_3^-]$. Values for $\delta^{15}N_{nitrate}$ varied seasonally during the high-discharge season (January through May) and low-discharge season (June through December) with weighted means of $1.0 \pm 1.0‰$ and $4.7 \pm 2.3‰$ respectively. This suggests that during the high-discharge season NO_3^- originates from nitrification of NH_4^+ fertilizer, and during the low-discharge season NO_3^- originates from mineralized soil organic nitrogen. The estimated travel time through the soil for nitrified NH_4^+ fertilizer leached during the high-discharge season is less than 6 months, from fall fertilizer application to leaching through the tile-drain. This study suggests that understanding the hydrology of a region is necessary before dominant nitrogen cycling processes can be reliably determined.

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1. Introduction

Nitrogen fertilizer leads to excess reactive nitrogen stimulating nitrogen cycle processes that transform and potentially release nitrogen from agricultural systems (Cey et al., 1999; Karr et al., 2001; Munoz et al., 2003; Deutsch et al., 2005). Processes of particular concern are nitrification and denitrification because of their potential to produce the greenhouse gases nitrous oxide (N_2O) and nitric oxide (NO), as well as controlling the abundances of ammonium (NH_4^+) and nitrate (NO_3^-) available for leaching to surface and groundwater systems. Most studies in agricultural systems account for nitrogen loss through nitrification and denitrification by assuming the rates are similar to previous field studies (Smith et al., 2000; Munoz et al., 2003; Ross et al., 2008) or that the rates are insignificant at the scale of the study (Soon and Clayton, 2003). Assumed rates can lead to misunderstanding of actual nitrogen cycle processes and pathways for nitrogen loss.

Developing a better understanding of nitrogen cycle processes and storage rates will lead to more precise nitrogen budgets and more efficient nitrogen management practices. The first step to achieve an accurate understanding of field-scale nitrogen cycling is to improve the understanding of nitrification and denitrification at these scales. In a dryland setting with strongly seasonal precipitation and temperature cycles, transitions between nitrification and denitrification regimes may be influenced by soil-vadose hydrologic dynamics. Prior to winter precipitation and the development of saturation, aerated profiles may promote nitrification. Later, saturated profiles may promote denitrification. These interactions of hydrologic and soil-biochemical processes could strongly affect the timing and rates of leaching losses of N as NO_3^-. Here we use nitrogen and oxygen isotopic compositions of NO_3^- to determine the seasonal timing of nitrification and denitrification, and how these processes influence the sources and fluxes of NO_3^- leached from a dryland agricultural field.

Natural abundance of N & O isotope ratios in NO_3^- ($\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$) have been shown to be useful tools to identify the sources of NO_3^- and dominant nitrogen cycle processes (Kendall et al., 1995; Lake et al., 2001; Robinson, 2001; Chang et al., 2002;

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http://dx.doi.org/10.1016/j.soilbio.2012.10.017

McKinney et al., 2002; Vander Zaden et al., 2005; Kendall et al., 2007; Wankel et al., 2007; Wankel et al., 2009). Nitrate deposited in precipitation has $\delta^{18}O_{nitrate}$ values from +23‰ to +75‰ and $\delta^{15}N_{nitrate}$ values from −15‰ to +15‰ (Durka et al., 1994; Kendall et al., 1995; Oelmann et al., 2007). Nitrate originating from synthetic NH_4^+ fertilizer and mineralized soil organic nitrogen has $\delta^{18}O_{nitrate}$ values from −10‰ to +10‰ (Durka et al., 1994; Kendall et al., 1995), and $\delta^{15}N_{nitrate}$ values from −1.7‰ to +3.9‰ and +2‰ to +10‰; respectively (Vitoria et al., 2004; Kendall et al., 2007; Oelmann et al., 2007). Synthetic NO_3^- fertilizer $\delta^{18}O_{nitrate}$ ranges from +18‰ to +22‰ and $\delta^{15}N_{nitrate}$ from −1.7‰ to +3.9‰, similar to $\delta^{15}N_{nitrate}$ of nitrified NH_4^+ fertilizer (Vitoria et al., 2004; Kendall et al., 2007; Oelmann et al., 2007).

Dominant nitrogen cycle processes can also be distinguished through changes in NO_3^- concentration ($[NO_3^-]$) and $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$. Denitrification results in a fractionation ratio for $\delta^{15}N_{nitrate}$:$\delta^{18}O_{nitrate}$ of 1:1 to 2.1:1 while also decreasing $[NO_3^-]$ (Cey et al., 1999; Durka et al., 1994; Kendall et al., 1995; Davis et al., 2008). Nitrification does not discriminate against ^{18}O isotopes, so the $\delta^{18}O_{nitrate}$ is a combination of the ratios of $\delta^{18}O_{water}$ and $\delta^{18}O_{atmospheric\ O_2}$ (Kumar et al., 1983; Hollocher, 1984; Durka et al., 1994; Kendall et al., 1995).

The objectives of this study were to use $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ to identify the roles of nitrification and denitrification in NO_3^- production/consumption in a 12 ha field catchment, and to identify the source(s) of NO_3^- leached from this catchment via a tile drain. This study was conducted to test the following hypotheses: 1) the dominant source of nitrate in tile-drain discharge is synthetic nitrogen fertilizer applied in the fall prior to the onset of the precipitation season, 2) the dominant nitrogen cycle process during the low-discharge season (June through December) is nitrification, 3) the dominant nitrogen cycle process during the high-discharge season (January through May) is denitrification.

2. Methods and analysis

2.1. Study site

Research was conducted at the 12 ha tile-drained section (TD-12) of the Washington State University Cook Agronomy Farm (CAF) in the semiarid Palouse Basin of eastern Washington and northern Idaho (46°46′44″ N, 117°05′19″ W). Annual precipitation is 310–580 mm yr^{-1} with the majority occurring during the winter (Donaldson, 1980). Previous hydrologic investigations at the CAF and in the larger Missouri Flat Creek Watershed have shown that winter precipitation stimulated water movement in the soil at CAF and winter precipitation is the source water of stream flow for the Missouri Flat Creek Watershed throughout the year (Keller et al., 2008; Moravec et al., 2010). Mean annual temperatures range from a mean summer high of 27 °C to a mean winter low of −7 °C (Geyer et al., 1992). The topography consists of rolling hills with up to 30 m of relief in loess deposits that overly Columbia River Basalt flows (Treasher, 1925). The soils are silt loam Mollisols that are mapped as part of the Palouse–Thatuna Association soil series (USDA, 1978). The Cook Agronomy Farm is within the Missouri Flat Creek Watershed, which has a land use of >95% dryland agriculture with the remaining land use being residential farmhouses, horse pasture, and a gravel pit. Crops grown at the CAF consist of a rotation between winter wheat, spring wheat, and chickpeas. Fertilization rates at CAF range from 123 kg N ha^{-1} yr^{-1} to 215 kg N ha^{-1} yr^{-1} (Keller et al., 2008). The nitrogen is currently applied as an ammonium–nitrate–urea blend. Fertilization for the winter wheat occurs during the fall, and in the spring for the spring wheat and chickpeas. Soil organic nitrogen mineralization rates in the Palouse Region are reported to range from 4.5 kg N ha^{-1} yr^{-1} to 110 kg N ha^{-1} yr^{-1} (Fiez et al., 1994).

2.2. Sample collection

Samples were collected from the TD-12 outlet (April 2005–April 2010) and from a precipitation catch (April 2006–April 2010) above the TD-12 outlet in Nalgene® bottles that were previously acid washed with 10% HCl and rinsed three times each with deionized water and ultra-pure deionized water. TD-12 event samples were collected in 250 mL bottles at ~1 week intervals during the high-discharge season (January–May) and at 2–3 week intervals during the low-discharge season (June–December). Precipitation samples were collected in a 1000 mL bottle draining a tipping-bucket precipitation collector at the southwest corner of CAF. The tipping bucket was mounted on a post, with its top ~1 m above the soil surface and ~5 m from the TD-12 outlet. Precipitation samples were collected after the bottle had accumulated at least 100 mL of water, which could take <1 week during the winter and up to ~3 weeks during the summer. The longer sampling intervals during the summer occurred because of long periods without precipitation. The precipitation catch was kept as clean as possible and samples were collected after events to limit biological activity in the precipitation catch. TD-12 discharge was measured with a 1 L bucket (during low flow) or 18 L bucket (during high flow) and stopwatch to determine flow rates out of the tile-drain system. After collection, samples were stored in a cooler on ice and brought back to the Washington State University School of the Environment Vadose and Groundwater Hydrology Laboratory, the same day. Aliquots were vacuum filtered through 0.45 μm cellulose nitrate membrane filters; filtration was typically within 24 h and never exceeded 48 h. Field blanks of ultra pure deionized water were also filtered alongside samples and analyses for $[NO_3^-]$; field blank $[NO_3^-]$ never exceeded the detection limits. Samples not filtered the same day were stored in a refrigerator until filtration. After filtration two sample aliquots were stored in 25 mL Nalgene® bottles with foil lined caps and frozen until analysis for $[NO_3^-]$ and ^{15}N and ^{18}O isotopic composition of NO_3^-.

2.3. Chemical and isotope analysis

Samples were analyzed for $[NO_3^-]$ by Washington State University United States Department of Agriculture-Agricultural Research Service Laboratory (WSU USDA-ARS) by continuous flow analysis with Model RFA300, Alpkem/OI Analytical and were reported as mg N L^{-1}.

Nitrate $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ were measured using the denitrifier method of Sigman et al. (2001) and Casciotti et al. (2002), with the bacterial strain *Pseudomonas choloraphis subs. aureofacies* (ATCC no. 13985) at the Washington State University Stable Isotope Core Laboratory. Samples were injected into 20 mL glass vials that were previously inoculated with 2 mL of condensed bacterial cultures in growth media, and then purged with N_2 to achieve anoxic conditions. Samples were then incubated overnight to ensure the entire sample NO_3^- was converted to N_2O. Prior to analysis samples were injected with 10 N NaOH to lyse cell walls releasing trapped N_2O. $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ of the produced N_2O were then measured on a Thermo Finnigan Delta Plus XP mass spectrometer with a Thermo Finnigan Gas Bench II (Bremen, Germany) connected to a GC Pal (CTC Analytics, Zwingen, Switzerland) auto sampler using He as a carrier gas. The original Gas Bench II was modified slightly to include an initial dry ice + ethanol water trap and Ascarite + magnesium perchlorate trap to scrub CO_2 from the mixture. The sampling needle was

replaced with a needle we fabricated which placed helium supply into the liquid media of sample vials, thereby pushing any dissolved N_2O into the sample line. Samples were normalized with USGS32 (180‰), USGS34 (−1.8‰), and $IAEANO_3$ (4.72‰) for ^{15}N and USGS32 (25.40‰), USGS34 (−27.78‰), USGS35 (56.81‰), and $IAEANO_3$ (25.32‰) for ^{18}O relative to VSMOW. Typical error of standards (1 sigma) was ≤0.2‰ for ^{15}N and ^{18}O.

2.4. Calculations and statistical analysis

Estimating conservatively high NO_3^-–N flux through precipitation was calculated by:

$$(\text{Yearly Precipitation Rate} \times \text{Precipitation Mean}[NO_3^-]) \quad (1)$$

where yearly precipitation rate was the high-end precipitation rate of 580 mm yr^{-1} and $[NO_3^-]_{mean\ precip}$ was the volume-weighted nitrate concentration of precipitation.

When nitrification occurs the expected $\delta^{18}O_{nitrate}$ can be calculated by Durka et al. (1994), Hollocher (1984), Kendall et al. (1995) and Kumar et al. (1983):

$$\text{Expected } \delta^{18}O_{nitrate} = 2/3\delta^{18}O_{water} + 1/3\text{soil } \delta^{18}O_{O_2} \quad (2)$$

Moravec et al. (2010) found that winter precipitation was the dominant source of water to both the TD-12 system and the rest of the Missouri Flat Creek watershed. Isotopic composition of water discharged from TD-12 varies little, with a mean $\delta^{18}O_{water}$ from October 2000 to December 2007 of −14.7‰ (s = 0.9‰) (Moravec et al., 2010). This study assumes that this mean value characterizes the water contributing oxygen in the nitrification process (2). We assume that soil $\delta^{18}O_{O_2}$ is equivalent to atmospheric $\delta^{18}O_{O_2}$ of +23.5‰ (Kroopnick and Craig, 1972).

Estimates of the mean monthly NO_3^- flux were calculated based on measured discharge and $[NO_3^-]$:

$$\text{Mean Monthly } NO_3^- - \text{N Flux} = \sum([NO_3^-] \times \text{Discharge})/(n \times \text{area}) \quad (3)$$

where n is the number of samples in a given calendar month over the period of record (2005–2010). $[NO_3^-]$ and *discharge* are the concentration and flow rate on the day of sampling, and *area* is the area of the tile-drained watershed (12 ha).

A z-test was used to determine if the seasonal differences in $\delta^{15}N_{nitrate}$ were statistically significant, and a t-test to assess whether the observed $\delta^{18}O_{nitrate}$ values were statistically distinguishable from expected $\delta^{18}O_{nitrate}$.

3. Results

3.1. Precipitation $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$

Precipitation $[NO_3^-]$ from 2007 through 2010 ranged from 0.1 to 5.7 mg L^{-1} with a volume-weighted mean of 1.7 ± 1.5 mg L^{-1} (Fig. 1A). Precipitation $\delta^{15}N_{nitrate}$ values ranged from −6.3‰ to +6.5‰ with a weighted mean of +0.2‰ (Fig. 1B). There were no observable seasonal variations for precipitation $[NO_3^-]$ or precipitation $\delta^{15}N_{nitrate}$ values (Fig. 1A and 2B). Precipitation $\delta^{18}O_{nitrate}$ values ranged from −4.9‰ to +91.4‰ with a weighted mean of +52.4‰ (Fig. 1C). Precipitation $\delta^{18}O_{nitrate}$ did have a seasonal trend with winter (October through March) and summer (April through September) weighted means of 69.5‰ and 44.3‰ respectively.

3.2. Leached $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$

$[NO_3^-]$ from 2005 to 2010 ranged from 2.9 mg L^{-1} to 25.5 mg L^{-1} with an overall volume-weighted mean of 19.3 ± 6.1 mg L^{-1} (Fig. 2A). The highest $[NO_3^-]$ occurred during the high-discharge season (January through May) and the lowest $[NO_3^-]$ occurred during the low-discharge season (June through December).

$\delta^{15}N_{nitrate}$ (Fig. 2B) for TD-12 ranged from −0.5‰ to 9.0‰ with a volume-weighted mean of 2.1‰, overlapping with precipitation $\delta^{15}N_{nitrate}$ values (Fig. 3). Values varied seasonally: volume-weighted means for the high-discharge and low-discharge seasons were 0.9‰ and 4.7‰ for $\delta^{15}N_{nitrate}$ respectively (Table 1). These means were statistically different (p-value <0.001). In general, $\delta^{15}N_{nitrate}$ was inversely related to $[NO_3^-]$ in time (Fig. 2A and B). TD-12 $\delta^{18}O_{nitrate}$ ranged from −6.6‰ to 3.8‰ with a volume-weighted mean of −1.3‰ (Fig. 2C). There were no clear seasonal fluctuations for $\delta^{18}O_{nitrate}$.

3.3. Seasonality of NO_3^- fluxes

Monthly NO_3^- fluxes during the high-discharge season ranged from 1.41 to 6.59 kg NO_3 ha^{-1} $month^{-1}$ and $\delta^{15}N_{nitrate}$ range from 0.5 to 1.6‰ (Table 1). These flux estimates for the high-discharge season are believed to be conservatively small, because the weekly to biweekly sampling frequency did not capture the peaks of surge events during spring runoff. Approximately 96% (~23 kg NO_3–N ha^{-1} yr^{-1}) of the yearly NO_3^- flux from the TD-12 system occurred during the high-discharge season (Fig. 4).

Monthly NO_3^- fluxes during the low-discharge season ranged from 0.01 to 0.53 kg NO_3 ha^{-1} yr^{-1} and $\delta^{15}N_{nitrate}$ ranged from 3.1 to 5.9‰ (Table 1). Approximately 4% (~1 kg NO_3^- ha^{-1} yr^{-1}) of the yearly flux occurred during this period (Fig. 4).

4. Discussion

4.1. Nitrate sources

Precipitation NO_3^- was determined to have no measurable influence on TD-12 $[NO_3^-]$, based on the relatively low mass of NO_3^- added to the system through precipitation events compared to fertilizer application rates. Using Equation (1), estimated yearly NO_3^- flux through precipitation to the tile-drained section of CAF is ~10 kg NO_3^- ha^{-1} yr^{-1}, which based on fertilizer application rates of 123 kg N ha^{-1} yr^{-1}–215 kg N ha^{-1} yr^{-1} (Keller et al., 2008) is 12–22 times smaller then the mass of inorganic nitrogen applied during fertilization. Also, $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ of precipitation NO_3^- allows it to be easily distinguished from TD-12 NO_3^-, and there was no evidence to suggest leached NO_3^- $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ were influenced by precipitation NO_3^- (Fig. 3). Winter precipitation $\delta^{18}O_{nitrate}$ was significantly more enriched than leached $\delta^{18}O_{nitrate}$ values (+69.5‰ and −1.3‰ respectively). If NO_3^- in winter precipitation did influence leached NO_3^-, we would expect to see more enriched leached $\delta^{18}O_{nitrate}$ values. Some precipitation samples collected during the spring and summer had unusually low $\delta^{18}O_{nitrate}$ values compared to the expected range (Fig. 3). This pattern could be connected to similar seasonal effects observed in precipitation $\delta^{18}O_{water}$ at CAF (Moravec, 2008). Similar seasonal variations have also been reported for precipitation $\delta^{18}O_{nitrate}$ at other locations (Kendall et al., 1995). Other possible causes of the unusually low $\delta^{18}O_{nitrate}$ are: nitrification occurring in the precipitation catch prior to sample collection, or contamination from windblown sediment. Especially during the summer, it was common for dust to accumulate in the bottom of the sample bottle. This study did not investigate the cause for the usually low $\delta^{18}O_{nitrate}$ values, because precipitation NO_3^- was determined to have no measurable influence on leached $\delta^{18}O$–NO_3^- values. The

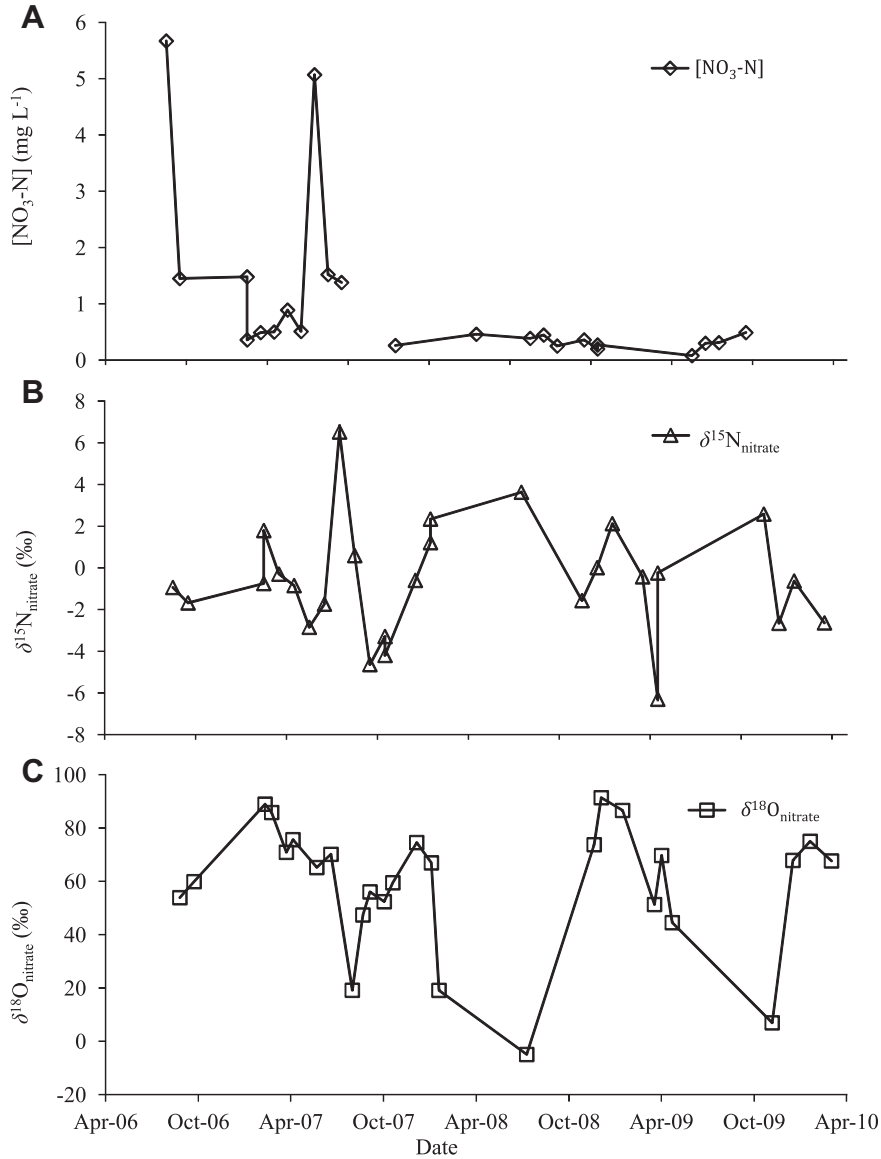

Fig. 1. Precipitation [NO_3^-], $\delta^{15}N_{nitrate}$, and $\delta^{18}O_{nitrate}$ over 3 water years. [NO_3^-] (panel A) (open diamonds), $\delta^{15}N_{nitrate}$ (panel B) (open triangles), and $\delta^{18}O_{nitrate}$ (panel C) (open circles) of precipitation from April 2006 through October 2010. Note different dimensions of the y-axes. Samples represent bulk collection over time intervals ranging from <1 week up to 3 weeks, depending on precipitation rates, to achieve at least 100 mL of precipitation water in collector. Error bars represent the standard error for samples that were analyzed on at least three different days. [NO_3^-–N] was typically below ~1.5 mg L^{-1} except for on 8/25/2006 and 7/31/2007, when [NO_3^-–N] exceeded 5 mg L^{-1}. Even considering the two high [NO_3^-] events, precipitation nitrate is a negligible source of NO_3^- to the Cook Agronomy Farm compared to fertilization.

relatively depleted precipitation $\delta^{18}O_{nitrate}$ events all occurred during the summer and fall when precipitation was shown by Moravec et al. (2010) to be lost due to evapotranspiration and not leached through the tile-drain system.

Measured TD-12 $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ (Fig. 3) were consistent with reported values for nitrified NH_4^+ fertilizer and mineralized soil organic matter NO_3^- sources (Chang et al., 2002; Durka et al., 1994; Kendall et al., 1995; Vitoria et al., 2004; Kendall et al., 2007; Oelmann et al., 2007; Lee et al., 2008). The distinctive seasonal $\delta^{15}N_{nitrate}$ variations, coinciding with seasonal discharge and flux variations, are interpreted as indicators of one source dominating leached NO_3^- during the high-discharge season, and another source dominating during the low-discharge season. The high-discharge $\delta^{15}N_{nitrate}$ range was within the expected values for NO_3^- produced from nitrification of NH_4^+ fertilizers (Kendall et al., 1995, 2007). Oelmann et al. (2007) showed that mineralization of leguminous organic matter in soils produces similar $\delta^{15}N_{nitrate}$ as nitrified NH_4^+ fertilizers. The CAF does produce chickpeas, a legume capable of N_2 fixation, on approximately 1/4 of the farm per year. However every third year the chickpeas are on the eastern edge of the farm that makes up less then 10% of the artificially drained section of the CAF, and when chickpeas are grown along the western edge they only account for ~1/3 of the artificially drained area. The chickpeas also receive a starter dose of fertilizer when they are planted in the spring to encourage root development, and depending on residual plant available nitrogen the rate of N_2 fixation would decrease (Doughton et al., 1993). Fatima et al. (2008) found chickpeas grown in rotation with fertilized wheat in a rain fed agricultural system resulted in an N balance of +38 kg N ha^{-1} and +35 kg N ha^{-1} during a two-year study. If we assume similar positive balances in our system and that all of the residual N were mineralized to NO_3^-, then mineralized chickpea residue could

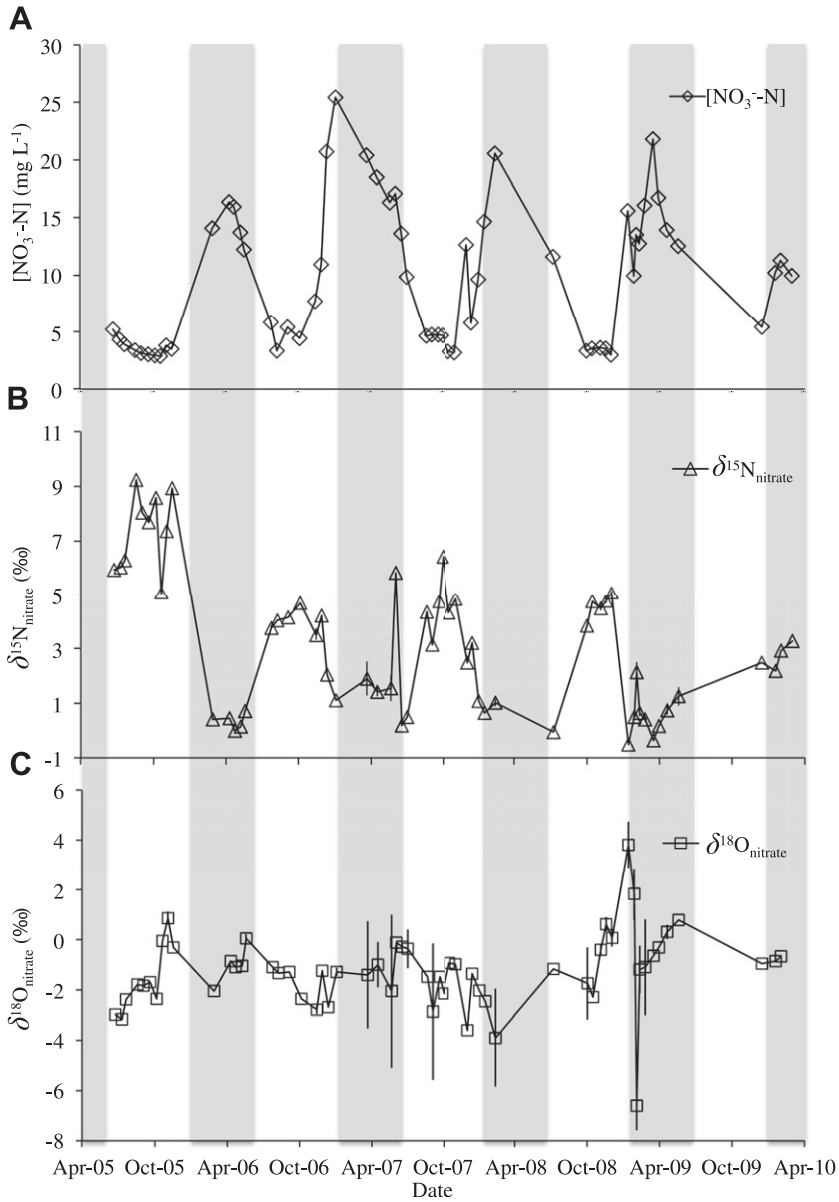

Fig. 2. Tile Drain [NO_3^-], $\delta^{15}N_{nitrate}$, and $\delta^{18}O_{nitrate}$ over 5 water years. [NO_3^-] (panel A) (open diamonds), $\delta^{15}N_{nitrate}$ (panel B) (open triangles), and $\delta^{18}O_{nitrate}$ (panel C) (open circles) of leached NO_3^- from April 2005 through October 2010. Error bars represent the standard error of the sample that were analyzed on at least three different days. Note different dimensions of the y-axes. Samples were event-based samples collected every 1–2 weeks. Shade areas represent the high-discharge season. Leached [NO_3^-–N] ranged from ~3 mgL^{-1} during the low-discharge season (June through December) to ~25 mg L^{-1} during the high-discharge season (January through May). Leached NO_3^- $\delta^{15}N_{nitrate}$ also varied during the high and low discharge seasons from −0.5 ‰ to +9 ‰. Leached NO_3^- $\delta^{18}O_{nitrate}$ did not vary during the high and low discharge seasons, averaging −1.3 ‰ throughout the study.

contribute approximately 10%–25% of the total nitrate load to the CAF with fertilizer applications accounting for the rest. But given crop rotations and the small size of the chickpea cultivation area in the TD-12 catchment, mineralization of chickpea based soil organic matter is unlikely to be a substantial source of nitrate during the high-discharge season. The low-discharge $\delta^{15}N_{nitrate}$ was within the expected range for nitrate produced from mineralized soil organic nitrogen (Kendall et al., 1995, 2007; Oelmann et al., 2007).

A linear mixing model (Fig. 5) was tested by plotting $\delta^{15}N_{nitrate}$ against 1/[NO_3^-]. Mixtures should plot on a line between the two end-member NO_3^- source isotope values (Mariotti et al., 1988). The linear relationship ($R^2 = 0.67$) supports the interpretation of two sources of nitrate, NH_4^+ fertilizer and soil organic nitrogen. This simplified mixing model is preferred to an interpretation based on Rayleigh fractionation (commonly represented by plotting $\delta^{15}N_{nitrate}$ or $\delta^{18}O_{nitrate}$ against ln[NO_3^-]). To attribute isotopic changes to this mechanism, we would have to assume that the system is closed, and that there is only one source of nitrate. Neither of these assumptions is reasonable in this case.

Based on our estimates of mean monthly fluxes (Table 1) calculated with Equation (3), approximately 23 kg leached NO_3^- ha^{-1} yr^{-1} originates from NH_4^+ fertilizer. Mineralized soil organic nitrogen accounts for ~1 kg leached NO_3^- ha^{-1} yr^{-1} leached during the low-discharge season with mean $\delta^{15}N_{nitrate}$ of 4.7‰. Reported N mineralization rates (Fiez et al., 1994) are more than adequate to generate this flux. The ~23 kg leached NO_3^- ha^{-1} yr^{-1} originating from NH_4^+ fertilizer accounts for approximately 20% of the mean yearly fertilizer application to the TD-12 section of the CAF over the last 10 years.

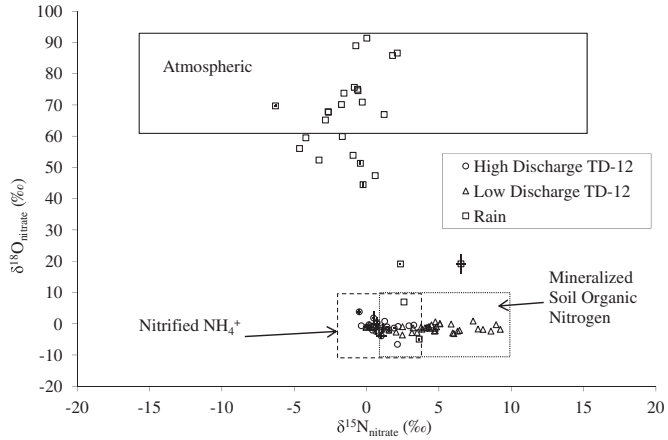

Fig. 3. NO_3^- Sources at Cook Agronomy Farm. $\delta^{15}N_{nitrate}$ (AIR) and $\delta^{18}O_{nitrate}$ (VSMOW) of precipitation NO_3^- (open squares), high-discharge season leached NO_3^- (open circles), and low-discharge season leached NO_3^- (open triangles). Error bars represent the standard error of the sample, typically smaller then the symbols of the mean, which were analyzed on at least three different days. Boxes represent typical isotope compositions of nitrate from the atmosphere, nitrification of NH_4^+ fertilizer, and in soil mineralization of soil organic nitrogen (SON) (Cey et al., 1999; Kendall et al. 1995; Kendall et al., 2007; Lee et al., 2008; Lake et al., 2001; McKinney et al., 2002; Oelmann et al., 2007; Vitoria et al., 2004).

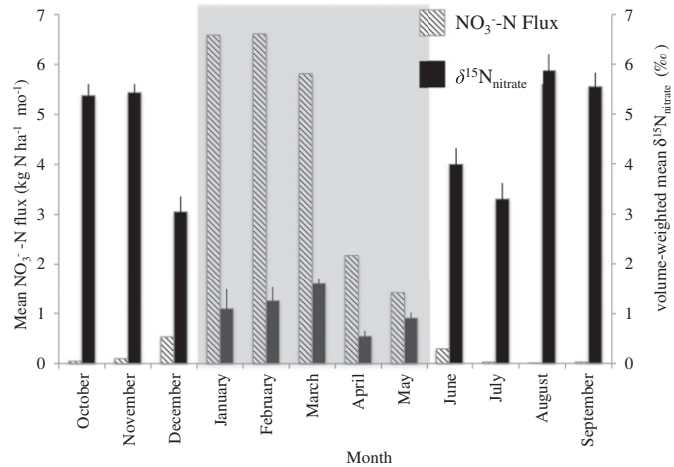

Fig. 4. 2005–2010 TD-12 monthly NO_3^-–N flux and volume-weighted mean $\delta^{15}N_{nitrate}$. 2005–2010 mean monthly NO_3^- Flux (stripped bars) as calculated by equation 3, and volume-weighted mean monthly $\delta^{15}N_{nitrate}$ (solid bars) over 5 years. The shaded region represents the high-discharge season, and the non-shaded region is the low-discharge season. Error bars represent the measured $\delta^{15}N_{nitrate}$ standard error. The majority of NO_3^- flux occurs during the high-discharge season (~96% of the yearly flux). The high-discharge season volume-weighted mean monthly $\delta^{15}N_{nitrate}$ ranged from 0.5 ‰ to 1.5 ‰, and the low-discharge season ranged from +3 ‰ to +6 ‰. The difference between high and low-discharge season volume-weighted mean monthly $\delta^{15}N_{nitrate}$ is interpreted to indicate a shift from nitrified NH_4^+ fertilizer (high-discharge) to mineralized soil organic nitrogen (low-discharge) sources of NO_3^-.

Because most leached NO_3^- originates from synthetic fertilizer, it follows that the leached flux can be reduced through improved fertilizer and crop efficiencies. Employing systems, such as precision agriculture, that match the fertilizer demand of an area to the appropriate application rate will reduce the total amount of fertilizer applied to the field. Because most NO_3^- is lost from January through May, the potential to remediate leached NO_3^- fluxes with buffer strips or other biological process is limited, because weather conditions during this time slow biologic activities.

Keller et al. (2008) proposed that soil NO_3^- accumulates in the soil profile at the depth of the tile-drain during summer and fall due to soil mineralization and nitrification of NH_4^+ fertilizer. This built-up pool of NO_3^- is what they believed to be responsible for the immediate appearance of high [NO_3^-] at the onset of tile-drain discharge. Our results suggest that, if at the onset of discharge high [NO_3^-] was based on the Keller et al. (2008) interpretations, the resulting $\delta^{15}N_{nitrate}$ values would resemble a mixture of the two sources (soil mineralization and nitrification of NH_4^+ fertilizer). Any such effect appears to be small (Figs. 2 & 4), suggesting that accumulation of mineralized soil N (during the summer and fall) contributes negligible amounts of NO_3^- during the high-discharge period. We suggest that redistribution of soil water during the wetting-up of the profile is essential for vertical transport of NO_3^- to the depth of the tile drain and that this transport is principally responsible for the instantaneous high [NO_3^-] at the onset of discharge.

Table 1
2005–2010 monthly mean NO_3^-–N Flux and $\delta^{15}N_{nitrate}$ values.

Month	NO_3^-–N flux (kg ha^{-1} Month^{-1})	$\delta^{15}N_{nitrate}$	
		Average (‰)	Standard error
October	0.05	5.4	0.2
November	0.10	5.4	0.2
December	0.53	3.1	0.3
January	6.59	1.1	0.4
February	6.61	1.3	0.3
March	5.82	1.6	0.1
April	2.18	0.5	0.1
May	1.41	0.9	0.1
June	0.29	4.0	0.3
July	0.03	3.3	0.3
August	0.01	5.9	0.3
September	0.03	5.6	0.3

The shaded area represents the high-discharge season, and the non-shaded area the low-discharge season.

4.2. Hydrologic controls on nitrogen cycling

Recent research at the CAF determined that seasonal precipitation and soil–water-flow variations were the primary drivers of NO_3^- loss (Keller et al., 2008). Typically, drainage through TD-12 and the adjoining ditches is not triggered until approximately 150 mm

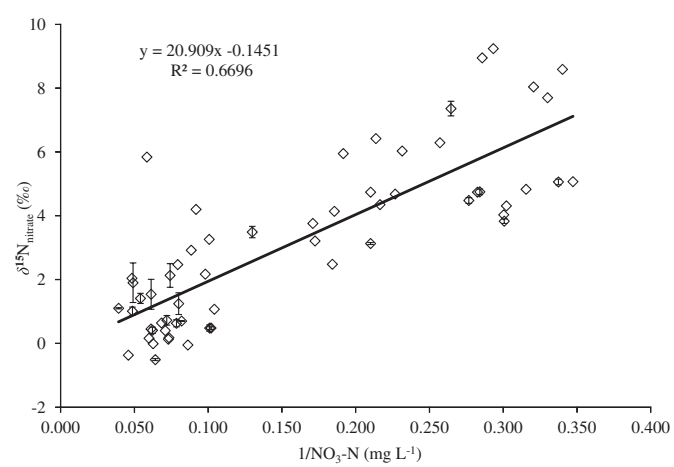

Fig. 5. Leached NO_3^- two-ended mixing model. Leached NO_3^- (open diamonds) linear mixing model, error bars represent the $\delta^{15}N_{nitrate}$ standard errors, for some samples the error bars were smaller then the symbol used to represent the sample mean. A linear mixing model is used to support the interpretation that there are two sources of NO_3^-, nitrified NH_4^+ fertilizer during the high-discharge season and mineralized soil organic nitrogen during the low-discharge season. The strong correlation ($R^2 = 0.6696$) supports the interpretation of mixing between two NO_3^- sources.

of fall and winter precipitation has saturated the soil profile above the drainage network (Keller et al., 2008). Here we build upon these ideas and suggest that fall fertilization primes the soil with NH_4^+, and the first 150 mm of winter precipitation stimulates nitrification and transports the produced NO_3^- throughout the soil-vadose profile prior to the onset of TD flow. Continued winter precipitation transports additional nitrified NH_4^+ fertilizer in the form of NO_3^- through the soil and into the tile-drain system, until winter precipitation ceases and soil water levels decrease to field capacity. Based on this conceptual model, we propose that leached N from fertilizer spends approximately 4–7 months in the soil between fall fertilizer application in early October and the end of high concentrations and leaching rates in April. This interval is similar to estimated mean residence times of soil water in fields in the Missouri Flat Creek watershed (Goodwin, 2006; Keller et al., 2008; Moravec et al., 2010).

Previous research on NO_3^- transport and denitrification potential (DNP) through vadose zones in the Missouri Flat Creek watershed found that NO_3^- is transported with infiltrating and recharging groundwater, and large masses of agricultural NO_3^- have reached depths up to 16 m (Geyer et al., 1992). Field measurements suggest that the soil pH, temperature [NO_3^-], and DOC levels were all within acceptable ranges to promote denitrification. Incubations of soil cores to measure DNP confirmed there is an active denitrifier community in the saturated and vadose zones (Geyer et al., 1992). In many situations with these conditions one might expect denitrification to be the dominant nitrogen cycle process during the high-discharge season, when profile saturation results in anoxic conditions. However [DO] measurements down to 8 m indicated the unsaturated and saturated zones were well aerated, suggesting that denitrification should be limited to microsites where [DO] is controlled by diffusion (Geyer et al., 1992). Given the observed volume-weighted mean $\delta^{18}O_{nitrate}$ for the TD-12 system of $-1.3 \pm 1.5‰$, which is not statistically different from the expected value due to nitrification (Equation (1)), we suggest that the dominant field-scale soil nitrogen cycle process at the CAF is nitrification. Possible ^{18}O abiotic exchange between NO_3^- and water was considered to be insignificant, because if exchange did occur we would expect the measured $\delta^{18}O_{nitrate}$ to be depleted toward $\delta^{18}O_{water}$ ($-14.7‰$) and not reflect the expected $\delta^{18}O_{nitrate}$ based on nitrification reaction stoichiometry (Kool et al., 2011). Our data suggest that if denitrification in microsites occurs, it is not important in governing N leaching dynamics at the field scale. The installation of tile-drains may be key to preventing the development of the conditions that favor denitrification, in bulk soil, throughout the water year.

5. Conclusion

This study, like previous studies (Geyer et al., 1992; Butturini et al., 2003; Cey et al., 1999), has found that the vadose zone is important in the assessment of nitrogen threats to surface water and groundwater quality. We focused on the impacts of vadose zone nitrogen cycle processes on surface water quality; similar impacts on shallow groundwater quality have also been documented (Schwille, 1969; Ritter and Chirnside, 1984; Berndt, 1990; Bohlke and Denver, 1995; Geyer et al., 1992).

Our results support our original hypotheses that nitrified NH_4^+ fertilizer is the dominant source of NO_3^- in leached TD-12 water and that nitrification, during mineralization of soil organic nitrogen to NO_3^-, is the dominant nitrogen cycle process during the low-discharge season. Our hypothesis that denitrification is the dominant process during high-discharge was not supported; nitrification appears to be dominant throughout the year while denitrification is negligible at our scale of observation. The interpretation that denitrification is not the dominant processes is based on the lack of observed ^{18}O discrimination in nitrate and is consistent with previous research suggesting denitrification would be limited, in well aerated profiles, to microsites. Under normal (not artificially drained) field conditions when soils reach full saturation denitrification rates would be expected to increase. During high discharge, when we expected denitrification, we speculate denitrification is limited because the tile-drain network increased drainage rates and lowers the DNP. By inserting artificial drains into naturally flooded soils, soil hydrology is altered to increasing the water discharge rates effectively aerating the soils and switching conditions from favoring denitrification to nitrification. The increased drainage also decreases the residence time of soil NO_3^-, which likely decreases potential denitrification. Further work is needed to test these ideas and their implications for precision agriculture, as well as for non-tile-drained fields in pedologic and climatic settings like the CAF.

Since most leached NO_3^- originates from NH_4^+ fertilizer in this system, dramatic reductions in leached NO_3^- rates to both surface and groundwater systems could be obtained through improved nitrogen efficiency.

Acknowledgments

We thank the three anonymous reviewers for their critical comments on earlier versions, especially the second reviewer who provided through and well thought-out comments on two earlier versions of this manuscript. We also thank the NSPIRE fellowship program at Washington State University, Washington State University School of the Environment and Department of Crop and Soil Science, all collaborators and staff, especially the previous graduate students who have contributed to building the hydrology data set, at the Cook Agronomy Farm.

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