Temporal trends in nitrogen isotope values of nitrate leaching from an agricultural soil

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Abstract

The concentration and $\delta^{15}N$ of NO_3^- in leachate from two undisturbed and unfertilized soil lysimeters, one conventionally tilled and one with no tillage, was determined on a bi-weekly basis from March through November, 1993 to assess the origins and transformations of NO_3^- leaching from an agricultural soil. Concentrations of NO_3^- in leachate from the tilled lysimeter were approximately twice those from the non-tilled lysimeter throughout the year and are consistent with observations that tilling favors the mineralization process in soils. Although no difference in $\delta^{15}N$ between lysimeters was evident, NO_3^- from both lysimeters exhibited considerable isotopic variability, ranging from -3.9‰ in March to a late summer maximum of 9.6‰. Low $\delta^{15}N$ values in the spring and fall were indicative of NO_3^- derived from soil organic matter and affected by fractionation during mineralization. High values in late summer indicated NO_3^- originating from soils that had been influenced to a small extent by fractionation during denitrification. Weighted mean $\delta^{15}N$ values for NO_3^- from the conventionally tilled and non-tilled lysimeters were 1.7 and 3.2‰, respectively, and are depleted in ^{15}N by greater than 4‰ relative to soil organic matter. These $\delta^{15}N$ values would normally be indicative of NO_3^- derived from fertilizers, however, in this study they reflect an origin from soil organic matter and are depleted in ^{15}N in response to fractionation during mineralization. The wide range of $\delta^{15}N$ values in this study illustrates that sampling of NO_3^- in soil leachate at one point in time is clearly not sufficient to assess origins or identify the predominant microbial processes occurring in soils. Our results indicate that NO_3^- in soil leachate is subject to considerable isotopic variation and that $\delta^{15}N$ may provide more information on the predominance of microbial processes in soils than on origins. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Stable isotope abundances of N (δ^{15}N) [1] have been used extensively to provide information on the origins and transformations of inorganic N in surface and ground waters (Letolle, 1980; Heaton, 1986; Hübner, 1986; Macko and Ostrom, 1994; Nadelhoffer and Fry, 1994). An assessment of the origins of NO_3^- using this technique, however, requires the assumption that the δ^{15}N of inorganic N behaves conservatively during movement through vadose and phreatic zones. The isotopic composition of inorganic N in soils and groundwater is not only controlled by its origin but may also be influenced by microbial or physical processes such as denitrification, nitrification, and ion exchange (Delwiche and Steyn, 1970; Bremner and Tabatabai, 1973; Blackmer and Bremner, 1977; Mariotti et al., 1981, 1982). A major difficulty associated with the use of δ^{15}N to assess origins is the inability to distinguish between isotope behavior that is conservative and that which is a reflection of processes that alter isotope abundances. Additional complexity may arise because source materials, such as soils, may contain several isotopically heterogeneous forms of N (Tiessen et al., 1984; Ledgard et al., 1984) and, therefore, may not behave functionally as a single pool of N with a distinct and characterizable isotope ratio.

Non-conservative behavior in the δ^{15}N of NO_3^- is most often the result of microbial processes. The direction of an isotope shift during microbial reactions can indicate the process that causes alteration. For example, loss of NO_3^- through denitrification can result in a marked enrichment in the ^{15}N content of the remaining NO_3^- (Blackmer and Bremner, 1977; Mariotti et al., 1981, 1982; Bryan et al., 1983). In contrast, during nitrification, the light isotope is preferentially incorporated into NO_3^- and a decrease in δ^{15}N during this process is frequently observed (Delwiche and Steyn, 1970; Mariotti et al., 1981;

Yoshida, 1988). Fractionation during nitrification is most marked when NH_4^+ is abundant in soils, as occurs following the application of NH_4^+ based fertilizers, and may be small or negligible when NH_4^+ is not readily available (Feigin et al., 1974a; Freyer and Aly, 1975; Heaton, 1986). Mineralization of soil organic matter is generally considered to favor incorporation of the light isotope in NH_4^+ (Nadelhoffer and Fry, 1994), although enrichment has been observed (Bremner and Tabatabai, 1973; Black and Waring, 1977). Long term soil incubation studies have shown that although the initial NO_3^- released may be depleted in ^{15}N relative to soils the net NO_3^- released is similar in δ^{15}N to the soil from which it was derived (Feigin et al., 1974b). An increase in the δ^{15}N of the residual NO_3^- is expected during plant uptake; however, the magnitude of this effect is small (Kohl and Shearer, 1980) and not likely to be a dominant control on the δ^{15}N of NO_3^-. Fractionation during ion exchange in soils has also been shown to be small (Delwiche and Steyn, 1970; Black and Waring, 1979). Therefore, the dominant processes affecting the δ^{15}N of NO_3^- in most soils are nitrification and mineralization, in which depletions in the ^{15}N content of NO_3^- are expected, and denitrification, which causes an enrichment in ^{15}N.

In this study we sought to (1) evaluate whether the isotopic composition of NO_3^- leaching from the upper horizon of an agricultural soil behaves conservatively over time, (2) characterize the extent and magnitude of non-conservative behavior, and (3) understand processes causing non-conservative behavior. We analyzed the δ^{15}N of NO_3^- in bi-weekly samples from two unfertilized, enclosed, zero tension, 5.6 m^3 ($2.29 \times 1.22 \times 2.03$ m deep) soil lysimeters that were constructed with a minimum of disturbance to the soil column and planted with corn. Neither lysimeter had been fertilized for three years; consequently we expected soil organic matter to be the primary source of NO_3^- in leachate. Deposition of N from wet and dry precipitation is approximately 13 to 15 kg/ha in our study area with NH_4^+ accounting for approximately 1/3 of total inorganic N deposition (Rheaume, 1990; Lovett, 1993). Precipitation, therefore, may be a secondary source of NO_3^- in leachate. To determine if the δ^{15}N of NO_3^- varies as a function of a standard tillage practices, one lysimeter was conventionally tilled (CT) while the other

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[1] N stable isotope ratios are expressed in per mil (‰) notation:

$$\delta^{15}N = \left[\left(R_{sample} / R_{standard} \right) - 1 \right] * 1000$$

where R is the abundance ratio of the heavy to light isotope. The internationally recognized standard for N is atmospheric N gas, which, by definition, has a per mil value of 0.

was not tilled (NT). If NO_3^- $\delta^{15}N$ is conservative and reflects origin, then the isotopic composition of NO_3^- should be similar between the lysimeters, and resemble that of the primary source or sources.

2. Methods

We collected leachate samples from unfertilized undisturbed monolith soil lysimeters on approximately a bi-weekly basis from March to November, 1993. The lysimeters were located 5 m from the edge of adjacent 27×40 m CT and NT permanent corn plots at the W.K. Kellogg Biological Station (KBS) in Hickory Corners, MI. Each lysimeter was constructed of stainless steel ($2.29 \times 1.22 \times 2.03$ m depth) and installed with a minimum of disturbance to the soil column approximately 5 cm above the soil surface in 1986 following the procedure of Brown et al. (1974). The lysimeters were grown in a corn/rye rotation prior to 1991 and have been planted continuously in corn since that time. Three rows of corn were planted within each lysimeter at a standard row spacing of 71.1 cm and 15 cm between plants within rows following planting and tilling practices typical of the area. The lysimeters were not fertilized prior to September, 1990, at which time 10 times the normal application rate of NH_4NO_3 fertilizer was accidentally applied (193 kg/ha). Chemical fertilizers were not applied between September 1990 and the end of this study. Concentration measurements suggested that there was no appreciable contribution of inorganic N from the fertilization event at the beginning of this study. Soils within the lysimeters consisted of a Kalamazoo series loam (fine–loamy mixed mesic Typic Hapludolf) typical of many agricultural glacial outwash regions surrounding the Great Lakes. The sequence of soils horizons is Ap (loam textured), Bt1 (clay and loam textured), Bt2 (sandy loam textured), and 2E/Bt (stratified sand and loamy sand textured). The top soil horizon contains 1% C and 0.1% N. The CT and NT lysimeters were in close proximity to each other and had similar soil characteristics and horizons.

Concentrations of NO_3^- in leachate were determined by Dionex ion chromatography, using chemically suppressed conductivity detection and a Dionex AS4A column. Samples for NO_3^- concentration were filtered within 3 h of collection, refrigerated at 4°C, and analyzed within 2 weeks. We have observed no significant change in NO_3^- concentration for up to 4 weeks of storage using this protocol. Samples for the determination of NO_3^- $\delta^{15}N$ were similarly filtered and stored frozen (-20°C) until analysis.

Owing to a desire to maintain the integrity of the lysimeters, only two samples of the surface 2 cm of soil within each lysimeter were collected for isotopic analysis. Soil samples were dried at 40°C and ground into a fine powder. Approximately 100 mg of dry soil was combusted using a modified Dumas sealed-tube method to obtain N_2 gas for isotopic analysis (Macko, 1981). This aliquot was placed in an ashed quartz tube to which excess pre-combusted copper oxide and pure copper were added. Evacuated samples were heated to 850°C and allowed to cool gradually overnight to prevent the formation of carbon monoxide and N oxides. N gas was separated cryogenically from carbon dioxide and other combustion products on a vacuum line and analyzed for isotopic abundances on a Micromass Prism stable isotope ratio mass spectrometer. Soil temperature data and concentrations of inorganic N in precipitation were obtained from the meteorological station at the KBS Pond Field Laboratory.

Extraction of NO_3^- from water samples for isotopic analysis was performed by standard steam distillation procedures (Bremner and Keeney, 1966; Velinsky et al., 1989; Ostrom, 1992). To convert NH_4^+ to volatile ammonia gas the pH of the distillate was shifted to 10 or greater by addition of 1 ml of NH_4^+-free 5 N NaOH. Finely ground Devardas alloy (50% Cu, 45% Al, 5% Zn), 0.3 g, was added to the distillate to reduce NO_3^- in the sample to ammonia (Kreitler, 1975). The condensate from a the distillation was collected in a flask containing 20 ml of 0.03 N HCl. NH_4^+ was bound by absorption onto 100 mg of a zeolite molecular sieve (Union Carbide Ionsiv W-85; Velinsky et al., 1989). The sieve containing the bound NH_4^+ was dried at 40°C and prepared for combustion following the procedure described above for isotopic analysis of soils. Distillation of NH_4^+-free water yielded a background contribution of NH_4^+ of 1.4 ± 0.6 μmol from the reagents. All samples were corrected for this background contribution using a mass balance equation (Cline, 1973; Ostrom, 1992). Accuracy in $\delta^{15}N$ during the distillation and back-

ground correction process was demonstrated by analysis of two international NO_3^- standards, IAEA-N3 and USGS-32, that have reported values of 4.72‰ and 180‰, respectively (Böhlke et al., 1993; Böhlke and Coplen, 1995). Analysis of three replicates of the IAEA-N3 and USGS-32 standards in our laboratory yielded respective $\delta^{15}N$ values of 4.1 ± 0.1‰ and 180.5 ± 0.9‰. Precision of replicate samples was 1‰ or less.

3. Results

The $\delta^{15}N$ of NO_3^- from the CT and NT lysimeters varied considerably, ranging from −3.9‰ to 9.6‰ (Fig. 1). The seasonal pattern of $\delta^{15}N$ variation in both lysimeters was remarkably similar and a series of maximum and minimum values are coincident. Minimum values were most common in the spring and fall and maximum values in late summer. Such strong seasonal variation was not apparent in NO_3^- concentration or flux (Fig. 2) and significant relationships were not found between these variables and $\delta^{15}N$. The $\delta^{15}N$ of NO_3^- in both the NT and CT lysimeters is, however, correlated with soil temperature (F-test, $p = 0.01$, $df = 25$). Concentrations of NO_3^- in the CT lysimeter were approximately twice those of the NT lysimeter throughout the study. Discharge of water was similar for both lysimeters and showed maximum values in late April, June and
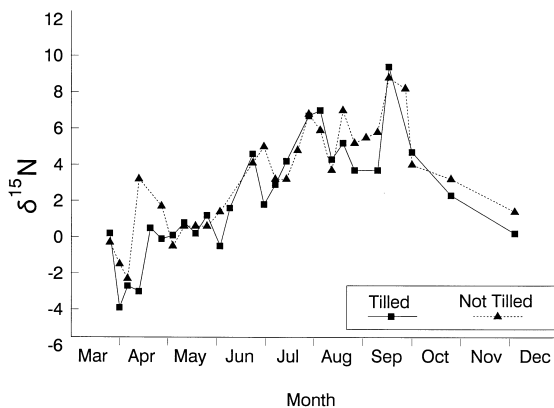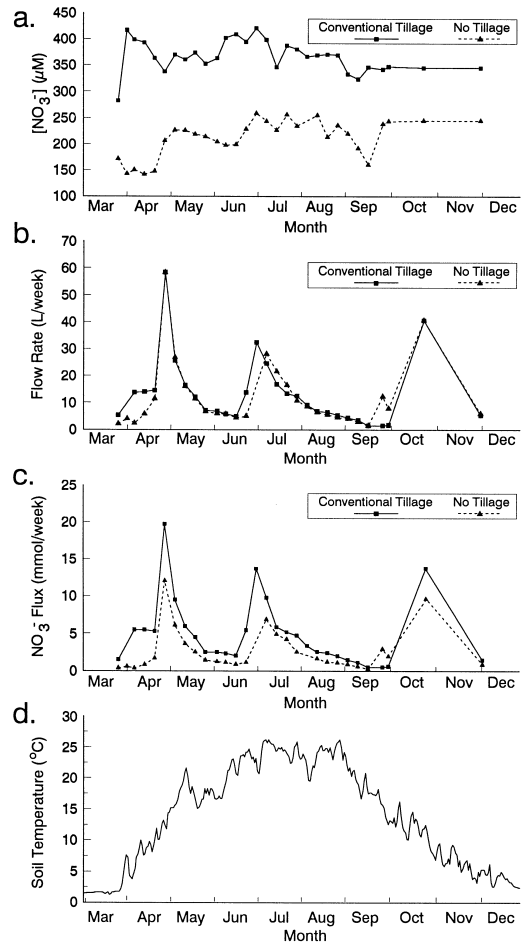
Fig. 2. Concentration and flow rate data for the tilled and untilled lysimeters. (a) Concentration of nitrate in leachate. (b) Rate of water flow to the base of the lysimeters. (c) Flux of nitrate to the base of the tilled and untilled lysimeters. (d) Soil temperatures for the KBS Pond Field Laboratory station.

October. The flux of NO_3^- at the base of the lysimeters closely followed the variation exhibited by the discharge indicating that water flow was the dominant control on NO_3^- flux.

The wide variation in the $\delta^{15}N$ of lysimeter NO_3^- complicates a comparison between NO_3^- and the primary source of N within this system, soil organic matter. Comparisons based on a simple mean do not take into account temporal variation in the flux of NO_3^- to the base of the lysimeter. For this reason, we relate the isotopic composition of lysimeter NO_3^-

Fig. 1. The nitrogen isotopic composition of nitrate in leachate from the tilled and untilled lysimeters.

to soil organic matter by use of weighted mean. [2] The weighted mean of $\delta^{15}N$ values for the CT and NT lysimeters were 1.7 and 3.2‰, respectively. These values should be an estimate of the isotopic composition of net annual NO_3^- in recharge since recharge in winter months is likely to be low relative to other times of the year. Despite the difference in weighted values the CT and NT were not significantly different in $\delta^{15}N$ (*F*-test, $p = 0.01$, $df = 22$). A consistent difference in the $\delta^{15}N$ of soil organic matter was not evident between lysimeters and the average isotopic composition of all four samples of lysimeter soils was 7.3 ± 1.4‰. Therefore, NO_3^- from the CT and NT lysimeters was depleted in ^{15}N by 5.6‰ and 4.1‰, respectively, relative to total soil organic N.

4. Discussion

The isotopic composition of NO_3^- in natural environments is controlled by that of its source and processes that cause its production, consumption, or exchange. The wide range in $\delta^{15}N$ values found in this study, -3.9‰ to 9.6‰ (Fig. 1) is much greater than would normally be expected for NO_3^- derived from a single source and, therefore, must be a consequence of multiple sources or non-conservative behavior. In this study, the probable sources of NO_3^- were limited to soil organic matter and precipitation, and the primary processes expected to cause variation in $\delta^{15}N$ were mineralization, nitrification, and denitrification.

Values for the $\delta^{15}N$ of NO_3^- in lysimeter leachate collected in the early spring were low and generally less than 0‰ (Fig. 1). Such low values could indicate an origin from chemical fertilizers that have been found to have low $\delta^{15}N$ values, primarily between -3 and 2‰ (Macko and Ostrom, 1994). A contribution of NO_3^- from fertilizers in these lysimeters would only be possible if the isotopic signal of this source had been preserved in microbial biomass or ion exchange sites within the soil and preferentially released during the spring. Given the length of time since fertilization (2.5 years), cooler temperatures in spring relative to summer, and the dynamic nature of N cycling in soils the influence of a fertilizer signal retained in soil in this study is unlikely.

The low $\delta^{15}N$ values evident for NO_3^- in the spring could also be a consequence of an origin in atmospheric deposition. Isotope values for NO_3^- in precipitation have been shown to vary considerably, however, in many studies $\delta^{15}N$ values less than 0‰ are common (Moore, 1977; Freyer, 1978; Heaton, 1987; Garten, 1991; Paerl and Fogel, 1994). NO_3^- in two samples of precipitation at KBS were found to have similarly low $\delta^{15}N$ values of -1.7 and -1.3‰. The average monthly concentrations of inorganic N in precipitation at KBS in 1993 ranged between 31.7 and 113.6 μM and averaged 53.8 NO_3^- μM annually. These concentrations are substantially lower that those found for NO_3^- in lysimeter leachate (Fig. 2) and indicate that atmospheric deposition may be an important, but not the primary, source of NO_3^- leaving KBS soils. NO_3^- leaching from these unfertilized lysimeters must primarily be derived from the mineralization of soil organic matter. Temporal variation in the isotopic composition of lysimeter NO_3^- is, therefore, largely a consequence of microbial processes.

Kinetic isotope effects favor the incorporation of the light isotope in the product of microbial reactions. The low $\delta^{15}N$ values for NO_3^-, therefore, must be a reflection of isotopic segregation during its production. Low $\delta^{15}N$ values for NO_3^- in spring could result from isotopic fractionation during nitrification, however, the expression of the isotope effect during nitrification is reduced or not observed if NH_4^+ is limiting to nitrifying bacteria (Feigin et al., 1974a; Mariotti et al., 1981). The observation of NH_4^+ concentrations in leachate that were near or at detection limits at all times suggested that isotopic

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[2] The isotopic composition of NO_3^- in each sample is weighted by the quantity of NO_3^- reaching the base of the lysimeter during the time period the leachate was collected and divided by the total amount of NO_3^- collected during the entire study:

$$\sum_{i=1}^{n} F_i \delta_i / \Sigma F_i \qquad (1)$$

where δ_i is the $\delta^{15}N$ of NO_3^- for a particular sample, and F_i is the flux of NO_3^-, in mol-N, reaching the base of the lysimeter at time *i*. F_i is obtained by multiplying the concentration of NO_3^- in a particular collection by the total volume of water collected during that period.

fractionation during nitrification was unlikely. Fractionation during mineralization is generally considered to favor incorporation of the light isotope in inorganic N (Nadelhoffer and Fry, 1994) and could explain the low values for NO_3^- $\delta^{15}N$. The majority (approximately two thirds) of $\delta^{15}N$ values for NO_3^- from soil incubations or extracted from soils has been shown to differ in $\delta^{15}N$ from that of soils by -4.5 to $2.5‰$ ($\delta^{15}N_{NO3 \text{ or } NH4} - \delta^{15}N_{soil}$) (Cheng et al., 1964; Bremner and Tabatabai, 1973; Feigin et al., 1974a; Rennie et al., 1976; Black and Waring, 1977; Ledgard et al., 1984; Binkley et al., 1985). The differences between the $\delta^{15}N$ of NO_3^- and that of soil organic matter for the CT and NT lysimeters of -5.6 and $-4.1‰$, respectively, suggest that fractionation during mineralization in KBS soils is large relative to other environments. The direction of the isotope effect observed in these KBS soils is consistent with the observation of increasing $\delta^{15}N$ values with depth in soil profiles that has previously been attributed to fractionation during mineralization (Nadelhoffer and Fry, 1994). The $\delta^{15}N$ values of lysimeter NO_3^- exhibited a general increase from early spring to late summer and reached a maximum value of $9.6‰$ in mid-September (Fig. 1). The high $\delta^{15}N$ values for NO_3^- in late summer are a strong indication of the influence of denitrification on soil NO_3^-, however, data on rates of denitrification in KBS soils were not available. During denitrification the light isotope is preferentially incorporated into the gaseous products of this reaction and the remaining NO_3^- is enriched in ^{15}N (Mariotti et al., 1981; Bryan et al., 1983). Losses of NO_3^- due to denitrification as low as 20% can result in an increase in $\delta^{15}N$ by $8‰$ (Heaton, 1984). Given that dramatic enrichments in ^{15}N, and $\delta^{15}N$ values much greater than that of soil organic matter, were not evident in lysimeter leachate, the amount of NO_3^- lost to denitrification is not likely to have been a large portion of the total NO_3^- available.

The marked decrease in the $\delta^{15}N$ of NO_3^- in leachate following late September suggests that much of the earlier microbial activity, particularly denitrification, had declined, most likely in response to cooler temperatures. Denitrification rates have been shown to be markedly influenced by temperature and may not be significant below $4–6°C$ (Ryden, 1986; Jordan, 1989; Ruz-Jerez et al., 1994). Temperatures

below this level were observed at KBS prior to April and after mid-November (Fig. 2). Indeed, the correlation of $\delta^{15}N$ values in both lysimeters with soil temperature is evidence of a microbial control on the isotopic composition of NO_3^-. This relationship furthermore suggests that the transport of NO_3^- through the lysimeters is rapid and occurs in a matter of days to a few weeks. NO_3^- released after mid-September may be derived from soil organic matter and influenced by fractionation during mineralization and/or nitrification. Although rates of mineralization and nitrification were reduced relative to spring and summer values they indicated that these processes were still active despite low soil temperatures (G.P. Robertson, unpublished data on Web site http://KBS.MSU.EDU/lter/data/000_toc.htmlx).

Although the seasonal variation in the isotopic composition of NO_3^- in lysimeter leachate was large, the pattern of variation between the two lysimeters was remarkably similar (Fig. 1) and, indeed, the CT and NT lysimeters were significantly correlated in $\delta^{15}N$ (F-test, $p = 0.01$, $df = 22$). This result indicates that the timing of and processes responsible for isotopic variation in the lysimeters were similar, despite the fact that one lysimeter was tilled and the other was not. Considerably more NO_3^- was leached from the CT lysimeter and this is consistent with the observation that tilling soils enhances the mineralization process (Winteringham, 1984). Although tillage resulted in the release of considerably more NO_3^- than the NT lysimeter, it did not cause a change in the pattern of seasonal isotopic variation. Because concentrations of NO_3^- in the NT lysimeter leachate were much less than those in the CT leachate, any input of N from a source in addition to soil organic matter, such as precipitation, would have affected the $\delta^{15}N$ values in this lysimeter to a greater extent and such a trend was not evident. These observations further emphasize that NO_3^- leaching from the soils in both lysimeters was derived from a common source, mainly soil organic matter via mineralization and nitrification.

The weighted mean $\delta^{15}N$ values for NO_3^- are an estimate of the annual isotope ratio of NO_3^- leaching from soils to groundwater. These values of $1.7‰$ and $3.2‰$ for the CT and NT lysimeters, respectively, would normally be interpreted as indicating an origin in fertilizers (Heaton, 1986; Macko and Ostrom,

1994) and clearly emphasizes the difficulties associated with interpreting N isotope data. The weighted mean isotope values in this study are a consequence of NO_3^- derived from soil organic matter and, to a minor extent, precipitation and altered, in a non-conservative manner, by microbial activity.

5. Conclusions

The wide range of isotope values for NO_3^- collected on a seasonal basis from unfertilized soil lysimeters in this study clearly illustrates the dynamic nature of the N cycle in soils. Even within relatively simple systems, the $\delta^{15}N$ of NO_3^- is controlled by complex interactions between sources and microbial processes. Sampling of NO_3^- in soil leachate at a single point in time, is clearly not sufficient to assess origins or identify the predominant microbial processes occurring in soils. The weighted mean $\delta^{15}N$ values for lysimeter NO_3^- in this study were depleted in ^{15}N by more than 4‰ relative to those of soil organic matter. This distinction was found to be primarily a consequence of isotopic fractionation during mineralization and is consistent with the conclusion that increasing soil organic matter $\delta^{15}N$ values with depth are the result of the loss of the light isotope during mineralization (Nadelhoffer and Fry, 1994). The observation of no significant difference in NO_3^- $\delta^{15}N$ between the CT and NT lysimeters, despite greater NO_3^- concentrations in the CT lysimeter, is consistent with a single common origin from soil organic matter via mineralization in both lysimeters, although minor contributions of NO_3^- from precipitation are likely. A correlation between soil temperature and $\delta^{15}N$ is strong evidence for a microbial control on isotopic variation. Temporal variation in $\delta^{15}N$ indicates that the isotopic composition of NO_3^- in spring and fall is controlled primarily by fractionation during mineralization and by denitrification in summer.

Determination of the origin of NO_3^- in groundwater on the basis of $\delta^{15}N$ is complicated by the multitude of sources within agricultural environments and the complexity of the N cycle within soil systems. A proper assessment of origins based on this technique requires the establishment of criteria for recognizing the microbial alteration of isotope ratios in NO_3^-. The recent use of $\delta^{18}O$ to track the fate of NO_3^- in forests (Hedin, 1994; Durka et al., 1994) and other systems may prove useful in this regard (Amberger and Schmidt, 1987; Böttcher et al., 1990; Aravena et al., 1993). N isotope data can, however, provide considerable insight into microbial processes in soils and the dynamics of the mineralization process and it may be this line of research that will be most fruitful for researchers using this technique.

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