Tracing sources of nitrate in agricultural catchments by natural stable isotopes

Outlet of the Mørdre agricultural catchment (Photo: J.R. Selvik)

Norwegian Institute for Water Research

– an institute in the Environmental Research Alliance of Norway

REPORT

Title	Serial No.	Date
Tracing sources of nitrate in agricultural catchments by natural stable isotopes	5240-2006	July 2006
	Report No. Sub-No. O-21601 05 O-24060 OKA	Pages Price 21
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	Geographical area Eastern Norway	Printed NIVA

Client(s)	Client ref.
Research Council of Norway; Norwegian Institute for Water Research	

Abstract

The report summarises the results from isotope studies performed as part of the Strategic Institute Programme 'Watershed-oriented management of large rivers' and the research programme INTRA – 'Integrated risk assessment for the transport of particles, nutrients and pesticides in agricultural catchments'. Abundances of stable isotopes of nitrogen and oxygen ($\delta^{15}N$ and $\delta^{18}O$) in the nitrate molecule (NO_3^-) were analysed in runoff from 9 small agricultural catchments in SE Norway. The main focus of the studies was to investigate the potential of the dual isotope technique of NO_3^- to classify drainage pipe water or small streams with regard to their dominant sources of NO_3^-. The data generally indicate that the methodology should be used with care in catchment-oriented studies, where multiple NO_3^- sources, a mosaic of land-use types and complex hydrology easily result in overlapping isotopic signals. On the other hand, it may still add value to more traditional chemical assays and source apportionment methods, especially in combination with N transformation studies.

4 keywords, Norwegian	4 keywords, English
1. Overflatevann	1. Surface water
2. Nitrat	2. Nitrate
3. Jordbruk	3. Agriculture
4. Stabile isotoper	4. Stable isotopes

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Ackowledgements

Grimstad, July 2006

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Contents

Abstract

Abundances of stable isotopes of nitrogen and oxygen (δ^{15}N and δ^{18}O) in the nitrate molecule (NO_3^-) were analysed in runoff from 9 small agricultural catchments in SE Norway. The main focus of the study was to investigate the potential of the dual isotope technique of NO_3^- to classify drainage pipe water or small streams with regard to their dominant sources of NO_3^-. On the basis of 3–6 isotope samples from each site during the growing season it was possible to distinguish between major land-use types as forest (range: δ^{15}N 2.6–3.4‰, δ^{18}O 12.3–23.4‰) and agricultural land (range: δ^{15}N 3.3–16.7‰, δ^{18}O 9.6–19.4‰). These values fall well within ranges previously reported in literature. The agricultural sub-catchments to a great extent showed overlapping isotopic signatures, although the catchments affected by animal manure show slightly different signatures at specific sampling dates. The data generally indicate that source-dependent isotopic signals can be masked by variation in external forcing (rainfall, temperature), hydrological flow paths, and soil processes (microbial transformations, plant uptake) throughout the growing season. The methodology should therefore be used with care in catchment-oriented studies, where multiple NO_3^- sources, a mosaic of land-use types and complex hydrology easily result in overlapping isotopic signals. Natural abundance isotope studies may therefore be more suitable as a complementary method than a stand alone method under such conditions. However, it may still add value to more traditional chemical assays and source apportionment methods, especially in combination with N transformation studies. Further, isotopic techniques will be highly useful and important tools in detailed studies of N transformation processes and pathways for nitrate under controlled field conditions.

1. Introduction

Several decades with high nitrogen (N) and phosphorus (P) loading from anthropogenic activity have led to widespread nutrient enrichment and eutrophication in rivers and coastal areas (Vitousek et al., 1997). In many lowland areas, a large fraction of nutrients entering the streams originates from non-point agricultural and urban sources (Howarth et al., 1996; Boyer et al., 2002). These sources are often difficult to quantify, and thereby complicate river basin management plans and programmes of measures as e.g. required by the European Union's (EU) Water Framework Directive (WFD). A reduction of the large uncertainties associated with diffuse nutrient sources and sinks would significantly improve the basis for river basin management and adequate abatement measures.

Different sources of nitrate (NO_3^-) often have distinct nitrogen and oxygen (O) isotopic compositions. Hence, identification of stable nitrogen and oxygen isotope ratios ($\delta^{15}N$ and $\delta^{18}O$) in NO_3^- can be a suitable tool for tracing sources for NO_3^- in surface water and in groundwater (Kendall, 1998). Biological cycling of NO_3^- often changes isotopic ratios in a predictable manner owing to isotope fractionation, i.e. discrimination between light and heavy isotopes during the transformation process. Hence, the origin and history of exported NO_3^- in many instances might be reconstructed from the isotopic compositions of N and O.

N isotope ratios have been applied in many studies to discriminate between point sources (manure, sewage) and non-point sources (atmospheric deposition, synthetic fertiliser, soil organic matter) in river basins (Mayer et al., 2002). However, N isotopes alone cannot differentiate the latter three sources, because they usually have overlapping $\delta^{15}N$ values. During the last 15 years, methods for measuring both N and O isotope ratios of NO_3^- (the dual-isotope technique) have gradually been improved (Amberger and Schmidt, 1987; Revesz et al., 1997, Silva et al., 2000). The combined analysis of both $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in many cases provides a valuable tool for differentiating between diffuse NO_3^- sources in lowland rivers (fertiliser vs. manure; natural soil N vs. fertiliser and/or manure) and in upland catchments enriched with atmospheric N (natural soil N vs. atmospheric deposition). Some studies, however, indicate that the dual isotope method also has some limitations when applied on agricultural catchments under biologically active conditions and where several sources may be potential contaminants (Mengis et al., 2001; Kellman and Hillaire-Marcel, 2003).

In this study, we have used N and O isotope ratios of NO_3^- in surface water samples from small agricultural catchments in south-eastern Norway to investigate whether the dual isotope method of NO_3^- can be used as a tool for source tracing in this area, and subsequently to group different sub-catchments in an agricultural landscape with regard to their dominant sources of NO_3^-.

2. Material and metods

2.1 Site description

2.1.1 Mørdre

The Mørdre catchment (680 ha) is located in south-eastern Norway (60°07'N, 11°24'E) at 150–240 m.a.s.l. (**Figure 1**). The soils consist of marine clay deposits with a thin silt layer on top of the flat plains. In the steep ravines clay soils dominate. Most soils in the catchment are artificially drained at a spacing of 8 m and a drain depth of 0.8 m below the soil surface. The annual mean air temperature and precipitation are 4 °C and 665 mm, respectively, measured at the adjacent meteorological station Hvam-Tolvhus (met.no, 2005).

Agricultural land covers 65% of the total Mørdre catchment area, while for the three sub-catchments selected for isotope analysis, 74–100% were covered by agricultural land use (**Table 1**). Cereals dominate the production, whereas potatoes and permanent grasslands cover a minor area. The two farm units within the catchment have swine production of 860 heads yr^{-1} and 500 heads yr^{-1}, respectively. About 3% of the catchment is covered by a small housing area. Point sources, based on standard conditions and number of person equivalents, were estimated to contribute 1.1 kg N ha^{-1} yr^{-1} to the catchment N losses (Bechmann et al., 2005). Given the annual runoff at Mørdre in 2003 (section 3.1) this roughly corresponds to a mean N concentration of 0.57 mg l^{-1} at the catchment outlet.
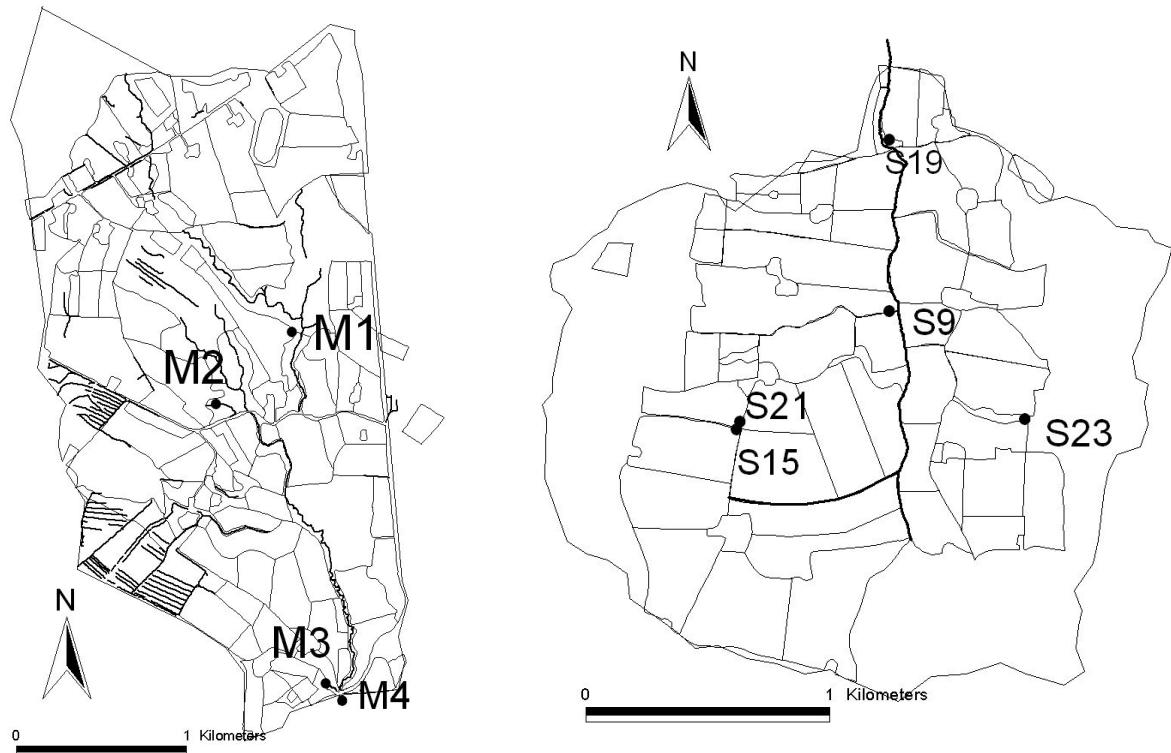

Figure 1. Map showing the Mørdre catchment (left) and the Skuterud catchment (right). Sampling points are indicated by black dots.

2.1.2 Skuterud

The Skuterud catchment (449 ha; 59º66'N, 10º78'E) is located at 91–146 m.a.s.l., about 80 km south of Mørdre and closer to the coast (**Figure 1**). The predominant soil types are developed on marine clay in most of the area and on coarser sandy shore deposits/moraine materials towards the water divide. The soil texture classes vary from coarse sand to silt clay loam, with sandy clay loam/clay loam as the dominant texture in the agricultural areas. Most of the agricultural land is artificially drained at a spacing of 8 m and a drain depth of 0.8 m below the soil surface. A bog in the south-western part of the catchment is artificially drained with open ditches. The annual mean air temperature and rainfall, measured at the adjacent meteorological station at the Norwegian University of Life Sciences (UMB), are 5.3 $^{\circ}$C and 785 mm, respectively.

Agricultural land covers 61% of the total Skuterud catchment area, while 0–100 % of the four sub-catchments selected for isotope analysis were covered by agricultural land use (**Table 1**). Cereals are the main crops in the catchment area and constitute about 80–90 % of the arable land. Other crops include potatoes and ley. Point sources within the Skuterud catchment are scattered dwellings from households and agricultural point sources from one cattle farm. Estimated point source contributions to N losses are 0.43 kg N ha^{-1} yr^{-1} (Deelstra et al. 2005). Given the annual runoff at Skuterud in 2004 (section 3.1) this roughly corresponds to a mean N concentration of 0.09 mg l^{-1} at the catchment outlet.

Table 1. Land use at Mørdre and Skuterud, including sub-catchments selected for isotope analysis. All numbers in ha.

Sub-catchment	Agriculture	Forest	Bogs	Other[1]	Total area
Mørdre:					
M1 Agricultural	5.5	-	-	-	5.5
M2 Agricultural	30.2	-	-	-	30.2
M3 Agricultural	35.0	-	7.0	5.0	47.0
M4 Main outlet	444	192	27	18	681
Skuterud:					
S9 Mixed	26.7	28.5	-	2.9	58.2
S15 Agricultural	7.2	-	-	-	7.2
S21 Agricultural	5.6	-	-	0.6	6.2
S23 Forest	-	16.6	-	-	16.6
S19 Main outlet	272	129	10	38	449

[1]Buildings, roads, etc.

2.2 Streamwater monitoring and land management assays

Water chemistry and streamwater flow at Mørdre and Skuterud have been monitored since 1991 and 1993, respectively, as part of the Norwegian Agricultural Environmental Programme (JOVA; e.g. Bechmann and Våje, 2002). Annual surveys of agricultural practices on each field within the catchments describe the land management (**Table 2**). In both catchments discharge measurements are carried out in a cross-section defined by a Crump weir. The flow depth is measured with pressure transducers, and data loggers record flow data every hour. Flow proportional composite samples are sampled and analysed every second week for a number of parameters, of which NO_3^- is used here.

Table 2. Land management in sub-catchments selected for isotope studies, with dates (in 2003 at Mørdre and 2004 at Skuterud) for manure/fertiliser application, ploughing and harvest of cereals.

	Manure appl.		Fertiliser appl.		Ploughing	Harvest
	Rate	Date	Rate	Date	Date	Date
	kg N/ha	dd.mm	kg N/ha	dd.mm	dd.mm	dd.mm
Mørdre (2003)						
M1 Agricultural	99	24.4	147	9.5, 9.7	25.4	27.8
M2 Agricultural	0	-	110	21-30.5	26.4-6.5	12.9
M3 Agricultural	0	-	118	29.5	2-12.5	1.9
Skuterud (2004)						
S9 Mixed	73	10-15.4	156	13.4, 10.8	27.8[1]	25.8
S15 Agricultural	0	-	147	14.4, 12.5	-	2.9
S21 Agricultural	0	-	168	14.4, 9.5	29.8	20.8

[1] The date is for 58% of agricultural area, the rest is no till or grassland

2.3 Sample collection and preparation for isotope analysis

Four sampling points in the Mørdre catchment and five sampling points in the Skuterud catchment were selected for analysis of NO_3^- isotopic composition in runoff water (**Figure 1**). The sites include the main outlets of each study catchment (Mørdre and Skuterud) and outlets from the sub-catchments within each of the study catchments (**Table 1**). The Mørdre sites include one sub-catchment treated with both animal manure and inorganic fertiliser (M1) and two sub-catchments treated with inorganic fertiliser only (**Table 2**). The M3 sub-catchment drains a small housing area. The Skuterud site includes one forested catchment (S23), one mixed forest/agricultural catchment treated with both animal manure and inorganic fertiliser (S9), and two catchments treated with inorganic fertiliser only (S15 and S21). The catchments S9 and S21 may also be affected by point-source pollution from cow barns.

Grab samples from the streams were collected in 5-litre plastic cans, 6 times at Mørdre (2003) and 4 times at Skuterud (2004) during the growing season (May-October), and transported directly to the laboratory at NIVA for further processing. In addition to regular water samples, four samples containing solutions of synthetic fertiliser (NPK and calcium nitrate), animal manure, and urban wastewater were also prepared for isotope analysis.

The sample preparation followed the main steps recommended by Silva et al. (2000). In the laboratory, a small sub-sample was taken from each sample and analysed for NO_3^- by a fast determination technique (Dr. Lange LT 100 photometer, detection limit: 0.23 mg N l^{-1}). The results were used to calculate the amount of water needed to retain about 2.5 mg NO_3-N on the anion exchange resin. For control, another sub-sample was analysed for low-level NO_3^- (detection limit: 1 µg N l^{-1}) and total organic carbon (TOC, detection limit: 0.1 mg C l^{-1}). After filtering through a 0.45 µm polycarbonate membrane filter to remove particles, the required volumes of water were added to a separatory funnel and dripped with a flow rate of 0.5–1 l h^{-1} through columns pre-filled with 2 ml Bio-Rad AG1-X8 (200–400 mesh) anion exchange resins in chloride form (**Figure 2**). Then, the columns were capped and stored at 2–4ºC until they were processed for isotope analysis. To verify that the anion column adsorbed at least 95% of the sample NO_3^-, the eluent that passed through the column were analysed for low-level NO_3^-.

Figure 2. Apparatus used (five parallels) to retain NO_3^- from water samples on anion exchange resins. Photo: J.R.Selvik, NIVA.

2.4 Isotope analysis

Isotope analysis was done at the Institute for Energy Technology (IFE) according to Silva et al. (2000). The method is given in short below, where also the changes from the original method are described. The analyses were done in four batches over two years, with standards included in each batch. After loading the ion exchange columns as above they were stored dark at 2ºC until analysis. NO_3^- was stripped from the columns by 5 x 3 ml 3M HCl. The eluded HNO_3 was neutralized immediately in the dark by rinsed silver oxide (Ag_2O), forming $AgNO_3$ and AgCl while kept on ice. AgCl was filtered out (Whatman GF/A), and remaining $AgNO_3$ in the filter was washed out with DI water until 40–50 ml filtrate was reached. Each sample was split in three aliquots for $\delta^{15}N$ and $\delta^{18}O$ analysis and a reserve and then frozen.

N isotope analysis: To remove water one aliquot of each sample was freeze-dried and redissolved in 2 ml DI water, transferred to a reagent tube, frozen and freeze-dried again. The samples where then dissolved in 400 µl DI water, split into four silver capsules and frozen by partly immersion of the rack containing the samples in liquid N_2. The capsules were closed and the samples were freeze-dried. One grain of sucrose was added to each capsule and the $\delta^{15}N$ was analysed on a ThermoQuest NCS 2500 elemental analyser (EA) coupled to a VG isotech continuous flow isotope ratio mass spectrometer (CF-IRMS).

O isotope analysis: To remove phosphate and sulphate 2 ml 1 M $BaCl_2$ was added to each (15 ml) sample aliquot, and left at 2ºC overnight. The samples were then filtered (Sartorius Minisart 0.2 µm using a VAC Master filtration unit) and percolated through 4 ml cation exchange resin (BIO-RAD AG 50W-X8 100–200 mesh) to exchange Ag^+ and Ba^+ with H^+. The sample was then neutralised by Ag_2O, and filtered again as above (2 µm). To remove organic C the samples were shaken 20 minutes with 10 mg activated carbon per 50 ml filtrate. The samples were filtered (2 µm), freeze-dried, dissolved (2 ml DI water) and pipetted into a baked (900ºC) quarts tube and freeze-dried. 4–5 mg spectrographic graphite was added to the quarts tube and the tube was evacuated and sealed. The samples were

combusted at 850°C and cooled overnight in the oven. CO_2 was concentrated on a vacuum line, and $\delta^{18}O$ was measured on a Finnigan MAT 251 dual inlet IRMS.

IAEA N3 (KNO_3) was used as isotopic standard. It was dissolved in DI water, loaded on the anion exchange resin, and otherwise treated as the samples. Standard deviation (SD) was 0.4 and 0.7‰ of $\delta^{15}N$ and $\delta^{18}O$ respectively for all standards pooled (n = 12). $\delta^{15}N$ of IAEA N3 is defined as 4.7‰ and Rèvèrez et al. (1997) found 22.7‰ ± 0.5‰ as reference value for $\delta^{18}O$, which are used as reference values for these samples.

2.5 Characteristic isotope ratios in NO_3^- from various sources

The stable isotopic composition of N ($\delta^{15}N$) in a sample X is generally reported in permil (‰) relative to N_2 in atmospheric air (eq 1). In the same manner, $\delta^{18}O$ is reported relative to a seawater standard.

$$\delta^{15}N (x) = \{ [(^{15}N/^{14}N)_X / (^{15}N/^{14}N)_{AIR}] - 1 \} * 1000 \qquad \text{(eq. 1)}$$

Cycling of N compounds often involves isotope fractionation; i.e. discrimination between the light and the heavy isotopes of N and O during the transformation process. Many field and laboratory studies have measured fractionation factors associated with various N transformation processes, and on this basis, specific ranges of fractionation factors can be defined for individual processes in the N cycle (for review, see e.g. Hogberg, 1997; Kendall, 1998).

$\delta^{15}N$ values of NO_3^- in streamwater often show a large variability, and overlap of $\delta^{15}N$ distributions often makes its difficult to discriminate between nitrogen from different origins. However, the combined use of N and O isotopes in NO_3^- provides a more powerful tool for differentiating between diffuse NO_3^- sources as animal manure, synthetic fertiliser, soil organic material, and atmospheric deposition. In **Figure 3**, compiled by Kendall (1998), normal ranges of $\delta^{18}O$ and $\delta^{15}N$ are showed for the dominant sources of NO_3^-.
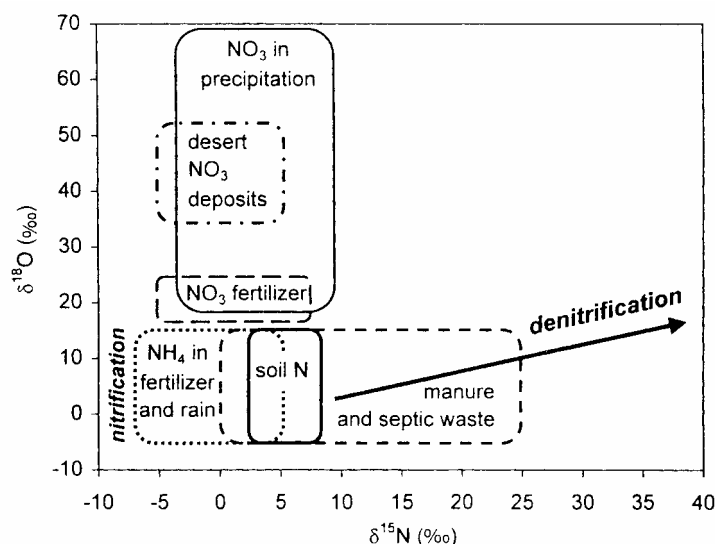
Figure 3. Typical ranges of $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- from various sources (from Kendall, 1998).

3. Results

3.1 Streamwater flow and nitrogen concentrations

Data from the regular monitoring of stream water flow and NO_3^- concentrations at the outlets of the catchments are shown in **Figure 4**. Annual runoff at Mørdre in 2003 and Skuterud in 2004 was 189 and 483 mm, respectively. The corresponding precipitation amounts measured at the nearest meteorological stations (Hvam-Tolvhus and UMB) were 693 and 856 mm, respectively, indicating approximately 400–500 mm as evapotranspiration. The catchments showed relatively similar runoff patterns, with the highest fluxes during autumn and spring snowmelt and relatively dry conditions during summer. Surface water NO_3^- concentrations were of similar magnitude at the catchment outlets with peaks reaching up to 8–9 mg N l^{-1} in flow-proportional composite samples. The highest concentrations appeared in conjunction with manure/fertiliser application in spring/early summer and after harvest in autumn. Both catchments had NO_3^- concentration minima during August.
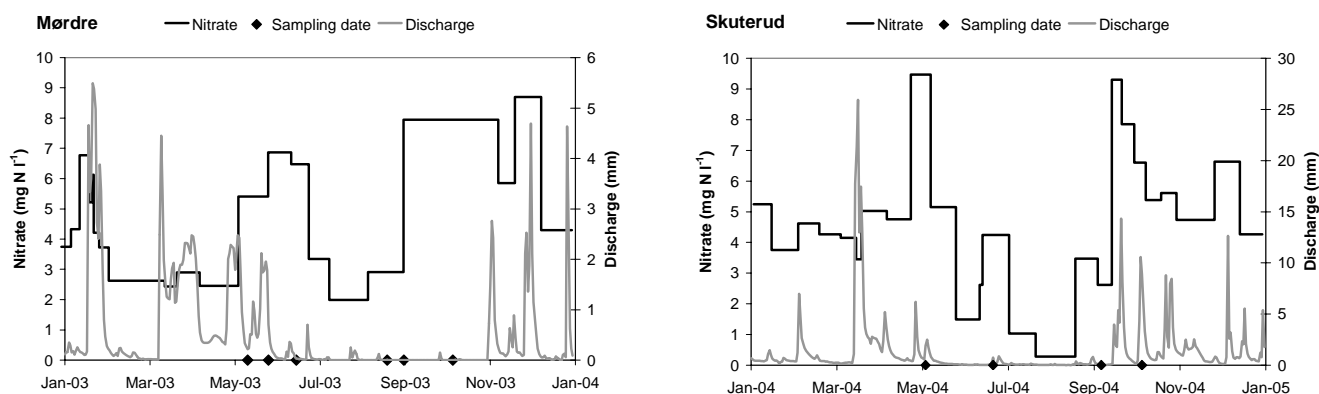

Figure 4. Water flow and nitrate concentrations in flow-proportional samples collected at the main outlets at Mørdre and Skuterud during 2003 and 2004, respectively (Vandsemb et al., 2004; 2005). Sampling dates for isotope analysis are indicated.

The point samples used for isotope analysis were collected from May to October under variable flow conditions (**Figure 4**). NO_3^- concentrations in these surface water samples also were highly variable, especially in the smallest agricultural sub-catchments (**Figure 5**), where land management and flow fluctuations readily affect the water quality (Bechmann et al., 2005). At Mørdre, the two small agricultural sub-catchments (M1 and M2) showed NO_3^- concentrations in the range of 2.7–32.5 mg N l^{-1}. The highest concentrations (>20 mg N l^{-1}) were recorded during very low flow conditions. In stream M3 and at the main outlet (M4) the NO_3^- concentrations were less variable and in the ranges of 2.2–5.1 mg N l^{-1} and 1.7–4.4 mg N l^{-1}, respectively. The forested parts of the catchment probably play a minor role in the overall catchment NO_3^- budget, at least during the growing season when productive forest usually show very high N retention and consequently low N runoff (Mulder et al., 1997). In early May 2003, the NO_3^- concentration of a small brook draining a forested area in the upper part of the Mørdre catchment already was below the detection limit of 1 µg l^{-1}.

At Skuterud, the NO_3^- dynamics were quite similar to Mørdre. The highest NO_3^- peak (25.9 mg N l^{-1}) was recorded at low flow conditions at site S9, which has a mixed land use (forest and agriculture). To the agricultural areas within S9, both manure and fertiliser are applied and the total N application rate is high (229 kg N ha^{-1}). The peak concentration may also indicate influence of a point source. The other two agricultural catchments (S15 and S21) had NO_3^- concentrations in the range 1.3–9.0 mg N l^{-1}

during the four sampling events in 2004. At the forested site S23, representing nearly 30% of the total catchment area at Skuterud, NO_3^- concentrations were much lower, 0.3–0.65 mg N l^{-1}. As a result of mixing of water from the forested area with water from agricultural fields, NO_3^- concentration levels at the main outlet (S19) was intermediate, 4.1–6.7 mg N l^{-1}.
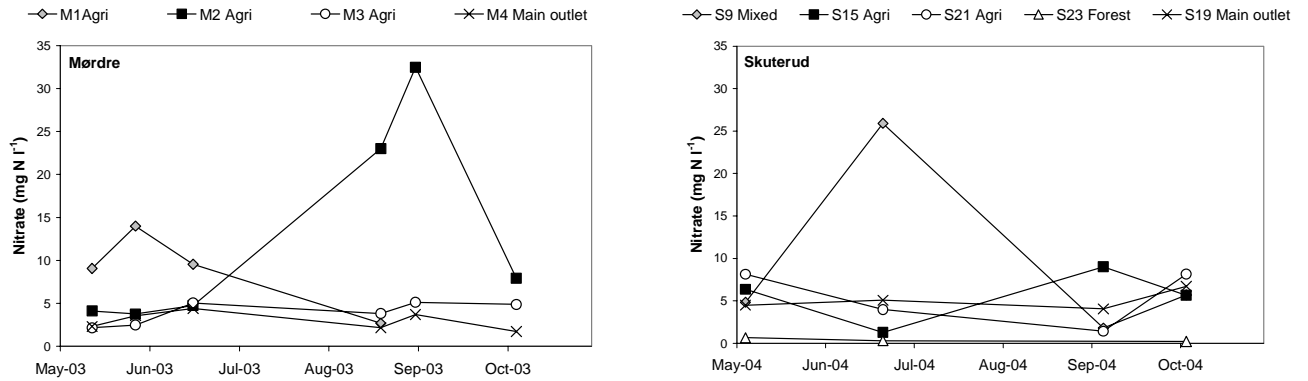

Figure 5. Nitrate concentrations in point samples collected for isotope analysis in the Mørdre catchment (left) and in the Skuterud catchment (right).

3.2 Isotopic composition of NO_3^- in source material

To indicate the isotopic composition of various anthropogenic NO_3^- sources, we prepared and analysed solutions containing commercial fertilisers, animal manure and urban wastewater (**Table 3**). The two types of fertiliser had almost identical $\delta^{15}N$ values of NO_3^-, 1.8–2.0‰. With NO_3^- $\delta^{18}O$ values of 20.2‰ the calcium nitrate was within the typical range of industrial fertilisers ($\delta^{15}N$ -4–7‰, $\delta^{18}O$ 17–23‰) reported by Kendall (1998). No $\delta^{18}O$ data exists for the NPK fertiliser.

The urban wastewater sample was taken from the oxidative stage in a biological sewage treatment plant ensuring that most of the nitrogen was present as NO_3^-. The isotopic composition of this sample ($\delta^{15}N$ 8.7‰, $\delta^{18}O$ 13.9‰) fell within the range previously reported for manure and septic waste sources ($\delta^{15}N$ 0–25‰, $\delta^{18}O$ -5–5‰) by Kendall (1998). In the solution containing animal manure the concentration of oxidised N was to low for further isotopic analysis.

Table 3. Isotopic composition of nitrate in solutions of commercial fertilisers (calcium nitrate, NPK) and urban wastewater collected from the oxidative stage of a biological sewage treatment plant, Grimstad, Norway.

Source	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
Fertiliser (calcium nitrate)	1.8	20.2
Fertiliser (NPK)	2.0	n.a.
Sewage water	8.7	13.9

n.a. – not analysed

3.3 Isotopic composition of NO_3^- in surface water samples

3.3.1 Temporal variation

There was no general seasonal pattern in the isotopic composition of NO_3^- at the study sites, and the observed variation may rather be attributed to climate, land management and site-specific factors than related to season (**Figure 6**). At some of the sampling dates (27 May 2003 at Mørdre and 6 September 2004 at Skuterud) there was an apparent synchronicity in $\delta^{15}N$ values from the various sub-catchments, indicating a possible influence of an external factor as e.g. climate and hydrology.

At Mødre, $\delta^{15}N$ values of NO_3^- varied within a relatively narrow range at individual sampling dates (little spatial variation) and were 7–15‰ for all dates. The majority of samples at Mørdre had $\delta^{15}N$ values above 10‰, whereas most samples at Skuterud were below this level. Skuterud also showed a much broader range in $\delta^{15}N$ values (2–17‰) compared to Mørdre. Also $\delta^{18}O$ values showed no clear seasonal pattern at the study catchments (**Figure 6**). Mørdre had the largest variation, 5–21‰, and the corresponding range at Skuterud was 10–23‰. Whereas the latter catchment had no values below 10‰, 25% of the samples from Mørdre had $\delta^{18}O$ values below 10‰.
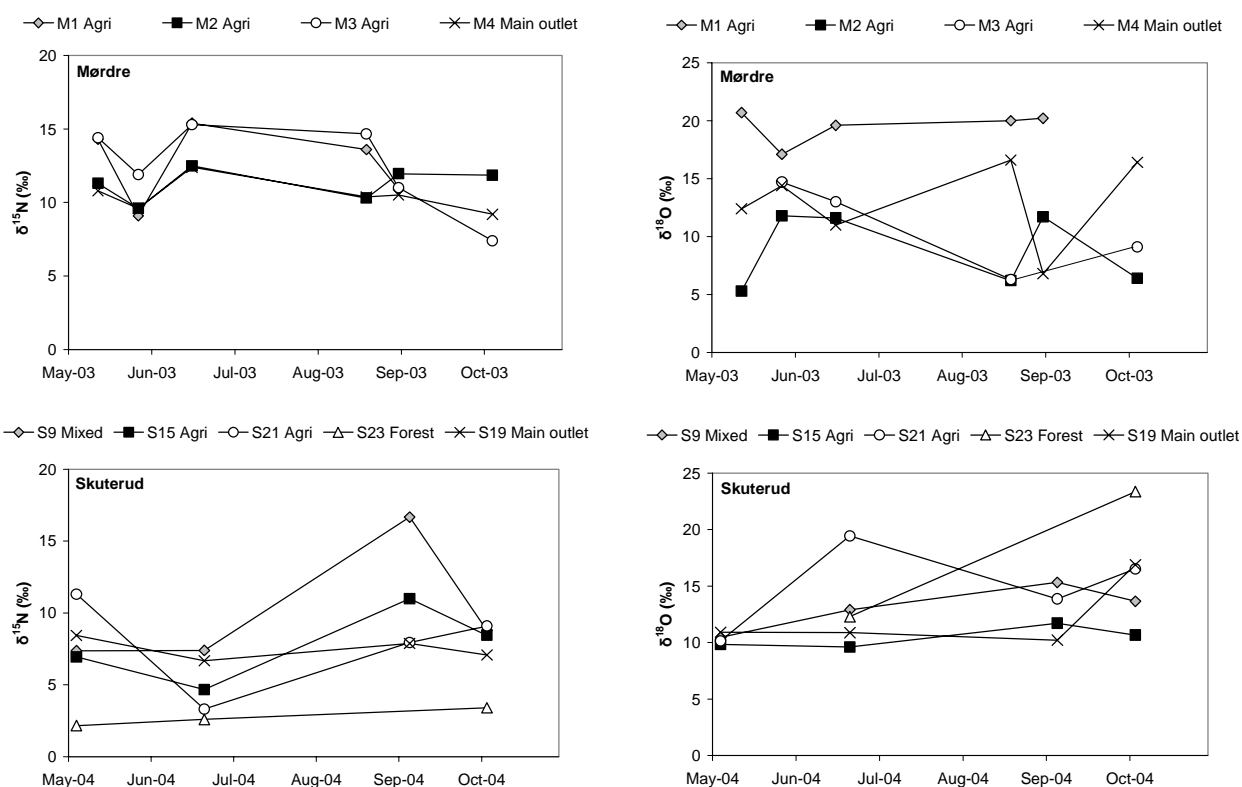

Figure 6. Time-dependent variation in isotopic composition of NO_3^- in runoff at the study catchments.

3.3.2 Spatial variation

In general, the temporal variation in isotopic composition of NO_3^- exceeded the spatial or inter-site variability (**Figure 7**). At Mørdre, the manure application site (M1) could be separated from the other sub-catchments by higher $\delta^{18}O$ values (t-test, $p < 0.01$). The three other sites had overlapping isotopic signals both with regard to $\delta^{15}N$ and $\delta^{18}O$. Both sub-catchments M2 and M3 were subjected to inorganic fertiliser but no manure application, while the main outlet (M4) integrates all NO_3^- sources present in the catchment.

Within Skuterud, the forested site S23 showed systematically lower $\delta^{15}N$ values than the other sites. Due to lack of water or low NO_3^- concentrations during the growing season only two $\delta^{18}O$ analyses are available from this site. These span a relatively wide range from 12 to 23‰; the first one (from June) indicative of soil-derived NO_3^- and the latter (from October) suggesting influence from atmospheric N sources (Kendall, 1998).

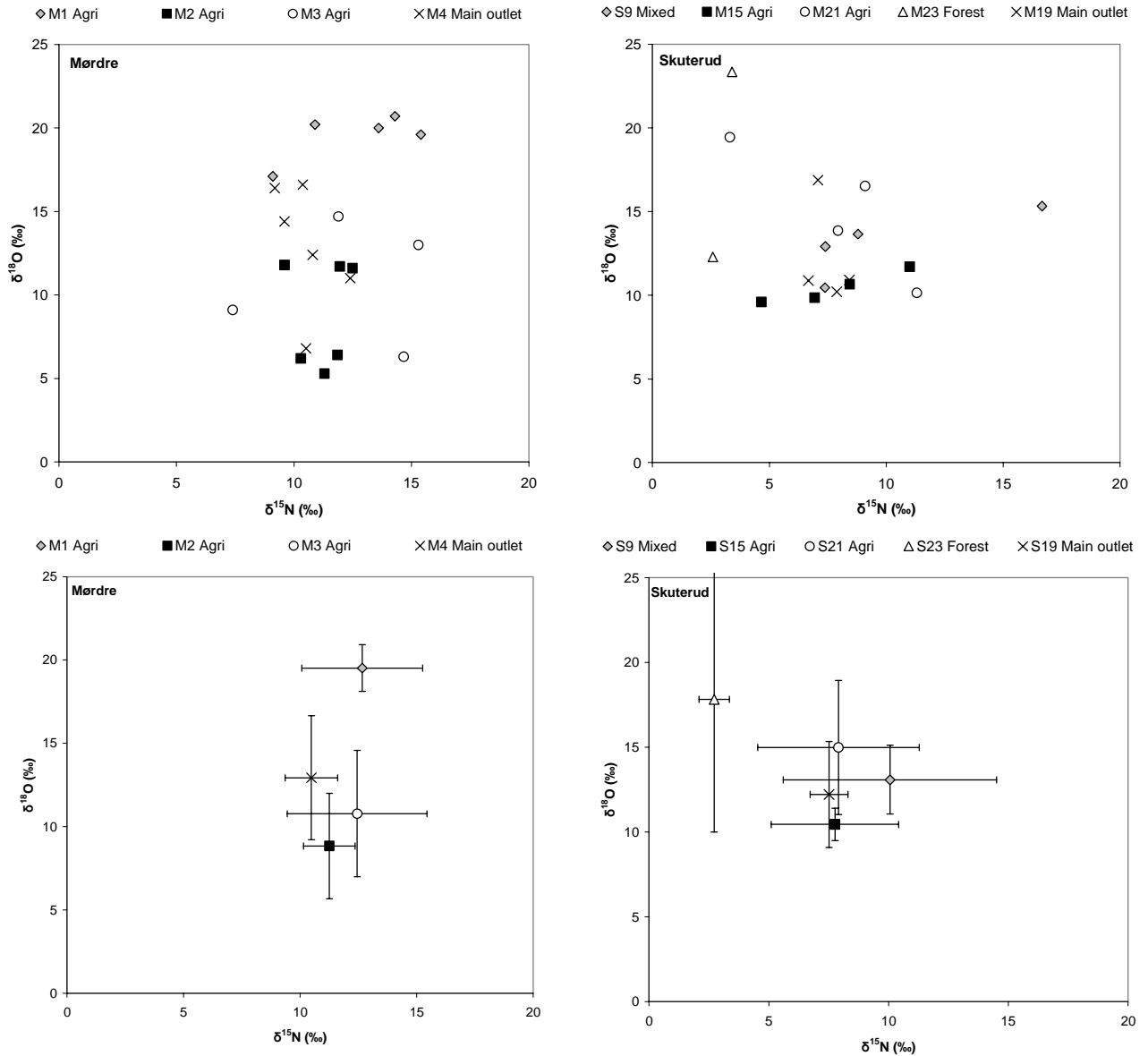**Figure 7.** Upper panels: Combined $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- in runoff from agricultural and forested sub-catchments at Mørdre and Skuterud. IF=Inorganic fertiliser, AM=animal manure, PS=Point source pollution. Lower panels: Mean values ± standard deviation.

3.4 Relation to flow, NO_3^- concentrations and land management

The large intra-site variability at Mørdre and Skuterud suggests that NO_3^- origins and pathways might vary significantly during the growing season. As illustrated in **Figure 4** samples for isotopic analysis were collected under highly variable flow conditions. The highest NO_3^- concentrations were generally recorded during periods with low flow, but only the M3 and S9 sites showed a significant negative relationship ($p<0.1$) between stream flow and NO_3^- concentrations at the sampling dates. At M1, there was a positive correlation between NO_3^- concentrations and water flow ($p<0.1$).

With a few exceptions, the isotopic composition of NO_3^- showed no general relationship to hydrology or streamwater NO_3^- concentrations at the study catchments. At the M1 sub-catchment $\delta^{18}O$ was negatively correlated to water flow ($r^2=0.73$, $p<0.1$), and at the S15 site $\delta^{15}N$ was positively correlated to NO_3^- concentrations ($r^2=0.86$, $p<0.1$). Also, there was a positive correlation between $\delta^{18}O$ values and water flow ($r^2=0.99$, $p<0.01$) and NO_3^- concentrations ($r^2=0.92$, $p<0.05$) at the main outlet of the Skuterud catchment (S19).

Site M1 at Mørdre was treated with both animal manure (24 April) and inorganic fertiliser (9 May and 9 July) in 2003 (**Table 2**). The first streamwater sample was taken 12 May, i.e. three days after the first fertiliser application. Hence, no sample from this site represents manure application alone, but rather a mixture of manure and fertiliser application. At the M2 and M3 sites, only synthetic fertilisers were applied (20–31 May 2003). Hence, it was expected that M2 and M3 would show the highest $\delta^{18}O$ values and M1 the highest $\delta^{15}N$ values. Surprisingly, it was M1 that showed the highest $\delta^{18}O$ values, whereas $\delta^{15}N$ values were around the same level at all the Mørdre sites (**Figure 7**). This might indicate that the manure signal at M1 was obscured by the following fertiliser applications in the catchment. Additionally, there are indications that the isotopic composition at M1 is affected by denitrification (see discussion chapter).

At Skuterud, site S9 was treated simultaneously with animal manure and inorganic fertiliser in mid-April 2004 and with inorganic fertiliser only in August (**Table 2**). At the other two agricultural catchments inorganic fertiliser was applied at the same time in April and in mid-May. In the first streamwater samples collected in early May, there were no differences in isotopic signals that could be related to the manure application in catchment S9. The highest $\delta^{15}N$ value in early May was found at S21, possibly due to pollution from a known point source in this catchment. Also the high $\delta^{15}N$ value recorded at S9 in September may be related to point source pollution.

It is difficult to identify any clear effects of harvest and ploughing on the isotopic data. All sub-catchments at Skuterud experienced an increase in $\delta^{15}N$ values in early September, but this also coincided with a period of very low flow.

4. Discussion

The studies of natural stable isotopes of NO_3^- in streams at Mørdre and Skuterud illustrates some of the possibilities, but also important limitations and problems with using the dual isotope method to trace different sources of NO_3^- in mixed agricultural catchments. The method enabled us to distinguish between major land-use types as forest (range: $\delta^{15}N$ 2.6–3.4‰, $\delta^{18}O$ 12.3–23.4‰) and agricultural land (range: $\delta^{15}N$ 3.3–16.7‰, $\delta^{18}O$ 9.6–19.4‰). These values fall well within ranges previously reported in literature, where forest runoff often shows influence from atmospheric N deposition ($\delta^{15}N$ -3–10‰, $\delta^{18}O$ 18–70‰) and agricultural runoff is typically influenced by a mixture of animal manure ($\delta^{15}N$ 0–25‰, $\delta^{18}O$ -5–15‰), industrial fertilisers ($\delta^{15}N$ -4–7‰, $\delta^{18}O$ 18–23‰) and soil N ($\delta^{15}N$ 2–8‰, $\delta^{18}O$ -5–15‰) (see review by Kendall, 1998). There were also indications that the isotopic composition at site M1 was affected by denitrification. Decreasing NO_3^- concentrations throughout the growing season and high $\delta^{18}O$ values, negatively related to water flow, points to this possible conclusion. Denitrification is likely to occur at this site as animal manure contains easily available organic C that increase respiration and thus decrease the O_2 availability in wet soils. Additionally, manure serves as a C source for denitrifiers.

In general, the isotopic data show large temporal variation at individual sites. In most cases the intra-site variability was much higher than variation among different sites. There were however no clear seasonal patterns, neither a general relationship with possible explanatory factors as stream flow or stream water NO_3^- concentrations. This indicates a more complex causal relationship, i.e. that the isotopic composition in the streams is a result of mixing from two or more NO_3^- sources (e.g., manure and synthetic fertilisers) under varying internal and external conditions (Kellman and Hillaire-Marcel, 2003). Examples here are drainage from different soil layers/compartments under varying flow regimes, plant succession and corresponding variation in uptake rates throughout the growing season, and temperature- and moisture-dependent microbial activity in soils and water. For manure N to contribute to NO_3^- runoff, the organic N must first be mineralised and further nitrified. It is known that kinetic fractionation varies with process rate (e.g. Mariotti et al., 1988), thus the $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- originating from manure cannot easily be estimated without additional processes studies performed at the site (Mengis et al., 2001; Søvik and Mørkved, submitted). Altogether, this large complexity of internal and external factors can easily obscure any source-dependent isotopic signals at the catchment scale.

The sampling programme conducted at Mørdre and Skuterud in many aspects represents a pragmatic approach to test the applicability and robustness of the dual isotope method on a full catchment scale and based on a relatively limited number of samples (3–6) from each site. As such, the lack of distinct isotopic signatures in our study catchments might well be a result of too low number of samples and too high complexity with regard to land-use and potential NO_3^- sources. On the other hand, it probably reflects a realistic level of economic input and sampling efforts in the context of a river basin management programme.

The method used for isotope analysis here requires relatively large amounts of NO_3^- (~2.5 mg N sample[-1]), and is very laborious. This limits the number of samples that can be taken and analysed, both technically (e.g. due to low concentrations) and economically. Although no direct comparison has been done, the 'denitrifier method' for $\delta^{15}N$ and $\delta^{18}O$ analysis of NO_3^- (Sigman et al., 2001; Casciotti et al., 2002; Mørkved et al., sumitted) yields better precision, is far less laborious than the method used in this paper, and requires only 1–5 µg NO_3-N sample[-1]. Anyhow, the precision achieved here is generally far below the variation within sampling sites and between the different sampling dates in the present data set.

Some of these difficulties mentioned above have been previously identified in other isotopic studies (Mengis et al., 2001; Kellman and Hillaire-Marcel, 2003), and it is generally accepted that the isotope techniques are most robust in areas with direct leaching of N from septic systems, animal manure, fertiliser or atmospheric deposition (Kendall et al., 1995; Aravena et al., 1993; Karr et al., 2001; Burns and Kendall, 2002). The methodology should therefore be used with care in catchment-oriented studies, often required by river basin managers, where multiple NO_3^- sources, a mixture of land-use types and complex hydrology easily result in mixed isotopic signals. Natural abundance isotope studies may therefore be more suitable as a complementary method than a stand alone method under such conditions. However, it may still add value to more traditional chemical assays and source apportionment methods, especially in combination with N transformation studies. Further, isotopic techniques will be highly useful and important tools in detailed studies of N transformation processes and pathways for nitrate under controlled field conditions.

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