

**Dual Isotope Tracing of Nitrate Contaminant Source in
Surface and Groundwater in the Great Miami River
Watershed, Southwestern Ohio**

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Abstract

Much of the Great Miami River Watershed's streams and aquifers in southwestern, Ohio are impacted by nitrate contaminants originating from anthropogenic sources. These include synthetic/inorganic fertilizers for agriculture, animal manure, and municipal wastewater. High nitrate concentrations cause ecological disturbances affecting organisms across all trophic levels. Nitrate levels greater than 10 mg/l also pose a danger to human health, if the contaminant reaches drinking water sources. Although networks of water quality monitoring stations in the watershed collect data on nitrate concentrations in surface and groundwater, a nitrate contaminant source has not been identified. In this study we used isotope ratios of nitrogen ($\delta^{15}\text{N}$) and oxygen ($\delta^{18}\text{O}$) in nitrates to identify nitrate sources for surface and groundwater. Initially we fingerprinted the isotopic composition of the main nitrate contaminant sources in the watershed. The results show a distinct low $\delta^{15}\text{N}$ for commercial synthetic fertilizers ($0.7\pm 4\text{‰}$) and high $\delta^{15}\text{N}$ for animal and human waste ($14.7\pm 2.5\text{‰}$). However, as $\delta^{15}\text{N}$ of human and animal waste overlaps, analysis of boron isotopes ($\delta^{11}\text{B}$) is used to distinguish anthropogenic sources from natural sources. Sampling along the Great Miami River, Mad River, and Stillwater River within the watershed provides insights into contaminant sources contributing to high levels of nitrate. In general, the $\delta^{15}\text{N}$ from river samples collected during low river flow lies within a range of animal manure and human waste, whereas $\delta^{15}\text{N}$ values of groundwater suggest that the nitrates might be derived from soil organic nitrogen and synthetic fertilizers. This research provides a regional baseline for nitrate contaminant source tracing and helps to better inform future studies.

Introduction

Nitrogen (N) is the most abundant element (~ 78%) in the earth's atmosphere. N is also an important constituent of living matter as well as a primary nutrient critical for the existence of all living organisms. Although N is abundant in the atmosphere it does not exist in a usable form for most organisms, which makes it a scarce resource and limiting factor for primary productivity (Bernhard, 2010). However, N converts to different usable forms as it moves through the nitrogen cycle and is subjected to physical, chemical and biological processes. In the nitrogen cycle, atmospheric N is converted to ammonia (NH₃) by the activity of microorganisms (particularly *Rhizobium* sp.). Organic nitrogen converts to inorganic forms such as ammonium (NH₄⁺) by the process of mineralization, followed by nitrification processes, which involves a two a step-oxidation of NH₃ into nitrite (NO₂⁻) and then to nitrate (NO₃⁻). Plants and animals can utilize these nitrogen compounds and any unused NO₃⁻ may be converted back to atmospheric molecular N by the process of denitrification in an anoxic zone.

In the past century, human activities have significantly affected the nitrogen cycle, particularly the rate of nitrogen fixation. Nitrogen fixation doubled the biologically usable form of nitrogen through industrial production of fertilizers, fossil fuel burning and increased cultivation of crops that host symbiotic *Rhizobium* bacteria (Vitousek et al., 1997). These conversions of N to NO₃⁻ are suitable as most of the nitrogen absorbed by plants is in that form, and its high solubility and mobility helps to move nitrogen through the soil profile easily. However, excess runoff and infiltration may carry nitrates into streams and aquifers.

In the Great Miami River Watershed, about 70% of the land is used for agriculture, and more than three-fourths of the farmland is used for crop production (U.S. Geological Survey, 2014). The dominant crops in the watershed are corn, soybeans and wheat. Hogs, pigs and cattle are the main livestock (Debrewer et al., 2000). To enhance crop production, nitrogen, phosphate and potash-based fertilizers as well as livestock manure are used. Fertilizers applied in excess amounts contribute to high nutrient loading in streams due to runoff from agricultural fields. Infiltration of water in agricultural areas can also bring nutrients into underlying aquifers. Thus, the Great Miami River has some of the highest nutrient yields in the nation (Rowe et al., 2004).

Although nonpoint sources of nutrients dominate nitrogen loading in the Great Miami River Watershed, point sources of nitrogen may also play an important role in controlling primary productivity of phytoplankton during periods of low river flow. Flow in the Great Miami River during the months of July through October is often dominated by baseflow and likely contains a higher percentage of wastewater discharged by municipal wastewater treatment facilities and industries than at other times of the year (Reutter, 2003).

More than 40% of streams in the Great Miami River Watershed do not meet Ohio's water quality standards (OEPA, 2011, 2012, and 2013). Nutrient enrichment is one of the primary causes of impairment in the watershed. On a larger scale, streams and rivers in the Ohio

River Valley are among the major contributors of nutrient enrichment that results in hypoxia in the Gulf of Mexico (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, 2008; Goolsby et al., 1999).

The U.S. Environmental Protection Agency (EPA) set the maximum contaminant level for nitrate in drinking water at (10-mg/l) nitrate-N, or (45-mg/l) nitrate-NO₃. Nitrate is by far the most common chemical contaminant in the world's groundwater (Spalding and Exner, 1993). In the continental US about half of the population (42%) relies on groundwater as a drinking water source, of which 85% gets the water from public water supplies. In the Great Miami River Watershed, nearly all people (about 97% of the population) depend on groundwater as the source of drinking water. Even though nitrate concentrations in most public water supplies are monitored and regulated, less is known about private wells, as no routine monitoring is required in the state of Ohio (Ward et al., 2005).

Excessive nitrate concentrations in drinking water are a public health concern (CDHS, 2000). The main concern of high nitrate levels in drinking water are related to a condition called methemoglobinemia¹ in infants (also referred as "blue baby syndrome"). Other health related problems associated with exposure to high nitrate levels in drinking water have been suggested (Ward et al., 2005). Some of the risks of ingesting nitrate-contaminated drinking water include a high risk of various types of cancers (Mirvish, 1992, 1995), disruption of thyroid function (Van Maanen et al., 1994) and adverse reproductive outcomes (Ward, 2009).

Contamination of groundwater by nitrate from multiple sources has been and remains a problem in the US, particularly in the Midwest (Motzer, 2006). There are various sources of nitrate that can cause groundwater pollution, but anthropogenic sources are thought to be significant in raising the nitrate concentration to an unsafe level for human consumption. In many cases the source of nitrate contamination can easily be defined if there is a single known source such as livestock confinement, sewer systems, or areas of manure storage. However, in urbanized areas source discrimination between natural and human sources as well as quantifying the relative contribution of those contaminant sources can be complex. While a routine water chemical analysis of water can provide quantitative data on the concentration of contaminant, it does not provide contaminant source information.

Aim and scope

Here we used stable isotope analysis of nitrate as a tracer to discriminate between different nitrate sources, which have unique isotopic signatures. The basic idea of using stable isotopes as a tracer is based upon the fact that nutrient elements from different sources (end-members) have a distinct isotopic composition and their range of variability is limited. If this assumption is true, the sources of nutrients can be positively identified.

¹ Methemoglobinemia (also known as "blue baby syndrome") is a condition where blood cells lack the ability to carry sufficient oxygen because of high amount of nitrate, which can be converted to nitrate and react/oxidize iron in the hemoglobin of the red blood cells.

Furthermore, if the nutrients come from multiple sources, relative contribution from end members can be quantitatively determined.

The main objectives of this study are:

1. Establish isotopic signatures and range of variations of possible nitrate contaminant sources.
2. Identify processes that control the nitrogen isotopes of nitrate in different environmental settings and determine applicability of the multi-isotope approach as a tracer in surface and groundwater.
3. Test established isotopic signature techniques in assessing sources of nitrate in selected streams and aquifers in the study area.

The isotopic composition of nitrogen can be changed or fractionated mainly by biochemical reactions mediated by bacteria. The most important processes that lead to the fractionation of nitrogen isotopes are nitrogen fixation in plants by bacterial action as well as nitrification and denitrification on and near the land surface. As indicated above, nitrate originating from different sources has characteristic isotopic ratios. The $\delta^{15}\text{N}$ values of nitrate originating from soil organic matter range between +4 to +9‰. The $\delta^{15}\text{N}$ of nitrate originating from animal or human waste is typically greater than +10‰. Finally, $\delta^{15}\text{N}$ values of nitrate originating from inorganic mineral fertilizers generally range between -4 to +4‰ (Heaton, 1986). The distinction of end members in the nitrogen isotope space has been the basis for the identification of sources of nitrate in groundwater (Komor and Anderson, 1993; Rolson et al., 1996; Densmore and Bohlke, 2000; Robertson et al., 2016).

Although this unique technique has been successfully used, denitrification processes in oxygen-limited environments and overlap of manure and human waste $\delta^{15}\text{N}$ values complicate the interpretation of source information. Denitrification processes enrich ^{15}N and cause $\delta^{15}\text{N}$ values of some nitrate sources such as ammonia fertilizers to overlap with the $\delta^{15}\text{N}$ values of nitrate from animal and human waste (Fukada et al., 2003). When using $\delta^{15}\text{N}$ values alone it may be difficult to differentiate among various nitrate sources (Barth, 1998, Eppich et al., 2012).

Overlap of $\delta^{15}\text{N}$ values due to mixing of sources and denitrification can be resolved by using dual-isotope tracing techniques where a more conservative isotope such as oxygen ($\delta^{18}\text{O}$) of nitrate is analyzed with $\delta^{15}\text{N}$ simultaneously. The dual-isotope tracing method provides a better source identification because of the wider range of separation of the isotope signatures of different sources (*i.e.*, the $\delta^{18}\text{O}$ range of nitrate is > 60‰). Some nitrate sources have overlapping ranges for $\delta^{15}\text{N}$ and are indistinguishable but they have distinct ranges for $\delta^{18}\text{O}$ (*i.e.*, nitrate in fertilizers versus soil organic matter, and nitrate in fertilizers versus atmospheric sources of nitrate) (Kendall and McDonnell, 1998). More importantly, during denitrification processes $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate vary systematically and are enriched in their heavier isotopes ^{15}N and ^{18}O respectively (Kellman and Hillaire-Marcel, 2003). Thus, the dual $\delta^{15}\text{N}$ - $\delta^{18}\text{O}$ approach is an effective way of discriminating sources of nitrate in groundwater.

Similar to oxygen isotopes in nitrate, boron isotopes behave conservatively and are characterized by large isotopic ranges. The $\delta^{11}\text{B}$ of different sources in surface and groundwater are distinct and are suitable for discriminating between animal manure (Komor, 1997) and municipal wastewater (Barth, 1998). Boron isotopes are particularly effective in tracing anthropogenic sources due to the addition of sodium-perchlorate enriched bleaching agent to detergents and cleaning products (Barth, 1998). This isotopic multi-tracer approach will appraise the respective potential of each isotope and solve some of the limitations of using individual isotopes.

Study Methodology

Study Approach

The study was designed to characterize ranges of nitrogen, oxygen and boron isotopes in different nitrate contaminant sources and use a multi-isotope approach to identify nitrate sources in the Great Miami River Watershed (Figure 1). Fieldwork for this study was conducted between May 2017 and September 2018. Specific sampling sites were selected to cover the range of nitrogen isotopic variation in the nitrogen cycle. The study also considers natural variations and anthropogenic inputs that induce changes in the nitrogen cycle. Anthropogenic sources include animal manure and synthetic/inorganic fertilizers applied to agricultural fields, livestock, household wastewater treatment systems, municipal wastewater treatment plants and residential application of fertilizers. Thus, soil samples were collected from farmlands using manure and synthetic/inorganic fertilizers, natural sites, and wetlands. Water samples were collected from wetlands, tile drainages, small streams, rivers, groundwater, rainfall/runoff, municipal wastewater effluent, and storm outfalls. In addition, manure and synthetic/inorganic fertilizers were also collected and analyzed.

Sample Collection

A. *Soil samples*

Soil samples were collected from forested, manure-use and synthetic/inorganic fertilizer-use farms. The samples were collected in 40 cm long cores at 10 cm increments using a 3.25" diameter soil auger. To prevent further microbial activity and ammonia volatilization the samples were acidified with 5% reagent-grade hydrochloric acid (HCl) and oven dried at 60°C for a minimum of 8 hours. The dried samples were pulverized with mortar and pestle, homogenized and split in to four fractions. The samples were then stored in a freezer until analyzed.

B. *Manure and inorganic fertilizer samples*

Manure samples (cow, pig and chicken) were collected from animal pens. Synthetic/inorganic synthetic fertilizers were purchased from different

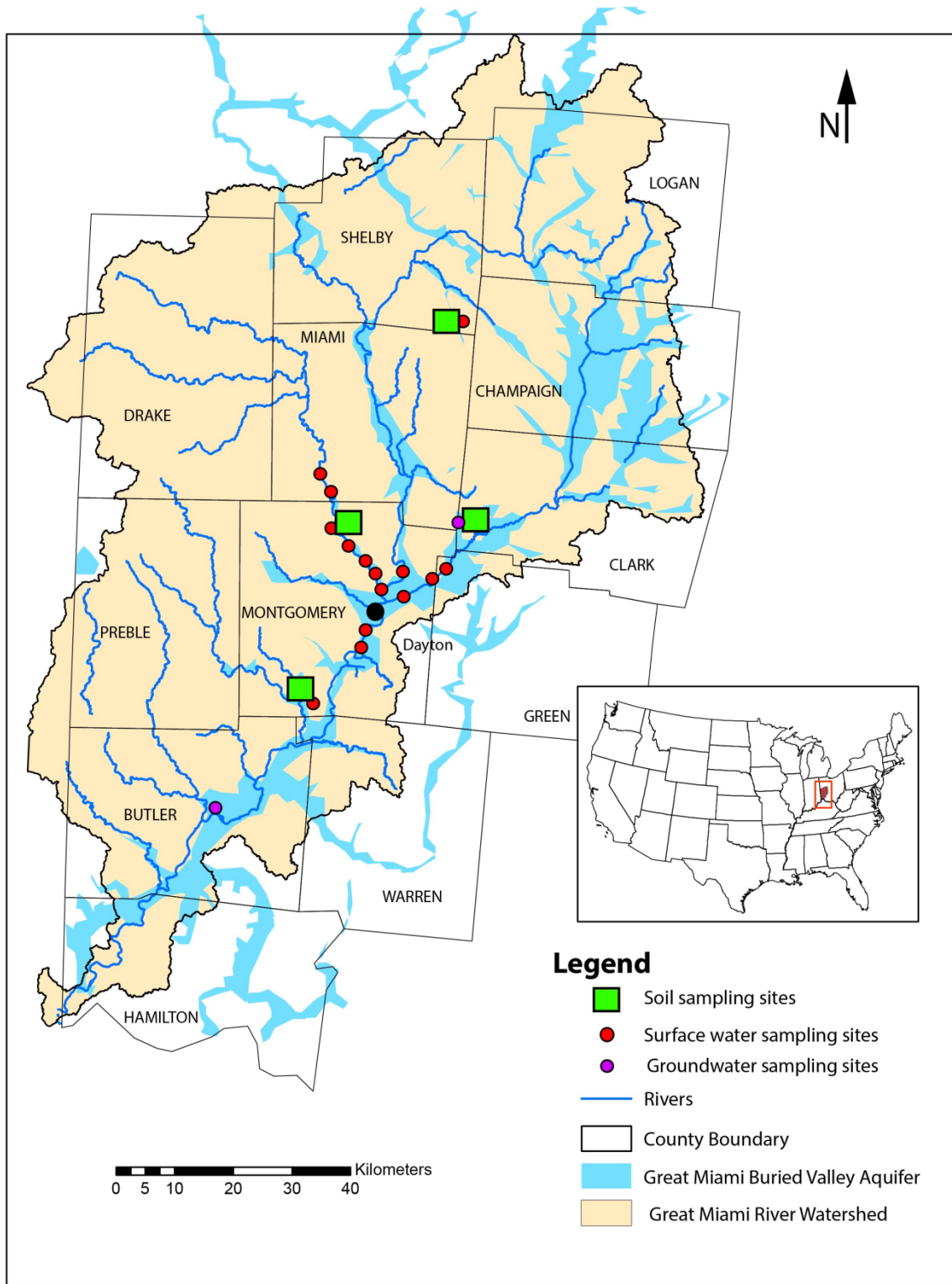


Figure 1. Location map of the study area showing sampling sites.

suppliers. The manure and synthetic/inorganic fertilizers samples were processed similar to the soil samples above.

C. *Water samples*

Surface water samples were collected from small streams, the Great Miami River, Mad River and Stillwater River. All water samples were filtered with 0.1/0.2- μm filters in the field or in the lab and treated with a reagent-grade HCL 0.5 vol. % to prevent further microbial action. The samples were packed in an ice cooler in the field and stored in a freezer in the lab until analyzed. During surface water sampling, in situ water quality parameters (i.e., pH, specific conductance, dissolved oxygen, temperature) were measured using a YSI meter. Groundwater samples were collected from one monitoring well in Butler County and one in Clark County and processed similar to surface water.

Isotope Sample Analysis

In all, 32 soil samples, 3 manure samples, 7 synthetic/inorganic fertilizer samples, and 59 water samples were analyzed for nitrogen ($\delta^{15}\text{N}$) and oxygen ($\delta^{18}\text{O}$). The $^{15}\text{N}:^{14}\text{N}$ and $^{18}\text{O}:^{16}\text{O}$ isotope ratios were measured by isotope ratio mass spectrometer (IRMS). Nitrate sample preparation for IRMS involves conversion of solid and liquid materials to a gaseous form (N_2) before introducing to the mass spectrometer following procedures described in Silva et al. (2000) and Sigman et al. (2001). The $^{15}\text{N}:^{14}\text{N}$ reference is N_2 in air, and the $^{18}\text{O}:^{16}\text{O}$ reference is Vienna standard mean ocean water (VSMOW). Individual sample analyses are referenced against automated injections of N_2O from a gas cylinder. International reference standards IAENO3-Potassium Nitrate ($\delta^{15}\text{N}=+4.7\text{‰}\text{N}_2$, $\delta^{18}\text{O}=+26.5\text{‰}\text{VSMOW}$, Böhlke and Coplen, 1995) and USGS 34-Potassium Nitrate ($\delta^{15}\text{N}=-1.8\text{‰}\text{N}_2$, $\delta^{18}\text{O}=-27.9\text{‰}\text{VSMOW}$, Böhlke et al. 2003) were used to standardize the isotope analyses.

Four water samples and seven soil samples were analyzed for boron (B) isotope composition and boron concentration. About 2g of soil was leached with 3ml of 1M acetic acid for 2 hours in an ultrasonicator. This process was repeated six times. The supernatant was collected after each leaching and combined for B concentration and isotope analysis. Elemental concentration analyses were performed on an Agilent 7500cx quadrupole inductively coupled plasma mass spectrometry (ICP-MS). Samples were diluted to signal match mixed calibration standards and unknown concentrations were calculated based on standard calibration curves, with standards run frequently between unknowns to monitor for drift in signal intensity.

Boron-isotope analyses were carried out on a Nu Plasma II multicollector inductively-coupled plasma mass spectrometer (MC ICP-MS). Accuracy is provided by certified SRM 951 values. The standard used to correct the sample for in-run plasma-induced mass fractionation is a 50-ppb solution of the NIST SRM 951 boric acid. In addition, background was monitored using the same nitric acid and deionized water that was used to dilute the samples and SRM 951 standards, and

subtracted from all analysis. The SRM 951 boric acid standard has a certified $^{11}\text{B}/^{10}\text{B}$ value of 4.0437 ± 0.003 . Results corrected for background, drift and fractionation, and final $^{11}\text{B}/^{10}\text{B}$ determined.

Results are reported as $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ and $\delta^{11}\text{B}$ values, which are defined as

$$\delta^i X = \left(\frac{R_{\text{sample}}}{R_{\text{Standard}}} - 1 \right) * 1000, \quad \text{Eq.1}$$

where X is N, O and B isotopes and R_{sample} and R_{standard} are the $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$ and $^{11}\text{B}/^{10}\text{B}$ ratios in the sample and standard expressed in delta notation (δ) as parts per thousand (‰).

Relative contribution of nitrate from multi-sources can be determined using Mass Isotope balance equations below-

$$Q_T = Q_1 + Q_2 \quad \text{Eq.2}$$

$$\delta^{15}\text{N}_{(\text{NO}_3)_T} Q_T = \delta^{15}\text{N}_{(\text{NO}_3)_1} Q_1 + \delta^{15}\text{N}_{(\text{NO}_3)_2} Q_2 \quad \text{Eq.3}$$

Where: Q_1 , and Q_2 -amounts of nitrate from different sources

Q_T -total amount of nitrate in well-mixed system

$\delta^{15}\text{N}_{(\text{NO}_3)_1}$ and $\delta^{15}\text{N}_{(\text{NO}_3)_2}$ -average end-member isotope values of contaminant sources

$\delta^{15}\text{N}_{(\text{NO}_3)_T}$ - average isotope value of contaminated media

Results and Discussion

Data Summary

The stable isotopic compositions of all samples analyzed for this study ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\delta^{11}\text{B}$) are presented in Table 1. The isotopic composition of samples from different land use and potential nitrate contaminant sources range from -1.4 to +20.8‰ for $\delta^{15}\text{N}$, -8.7 to +40.4‰ for $\delta^{18}\text{O}$ and -3.3to +20.51‰ for $\delta^{11}\text{B}$. Table 1 shows the average value for each sample category as well as the standard deviation.

Table 1. Summary of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\delta^{11}\text{B}$ values for sources of nitrate.

Sample type	n	$\delta^{15}\text{N}_{\text{Air}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)	$\delta^{11}\text{B}_{\text{SRM951}}$ (‰)
Organic farm	5	17.0 ± 4.1	8.9 ± 9.4	10.1 (n=1)
Inorganic farm	7	8.7 ± 3.5	6.56 ± 5.8	15.8 (n=1)
Wetland	3	10.9 ± 0.8	4.5 ± 8.8	10.9 (n=1)
Natural site	4	7.2 ± 3.3	12.4 ± 2.4	19.1 ± 2.0 (n=2)
Manures	3	14.7 ± 2.5	4.2 ± 3.2	14.0 ± 1.7 (n=3)

Inorganic fertilizers	6	0.7 ± 4.0	11.8 ± 4.9	N/A
Wastewater	4	11.6 ± 1.3	-3.2 ± 4.3	5.2 ± 2.8 (n=2)
Rain water	1	8.1	40.4	N/A

n is number of samples collected and analyzed to establish isotopic ranges for sources $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\delta^{11}\text{B}$. Values are reported in ‰ units, relative the respective international standards.

The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ Water samples collected from rivers, groundwater, runoff and storm outfalls range from -0.6 to +15.6‰ and -1.2 to +17.3‰ respectively. Average values with standard deviation for each sample type are summarized in Table 2.

Table 2. Summary of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values for surface and groundwater samples.

Sample type	n	$\delta^{15}\text{N}_{\text{Air}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)
Groundwater	4	4.8 ± 1.3	0.0 ± 1.6
Great Miami River	16	9.8 ± 4.2	6.9 ± 5.0
Mad River	11	10.1 ± 1.0	3.1 ± 0.8
Stillwater River	6	15.2 ± 0.7	7.0 ± 0.8
Dayton storm water	4	8.0 ± 0.4	2.0 ± 0.4

n is number of samples collected and analyzed. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values are reported in ‰ units relative to the respective international standards.

Isotope Signatures of Potential Nitrate Sources

Synthetic/inorganic fertilizers are considered to be one of the major sources of nitrate to rivers, streams, and aquifers in the Great Miami River Watershed. Nitrate from synthetic/inorganic fertilizers originates from the fertilizer itself or by nitrification of ammonia based fertilizers in the soil. The $\delta^{15}\text{N}$ values of fertilizers analyzed in this study range from -1.4 to 8.8 ‰ (average 0.7‰, Table 1, Figure 2) and lies within the range of reported values in previous studies. The typical $\delta^{15}\text{N}$ values for synthetic/inorganic fertilizers is between -4 to +4‰ (Sharp, 2007), however some fertilizer samples show a total range of -8 to +7‰ (Kendall, 1998). A recent study by Michalski et al. (2015) reported a range of $\delta^{15}\text{N}$ values for urea-ammonia-nitrate fertilizers -4 to +8‰, with 80% of the values between -3 to +3‰ (Figure 2). The $\delta^{18}\text{O}$ values for fertilizers analyzed in this study range from +6.9 to +20.9‰ (average +11.8 ‰, Table 1), and within the range reported for nitrate and ammonia based nitrates. However, the $\delta^{18}\text{O}$ values for synthetic/inorganic fertilizers show a wide range of values from about -10 to +80 ‰.

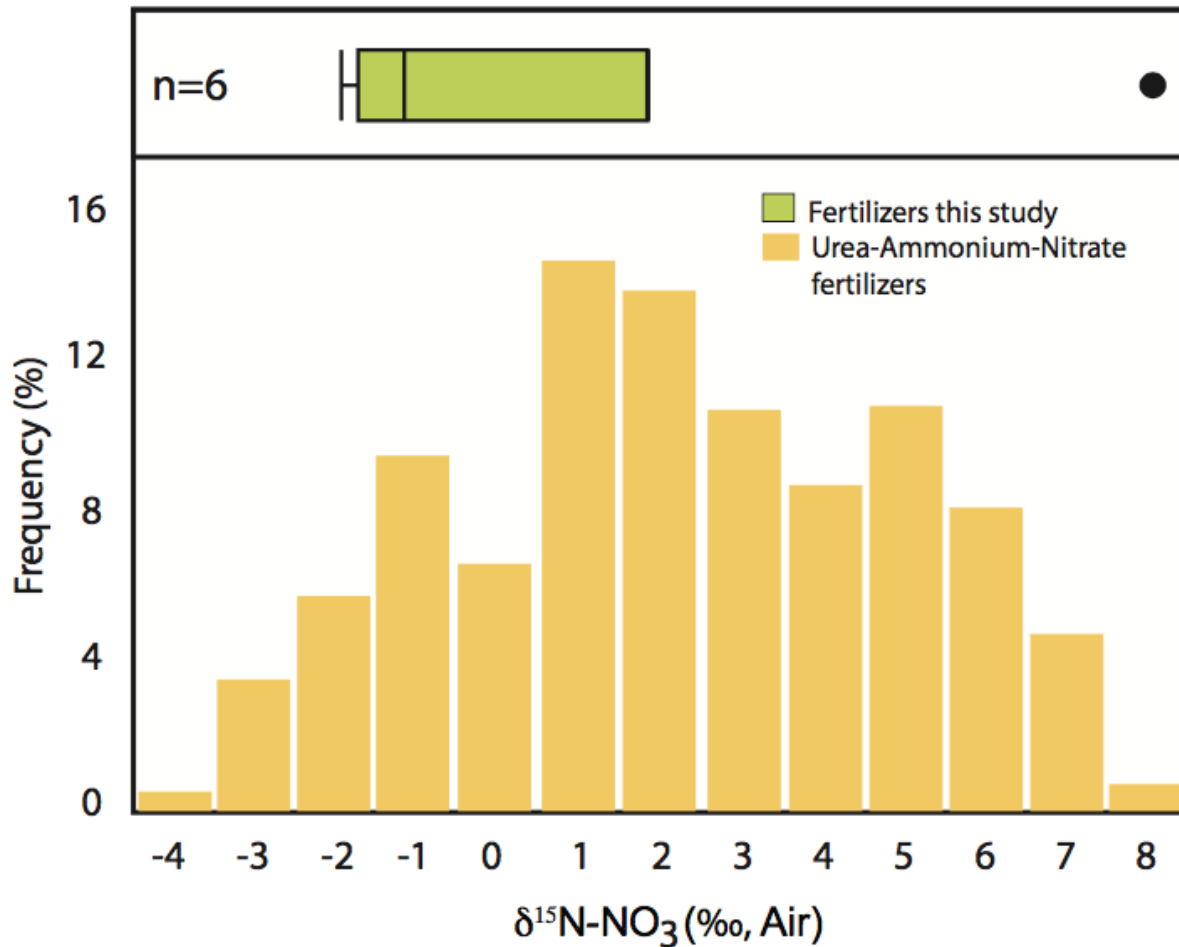


Figure 2. Comparison of $\delta^{15}\text{N}$ commercial synthetic/inorganic fertilizers analyzed in this study with the $\delta^{15}\text{N}$ percentage distribution of 270 samples of Urea-Ammonia-Nitrate (U-R-A) fertilizer. Modified after Michalski et al. (2015).

A second source of nitrate to natural water resources is animal manure. Manure from cows, pigs and chicken is an organic fertilizer widely used in agriculture. Manure is relatively ^{15}N enriched compared to the food the animal consumes and the animal tissue. Manure may be further enriched in ^{15}N through loss of the lighter nitrogen isotope- ^{14}N during ammonia volatilization (Lee et al., 2011). Thus, $\delta^{15}\text{N}$ values of manure are generally in the range of +10 to +20‰. The $\delta^{15}\text{N}$ values of manure samples from cow, pig and chicken in this study range from +12.9 to 17.6‰ (average +14.7‰, Table 1, Figure 3). The $\delta^{18}\text{O}$ values for manure analyzed in this study range from +2.3 to +7.7‰ (average 4.2 ‰, Table 1, Figure 4), and within previously reported ranges from -4 to +12‰ (e.g. Montzer, 2006). ($\delta^{11}\text{B}$). The $\delta^{11}\text{B}$ of animal manure ranged from +13 to 16 ‰ (average +14‰, Table 1, Figure 5).

A third potential source of nitrate for natural water resources in southwest Ohio is municipal wastewater treatment plant effluents. The $\delta^{15}\text{N}$ values for treated wastewater

effluent from the Englewood and Miamisburg wastewater treatment plants ranged from +9.8 to 11.9‰ (average +11.6‰, Table 1, Figure 3). The range of values for $\delta^{15}\text{N}$ in treated wastewater effluent depends upon the efficiency of the water treatment technology and level of treatment used. In secondary treatment processes, the $\delta^{15}\text{N}$ becomes higher with increasing efficiency in the conversion of ammonia to nitrates. With tertiary treatment (denitrification) ^{15}N is further enriched and may result in $\delta^{15}\text{N}$ values higher than 30‰ (Mayer et al., 2013). The $\delta^{11}\text{B}$ of wastewater ranged from +3.3 to 7.2 ‰ (average +5.2‰, Table 1, Figure 5). The $\delta^{11}\text{B}$ of wastewater from this study are within the ranges reported in the country (Barth, 1998).

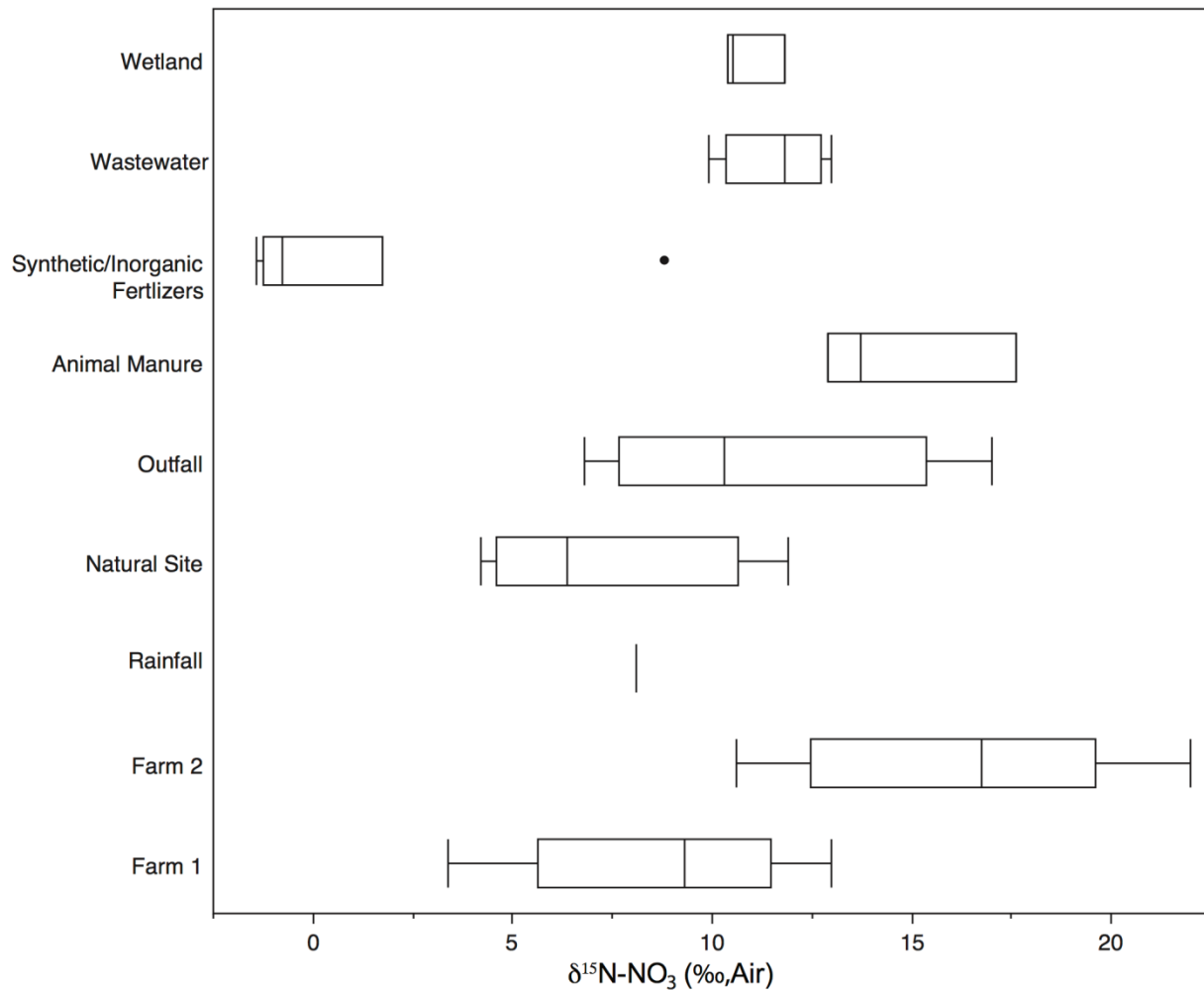


Figure 3. Box and whisker plot summarizing $\delta^{15}\text{N}$ from this study. Each box encloses 50% of the data with the median value represented by a vertical line in the box. Each box is thus bounded by the first quartile (25%) and the third quartile (75%) of the variable distribution. The acceptable range of data is represented by the horizontal line extending from the sides of the box, while outliers are displayed as individual points.

Natural soils are a fourth source of nitrate to the water resources of the Great Miami River Watershed. Soil $\delta^{15}\text{N}$ show a wide range values and can be affected multiple factors such as vegetation, climate, soil depth, land use etc. In this study, we analyzed soil samples from natural forest areas, wetlands and inorganic and organic farms. The $\delta^{15}\text{N}$ values of natural forest soils range from +4.2 to +11.9‰ (average 7.2 ‰, table 1, Figure 3) and wetland soils range from +10.4 to +11.8‰ (average 10.9 ‰, table 1, Figure 3). These values are consistent with previously reported $\delta^{15}\text{N}$ values of soil organic nitrogen (i.e., +4 to +9‰, e.g., Kendall et al., 2007).

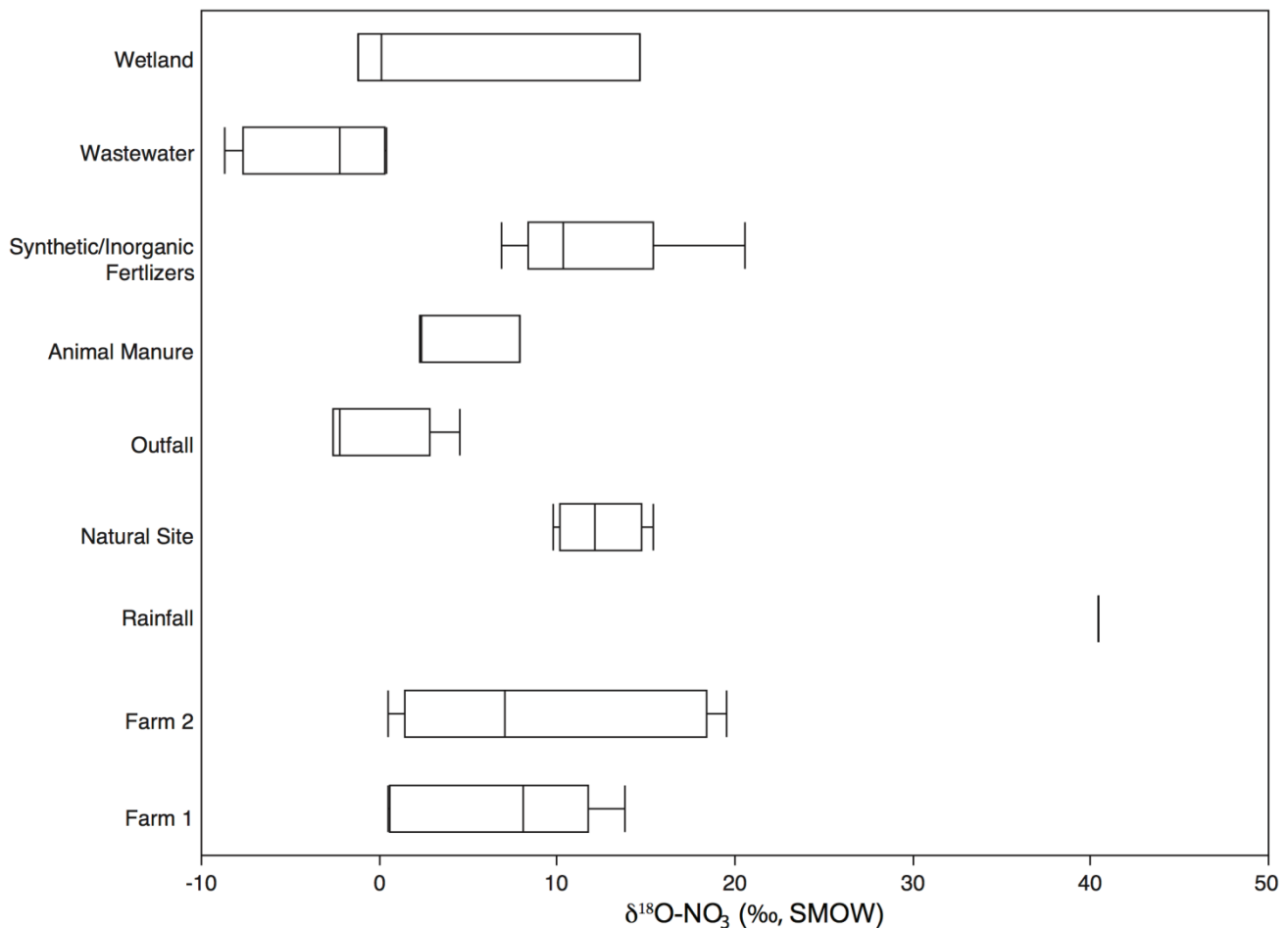


Figure 4. Box and whisker plot summarizing $\delta^{18}\text{O}$ from this study. Each box encloses 50% of the data with the median value represented by a vertical line in the box. Each box is thus bounded by the first quartile (25%) and the third quartile (75%) of the variable distribution. The acceptable range of data is represented by the horizontal line extending from the sides of the box, while outliers are displayed as individual points.

The $\delta^{15}\text{N}$ value of farm soils is affected by the type of fertilizers (synthetic/inorganic vs. manure used). In this study, the $\delta^{15}\text{N}$ values of soil samples from the inorganic farms ranged from +3.4 to +13‰ (average 8.7 ‰, table 1 Figure 3) whereas $\delta^{15}\text{N}$ from organic farm soils ranged from +10.6 to +22‰ (average 16.3 ‰, Table 1, Figure 3). The $\delta^{15}\text{N}$ values from inorganic and organic farm soils are distinct but they overlap with the $\delta^{15}\text{N}$ values of the other soils in this investigation (Figure 3). The $\delta^{18}\text{O}$ values from natural soils range from +4.2 to +11.9‰ (average +7.2‰, Table 1, Figure 4). The $\delta^{11}\text{B}$ values from natural soils range from +17.6 to 20.5 ‰ (average +19.1‰, Table 1, Figure 5).

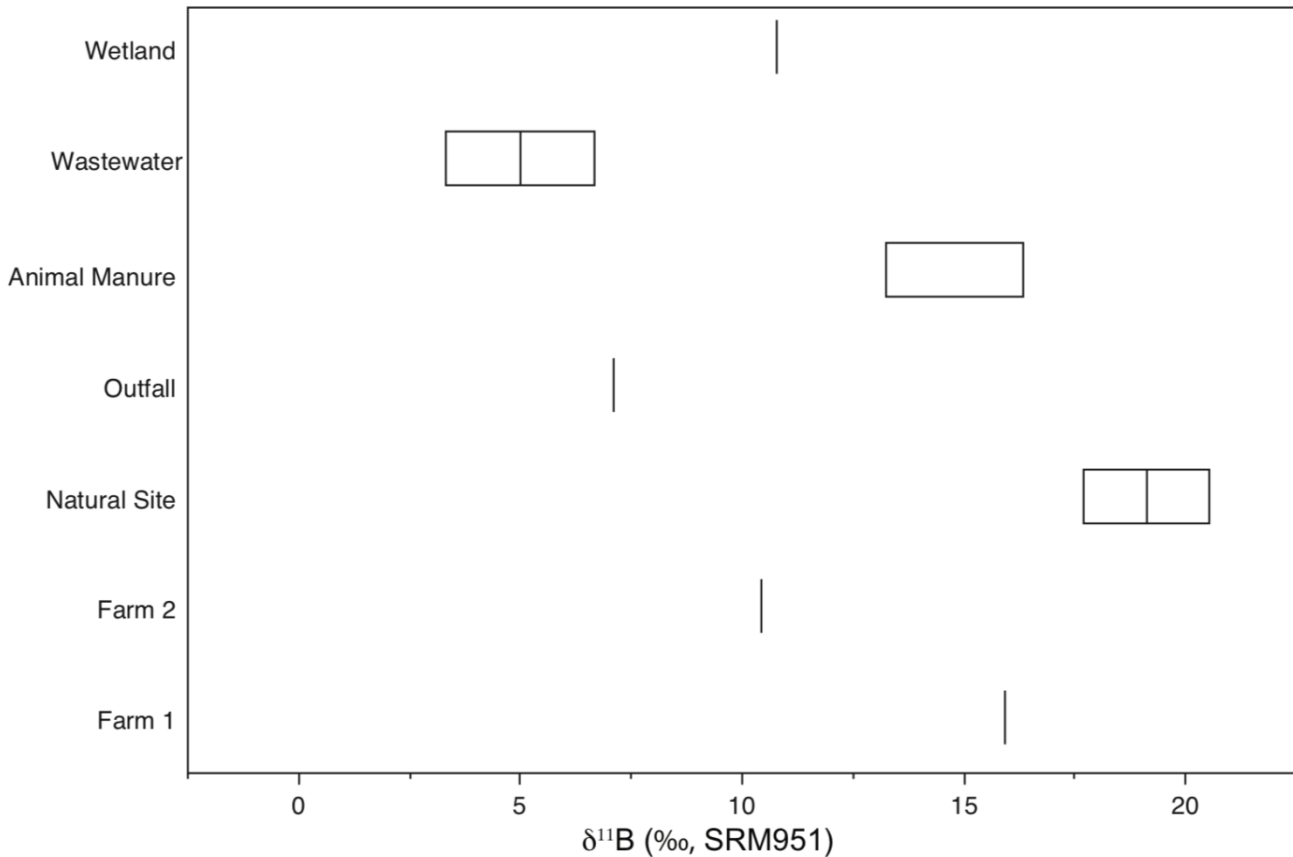


Figure 5. Box and whisker plot summarizing $\delta^{11}\text{B}$ from this study. Each box encloses 50% of the data with the median value represented by a vertical line in the box. Each box is thus bounded by the first quartile (25%) and the third quartile (75%) of the variable distribution.

Dual Isotope Signatures of Potential Nitrate Sources

For this investigation, the dual isotope approach of identifying source of nitrate involved determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate in a sample.

The key advantage of dual isotope tracing of nitrate source is that some of the nitrate sources show overlapping $\delta^{15}\text{N}$ ranges, (e.g. nitrate from rain and fertilizer sources, Figure 3) making it difficult to distinguish between different sources. However, simultaneous measurement of nitrate $\delta^{18}\text{O}$ helps to differentiate between unique nitrate sources

Figure 6 is a dual isotope plot showing how sample $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values for various nitrate sources in the Great Miami River Watershed plot in distinct boxes or zones depending upon the level of heavy isotope enrichment. Figure 7 is the same dual isotope plot with expanded dual isotope boxes or zones showing typical ranges of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ nitrate derived from other studies (Kendall et al., 2007).

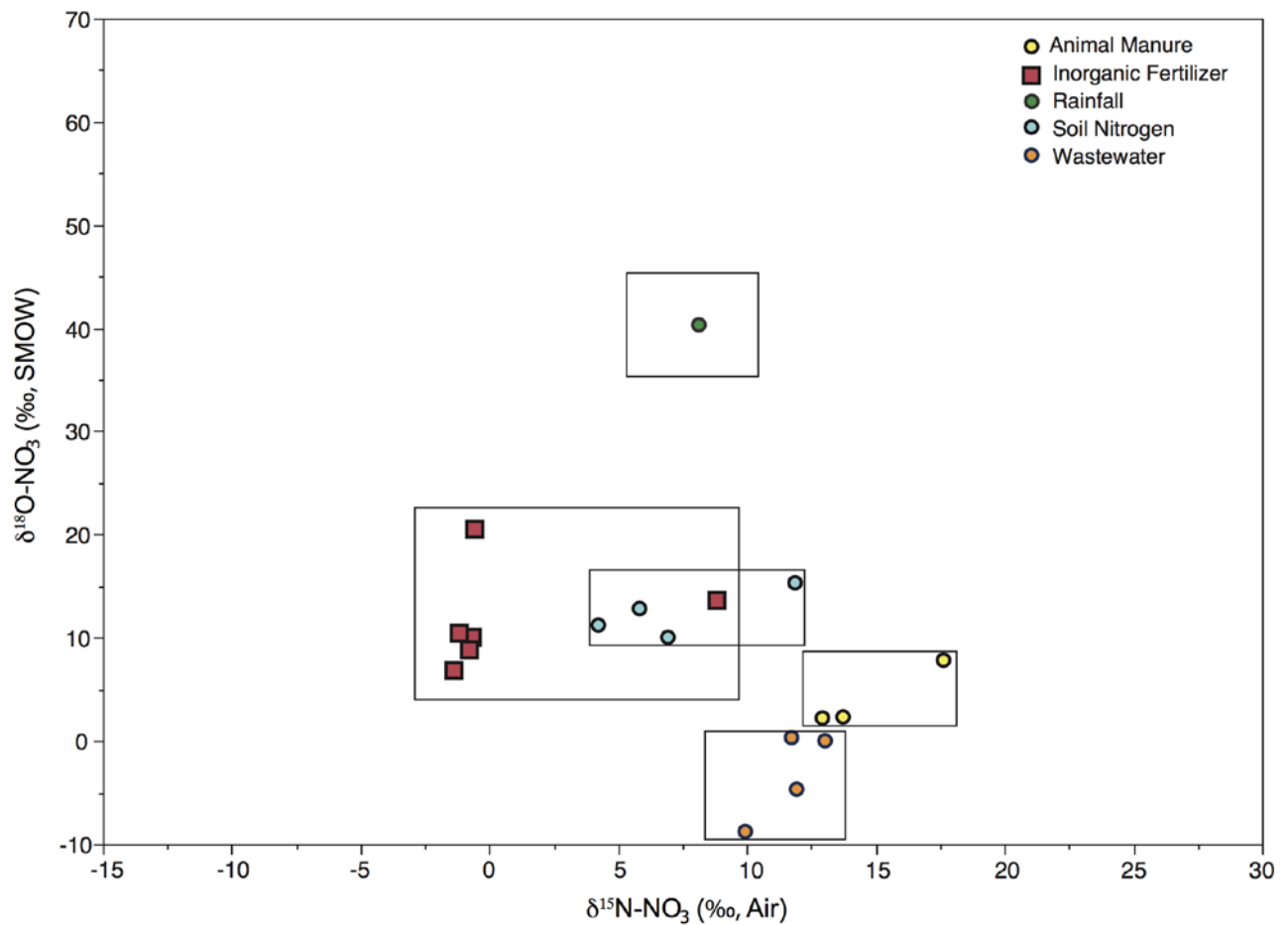


Figure 6. Scatterplot of $\delta^{15}\text{N-NO}_3$ (‰ Air) and $\delta^{18}\text{O-NO}_3$ (‰ VSMOW) for samples of potential nitrate sources in the Great Miami River Watershed.

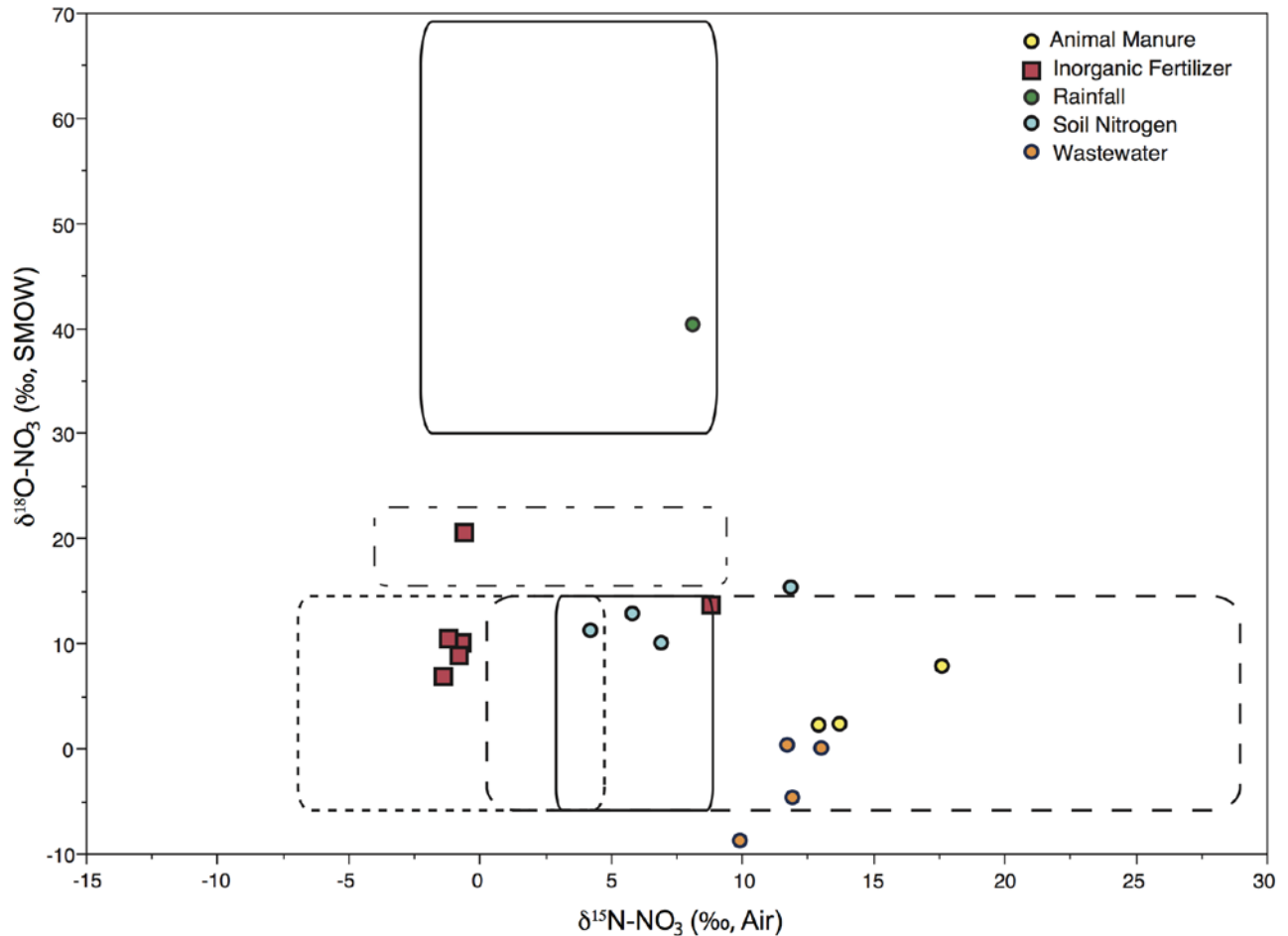


Figure 7. Comparison of $\delta^{15}\text{N} - \text{NO}_3$ (‰ Air) and $\delta^{18}\text{O} - \text{NO}_3$ (‰ VSMOW) for samples of potential nitrate sources in the Great Miami River Watershed with typical ranges of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate from published sources. Modified from Kendall (1998).

In this study, $\delta^{15}\text{N}$ of nitrate derived from synthetic/inorganic fertilizers (average 0.7‰) can easily be distinguished from animal manure (average 14.7‰). However, overlapping $\delta^{15}\text{N}$ values of nitrate sources such as rain, synthetic/inorganic fertilizers and nitrate produced by nitrification processes in the soil, make it impossible to distinguish between different nitrate sources using nitrogen isotope analysis alone. In this study, the range of $\delta^{18}\text{O}$ values of nitrate from inorganic fertilizers (ammonium) and soil nitrogen shows some overlap, but both nitrate sources are more depleted than atmospheric sources of nitrate

In addition to identifying nitrate sources, dual isotope tracing is very important to assess the occurrence and importance of microbial denitrification in soils, rivers and groundwater. Denitrification is the process of conversion of nitrate to N_2 under reducing conditions, during which lighter isotopes of ^{14}N and ^{16}O are preferentially removed from the system leading to enrichment with respect to the heavier respective isotopes. As a

result, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the remaining nitrate increases (Kendall et al., 2007) in a 1:1 to 2:1 ratio (Granger et al., 2008).

One of the limitations of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ dual isotope contaminant tracing technique is its inability to distinguish between animal manure and municipal wastewater. The $\delta^{15}\text{N}$ values of animal manure and human waste fall in a similar range. Here, we used boron isotopes to distinguish the two sources. Our result shows that municipal wastewater, animal manure and samples from natural soils plotted in distinct fields in the $\delta^{15}\text{N}$ and $\delta^{11}\text{B}$ dual isotope plot (Figure 8). This technique could potentially be used in Great Miami River Watershed to identify nitrate sources in surface and groundwater. However, due to the limited number of $\delta^{11}\text{B}$ samples analyzed in this study, additional $\delta^{11}\text{B}$ analyses of nitrate source samples is required to use this technique with confidence.

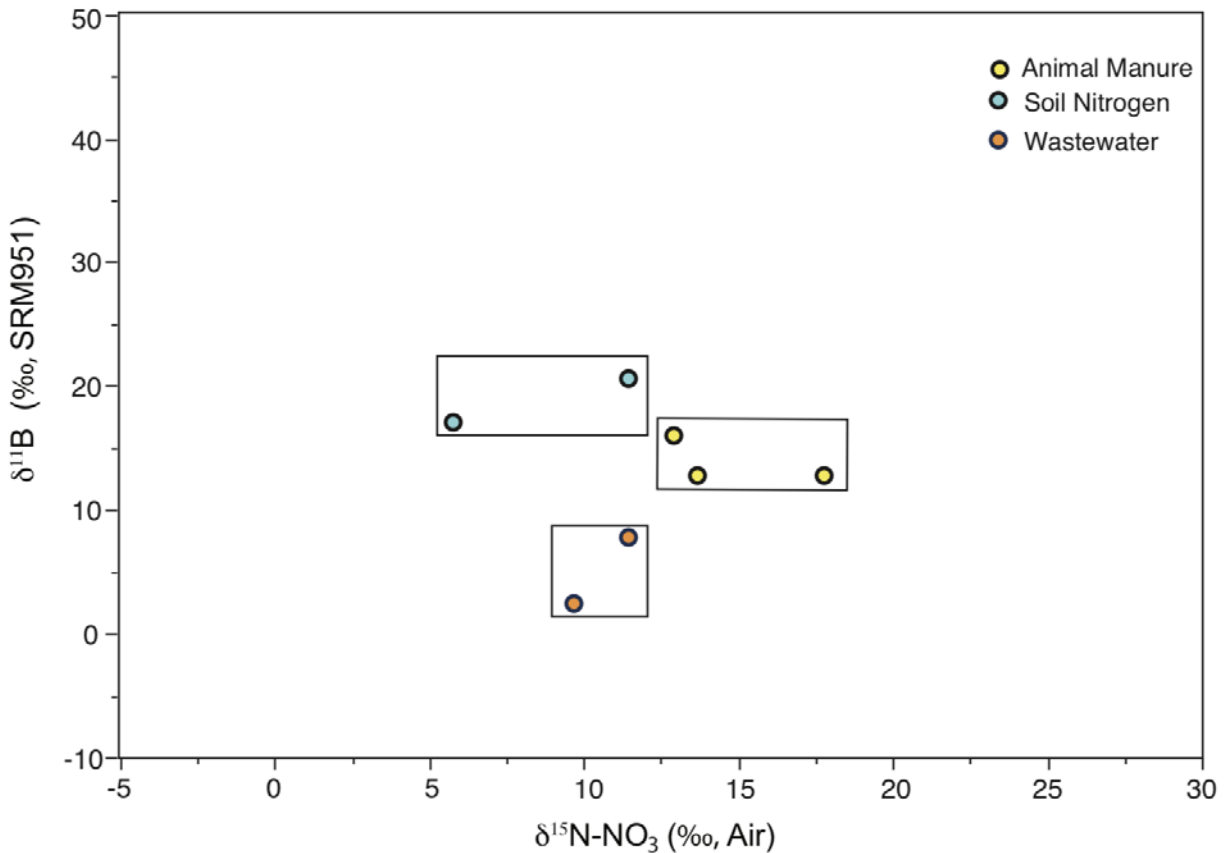


Figure 8. Scatterplot of $\delta^{15}\text{N-NO}_3$ (‰ Air) and $\delta^{11}\text{B}$ (‰ SRM951) for samples of from animal manure, municipal wastewater and natural soil in the Great Miami River Watershed.

Dual Isotope Tracing of Sources of Nitrate in Surface and Groundwater

Nitrate source identification in surface and groundwater was carried out by comparing nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in the water samples with nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values expected from various sources in the Great Miami River Watershed (Figures 9 and 10, Table 2). We also combined our results with the most commonly reported range of nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values from different nitrate sources (Table 3).

Table 3. Typical $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate from different nitrate sources (Adopted from Montzer, 2006)

Nitrate contaminant sources	$\delta^{15}\text{N}_{\text{Air}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)
Synthetic/inorganic fertilizer	-4 to +4 \pm 4.1	+18 to +26
Municipal wastewater	>+10	-4 to +12
Precipitation	-3	+18 to +60
Soil organic nitrogen	+4 to +9	+1 to -4

The observed surface and groundwater nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in Figures 9 and 10 overlapped with the expected ranges from the three sources: inorganic fertilizers, soil organic nitrogen and animal manure/wastewater effluent. The $\delta^{15}\text{N}$ values of nitrate for groundwater samples plot between the upper limit of inorganic fertilizer and the lower limit of soil organic nitrogen. This 3-5‰ $\delta^{15}\text{N}$ range could be indicative of sources of nitrate from nitrification of soil organic nitrogen and incorporation of ^{15}N - depleted nitrate sources such as synthetic/inorganic fertilizer that leached and infiltrated during groundwater recharge.

On the other hand, the $\delta^{15}\text{N}$ values of nitrate in samples collected from the Great Miami River, Mad River and Stillwater River plot between the upper limit of soil organic nitrogen and animal manure/municipal wastewater. The 8-16‰ $\delta^{15}\text{N}$ range in the samples could be indicative of nitrate from erosion of natural soils, manure, or wastewater effluent discharged in the rivers (Figure 11). The differences between the $\delta^{15}\text{N}$ values in the three rivers may be a result of spatial variations in nitrate sources, relative contribution of nitrate from different sources and/or the degree of mixing and interaction between surface and groundwater.

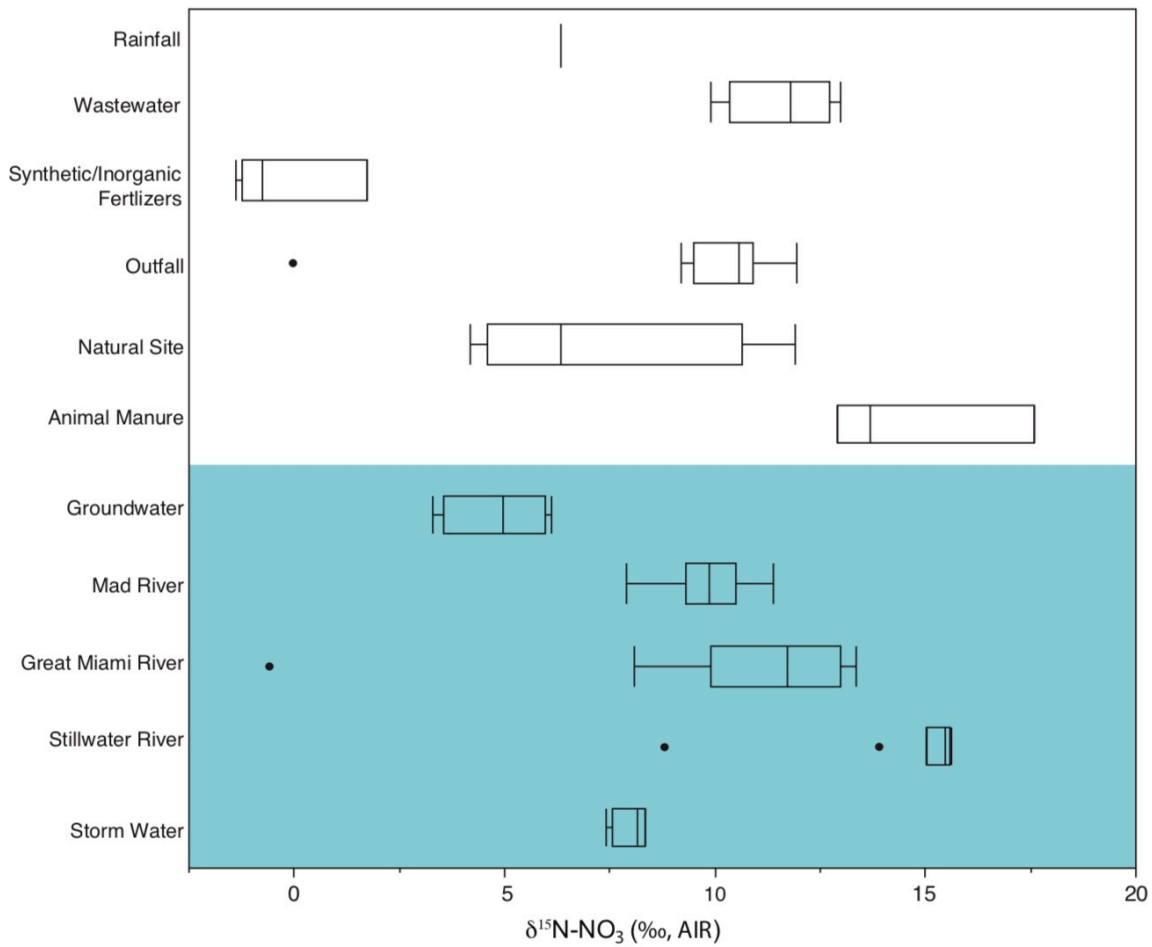


Figure 9. Comparison of surface and groundwater $\delta^{15}\text{N}$ values (shaded area) with potential nitrate sources in the Great Miami River Watershed. Each box encloses 50% of the data with the median value represented by a vertical line in the box. Each box is thus bounded by the first quartile (25%) and the third quartile (75%) of the variable distribution. The acceptable range of data is represented by the horizontal line extending from the sides of the box, while outliers are displayed as individual points.

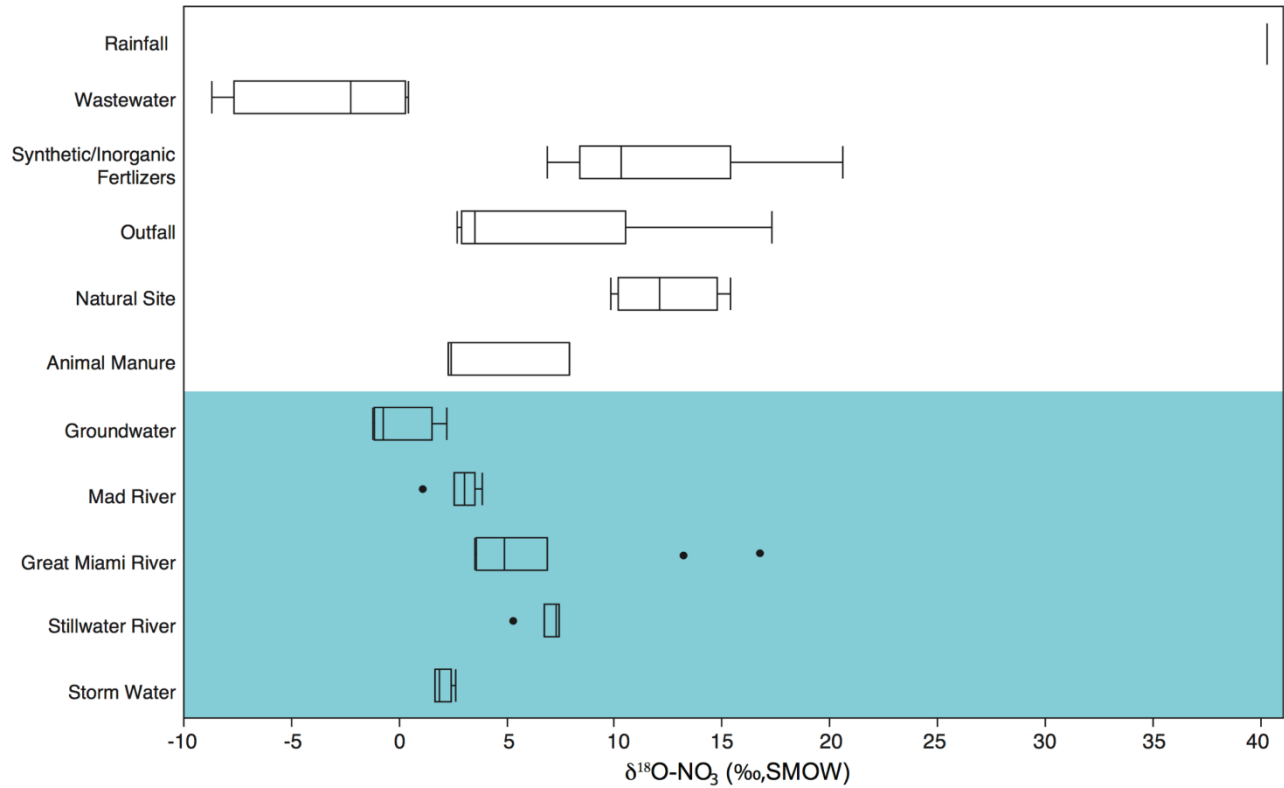


Figure 10. Comparison of surface and groundwater $\delta^{18}\text{O}$ values (shaded area) with potential nitrate sources in the Great Miami River Watershed. Each box encloses 50% of the data with the median value represented by a vertical line in the box. Each box is thus bounded by the first quartile (25%) and the third quartile (75%) of the variable distribution. The acceptable range of data is represented by the horizontal line extending from the sides of the box, while outliers are displayed as individual points.

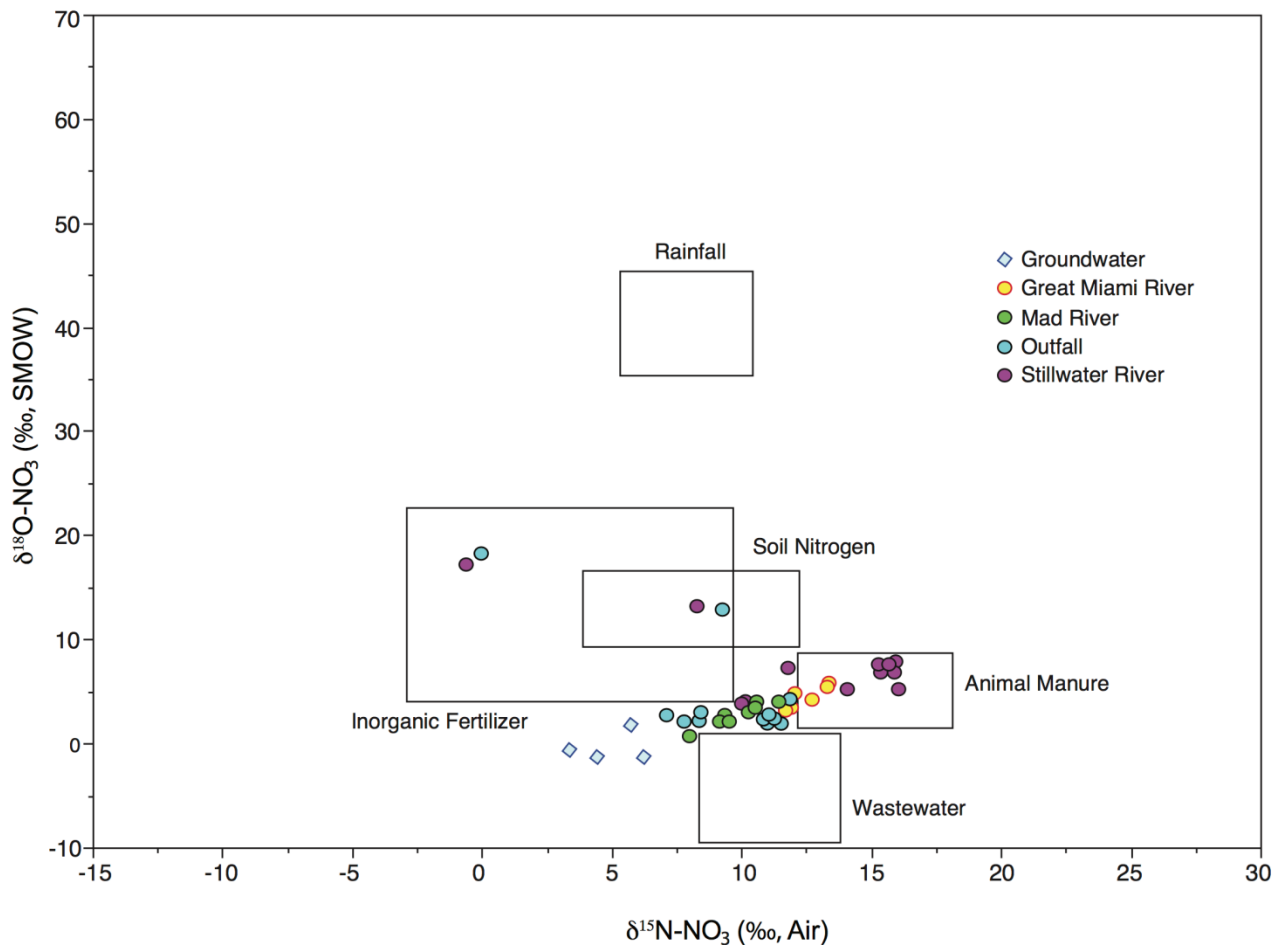


Figure 11. Scatterplot of $\delta^{15}\text{N}-\text{NO}_3$ (‰ Air) and $\delta^{18}\text{O}-\text{NO}_3$ (‰ VSMOW) for surface and groundwater samples collected in the Great Miami River Watershed. Each box represents the range of isotopic composition of potential nitrate sources.

Our interpretation of the sources of nitrate in surface and groundwater needs to consider denitrification processes that can shift both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ to higher values. In this study, excluding the outlier values, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in surface and groundwater show a trend with a slope ~ 0.67 , which might suggest some degree of denitrification (Figure 12). Measured dissolved oxygen values in the groundwater were relatively low (< 2 mg/l) and possibly favor denitrifying processes (Rivett et al., 2008). However, all three rivers have dissolved oxygen levels above 5 mg/l and are less likely to have anoxic conditions that promote denitrification processes. The other potential process that affects nitrate source identification is volatilization of NH_3 . NH_3 volatilization can occur in animal manure during storage (Lee et al., 2011) and also during storage and handling of liquid ammonia based fertilizers (http://www.fluidfertilizer.com/newsletters/fertilizer_newsletter2.html), and in soils after inorganic fertilizer application (Zhao et al., 2016). One of the fertilizer samples

(10-34-0 liquid ammonium phosphate) we analyzed in this study had a nitrate $\delta^{15}\text{N}$ value of 21.6‰ and a $\delta^{18}\text{O}$ value of -1.9‰. These values are outside of the range of expected isotopic values for synthetic/inorganic fertilizer and are attributed to volatilization of ammonia during storage. This fertilizer sample was excluded from the dataset and further statistical analysis in this study.

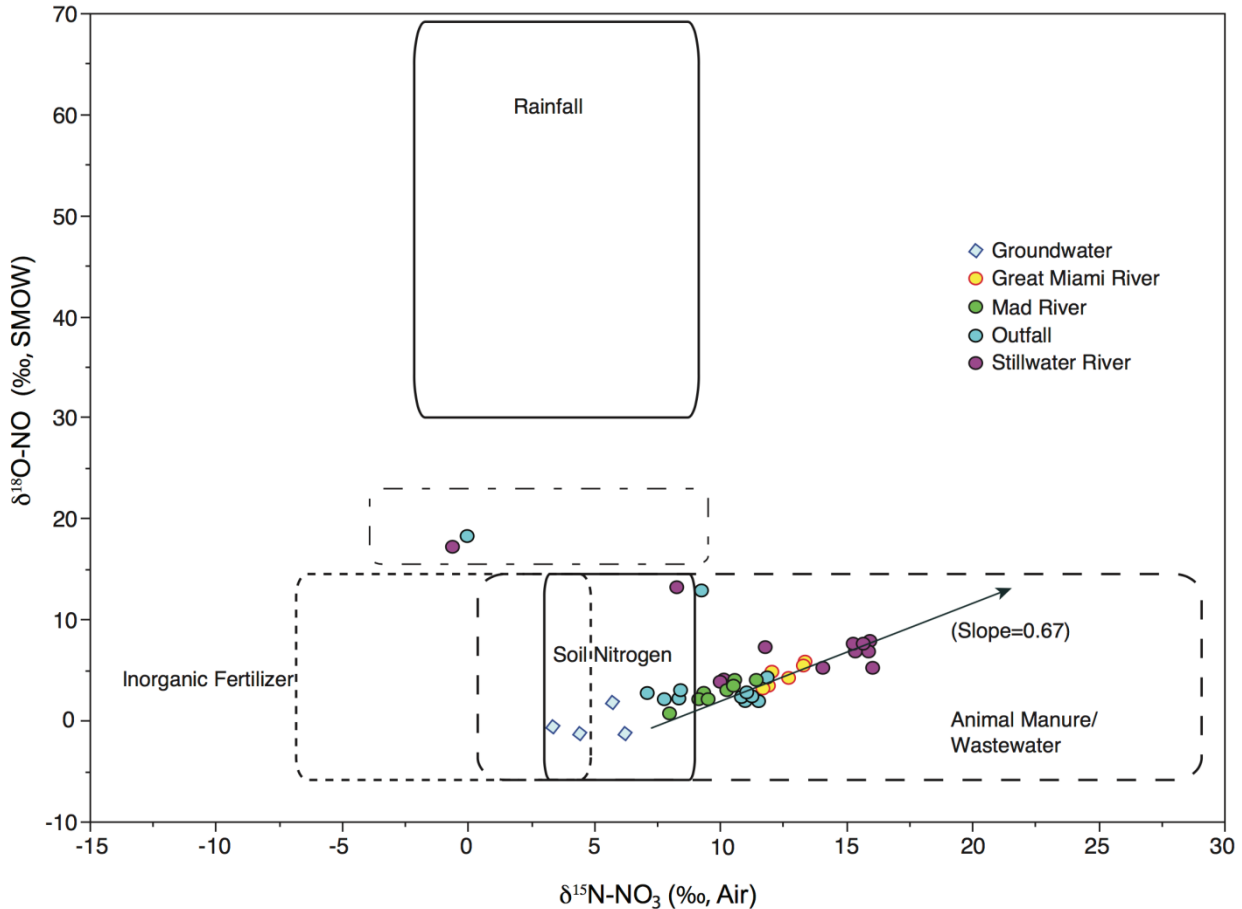


Figure 12. Comparison of $\delta^{15}\text{N-NO}_3$ (‰ Air) and $\delta^{18}\text{O-NO}_3$ (‰ VSMOW) for surface and ground water in this study with typical ranges of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate from various sources. Modified from Kendall (1998).

Conclusions

Water resources in the Great Miami River Watershed are impacted by enriched levels of nitrate originating from anthropogenic sources including use of fertilizers for agriculture and municipal wastewater. We conducted a study between May 2017 and October, 2018 to 1) determine the range of nitrate isotopic ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) values from potential nitrate contaminant sources in the Great Miami River Watershed and 2) identify sources of nitrate in surface and groundwater in

the watershed. A total of 101 samples (soil, synthetic/inorganic fertilizer, manure, wastewater and surface and groundwater samples) were collected and analyzed for nitrate $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\delta^{11}\text{B}$.

In general, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\delta^{11}\text{B}$ values of the nitrate contaminant sources sampled in this investigation are within the range of nitrate isotopic compositions reported in previous studies. The $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\delta^{11}\text{B}$ values in the Great Miami River watershed ranged from -1.4 to +17.6‰, -8.4 to +40.4‰ and +3.3 to +20.5‰ respectively.

The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of groundwater samples plot within the typical ranges of soil organic nitrogen and synthetic inorganic fertilizers. However, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of river samples show a wider range and plot within the ranges of animal waste/human waste. The wider isotopic range among the rivers might suggest contribution of nitrate contaminants from multiple sources. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ ratio (0.67) might also suggest the importance of denitrification processes and hint at the importance of ^{15}N depleted sources. Seasonal sampling of river and groundwater is required to fully identify nitrate sources. In addition to $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ analysis, boron isotope analysis of surface and groundwater is important to distinguish animal waste from human waste.

Recommendations for Further Study

This study made significant progress in establishing isotopic signatures of potential sources of nitrate to surface and ground waters in the Great Miami River Watershed. However, funding constraints limited the number of water samples analyzed for dual isotope tracing. Therefore, we offer the following recommendations for building upon the results of this study.

1. Conduct dual isotope tracing with Boron to differentiate wastewater from animal manure in surface water samples. Since the cost of Boron isotope analysis is high, conduct this analysis on samples collected during low flow conditions when wastewater is expected to be a major if not dominant source of nitrate.
2. Analyze surface water samples for dual isotopes of nitrate across all seasons and a broad spectrum of river flow conditions in order to establish seasonal mass isotope balance equations for differentiating between synthetic fertilizers, manure, and wastewater sources.
3. Conduct more targeted water sampling and analyses of nitrogen, oxygen and boron isotopes from areas with known high nitrate concentrations to identify nitrate sources and suggest remedial measures.

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