

REVIEW OF ISOTOPIC METHODS FOR NITRATE SOURCES IDENTIFICATION IN
SURFACE AND GROUNDWATER

A Thesis

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ABSTRACT

Nitrate pollution in surface and groundwater has become a worldwide environmental problem. For better water quality management, it's important to know total NO_3^- loads and identify the proportional contributions from multiple NO_3^- sources. Stable isotopes of NO_3^- , the $\delta^{15}\text{N}$ value and the $\delta^{18}\text{O}$ value, have been frequently used to identify NO_3^- sources in water. However, due to complexity in the N cycle, mixing multiple sources and fractionation during biological processes often caused biased and uncertain results. One possible solution is to use SIAR, a linear mixing model running in R, to estimate the proportional contribution from multiple NO_3^- sources. Unfortunately, SIAR cannot distinguish manure and septic sources, which requires the analysis of other chemical components, such as sodium, caffeine. A research approach is proposed to pilot a unified method to identify NO_3^- sources, determine their isotopic signature, and estimate each source's contribution by using the Fall Creek Watershed as a proof-of-concept study area.

BIOGRAPHICAL SKETCH

Yunxuan Pei was born in Urumqi, China on May 15th, 1998. This place used to be a part of the ancient Mediterranean and has undergone long geological changes, and now it has become a desert in the center of Eurasia. Yunxuan has been interested in natural science from the moment she discovered shellfish fossils in the desert. At China Agricultural University, she took Dr. John-James Wilson's introductory course in environmental science and developed a keen interest in soil science. Then she went to the University of Maryland via a 2+2 international exchange student program between CAU and UMD. In UMD, encouraged by Professor Ray R. Weil and mentor Chenglin Zhu, Yunxuan joined the UMD Soil Judging team and gained her most meaningful experience in university. UMD Soil Judging team participated in the northeast regional competition twice in Ohio and Maryland respectively and national competition once in California. They won the championship in these three competitions. In the regional competition held in Maryland in 2019 fall, Yunxuan got the third place in the individual competition. She not only won awards in the competition, but also gained practical knowledge in soil morphology learning. In 2020, Yunxuan enrolled in Cornell's MPS degree program in the field of Soil and Crop Sciences under the guidance of Dr. Jonathan Russell-Anelli. At Cornell, when took the Nutrient Cycling in Natural and Managed Ecosystems course, she developed a passion for nitrogen isotope, which is also the research direction of her graduation project. Yunxuan's experience at Cornell enabled her to gain expertise in the application of geospatial technology, and cultivate critical thinking and the ability to complete projects independently.

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I want to appreciate my advisor, Dr. Jonathan Russell-Anelli, for his patience and understanding in guiding me through my MPS degree in Cornell. For my graduation project, from topic selection to development of ideas to completion of the article, he gave me the greatest support and encouragement with enough patience. In every week communications, he can always put forward points that I have not considered or paid attention to, prompting me to continuously improve my project. He always reminds me “Remember to be awesome!”, which motivates me a lot.

I am also very grateful to my parents for supporting me even if they don't understand why I want to study this major. They hope I become a certified public accountant. They couldn't understand why I, a person who is afraid of insects and has allergic rhinitis to dust, would study soil science. Even so, they supported me in applying for the Soil and Crop Sciences program of Cornell and provided my tuition. Additionally, they are my psychological comfort. Since January 2020, after the COVID-19 outbreak, I have lived alone in the United States and never returned home. They often made video calls with me to relieve my occasional anxiety during my studies.

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LIST OF ABBREVIATIONS

SIAR: Stable Isotope Analysis in R

SWRP: Stickney Wastewater Reclamation Plant

USGS: the United States Geological Survey

VSMOW: Vienna Standard Mean Ocean Water

1. Introduction

Nitrogen is an extremely abundant element in the earth's atmosphere. As a foundation for metabolism and a major nutrient for plant growth, nitrogen is essential for all life on earth (UNEP, 2019). However, at the same time, most of the waste from human and animal metabolism as well as the fertilizers supplied to increase crop yields can also contaminate surface and groundwater, causing serious water pollution, reducing biodiversity, and destroying the ecosystem (UNEP, 2019). As a result, nitrogen contamination in water has become a worldwide environmental problem. In Frontiers 2018-2019 Emerging Issues of Environmental Concern, the United Nations Environment Programme advocated that global society needs a holistic approach for nitrogen management requiring multiple fields to work together, in an effort to precisely identify nitrate sources to formulate and improve relevant policies (UNEP, 2019).

The nitrate sources have great diversity, mainly from industrial fertilizer applied on agricultural land, animal waste, septic system, atmospheric precipitation, and biologically fixed nitrogen in the soil (Zhang et al., 2014). Quantifying the sources and loads has become increasingly important. Stable isotope data of NO_3^- is a great approach to estimate NO_3^- sources because the isotopic composition ratio of NO_3^- are generally distinctive among different sources. (Xue et al., 2009). Initially, N isotope data were used to determine the main NO_3^- sources, but when multiple sources' isotope values overlap each other, it is difficult to use a single isotope to distinguish (Kohl, 1971; Heaton, 1986; Wells & Krothe, 1989). O isotopes can distinguish some sources that are indistinguishable by N isotopes. Therefore, using both N and O isotopes have been widely used together to determine the main source of NO_3^- (Wassenaar, 1995). However, the isotopic analysis method cannot generate a quantitative result for two reasons. One is that NO_3^- in surface water or groundwater is a mixture coming from multiple sources. The isotopic

composition ratio of a mixture may be an intermediate value, which does not belong to any typical isotope value of NO_3^- sources, making it hard to identify and quantify the source. The other is that nitrogen cycling varies based on climatic and biological conditions. Various fractionation processes can change the original isotopic composition (Heaton, 1986; Xue et al., 2009; Xue et al., 2015).

One possible solution is Stable Isotope Analysis in R (SIAR), a stable isotope mixing model package running in R statistical computing program, which can estimate the probability distribution for the proportional contribution of each source to the mixture (Parnell & Jackson, 2008; Xue et al., 2009). Even if there are multiple sources of NO_3^- , SIAR can still identify their proportional contribution, as the fractionation parameter is considered in the calculation process. However, when inputting N and O isotope values into SIAR to calculate the proportional contribution of sources, most studies so far put manure and septic in one group (Yang et al., 2013; Ding et al., 2014; Matiatos, 2016; M. Zhang et al., 2018; Y. Zhang et al., 2018). The main reason is that the N and O isotope values of manure and septic basically coincide. To solve this problem, SIAR can be further assisted by the hydrochemistry approach that finds the unique characteristics for manure and septic respectively.

To discuss these issues and present some possible solutions this paper will address three critical components of using isotope tracking : (1) the fundamental principle of the isotope, (2) a literature review and evaluation about isotopic methods used for NO_3^- sources identification, and (3) a research proposal to illustrate a possible approach to overcome our present approaches. The main purpose of this article is to find gaps in existing methods on NO_3^- sources identification and provide effective information for future research.

2. Fundamental Principle of the Isotope

2.1 Definition

Isotopes are atoms of the same element that have the same numbers of protons and electrons but different numbers of neutrons. It means that various isotopes have similar charges but different masses. The stable isotopic compositions of elements are "delta" (δ) values, per thousand (denoted as ‰, pronounced as per mille) enrichments or depletions relative to a standard of known composition. δ values are calculated by:

$$\delta(\text{in } \text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) 1000$$

where "R" is the ratio of the heavy to light isotope. Comparing sample's ratio to a standard ratio would be more precise. A positive δ value indicates that there are more heavy isotopes in the sample than the standard, and vice versa. For example, if $\delta^{15}\text{N}$ of one sample equals to 10‰, this sample contains 10‰ more ^{15}N relative to the standard. (Kendall & Caldwell, 1998)

2.2 Fractionation

Although different isotopes of the same element have similar physical and chemical properties, because of slight mass differences, for some elements of light atomic number, these small mass differences will greatly impact their physical, chemical, and biological processes or their reactions to fractionate the relative proportions of isotopes. As a result of fractionation processes, waters and solutes often develop unique isotopic compositions (the δ value) that may be indicative of their source or the processes that formed them. There are two types of fractionations: equilibrium fractionation and kinetic fractionation. (Kendall & Caldwell, 1998)

For reversible equilibrium reactions, the heavier isotope generally prefers accumulating in the compound with a higher oxidation state. For example, during the reversible equilibrium

reaction between ammonia and ammonium, ammonium has a higher oxidation state so it tends to be ^{15}N -enriched. The difference between $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NH}_3$ can be as high as 25‰ to 35‰. For irreversible unidirectional kinetic reactions, the remaining substrate becomes gradually more enriched with the heavy isotope, and products are depleted. For example, after the denitrification process, the remaining NO_3^- will be $\delta^{15}\text{N}$ -enriched, and the difference between $\delta^{15}\text{N-N}_2$ and $\delta^{15}\text{N-NO}_3^-$ can be as low as -35‰. (Heaton, 1986, Xue et al., 2009, Kendall & Caldwell, 1998)

3. Different Methods

3.1 Single Isotope: $\delta^{15}\text{N}$ Value of NO_3^-

There are two stable nitrogen isotopes: ^{14}N and ^{15}N . Nitrogen in the atmosphere is mainly consists of ^{14}N (99.6337%) and ^{15}N (0.3663%), and the ratio of ^{15}N to ^{14}N is a constant number 0.366% (Junk & Svec, 1958). Therefore, air is set the standard to determine $\delta^{15}\text{N}$ value, which is distinct among different nitrogen sources.

Typical $\delta^{15}\text{N}$ value ranges of different sources are displayed in Figure 1. Artificial fertilizers, such as NH_4^+ , NO_3^- , Urea, are derived by industrial fixation of atmospheric nitrogen with little isotopic fractionation, so the $\delta^{15}\text{N}$ value of fertilizer is close to zero, ranging from -3‰ to 3‰ (Heaton, 1986). $\delta^{15}\text{N}$ value is light in precipitation, but has a wider range typically from -10‰ to 8‰, because it is determined by complicated atmospheric chemical reactions (Xue et al., 2009; Zhang et al., 2019). Soil N is mainly affected by mineralization and nitrification, and it also reflects the site's history, climate, and vegetation. The $\delta^{15}\text{N}$ value of soil N typically ranges from 0‰ to 8‰ (Xue et al., 2009). Compared with the sources mentioned above, the $\delta^{15}\text{N}$ value of manure and septic are relatively high. Animal feces will volatilize during storage and

transportation. NH_4^+ has a higher oxidation state so it tends to be ^{15}N -enriched and consequently results in ^{15}N -enriched NO_3^- . The $\delta^{15}\text{N}$ value of manure ranges from 5‰ to 25‰, and the value of sewage ranges from 4‰ to 19‰ (Xue et al., 2009).

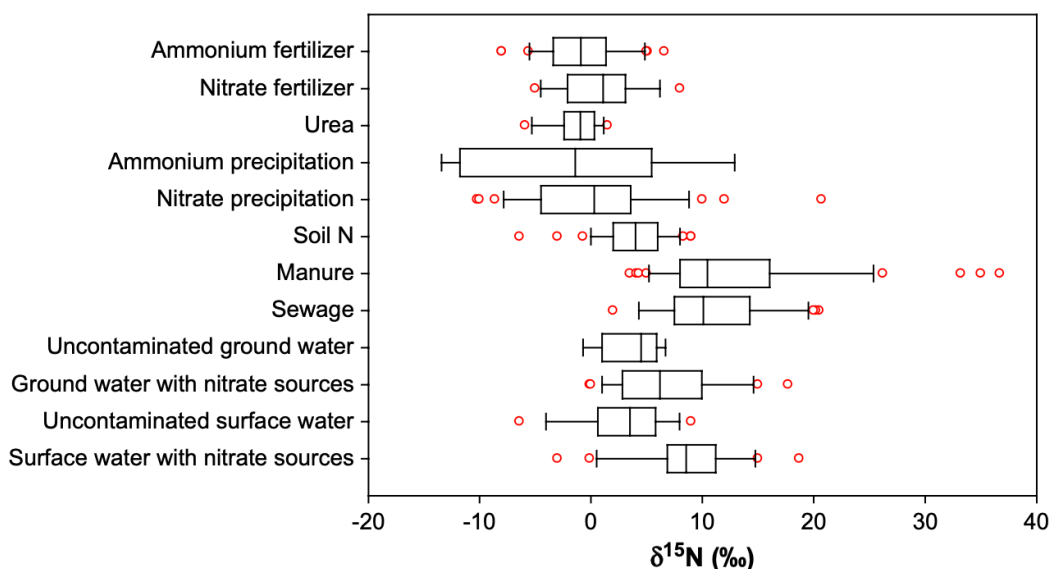


Figure 1 Box plots of $\delta^{15}\text{N}$ values of NO_3^- from various sources and sinks. Box plots illustrate the 25th, 50th, and 75th percentiles; the whiskers indicate the 10th and 90th percentiles; the circles represent outliers. (Xue et al. 2009)

In the 1970s, when the NO_3^- content of surface water in the United States continued to increase, a question came up: “What fractional responsibility must the rising rate of application of inorganic nitrogen fertilizers bear for the increase in the nitrate concentration” (Kohl et al., 1971). To answer this question, the first study of using $\delta^{15}\text{N}$ Value to identify dominant NO_3^- was done by Kohl et al., and it was plausible to give a quantitative estimation of the contribution of fertilizer to NO_3^- contamination in a large area in Illinois corn land. Kohl’s study only considered two possible sources of NO_3^- found in surface water. One is soil nitrogen with $\delta^{15}\text{N}$ value 13‰, and the other is the fertilizer with $\delta^{15}\text{N}$ value 3‰. The $\delta^{15}\text{N}$ value of surface water was 7.5‰. A linear interpolation of $\delta^{15}\text{N}$ value between soil nitrogen and fertilizer indicates

55% NO_3^- was provided by fertilizer. This was a good attempt, but the result was based on many assumptions and there were many uncertainties. First, it was mostly that there were more than two potential sources of NO_3^- . Second, the interpolation method used the estimated mean $\delta^{15}\text{N}$ value to calculate sources' contribution, but the deviation and distribution of $\delta^{15}\text{N}$ value were not considered in this study, which led to a biased result. Kohl's research results attracted concern and criticism, and consequently a large number of related experiments using N isotopes to determine the sources of NO_3^- were carried out. It turned out that the $\delta^{15}\text{N}$ value can indeed be used in determining the sources of NO_3^- pollution, but it requires more detailed work than what Kohl did, and it usually gets semi-quantitative results rather than quantitative results (Heaton, 1986).

Merely the $\delta^{15}\text{N}$ value signature alone cannot identify NO_3^- sources. Even if the possible ranges of different sources' $\delta^{15}\text{N}$ value are collected accurately and precisely, the ranges overlap with each other. With an increasing number of studies, possible ranges of $\delta^{15}\text{N}$ value become much broader. Sources from fertilizer, atmosphere, and soil have similar value; sources from manure and sewage have similar value. What's worse, the typical ranges of $\delta^{15}\text{N}$ value may not be a suitable reference when NO_3^- comes from mixing sources or kinetic isotope fractionation occurs. If NO_3^- comes from more than two sources, using $\delta^{15}\text{N}$ values alone cannot identify which source is the dominant one. Further with kinetic fractionation, some processes such as denitrification, will result with the remaining NO_3^- tending to have a much higher $\delta^{15}\text{N}$ value than what it should have. $\delta^{15}\text{N}$ value alone is not enough, and other methods are needed to assist the identification of NO_3^- sources.

3.2 Dual Isotopes: $\delta^{15}\text{N}$ Value and $\delta^{18}\text{O}$ Value of NO_3^-

There are three stable oxygen isotopes: ^{16}O (99.759%), ^{17}O (0.037%), and ^{18}O (0.204%). Vienna Standard Mean Ocean Water (VSMOW) is the standard for $\delta^{18}\text{O}$ evaluation (Xue et al., 2009). The combination uses of $\delta^{15}\text{N}$ value $\delta^{18}\text{O}$ value of NO_3^- can date back to the late 1980s and early 1990s. Böttcher et al. used ^{15}N and ^{18}O of NO_3^- for evaluation of microbial denitrification in 1990; Durka et al. used $\delta^{15}\text{N}$ value $\delta^{18}\text{O}$ value to determine the effects of forest decline on uptake and leaching of deposited NO_3^- in 1994 (Wassenaar, 1995). Their results indicated that oxygen might be a useful tracer of NO_3^- sources and then oxygen isotopes became widely used as an additional mean in NO_3^- sources identification.

The typical $\delta^{18}\text{O}$ value ranges of different sources are displayed in Figure 2. O is more useful than N to separate microbiologically fixed NO_3^- from atmospheric deposition and NO_3^- fertilizer. The $\delta^{18}\text{O}$ value of microbial-produced NO_3^- is determined by oxygen in both H_2O and O_2 . Microbial-produced NO_3^- is composed of two oxygen atoms from H_2O and one oxygen atom from O_2 (Xue et al., 2009). Theoretically by calculation, the $\delta^{18}\text{O}$ value of NO_3^- derived from nitrification should range from -10‰ to 10‰. The $\delta^{18}\text{O}$ value of atmospheric O_2 is around 23.5‰. The ^{18}O in atmospheric deposition has great spatial and temporal variability and is differentially enriched by fractionation reactions. The fractionation might be caused by reactive oxygen combining with NO_x to form NO_3^- , or some highly uncontrolled reactions happened during lighting. As a result, the $\delta^{18}\text{O}$ value of atmospheric deposition has a quite broad range from 25‰ to 75‰, higher than the $\delta^{18}\text{O}$ value of atmospheric O_2 (Xue et al., 2009). Industrial NO_3^- fertilizer is made of atmospheric O_2 so its $\delta^{18}\text{O}$ value is close to 23.5‰. It has a narrow range from 17‰ to 25‰ because fertilizer is made under a regulated process.

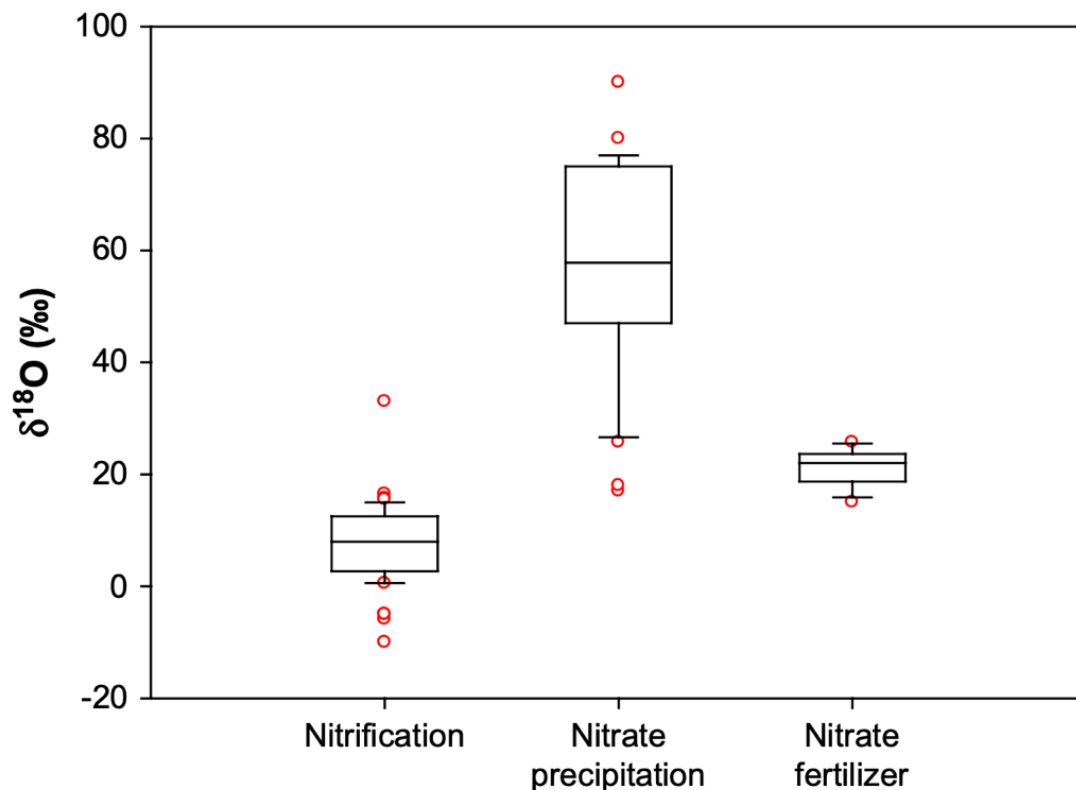


Figure 2 Box plots of $\delta^{18}\text{O}$ values of NO_3^- generated during nitrification, nitrate precipitation, and nitrate fertilizer. Box plots illustrate the 25th, 50th, and 75th percentile; the whiskers indicate the 10th and 90th percentiles; the circles represent data outliers. (Xue et al., 2009)

Figure 3 shows typical ranges of $\delta^{15}\text{N}$ value and $\delta^{18}\text{O}$ value of NO_3^- from air, fertilizer, soil, manure, and sewage. They are summarized from Xue et al. review paper and have been explained above. After using both nitrogen and oxygen isotope value, NO_3^- from air and industrial fertilizer are distinguishable, but manure and sewage sources are still indistinguishable. Additionally, the remained problem by single nitrogen method that NO_3^- comes from mixing sources or isotope fractionation occurs, still cannot be solved.

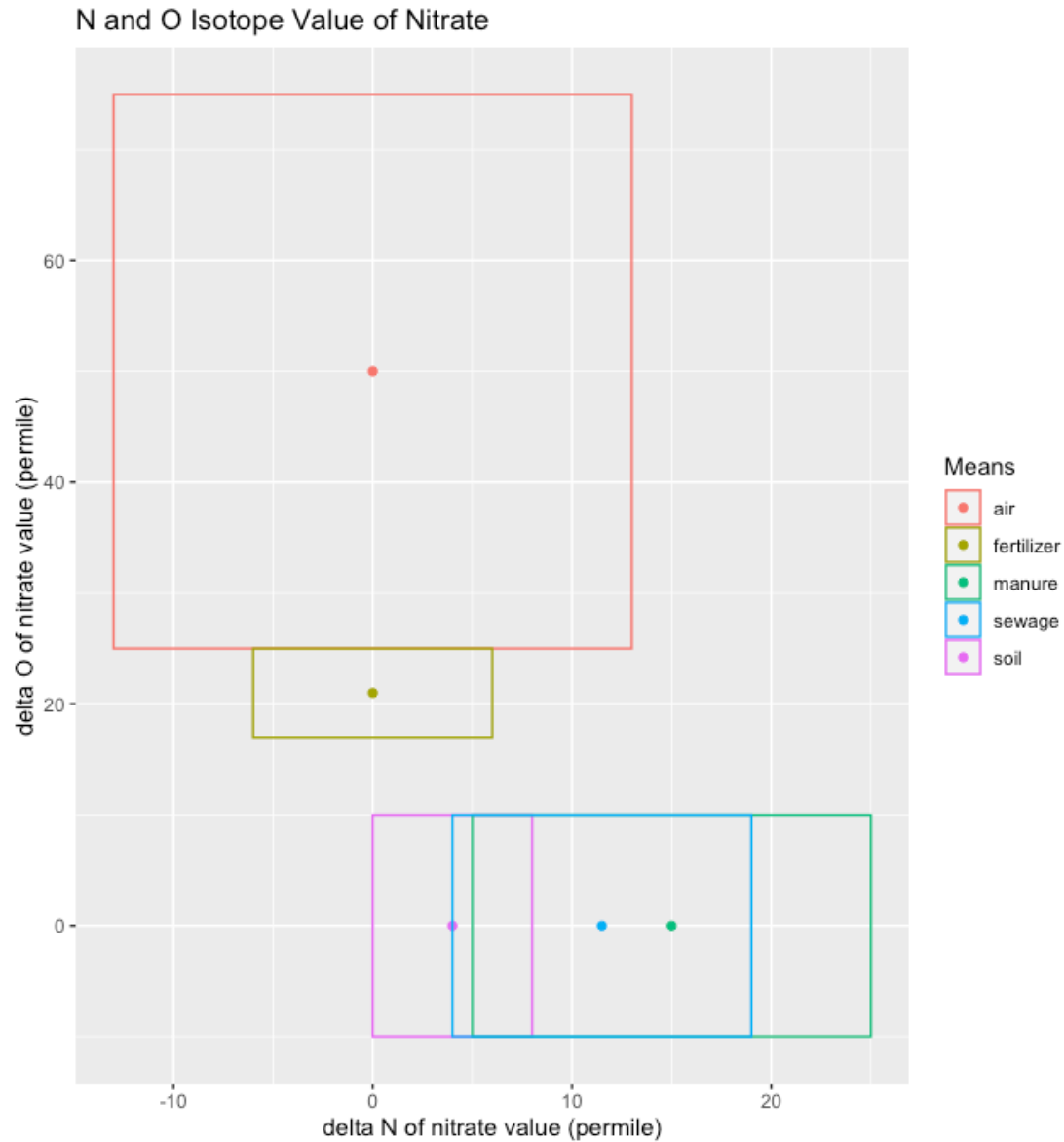


Figure 3 Typical ranges of $\delta^{15}\text{N}$ value and $\delta^{18}\text{O}$ value of NO_3^- from atmospheric deposition, industrial NO_3^- fertilizer, manure, sewage, and soil nitrogen.

3.3 Stable Isotope Analysis in R

In 2002, Phillis and Koch developed a concentration-weighted linear mixing model which assumes that for each element, a source's contribution is proportional to the contributed mass times the elemental concentration in that source. The model is outlined for two elements and

three sources and can be generalized to n element and n+1 source (Phillis & Koch, 2002). In 2006, Deutsch used this model on NO_3^- identification. $\delta^{15}\text{N}$ value and $\delta^{18}\text{O}$ value of NO_3^- were used to address three sources' contribution, drainage water from agricultural land, groundwater, and atmospheric deposition (Deutsch, 2006). Based on this model, in 2008, Parnell and Jackson developed the SIAR package (Stable Isotope Analysis in R). It's a Bayesian isotope mixing model running in R, which can estimate the possible proportional contribution of many sources to a mixture (Parnell et al., 2013). One improvement is that this model takes isotope fractionation into consideration. The theory behind this mixing model can be expressed as follows:

$$X_{ij} = \sum_{k=1}^K P_k (S_{jk} + C_{jk}) + \varepsilon_{ij}$$

$$S_{jk} \sim N(\mu_{jk}, \omega_{jk}^2)$$

$$C_{jk} \sim N(\lambda_{jk}, \tau_{jk}^2)$$

$$\varepsilon_{ij} \sim N(0, \sigma_j^2)$$

where X_{ij} is the observed isotope value j of mixture i, in which $i = 1, 2, 3, \dots, N$ and $j = 1, 2, 3, \dots, J$; S_{jk} is the source value k on isotope j, in which $k = 1, 2, 3, \dots, K$ and is normally distributed with mean μ_{jk} and standard deviation ω_{jk} ; C_{jk} is the fractionation factor for isotope j on source k, and is normally distributed with mean λ_{jk} and standard deviation τ_{jk} ; ε_{ij} is the residual error representing the additional unquantified variation between individuals, and is normally distributed with mean=0 and standard deviation σ_j ; P_k is the proportion of source k needs to be estimated by this model (Jackson et al., 2009).

So far, research about NO_3^- sources apportionment often uses hydrochemistry approach assisted with SIAR. Though in most cases the quantitative results generated by SIAR were reasonable and matched with previous analysis, there are still many uncertainties and room for

improvement. First, SIAR assumes that sources have a Gaussian distribution. Biased results will be generated if the real sources' isotope values are not normally distributed. Second, as the input value, a broad range of sources' isotope value will cause a broad possibility of sources' contribution (Yang et al., 2013). Consequently, there are great uncertainties in the distribution results. Third, as previously stated, most studies using SIAR grouped manure and sewage into one NO_3^- source. The main reason is that the $\delta^{15}\text{N}$ value and $\delta^{18}\text{O}$ value of NO_3^- don't allow manure and sewage distinguishment. To better manage nitrate pollution caused by manure and sewage, it's necessary to distinguish these two sources and identify their contributions respectively. Using unique characteristics of different sources might be an effective approach.

3.4 Combine Isotopic Method with Hydro-chemical Approach

Researchers often used spatial-temporal analysis combined with hydrochemistry to study NO_3^- sources and process in stream water. Lin et al. analyzed NO_3^- concentration data of the Upper Illinois River Basin of October in 2004. The Chicago Sanitary and Ship Canal River and the Des Plaines River are two major tributaries, above the Illinois River, flowing through Chicago and receive treated wastewater from the Stickney Wastewater Reclamation Plant. High NO_3^- concentration in the Chicago Sanitary and Ship Canal River and the Des Plaines River mainly came from the urban source. The Kankakee River joins the Illinois River 20 km downstream from Lockport where the NO_3^- concentration was diluted. The Kankakee River is an agriculture-urban mixed tributary, as a result, NO_3^- concentration decreased as agriculture source increased. In addition, this article also analyzes the seasonal variability of NO_3^- sources, using data collected in October of 2004, May, August, and October of 2005. The Upper Illinois River Basin in May has higher rainfall amount and more surface flow; in October and August, it has a

lower rainfall amount and less surface flow. Wastewater treatment plant discharge is constant all over the year, but the drainage water from agricultural areas is greatly affected by rainfall as precipitation brings more NO_3^- fertilizer into the river. During a large flow increase due to precipitation in May, the $\delta^{15}\text{N}$ value of the Illinois River decreased as the river moved downstream and at the same time, the $\delta^{18}\text{O}$ value increased. In contrast, during the small net flow in August and October, both the $\delta^{15}\text{N}$ value and the $\delta^{18}\text{O}$ value of the Illinois River increased. It shows that the NO_3^- concentration in the watershed is affected by the drainage of agricultural land when the precipitation is high. (Lin et al. 2019)

In addition, the concentration of anions and cations can also provide useful information for NO_3^- identification. Spruill in 2002 built a Classification and Regression Tree model to predict NO_3^- with a better than 80% overall success. In this study, part of the data was used to make model and the other part was used to test the model. The model eventually selected four predictors ($\delta^{15}\text{N}$ value, nitrate to ammonia ratio, sodium to potassium ratio, and zinc concentration) from 32 variables to distinguish five NO_3^- sources: fertilizer on crops, fertilizer on golf courses, spray from hog wastes, and leachate from poultry litter and septic systems. They found the $\delta^{15}\text{N}$ value can tell whether this source was related to animal or human waste because the $\delta^{15}\text{N}$ value of fertilizer is much lower than animal or human waste. The nitrate to ammonia ratio was used to distinguish fertilizer applied on crops or golf courses. The sodium to potassium ratio was used to distinguish human waste and animal waste because sodium is a special characteristic for human use, cooking salts and water softener. Zinc content is much higher in pig manure than in chicken manure, which is caused by pig feed additive content and longer growth cycles in pigs, so it can be used to distinguish between pig manure and chicken manure. (Spruill, 2002)

Some recent research also used pharmaceuticals in human and veterinary treatment as tracers to identify NO_3^- sources, especially for manure and sewage distinction. Drugs that satisfy the following requirements might be good tracers for NO_3^- identification. First, most have unique characteristics that can be detected after passing through the digestive system. Second, it can persist after going through the wastewater treatment plants and remain relatively stable in the natural environment. Third, the drug must be used only by humans or by animals so that it can be used to distinguish between them. Finally, the drug should present in different regions. According to many studies, Caffeine is currently studied in-depth and might be a good tracer. Though there are some uncertainties with these methods, they show promise to distinguish between these sources and can be used as supplementary methods. (Fenech et al., 2012)

After using SIAR to estimate the proportion of different sources, the proportion distribution and the classification of sources can be further refined with other chemical components in the water. For example, sodium ion concentration or caffeine concentration can be used to distinguish between human and animal feces. Additionally, it is worth noting that when using the concentration of chemical components, the total amount should also be considered, especially in areas with large seasonal differences. In the rainy season, the concentration of NO_3^- may be diluted by larger runoff, but this does not mean that the total NO_3^- content is reduced. For more effective water quality management, the total loads and proportional contribution of each source of NO_3^- , as well as seasonal differences, need to be considered. This is also the research direction of this experimental proposal.

4. Research Proposal

4.1 Objective

The goal of this research is to pilot a unified approach to identify NO_3^- sources, determine their isotopic signature, and estimate each source's contribution by using the Fall Creek Watershed as the proof-of-concept study area. Objective 1: Determine the main source of NO_3^- based on the concentration and isotopic composition of NO_3^- in Fall Creek. Objective 2: Estimate total loads and the main source of NO_3^- regarding seasonal variability of isotopic composition. Objective 3: Estimate the possible numerical distribution of local isotopic composition of NO_3^- based on the sample data of each source, and substitute this into the SIAR model to estimate the proportion of each source. Objective 4: If agriculture source and urban source have quite close isotopic composition value, use chemical tracers to further distinguish manure and septic sources.

4.2 Study Area

Fall Creek is a major tributary of the Cayuga Lake located in Ithaca, New York. It begins near Lake Como and then meanders for approximately 33 miles to Ithaca city, where it enters into Cayuga Lake. The Beaver Creek, the Mud Creek, the Virgil Creek, the Lake Como Outlet, the Webster Brook, and the Mill Creek are the six main tributaries of the Fall Creek. Fall Creek provides drinking water for more than 30 000 people (Carpenter et al., 2018). There is a U.S. Geological Survey (USGS) stream gage (Lat 42°27'12", long 76°28'22" referenced to North American Datum of 1983) about 0.2 mi upstream of Beebe Lake dam. According to its 95-year (1925-2020) historical monthly average water flow record, March has the highest average water flow 403 ft³/s, and August has the lowest 57 ft³/s (USGS). Based on Cornell University Geospatial Information Repository's data, Figure 4 was made to show the land cover of the Fall Creek Watershed in 2004 and the proportion of each type within the watershed. The three main

types of land use in this area are agricultural land (46.2%), forest land (41.4), and urban land (9.08%). If further study is needed, agricultural land can be divided into alfalfa, corn, soybean, hay, orchard, and dairy.

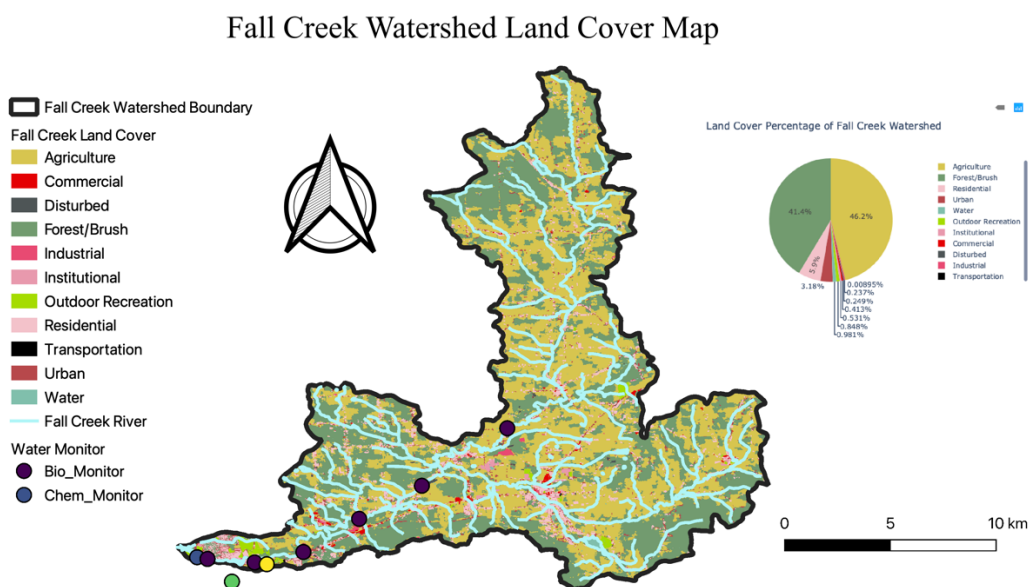


Figure 4 Land cover map of Fall Creek watershed, upstream of the Cayuga Lake. Dots represent biomonitoring sites and monitoring stations.

The Beaver Creek, the Mud Creek, the Virgil Creek, the Lake Como Outlet, the Webster Brook, and the Mill Creek are six main tributaries in the watershed that join the Fall Creek. The main one is the Virgil Creek in the east part of the watershed, which passes through the Dryden urban area and merges into Fall Creek. From the north of the confluence point to the upstream source area, agricultural and forestry land is the main area with minor spots of urban land. South of this confluence point, the Fall Creek flows through the Beebe Lake in Cornell University, passing the urban land of Ithaca, and finally into the Cayuga Lake.

4.3 Sample Collection

For this study, two different types of samples will be collected. One is from the sources area as the reference sample, and the other is from the Fall Creek mainstream as the target sample. The watershed has three major land use with agriculture (fertilizer and manure) and forests dominant at 46.2% and 41.4% respectively, as well as urban (septic system). Reference samples for agriculture and forest will be collected from 9 locations in three tributaries that originated from these two land types in the watershed, and 5 samples are from each location. Reference samples for the urban areas will be directly collected from local wastewater treatment plants. The target sample will be divided into two groups. The first group at the intersection of the tributaries into the Fall Creek and the second group at the main flow of the Fall Creek are displayed in Figure 5. Taking into account the seasonal differences, all sampling will be repeated in April-May when the runoff is the largest and in August when the runoff is the smallest. April-May is also the beginning of the growing season when fertilizer and manure have already been applied. Sampling will be carried out after rainy days to avoid the influence of precipitation. When it is raining, the detection values are not accurate. The total amount and intensity of precipitation will affect the peak runoff and the concentration of nitrate brought into the runoff. All of the stream water samples will be collected at the surface of the river by using a submersible pump (Lin et al., 2019). The water samples will be filtered on-site through a 0.45 μ m filter, sealed in an HDPE bottle, and then cooled during transport to the laboratory, and stored frozen until thawed to analyze the nitrate concentration, isotopic composition, and other needed hydro-chemical properties (Lin et al. 2019).

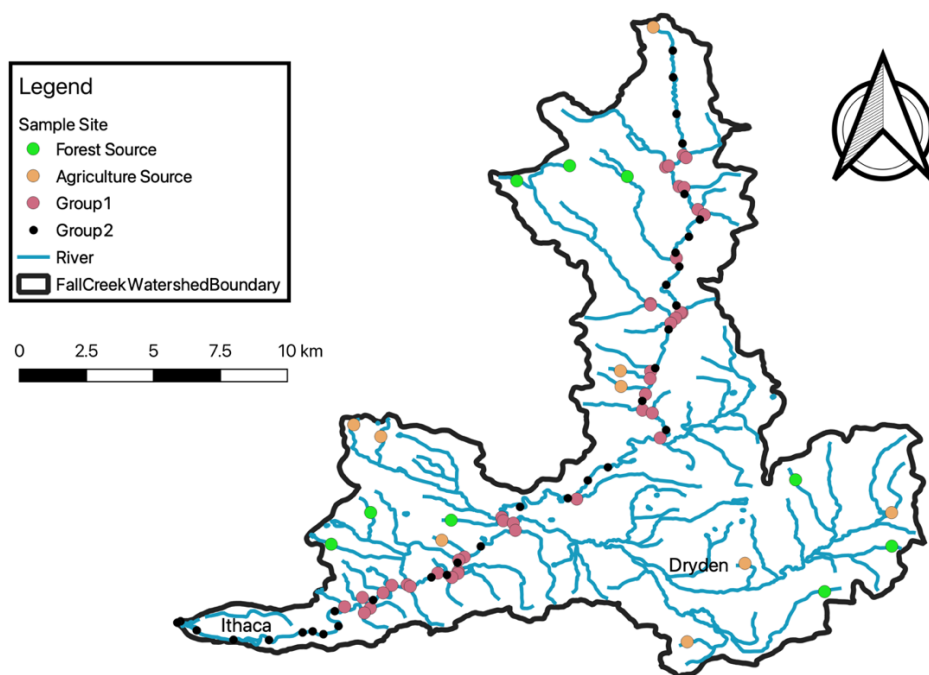


Figure 5 Locations of sample sites. Green circles are sample sites for forest sources; yellow circles are sample sites for agriculture sources; red circles are target samples collected near where tributaries enter Fall Creek; black dots are target sample sites in Fall Creek.

4.4 Discussion

Objective 1 is to determine the main source of NO_3^- based on the concentration and isotopic composition of NO_3^- in Fall Creek. The first objective does not require a quantitative conclusion but serves to predict which source has a greater impact on this watershed. Based on land use distribution, the expected result is that the upstream of the Fall Creek is dominantly contributed by agricultural fertilizers with lower $\delta^{15}\text{N}$ value, and the downstream is dominantly contributed by urban sewage with a higher $\delta^{15}\text{N}$ value. Although the forest area is widely distributed, the proportion of NO_3^- pollution caused by the forest should be the smallest.

Compared with fertilizer and manure applied on agricultural land and septic system in the urban regions, the amount of NO_3^- in forests derived from microbiological fixation is very small.

To verify the expected conclusion of Objective 1, Objective 2 will estimate total loads and the main source of NO_3^- regarding seasonal variability of isotopic composition. In this area, April-May is the beginning of the crop growing season and the month with heavy precipitation. Agricultural land is mainly distributed in the upper and middle stream, but as streamflow moves downstream it will have an impact on the entire watershed. To determine the impact, the total amount of NO_3^- will be calculated using the concentration of NO_3^- and the runoff of the river. The difference between the total amount in April-May and August can be used to predict the amounts of agricultural NO_3^- fertilizers brought into the watershed.

Objective 3 and Objective 4 will advance to predict the specific proportion of each source by using SIAR. As related experiments have not been conducted in this area, it is difficult to predict the specific contribution percentage of each source. If the source's $\delta^{15}\text{N}$ value and $\delta^{18}\text{O}$ value overlap, it would be impossible to distinguish between manure and septic source, so using the caffeine concentration we hope to calculate the relevant value of urban septic.

4.5 Expected Outcomes

This project is important for nitrate pollution control and water quality management because it focuses on nitrate sources identification and apportionment. Firstly, it can determine the predominant nitrate source by using a single isotope. Secondly, it can estimate total nitrate loads and identify sources at high and low surface flow. Last, the proportional contribution from multiple sources will be estimated by the SIAR model. However, the unavoidable question is how reliable this method is. When errors occur in this method, can other methods make up for it?

In the case of the Fall Creek Watershed, according to historical recording, caffeine was detected by the local monitoring station, so this should be a feature that can be used as a local septic source.

5. Conclusion

Half a century ago, the problems of water pollution, reduction of biodiversity, and ecological destruction caused by excessive nitrate in surface and groundwater, gradually attracted attention and became an important issue all over the world. It's important to know where the nitrate comes from and each source's contribution. The stable isotope value of nitrate has been frequently used to trace nitrate sources. This paper summarized several methods using the isotopic approach to nitrate sources identification and apportionment. This paper attempts to make progress, propose an improvement on the existing methods, and explore future research directions, that is the use of the hydrochemistry approach to make up for the shortcomings of SIAR, and the use of caffeine to distinguish between manure and septic sources.

Reference

- Bailes, I. R., & Gröcke, D. R. (2020). Isotopically labelled macroalgae: A new method for determining sources of excess nitrogen pollution. *Rapid Communications in Mass Spectrometry*, 34(24), e8951.
- Carpenter, C. M., Wong, L. Y. J., Johnson, C. A., & Helbling, D. E. (2018). Fall Creek Monitoring Station: Highly resolved temporal sampling to prioritize the identification of nontarget micropollutants in a small stream. *Environmental science & technology*, 53(1), 77-87.
- Deutsch, B., Mewes, M., Liskow, I., & Voss, M. (2006). Quantification of diffuse nitrate inputs into a small river system using stable isotopes of oxygen and nitrogen in nitrate. *Organic geochemistry*, 37(10), 1333-1342.
- Ding, J., Xi, B., Gao, R., He, L., Liu, H., Dai, X., & Yu, Y. (2014). Identifying diffused nitrate sources in a stream in an agricultural field using a dual isotopic approach. *Science of the total environment*, 484, 10-18.
- Fenech, C., Rock, L., Nolan, K., Tobin, J., & Morrissey, A. (2012). The potential for a suite of isotope and chemical markers to differentiate sources of nitrate contamination: a review. *Water Research*, 46(7), 2023-2041.
- Heaton, T. H. (1986). Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology: Isotope Geoscience Section*, 59, 87-102.
- Jackson, A. L., Inger, R., Bearhop, S., & Parnell, A. (2009). Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008). *Ecology letters*, 12(3), E1-E5.

- Junk, G., & Svec, H. J. (1958). The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. *Geochimica et Cosmochimica Acta*, 14(3), 234-243.
- Kendall, C., & Caldwell, E. A. (1998). Fundamentals of isotope geochemistry. In *Isotope tracers in catchment hydrology* (pp. 51-86). Elsevier.
- Kohl, D. H., Shearer, G. B., & Commoner, B. (1971). Fertilizer nitrogen: contribution to nitrate in surface water in a corn belt watershed. *Science*, 174(4016), 1331-1334.
- Lin, J., Böhlke, J. K., Huang, S., Gonzalez-Meler, M., & Sturchio, N. C. (2019). Seasonality of nitrate sources and isotopic composition in the Upper Illinois River. *Journal of Hydrology*, 568, 849-861.
- Matiatos, I. (2016). Nitrate source identification in groundwater of multiple land-use areas by combining isotopes and multivariate statistical analysis: A case study of Asopos basin (Central Greece). *Science of the Total Environment*, 541, 802-814.
- Parnell, A., Jackson, A., & Parnell, M. A. (2013). Package ‘siar’. *Stable Isotope Analysis in R*, 4.
- Phillips, D. L., & Koch, P. L. (2002). Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, 130(1), 114-125.
- Spruill, T.B., Showers, W. J., & Howe, S. S. (2002). Application of classification-tree methods to identify nitrate sources in ground water. *Journal of Environmental Quality*, 31(5), 1538-1549.
- UNEP (2019). *Frontiers 2018/2019 Emerging Issues of Environmental Concern*. United Nations Environment Programme. Nairobi.
- U.S. Geological Survey. National Water Information System data.
https://waterdata.usgs.gov/nwis/monthly/?referred_module=sw&site_no=04234000&

[amp;por_04234000_105681=1050457,00060,105681,1925-02,2020-11&format=html_table&date_format=YYYY-MM-DD&rdb_compression=file&submitted_form=parameter_selection_list](#)

- Wassenaar, L. I. (1995). Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^- . *Applied geochemistry*, 10(4), 391-405.
- Wells, E. R., & Krothe, N. C. (1989). Seasonal fluctuation in $\delta^{15}\text{N}$ of groundwater nitrate in a mantled karst aquifer due to macropore transport of fertilizer-derived nitrate. *Journal of Hydrology*, 112(1-2), 191-201.
- Xue, D., Botte, J., De Baets, B., Accoe, F., Nestler, A., Taylor, P., ... & Boeckx, P. (2009). Present limitations and future prospects of stable isotope methods for nitrate source identification in surface-and groundwater. *Water research*, 43(5), 1159-1170.
- Xue, D., Pang, F., Meng, F., Wang, Z., & Wu, W. (2015). Decision-tree-model identification of nitrate pollution activities in groundwater: A combination of a dual isotope approach and chemical ions. *Journal of contaminant hydrology*, 180, 25-33.
- Yang, L., Han, J., Xue, J., Zeng, L., Shi, J., Wu, L., & Jiang, Y. (2013). Nitrate source apportionment in a subtropical watershed using Bayesian model. *Science of the Total Environment*, 463, 340-347.
- Zhang, M., Zhi, Y., Shi, J., & Wu, L. (2018). Apportionment and uncertainty analysis of nitrate sources based on the dual isotope approach and a Bayesian isotope mixing model at the watershed scale. *Science of the Total Environment*, 639, 1175-1187.
- Zhang, Y., Li, F., Zhang, Q., Li, J., & Liu, Q. (2014). Tracing nitrate pollution sources and transformation in surface-and ground-waters using environmental isotopes. *Science of the Total Environment*, 490, 213-222.

- Zhang, Y., Shi, P., Li, F., Wei, A., Song, J., & Ma, J. (2018). Quantification of nitrate sources and fates in rivers in an irrigated agricultural area using environmental isotopes and a Bayesian isotope mixing model. *Chemosphere*, 208, 493-501.
- Zhang, Y., Shi, P., Song, J., & Li, Q. (2019). Application of nitrogen and oxygen isotopes for source and fate identification of nitrate pollution in surface water: a review. *Applied Sciences*, 9(1), 18.