

# A stable isotope approach and its application for identifying nitrate source and transformation process in water

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**Abstract** Nitrate contamination of water is a worldwide environmental problem. Recent studies have demonstrated that the nitrogen (N) and oxygen (O) isotopes of nitrate ( $\text{NO}_3^-$ ) can be used to trace nitrogen dynamics including identifying nitrate sources and nitrogen transformation processes. This paper analyzes the current state of identifying nitrate sources and nitrogen transformation processes using N and O isotopes of nitrate. With regard to nitrate sources,  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values typically vary between sources, allowing the sources to be isotopically fingerprinted.  $\delta^{15}\text{N}-\text{NO}_3^-$  is often effective at tracing  $\text{NO}_3^-$  sources from areas with different land use.  $\delta^{18}\text{O}-\text{NO}_3^-$  is more useful to identify  $\text{NO}_3^-$  from atmospheric sources. Isotopic data can be combined with statistical mixing models to quantify the relative contributions of  $\text{NO}_3^-$  from multiple delineated sources. With regard to N transformation processes, N and O isotopes of nitrate can be used to decipher the degree of nitrogen transformation by such processes as nitrification, assimilation, and denitrification. In some cases, however, isotopic fractionation may alter the isotopic fingerprint associated with the delineated  $\text{NO}_3^-$  source(s). This problem may be addressed by combining the N and O isotopic data with other types of, including the concentration of selected conservative elements, e.g., chloride

( $\text{Cl}^-$ ), boron isotope ( $\delta^{11}\text{B}$ ), and sulfur isotope ( $\delta^{35}\text{S}$ ) data. Future studies should focus on improving stable isotope mixing models and furthering our understanding of isotopic fractionation by conducting laboratory and field experiments in different environments.

**Keywords** Stable isotopes · Nitrate sources · Isotope mixing models · N transformation processes

## Introduction

Nitrate contamination of water is a worldwide environment problem that, in most cases, can be attributed to human activities. Ecologically, excess nitrate ( $\text{NO}_3^-$ ) in surface waters can cause eutrophication, a condition that leads to excessive algal and/or plant growth and the severe degradation of the aquatic ecosystem. The consumption of  $\text{NO}_3^-$  polluted water is also of concern as it can (1) induce methemoglobinemia in humans, particularly in infants, and (2) increase the risk of cancer and infectious disease (Galloway et al. 2008). As a result, the World Health Organization has set a maximum limit of 10 mg/L of  $\text{NO}_3^-$ -N for drinking water, whereas the EU (EC 1998) established a threshold of 50 mg/L of  $\text{NO}_3^-$ -N for human consumption. An EU Council Directive (EEC 1991) has also been formulated to protect freshwater against  $\text{NO}_3^-$  contamination. Nitrate contamination of water is difficult to control. Data from an ETC Water Technical Report (EEA 2010) indicated that from 1992 to 2008,  $\text{NO}_3^-$  concentrations in European rivers declined slightly, although some rivers and aquifers showed significantly increasing  $\text{NO}_3^-$  concentrations. In the USA,  $\text{NO}_3^-$  is also perceived as a problem. For example,  $\text{NO}_3^-$  concentrations have increased since 2000 within waters of the upper Mississippi and Missouri Rivers (Murphy et al. 2013), and a study released by UC Davis

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(Thomas and Lund 2012) warned that the  $\text{NO}_3^-$  contamination of groundwater would likely worsen in the coming years, potentially resulting in an 80 % increase in human health risks by 2050.

In light of the above, the protection of surface and drinking water supplies from  $\text{NO}_3^-$  contamination is a vital component of any water resource management plan. The protection of surface and groundwater from  $\text{NO}_3^-$  pollution requires the identification of the primary  $\text{NO}_3^-$  sources within the catchment and the relative contributions of  $\text{NO}_3^-$  to the water body. Simply put, it is impossible to control the influx of  $\text{NO}_3^-$  to the water if the source(s) is unknown. However, given the wide variety of potential  $\text{NO}_3^-$  sources that may exist within a catchment, and the non-conservative behavior of nitrogen (N) as it is dispersed from a source, it is often difficult to determine the predominant  $\text{NO}_3^-$  sources using conventional water quality monitoring techniques.

In order to overcome these shortcomings of source identification using monitoring methods, investigators have commonly used three alternative approaches to identifying N sources: (1) export coefficient modeling (Johnes 1996), (2) the analysis of an agricultural pollution potential index (APPI) (Petersen 1991), and (3) non-point source pollution models. Export coefficient modeling is used to identify N sources by calculating N loads from all potential sources. The key step is to determine export coefficients for each source, a process that requires significant observational data. The APPI is a compressive function based on natural and anthropogenic factors, including a runoff index (RI), sediment production index (SPI), chemical use index (CUI), and a people and animal load index (PALI). A shortcoming of this method is that weight determinations of the four indices are subjective and there is no standard approach to assigning the subjective values. Both the export coefficient modeling and APPI methods calculate total N loads rather than the inorganic form of N and neither consider N transformation processes. Non-point source pollution models require a large amount of observational data although they involve N transformation processes. Thus, they are difficult to apply to the areas with limited data.

An approach that is growing in popularity is the use of the N and oxygen (O) isotope systems ( $^{15}\text{N}\text{-NO}_3^-$  and  $^{18}\text{O}\text{-NO}_3^-$ ) to determine both the predominant sources and their relative contributions to a water body. The approach may also serve as a valuable tool to understand N transformation processes (Sun et al. 2011). This approach has been successfully applied during the past several decades to streams and rivers (Mayer et al. 2002; Kaushal et al. 2011; Ma et al. 2015), groundwater (Li et al. 2007; Barnes and Raymond 2010; Jin et al. 2015), and coastal and estuarine systems (Wankel et al. 2009; Korth et al. 2013, 2014; Wong et al. 2015). The approach is based on the realization that  $\text{NO}_3^-$  originating from different sources will exhibit differing isotopic compositions. These distinct isotopic

“fingerprints” can then be used to quantitatively determine the amount of  $\text{NO}_3^-$  that is coming from each of the potential N sources. For example, fertilizers and sewages have been shown to possess a unique range of  $^{15}\text{N}\text{-NO}_3^-$  values (Kendall et al. 2007; Xue et al. 2009), while soil microbial N and atmospherically deposited N often exhibit a unique range of  $^{18}\text{O}\text{-NO}_3^-$  values (Kendall 1998). N transformation processes such as nitrification, assimilation, and denitrification may also lead to distinct isotopic compositions.

This paper summarizes the current state of the N and O isotopes of nitrate as applied to  $\text{NO}_3^-$ -contaminated water, including (1) identification of  $\text{NO}_3^-$  sources in water; (2) assessment of N transformation processes; and (3) the combined use of chloride ( $\text{Cl}^-$ ), boron ( $^{11}\text{B}$ ), and sulfur ( $^{35}\text{S}$ ) isotopes with the isotopes of N and O.

## Utilization of stable isotope approach to determine nitrate sources

### Isotope values of nitrate sources

Natural N has two stable isotopes,  $^{14}\text{N}$  and  $^{15}\text{N}$ . Natural O has three stable isotopes,  $^{16}\text{O}$ ,  $^{17}\text{O}$ , and  $^{18}\text{O}$ . The abundances of these isotopes in the atmosphere are as follows:  $^{14}\text{N}$  (99.6337 ‰),  $^{15}\text{N}$  (0.3663 ‰) (Junk and Svec 1958),  $^{16}\text{O}$  (99.759 ‰),  $^{17}\text{O}$  (0.037 ‰), and  $^{18}\text{O}$  (0.204 ‰) (Cook and Lauer 1968). The isotopic ratios of both N and O are expressed using the per mille (‰) notation which presents the isotopic ratio in a sample relative to the ratio in a standard, such that

$$\delta(\text{‰}) = \frac{(R)_{\text{sample}} - (R)_{\text{standard}}}{(R)_{\text{standard}}} \times 1000 \quad (1)$$

where  $R$  is the  $^{15}\text{N}/^{14}\text{N}$  or  $^{18}\text{O}/^{16}\text{O}$  ratio of the sample and standard and  $\delta^{15}\text{N}$  values are reported relative to atmospheric air (AIR) and  $\delta^{18}\text{O}$  values are reported relative to the Vienna Standard Mean Ocean Water (VSMOW) (Gonfiantini 1978).

Nitrate in water comes from a variety of sources, including atmospheric deposition, fertilizers and soil N, industrial wastewater, sewage, and manures. All these sources tend to have unique  $\delta^{15}\text{N}\text{-NO}_3^-$  values or  $\delta^{18}\text{O}\text{-NO}_3^-$  values (Table 1).

Atmospheric N is transferred to the Earth's surface in aqueous solution through rain, snow, fog, and gaseous and particulate species.  $\delta^{15}\text{N}$  values measured within atmospheric  $\text{NO}_3^-$  are usually in the range of  $-15$  to  $15\text{‰}$  (Kendall et al. 2007). The  $\delta^{18}\text{O}$  values of atmospheric  $\text{NO}_3^-$  are higher than the  $\delta^{15}\text{N}$  values ranging from  $+14$  to  $+75\text{‰}$  (Kendall 1998).

Fertilizers applied to agriculture and urban green spaces are important  $\text{NO}_3^-$  sources in water.  $\delta^{15}\text{N}$  values of inorganic fertilizers have been found to be lower than those of organic fertilizers (Kendall et al. 2007), the former generally ranging from  $-5.9$  to  $+6.6\text{‰}$  (Bateman and Kelly 2007), whereas the

**Table 1** The reported  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  values

Nitrate sources	$\delta^{15}\text{N-NO}_3^-$ (‰)	Reference	$\delta^{18}\text{O-NO}_3^-$ (‰)	Reference
Atmospheric nitrate	-15~+15	Kendall (1998); Kendall et al. (2007); Elliott et al. (2007);	+14~+75	Kendall (1998); Kendall et al. (2007);
Ammonium fertilizer	-5.9~+6.6	Bateman and Kelly (2007) Kendall (2007); Li et al. (2007); Choi et al. (2007);	-5~+15	Amberger and Schmidt (1987); Kendall et al. (2007);
Nitrate fertilizer	-4~+5	Bateman and Kelly (2007); Kendall et al. (2007); Heaton et al. (2012);	+17~+25	Amberger and Schmidt (1987); Kendall et al. (2007);
Soil nitrate	0~+8	Aravena et al. (1993); Heaton (1986); Kendall (1998); Kendall et al. (2007);	-10~+15	Kendall et al (2007); Wassenaar (1995); Mayer et al. (2001); Umezawa et al (2008);
Animal waste	+10~+20	Aravena et al. (1993); Heaton (1986); Kendall et al. (2007); Wassenaar (1995);	-10~+15	Kendall et al. (2007); Umezawa et al. (2008); Wassenaar (1995); Widory et al. (2005)
Sewage	+4~+19	Kendall (1998); Widory et al. (2005); Li et al. (2007)	-10~+15	Kendall et al. (2007); Umezawa et al. (2008);
Industrial wastewater	<10	Li et al. (2005); Wang et al. (2013); Ma et al. (2015)	-	-

latter from +2 to +30‰ (Kendall et al. 2007), respectively.  $\delta^{15}\text{N-NO}_3^-$  values of most soil are low ranging from 0 to +8‰, while soils polluted by fertilizer and animal waste exhibited average  $\delta^{15}\text{N-NO}_3^-$  values of  $+4.7\pm 5.4$  and  $+14.0\pm 8.8$ ‰ (Kendall 1998).  $\delta^{15}\text{N-NO}_3^-$  values of industrial wastewater are reported less than 10‰ (Li et al. 2005). Compared to fertilizers and soils, sewage and animal waste have higher  $\delta^{15}\text{N-NO}_3^-$  values as shown in Table 1.

The  $\delta^{18}\text{O-NO}_3^-$  values of soil and animal waste are low. The  $\delta^{18}\text{O-NO}_3^-$  values of nitrate fertilizers are distinct. Amberger and Schmidt (1987) reported that  $\delta^{18}\text{O-NO}_3^-$  values of  $\text{NO}_3^-$  generated from nitrification of ammonium fertilizers are in the range of -5 to +15‰, while  $\delta^{18}\text{O-NO}_3^-$  values of synthetic fertilizers are in the range of +17 to +25‰.

**Stable isotope approach for identifying nitrate sources**

As discussed above, nitrate in water comes from a variety of sources. During runoff and infiltration events,  $\text{NO}_3^-$  from these sources is transported into surface water and groundwater. Once in the water, the  $\text{NO}_3^-$  represents a mixture from all of the differing sources, and determining the amount from each source has proven to be a difficult problem.

The stable isotope approach has been applied to solve this problem. Due to different ways in which N is formed within these potential sources, they are likely to have distinct  $\delta^{15}\text{N-NO}_3^-$  values. For example, the  $\delta^{15}\text{N-NO}_3^-$  values in fertilizers are low because they are produced by the fixation of atmospheric  $\text{N}_2$  (Kendall et al. 2007); manure and sewage have higher  $\delta^{15}\text{N-NO}_3^-$  values attributed to ammonia ( $\text{NH}_4^+$ ) volatilization (Kendall et al. 2007), a process that results in a large enrichment of  $^{15}\text{N}$  in the residual  $\text{NH}_4^+$  (which is subsequently converted into  $^{15}\text{N}$ -enriched  $\text{NO}_3^-$ ). Thus, natural waters with limited N pollution from manure and sewage will exhibit lower  $^{15}\text{N}$  values (Mayer et al. 2002; Deutsch et al. 2006; Hales et al. 2007). In contrast, if  $\text{NO}_3^-$  from manure and sewage enters into the water, the  $^{15}\text{N}$  values will increase, as the isotopic composition of water will move toward that of the  $\text{NO}_3^-$  source if no N transformation occurs. As for  $\delta^{18}\text{O-NO}_3^-$ , atmospheric  $\text{NO}_3^-$  has higher  $\delta^{18}\text{O-NO}_3^-$  values compared with other nitrate sources (e.g., fertilizers, soil nitrogen). Thus, if unprocessed atmospheric  $\text{NO}_3^-$  enters into water, the  $^{18}\text{O-NO}_3^-$  values will increase. As a result, the source of the  $\text{NO}_3^-$  can be identified by analyzing the isotopic composition of nitrate in the water. Similarly, if  $\text{NO}_3^-$  from multiple sources enters into water, the isotopic composition of water is a product of the physical mixing of  $\text{NO}_3^-$  from these

sources, and all these sources can, at least theoretically, be identified and quantified using an isotope mixing model.

The use of N isotope data to determine  $\text{NO}_3^-$  sources in water can be traced back to the 1970s. Kohl et al. (1971) estimated the source of  $\text{NO}_3^-$  in waters of the Sangamon River in the USA on the basis of natural  $\delta^{15}\text{N}$  values. The results showed that half of the  $\text{NO}_3^-$  was derived from soil and half from unfractionated  $\text{NO}_3^-$  found in fertilizer. However, the study by Kohl et al. (1971) was questioned because they discarded potential effects of isotope fractionation on the identified fingerprint as well as the variations in isotopic values within the soil (Hauck et al. 1972). Shearer and Kohl (1988) later suggested that  $^{15}\text{N}$  was better suited to study  $\text{NO}_3^-$  transformation processes than to trace  $\text{NO}_3^-$  sources. Consequently, the use of  $^{15}\text{N}$  to identify  $\text{NO}_3^-$  sources in river waters draining agriculture areas in the USA was limited until the late 1990s. Similarly,  $\delta^{18}\text{O}$  was rarely used to identify  $\text{NO}_3^-$  sources, primarily because of analytical limitations in the technique at the time. With the advancement of isotope technology, of particular importance was the development of the microbial denitrified method (Sigman et al. 2001; Casciotti et al. 2002) which appeared in the early 2000s, a method which can simultaneously analyze for both N and O isotopes, and N and O isotopes were frequently used in identifying  $\text{NO}_3^-$  source and have been shown to serve as a powerful tool to identify  $\text{NO}_3^-$  sources in water.

During the past decade, many studies have used N and O isotopes to investigate the contributions of  $\text{NO}_3^-$  to streams, rivers, and groundwater from terrain characterized by differing land use (Mayer et al. 2002; Burns et al. 2009; Barnes and Raymond 2010; Ohte et al. 2010; Kaushal et al. 2011; Liu et al. 2014). These investigations found that rivers draining areas of agricultural and urban land had higher  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}\text{-NO}_3^-$  values (>5%), indicating that sewage and manure were the main  $\text{NO}_3^-$  sources. As expected, both  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}\text{-NO}_3^-$  values are typically lower (<5%) in forested watersheds (Barnes et al. 2008; Ohte et al. 2010; Schwarz et al. 2011). It has been suggested that these lower values for  $\text{NO}_3^-$  in stream waters in forested watersheds result from the atmospheric deposition of  $\text{NO}_3^-$ , a process that also leads to stream water acidification (Durka et al. 1994), which refers to  $\text{NH}_4^+$  assimilated and nitrified by microorganisms and contributes to  $\text{NO}_3^-$  in runoff and soil nitrification (Burns et al. 2009). Recent studies (Wang et al. 2013; Ma et al. 2015) found that groundwater and rivers receiving industrial wastewater also have lower  $\delta^{15}\text{N}\text{-NO}_3^-$  (<5%). High  $\delta^{18}\text{O}\text{-NO}_3^-$  values derived from atmospheric nitrate are always reduced through nitrogen biological processes and are similar to those values derived from fertilizers, sewage, and soils. Thus, oxygen isotope may be not effectively used to identify nitrate sources derived from human activities but may be used to distinguish nitrate derived from unprocessed atmospheric nitrate from nitrogen biochemical processes such as

nitrification. Koszelnik and Gruca-Rokosz (2013), for example, argued that  $\text{NO}_3^-$  from wells and ditches located in hardwood forests was derived from the soil where nitrification generated  $\delta^{18}\text{O}\text{-NO}_3^-$  values in the range of those typically associated with nitrification. Similarly, Tobari et al. (2010) estimated the contribution of atmospheric  $\text{NO}_3^-$  in streams draining forest watersheds in Japan by using O isotopes. The results indicated that the contribution of atmospheric  $\text{NO}_3^-$  in stream water was higher in young forests than in old forests. The higher  $\delta^{18}\text{O}\text{-NO}_3^-$  values discharged from young forests were attributed to atmospheric  $\text{NO}_3^-$  that was not altered because of the lower level of plant  $\text{NO}_3^-$  uptake by the younger trees. In shallow groundwater,  $\text{NO}_3^-$  distributions are also influenced by land use. In the West Lake watershed, eastern China (Jin et al. 2015), nitrogen and oxygen isotopic data indicated that nitrate sources in groundwater were soil N from forest, chemical fertilizers and manure in the tea garden, and domestic sewage from the old residential area in forest and urban area. In deep groundwater, Murgulet and Tick (2013) found that  $\delta^{18}\text{O}\text{-NO}_3^-$  and  $\delta^{15}\text{N}\text{-NO}_3^-$  values in the coastal aquifer of the Alabama, USA, indicated that the mixing ratios of sewage and fertilizer were consistent with the study area's land use. Singleton et al. (2005) found that industrial sources contributed to the major nitrate plumes in groundwater below Hanford chemical processing facilities in south-central Washington, USA. Human activities have a direct effect on  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values in water. Mayer et al. (2002) found that mean  $^{15}\text{N}\text{-NO}_3^-$  values in water increased directly with increases in the area of agricultural and urban lands in the watershed. Burns et al. (2009) also found that in the urban watershed of Lisha Kill in the USA,  $\delta^{18}\text{O}\text{-NO}_3^-$  values were significantly related to the amount of runoff from impervious areas.

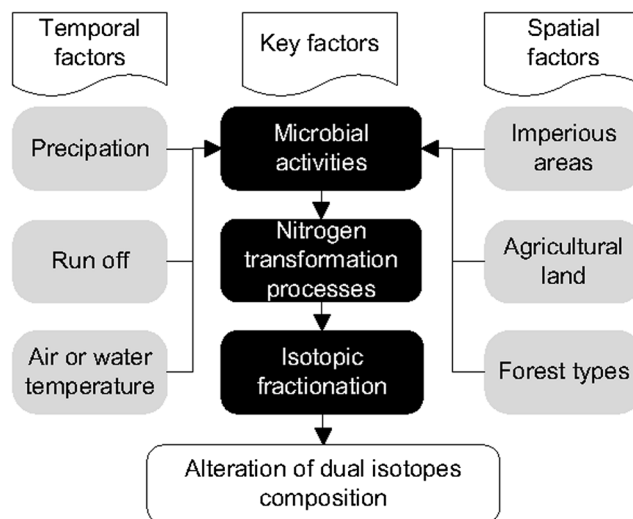
Temporal changes in system hydrology have been shown to significantly influence  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values, potentially complicating the use of N and O isotopes as tracers of  $\text{NO}_3^-$  provenance. Kaushal et al. (2011), for example, found that  $\delta^{15}\text{N}\text{-NO}_3^-$  values were higher during low-flow conditions than during high flow in urban watersheds, suggesting that  $\delta^{15}\text{N}\text{-NO}_3^-$  values decreased with increasing runoff from agricultural watersheds. These results were consistent with those of Silva et al. (2002). In contrast to  $\delta^{15}\text{N}\text{-NO}_3^-$ ,  $\delta^{18}\text{O}\text{-NO}_3^-$  values were higher during high-flow conditions than during low-flow conditions (Barnes and Raymond 2010; Kaushal et al. 2011; Ding et al. 2014). These differences can largely be attributed to the greater proportion of unprocessed atmospheric  $\text{NO}_3^-$  entering into streams or rivers directly because high recharge rates shortened residence times. In other words,  $\text{NO}_3^-$  enters the runoff without being dissolved in the soil solution and, in turn, without being denitrified as there is a decrease of soil  $\text{NO}_3^-$  dissolution. In groundwater, however, temporal changes seem to have few influence on  $\delta^{15}\text{N}\text{-NO}_3^-$

and  $\delta^{18}\text{O}-\text{NO}_3^-$  values (Baily et al. 2011; Wexler et al. 2012; Jin et al. 2015), which may be due to surface runoff entering shallow aquifers and mixing with groundwater there (Wexler et al. 2012). But, in the groundwater body of the Brussels sands (Belgium),  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values displayed a clear temporal pattern due to groundwater recharge dynamics and cycling process of nitrogen in the soil nitrogen pool (Mattern S et al. 2011). Temperature also appears to effect  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values. Burns et al. (2009) found that  $\delta^{15}\text{N}-\text{NO}_3^-$  values are positively related to air temperature in two agricultural watersheds with the correlation coefficient of 0.87 and 0.84, respectively. Barnes and Raymond (2010) found that  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values were positively related to water temperature. These values are explained by higher rates of denitrification at warm temperatures resulting in increases in  $\delta^{15}\text{N}-\text{NO}_3^-$  values and  $\delta^{18}\text{O}-\text{NO}_3^-$  values by isotope fractionation (discussed in the “Denitrification” section). Catchment size influences  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  heterogeneities. For example, Danielescu and MacQuarrie (2013) found that  $\delta^{15}\text{N}-\text{NO}_3^-$  values in the smaller watershed of McIntyre Creek were less variable than those in the larger Trout River, whereas  $\delta^{15}\text{N}-\text{NO}_3^-$  values exhibited a logarithmic relationship with the catchment size in Canada. Ohte (2013) also found that  $\delta^{18}\text{O}-\text{NO}_3^-$  values in a river in central Japan were 10‰ lower than that those observed in the northeastern USA, where the former’s area is 100 times smaller than the latter’s.

The data highlighted above show that seasonal and spatial changes are the primary factors influencing  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values. Seasonal factors include precipitation, runoff, and air or water temperature, and spatial factors include land use type such as impervious urban areas, agricultural farmland, and forests of varying age. These factors may not cause the alteration of isotopic compositions directly; rather, they may be the result of changes associated with microbial activities (Fig. 1) that control N transformation processes. In other words, the signatures are produced by isotopic fractionation (discussed in “Utilization of stable isotope approach to determine nitrogen transformation processes” section) that alters  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values. Consequently, the isotopic composition (or fingerprint) of the  $\text{NO}_3^-$  within a particular source does not remain constant but may be altered when being transported into water.

**Quantification of contributions of nitrate sources**

The traditional method for quantifying the relative contributions of  $\text{NO}_3^-$  from individual sources requires monitoring  $\text{NO}_3^-$  concentration and stream flow, which are used to estimate  $\text{NO}_3^-$  load in a particular period (e.g., day, month, season, or year).  $\text{NO}_3^-$  concentration is usually monitored less frequently than stream flow because of the higher costs of sampling and laboratory analyses (Quilbé et al. 2006).



**Fig. 1** The main factors influencing isotopic fractionation of nitrogen and oxygen isotopes of nitrate

Moreover, monitor stations are usually sited at the outlet of the basin or watershed where waters contain  $\text{NO}_3^-$  from multiple sources. Thus, it may not be possible to use the traditional method to quantitatively estimate the contribution of each  $\text{NO}_3^-$  source. In these instances, contributions may be estimated using statistical mixing models.

Since the isotopic signature of the  $\text{NO}_3^-$  sources is distinct, it is possible to determine the source of the  $\text{NO}_3^-$  as the isotopic values in the water reflect the physical mixing of isotopes from two or more sources.

*Basic mass balance isotope mixing models*

N and O isotopes can be used to quantify the contributions of  $\text{NO}_3^-$  sources to water based on the assumption that any change in isotopic composition is the direct result of mixing of two or more sources of known composition.

A basic mass balance mixing model (Moore and Semmens 2008) is defined as follows:

$$\delta_M = f_1(\delta_1 + \gamma_1) + f_2(\delta_2 + \gamma_2) + f_n(\delta_n + \gamma_n) \tag{2}$$

where  $f_n$  is the proportional contribution of the source to the mixture,  $\delta_n$  is the isotopic signature of the source, and  $\gamma_n$  is the isotope-specific fraction of the source. According to the model, these equations can be solved exactly for the contributions of the different sources when the number of sources is less than or equal to the number of isotopes+1. When applied to the combined use of N and O isotopes to determine the relative contributions of  $\text{NO}_3^-$  sources in waters, the model takes the following form as described by Xue et al. (2009):

$$\begin{aligned} \delta^{15}N_M &= f_1\delta^{15}N_1 + f_2\delta^{15}N_2 + f_3\delta^{15}N_3 \\ \delta^{18}O_M &= f_1\delta^{18}O_1 + f_2\delta^{18}O_2 + f_3\delta^{18}O_3 \\ 1 &= f_1 + f_2 + f_3 \end{aligned} \tag{3}$$

Deutsch et al. (2006) used the model successfully to estimate relative source contributions of  $\text{NO}_3^-$  to tile drainage water. Voss et al. (2006) also demonstrated the usefulness of the mixing model for identifying  $\text{NO}_3^-$  sources by estimating the contributions of  $\text{NO}_3^-$  from various sources (including agriculture, pristine soil, and atmospheric deposition) in catchments of the Baltic Sea. Kaushal et al. (2011) used the model to estimate the contributions of  $\text{NO}_3^-$  from sewage and atmospheric deposition in an urban watershed in Baltimore, Maryland (USA).

### Bayesian mixing models

The three-equation model (Eq. 3) discussed above can only estimate the contributions of two or three  $\text{NO}_3^-$  sources. In addition, the particular mass balance mixing model does not include an analysis of the potentially substantial and multiple sources of uncertainty (Moore and Semmens 2008) (e.g., those associated with the temporal and spatial variability in  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values described earlier). To overcome these shortcomings, Moore and Semmens (2008) developed a statistical stable isotope mixing model based on a Bayesian framework referred to as MixSIR. MixSIR considers the temporal and spatial variability of isotopes in the source materials (including those associated with fractionation), and the number of sources solved by several statistical treatments was unlimited.

Nevertheless, Jackson et al. (2009) argued that the MixSIR model could not accurately quantify contributions of  $\text{NO}_3^-$  from the sources, since the model could not provide a reliable estimate for additional unquantified error and beta distributions misled the prior proportions. So, he developed another statistical model also based on Bayesian framework referred to as SIAR, which used the Dirichlet distribution instead of beta distribution as the prior distribution. The model by Jackson et al. (2009) takes the form of the following:

$$\begin{aligned}
 X_{ij} &= \sum_{k=1}^K p_k (S_{jk} + c_{jk}) + \varepsilon_{ij} \\
 S_{jk} &\sim N(\mu_{jk}, \omega_{jk}^2) \\
 c_{jk} &\sim N(\lambda_{jk}, \tau_{jk}^2) \\
 \varepsilon_{jk} &\sim N(0, \sigma_j^2)
 \end{aligned} \quad (4)$$

where  $X_{ij}$  is the observed isotope value  $j$  of the mixture  $i$ , in which  $i=1, \dots, N$ ;  $j=1, \dots, J$ ;  $S_{jk}$  is the source value  $k$  on isotope  $j$  ( $k=1, \dots, K$ ) that is normally distributed with a mean  $\mu$  and standard deviation  $\omega$ ;  $p_k$  is the proportion of source  $k$  which is to be estimated by the model;  $c_{jk}$  is a fractionation factor for isotope  $j$  on source  $k$  that is normally distributed with mean  $\lambda$  and standard deviation  $\tau$ ; and  $\varepsilon_{jk}$  is the residual error representing additional unquantified variations between individual normally distributed with mean=0 and standard deviation  $\sigma$ .

The SIAR model was used by Xue et al. (2012) to estimate the contributions of  $\text{NO}_3^-$  from several sources in the Flanders watershed, Belgium. The results showed that the contribution of manure and sewage was highest from 32 to 49 % to the water, whereas the contribution of ammonium fertilizer and  $\text{NO}_3^-$  precipitation was minimal, ranging between 2 and 8 %.

Compared with the basic mixing model, the MixSIR and SIAR models have advantage of considering isotope variations in the  $\text{NO}_3^-$  sources and fractionation processes. However, some factors may influence the accuracy of the models. First, the basic assumption that the isotopic values of the sources are unique and can be used to fingerprint a source may be violated since the isotope values of different sources may overlap (e.g., fertilizer and soil N, manure, and sewage). Second, the accuracy of the two models decreases with the increasing number of sources. Third, the larger range of isotope values within the sources, as a result of the discussed temporal and spatial factors, will reduce the accuracy of the models. In contrast, a smaller range of isotope values will increase the accuracy of the results.

In order to reduce and quantify the uncertainty of the models, the study area should be divided into multiple, smaller sections to reduce  $\text{NO}_3^-$  sources and the variability of isotopic values resulting from spatial factors such as land use. In addition, the isotopic composition of each potential source within the study area should be analyzed instead of using reported isotopic  $\text{NO}_3^-$  data provided by other studies.

### Utilization of stable isotope approach to determine nitrogen transformation processes

Nitrogen exists in various chemical forms. Transformations between these chemical forms are often induced by microbes, during which N and O isotopic fractionation may occur. In most cases, isotopic fractionation results in an increase in the heavy isotopes within the residue N pool. As discussed above, isotopic fractionation can complicate their use for purposes of  $\text{NO}_3^-$  source identification because the original isotopic fingerprint of the source materials is altered. However, on the other hand, the nature and degree of isotope fractionation can also provide a powerful tool (Wexler et al. 2011) for tracing N transformation processes.

### Stable isotope fractionation

Stable isotopes are subjected to kinetic isotope fractionation, which is the variations of isotope composition in different phase and compounds due to geochemical processes. Kinetic fractionations often result in the light isotope accumulating in

the product and heavy isotope accumulating in the substrate (Sulzman 2007). The fractionation factor is defined as follows:

$$a = R_p/R_s \tag{5}$$

where  $R$  is the ratio of heavy to light isotopes in the instantaneous product ( $R_p$ ) and substrate ( $R_s$ ). The enrichment in the heavy isotope in the remaining substrate is characterized by an enrichment factor ( $\epsilon$ ) in ‰ which is defined as (Xue et al. 2009) follows:

$$\epsilon = 10^3(a-1) \tag{6}$$

In a closed system, isotopic enrichment can be expressed by the Rayleigh equation (Xue et al. 2009) as follows:

$$\delta_{S(t)} = \delta_{S_0} + \epsilon \ln(S_t/S_0) \tag{7}$$

$\delta_{S_0}$  and  $\delta_{S(t)}$  are the isotopic compositions of the substrate at time 0 and  $t$ , and  $S_0$  and  $S_t$  are the concentrations of the substrate at time 0 and  $t$ , respectively.

Nitrogen does not always behave in a conservative manner (Li et al. 2013; Badruzzaman et al. 2012) when transporting through aquatic systems, during which N transformation processes may occur. Both nitrogen and oxygen isotopic fractionations exist during N transformation processes, and the fractionation factors in various N transformation processes are different. For example, the fractionation factor during nitrogen fixation is small about 1.004 (Delwiche and Steyn 1970), whereas the factors during nitrification and denitrification are high about 1.035 (Mariotti et al. 1981) and from 1.01 to 1.03 (Mariotti et al. 1982), respectively. In addition, the changes of relationship between nitrogen and oxygen isotopes originated from isotope fractionation are distinct during various N transformation processes. For example,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of residual  $\text{NO}_3^-$  increase in both processes (denitrification: 2:1, Burns et al. 2009; assimilation: 1:1, Granger et al. 2004). Nitrogen and oxygen isotopes of nitrate can be used to assess N transformation processes since different N transformation processes lead to a distinct isotopic fractionation signature. Lehman et al. (2004) pointed out that the N and O isotopes of nitrate allow the effects of simultaneous nitrification and denitrification to be separated and used isotope analysis of nitrate (N and O) in benthic chambers deployed in Santa Monica Bay to show that benthic nitrification co-occurred with denitrification. A recent study (Dale et al. 2014a, b) used an isotope box model to calculate nitrate fractionation in bottom water of offshore NW Africa and found low benthic isotope fractionation during nitrification and high isotope fractionation during denitrification.

## Stable isotope approach for identifying nitrogen transformation processes

### Denitrification

Denitrification refers to microbial reduction process that converts  $\text{NO}_3^-$  to gaseous products. It occurs in soils, aquifers, riparian zones, and benthic and river sediment where  $\text{O}_2$  concentrations are less than 20  $\mu\text{M}$  (Kendall et al. 2007). Denitrification reduces  $\text{NO}_3^-$  in water from terrestrial ecosystems, thereby reducing the degree of  $\text{NO}_3^-$  pollution.

During denitrification, the fractionation of both N and O isotopes occurs. Laboratory experiments (Vidal-Gavilan et al. 2013; Vavilin and Rytov 2015) showed that the N and O isotope fractionations were  $-13.0$  and  $-17.1$ ‰ for  $\epsilon_N$  and  $-8.9$  and  $-15.1$ ‰ for  $\epsilon_O$ . In riparian zones, nitrogen isotopic fractionation caused by denitrification is more apparent being on the order of about  $-18$ ‰ (Sebilo et al. 2003). In groundwater, N isotope enrichment factors are reported in the range of  $-13.9$  to  $-30$ ‰ (Li et al. 2014 and references therein). Nitrogen enrichment factors associated with benthic denitrification appears to be smaller, ranging from  $-1.5$  to  $-6$ ‰ (Alkhatib et al. 2012), which is influenced by  $\text{NO}_3^-$  diffusion across the water-sediment interface, a process that is the rate-determining step (Sebilo et al. 2003; Lehman et al. 2004). As denitrification is a redox reaction, during which process, two types of electron donor including organic carbon and sulfur compounds participate in the reaction, so the denitrification fate and isotopic fractionation factors depend on the enrichment of electron donors besides  $\text{NO}_3^-$  concentration and anoxic conditions. For example, in the bottom of Pétrola lake with organic matter-rich sediment, isotopic enrichment factors are high ( $\epsilon_N=-14.7$ ‰;  $\epsilon_O=-14.5$ ‰) (Carrey et al. 2014), while in shallow saturated zone, Clague et al. (2015) found that low enrichment factors ( $\epsilon_N=-1.1$  to  $-9.6$ ‰,  $\epsilon_O=-1.0$  to  $-7.2$ ‰) caused by denitrification are a result of denitrification primarily occurring in the small pores, rather than in the freely moving bulk of the groundwater system, because of longer contact time between microbes, electron donors, and  $\text{NO}_3^-$ .

Denitrification causes both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of the residual  $\text{NO}_3^-$  to increase. Sigman et al. (2005) and Granger et al. (2008) found that  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of residual  $\text{NO}_3^-$  increased at a ratio of 2 during denitrification. However, some other surface and groundwater studies found that denitrification caused  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  to increase at a ratio of more than 1:1 (Minet et al. 2012; Dale et al. 2014a, b) and some closed to 2 (Burns et al. 2009; Baily et al. 2011; Critchley et al. 2014; Wexler et al. 2014) in agricultural watersheds. However, these lower ratios were not found in urban watersheds (Kaushal et al. 2011), presumably because denitrification was limited by less organic carbon (Barnes and Raymond 2010; Wong et al. 2015). The reported ratio of  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values (1.13 to 2.1) (Vidal-

Gavilan et al. 2013; Wexler et al. 2014) as being indicative of denitrification is widely used in groundwater system and groundwater surface-water interactions. The relationship between enriched  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of the residual  $\text{NO}_3^-$  is used by Critchley et al. (2014) to provide evidence of in situ denitrification after a cross-injection experiment in glacial sand gravel aquifer. Izbicki et al. (2015) pointed out that storage of nitrate in thick unsaturated zones and dilution with low-nitrate groundwater rather than denitrification are the primary attenuation mechanisms for nitrate from septic discharges in an arid area in California, based on the weak relationship between  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values, even though denitrifying bacteria are abundant. Gómez-Alday et al. (2014) also found that weak relationship between  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values in regional groundwater flow from recharged areas to the lake indicates that  $\text{NO}_3^-$  is only partially denitrified. It should also be remembered that the identification of denitrification may also be hidden by the mixing of N and O isotopes from multiple sources. In groundwater, long groundwater transit times lead to the water being mixed sufficiently with seasonality infiltration events, which covered the nitrogen and oxygen isotopic signature of nitrate during denitrification process (Clague et al. 2015). The same case also occurs in the northern hardwood forests at the Hubbard Brook Experimental Forest in the White Mountains of New Hampshire, USA. Wexler et al. (2014) found that both  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values of a few samples fell below the denitrification line, which might be attributed to a mix of partially denitrified  $\text{NO}_3^-$  in groundwater and the  $\text{NO}_3^-$  in stream water when a hyporheic flow path brought water from the stream to the shallow groundwater in the riparian zone. One possible means of differentiating denitrification from the mixing of source materials is to analyze the relationship between  $\delta^{15}\text{N}\text{-NO}_3^-$  values (or  $\delta^{18}\text{O}\text{-NO}_3^-$ ) and  $\text{NO}_3^-$  concentrations (Kendall et al. 2007). According to the Rayleigh equation (Eq. (7)), denitrification leads to an exponential relation between  $\delta^{15}\text{N}\text{-NO}_3^-$  values and  $\text{NO}_3^-$  concentrations. The  $\delta^{15}\text{N}\text{-NO}_3^-$  values and  $\text{NO}_3^-$  concentrations of mixtures of two sources must plot on a line between those values of two sources in  $\delta^{15}\text{N}\text{-NO}_3^-$  values versus  $\text{NO}_3^-$  concentration plots, if the  $\text{NO}_3^-$  concentrations of the two sources are identical, the mixing line is straight, and, otherwise, the mixing line is hyperbolic. Hence, if two sources are mixed,  $\delta^{15}\text{N}\text{-NO}_3^-$  (or  $\delta^{18}\text{O}\text{-NO}_3^-$ ) is linearly positively related to the reciprocal of  $\text{NO}_3^-$  concentration. In contrast, if denitrification occurred,  $\delta^{15}\text{N}\text{-NO}_3^-$  (or  $\delta^{18}\text{O}\text{-NO}_3^-$ ) is linearly negatively related to the logarithm of  $\text{NO}_3^-$  concentration. Wong et al. (2015) pointed out that the distribution patterns of the  $\delta^{15}\text{N}\text{-NO}_3^-$  versus  $\text{NO}_3^-$  concentrations and the inverse ( $1/\text{NO}_3^-$ ) and logarithmic forms of  $\text{NO}_3^-$  in an agricultural-urban coastal aquifer in Werribee Plain, Australia, are indicative of conservative mixing nitrate sources from sewage and fertilizers. Lohse et al. (2013) confirmed that  $\text{NO}_3^-$  sources in a small

catchment in California were primarily derived from mixing of microbial  $\text{NO}_3^-$  and denitrification. Peng et al. (2012) analyzed  $\text{NO}_3^-$  sources and denitrification within the Chi-Chia-Wan watershed of Taiwan and pointed out that spatial variation of  $\text{NO}_3^-$  was attributed to mixing of different sources rather than denitrification.

#### Assimilation

Assimilation refers to the process during which inorganic nitrogen is transformed into an organic form by living organisms during biosynthesis. Creatures prefer to assimilate light  $\delta^{14}\text{N}$  rather than heavy  $\delta^{15}\text{N}$ . Some experimental laboratory and field results (e.g., Granger et al. 2004; Montoya et al. 1991) have shown that nitrogen enrichment factors fall within a large range of  $-30$  to  $0\%$  during phytoplankton assimilation of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . In the estuary, the nitrogen enrichment factors fall within a narrow range of  $-13$  to  $-6\%$  (Kendall et al. 2007). Algae prefer to assimilate  $\text{NH}_4^+$  rather than  $\text{NO}_3^-$  even when  $\text{NO}_3^-$  concentrations are higher than  $\text{NH}_4^+$  concentrations in the system (Dortch 1990). Consequently,  $\text{NO}_3^-$  assimilation is prevented by  $\text{NH}_4^+$  (Sugimoto et al. 2011). The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of residual  $\text{NO}_3^-$  are enriched at a ratio of 1:1 during  $\text{NO}_3^-$  assimilation (Granger et al. 2004). Wankel et al. (2009) point out that combined use of  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  allows the separation between the enrichment of  $\delta^{15}\text{N}$  through assimilation processes, which would cause a positive covariance, the production of  $\text{NO}_3^-$  through regeneration, and the sewage-derived nutrients.

Deutsch et al. (2009) analyzed  $\text{NO}_3^-$  assimilation in Elbe River in German. The results suggested that  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  increased at a ratio of 0.89:1, indicating that  $\text{NO}_3^-$  assimilation was the main N transformation process. Korth et al. (2013) found that in Szczecin lagoon,  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values near the surface of the outflowing water increased at a ratio of 1.2:1 indicating that  $\text{NO}_3^-$  assimilation was occurring. In a groundwater-fed estuary, Wong et al. (2014) used  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  in the Werribee River estuary to show that the shallow groundwater contributed 60 to 76 % of the  $\text{NO}_3^-$  to the estuary, and assimilation removed  $\sim 70\%$  of this groundwater-derived  $\text{NO}_3^-$  during dry periods. Swart et al. (2013) provided evidence that wastewater had been injected into the aquifer and leaked back to the surface based on the monitored  $\delta^{15}\text{N}\text{-NO}_3^-$  values.  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values in their study indicate that dissolved nitrate in the Biscayne Bay originated from anthropogenic sources and experience assimilation process.

#### Nitrification

Nitrification is an oxidation process in which  $\text{NH}_4^+$  is transformed to  $\text{NO}_3^-$ , a process mediated by several different kinds



of autotrophic bacteria or Archaea. Nitrification has two steps. The first is  $\text{NH}_4^+$  oxidation to  $\text{NO}_2^-$ , and the second is  $\text{NO}_2^-$  oxidation to  $\text{NO}_3^-$ . Nitrification often occurs in aerobic conditions. At the Massachusetts Military Reservation (MMR), chemical and isotopic evidence indicated that nitrification occurred near the contact between the plume and the oxic overlying groundwater, whereas it did not occur in the anoxic zone (Böhlke et al. 2006). Wexler et al. (2012) found that nitrification occurred in surface after mixing of shallow groundwater and flow from surface drainage based on isotope composition of nitrate. In groundwater underlying the Civic Center area of Malibu, CA, isotope of nitrate associated with nitrogen concentrations indicated that nitrification also occurred in the saturated zone after mixing of treated sewage discharges and native groundwater (Izbicki, 2014). Savard et al. (2007) pointed out that in groundwater, nitrification takes place throughout all seasons. Some studies have indicated that the rate of nitrification was equal to or even higher than the rate of denitrification in streams (Webster et al. 2003; Arango and Tank 2008), rivers (Richardson et al. 2004), and valleys (Lohse et al. 2013).

Nitrification causes isotope fractionation; in this case, there is a decrease in  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values in the residual pool. The most nitrogen fractionation is associated with the first step during which  $\text{NH}_4^+$  is transformed to  $\text{NO}_2^-$  (Kendall et al. 2007). Nitrogen enrichment factors during the nitrification process are in the range of  $-38$  to  $-14\%$  (Casciotti et al. 2003). The extent of N fractionation is influenced by  $\text{NH}_4^+$  concentrations in the system (Casciotti et al. 2003). Large amounts of  $\text{NH}_4^+$  stimulate nitrification and lead to a large fractionation of nitrogen isotope. However, as the  $\text{NH}_4^+$  pool is consumed, the nitrification rate decreases resulting in a slight fractionation of N isotope and a minor  $\delta^{15}\text{N}$  enrichment in  $\text{NO}_3^-$  (Böhlke et al. 2006). Böhlke et al. (2006) found that nitrification reaction was highly localized within a narrow zone of mixing between contaminated and uncontaminated water with an enrichment factor in the range of  $-20$  to  $-9\%$  in shallow groundwater. In unsaturated zone, nitrification rate and nitrogen isotope fractionation factors are affected by soil moisture. Laboratory experiment (Yun and Ro 2014) showed that with soil water potentials from  $-1100$  to  $-11$  kPa, nitrification rate increased from 2.3 to 9.8 mg N  $\text{kg}^{-1} \text{day}^{-1}$ , and nitrogen isotope enrichment factor increased from  $-14.7$  to  $-33.8\%$ .

Oxygen isotope fractionation is complex during nitrification. Previous studies have shown that O atoms within  $\text{NO}_3^-$  originated from water and dissolved O (Andersson and Hooper 1983; Hollocher 1984) formed during nitrification, one atom from dissolved  $\text{O}_2$  and two atoms from water. Thus, the formed  $\text{NO}_3^-$  exhibits an isotopic signature equal to  $2/3 \delta^{18}\text{O}$  of water and  $1/3 \delta^{18}\text{O}$  of dissolved O (Eq. (8)) if no kinetic fractionation occurs. Consequently,

$\delta^{18}\text{O}-\text{NO}_3^-$  values formed during nitrification are in the range of  $-10$  to  $+10\%$  (Kendall et al. 2007).

$$\delta^{18}\text{O}_{\text{NO}_3} = 2/3(\delta^{18}\text{O}_{\text{H}_2\text{O}}) + 1/3(\delta^{18}\text{O}_{\text{O}_2}) \quad (8)$$

Equation (8) has been used by some researchers to identify nitrification in water. For example, Thibodeau et al. (2013) found that nitrification was not the main N transformation process in the St. Lawrence River since  $\delta^{18}\text{O}-\text{NO}_3^-$  was negatively related to  $\delta^{18}\text{O}-\text{H}_2\text{O}$ . Lohse et al. (2013) found that the measured  $\delta^{18}\text{O}-\text{NO}_3^-$  values in a stream in Marin, CA, were consistent with calculated values based on Eq. (8); thus, so they concluded that nitrification occurred. However, some studies (Casciotti et al. 2010; Buchwald and Casciotti 2010) found that  $\delta^{18}\text{O}-\text{NO}_3^-$  values produced by nitrification were significantly higher than theoretical values in surface water. This might be attributed to evaporation (Böhlke et al. 1997), respiration (Kendall 1998), and higher  $\delta^{18}\text{O}$  values in soil (Wassenaar 1995) that are caused by seasonal changes or different nitrobacteria (Mayer et al. 2001). In groundwater, however, Mongelli et al. (2013) found that  $\delta^{18}\text{O}-\text{NO}_3^-$  values of groundwater in the central-southern Italy during nitrification process are lower than the expected  $\delta^{18}\text{O}-\text{NO}_3^-$  values, which might be explained by that the relative contribution of ambient O from surrounding water and  $\text{O}_2$  did not always keep the rule of 2:1 (Mayer et al. 2001). In addition, a recent study (Kool et al. 2011) confirmed the exchange of O atoms between nitrite and water during nitrification in soil which also resulted in a lighter measured  $\delta^{18}\text{O}-\text{NO}_3^-$  values than predicted  $\delta^{18}\text{O}-\text{NO}_3^-$  values. Temperature and runoff are fundamental controls on  $\delta^{18}\text{O}-\text{NO}_3^-$  values from nitrification. Higher temperature stimulates microbial respiration with the enrichment of  $\delta^{18}\text{O}-\text{O}_2$  (Kendall 1998), and produced  $\text{NO}_3^-$  is transported into waters. Savard et al. (2007) also noted that in temperate regions,  $\delta^{18}\text{O}-\text{NO}_3^-$  values produced by nitrification were largely determined by hydrological and climatic conditions (hydroclimatic index) and were significantly positively related to  $\delta^{18}\text{O}-\text{NO}_3^-$  values. Gammons et al. (2011) pointed out that diurnal variability of oxygen isotopic compositions of dissolved oxygen ( $\delta^{18}\text{O}-\text{DO}$ ) should be considered in the analysis of  $\delta^{18}\text{O}-\text{NO}_3^-$  derived from nitrification, because  $\delta^{18}\text{O}-\text{DO}$  caused by plant or algae respiration would directly influence  $\delta^{18}\text{O}-\text{NO}_3^-$  values.

### Combining nitrogen and oxygen isotopes of nitrate with chloride, boron, and sulfur isotope

From the above analysis, we have shown that determining the nature and degree of isotopic fractionation provides a means of assessing N transformation processes as well as  $\text{NO}_3^-$  source(s). However, other types of data may be combined with O and N isotopic information to more fully identify  $\text{NO}_3^-$

source(s) and N transformation processes. The following sections describe the use of chloride, boron isotope, and sulfur data to further our analysis.

### Combining nitrogen and oxygen isotopes of nitrate with chloride

Chloride ( $\text{Cl}^-$ ) is a conservative substance, meaning that it moves with the water without any loss in elemental mass. It exists in a wide range of fertilizers, animal waste, and sewage. Thus,  $\text{Cl}^-$  can be combined with  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  to identify  $\text{NO}_3^-$  sources and N transformation processes. Silva et al. (2002) found that  $\delta^{15}\text{N-NO}_3^-$  values were positively related to  $\text{Cl}^-$  concentrations in an urban watershed, indicating that the main  $\text{NO}_3^-$  source was mixing between baseflow and stormflow. Chen et al. (2014) found that mixing processes played an important role in Taihu Lake in China by combining  $\text{NO}_3^-/\text{Cl}^-$  data with  $\delta^{15}\text{N-NO}_3^-$ . In a study on groundwater  $\text{NO}_3^-$  contamination in Osona, Spain (Vitòria et al. 2008),  $\delta^{18}\text{O-NO}_3^-$  values increased with a decrease in  $\text{NO}_3^-/\text{Cl}^-$  ratios and exhibited a relative decrease in  $\text{NO}_3^-$  concentrations compared to  $\text{Cl}^-$ . These trends indicated that denitrification was occurring. However, without the linear relationship between  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$ ,  $\text{NO}_3^-/\text{Cl}^-$  ratios instead of the  $\text{NO}_3^-$  concentrations dispense with the effect of other  $\text{NO}_3^-$  sources. Some studies on  $\text{NO}_3^-$  sources in rivers in Korea (Min et al. 2002; Shin et al. 2013) have found that water chemistry changed from a Ca- $\text{HCO}_3^-$ - to Na- $\text{Cl-NO}_3^-$ -dominated system when  $\text{NO}_3^-$  sources changed from soil organic N to sewage and manure. Dun et al. (2014) calculated the contribution of Xiao River to groundwater combining  $\delta^{15}\text{N}$  with  $\text{Cl}^-$ .

### Combining nitrogen and oxygen isotopes of nitrate with boron isotope

Boron (B) has two natural stable isotopes,  $^{11}\text{B}$  and  $^{10}\text{B}$ , whose relative abundances are 80 and 20%, respectively. B is widely used in fertilizer, manure, and household products (Kendall et al. 2007) and is difficult to remove by sewage treatment (Barth 2000). B isotopes never change with physical, chemical, and microbiological processes in water.

$\delta^{11}\text{B}$  values vary between  $\text{NO}_3^-$  sources. Xue et al. (2009), for example, found by summarizing existing data that  $\delta^{11}\text{B}$  values in manures, fertilizers, and sewage were in the range of +6.9 to 42.1%, +8 to 17%, and -7.7 to 12.9%, respectively.

Combining  $\delta^{11}\text{B}$  with  $\delta^{15}\text{N-NO}_3^-$  can, in some cases, provide a clear understanding of  $\text{NO}_3^-$  sources. Widory et al. (2005) used a combination of  $\delta^{11}\text{B}$  and  $\delta^{15}\text{N-NO}_3^-$  data to conclude that  $\text{NO}_3^-$  in groundwater in the Pyrenees region of France was derived from wastewater. Xue et al. (2013) identified the  $\text{NO}_3^-$  sources in five polluted areas of the Flanders watershed in Belgium. The results showed that

relatively high  $^{11}\text{B}$  values, which were consistent with those found in agricultural manure, were associated with groundwater beneath agricultural areas and in groundwater below areas of agriculture and horticulture. In contrast, lower  $\delta^{11}\text{B}$  values that were within the range typically associated with fertilizers were found in groundwater associated with households.

As a conservative substance, B can also provide useful information for distinguishing the mixing of multiple sources from denitrification. Widory et al. (2013) found by analyzing  $\delta^{15}\text{N-NO}_3^-$ ,  $\delta^{18}\text{O-NO}_3^-$ , and  $\delta^{11}\text{B}$  values that  $\text{NO}_3^-$  in the Alsace region of the Rhine basin was the result of mixing of multiple sources accompanied with denitrification. More specifically, they found that  $^{15}\text{N-NO}_3^-$  and  $^{18}\text{O-NO}_3^-$  values increased by following a 2:1 ratio, indicating that denitrification was occurring whereas  $^{11}\text{B}$  values varied between values typically associated with different source materials (e.g., fertilizer in the range of 2.4‰ and pig manure in the range 44‰).

### Combining nitrogen and oxygen isotopes of nitrate with sulfur isotope

Sulfur (S) has four natural stable isotopes,  $^{32}\text{S}$ ,  $^{33}\text{S}$ ,  $^{34}\text{S}$ , and  $^{36}\text{S}$ , of which  $^{32}\text{S}$  and  $^{34}\text{S}$  are widely studied, whose natural abundances are 95.02 and 4.21 %, respectively. The general terrestrial range of stable S isotope values ( $\delta^{34}\text{S}$ ) is -50 to +50 ‰ (Kendall et al. 2007). It is useful to identify natural and anthropogenic sources by using  $^{34}\text{S-SO}_4^{2-}$  (Pauwels et al. 2010; Urresti-Estala et al. 2015), since  $\delta^{34}\text{S-SO}_4^{2-}$  values in natural and anthropogenic sources are distinct.  $\delta^{34}\text{S-SO}_4^{2-}$  values in anthropogenic sources are in the range of -2 to +11‰, while those values in natural sources are in the range of +13 to +16‰ (Urresti-Estala et al. 2015). As to anthropogenic sources,  $\delta^{34}\text{S-SO}_4^{2-}$  values in various sources are different. Sewage is characterized by a  $\delta^{34}\text{S-SO}_4^{2-}$  value of 9.6‰ (Otero et al. 2008). Chemical fertilizers have lower  $\delta^{34}\text{S-SO}_4^{2-}$  values in the range of -1 to 8.7‰, whereas those values of chemical detergents are higher with average value of 12.1 ‰ (Vitòria et al. 2004). For natural sources, mineralized soil organic sulfate has low  $\delta^{34}\text{S-SO}_4^{2-}$  values in the range of -1.5 to +8‰ (Vitòria et al. 2004). The  $\delta^{34}\text{S-SO}_4^{2-}$  values of atmospheric deposition are in the intermediate field between sulfate from anthropogenic sources (fertilizers and detergents) and that of geological origin (Krouse and Mayer 2000;  $\delta^{34}\text{S-SO}_4^{2-} = -3$ –9‰).

Due to the difference of sulfur isotopic compositions in natural and anthropogenic sources, a combination of sulfur isotope and nitrogen and oxygen isotopes of nitrate is successfully used to identify contamination sources in surface water and groundwater. Kaown et al. (2011) found that in rural areas of Yupori, Korea, a mixture of chemical fertilizers and manure is the main contamination sources in groundwater by analyzing stable isotopes of nitrate and sulfate in soils and shallow groundwater. In Taipei City of Taiwan,

Hosono et al. (2011) used N and O isotopes of nitrate and sulfate to identify these ions sources and found that municipal sewage leaking from sewer pipes contribute in urban area, while chemical fertilizers contribute in the suburb area. In coastal regions, the combined analysis of stable isotope of nitrate and sulfate can not only be used to determine nitrate and sulfate sources, but also be used to distinguish these source in seawater from freshwater (Hosono et al. 2011; Saccon et al. 2013), which is not be understood by only analyzing stable isotopes of nitrate.

Besides identification of contamination sources, the nitrogen and oxygen isotopes of nitrate combined with  $^{34}\text{S-SO}_4^{2-}$  can also provide a clear understanding of nitrogen transformation processes (Moncaster et al. 2000). Pyrite is an abundant sulfide mineral in natural aquifers which represents one of the potential electron sources (Hosono et al. 2014; Schwientek et al. 2008) for denitrification process. Pyrites existed in aquifer are sensitive to redox conditions and promote autotrophic denitrification processes in groundwater. During autotrophic denitrification process, the  $\text{SO}_4^{2-}$  produced through sulfide oxidation has a  $\delta^{34}\text{S}_{\text{SO}_4}$  close to the  $\delta^{34}\text{S}_{\text{FeS}_2}$  (Pauwels et al. 2010), and  $\text{SO}_4^{2-}$  concentration,  $\delta^{15}\text{N-NO}_3^-$ , and  $\delta^{18}\text{O-NO}_3^-$  values increase while  $\text{NO}_3^-$  concentration and  $\delta^{34}\text{S}_{\text{SO}_4}$  values decrease (Vitòria et al. 2008; Pauwels et al. 2010). Pauwels et al. (2010) pointed out that it was only from the association of both  $\delta^{34}\text{S-SO}_4^{2-}$  and  $\delta^{34}\text{N-NO}_3^-$  values that the occurrence of denitrification had become clear.  $\delta^{18}\text{O-NO}_3^-$  associated with  $\delta^{34}\text{S-SO}_4^{2-}$  is also used by Vitòria et al. (2008) to prove

the relationship between sulfide oxidation and denitrification in the Osona region of NE Spain. A number of field studies (Vitòria et al. 2008; Otero et al. 2009; Pauwels et al. 2010; Hosono et al. 2014) have demonstrated the occurrence of autotrophic denitrification coupled to oxidation of pyrite in aquifers based on isotope method by a combined analysis of sulfur and nitrogen and oxygen isotopes of nitrate. Hosono et al. (2014) studied a type of denitrification and occurrence of sequential anaerobic processes along the flow systems with N and S isotopes in Midori River watershed in the west of Japan and developed a model of N and S isotopic evolutionary patterns, which is useful in understanding denitrification processes.

Overall,  $\text{Cl}^-$ ,  $^{11}\text{B}$ , and  $^{34}\text{S}$  can be used to address the shortcomings inherent in the application of nitrogen and oxygen isotopes of nitrate alone. Combining  $\text{Cl}^-$ ,  $^{11}\text{B}$ , or  $^{34}\text{S}$  with  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  will provide more information on potential  $\text{NO}_3^-$  sources and represents a fresh approach to distinguish between the changes in  $\delta^{15}\text{N}$  values caused by the mixing of N from different sources and denitrification. While these iron and isotopes are useful tools for studies on nitrogen dynamics, there is still some weakness of this method. For example,  $\text{Cl}^-$  may be more useful in those areas in which groundwater anion is mainly  $\text{Cl}^-$ . As for boron isotope, various nitrogen sources have large ranges of  $\delta^{11}\text{B}$  values (Pennisi et al. 2013) and the overlaps in isotopic values among these nitrate sources may increase the uncertainty for nitrate source identification; thus, a well-characterized  $\delta^{11}\text{B}$  values for each nitrate source are

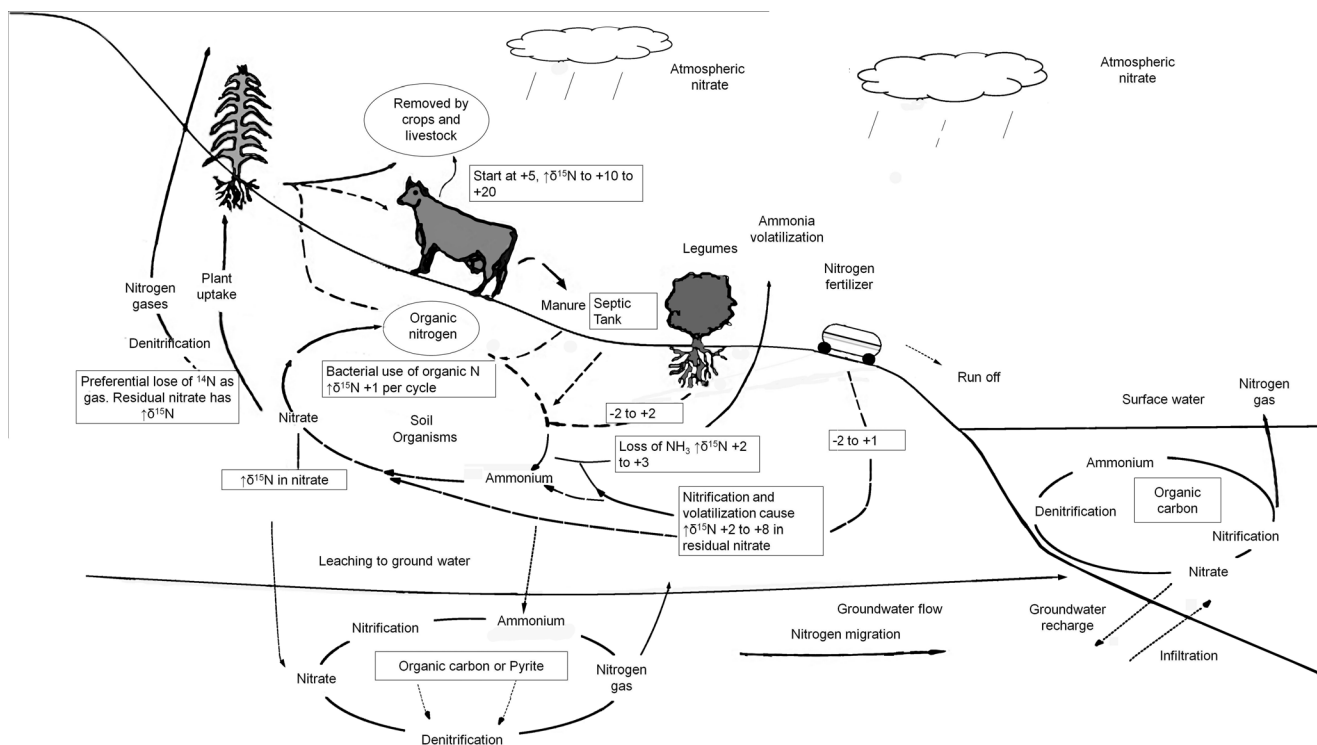


Fig. 2 The potential nitrate sources and the main nitrogen transformation processes in soil (Heaton 1986), surface water, and groundwater

required in a special area. As for sulfur isotope, some sulfates in aquifers do not derived from denitrification but from pyrite oxidation by newly formed ferrous ion ( $\text{Fe}^{3+}$ ) (Tarits et al. 2006) which result in a little unclear about understanding the occurrence of denitrification process.

## Conclusions

The stable isotope approach can be used to identify both  $\text{NO}_3^-$  sources and N transformation processes without requiring the collection of a large amount of monitoring data. With regard to determining  $\text{NO}_3^-$  sources in water, as the  $\text{NO}_3^-$  moves into the receiving waters, the various  $\delta^{15}\text{N}\text{-NO}_3^-$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values of the natural and anthropogenic sources of nitrate lead to receiving waters having different isotopic composition of nitrate from original waters, and the isotope changes are attributed to the mixing of water from different  $\text{NO}_3^-$  sources, each characterized by a specific isotopic signature. By doing so,  $^{15}\text{N}\text{-NO}_3^-$  is effective at tracing  $\text{NO}_3^-$  sources from areas with different land use (Fig. 2). For example,  $\delta^{15}\text{N}\text{-NO}_3^-$  values are typically higher in agricultural and urban watersheds (where the  $\text{NO}_3^-$  is primarily derived from fertilizers, sewage, and manure) than those in forest watersheds, where the  $\text{NO}_3^-$  is from soils and atmospheric deposition.  $\delta^{18}\text{O}\text{-NO}_3^-$  values are more useful to identify  $\text{NO}_3^-$  sources from atmospheric  $\text{NO}_3^-$ . Thus, the fingerprint based on N and O isotopes can be used to identify the predominant sources, and their relative contributions, in surface water and groundwater. Nitrogen and oxygen isotopes of nitrate can also be used to identify nitrogen transformation processes, such as nitrification, assimilation, and denitrification, since, during these nitrogen transformation processes, isotope fractionation leads to various changes in the isotopic composition of the residual N pool. However, isotopic fractionation associated with N transformation processes (described as in Fig. 2) is often temporally and spatially variable because they are largely driven by microbial activity, which reduces the accuracy of identifying  $\text{NO}_3^-$  sources and assessing N transformation processes. This problem may be reduced by combining the N and O isotopic data with other types of information including  $\text{Cl}^-$  concentrations and  $^{11}\text{B}$  and  $^{35}\text{S}$  isotopes, which widely exist in  $\text{NO}_3^-$  sources and are not influenced by physical, chemical, and microbiological process. Nevertheless, there is much room for improvement in the methods used to quantify  $\text{NO}_3^-$  sources, including the following.

1. Stable isotope mixing models need to be further improved. The three models presented here can quantify the relative contributions of  $\text{NO}_3^-$  from delineated sources but have not been widely applied or tested. The reason may be that the accuracy of the models is low because variations in the isotope values of  $\text{NO}_3^-$  sources and fractionation factors have significant effect on results. The two Bayesian models are statistical in nature and are not based on analytical solutions but consider temporal and spatial variability in the isotopes and fractionation factors. The models might be improved by reasonably dividing target areas and by using specific isotopic compositions of measured for potential  $\text{NO}_3^-$  sources within these target areas instead of those values that have been reported by other studies.
2. The fractionation of N and O isotopes in various environmental surroundings requires further study. Stable N and O isotope methods can be used for qualitative analysis but are not suitable to quantify N transformation processes accurately. Isotope fractionation factors associated with N transformation vary in different environments as a result of many factors. Consequently, further laboratory and field experiments on isotope fractionation processes in different environments are clearly needed.

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