Review

# Isotopic composition of nitrogen species in groundwater under agricultural areas: A review

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HIGHLIGHTS

- isotopic signatures of N species in groundwater (GW) under agricultural areas are reviewed
- N isotopic signature of GW samples is the result of combined effects of N sources processes, and environmental factors
- interpretation of isotopic signatures of GW samples is not a straightforward process
- ambiguity in interpreting isotopic signatures of N species can be addressed by analyses of O, B, C, S, Sr isotopes in GW

GRAPHICAL ABSTRACT
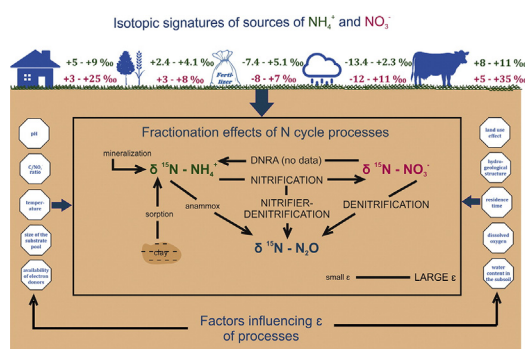
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ABSTRACT

This work reviews applications of stable isotope analysis to the studies of transport and transformation of N species in groundwater under agricultural areas. It summarizes evidence regarding factors affecting the isotopic composition of NO_3^-, NH_4^+ and N_2O in subsurface, and discusses the use of ^{11}B, ^{18}O, ^{13}C, ^{34}S, $^{87}Sr/^{86}Sr$ isotopes to support the analysis of $\delta^{15}N$ values. The isotopic composition of NO_3^-, NH_4^+ and N_2O varies depending on their sources and dynamics of N cycle processes. The reported $\delta^{15}N$-NO_3^- values for sources of NO_3^- are: soil organic N – $+3$‰–$+8$‰, mineral fertilizers – -8‰–$+7$‰; manure/household waste – $+5$‰ to $+35$‰. For NH_4^+ sources, the isotopic signature ranges are: organic matter – $+2.4$–$+4.1$‰, rainwater – -13.4–$+2.3$‰, mineral fertilizers – -7.4–$+5.1$‰, household waste – $+5$–$+9$‰; animal manure – $+8$–$+11$‰. For N_2O, isotopic composition depends on isotopic signatures of substrate pools and reaction rates. $\delta^{15}N$ values of NO_3^- are influenced by fractionation effects occurring during denitrification ($\varepsilon = 5$–40‰), nitrification ($\varepsilon = 5$–35‰) and DNRA (ε not reported). The isotopic signature of NH_4^+ is also affected by nitrification and DNRA as well as mineralization ($\varepsilon = 1$‰), sorption ($\varepsilon = 1$–8‰), anammox ($\varepsilon = 4.3$–7.4‰) and volatilization ($\varepsilon = 25$‰). As for the N_2O, production of N_2O leads to its depletion in ^{15}N, whereas consumption – to enrichment in ^{15}N. The magnitude of fractionation effects occurring during the considered processes depends on temperature, pH, DO, C/NO_3^- ratio, size of the substrate pool, availability of electron donors, water content in subsoil, residence time, land use, hydrogeology. While previous studies have accumulated rich data on isotopic composition of NO_3^- in groundwater, evidence remains scarce in the cases of NH_4^+ and N_2O. Further research is required to consider variability of $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-N_2O in groundwater across agricultural ecosystems.

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1. Introduction

Cropland and pasture cover about 40% of the Earth's ice-free land surface (Foley et al., 2005). Intensive influx of nitrogen (N) compounds from agricultural areas into groundwater and surface water is an issue of worldwide concern, since it leads to disruption of multiple vital water-related environmental services (Robertson and Vitousek, 2009; Sutton et al., 2011; Keuskamp et al., 2012). In particular, leaching of N-containing pollutants from arable lands into subsurface frequently has adverse effects on groundwater quality (Strebel et al., 1989; Directive, 1991; Di and Cameron, 2002; Ledoux et al., 2007). Moreover, it also considerably influences global N cycling because long groundwater residence time stimulates accumulation of N species and their biogeochemical transformations (Viers et al., 2012).

Pollution of aquifers in agricultural regions with reactive N poses multiple threats to sustainable development of global population. Since groundwater resources are intensively used for potable water supply, their contamination with reactive N can have negative impact on dependent communities. For instance, long-term exposure to high nitrate (NO$_3^-$) drinking water (>50 mg/l of NO$_3^-$) might increase human health risks associated with methemoglobinemia and cancer (WHO, 2008; Fewtrell, 2004; Xue et al., 2016). At the same time, N-polluted aquifers are the indirect sources of emission of nitrous oxide (N$_2$O) (Organization of Economic Co-operation and Development, 2009), produced as an obligatory intermediate of denitrification or as a by-product of nitrification. Since N$_2$O is a greenhouse gas (GHG) that possesses the capacity to trap large amount of heat and destroy the stratospheric ozone layer, such emissions contribute to global climate change (Knowles, 2000; Bernstein et al., 2008; Weymann et al., 2008).

Concentrations of different N species in groundwater could vary due to heterogeneity of N sources across the water bodies and shifting dynamics of N transport and transformation in the subsurface. In agricultural areas, aquifer pollution by N compounds might be attributed to various sources: intensive application of N-containing organic and inorganic fertilizers, inflow from animal manure and sewage discharge (Anderson et al., 2014; Böhlke, 2002; Ostrom et al., 1998). In subsurface environments, leached N compounds are further transformed by complex dynamics of different biochemical and chemical processes of the N cycle such as denitrification, dissimilatory nitrate reduction to ammonium (DNRA), nitrification, anammox (anaerobic ammonium oxidation), nitrifier denitrification, sorption and mineralization of organic matter (Fig. 1), which change their initial concentrations and produce new N species (Burgin and Hamilton, 2007; Jurado et al., 2017).

Denitrification is a microbial respiratory process where NO$_3^-$ is used as a terminal electron acceptor and reduced to N$_2$. It is considered to be the main process of NO$_3^-$ attenuation under anaerobic conditions in groundwater systems. Intermediates in this reaction might include nitrite (NO$_2^-$), nitric oxide (NO) and N$_2$O (Tesoriero et al., 2000).

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \tag{1}$$
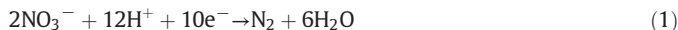
Similar to denitrification, DNRA is also an anaerobic reduction process that leads to consumption of NO$_3^-$. It is assumed that partitioning of NO$_3^-$ consumption between denitrification and DNRA is controlled by availability of organic matter: denitrification dominates when carbon (electron donor) supplies are limiting and DNRA dominates when NO$_3^-$ (electron acceptor) supplies are limiting (Korom, 1992; Kelso et al., 1997).

$$2H^+ + NO_3^- + 2CH_2O \rightarrow NH_4^+ + 2CO_2 + H_2O \tag{2}$$

Biodegradation of ammonium (NH$_4^+$) occurs during the processes of nitrification, nitrifier denitrification and anammox. There are two types of nitrification: 1) autotrophic nitrification and 2) heterotrophic nitrification. These two processes use the same substrate and produce the same intermediates and products but they differ in the enzymes involved into the reactions (De Boer and Kowalchuk, 2001). Autotrophic nitrification is carried out by two groups of microorganisms, collectively designated as Nitrobacteriaceae: 1) NH$_4^+$-oxidizers, or primary nitrifiers, and 2) NO$_2^-$ oxidizers, or secondary nitrifiers (Bock et al., 1986). Heterotrophic nitrification is conducted by bacteria (e.g. *Paracoccus denitrifcans*, *Thiosphaera pantotropha*, *Pseudomonas putida and Alcaligenes faecalis*) or fungi (De Boer and Kowalchuk, 2001). Odu and Adeoye (1970) showed that heterotrophic nitrification is more common among fungi than bacteria. In general, nitrification, which is a strictly anaerobic reaction, consists of two steps: 1) NH$_4^+$ oxidation to NO$_2^-$ and 2) NO$_2^-$ oxidation to NO$_3^-$ (Buss et al., 2004).
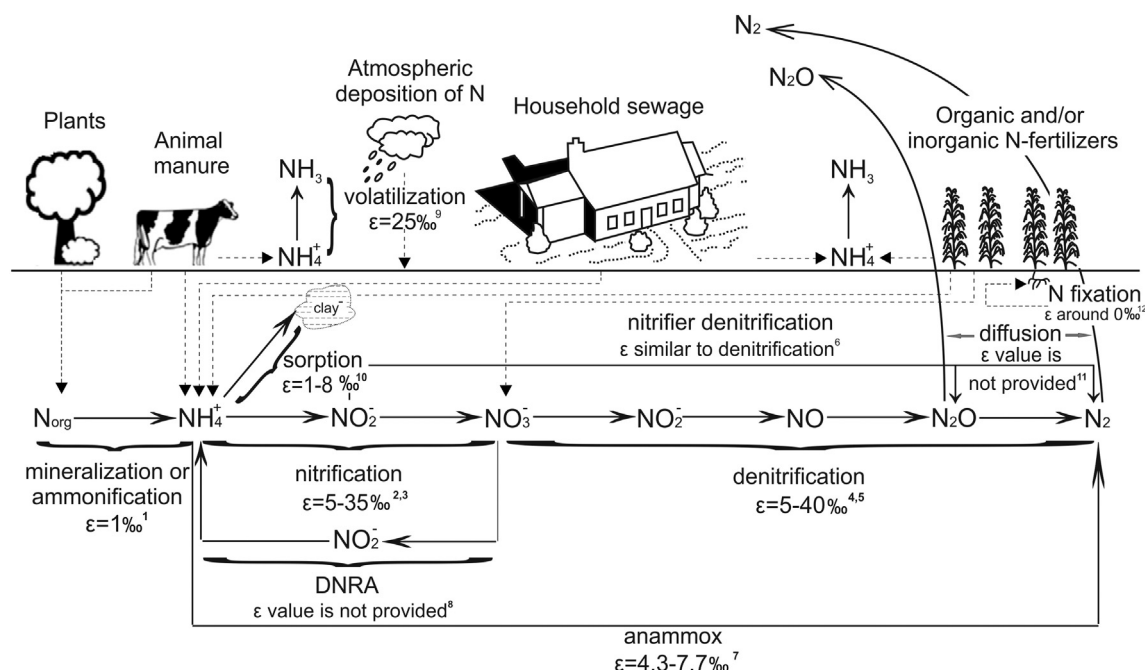
$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \tag{3}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \tag{4}$$

Fig. 1. N sources and transformation processes that affect N species in the subsurface. The enrichment values (^{15}N-NO$_3^-$, ^{15}N-NH$_4^+$) of such processes are also provided. [⟶ shows the transformation of the initial N compound; - - -▶ shows sources of different N species. References: 1 – Sharp, 2007; 2 – Kendall and Aravena, 2000; 3 – Mariotti et al., 1981; 4, 7 – Clark, 2015; 5 – Kendall, 1998; 6 – Well et al., 2012; 8 – Michener and Lajtha, 2007; 9 – Bedard-Haughn et al., 2003; 10 – Hübner, 1981; 11 – Minamikawa et al., 2011; 12 – Brandes and Devol, 2002].

Nitrifier denitrification is one of the nitrification pathways consisting of two following reactions: 1) NH$_3$ oxidation, which is attributed to nitrification, and 2) NO$_2^-$ reduction via NO to N$_2$O or N$_2$, which is regarded as denitrification (Zhu et al., 2013). The organisms involved in nitrifier denitrification are mostly NH$_3$-oxidizers (Wrage et al., 2001).

As for the anammox, it occurs in the presence of NO$_2^-$ or NO$_3^-$, which play the role of electron acceptors, and leads to conversion of NH$_4^+$ to diatomic nitrogen (N$_2$) and water (Burgin and Hamilton, 2007; Kuenen, 2008). Currently, five genera of anammox bacteria have been identified: *Brocadia*, *Kuenenia*, *Anammoxoglobus*, *Jettenia* and *Scalindua* (Wang et al., 2012).

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad (5)$$

$$3NO_3^- + 5NH_4^+ \rightarrow 4N_2 + 9H_2O + 2H^+ \qquad (6)$$
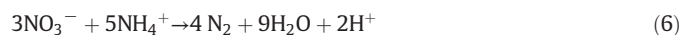
Though there are several microbial reactions leading to attenuation of NH$_4^+$, it is considered that the key reactive process controlling subsurface transport of NH$_4^+$ is sorption, which occurs as a result of cation exchange (Buss et al., 2004). Mineralization of organic matter, or ammonification, is the process that leads to conversion of organic N to NH$_4^+$. It occurs under oxidizing conditions and is carried out by virtually all microorganisms involved in the decay of dead organic matter (Schimel and Bennett, 2004; Bernhard, 2012).

N-fixation is the process by which atmospheric nitrogen is converted into ammonia (NH$_3$) by N$_2$-fixing organisms called diazotrophs. Some of them can fix N$_2$ in the free-living state, while others fix N$_2$ in association with plants (Brandes and Devol, 2002; Virginia and Delwiche, 1982). In agricultural systems the free-living bacteria is represented by: 1) anaerobic diazotrophs (*Clostridium*, *Methanosarcina*); 2) microaerophilic diazotrophs (*Frankia*, *Bra'dyrhizobium* etc.) and 3) aerobic diazotrophs (*Bradyrhizobium*, *Azobacter*, *Derxia* etc). N-fixing symbiotic associations between diazotrophs and plants can be represented by two groups according to the energy obtaining pathways of diazotrophs: 1) heterothrophic diazotrophs and plants: *Bradyrhizobium* or *Mesorhizobium* with legumes (Fabaceae family) and *Parasponia*; *Azorhizobium* with *Trifolium* sp.; *Phaseolus* sp. with *Allorhizobium* or *Devosia*; *Aeschynomene* sp. with *Ochrobactrum*, etc. and 2) autotrophic

diazotrophs and plants: *Anabaena azollae* with *Azolla* sp.; *Cyanobacteria* with fungi (lichens) or cycads; *Bradyrhizobium* with *Gunnera*, etc. (Unkovich et al., 2008; Okito et al., 2004; Postgate, 1982).

In order to address the risks imposed by contamination of groundwater with N species, it is essential to develop comprehensive scientific understanding of N species transport and transformation in subsurface. However, this is a challenging task, since various aquifers could be simultaneously exposed to multiple contamination sources and characterized with occurrence of different N-cycle processes along groundwater flow paths. Moreover, analysis of subsurface N fluxes in agricultural areas could appear even more complicated due to predominance of diffusive N pollution, which makes it difficult to calculate the total pollutant input into the aquifers. Under such circumstances, understanding of pollution transfer between different parts of aquifer and across environmental compartments of the given catchment, such as atmosphere, soil, sediment, groundwater, surface water and biota, might become especially difficult.

To obtain information regarding origin, transport and transformation of N compounds in groundwater, many environmental researchers apply stable isotope analysis. This method helps to understand migration and mixing of N derived from multiple sources, to identify various chemical and biochemical processes involving N species and to explore the dynamics and effects of occurring reactions (Kaushal et al., 2011; Robinson, 2001). Throughout several decades analysis of N isotopes in groundwater has been employed in denitrification studies in order to identify the origin of N pollution and estimate its attenuation. Nowadays, with the rising interest towards climate change, N stable isotope analysis method also becomes more frequently applied to studies of transport and production/consumption of N$_2$O in subsurface. It is expected that applications of this approach in such domain should help to understand mechanisms controlling indirect N$_2$O emissions via groundwater pathway, improve quantification of N$_2$O fluxes and reveal the sites which are prone to such emissions, thus contributing to better constraint and more realistic detalization of N budget and GHG emission both on regional and global level.

While analysis of variations in stable N isotope ratios (^{15}N/^{14}N) can potentially provide valuable information regarding the N fluxes in agro-ecosystems, interpretation of the obtained experimental evidence

is challenging. Besides the continuous simultaneous mixing of N species derived from various N pools such as atmospheric precipitation, soil organic matter, synthetic fertilizers and manure characterized with different isotope compositions (Kendall, 1998), the observed patterns of isotopic enrichment factor (enrichment/depletion of a reaction product relative to that of the substrate) of N species are considerably influenced by shifting dynamics of various microbiological (denitrification, nitrification, DNRA, anammox, etc.) and physicochemical processes (upward diffusion, sorption, volatilization, etc.) resulting in isotopic fractionation – enrichment of one isotope relative to another in an element during a chemical or physical process. Consequently, for proper interpretation of isotope signatures variability it is crucial to: 1) understand the factors and processes that may cause it, 2) consider the probable magnitude of the potential alterations; 3) verify the results of observations across a range of ecosystems with contrasting environmental settings; 4) support the interpretation of observed $\delta^{15}N$ values with results obtained using other experimental methods: analyses of other stable isotopes, concentration studies, microbiological analyses.

So far, considerable research effort has been devoted in order to accomplish these goals and improve the reliability of conclusions derived using experimental data provided by stable isotope analysis. Up to now few review articles have been published which summarize the evidence regarding the NO_3^- isotopic signatures of different contamination sources (Choi et al., 2003), the variability of $\delta^{15}N$-NO_3^- through landscapes (Bedard-Haughn et al., 2003) and the isotopic values of biologically produced N_2O in different environments, including groundwater (Toyoda et al., 2017). However, there is a lack of comprehensive review which would concentrate on the use of stable isotopes for studies of N species transport and transformation in groundwater under agricultural lands and summarize the evidence regarding factors determining the isotopic composition of NO_3^-, NH_4^+ and N_2O in subsurface in such environmental settings. The objectives of this review are: 1) summarizing the data about the $\delta^{15}N$-NO_3^-, $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-N_2O values of various N sources; 2) describing the fractionation effects of different biochemical and physicochemical processes that alter ^{15}N composition of NO_3^-, NH_4^+ and N_2O; 3) characterizing the influence of multiple environmental factors on the extent/intensity of fractionation effects; 4) discussing the application of additional stable isotopes (^{11}B, ^{18}O, ^{13}C, ^{34}S, $^{87}Sr/^{86}Sr$) analyses to support the data obtained from the ^{15}N studies. In doing so, this review summarizes evidence available from a range of case studies conducted in various hydrogeological conditions (confined, unconfined or semiconfined aquifers; different aquifer materials and properties) and in areas with different agricultural practices (type of applied fertilizer, degree of integration of livestock and crops production etc.). Section 2 describes the $\delta^{15}N$ values of various N sources and their change due to different surface and subsurface processes involving the various N species. Also, it discusses the environmental factors that affect intensity of $^{15}N/^{14}N$ isotope ratios variation. Section 3 provides brief information about the methods that could be employed in order to address the potential ambiguities during interpretation of N isotopic signatures of the groundwater samples and sustain reliability of derived conclusions regarding the process dynamics in the subsurface by discussing the application of oxygen (O), boron (B), carbon (C), sulfur (S) and strontium (Sr) isotopes analysis as tools for identification of N sources and tracing of certain chemical processes.

2. Isotopic composition of nitrogen compounds in groundwater under agricultural areas

According to previous studies conducted under various environmental settings across the globe, the isotopic signatures of N species (NO_3^-, N_2O, NH_4^+) in groundwater under agricultural lands exhibit different ranges depending on variability of N sources, transformation processes and migration pathways (Hosono et al., 2013; Well et al., 2012; Liu et al., 2006). In the cases when observed isotopic signatures of NO_3^-, N_2O, NH_4^+ in groundwater are simultaneously influenced by

multiple sources and occurrence of several N-cycle processes, interpretation of $\delta^{15}N$ values demands thorough attention. While identification of the origin of N compounds in most cases still remain a relatively straightforward task, it might be more challenging to distinguish precisely the subsurface processes that cause different fractionations of N isotopes. The following section discusses the variability of isotope signals of $\delta^{15}N$-NO_3^-, $\delta^{15}N$-N_2O and $\delta^{15}N$-NH_4^+ in groundwater, with particular emphasis on the agricultural areas, taking into account diversity of N sources, variety of N cycle processes and impact of multiple environmental parameters.

2.1. Variability of $\delta^{15}N$-NO_3^- in groundwater

According to previous studies, the isotopic signature of $\delta^{15}N$-NO_3 in groundwater under agricultural areas shows a considerably wide range from – 8.3‰ to + 65.5‰ (Table 1, Fig. 2), depending on the heterogeneity of N sources, geochemical conditions and groundwater flow patterns as well as on the peculiarities of agricultural practices in the explored regions.

2.1.1. Isotopic signatures of nitrate sources
The observed inflow of N into groundwater in agricultural areas can be attributed to multiple sources such as organic and inorganic fertilizers, manure, soil organic N, sewage (e.g. septic wastewater), and atmospheric precipitations. N originating from each source is characterized with distinct intervals of ^{15}N-NO_3 enrichment values (Fig. 3), which can be used to determine the origin of observed NO_3^- and estimate the relative contribution of NO_3^- sources to its content in the groundwater.

In particular, it has been observed that the organic and inorganic fertilizers are characterized with different isotopic signatures, which is explained by their production processes. For example, synthetic fertilizers, such as urea or NH_4^+ and NO_3^- fertilizers, are usually produced by fixation of atmospheric N_2 which has $\delta^{15}N$ $0 \pm 3‰$ (Kendall, 1998). This process only slightly fractionates the isotope composition resulting in low $\delta^{15}N$ range of inorganic fertilizers, from -4 to $+4‰$ (Sharp, 2007), -8 to $+7‰$ (Kendall, 1998) or -6 to $+6‰$ (Xue et al., 2009). However, in groundwater, this typical isotopic composition of inorganic fertilizers frequently changes because of N isotope fractionation during various physicochemical or biochemical reactions (e.g. NH_3 volatilization, nitrification or denitrification).

In line with these suggestions, further studies demonstrated that the $\delta^{15}N$-NO_3 in groundwater of cropping areas with mineral fertilizer application may be in the range of $+4.5$–$+8.5‰$ (Choi et al., 2007) or -7–$+5‰$ (Danielescu and MacQuarrie, 2013). At the same time, organic fertilizers, such as plant compost or liquid and solid animal waste, generally are characterized with higher initial $\delta^{15}N$ values and a broader range of isotopic composition ($+6$ to $+30‰$) than inorganic fertilizers. This is explained by the processes occurring in animal wastes such as excretion of isotopically light N in urine and accumulation of heavy ^{15}N isotope in the residual waste as well as volatilization of ^{15}N depleted ammonia with subsequent oxidation of the residual waste (Sharp, 2007).

In comparison to both organic and inorganic fertilizers, NO_3^- produced by nitrification of manure-N has higher $\delta^{15}N$-NO_3^-, since during its storage, treatment and application, the volatilization of NH_3 causes significant enrichment of ^{15}N in the residual NH_4^+, while most of this NH_4^+ is subsequently oxidized to ^{15}N-enriched NO_3 (Widory et al., 2004). Consequently, $\delta^{15}N$ values of NO_3^- originating from manure usually range between $+5$ to $+25‰$ (Xue et al., 2009), $+10$ to $+22‰$ (Bateman et al., 2005), $+5$ to $+35‰$ (Widory et al., 2005).

Soil organic-derived NO_3^- is a product of bacterial decomposition of organic matter originated from degradation of plants and animal wastes. The $\delta^{15}N$-NO_3 of soil NO_3^- may be between $+3‰$ and $+8‰$ (Kendall and Aravena, 2000). It is also particularly important to consider, in groundwater polluted by fertilizers, the possible mixing of N

Table 1

Analysis of NO$_3^-$ isotopic signatures in groundwater: an overview of case studies (UA – unconfined aquifer, CA – confined aquifer, IF – inorganic fertilizers, OF – organic fertilizers, SON – soil organic N, AM – animal manure, Ww – wastewater, D – denitrification, N – nitrification, Dom – decomposition of organic matter, Mix – mixing, Dl – dilution, V – volatilization).

Site	δ^{15}N (‰)	δ^{18}O (‰)	Aquifer type	Aquifer material	Potential NO$_3^-$ source	DO (mg/l)	NO$_3^-$ (mg/l)	pH	Processes altering the δ^{15}N and δ^{18}O of NO$_3^-$
The Chalk aquifer (France) (Mariotti et al., 1988)	+3–+7 (s. d. 1.6)		UA	Limestone	IF		3–10		—
	+5–+20		Boundary between UA & CA				0.37–12.2		D
	+0.9–+5.8		CA				0.01–0.05 (s. d. 0.06)		Dom
The Arguenon watershed (Brittany, France) (Widory et al., 2005)	+2.7–+21 (s. d. 0.2)			Granitic gneiss and mica schist	AM, Ww		3.2–245 (mean value 106 (s. d. 78))	4.8 – 7.8	D, mix (Dl)
The "Roussillon" aquifer (Pyrénées, France) (Widory et al., 2005)	+5.4–+23.9			Deep alluvial formation; three aquifer levels due to the presence of clay layers	Ww, IF		10–139 (mean value 51 (s. d. 39))	6.5 – 7.9	Mix
The "Ile du Chambon" Catchment (the Allier Valley, France) (Widory et al., 2005)	+5.1–+42.4			Sand and gravel, subsurface alluvial formation	IF, Ww		<0.2–53 (mean value 30 (s. d. 13))		D
Fuhrberger Feld aquifer (Lower Saxony, Germany) (Well et al., 2012)	−2.1–+65.5 (mean + 6.9 (s. d. 11.7))	−5.0 – +33.5 (mean + 1.6 (s. d. 5.9))	UA	Carbonate-free sand and gravel	IF	0.0–9.6 (mean 2.4 (s. d. 2.9))	0.0–43.4 (mean 21.9 (s. d. 10.3))	4.1–6.3	D
Großenkneten aquifer (Lower Saxony, Germany) (Well et al., 2012)	−1.8–+65 (mean + 8.6 (s. d. 18.9))	−8.1 – +38 (mean + 0.5 (s. d. 12.8))	UA	Carbonate-free sand and gravel	IF	0.1–9.0 (mean 2.8 (s. d. 3.2))	0.0–57.6 (mean 15.2 (s. d. 18.5))	4.1–5.8	D
Osona region (Spain) (Vitòria et al., 2008)	+2.2–+20.9 (mean + 13)	+4.6 − +9.7 (mean + 7.4)	CA	Carbonate and carbonate sandstone; presence of pyrite	AM, IF		0.0–366 (mean 90)	>7	D
The alluvial aquifer of the Vibrata plain (Italy) (Di Lorenzo et al., 2012)	In summer: +4.9–+22.8 In winter: +3.8–+18.9	In summer: +1.3 − +11 In winter: +3.7–+14.7	UA	Gravel and sand with silty lenses	IF (NH$_4^+$ salts)		In summer: 0.1–148 (mean value 77.2); In winter: 2–151 (mean value 66.3)		Patchy D
The Maresme groundwater (Spain) (Vitòria et al., 2005)	+6.8–+9.4	+5.1–+10.2		Sand	IF		235–482 (mean value 334)		V
The Zunyi area groundwater (China) (Li et al., 2010)	In summer: −1.8–+20.7 (mean + 7) In winter: −4.3–+22.7 (mean + 10.4)			Carbonate rocks (limestone and dolomite) and clastic rocks; sulfate evaporite (gypsum) and coal occur locally	OF, IF, Ww		In summer: 0–90.5 In winter: 0–107.9	6.8–8.4	Mix, N
The Sichuan Basin (China) (Li et al., 2007)	Well in farmland: −0.1–+8.9 (mean value +3.7 (s. d. 2.1)) Well in farmyard: mean value +9.7 (s. d. 4.7) Spring: −8.3–+6.4 (mean value −0.2 (s. d. 3.7))		UA	Redbeds and mudstone interbedded with sandstone	IF, Ww which might contain AM		Well in farmland: 42.94 (s. d. 47.2) Well in farmyard: 39.8 (s. d.42.1) Spring: 16.4 (s. d. 13.7)		N
Guiyang, (China)	In summer:	In summer:		Carbonate rocks	In summer:		In summer:		Suburban

(continued on next page)

Table 1 (*continued*)

Site	δ15N (‰)	δ18O (‰)	Aquifer type	Aquifer material	Potential NO$_3^-$ source	DO (mg/l)	NO$_3^-$ (mg/l)	pH	Processes altering the δ15N and δ18O of NO$_3^-$
(Liu et al., 2012)	−1.4–+14.9 (mean 4.1) In winter: −0.1–+15.4 (mean 7.0)	+2.8–+18.2 (mean 10.7) In winter: +4.3–+23.5 (mean 12.5)		(limestone, dolomite) and clastic rocks (shale, sandstone); sulfate evaporite (gypsum) and coal occur locally	IF (urea, (NH$_4$)$_2$SO$_4$, N/P/K mix) In winter: IF, Ww		0.29–11.7 (mean 5.0) In winter: 0–8.9 (mean 3.1)		areas: N Urbanized areas: D
The Wensum catchment (East Anglia, UK) (Wexler et al., 2011)	+6.2 (s. d. 0.6)	+0.8 (s. d. 0.5)	UA/CA	Limestone	OF,IF		56.1 (s. d. 6.8)		Mix and N
Kumamoto groundwater area (Japan) (Hosono et al., 2013)	−6–+46	−3–+48	UA CA	Pyroclastic and alluvial sedimentary deposits Porous andesitic lava and pyroclastic deposits	IF, AM		0–73		Dl and D
The Cretaceous Chalk aquifer (Cambridge shire and Norfolk, UK) (Feast et al., 1998)	+3.6 (s. d. 1.8)	+8.5 (s. d. 2.8)	UA	Limestone	IF, OF		39.2 (s. d. 14.3)	7.2 (s. d. 0.1)	N, minor D
La Pine, (Oregon, USA) (Hinkle et al., 2007)	+3.3 − +12.8 (mean 7.5)			Sand	Ww, IF	ˉ0.1–10.7 (mean 1.2)		6.7–8.2 (mean 7.4)	D
Ichikawa city (Japan) (Li et al., 2014a, 2014b)	+5.7		Upland shallow ground water	Sand	IF, OF, AM	In summer: 9.3 In winter: 5.7	In summer: 76.6 In winter: 153.8	In summer: 6.9 In winter: 6.1	N
Sacramento Valleys (USA) (Fogg et al., 1998)	+1–+6		SCA/UA	Sand and gravel	IF, AM, Ww				—
Salinas Valley (USA) (Fogg et al., 1998)	+4.1–+5.1		SCA/UA	Sand and gravel	IF, AM, Ww		32–74		Possible D
Wexford (Ireland) (Baily et al., 2011)	+6–+32.4	+1.4–+21.2	Shallow ground water	Greywacke, schist and massive schistose quartzites	M, IF		0–66.4		N, D
The Bure River valley (Norfolk, UK) (Feast et al., 1998)	−2.1–+13.7	−7.01 to −8.2	UA/CA	Limestone	IF		ˉ0.1–95.4 (mean 18.7)		Mix, D
the Cedar River Watershed (Iowa, USA) (Gautam and Iqbal, 2010)	+0.5–+5.4			Shallow aquifer: sand and gravel deep aquifer: limestone and dolomite	IF, SON	shallow aquifer: 4.9–7 Deep aquifer: 2.9–6.9	0–75.5 (mean 35.8)		—
Sandstone catchment (Ireland) (McAleer et al., 2017)	+6.0–+18.2 (mean + 9.6)	+3.0–+11.6 (mean 6.3)	UA	Mudstone, sandstone and minor siltstone	AM	2.0–9.6 mean 5.5	0–43.4 mean 21.7		D
Slate catchment (Ireland) (McAleer et al., 2017)	+1.9–+6.8 (mean + 3.3)	−0.5–+3.8 (mean + 0.8)	UA	Slate, siltstone	IF	4.5–11.8 mean 8.9	32.8–51.4 mean 35.9		Dl

originating from the addition of fertilizers and N mineralized from soil organic matter which might not be taken up by crops if their demands are already satisfied (Li et al., 2007). For example, Danielescu and MacQuarrie (2013) revealed that 72% of their surface- and groundwater samples of the Trout catchment fell into the overlapping interval of +3 to +5‰. This indicates that the detected concentrations could be derived either from the use of NH$_4^+$ fertilizers or from the presence of soil organic-derived NO$_3^-$. The studies in the Cedar river basin (USA) (Gautam and Iqbal, 2010) (Table 1) also demonstrated that the δ15N-NO$_3^-$ range, between +0.45‰ and +5.35‰, was the result of the joint effect of fertilizers and soil organic N on groundwater quality. On the contrary, the isotopic signature of NO$_3^-$ originated from animal or sewage waste is commonly less influenced by interaction with soil N because the distribution of waste is often localized at point sources with high concentrations. In some cases, the observation of the distribution

of point and non-point sources of pollution can help to identify the origin of NO$_3^-$ more precisely.

Another significant source of NO$_3^-$ in groundwater under agricultural lands is household sewage whose δ15N-NO$_3$ range vary between +4‰ and +19‰ (Xue et al., 2009). In many cases, experimental studies have revealed similar ranges of δ15N for both animal manure and sewage, for instance: +3‰–+25‰ (Di Lorenzo et al., 2012), +8–+18‰ (Vitòria et al., 2008), and others. Consequently, it is often difficult to determine exactly the origin of NO$_3^-$ in areas characterized with simultaneous occurrence of groundwater pollution from livestock manure and household wastes.

The amount of N contained in atmospheric precipitation is influenced by several factors: volatilization of NH$_3$, nitrification and denitrification occurring in the soils and the impact of various anthropogenic sources. In general, the δ15N-NO$_3^-$ composition of rain is higher than
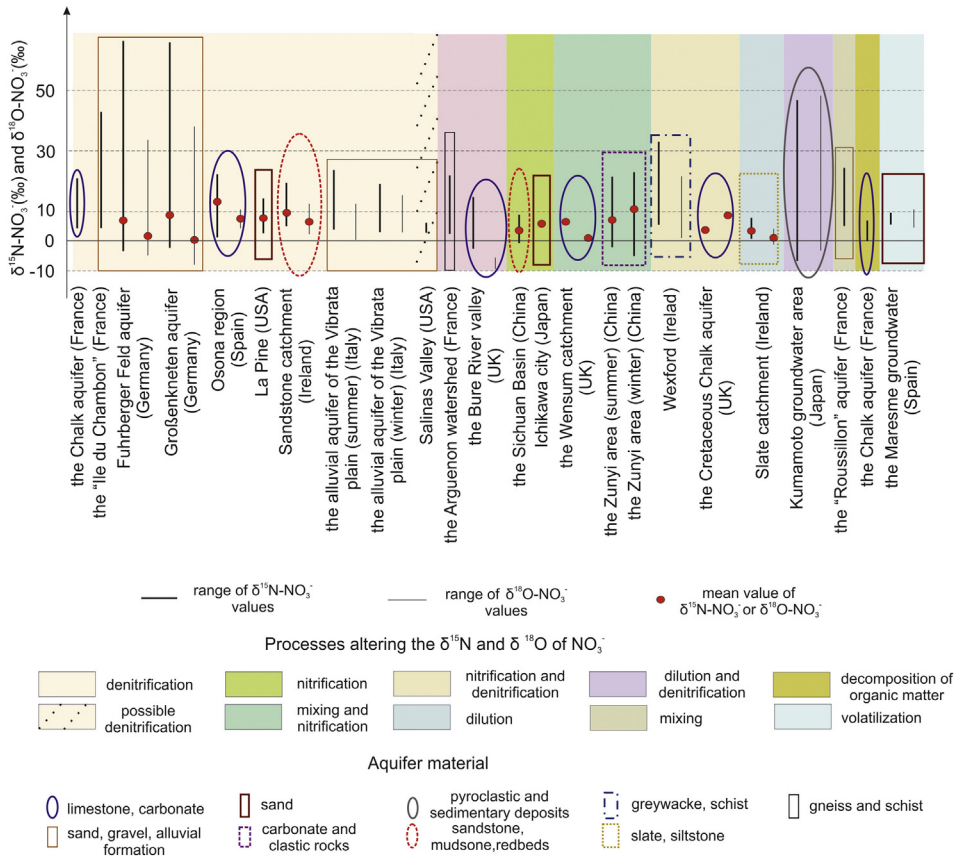
Fig. 2. NO_3^- isotopic signatures in groundwater: a summary of case studies in agricultural areas.

that of the co-existing $\delta^{15}N\text{-}NH_4^+$ (Bedard-Haughn et al., 2003). The $\delta^{15}N\text{-}NO_3^-$ isotopic signature of rain might vary between $-10‰$ and $+9‰$ – based on various case studies (Sharp, 2007), $-11.8‰$ and $+11.4‰$ – reported for eastern Canada (Savard et al., 2010) and -10.2 and -4.4 – reported for central China (Li et al., 2007).

This overview demonstrates that the sources of NO_3^- pollution are characterized with relatively different $\delta^{15}N\text{-}NO_3^-$ isotope ranges: rain water – from -12 to $+11‰$, inorganic fertilizers – from -8 to $+7‰$,

organic fertilizers – from $+6$ to $+30‰$, soil organic matter – from $+3$ to $+8‰$, manure – from $+5$ to $+35‰$, and household sewage – from $+3$ to $+25‰$. The lowest values of $\delta^{15}N\text{-}NO_3^-$ are typical for inorganic fertilizers followed by NO_3^- derived from soil organic matter, while the highest values are usually related to the impact of manure or household wastes, both of which may overlap. However, the isotope composition of NO_3^- from different sources might be subject to considerable alterations due to fractionation processes occurring under certain

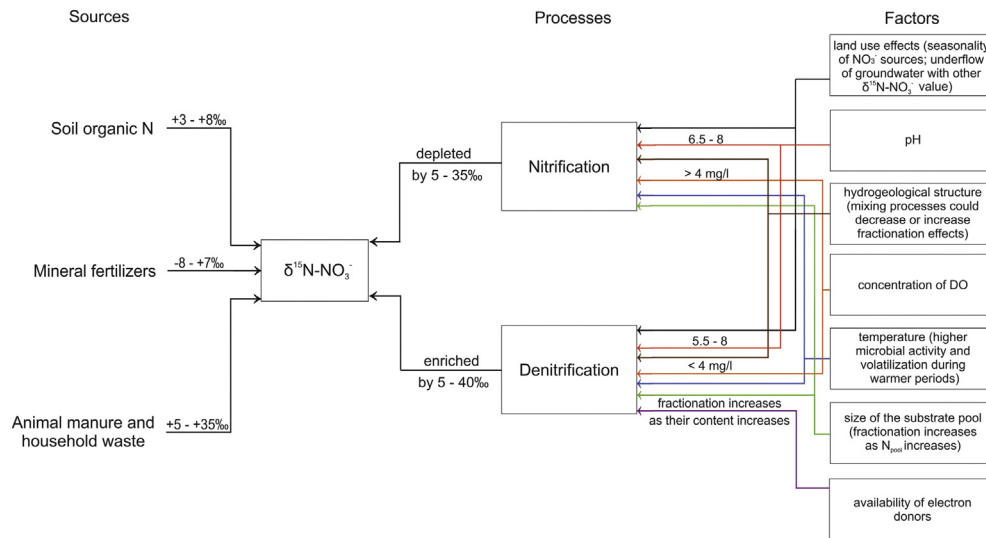**Fig. 3.** Sources, processes and factors that influence the $\delta^{15}N\text{-}NO_3^-$ values: summary (the following arrows connect processes with factors that have decisive effect on their dynamics and, consequently, on resulting fractionation effects: ⟶ availability of electron donors; ⟶ size of the substrate pool; ⟶ temperature; ⟶ concentration of DO; ⟶ hydrogeological structure; ⟶ pH; ⟶ land use).

biochemical or physicochemical reactions during the migration to or within the aquifer.

2.1.2. Isotopic effects of nitrate production/consumption processes

Previous studies showed that denitrification and nitrification alter the original $\delta^{15}N$-NO_3^- isotopic composition of NO_3^- in groundwater under agricultural areas (Fig. 1). Isotope effects of the considered N processes are presented in terms of their enrichment factors which show isotope enrichment of a reaction product relative to that of the substrate and are determined by means of the Rayleigh equation (Mariotti et al., 1981):

$$\varepsilon = \frac{10^3 \ \ln \frac{10^{-3}\delta(NO_3^-)_{measured} + 1}{10^{-3}\delta(NO_3^-)_{initial} + 1}}{\ln \left[C(NO_3^-)_{measured} / C(NO_3^-)_{initial} \right]} \tag{7}$$

where ε is the isotopic enrichment factors for N or O, δ is the $\delta^{15}N$ and $\delta^{18}O$ values, respectively and C–NO_3^- concentration.

Denitrification has attracted most considerable research effort as it plays a significant role in the attenuation of NO_3^- pollution in the subsurface (Rivett et al., 2008). Experimental results suggest that it is a strongly fractionating process responsible for preferential conversion of the lighter isotope ^{14}N to N_2O and N_2. Consequently, the corresponding enrichment of the residual (unreacted) NO_3^- with the heavy isotope ^{15}N is observed (Knöller et al., 2011; Fukada et al., 2003). During this process the $\delta^{15}N$ value of the initially produced NO_3^- might be enriched in comparison to N_2 or N_2O by approximately 20–30‰ (Clark, 2015), or 5–40‰ (Kendall, 1998). For example, denitrification of NO_3^- fertilizer that originally had a distinctive $\delta^{15}N$ value of $+1‰$ can yield residual NO_3^- with a $\delta^{15}N$ value of $+15‰$ which is within the range of composition expected for a NO_3^- from a manure or septic-tank source (Kendall, 1998). Among the case studies considered in this review (Table 1) the most pronounced effects of denitrification were reported for the unconfined sand and gravel aquifers of Fuhrberger Feld (Lower Saxony, Germany) and Großenkneten (Lower Saxony, Germany) (Well et al., 2012), for the Chalk aquifer (France) at the boundary between confined and unconfined zones (Mariotti et al., 1988) and for the alluvial aquifer of the Vibrata plain (Italy) (Di Lorenzo et al., 2012). These effects originate from: 1) microorganisms' activity within the pore spaces of sediments in case of Fuhrberger Feld and Großenkneten; 2) local physicochemical conditions (e.g. availability of the substrate pool and electron donors, concentration of the electron donors) in case of the Chalk aquifer and 3) the extent of hyporheic zone (groundwater/surface water flow exchange) in case of alluvial aquifer in the Vibrata plain. However, it should be emphasized that the rate and extent of denitrification processes in the considered cases as well as other cases depend of the combination of multiple environmental factors (Section 2.1.3) and their mutual interaction.

In contrast, nitrification reaction results in the preferential incorporation of the lighter isotopes into NO_3^- and often leads to decrease in the $\delta^{15}N$-NO_3^- (Barnes and Raymond, 2010). In average the difference between initial $\delta^{15}N$-NH_4^+ and produced $\delta^{15}N$-NO_3^- can reach 12–29‰ (Kendall and Aravena, 2000), or 5–35‰ (Mariotti et al., 1981). However, evidence has been also obtained that both $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-NO_3^- will increase as the NH_4^+ reservoir is converted to NO_3^-, with $\delta^{15}N$-NO_3^- evolving towards the initial $\delta^{15}N$-NH_4^+ value (Clark, 2015). In general, it appears that the final $\delta^{15}N$ of NO_3^- derived via nitrification from manure-N would be more positive than that from fertilizer-N (Choi et al., 2003). The influence of the nitrification on the $\delta^{15}N$-NO_3^- of groundwater was detected in the Sichuan Basin (China) (Li et al., 2007), Ichikawa city (Japan) (Li et al., 2014a, 2014b), shallow groundwater in Wexford (Ireland) (Baily et al., 2011), in the Cretaceous Chalk aquifer in Cambridgeshire and Norfolk, UK (Hiscock et al., 2003) and in the hydrogeological formation in Zunyi (China) (Li et al., 2010).

2.1.3. Factors controlling nitrate production/consumption processes and their impact on $\delta^{15}N$-NO_3^- variability

The magnitude of fractionation related to nitrification, denitrification and anammox processes is influenced by ambient conditions of hydrogeological systems where they occur, e.g. substrate concentration, availability of electron donors, concentration of dissolved oxygen, temperature, pH, residence time, etc. (Böttcher et al., 1990).

In particular, it has been demonstrated that the size of the substrate pool (the amount of the chemical species which reacts with a reagent to generate a specific product) determines the extent of fractionation by minimizing it in N-limited systems and maximizing in systems with constant and high supply of N compounds (Li et al., 2007). For example, nitrification processes will be more intensive under the presence of a large amount of NH_4^+ (e.g. due to application of artificial fertilizers), which would likely cause considerable fractionation (Kendall, 1998). However, as the NH_4^+ pool is consumed, the overall nitrification fractionation gradually decreases. It has also been revealed that excessive concentrations of NO_3^- might induce a termination of denitrification with the formation of N_2O (Rivett et al., 2008). The threshold concentrations for the occurrence of this effect appear to be case-specific, since in some cases it has been reported that even low concentrations affected the ratio between produced N_2O and N_2. For example, an increase in the N_2O:N_2 ratio from 0.11 to 0.34 associated with an addition of 0–4 mg-N/l was reported by Magalhaes et al. (2003). That is why it is essential to consider the initial concentration of the substrate in order to achieve more accurate conclusions concerning the production/consumption of NO_3^- and related changes in its isotopic composition.

Availability of electron donors is mostly discussed in the context of fractionation effects caused by denitrification. In general, it is suggested that denitrification may not play an important role in increasing $\delta^{15}N$ of NO_3^- under the conditions of low contents of electron donors (Choi et al., 2003). Electrons needed for denitrification can originate from the microbial oxidation of organic C or reduced S which might be present in water as the S^{2-} state in H_2S, S^{1-} in FeS_2, S^0 in elemental sulfur, S^{2+} in thiosulfate ($S_2O_3^{2-}$) or S^{4+} in sulfite (SO_3^{2-}), (to the S($+$VI) state as sulfate) (Rivett et al., 2008). To consider the potential impact of limited availability of electron donors on isotopic composition of NO_3^- it has been proposed to monitor their concentrations throughout the periods of observation of the ^{15}N isotopic signatures. For example, the presence of DOC in waters has been used as an indicator of an available carbon source for denitrification. Moreover, concentrations of sulfate ion have also been measured to test for consistency with denitrifying environment (Kellman and Hillaire-Marcel, 2003). It should be mentioned that the amount of DOC has been shown to decrease in conjunction with an increase in sulfate concentration. This effect is related to the reduced solubility of DOC under conditions of increased ionic strength and acidity of water (Evans et al., 2006; Clark et al., 2005).

Concentration of dissolved oxygen (DO) in hydrogeological systems can also have a crucial impact on observed NO_3^- isotopic signatures. It may determine the type of N biochemical transformations occurring, which can alternatively lead either to decrease or increase of $\delta^{15}N$ of NO_3^-. As a common rule, the low content of oxygen is associated with denitrification reactions which lead to the increase of $\delta^{15}N$-NO_3^-. On the contrary, higher content of oxygen usually accompanies nitrification reactions which result in low $\delta^{15}N$-NO_3^- values. From previous studies, it has become obvious that the occurrence of denitrification and nitrification processes could not be associated with clearly defined values (or narrowly constrained intervals) of DO concentrations. In particular, there is the range of DO concentration where both nitrification and denitrification can occur. For instance, denitrification cannot occur if the content of DO is above 0.2 mg/l according to Feast et al., 1998, above 2 mg/l according to Rivett et al. (2008) or above 4 mg/l according to Baily et al. (2011). At the same time, it has been reported that the rate of nitrification reactions is maximized for a range of DO concentrations between 0.3 mg/l and 4 mg/l (Stenstrom and Poduska, 1980). However, the experimental evidence is not conclusive, as in some cases it has been

determined that a dissolved oxygen concentration in excess of 4.0 mg/1 was required to achieve the highest nitrification rates (Stenstrom and Poduska, 1980). That is why, in order to be able to distinguish these two processes it is important to consider thoroughly the data about pH, availability of electron donors etc.

As the water temperature controls microbial activity and, consequently, DO content in groundwater, any seasonal changes could affect the $\delta^{15}N$ of NO_3^-, resulting in higher values of isotopic enrichment in the summer periods in aquifers where denitrification occurs, or lower values in groundwater influenced by nitrification activity. However, evidence about the impact of water temperature is not yet conclusive, as some reports suggested that $\delta^{15}N–NO_3^-$ values might not exhibit seasonal trends (Danielescu and MacQuarrie, 2013). So it is essential to study microbial communities and distribution of potential denitrifying genera, as this will allow to get better insight into the nature of NO_3^- production/consumption processes and, in particular, into the impact of temperature on their dynamics (Hernández-del Amo et al., 2018).

The pH range is another important factor that affects the intensity of microbiological reactions and influences the magnitude of fractionation effect. It has been reported that pH ranging between 6.5 and 8 is the optimal range for nitrification, and reaction rates are likely to be significantly decreased below pH 6.0 and above pH 8.5 (Buss et al., 2004). Denitrification processes typically occur under a pH range be between 5.5 and 8, but the optimal pH is site-specific because of the effects of adaptation on the microbial ecosystems (Feast et al., 1998). Anammox activity is observed in a pH range from 6.5 to 9.3 with the optimum pH at 8 (Tomaszewski et al., 2017; Jin et al., 2012).

Furthermore, the hydrogeological structure of the area predetermines the processes of mixing of waters derived from different sources (see Section 2.1.1.) and of different age. Therefore, it also profoundly affects the dynamics of $\delta^{15}N$ isotopic signature (as demonstrated by the vast majority of considered case studies – see Table 1) (Einsiedl and Mayer, 2006). Therefore, comprehensive analysis of $\delta^{15}N-NO_3^-$ distribution in groundwater should be supported by in-depth consideration of hydrogeological features of the examined territories, for instance - the extent of confined and unconfined zones in the subsurface system, their connection and location of the recharge areas along the aquifer.

While studying variations of $\delta^{15}N-NO_3^-$ in agricultural areas, it is particularly important to consider agricultural practices and the types of adjacent land uses, as they might significantly alter the isotopic signature of NO_3^- in groundwater samples. In agricultural areas where it is common to leave crop residues on the fields over the winter period it is necessary to consider the seasonality of NO_3^- sources. Previous studies which analyzed the influx of N from inorganic fertilizers into aquifer systems under intensive row-cropping and fertilization highlighted the significance of the intermediate N cycling processes of mineralization and nitrification of soil organic matter, such as crop residue, in the overall N cycling (Savard et al., 2010). Since resulting winter and spring load of NO_3^- is attributed to slow mineralization and nitrification during soil organic matter degradation, it is hard to identify precisely the source of NO_3^- in groundwater using its isotopic signature, since $\delta^{15}N–NO_3^-$ values are close to those typical for fertilizers. Moreover, Sebilo et al. (2013) showed that the isotopic composition of NO_3^- in groundwater might be considerably influenced by mineralization of N fertilizers incorporated into the soil organic matter pool several decades ago. Therefore, the evidence regarding the dynamics of isotopic signatures should be supported by the expert knowledge about the local agricultural practices.

To summarize, the previous studies considered in this review have demonstrated that aquifers under agricultural areas are characterized with a wide range of $\delta^{15}N-NO_3^-$ determined by the variability of N sources and N transformation processes, intensity of which is controlled by the ambient geochemical conditions and hydrogeological settings (Fig. 2).

In general, mineral fertilizers typically show the lowest $\delta^{15}N-NO_3^-$ values, followed by the isotopic signatures of soil-derived organic NO_3^-

. The highest $\delta^{15}N-NO_3^-$ are commonly observed in animal manure or household sewage. Among the microbiological and physicochemical processes influencing isotopic composition of NO_3^- in groundwater, the highest $\delta^{15}N-NO_3^-$ values are associated with the denitrification activity. On the contrary, nitrification is responsible for the occurrence of NO_3^- with the ^{15}N isotopic signature on 5–35‰ lower in comparison to the ^{15}N of initial NH_4^+. While exploring the variability of ^{15}N in groundwater systems, it is important to account for possibilities of physical mixing of water of different origins and the impact of multiple environmental parameters on the intensity of transformation processes as they might lead to change in the isotopic signature of initial N pollutants.

2.2. Variability of $\delta^{15}N-NH_4^+$ in groundwater

In comparison to the amount of information regarding $\delta^{15}N-NO_3^-$ in groundwater under the agricultural areas, the data about distribution of $\delta^{15}N-NH_4^+$ are less abundant. In general, conducted studies revealed that the $\delta^{15}N$ values of NH_4^+ in aquifers cover the range from −8.5‰ to +23.8‰ (Table 2), being significantly lower than the corresponding $\delta^{15}N$ values of NO_3^- (Li et al., 2010; Li et al., 2007; Hinkle et al., 2007; Liu et al., 2006).

2.2.1. Isotopic signatures of ammonium sources

Overall, fertilizers, manure and sewage effluent are the principal anthropogenic sources of the NH_4^+ in groundwater under agricultural areas. Rainwater and organic matter may also substantially contribute to NH_4^+ concentration in groundwater (Hinkle et al., 2007). The comparison of $\delta^{15}N-NH_4$ values of different pollution sources with the isotopic signatures of groundwater samples is widely used for identification of the origin of detected NH_4^+.

NH_4^+ fertilizers usually have $\delta^{15}N$ values of 0‰ or lower (Kendall, 1998). Available data provide the following ranges: from −1.5‰ to −0.7‰ (Wassenaar, 1995); from −7.4‰ to +3.6‰ (median value −0.6‰) (Vitòria et al., 2004a, 2004b); from +2.7‰ to +5.1‰ (mean value +4.2 ± 0.8‰) (Li et al., 2007); −3.9‰ (±0.3‰) (Choi et al., 2007), −0.91‰ (±1.88‰) (Kendall, 1998). In general, the isotopic signature of $\delta^{15}N-NH_4^+$ is reported to be 2.5‰ lower than the isotopic signatures of $\delta^{15}N-NO_3^-$ of synthetic fertilizers.

Application of manure in agricultural fields or animal waste effluents from farms might increase the isotopic signature of $\delta^{15}N-NH_4^+$ in the groundwater located under such areas in comparison to the aquifers effected by the fertilizer use, as animal waste is characterized by higher level of $\delta^{15}N$ enrichment of NH_4^+ (Fig. 4). It appears that the higher $\delta^{15}N$ values observed in animal wastes are related to the increase in $\delta^{15}N$ by 3–4‰ at each successive trophic level (step in a nutritive series, or food chain, of an ecosystem). The most important factor contributing to this increase is the excretion of isotopically light urine: animal waste gets further enriched in ^{15}N by the subsequent volatilization of isotopically light NH_3 (Sharp, 2007). The initial $\delta^{15}N-NH_4^+$ values of manure may vary between +8‰ and +10‰ for pig waste (Vitòria et al., 2003) and around +7.4‰ ± 3.8‰ for cow waste (Maeda et al., 2016).

NH_4^+ is also one of the major components in groundwater contamination plumes originating from septic tank effluents or wastewater release from treatment plants. In untreated sewage, the isotopic signature of $\delta^{15}N-NH_4^+$ is typically between +5‰ and +9‰ (Cole et al., 2006). The sewage effluent in Guiyang (China) showed the mean value of $\delta^{15}N-NH_4$ at +5.3‰ (Liu et al., 2006), and Robertson et al. (2012) detected the $\delta^{15}N-NH_4^+$ value of +4.4‰ ± 4.6‰ in the septic system of the Long Point campground located on the shore of Lake Erie (USA and Canada). Usually, the contamination plumes exhibit clear stratification between the differently enriched NH_4^+ species. The top of the plume is typically characterized with more enriched $\delta^{15}N-NH_4^+$ values, caused by ongoing nitrification, in comparison to the core of the plume, where NO_3^- and NH_4^+ coexist and anammox reaction

Table 2
Analysis of NH$_4^+$ isotopic signatures in groundwater: an overview of case studies (Vu – volatilization of urea, N – nitrification, V – volatilization, M – mineralization of organic N, s. d. – standard deviation, bdl – below detection level).

Site	δ^{15}N (‰)	Processes altering the δ^{15}N of NH$_4^+$	NH$_4^+$ (mg/l)
The Sichuan Basin (China) (Li et al., 2007)	Well in farmland: −6.7–+5.1 mean value (−1.2 (s. d. 3) Well in farmyard: +5.4–+23.8 (mean value +9.7 (s. d. 6.1)) Spring: mean value −8.5 (s. d. 1.5)	Vu	0.1–0.3
Guiyang (China) (Liu et al., 2006)	In summer: +0.04–+1. (mean + 0.64) In winter: −1.7–+3.9 (mean + 1.2)	N, V	In summer: 0.04–3.6 (mean 0.8) In winter: 0.04–18 (mean 4.1)
La Pine, (Oregon, USA) (Hinkle et al., 2007)	+2.5–+3.9 (mean 3.5)	M	>0.02–38 (mean 4.3)
The Zunyi area groundwater (China) (Li et al., 2010)	−1.1–+5.2 (mean + 1.9)	N	In summer: bdl – 1.7 In winter: bdl – 1.3

enriches both compounds, and below plume where only NO$_3^-$ attenuated by denitrification remains (Clark, 2015).

NH$_4^+$ is also the most abundant N compound in rainwater which commonly exhibits negative δ^{15}N values. In particular, experimental data provided by Li et al. (2007) in the Sichuan river basin (China) showed that δ^{15}N-NH$_4^+$ in atmospheric precipitation vary from −13.4‰ to +2.3‰ (mean value −6.6‰ ± 4.0‰). Isotope analyses conducted on rainwater samples from Zunyi in China, also demonstrated negative (approximately −12‰) δ^{15}N-NH$_4^+$ values (Li et al., 2010). The inflow of NH$_4^+$ originating from decomposition of organic matter in sediments and soils may also influence the isotopic signature of δ^{15}N-NH$_4^+$ in groundwater. In general, δ^{15}N-NH$_4^+$ in soil or sediments usually differs from the isotopic composition of total organic N in such samples only by ±1‰ (Kendall, 1998). This is explained by the small magnitude of fractionation effect occurring during mineralization of organic matter. Norrman et al. (2015) revealed that NH$_4^+$ detected in groundwater of the Nam Du area (Hanoi, Vietnam) originated from the overlaying peat which exhibited the isotopic signature of total N in the range of +2.4 to +4.1‰. In addition, Hinkle et al. (2007) (Table 2) during the studies of groundwater in La Pine (Oregon, USA)

concluded that the observed groundwater NH$_4^+$ concentration of 38 mg/l were likely due to mineralization of organic N, with measured δ^{15}N-NH$_4^+$ of 2.5–3.9‰.

To sum up, the most negative values of δ^{15}N-NH$_4^+$ could be observed in rainwater, while the highest positive isotopic signatures are typical for animal manure and sewage. At the same time, organic matter exhibits slightly higher δ^{15}N-NH$_4^+$ isotopic composition in comparison to synthetic fertilizers. However, the available experimental evidence also suggests that in practice the isotopic signals of various NH$_4^+$ sources (Fig. 4) might overlap due to the peculiarities of environmental settings in certain areas.

2.2.2. Isotopic effects of ammonium production/consumption processes

The existing body of research devoted to exploration of δ^{15}N-NH$_4^+$ variability in groundwater of agricultural areas demonstrate that during the transport of contaminants within the hydrogeological system the initial δ^{15}N values of NH$_4^+$ pollution sources can undergo considerable changes due to mineralization, sorption, volatilization, nitrification, anammox and dissimilatory NO$_3^-$ reduction to NH$_4^+$ (DNRA). So far, significant research efforts have been devoted to estimation of
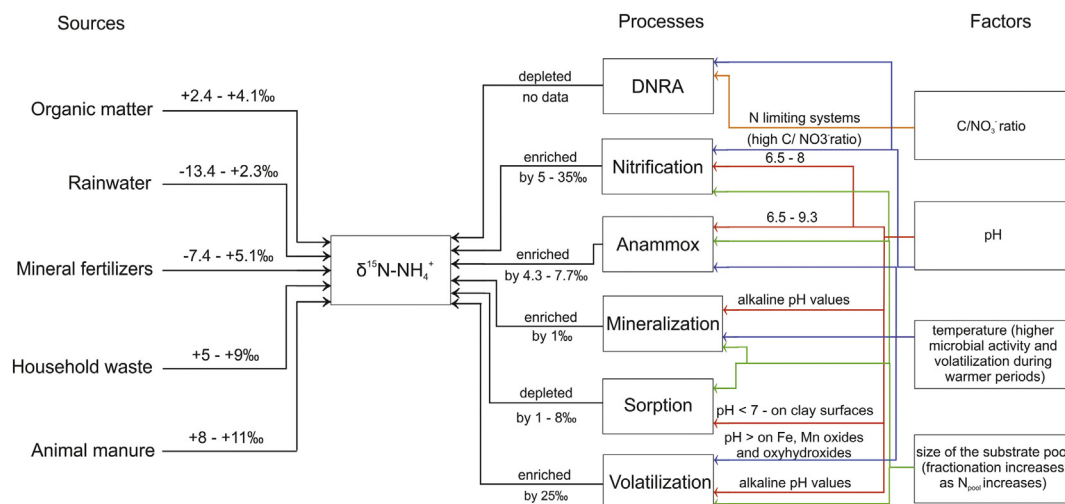

Fig. 4. Sources, processes and factors that influence the δ^{15}N-NH$_4^+$ values: summary (the following arrows connect processes with factors that have the decisive effect on their dynamics and, consequently, on resulting fractionation effects: ⟶ C/NO$_3^-$ - ratio; ⟶ pH; ⟶ temperature; ⟶ size of the substrate pool).

fractionation effects of different processes which underlie the observed δ^{15}N-NH$_4^+$ variability (Norrman et al., 2015; Zhu et al., 2013; Jin et al., 2012; Michener and Lajtha, 2007; Böhlke et al., 2006, Buss et al., 2004).

The conducted analysis showed that mineralization or ammonification usually causes only small fractionation (nearly ± 1‰) between soil organic matter and soil NH$_4^+$ (Sharp, 2007). According to Michener and Lajtha (2007), the term mineralization might be used to describe the overall process of production of NO$_3^-$ from organic matter, which usually involves several reaction steps. Under such definition, observed fractionation ranged from − 35 to 0‰, depending on which step was considered as the limiting one (Michener and Lajtha, 2007). However, the results of such observations should be used cautiously, since such large and variable range might be attributed not to the mineralization step itself, but rather to nitrification of NH$_4^+$ to NO$_3^-$.

Small isotopic fractionations have been reported for NH$_4^+$ sorption/ desorption processes on charged surfaces of clays and other minerals. According to laboratory studies, NH$_4^+$ sorbed from solutions by clays commonly is enriched in ^{15}N relative to the NH$_4^+$ that remains in solution (Böhlke et al., 2006). These results support the findings of the research accomplished by Delwiche and Steyn (1970) which showed that ion-exchange fractionations between kaolinite and solution are in the range of 0.7–0.8‰. Also, Hübner (1981) showed that ion-exchange fractionations are commonly in the range of 1 to 8‰ and stated that the actual fractionation is dependent on concentration and the fractionation factor for the exchange with the clay material. According to Kendall (1998) the fractionation factor will probably vary with depth in the soil because of changes in clay composition and water chemistry (Kendall, 1998). These factors might retard or intensify sorption processes leading, respectively, to enrichment or depletion of ^{15}N-NH$_4^+$ in groundwater.

Volatilization is a highly fractionating process in which the produced NH$_3$ gas has a lower δ^{15}N value than the residual NH$_4^+$. It involves several steps that cause fractionation, including: 1) equilibrium fractionation between NH$_4^+$ and NH$_3$ in solution, and between aqueous and gaseous NH$_3$; 2) kinetic fractionation caused by the diffusive loss of ^{15}N-depleted NH$_3$. In general, the overall dynamics of the process leads to the enrichment of the remaining NH$_4^+$ in ^{15}N on the order of 25‰ in comparison to the volatilized NH$_3$. However, it is noticed that the actual fractionation could depend on the pH and temperature (Bedard-Haughn et al., 2003).

Nitrification of NH$_4^+$ is a two-step process which yields ^{15}N-depleted products and commonly results in a substantial increase of δ^{15}N-NH$_4^+$ value. As was mentioned in the previous section (Section 2.1.2), the oxidation of NH$_4^+$ to NO$_3^-$ enriches the remaining NH$_4^+$ by approximately 30‰ in comparison to produced NO$_3^-$. In general, the total fractionation associated with nitrification depends on which step is rate determining. Because the oxidation of NO$_2^-$ to NO$_3^-$ is rapid in natural systems, this step is usually not considered as the rate-determining one, and most of the observed N fractionation is caused by the slower oxidation of NH$_4^+$ to NO$_2^-$ (Michener and Lajtha, 2007). The extent of fractionation during nitrification is also evidently dependent on the fraction of the substrate pool that is consumed during the process (refer to Section 2.2.3. for further details).

Anammox or anaerobic oxidation of NH$_4^+$ to N$_2$ leads to a slight enrichment of the residual NH$_4^+$ by 4–8‰ (Clark, 2015; Robertson et al., 2012). The low fractionation effect of anammox process, usually observed during field studies, could probably be caused by the presence of greater reservoir of NH$_4^+$ sorbed on the aquifer that buffers the enrichment of δ^{15}N in the dissolved NH$_4^+$ in the explored cases (Clark, 2015). So far, the anammox process was detected mostly within the long pollution plumes (i.e., from several hundred meters to 1 km in length) originating from point pollution sources (septic tanks, industrial or residential effluents). For example, Smith et al. (2015) and Böhlke et al. (2006) explored anammox activity in the contaminated groundwater plume created by land disposal of treated wastewater which appeared at the location of Cape Cod (Massachusetts, USA). Similary,

Robertson et al. (2012) explored the possibilities for occurrence of anammox conditions in a septic system plume originating from the washroom facility located on the north shore of Lake Erie (between USA and Canada).

Since it has been discovered that, under anaerobic conditions, NO$_3^-$ may also be reduced to NH$_4^+$ by a process known as DNRA, it is necessary to consider its potential impact on δ^{15}N-NH$_4^+$ as well. In general, this process occurs under the same conditions as denitrification, but is less commonly observed in practice. While, to the best of our knowledge, the reports devoted exclusively to the investigation of the N isotope fractionation occurring during DNRA are yet not available, broader studies conducted so far have demonstrated that NH$_4^+$ produced by DNRA has much lower δ^{15}N that the substrate NO$_3^-$, which suggests an ongoing kinetic fractionation (Michener and Lajtha, 2007).

2.2.3. Factors controlling ammonium production/consumption processes and their impact on δ^{15}N-NH$_4^+$ variability

The extent of fractionation effect caused by NH$_4^+$ transformation processes depends on multiple environmental factors (Fig. 4) which, therefore, can substantially influence the observed dynamics of δ^{15}N values of NH$_4^+$ in the subsurface. Among these factors, pH, temperature and size of the substrate pool are the ones most discussed in the available research literature.

The pH parameter defines the intensity of not only microbiological reactions, but also affects the rate of volatilization: it is proved that this process is intensified under the alkaline soil pH (Witter and Lopez-Real, 1988). For this reason, the observed high rates of NH$_3$ volatilization are associated with the high carbonate content of soils (Bedard-Haughn et al., 2003). For example, in the unconfined High Plains aquifer (USA) NH$_3$ volatilization was promoted by the calcareous soils of the area (McMahon and Böhlke, 2006). At the same time, the pH values which support the development of DNRA are unclear. Some studies indicated that high rates of DNRA are associated with alkaline conditions, while the other ones revealed the negative correlation between DNRA occurrence and pH parameter (Rütting et al., 2011). As for N mineralization process, it tends to become more intensive with an increase of pH values towards more alkaline range (Curtin et al., 1998; Fu et al., 1987). At pH ˂ 7, NH$_4^+$ is predominantly sorbed on clay surfaces, and at higher pH values it starts to be sorbed by metal oxides and oxyhydroxides (e.g. FeOOH, MnO$_2$) (Buss et al., 2004).

The temperature variability can also have an impact on the changes in dynamics of δ^{15}N-NH$_4^+$ values. It should be particularly noticed that higher temperatures are also associated with the increasing rate of ongoing NH$_3$ volatilization, since they stimulate growth and activity of bacteria. Consequently, it can be expected that the isotopic composition of N species exhibits pronounced seasonal patterns (Bedard-Haughn et al., 2003). The optimal temperature range for mineralization is 25–40°, for nitrification – 15–35° and for anammox – 30–40° (Li et al., 2014a, 2014b; Guntiñas et al., 2012; Shammas, 1986; Jin et al., 2012).

In addition, the extent of observed fractionation effects is assumed to be dependent on the size of the substrate pool (reservoir). Usually, in N-limited systems, fractionation associated with nitrification is comparatively small. For instance, NH$_4^+$ concentration in groundwater of the Sichuan basin in China (Table 2) were low (and even occasionally below the detection limit (0.05 mg/l)), suggesting minimal isotopic fractionation during nitrification in groundwater (Li et al., 2007).

Finally, it should also be noticed that the relative concentrations of NO$_3^-$ to organic C (C/NO$_3^-$ ratio) control whether NO$_3^-$ is reduced by denitrification or DNRA. In general, DNRA, which leads to the production of isotopically depleted NH$_4^+$, is favored when NO$_3^-$ is limiting, while denitrification is favored when C (electron donor) is limiting (Vidal-Gavilan et al., 2013).

The presented evidence suggests that the variability in the δ^{15}N-NH$_4^+$ in groundwater heavily depends both on the type of pollution sources as well as on the dynamics of microbiological and physicochemical processes (Fig. 4).

In general, δ^{15}N-NH$_4^+$ values in groundwater are lower and less variable in comparison to δ^{15}N-NO$_3^-$, which is probably explained by the high sorption potential of NH$_4^+$ and it intensive involvement into oxidation processes. Among the pollution sources, animal wastes and household sewage contribute to the highest enrichment of NH$_4^+$ in groundwater with ^{15}N isotope. As for the processes resulting in isotope fractionation and respective changes in isotopic signatures of groundwater samples, it is revealed that volatilization and nitrification significantly contribute to higher accumulation of ^{15}N in the residual NH$_4^+$. However, the extent of fractionation effects due to these processes may depend on the environmental conditions. On the contrary, mineralization and sorption usually show small isotopic effects. Finally, there is still not much evidence available about the quantitative alterations in the isotopic composition of NH$_4^+$ during DNRA (Michener and Lajtha, 2007).

2.3. Variability of δ^{15}N-N$_2$O in groundwater

The information about the isotopic composition of δ^{15}N-N$_2$O in aquifers affected by agricultural activity is also scarce, as in the case of data regarding the natural abundance of ^{15}N-NH$_4^+$. In general, it has been reported that the values of δ^{15}N-N$_2$O could vary from −55.4‰ to +89.4‰ (Table 3). So the isotopic signatures of N$_2$O in groundwater samples demonstrate the largest variability among different isotopic compositions of N compounds considered in this review. It appears that such wide range of observed δ^{15}N-N$_2$O values is related to the fact that the production of N$_2$O involves many reactions steps (Fig. 4) which presume diverse fractionation effects depending on chemical processes kinetics and heterogeneous conditions of the subsurface environment along the vertical and lateral groundwater flow paths. Evidently, it also reflects the impact of the diversity of isotopic signatures of the initial substrates (e.g., NO$_3^-$, NH$_4^-$) and their involvement into microbial processes. In particular, according to previous studies, δ^{15}N values of N$_2$O emitted from fertilized soils are predominantly negative, which is explained by ^{15}N depletion during N$_2$O production by nitrification and denitrification. At the same time, positive δ^{15}N-N$_2$O values are likely to be attributed to ongoing N$_2$O reduction during denitrification (Well et al., 2005). Further discussion of the factors influencing variability of δ^{15}N-N$_2$O in groundwater will be devoted predominantly to shifting dynamics of various hydrobiogeochemical processes that affect the isotopic composition of N$_2$O. The isotopic signatures of NO$_3^-$ and NH$_4^+$ derived from various pollution sources have been described in more detail in the previous sections (namely, Sections 2.1.1. and 2.2.1).

2.3.1. Isotopic effects of nitrous oxide production/consumption and transport processes

The experimental evidence suggests that changes in N$_2$O isotopic signatures are caused by both physical and microbial processes. It is generally assumed that the enrichment factors of microbial processes tend to be large than those related to physical processes (Goldberg et al., 2008). Among the bacterial transformations, denitrification,

nitrification and nitrifier denitrification are the processes that seem to be the most discussed in the research literature in the context of the isotopic composition of δ^{15}N-N$_2$O (Jurado et al., 2017; Well et al., 2012; Clough et al., 2005). As for the impact of physical processes, it appears that diffusion frequently might be responsible for the alterations of detected δ^{15}N–N$_2$O values.

In the denitrification pathway, N$_2$O is produced as well as consumed during the subsequent reduction of NO$_3^-$ to N$_2$ (NO$_3^- \rightarrow$ NO$_2^- \rightarrow$ NO $\rightarrow$ N$_2$O $\rightarrow$ N$_2$) (Fig. 1). The δ^{15}N values of N$_2$O derived from denitrification depends upon the isotope fractionation during its production and consumption. N$_2$O originated from the reduction of NO$_3^-$ is typically depleted in ^{15}N in comparison to the initial substrate (NO$_3^-$). The reduction of N$_2$O to N$_2$ results in the enrichment of the residual N$_2$O. It is reported that the isotope fractionation factors for N during both processes are of comparable order of magnitude (Ueda et al., 1991). If N$_2$O is accumulated as the intermediate product of steady-state denitrification, it is observed that, its δ^{15}N value should become close to the value of the initial substrate NO$_3^-$. Correspondingly, significant N isotope discrimination between N$_2$O and NO$_3^-$ in groundwater might suggest that a large portion of N$_2$O may originate from nitrification (Ueda et al., 1991).

Nitrification, which is also a multistep reaction (NH$_3$ / NH$_4^+ \rightarrow$ H$_2$N-OH $\rightarrow$ NO$_2^- \rightarrow$ NO$_3^-$), yields N$_2$O which is isotopically light in comparison to its precursors. N$_2$O derived during this process could be produced as a byproduct from the complete or partial direct oxidation of H$_2$N-OH to NO or N$_2$O (Schmidt et al., 2004).

In addition, at low DO level, N$_2$O production is likely to proceed via nitrifier denitrification, i.e. NO$_2^-$ reduction to N$_2$O, which yields isotopic signatures similar to bacterial denitrification (Well et al., 2012). Consequently, these two processes cannot be distinguished using solely the data regarding ^{15}N isotope natural abundance, and additional evidence is necessary (Wells et al., 2016; Zhu et al., 2013).

The isotopic composition of N$_2$O detected in the groundwater samples can also be significantly influenced by its upward diffusion and volatilization from shallow groundwater to the atmosphere (Minamikawa et al., 2011). Available experimental data indicate that in the subsoil environment characterized with high diffusivity exchange with atmospheric N$_2$O may diminish the effects of isotopic fractionations expected from the previously described microbial processes (Goldberg et al., 2008). The rate of occurring diffusion depends mainly on the water content in the subsoil. The higher water content suggests that the time required for N$_2$O to diffuse from the soil profile to the surface is also increased, since diffusion of N$_2$O in water is approximately 4 orders of magnitude lower than in air (Clough et al., 2005. In addition, it should be highlighted that the macropores and cracks can also enhance the upward N$_2$O diffusion (Minamikawa et al., 2011).

To summarize, the research accomplished so far has demonstrated that both nitrification and denitrification processes are responsible for the depletion of ^{15}N value of N$_2$O in comparison to its substrates (Toyoda et al., 2017; Schmidt et al., 2004; Ueda et al., 1991). However, further reduction of N$_2$O to N$_2$ during denitrification leads to the enrichment of the remaining N$_2$O with ^{15}N (Clark, 2015; Knöller et al., 2011).

Table 3
Analysis of N$_2$O isotopic signatures in groundwater: an overview of case studies (D – denitrification; s. d. – standard deviation).

Site	δ^{15}N (‰)	δ^{18}O (‰)	Processes altering the δ^{15}N and δ^{18}O of N$_2$O	N$_2$O (mg/l)
Fuhrberger Feld aquifer (Lower Saxony, Germany) (Well et al., 2012)	−55.4–+89.4 (mean −11.0 (s. d. 21.0))	+17.6–+113.2 (mean +57.5 (s. d. 24.9))	D	0.001–3.7 (mean 0.08)
Großenkneten aquifer (Lower Saxony, Germany) (Well et al., 2012)	−40.5–+11.7 (mean −9.7 (s. d. 11.2))	+32.6–+87.6 (mean +46.1 (s. d. 13.9))	D	0.005–0.2 (mean 0.03)
Northwest German lowland, (Lower Saxony, Germany) (Well et al., 2005)	−41.6–+86.1	+20.7–+89.8	D	0.008–4.2
shallow groundwater under the lysimeter facility (Japan) (Minamikawa et al., 2011)	−44.7 to −16.8	+39.1–+49.4	D	—

In comparison to biochemical processes occurring in aquifers, diffusion usually results in less pronounced isotopic effects. However, the distribution of the δ^{15}N-N_2O values in groundwater cannot be comprehensively analyzed and clearly interpreted without referring to the heterogeneity of environmental factors (Fig. 5) of the studied hydrogeological systems.

2.3.2. Factors controlling nitrous oxide production/consumption processes and their impact on δ^{15}N-N_2O variability

Among the factors controlling the dynamics of N_2O production/consumption processes and resulting variations in δ^{15}N-N_2O values, the residence time, DO concentration, availability of substrate and pH are typically considered as the most decisive in the literature.

As the concentration of NO_3^- within a denitrifying layer diminishes with increasing residence time of groundwater, it appears, that with longer residence time, NO_3^- reduction to N_2 is more likely to be complete (provided the is no additional supply of NO_3^- and a sufficient amount of electron donors), which means that the isotopic compositions of δ^{15}N-NO_3 and δ^{15}N-N_2O become closer. At the same time, the instantaneously produced N_2O is typically depleted with respect to the NO_3^- signature (Well et al., 2005).

The DO concentration significantly impacts the isotopic signatures of N_2O in groundwater, because it determines the type of dominant microbial processes in the aquifer and it also affects the completeness of their reaction steps. In particular, under anaerobic conditions, microbial nitrification is unlikely to occur, at least the groundwater table (Goldberg et al., 2008), and denitrification usually prevails under such conditions. In particular, it is reported that denitrification might yield the highest N_2O amounts at intermediate O_2 concentrations (below 3.15 to 4 mg/l) as most denitrifiers are facultative anaerobes (Deurer et al., 2008). That is why it is frequently reported that the NO_3^- consumption, which is associated with the formation of excess N_2 and intermediate accumulation of N_2O, increases with the depth (Well et al., 2012).

In sequential reaction processes, such as denitrification, the supply of the members of the denitrification pathways, i.e., NO_3^-, NO_2^-, NO, N_2O, N_2, depends on the rate of previous reaction steps, except for NO_3^- which can be introduced to the system from the external sources. The availability of substrate, therefore, seems to have considerable impact on the magnitude of isotopic fractionation occurring during N_2O production/consumption processes. In particular, if NO_3^- supply is high in relation to reduction capacity of the subsurface system, substantial isotope fractionation effect occurs, whereas the effect is low or negligible in the opposite case. Overall, the same fractionation control principle appears to be relevant for the other N species subject to reduction during further stages of denitrification, namely NO_2^-, NO, and N_2O. However, for these species the situation is even more complicated, not only because their respective pool sizes depend on the rates of the previous reactions, but also because some microbes might lack enzymes for some of the reduction steps, which implies that transport within denitrifying species will be a necessary precondition for further reduction in such cases (Well et al., 2005). As a result, the isotopic signature of N_2O as an intermediate is influenced both by the kinetics of its production during NO reduction and consumption during N_2O reduction to N_2 affected by the availability of reaction substrates on the corresponding transformation steps.

It has been found that pH values below 5.5 seem to promote accumulation of N_2O, most probably because N_2O reductase is mostly inhibited by acid conditions that enable the build-up of N_2O in the subsurface environment (Deurer et al., 2008), and the denitrification process does not proceed to the final step.

Overall, since N_2O is an intermediate product of microbial reactions, its isotopic composition is determined by the rates of previous reactions as well as biological and physicochemical conditions of the aquifer (Fig. 4). It could be summarized that production processes of N_2O (e.g., nitrification, denitrification, etc.) lead to its depletion in the δ^{15}N value, whereas consumption processes, such as reduction of N_2O to

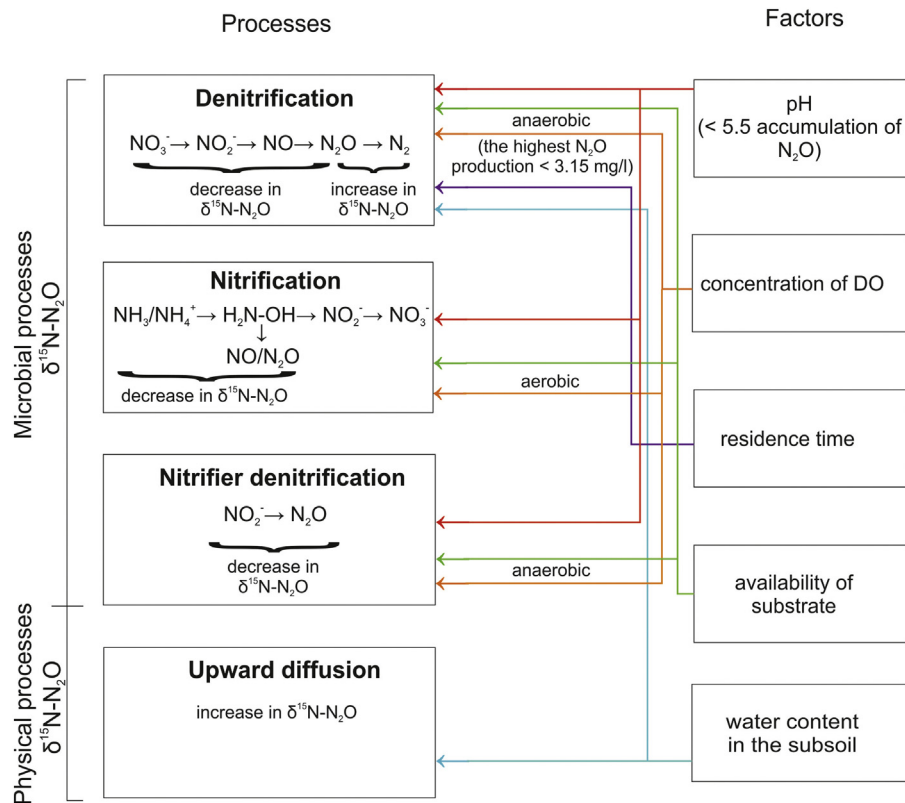**Fig. 5.** Sources, processes and factors that influence the δ^{15}N-N_2O values: summary (the following arrows connect processes with factors that have decisive effect on their dynamics and, consequently, on resulting fractionation effects: ⟶ water content in the subsoil; ⟶ availability of substrate; ⟶ residence time; ⟶ concentration of DO; ⟶ pH).

N_2, enrich it with ^{15}N. Residence time, DO concentration, substrate availability and pH are important parameters that affect the intensity of N_2O isotope fractionation processes. The large variability of $\delta^{15}N$ value of N_2O in the groundwater (Table 3) implies that N_2O production and consumption processes in the hydrogeological system occur simultaneously. However, the isotopic fractionation effects of these processes might be diminished by the effects of upward diffusion.

3. Complementary investigations based on other stable isotopes

Measurements and analysis of $\delta^{15}N$ values in groundwater are commonly complemented with analysis of isotope enrichment values of other isotopes in order to address and constrain the potential ambiguity in the interpretation of $\delta^{15}N$ variation associated with overlapping of $\delta^{15}N$ isotopic signatures resulting from different sources and processes. O, B, C, S, Sr isotopes are among the isotopes most frequently considered for such purpose (Hosono et al., 2014; Well et al., 2012; Di Lorenzo et al., 2012; Otero et al., 2009; Knöller et al., 2005; Widory et al., 2004, Choi et al., 2003; Böhlke and Horan, 2000). In the following section, discussion will be focused on their application to identification of N transformation processes and potential sources of N pollution, respectively.

3.1. Analysis of $\delta^{18}O$ values of nitrogen species in groundwater

Combined use of the $\delta^{18}O$ and $\delta^{15}N$ of NO_3^- may allow better separation of atmospheric and terrestrial NO_3^- sources, including the possible separation of different anthropogenic sources (Xue et al., 2009). In addition, oxygen isotope ratios could be used for distinguishing N_2O originating from nitrification and denitrification (Kendall, 1998). Table 1 shows that the isotopic signature of $\delta^{18}O\text{-}NO_3^-$ in groundwater might vary in the range between −8.1‰ to +48‰, which reflects the variability of NO_3^- sources.

In particular, the isotopic signature $\delta^{18}O\text{-}NO_3^-$ could help to separate NO_3^- originated from the fertilizers application from NO_3^- inflow originating from other sources which deliver NO_3^- produced by nitrification of NH_4^+ or organic N. It is observed that synthetic NO_3^- fertilizers, which are derived from the atmospheric N_2, have $\delta^{18}O$ value close to the atmospheric value of +23.5‰ (Moore et al., 2006). In particular, their isotopic composition of $\delta^{18}O\text{-}NO_3^-$ might vary from +17‰ to +25‰ (Xue et al., 2009). Meanwhile, NO_3^- from other sources tend to have lighter $\delta^{18}O$ values because the NO_3^- derived from nitrification processes incorporates only one O atom from dissolved atmospheric O_2 and the other two atoms from water (Kendall and Aravena, 2000). In general, isotopic signature of $\delta^{18}O\text{-}NO_3^-$ originated from nitrification can be calculated using the following equation (e.g. Hollocher, 1984):

$$\delta^{18}O_{NO_3^-} = 1/3 * \delta^{18}O_{O_2} + 2/3 * \delta^{18}O_{H_2O}$$

Nitrification has been associated with the $\delta^{18}O\text{-}NO_3^-$ values in a range between −2‰ to +6‰ (Liu et al., 2006; Sebilo et al., 2006; Smith et al., 2006) or approximately 0‰ (Böhlke et al., 2006). However, it should be emphasized that the isotopic composition of NO_3^- produced by nitrification depends on a range of factors which might alter those numbers: 1) H_2O might be enriched in ^{18}O isotope because of evaporation (Hoefs and Hoefs, 2015; Sharp, 2007), 2) O isotope fractionation during respiration can increase the $\delta^{18}O$ value of soil O_2 in comparison to that of atmospheric O_2 (Mayer et al., 2001), 3) the ratio of O incorporation from H_2O and O_2 is not exactly 2:1 (e.g. more O_2 may be derived from atmospheric O_2 when NH_4^+ is limiting) (Knöller et al., 2011; Kool et al., 2011), 4) low pH conditions might support the occurrence of another microbial process that consume atmospheric O_2 more intensively than nitrification consequently resulting in suppression of nitrification (Xue et al., 2009; Liu et al., 2006), and 5) oxygen isotope exchange of intermediates (especially NO_2) with ambient water might occur (Granger and Wankel, 2016; Casciotti et al., 2010; Kool et al., 2011).

Oxygen isotopes can also be used to trace denitrification in groundwater, as ^{18}O and ^{15}N become concurrently enriched in the remaining NO_3^- during bacterial denitrification (Petitta et al., 2009). Several studies reported constant isotope ratios that indicate enrichment of ^{15}N relative to ^{18}O as the evidence of denitrification occurrence: 2:1 (Kendall and Aravena, 2000), 1.5:1 (Baily et al., 2011), 2.1:1 (Aravena and Robertson, 1998) and 1.4:1 (Knöller et al., 2011; Mengis et al., 1999). During denitrification, the isotopic signature of the residual $\delta^{18}O\text{-}NO_3^-$ tends to be enriched by nearly 10‰ or 8–18‰ in comparison to the produced N_2O (Clark, 2015; Xue et al., 2009). Therefore, N_2O that is instantaneously produced is depleted in ^{18}O. According to Casciotti et al. (2002), the value of $\delta^{18}O$ is also affected by oxygen exchange with water, with the exchange ratio varying across different microbial species (Well et al., 2005).

It is also important to take into account that the isotopic expression of $\delta^{18}O\text{-}NO_3^-$ in groundwater might be influenced by atmospheric precipitation. Its $\delta^{18}O$ values can vary within an interval between +30 and +70‰ (Choi et al., 2003). Williard et al. (2001) demonstrated a seasonal variation of $\delta^{18}O\text{-}NO_3^-$ in atmospheric NO_3^- deposition. Durka et al. (1994) and Voerkelius (1990) have associated atmospheric NO_3^- with values of $\delta^{18}O$ between 52.5‰. and 73.4‰. However, usually such high values of $\delta^{18}O$ are found in groundwater under forest ecosystems that are not undergoing significant anthropogenic impact, and are not typical for the case of arable lands (Böttcher et al., 1990).

In general, it is clear that typical $\delta^{18}O$ values of NO_3^- originated from nitrification (including $\delta^{18}O$ values of NO_3^- derived from NH_4^+ in fertilizers and precipitation, NO_3^- derived from soil N and NO_3^- derived from manure and sewage) are lower than that of NO_3^- from precipitation and NO_3^- from application of fertilizers. Denitrification is responsible for the simultaneous enrichment of the remaining NO_3^- with ^{18}O and ^{15}N isotopes which might be traced in accordance to certain constant ratios. Therefore, application of O isotopes analysis along with N isotopes measurement can help to understand better the nature of $\delta^{15}N$ variability in groundwater.

3.2. Boron as a tracer for identification of nitrogen sources

Boron isotopes (i.e., ^{11}B and ^{10}B) have been used to trace sewage contamination in groundwater in a range of studies (Xue et al., 2009). Since the isotopic composition of B is not affected by the denitrification process, it also can be used as an indicator of mixing processes in hydrogeological systems (Widory et al., 2004). For instance, analysis of B isotopes was used for identification of pollution sources in the Arguenon watershed, the "Roussillon" aquifer and the "Ile du Chambon" catchment (Table 1) in France (Widory et al., 2005).

At the unpolluted sites B originates either from mixing with seawater, or from weathering of sandstones and igneous rocks, or could be found in certain evaporates, such as borax $(Na_2B_4O_5[OH]_4 \cdot 8H_2O)$ (Clark, 2015). In such context, natural B concentrations are typically only a few ppb in groundwater. However, they are significantly higher in liquid manure and septic tank effluents.

The isotopic signature of $\delta^{11}B$ of sewage reported in the literature ranges from −7.7‰ to +12.9‰ (Xue et al., 2009). Widory et al. (2004) distinguished two types of sewage: a high-B/low-NO_3^-/low-$\delta^{11}B$ type that is derived from washing powders, and a moderate-B/moderate-NO_3^- type with an isotopic signature close to animal manure (probably human excrement).

The $\delta^{11}B$ value of animal manure covers the interval from +14.5‰ to +42.5‰ (Widory et al., 2005). These values are, generally, higher than the ones reported for fertilizers whose $\delta^{11}B$ isotopic expression might fluctuate between +8‰ and +17‰.

It should be mentioned that sorption on clay minerals, iron and aluminum oxides along groundwater flow can enrich the residual B in solution with ^{11}B isotope at the pH value above 8, when the anion $B(OH)_4^-$ becomes important (Clark, 2015). However, Kloppmann et al. (2009) showed that at neutral pH, B transport characterized with

predominance of $B(OH)_3$ is nonfractionating, and could therefore be used as a reliable tracer of source and mixing processes.

Thus, analysis of abundance of B isotopes appears to be useful in identification the sources of N contamination. The combined use of $\delta^{11}B$ and $\delta^{15}N$ values along with the data regarding concentrations of the respective compounds can help to distinguish between multiple NO_3^- sources as well as to reveal the occurrence of mixing processes. Nevertheless, during the studies the possibility of the adsorption-desorption interaction with clay and other material should be considered as it might affect B isotopic composition.

3.3. Analysis of carbon and sulfur isotopes in groundwater systems

It is a common practice to support the results of studies of N isotope in groundwater, which indicated the occurrence of denitrification, with additional measurement of the $\delta^{13}C$-DIC and $\delta^{34}S$-SO_4^{2-} values in order to identify which type of denitrification is governing the dynamics of N species (Hosono et al., 2014; Otero et al., 2009; Aravena and Robertson, 1998). This experimental approach could be employed to distinquish between two main denitrification pathways that are observed in aquifers: heterotrophic denitrification, which requires organic C source, and autotrophic denitrification, which uses zero-valent iron, ferrous ions, elemental sulfur or reduced sulfur compounds such as pyrite (FeS_2) as an electron donor (Hosono et al., 2014). While the former one generates CO_2 as one of the reaction products, the later one produces SO_4^{2-} through elemental sulfur or FeS_2 (Rivett et al., 2008).

Heterotrophic denitrification is associated with the decrease in the $\delta^{13}C$-DIC and increase in $\delta^{15}N$-NO_3^- values. The decrease in $\delta^{13}C$-DIC is related to the fact that the organic source of carbon is isotopically more depleted in ^{13}C compared to that of the dissolved inorganic carbon pool (e.g. carbonate, bicarbonate). That is why the $\delta^{13}C$ values of DIC derived from organic matter are more negative than the values of DIC originated from non-organic sources (Nascimento et al., 1997). The values of $\delta^{13}C$-DIC originated from organic carbon are reported to vary in the range between $-29‰$ to $-25‰$ (Aravena and Robertson, 1998). However, in the aquifer these values can be buffered by dissolution of carbonate minerals which have higher isotopic signature of C. For example, Aravena & Robertson attributed the decrease in the $\delta^{13}C$-DIC values (from -1.9 to $-8.6‰$) in the groundwater system to denitrification processes, the occurrence of which was evidenced by substantial rise in $\delta^{15}N$-NO_3^- values (from 6.4 to 58.3‰).

Autotrophic denitrification, through FeS_2 oxidation, produces SO_4^{2-} depleted in ^{34}S, since sulfur in sulphide minerals is typically characterized with smaller $\delta^{34}S$ values in comparison to that of sulfate pools in earth surface environments (Krouse and Grinenko, 1991). For instance, Otero et al. (2009) explained the detected decrease in the $\delta^{34}S$-SO_4^{2-} values (from 10 to $-20‰$) accompanied by the increase in the isotopic signature signals of NO_3^- as the result of progress of autotrophic denitrification in the polluted deep aquifer in eastern Spain. Similar changes of the sulfate sulfur isotopic composition (from $+10$ to $-10‰$) due to the impact of autotrophic denitrification in an aquifer used for drinking water production were reported by Knöller et al., 2005.

While the decline in the $\delta^{13}C$-DIC or $\delta^{34}S$-SO_4^{2-} values in groundwater is the sign of heterotrophic or autotrophic denitrification, respectively, their increase is usually the evidence of other bacterial processes which typically occur in the anaerobic conditions after denitrification (denitrification → sulfate reduction → methanogenesis) (Korom, 1992). Studying the limestone aquifer in the eastern England, Moncaster et al. (2000) detected significant enrichment of SO_4^{2-} with ^{34}S (up to $+30‰$) as a result of sulfate reduction. Hosono et al. (2014) related the enriched isotopic values of ^{13}C-DIC ($+8‰$) in groundwater under the Kumamoto area (Japan) to the occurrence of methanogenesis. This idea was supported by the fact that high CH_4 concentrations (up to 1 mg/l) were detected at the studied locations.

Therefore, it is obvious that additional analysis of $\delta^{13}C$-DIC and $\delta^{34}S$-SO_4^{2-} in groundwater can help to identify certain hydrogeochemical processes (denitrification, DNRA, sulfate reduction or methanogenesis) in the aquifers and understand their intensity. It is especially helpful to include the measurements of these isotopes into experimental studies in the cases when the occurrence of denitrification processes is suspected, since such approach will help not only to differentiate between different types of denitrification pathways, but also reveal other bacterial processes that follow denitrification in groundwater heavily depleted in oxygen.

3.4. Strontium isotope as a tracer of mixing processes in subsurface environment

In contrast to N, O, B, C and S isotopes, Sr isotopes are characterized with a low biological and/or geological fractionation which make them effective tracers of transport (mixing) processes in the environment (Vilomet et al., 2001). The $^{87}Sr/^{86}Sr$ ratios in groundwater are predetermined by:

1) natural sources of Sr (e.g., mineral dissolution or cation exchange in soils and aquifer);
2) anthropogenic sources of Sr (e.g., mineral fertilizers or manure) (Widory et al., 2004; Böhlke & Horan, 2000).

During the study of groundwater in the Brittany region (France) Widory et al. (2004) detected that $^{87}Sr/^{86}Sr$ ratios of the anthropogenic sources vary from 0.7078 to 0.7145 with the lowest values corresponding to mineral fertilizers and the highest values to animal manure. However, this study showed the difficulties in distinguishing between different types of animal manure, which exhibited overlapping ranges from 0.709 to 0.712. The groundwater of the studied area showed varying $^{87}Sr/^{86}Sr$ ratios (from 0.7146 to 0.7196) suggesting the occurrence of mixing between different Sr sources, in particular Sr derived from animal manure and from water-rock interaction.

Böhlke & Horan (2000) examined the relationship between the age of groundwater and the distribution of Sr. It was revealed that higher $^{87}Sr/^{86}Sr$ ratios (0.713–0.715) are associated with younger oxic groundwater which is affected by anthropogenic activity, and the lower $^{87}Sr/^{86}Sr$ ratios (0.708–0.710) are typical for older suboxic groundwater where Sr is originated from calcareous glauconitic sediments.

To summarize, Sr isotope ratio is the useful parameter for studying mixing processes in the groundwater system, as it helps to determine the behavior of pollutants from different sources. In general, natural sources of Sr are typically characterized with lower $^{87}Sr/^{86}Sr$ ratio compared to anthropogenic ones usually exhibiting higher values of this parameter.

4. Conclusions

The versatility of the stable isotope analysis method enables obtaining a comprehensive insight into transport and transformation of NO_3^-, NH_4^+ and N_2O in the subsurface: from the assessment of relative contributions of different N sources into the system (using distinctions between their respective isotopic signals) to the identification of simultaneously occurring N cycle reactions and physicochemical processes affecting the isotopic composition of N species. Such information is especially valuable for sustainable management of groundwater resources in agricultural areas typically characterized with considerable N loadings and frequently exhibiting adverse effects of N pollution.

In order to capture the dynamics of N cycling using stable isotope analyses, it is necessary to understand the ranges and causes of variability of isotopic composition of NO_3^-, NH_4^+ and N_2O in various environmental settings. This review summarizes the data regarding the ranges of isotopic compositions of these N species in groundwater under agricultural areas and provides information about the impact of N sources, microbiological/physicochemical processes and environmental factors on the variability of NO_3^-, NH_4^+, N_2O isotopic signatures.

It also discusses the application of additional isotopes techniques, frequently used to support the analysis of $\delta^{15}N$ values for various N compounds.

According to the reviewed literature, the isotopic signatures of NO_3^- in groundwater are characterized with the following $\delta^{15}N\text{-}NO_3^-$ isotope ranges: soil organic N – from +3‰ to +8‰, mineral fertilizers – −8‰ to +7‰, animal manure or household waste – +5‰ to +35‰. The NH_4^+ sources are characterized with the following $\delta^{15}N$ values: organic matter – +2.4–+4.1‰, rainwater – −13.4–+2.3‰, mineral fertilizers – −7.4–+5.1‰, household waste – +5–+9‰, and animal manure – +8–+11‰. The isotopic composition of N_2O is determined by the rates of previous reactions as well as biological and physicochemical conditions of the aquifer.

Moreover, the $\delta^{15}N\text{-}NO_3^-$ values are influenced by fractionation effects caused by denitrification ($\varepsilon = 5$–40‰), nitrification ($\varepsilon = 5$–35‰) and DNRA (range of ε not available in literature). As for the isotopic signature of NH_4^+, it is also affected by nitrification and DNRA, as well as mineralization ($\varepsilon = 1$‰), sorption ($\varepsilon = 1$–8‰), anammox ($\varepsilon = 4.3$–7.4‰), and volatilization ($\varepsilon = 25$‰). $\delta^{15}N\text{-}N_2O$ values in the groundwater derive from: 1) production processes of N_2O (e.g., nitrification, denitrification, etc.) which lead to its depletion in ^{15}N, and 2) consumption processes, such as reduction of N_2O to N_2, which enrich it with ^{15}N. However, it should be emphasized that multiple environmental parameters regulate the extent of fractionation effects caused by the processes mentioned above, so the observed changes in isotopic composition of NO_3^-, NH_4^+ N_2O could vary.

Due to overlapping of the isotopic signatures of N sources and N cycle processes, interpretation of isotopic signatures of collected groundwater samples is not a straightforward process, and is associated with uncertainties. Moreover, the difficulty in interpretation of the results of N isotopes analyses are exacerbated by the lack of experimental data regarding variability of $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}N_2O$. Therefore, further research is required in order to address this issue and consider the isotopic composition of NH_4^+ and N_2O in different hydrogeological contexts. In addition, during interpretation of N isotopic signatures it is important to consider thoroughly the data obtained from hydrogeological, hydrochemical and microbiological studies which might help to elucidate N transformation and transport processes occurring in the hydrogeological systems.

Though such inclusive interpretation requires extensive amount of data, it is crucial to integrate all these insights into a flexible interpretative framework for the studies N transport and transformation processes. This could help to address the limitations of stable isotope analysis method in the complicated study cases characterized with possible occurrence of overlapping isotopic signals from different N sources and simultaneous progress of different multistep reactions with a range of intermediate products in the considered aquifer.

As the analysis of distribution of $\delta^{15}N$ values observed across the aquifer should rely on precisely determined estimations of signatures of N sources and expected fractionation effects caused by N cycle processes, it is crucial to facilitate the comparative component of the research strategies employing stable isotope analysis. There is a need to systematize the experimental evidence obtained from stable isotope analysis of groundwater samples in different studies exploring the same biogeochemical processes or similar issues.

With further advancements in these areas, stable isotope analysis will allow researchers to capture more precisely the dynamics of N species transformations in the subsurface. Therefore, it will help not only to understand better the processes of attenuation of N pollution in agricultural landscapes, but also to address efficiently the emerging environmental concerns regarding estimation of the indirect effects of anthropogenic impact in such areas. In particular, this approach will yield valuable information for the studies of N_2O production/consumption in subsurface environment and its subsequent emissions on the river-atmosphere interface. Therefore, it will enhance the understanding of N_2O cycle and, correspondingly, of the global N cycle in general.

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