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Comparison of two methods for the determination of nitrogen and oxygen isotope composition of dissolved nitrates

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Abstract. Two methods of sample preparation for isotopic analyses of nitrates dissolved in water are presented: (i) chemical conversion of NO_3^- to N_2 and CO_2, and (ii) bacterial reduction of NO_3^- to N_2O, followed by the measurement of nitrogen and oxygen isotope composition of these gases. Both methods have been successfully used for routine isotope analyses of dissolved nitrates in different types of water. The chemical conversion method, requiring ca. 300 μmol of NO_3^- yields relatively good precision, in the order of 0.3‰ for $\delta^{15}N$, and 0.6‰ for $\delta^{18}O$. The bacterial reduction method was modified in this study to accommodate samples of intermediate size (ca. 30 μmol of NO_3^-) to provide sufficient amount of N_2O gas for isotope analysis using conventional IRMS. The method demonstrated satisfactory results, although sample preparation was more complicated and required access to microbiological laboratory.

Key words: nitrates • isotope composition • denitrification • nitrogen-15 • oxygen-18

Introduction

Nitrates constitute a growing thread to quality of drinking water worldwide [17]. Both surface water and groundwater systems are affected. Increasing demand for drinking water results in growing importance of local groundwater resources. Adequate quality of water from those resources is a serious economic problem for local authorities due to high costs of water treatment. Elevated concentrations of nitrates often appear as the most important pollutant in groundwater. Increased concentration of NO_3^- poses a serious health risk, especially for children. The World Health Organisation defined the maximum permissible concentration of nitrates in drinking water at 10 mg $N/NO_3^-/dm^3$ (44 mg NO_3^-/dm^3), while the European Community Directive 98/83/EC set a maximum at 50 mg/dm^3 as NO_3^- [6]. The value of 50 mg NO_3^-/dm^3 (11.3 mg/dm^3 as N) is recommended by the Polish law [5].

Nitrate contamination in groundwater originates from various sources of both diffusive and point character, such as excessive use of fertilizers in agriculture, human and animal wastes also used as fertilizers, leakages from landfills and septic tanks, etc. [10]. Effective management practices directed towards preservation of water quality, and, if necessary, involving adequate remediation measures, require identification of major sources of nitrates and their pathways to the affected groundwater systems. Isotopic composition of nitro-

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Table 1. Published methods of nitrogen and oxygen isotope analyses of dissolved nitrates

Method	References
– separation of NO_3^- on cation exchange resin – conversion of NO_3^- to KNO_3 – reduction of KNO_3 to NH_3 with the aid of Devarda's alloy – reduction of NH_3 to N_2 with the aid of bromides – conversion/roasting of KNO_3 to CO_2 in presence of $Hg(CN)_2$ – cryogenic purification of CO_2	[1]
– separation of NO_3^- on cation exchange resin – conversion of NO_3^- to KNO_3 – conversion/roasting of KNO_3 in presence of graphite and catalyst – cryogenic separation of N_2 and CO_2 – thermal purification of N_2 in presence of CaO, Cu and Cu_2O	[14]
– extraction of NO_3^- on anion exchange resin – conversion of NO_3^- to KNO_3 – conversion/roasting of KNO_3 in presence of guanidine hydrochloride to CO_2 and NH_3 – purification of CO_2	[2]
– extraction of NO_3^- on anion exchange resin – conversion of NO_3^- to $AgNO_3$ – conversion/roasting of $AgNO_3$ in presence of graphite to CO_2 – conversion/roasting of $AgNO_3$ in presence of CaO, Cu and CuO to N_2 – cryogenic purification of CO_2 and N_2	[7, 16]
– reduction of NO_3^- to N_2O by denitrifying bacteria – (*Pseudomonas aureofaciens, Pseudomonas chlororaphis*) – extraction of N_2O from water samples – chemical and chromatographic purification of N_2O	[3, 8, 15]
– reduction of NO_3^- to NO_2^- in presence of Cd – reduction of NO_2^- to N_2O in presence of NaN_3 – elution of N_2O from water samples – chemical and chromatographic purification of N_2O	[11]

gen ($\delta^{15}N$) and oxygen ($\delta^{18}O$) in nitrates dissolved in groundwater proved to be a useful tool for identification of the sources of nitrate and to monitor the extent of denitrification process [18].

The isotopic composition of both nitrogen and oxygen in nitrate molecules was for the first time measured by Amberger and Schmidt [1]. Their method was based on conversion of dissolved nitrates to KNO_3 and its subsequent transformation to N_2 and CO_2 gases. Isotopic composition of N_2 and CO_2 was then measured using conventional isotope ratio mass spectrometry (IRMS). In the following years several modifications of Amberger and Schmidt method were proposed ([2, 7, 14, 16] – cf. Table 1).

An entirely different approach to analysis of nitrogen and oxygen isotope composition of nitrates was proposed by Sigman *et al*. [15] and Casciotti *et al*. [3]. They used two species of denitrifying bacteria (*Pseudomonas aureofaciens, Pseudomonas chlororaphis*) to convert dissolved nitrate to N_2O gas whose nitrogen and oxygen isotope composition was subsequently analyzed using continuous-flow isotope ratio mass spectrometry (CF-IRMS). The main advantage of the method was dramatic reduction of the sample size, from ca. 300 μmol NO_3 required by the conventional method down to ca. 20 nmol NO_3^- using denitrifying bacteria. However, such reduction of sample size was possible only with CF-IRMS. Recently, still another method was proposed by McIlvin and Altabet [11], which utilises chemical conversion of NO_3^- to N_2O (cf. Table 1).

The presented study was focused on comparison of the above-outlined analytical methods for the determination $\delta^{15}N$ and $\delta^{18}O$ in nitrates dissolved in water. The bacterial reduction method as described by Sigman *et al*. [15] and Casciotti *et al*. [3] was modified in order to accommodate samples of intermediate size (ca. 30 μmol of NO_3^-) which would provide sufficient amount of N_2O gas for isotope analysis using the conventional IRMS technique. The modification of the bacterial reduction method presented in this study allows adaptation of this method also by laboratories which do not have access to CF-IRMS instruments.

Methods

Methodological aspects of two different methods of sample preparation for measurement of isotopic composition of dissolved nitrates are here discussed in details: (i) chemical conversion of NO_3^- to N_2 and CO_2, followed by mass-spectrometric analysis of both gases, and (ii) bacterial reduction of NO_3^- to N_2O, followed by measurement of nitrogen and oxygen isotope composition of this gas. Both methods have been adopted for routine isotope analyses of dissolved nitrates in water samples of various origin. A schematic flow-chart of both discussed methods is presented in Fig. 1.

The adopted analytical protocol of the first method (called subsequently 'conventional method') was largely based on the work of Silva *et al*. [16]. The bacterial denitrifying method, as proposed by Sigman *et al*. [15] and Casciotti *et al*. [3], was modified in order to accommodate samples of intermediate size (ca. 30 μmol of NO_3^-) which would yield sufficient amount of N_2O gas

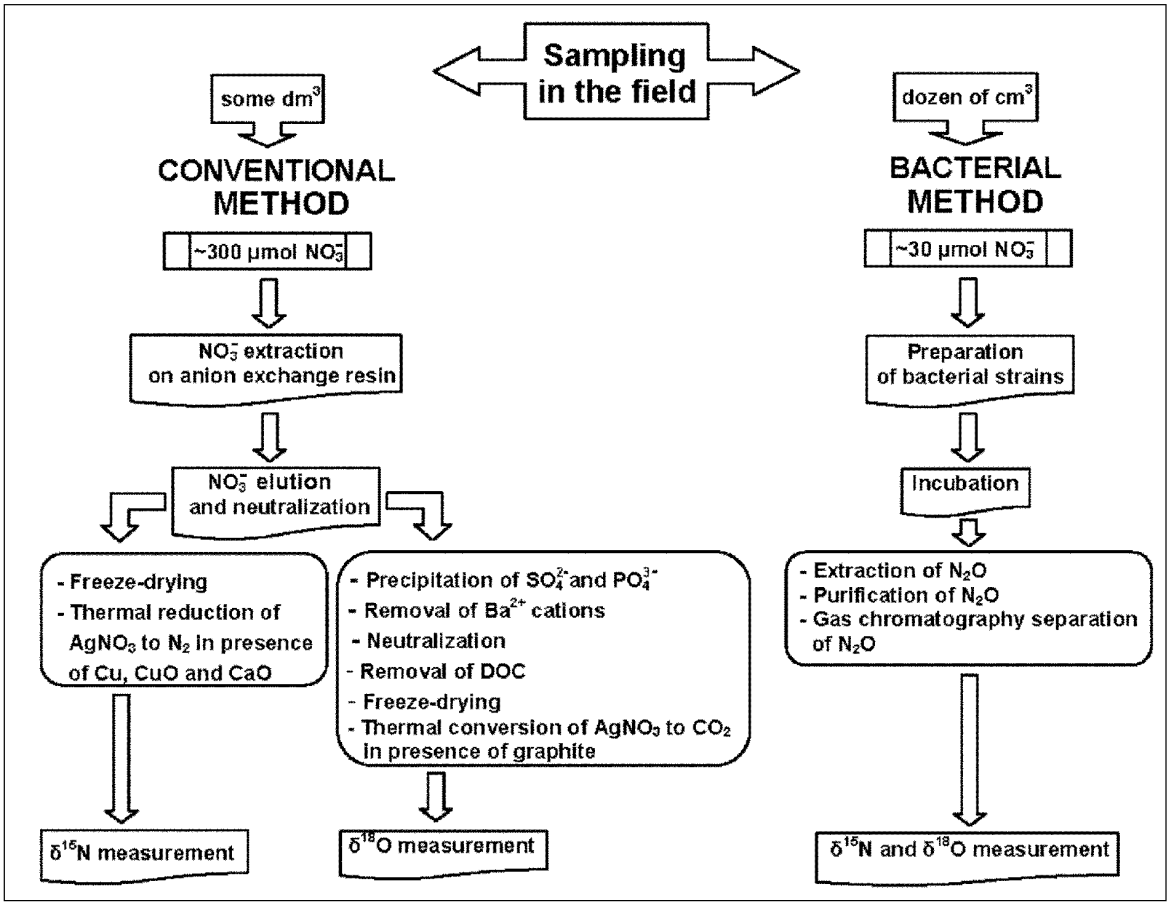

Fig. 1. Schematic flow-charts of two different methods of sample preparation for $\delta^{15}N$ and $\delta^{18}O$ analysis of dissolved nitrates described in details in the text.

for isotope analysis using the conventional IRMS. The results of isotope analyses are reported using generally accepted δ notation, defined as relative deviation of the measured isotope ratios ($^{15}N/^{14}N$, $^{18}O/^{16}O$) from the generally accepted standard. The standards in use here are atmospheric N_2 for $\delta^{15}N$ and VSMOW for $\delta^{18}O$.

Sampling in the field

The volume of water sample required for isotope analyses of nitrates was determined in the field on the basis of the measured NO_3^- content. For the conventional method, ca. 300 µmol of NO_3^- is required, while for the bacterial denitrifying method 10 times smaller sample is sufficient. Water samples can be collected in the field in plastic containers and delivered to the laboratory where, after filtration, the dissolved nitrate is extracted on an anion exchange resin (AG1-X8, Bio-Rad Laboratories). Alternatively, NO_3^- can be extracted on the resin directly in the field.

Laboratory experiments [4] showed that the efficiency of nitrate extraction using an anion exchange resin is close to 100% for a wide range of flow rates of water sample through a column (tested range from ca. 200 to 1700 ml/h) and the actual nitrate content in the sample (tested range from 1 to 120 mg/dm^3). Samples of NO_3^- deposited on resin packages can be stored at low temperature for up to one year.

Conventional method

Nitrates stored on the anion exchange resin were eluted from the column using 3 M HCl solution. HNO_3 was then converted to $AgNO_3$ according to the reaction:

$$(1)\ HCl + HNO_3 + Ag_2O \rightarrow AgCl\downarrow + AgNO_3 + H_2O$$

AgCl was subsequently separated on a Whatman filter (0.2 µm) and the remaining solution was split into two portions, for conversion of $AgNO_3$ to N_2 (measurement of $\delta^{15}N$) and to CO_2 (measurement of $\delta^{18}O$), respectively.

The portion of the solution envisaged for $\delta^{15}N$ determination was first freeze-dried under reduced light exposure. The remaining precipitate ($AgNO_3$ powder) was placed in a quartz tube together with appropriate reagents (Cu, CaO, CuO). The quartz tube was flame-sealed under vacuum, placed in a muffle oven and heated for 2 h at 850°C with following slow cooling over night (ca. 10 h) to convert $AgNO_3$ to N_2.

The portion of the solution envisaged for $\delta^{18}O$ measurement was first the subject for the procedure removing other ions containing oxygen (SO_4^{2-} and PO_4^{3-}). This was done by precipitation with $BaCl_2$. The precipitate was removed via filtration (Whatman, 0.2 µm). Then, the eluate was passed through a cation exchange resin (50W-X8, Bio-Rad Laboratories) to remove Ba^{2+} ions. Afterwards, the solution was neutralized using Ag_2O

Table 2. Parameters of the conventional method of nitrate conversion to N_2 and CO_2 for isotope analysis

Step	Duration	Efficiency
Separation of NO_3^- from water sample on anion exchange resin	from 2 to 48 h, depending on the sample volume	98–100%
Eluting of NO_3^- from anion exchange resins	ca. 1 h	85–100%, depending on NO_3^- content
Conversion of $AgNO_3$ to N_2:	ca. 17 h	
– chemical preparation	1 h	
– freeze-drying	6 h	
– thermal reduction to N_2	2 h at 850°C, 8 h of cooling	
Conversion of $AgNO_3$ to CO_2:	ca. 23 h	
– precipitation of SO_4^{2-} and PO_4^{3-}	1 h	
– removal of Ba^{2+}	1 h	
– neutralization	1 h	
– removal of DOC	1 h	
– freeze-drying	6 h (1st step) + 2 h (2nd step)	
– thermal decomposition and oxidation to CO_2	2 h at 850°C, 8 h of cooling	
– cryogenic purification of CO_2	1 h	
Total:	7–9 days (set of 8 samples)	~ 92% ($AgNO_3 \rightarrow N_2$) ~ 90% ($AgNO_3 \rightarrow CO_2$)

and filtered (Whatman, 0.2 μm). In order to remove dissolved organic carbon (DOC), which constitutes additional source of oxygen, ca. 10 mg of activated charcoal was added to the solution and stirred for ca. 20 min. Activated charcoal was removed from the solution immediately afterwards. The solution containing $AgNO_3$ was then freeze-dried in two steps. The remaining $AgNO_3$ powder was transferred to quartz break-seals, approximately 8 mg of spectrographic graphite was added, the quartz tube was flame-sealed under vacuum and heated in muffle oven at 850°C for 2 h, then cooled overnight to convert $AgNO_3$ to CO_2.

Isotopic composition of gaseous nitrogen and carbon dioxide obtained from nitrates extracted from the analyzed water sample using the procedures outlined above was analyzed using the conventional IRMS. The basic characteristics of sample preparation procedure outlined above are summarized in Table 2.

Denitrifier method

The method uses specific strains of denitrifying bacteria which convert nitrate dissolved in water to N_2O gas through several intermediate steps:

$$(4) \qquad NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O$$

The modification of the original method of Sigman *et al*. [15] and Casciotti *et al*. [3] undertaken in this work, was constrained by two requirements: (i) obtaining sufficiently large amount of N_2O gas for isotope analyses using the conventional IRMS, (ii) the maximum volume of water sample to be processed should not be larger than ca. 80 ml. Thus, water samples with low nitrate content had to be concentrated prior to the bacterial treatment. This was done through partial evaporation of the sample. Laboratory tests revealed that this way of reducing the initial volume of the sample does not modify in any noticeable way isotopic composition of the dissolved nitrates [4].

Bacterial strains of *Pseudomonas aureofaciens* (ATCC) were cultivated following the procedure described in details by Sigman *et al*. [15]. Portions of concentrated bacterial solution (ca. 20 ml) were transferred to the sterilized reaction vials (volume of 125 ml) with septa caps. After closing the vials and removing the gases from head-space using stream of high-purity helium, the analyzed water samples were added to each reaction vial using sterilized syringes. Depending on nitrate content, the volume of each water sample was adjusted to contain ca. 30 μmol of NO_3^- in 80 cm^3 of water. The vials, wrapped in an aluminium foil, were placed upside down in an autoclave. After the adopted incubation period (14 days), 2 ml of 10 M NaOH was added to each vial to stop bacterial activity. The resulting high pH (10–12) of the solution reduced the release of CO_2, produced along with N_2O by the bacteria.

Mixture of CO_2 and N_2O was extracted from the solution using a high-purity helium stream (Fig. 2). After passing through trap containing $Mg(ClO_4)_2$, the mixture was collected cryogenically in trap T1. Then, the mixture was sent to the GC column (Carboxen 1000) where CO_2 was separated from N_2O which was captured

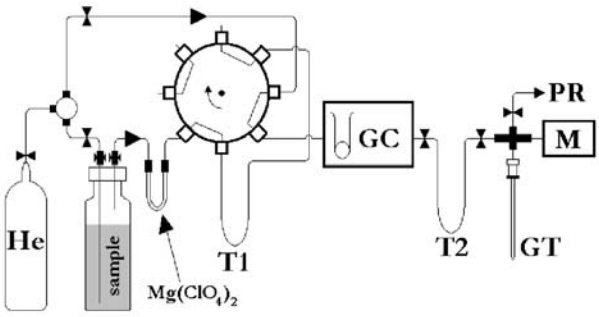

Fig. 2. Laboratory line used for the extraction and purification of N_2O from solution. Symbols: T1, T2 – cryogenic traps; GC – gas chromatography system; PR – rotary pump; M – manometer. Multi-port valve is at the position of flushing out N_2O from the sample. When flashing is completed, the valve is turned one step clockwise and N_2O collected in T1 is introduced to a chromatograph (GC) which detects N_2O peak enabling collection of this gas in T2. The N_2O gas is finally closed in a glass break-seal (GT).

Table 3. Parameters of the bacterial denitrification method of nitrate conversion to N_2O for isotope analysis

Step	Duration
Cultivation of bacteria (*Pseudomonas aureofaciens*)	10–14 days
Adding concentrated bacteria solution to water samples and incubation	14 days
Extraction of N_2O +CO_2 mixture from water samples, separation and purification of N_2O	ca. 3 h
Total:	Duration: ~28 days (set of 12 samples) Efficiency: 82–88% (for N)

in trap T2. Finally, N_2O was transferred cryogenically to a break seal and flame-sealed, ready for mass spectrometric measurement.

The main characteristics of the method are summarized in Table 3. The overall efficiency of the conversion of N-NO_3^- to N-N_2O was in the range of 82–88%. For oxygen, this efficiency cannot be determined since only part of the oxygen atoms in N_2O originates from sample nitrate (cf. discussion below).

Isotope analyses

The analyses of $\delta^{15}N$ and $\delta^{18}O$ of gases obtained from dissolved nitrates using the sample preparation procedures outlined above (N_2, CO_2, N_2O) have been performed on a MAT 250 isotope ratio mass spectrometer equipped with collectors allowing measurement of ion currents corresponding to masses 28 and 29 (N_2) as well as 44, 45 and 46 (CO_2 and N_2O).

N_2 and CO_2

The isotopic composition of nitrogen is reported against atmospheric N_2. The working standard (N_2 99.999% from Messer) was calibrated against samples of atmospheric nitrogen prepared according to the procedure recommended by Mariotti [12]. The reproducibility of $\delta^{15}N$ determinations for samples of atmospheric N_2 was better than 0.02‰. Oxygen isotope composition of CO_2 gas obtained from dissolved nitrate samples was analyzed against working standard calibrated on VSMOW-SLAP scale.

To assess the overall precision and accuracy of $\delta^{15}N$ and $\delta^{18}O$ analyses of dissolved nitrates with the aid of conventional method, IAEA-NO-3 intercomparison material (KNO_3) of known isotopic composition has been used [9]. Water solution of this material was prepared, and repeated $\delta^{15}N$ and $\delta^{18}O$ analyses of the dissolved NO_3^- were performed. The average $\delta^{15}N$ obtained from seven independent determinations (4.90 ± 0.22‰) agrees within one standard deviation of single measurement with the consensus the value assigned to this material (4.7 ± 0.2‰). For $\delta^{18}O$, the average value of seven independent determinations was 23.0 ± 0.6‰, to be compared with 25.6 ± 0.4‰ indicated in the IAEA report [9]. The apparent shift between the IAEA consensus value and the mean $\delta^{18}O$ value obtained from the repeated analyses of IAEA-NO-3 amounts to 2.6‰. On the other hand, Révész and Böhlke [13] pointed out that the $\delta^{18}O$ values reported for this intercomparison in the literature vary between 21‰ and 25‰, which would lead to a significantly lower consensus value than that reported in the IAEA publication. Therefore, no correction of measured $\delta^{18}O$ values was attempted. The obtained standard deviations of single measurement (0.3‰ for $\delta^{15}N$ and 0.6‰ for $\delta^{18}O$) were adopted as overall precision of isotope analyses in the conventional method.

N_2O

Nitrous oxide gas (99.9% from Linde), cryogenically purified, was used as a working standard. Repeated analyses of this gas revealed machine reproducibility of the order of 0.1‰ for both $\delta^{15}N$ and $\delta^{18}O$. The N_2O gas was calibrated against N_2O derived from the IAEA-NO-3 intercomparison material.

The oxygen isotope composition of N_2O produced by denitrifying bacteria differs from that of the dissolved nitrate due to isotope exchange between water and intermediate products of the denitrification process, as well as due to the presence of blank. The effect of isotope exchange and blank on the measured $\delta^{18}O$ values of N_2O gas can be quantified either using two nitrate standards of contrasting $\delta^{18}O$ values, or one nitrate standard dissolved in two water samples differing in ^{18}O content [3]. In this work the two-standards method was adopted (see Annex 1).

To assess the overall precision and accuracy of $\delta^{15}N$ and $\delta^{18}O$ analyses using the denitrifier method, three different intercomparison materials (IAEA-NO-3, USGS-32 and USGS-35), as well as a laboratory KNO_3 standard (purity > 99%, Merck) previously calibrated using the conventional method, have been used. Repeated analyses of IAEA-NO-3 yielded the reproducibility of the denitrifier method in the order of 0.7‰ for $\delta^{15}N$ and 1.0‰ for $\delta^{18}O$. For each of the other three materials (USGS-32, USGS-35 and KNO_3 laboratory standard), two independent determinations of $\delta^{15}N$ and $\delta^{18}O$ have been carried out. The mean values are summarized in Table 4. If the two sigma range is adopted, it is apparent from Table 4 that $\delta^{15}N$ and $\delta^{18}O$ values obtained using the denitrifier method are non-distinguishable from the assigned values. However, it should be noted that the apparent differences have the same sign (measured values are slightly higher than the assigned values for $\delta^{15}N$ and slightly lower for $\delta^{18}O$ values, which would suggest some systematic effects in work.

Field comparison

Two groundwater samples, differing in nitrate content were analyzed using both methods described above. The results of parallel analyses are reported in

Table 4. Isotopic composition of IAEA intercomparison materials (IAEA-NO-3, USGS-32, USGS-35) and a laboratory KNO_3 standard, as determined by the bacterial denitrification method. The reported numbers are mean values of two independent determinations. Quoted uncertainties represent one standard deviation

Standard	$\delta^{15}N$ (‰)		$\delta^{18}O$ (‰)	
	Assigned value [9]	Denitrifier method	Assigned value [9]	Denitrifier method
IAEA-NO-3	4.69 ± 0.2	5.0 ± 0.7	25.6 ± 0.4	24.7 ± 1.0
USGS-32	180 ± 1	182.6 ± 0.7	25.7 ± 0.4	23.1 ± 1.0
USGS-35	2.7 ± 0.2	2.9 ± 0.7	57.5 ± 0.6	55.9 ± 1.0
KNO_3	7.2 ± 0.2	8.1 ± 0.7	56.4 ± 0.9	52.4 ± 1.0

Table 5. Comparison of isotopic composition of nitrate in two groundwater samples analyzed using the two methods described in the text (conventional method and bacterial denitrification method)

Groundwater samples	Nitrate content (mg/dm^3)	$\delta^{15}N$ (‰)		$\delta^{18}O$ (‰)	
		Conventional method	Denitrifier method	Conventional method	Denitrifier method
Exploitation well, Kraków	19.0 ± 0.3	1.5 ± 0.3	10.0 ± 0.7	21.3 ± 0.6	19.7 ± 1.0
Borehole GZWP451/8, Bogucice	4.0 ± 0.1	16.9 ± 0.3	15.7 ± 0.7	22.5 ± 0.6	21.7 ± 1.0

Table 5. Two independent determinations of $\delta^{15}N$ and $\delta^{18}O$ values were performed for each groundwater type. The $\delta^{15}N$ and $\delta^{18}O$ values of dissolved nitrate obtained with both methods are similar, although in this case the denitrifier method yielded lower values (ca. 1.5‰, and 0.8‰ for $\delta^{15}N$ and $\delta^{18}O$, respectively) when compared to the conventional method.

Conclusions

Isotopic composition of nitrogen ($\delta^{15}N$) and oxygen ($\delta^{18}O$) in nitrates dissolved in groundwater proved to be a useful indicator of the origin of nitrate and a tool to monitor the extent of the denitrification process. Wider application of this isotopic tool in hydrological practice requires, however, that reliable analytical protocols yielding $\delta^{15}N$ and $\delta^{18}O$ values of dissolved nitrates are on hands.

The conventional method of $\delta^{15}N$ and $\delta^{18}O$ determination adopted in this work yields a relatively good precision (ca. 0.3‰ for $\delta^{15}N$ and 0.6‰ for $\delta^{18}O$). It requires ca. 300 μmol of NO_3^-. The main drawback of the method is a relatively large volume of water required for analysis, particularly for nitrate contents close to natural background.

The denitrifier method, as proposed by Sigman *et al*. [15] and Casciotti *at al*. [3], was modified in order to accommodate samples of intermediate size (ca. 30 μmol of NO_3^-) which provide sufficient amount of N_2O gas for isotope analysis using the conventional IRMS. It has been demonstrated that satisfactory results can be obtained, although sample preparation requires access to the microbiological laboratory.

Field comparison of both methods revealed satisfactory agreement, although the bacterial method tends to produce slightly lower delta values (between 0.8 and 1.6‰, respectively).

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Annex I

The oxygen isotope composition of N_2O produced by denitrifying bacteria differs from that of the dissolved nitrate due to isotope exchange between water and intermediate products of the denitrification process, as well as due to the presence of blank. The following mass and isotope balance equations can be formulated [3]:

(A1) $$m = s + b$$

(A2) $$\delta^{18}O_m \times m = (\delta^{18}O_s + \varepsilon) \times s \times (1-x) + \delta^{18}O_{H_2O} \times s \times x + \delta^{18}O_b \times b$$

where: m – final nitrogen content in N_2O gas; s – nitrogen content in the sample of nitrate; b – nitrogen content in the blank sample; ε – effective ^{18}O isotope fractionation associated with bacterial reduction of NO_3 to N_2O; x – contribution of oxygen atoms incorporated in N_2O molecules as a result of isotope exchange with water; $\delta^{18}O_m$ – measured oxygen isotopic composition of N_2O; $\delta^{18}O_s$ – oxygen isotope composition of the sample NO_3^-, $\delta^{18}O_{H_2O}$ – oxygen isotope composition of water; $\delta^{18}O_b$ – oxygen isotope composition of NO_3^- in the blank sample.

When two nitrate standards of known, contrasting $\delta^{18}O$ values are analyzed, Eq. (A2) can be rewritten:

(A3) $$\delta^{18}O_{m_1} \times m_1 = (\delta^{18}O_{s_1} + \varepsilon) \times s_1 \times (1-x) + \delta^{18}O_{H_2O} \times s_1 \times x + \delta^{18}O_b \times b$$

(A4) $$\delta^{18}O_{m_2} \times m_2 = (\delta^{18}O_{s_2} + \varepsilon) \times s_2 \times (1-x) + \delta^{18}O_{H_2O} \times s_2 \times x + \delta^{18}O_b \times b$$

If the same amount of nitrate standard is used for analysis ($s_1 = s_2$), the following expression can be derived from Eqs. (A3) and (A4):

(A5) $$\delta^{18}O_{s_1} - \delta^{18}O_{s_2} = k(\delta^{18}O_{m_1} - \delta^{18}O_{m_2})$$

where:

(A6) $$k = \frac{s+b}{s(1-x)}$$

The original ^{18}O isotope composition of nitrate in the analyzed water sample can then be expressed by the following formula:

(A7) $$\delta^{18}O_s = \delta^{18}O_{s,std} + k \times (\delta^{18}O_m - \delta^{18}O_{m,std})$$

where: $\delta^{18}O_{s,std}$ stands for the assigned $\delta^{18}O$ value of nitrate standard used in routine analyses, $\delta^{18}O_m$ represents the measured $\delta^{18}O$ of N_2O gas produces from the analyzed sample and $\delta^{18}O_{m,std}$ stands for the measured $\delta^{18}O$ of N_2O gas produced from nitrate standard. The k factor accounts for the effects of isotope exchange and blank. The value of k was derived from isotope analyses of six different pairs of nitrate standards (ca. 1.021 – [4]) and was further adopted for calculations of $\delta^{18}O$ values of nitrate in the analyzed water samples.