ORIGINAL ARTICLE

A decision support tool for the selection of ^{15} N analysis methods of ammonium and nitrate

Mengru Jia [ORCID] **· Roland Bol ·
Annemieke Kooijman · Wim W. Wessel ·
Albert Tietema**

Abstract The stable nitrogen isotope (^{15} N) analysis of ammonium (NH_4^+) and nitrate (NO_3^-) is widely used in ecological research, providing insights into N cycling and its underlying regulating mechanisms in both aquatic and terrestrial ecosystems. To date, a large number of methods have been developed for the preparation and measurement of ^{15} N abundance of NH_4^+ and NO_3^- in liquid environmental samples at either natural abundance or enriched levels. However, these methods are all subject to certain specific advantages and limitations, and ecologists might be looking for an efficient way to select the most suitable methods in face of shifting sampling and analytical conditions. Based on our extensive review of these ^{15} N analysis methods we developed a decision support tool (DST) to provide quick and proper guidance for environmental researchers in finding the optimal method for preparing their liquid samples for ^{15} N analysis in NH_4^+ or NO_3^-. The DST is a decision tree based on several key criteria that users need to take into account when choosing the preferred sample preparation method for their samples. The criteria concern: the sample matrix, the ^{15} N abundance and the concentration of the target N species, the contamination by other N-containing chemicals, the isotopic fractionation, the availability of equipment, concerns about toxicity of reagents, and the preparation time. This work links field-scale experiments and laboratory ^{15} N analysis. Potential applications of our decision trees include ^{15} N studies ranging from natural abundance to tracer level in a wide range of terrestrial, freshwater and marine ecosystems.

Keywords Decision support tool · ^{15} N analysis · Ammonium · Nitrate · Liquid samples

M. Jia (✉) · R. Bol · A. Kooijman · W. W. Wessel ·
A. Tietema
Institute for Biodiversity and Ecosystem Dynamics,
University of Amsterdam, Science Park 904, 1098, XH,
Amsterdam, The Netherlands
e-mail: m.jia@uva.nl

R. Bol
Institute of Bio- and Geosciences, IBG-3: Agrosphere,
Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Introduction

The increasing world-wide concern about the impact of high N inputs in ecosystems and their contribution to climate change has highlighted the need for a deeper understanding of N cycling and its underlying regulating mechanisms in ecosystems. Ammonium (NH_4^+) and nitrate (NO_3^-) are the two dominant forms of bioavailable N, driving productivity and affecting biodiversity in both terrestrial and aquatic ecosystems (Canfield et al. 2010; Zhu et al. 2015). In addition, they contribute to global N contamination

as important forms of anthropogenic N input through fertilizer and atmospheric deposition (Galloway 2005; Xue et al. 2009; Mayer et al. 2013). Due to the crucial roles of NH_4^+ and NO_3^- in N transformations, the two N species have been widely investigated using stable N isotope techniques to better quantify N dynamics.

Nitrogen is composed of two stable isotopes, the abundant ^{14}N and the rare ^{15}N. The stable N isotopic composition in a N-compound is usually expressed as either an "absolute value (x^{15}N)" or a "relative value (δ^{15}N)" in agricultural and biological research (Fry 2006; Coplen 2011; Mayer et al. 2013; Chalk et al. 2015). The former is preferred in applications where ^{15}N is used as a tracer (Hauck and Bremner 1976; Smith and Mullins 2000; Fillery and Recous 2001; Scrimgeour and Robinson 2003). The latter denotes a difference measurement made relative to a standard reference material (usually atmospheric N_2) during the actual analysis, and is more appropriate for samples close to natural abundance (Mariotti 1983; Shearer and Kohl 1993; Peoples et al. 2001; Stewart 2001; Scrimgeour and Robinson 2003; Fry 2006; Michener and Lajtha 2008; Coplen 2011; Kendall and McDonnell 2012; Mayer et al. 2013; Chalk et al. 2015). Measuring ^{15}N abundance of important N compounds like NH_4^+ and NO_3^- can provide valuable information on their sources, flow-paths, transformations and fates, and the N cycle of the ecosystems (Hauck 1982; Shearer and Kohl 1993; Kendall and McDonnell 2012; Ito et al. 2013). The ^{15}N analysis of NH_4^+ and NO_3^- is commonly involved in ^{15}N-related ecological investigations such as ^{15}N pool dilution (Davidson et al. 1991; Wessel and Tietema 1992; Laine et al. 2018), ^{15}N tracing (Mulholland et al. 2000, 2008; Rütting et al. 2011; Björsne et al. 2014) and natural abundance studies (Gathumbi et al. 2002; Houlton et al. 2007; Denk et al. 2017). The ^{15}N pool dilution and tracing assays are both based on ^{15}N labelling of pools of N species, providing information about gross rates of main N transformation processes, and N sources, influxes and sinks in catchments and food webs (Hauck 1982; Rütting and Müller 2007; Braun et al. 2018). The natural ^{15}N abundance approach, on the other hand, makes use of the isotopic discrimination that occurs when nutrients move through pathways, and provides an important tool for identifying and quantifying N sources and fluxes in soil and vegetation on a larger spatial and temporal scale (Owens 1988; Kendall 1998; Robinson

2001; Pörtl et al. 2007; Kendall and McDonnell 2012; Ito et al. 2013; Granger and Wankel 2016; Nikolenko et al. 2018).

In field experiments where the cycling and flows of N are studied using ^{15}N techniques, the ^{15}N analysis of NH_4^+ or NO_3^- is commonly conducted in an environmental solution, such as soil extracts, freshwater or seawater samples. To acquire reliable isotopic data it is important to prepare and measure the samples in an appropriate way. A wide range of sample preparation and measurement methods have been developed, and they commonly involve converting NH_4^+ or NO_3^- to an isotopic representative gas (e.g., N_2 or N_2O) and then quantifying ^{15}N abundance of the analyte. The gas conversion is necessary because most ^{15}N ratio measurements use either a mass spectrometer, an emission spectrometer, or a laser spectrometer, which accept only simple gas analytes (Bremner 1965a, 1996; Hauck 1982; Preston and Owens 1983; Mulvaney 1993; Brand 1996; Brenna et al. 1997; Mayer et al. 2013). Alternatively, a few sample preparation methods without gas conversion have also been reported. For example, the ^{15}N ratio of NH_4^+ in aquatic samples can be analyzed directly using HPLC (Gardner et al. 1991, 1995, 1997).

With so many ^{15}N analysis techniques available, it can be tedious and time-consuming for researchers to select a method in agreement with their analytical needs among a wide range of published methodologies. During past decades there have been great and valuable reviews published regarding the classic sample preparation and ^{15}N measurement approaches (Bremner 1965a, 1996; Fiedler and Proksch 1975; Hauck and Bremner 1976; Bremner and Hauck 1982; Hauck 1982; Owens 1988; Mulvaney 1991, 1993, 1996, 2008; Knowles and Blackburn 1993; Shearer and Kohl 1993; Sparks et al. 1996; Kendall 1998; Smith and Mullins 2000; Scrimgeour and Robinson 2003; Chang et al. 2004; Groot 2004). The well-reviewed sample preparation methods such as diffusion, bacterial denitrifier and azide techniques have been widely adopted for ^{15}N analysis in NH_4^+ and NO_3^- in ecological solutions (Christensen and Tiedje 1988; Bremner 1996; Mulvaney 1996; Mulvaney et al. 1997; Sigman et al. 1997, 2001; Holmes et al. 1998; Scrimgeour and Robinson 2003; Chang et al. 2004; McIlvin and Altabet 2005; Xue et al. 2009; Mayer et al. 2013). However, they either require laborious incubation

and maintenance of special bacteria cultures, or use highly toxic chemicals. In recent years, a number of novel methods and modifications to traditional methods have been reported (Houlton et al. 2007; Zhang et al. 2007, 2015; Isobe et al. 2009; Xing and Liu 2011; David Felix et al. 2013; Liu et al. 2014; Kiba et al. 2016; Tu et al. 2016; Jin et al. 2020; Gebus-Czupyt et al. 2020; Kawashima et al. 2021; Hilkert et al. 2021), leading to improvements in concentration ranges and analytical precision while reducing time, cost and environmental risk. While they are less well-known and documented, such advances have the potential to promote the more widespread practical application of isotope techniques in the management of nutrient issues in the future.

To assist method users select the appropriate sample preparation approach for the ^{15}N analysis of NH_4^+ and NO_3^- in their environmental samples, we developed a "Decision Support Tool (DST)" based on a review and comparison of the well-known "classic" and novel methods in terms of several key criteria that need to be considered when selecting methods for ^{15}N analysis in liquid samples. These criteria concern: the composition of the sample matrix, the ^{15}N abundance and concentration of the target N, the contamination by other N-containing chemicals, the isotopic fractionation, the availability of equipment, concerns about toxicity of reagents, and the preparation time. We organize the sections below in the following order: an overview of the methods, the development of the DST, and finally a discussion of two application cases and of the benefits and drawbacks of the present DST.

Overview of sample analysis techniques

In this section, we give a brief overview of methods reported in the literature to analyze ^{15}N in dissolved NH_4^+ and NO_3^-, arranged according to their final analytes and instruments. The most popular off-line gas methods are presented first, followed by on-line gas methods, and finally non-gas approaches. A more detailed description of the overall procedures, sample requirements, advantages and limitations of the methods can be found in Tables 1 and 2, and in the supplementary information.

Methods producing N_2 as the analyte (N_2 methods)

In the gas methods, the NH_4^+ or NO_3^- is converted into analytical gases such as N_2, N_2O, NO or volatile derivatives. Most gas methods involve isolating the NH_4^+ or NO_3^- from the liquid sample, converting it into N_2, and determining the ^{15}N abundance of the produced N_2 by an isotope ratio mass spectrometer (IRMS) (Hauck and Bremner 1976; Hauck 1982; Mulvaney 1993, 2008; Shearer and Kohl 1993; Bremner 1996; Smith and Mullins 2000; Groot 2004; Michener and Lajtha 2008; Cook et al. 2017). Previously, the conversion into N_2 and subsequent purification were achieved using either hypobromite oxidation (i.e., the Rittenberg technique) or quartz-tube Dumas combustion, and was determined via a dual-inlet isotope ratio mass spectrometer (DI-IRMS) or an emission spectrometer (Fiedler and Proksch 1975; McInteer et al. 1981; Bremner and Hauck 1982; Fisher and Morrissey 1985; Mulvaney et al. 1990; Kendall and Grim 1998; Mulvaney and Liu 1991; Liu and Mulvaney 1992a; Mulvaney 1993; Shearer and Kohl 1993; Bremner 1996). Révész et al (1997) also reported an off-line combustion technique using catalyzed graphite to convert potassium nitrate (KNO_3) into CO_2, K_2CO_3 and N_2 for simultaneous N and O isotope analysis. The Rittenberg oxidation was later automated by coupling a Rittenberg apparatus to the IRMS (Griffiths et al. 1981; McInteer et al. 1981, 1984; Mulvaney et al. 1990; Mulvaney 1991; Mulvaney and Liu 1991; Liu and Mulvaney 1992b), but this is no longer commonly used. The workhorse in most modern isotope ratio laboratories is the combination of an elemental analyzer (EA) with a continuous-flow IRMS (EA-CF-IRMS, EA-IRMS), in which isolated N-bearing salts from various sample preparation procedures are sealed into tin capsules and loaded into an autosampler, followed by online combustion in the EA and subsequent sweeping of the produced N_2 into the IRMS with an He carrier gas (Preston and Owens 1983; Barrie and Workman 1984; Marshall and Whiteway 1985; Owens 1988; Barrie et al. 1989; Owens and Rees 1989; Egsgaard et al. 1989; Craswell and Eskew 1991; Mulvaney 1993; Barrie and Prosser 1996; Brand 1996; Bremner 1996; Chang et al. 2004; Horita and Kendall 2004).

Prior to N_2 conversion and isotope measurements, the NH_4^+ or NO_3^- in samples can be concentrated and isolated from solutions by a number of

Table 1 Methods reported for ^{15}N analysis of NH_4^+ in liquid samples in case studies

Method	Analyte	Instrument	^{15}N abundance	Sample matrix	N requirement (N-NH_4^+)
Distillation	N_2	(DI-/EA-) IRMS; ES	^{15}N-enriched; natural abundance	Water samples, soil extracts	4–400 µmol
Diffusion	N_2	EA-IRMS; ES	^{15}N-enriched; natural abundance	Water samples, soil extracts	2–15 µmol
Ion exchange	N_2	EA-IRMS	^{15}N-enriched; natural abundance	Freshwater samples	1–2 µmol
Hg precipitation	N_2	ES	^{15}N-enriched (pool dilution samples)	Freshwater samples	~10 µM
TPB precipitation	N_2	EA-IRMS	^{15}N-enriched; natural abundance	Freshwater samples	~70 µmol
Derivatization	N_2	EA-IRMS; ES	^{15}N-enriched; natural abundance	Water samples	2–10 µM
	dye derivative	GC-QMS	^{15}N-enriched (pool dilution samples)	Water samples	10–200 nmol/kg
OX-MIMS	N_2	MIMS	^{15}N-enriched	Water samples	> 0.1 µM
SPIN-QMS	N_2	QMS	^{15}N-enriched	Water samples, soil extracts	> 140 µM (700 nmol)
SPIN-MIMS	N_2	MIMS	^{15}N-enriched	Water samples, soil extracts	> 70 µM (100 nmol)
BO-azide	N_2O	PT-IRMS	^{15}N-enriched; natural abundance	Water samples	0.5–10 µM
BO-NH_2OH	N_2O	PT-IRMS	^{15}N-enriched; natural abundance	Water samples, alkaline soil extracts	10–20 µM
Diffusion-BO-azide	N_2O	PT-IRMS	^{15}N-enriched; natural abundance	Water samples, soil extracts	20–50 µM
Diffusion-BO-NH_2OH	N_2O	PT-IRMS	^{15}N-enriched; natural abundance	Water samples, soil extracts	15–60 µM
Diffusion-BO-denitrifier	N_2O	PT-IRMS	^{15}N-enriched; natural abundance	NH_3 emissions, water samples, soil extracts	10 µM
Ion exchange-BO-denitrifier	N_2O	PT-IRMS	^{15}N-enriched; natural abundance	NH_3 emissions, freshwater samples	30 µM
Diffusion-PO-azide	N_2O	PT-IRMS	^{15}N-enriched; natural abundance	Water samples, soil extracts	12.4–250 µM
Diffusion-PO-denitrifier	N_2O	PT-IRMS; GC-QMS	^{15}N-enriched; natural abundance	Water samples, soil extracts	1–100 µM (GC–MS) 20–60 nmol (PT-IRMS)
AIRTS-HPLC	NH_4^+	HPLC	^{15}N-enriched ($x(^{15}$N$) = 25$–75%)	Water samples	2–10 µM

Method	Optimal sample volume [a]	Time [b]	Toxicity	^{15}N dilution by chemicals	Fractionation
Distillation	30–500 ml	5–40 min/sample	×	Prone to ^{15}N dilution by DON and reagents	Prone to low recovery and fractionation
Diffusion	10–100 ml	5–10 d/batch	×	Less prone to ^{15}N dilution by DON and reagents than distillation	Neglectable at high recovery
Ion exchange	50 ml–2 L	3–5 d/batch	×	Prone to DON interferences Small ^{15}N dilution by reagents	Neglectable at high recovery

Table 1 (continued)

Method	Optimal sample volume [a]	Time [b]	Toxicity	^{15}N dilution by chemicals	Fractionation
Hg precipitation	< 300 ml	1 d/batch	√	Large ^{15}N dilution by reagents	Not reported
TPB precipitation	5 ml after freeze-drying	1 d/batch	√	Small ^{15}N dilution by reagents	Significant for freeze-drying of > 100 ml
Derivatization	400–500 ml	1–2 d/batch	√	Large ^{15}N dilution by reagents	Small and constant
OX-MIMS	12 ml	10 samples/h	×	Not reported	Not reported
SPIN-QMS	≤ 10 ml	5–10 min/sample	×	Not reported	Not reported
SPIN-MIMS	1.5 ml	15 min/sample	×	Not reported	Not reported
BO-azide	20 ml	2–3 d/batch	√	Small ^{15}N dilution by reagents	Small
BO-NH$_2$OH	4 ml	2–3 d/batch	×	Small ^{15}N dilution by reagents	Small
Diffusion-BO-azide	10 ml	5–7 d/batch	√	Small ^{15}N dilution by DON and reagents	Not reported
Diffusion-BO-NH$_2$OH	5 ml	5–7 d/batch	×	Small ^{15}N dilution by DON and reagents	Small
Diffusion-BO-denitrifier	20 ml	5–7 d/batch	×	Small ^{15}N dilution by DON and reagents	Not reported
Ion exchange-BO-denitrifier	50–150 ml	2–3 d/batch	×	Neglectable	No fractionation
Diffusion-PO-azide	10 ml	5–7 d/batch	√	Small ^{15}N dilution by DON and reagents	Large at < 20 μM N
Diffusion-PO-denitrifier	10–20 ml	5–7 d/batch	×	Small ^{15}N dilution by DON and reagents	Not reported
AIRTS-HPLC	0.4 ml	6–7 samples/day	×	Neglectable	Neglectable

Method	Advantages	Disadvantages
Distillation	Simple, low-cost, non-toxic, matrix versatility	Time and labor consuming for large batches, high N requirement, require skills and special apparatus, prone to ^{15}N dilution by DON and reagents
Diffusion	Simple, low cost, non-toxic, matrix versatility, ease of batch processing	Time and labor consuming, high N requirement, potential fractionation and ^{15}N dilution by DON and reagents
Ion exchange	Simple, non-toxic, low reagent contamination, ease of transport and storage	High N requirement, time-consuming, salinity-sensitive, high cost, potential fractionation
Hg precipitation	Simple, stable, rapid	Toxic, high N requirement, large sample volumes, salinity-sensitive, large ^{15}N dilution by reagents
TPB precipitation	Simple, rapid, low cost, low reagent contamination, ease of batch processing	High N requirement, salinity-sensitive, potential fractionation, require large volumes and time-consuming freeze-drying for low N samples
Derivatization	Stable, rapid, high selectivity, low N requirement by GC–MS	High N requirement by IRMS, toxic, large ^{15}N dilution by reagents
OX-MIMS	rapid, low cost, small sample volumes, ease of batch processing	Low precision
SPIN-QMS		
SPIN-MIMS	Simple, rapid, fully on-line, ease of batch processing, simultaneous determination of N concentration and ^{15}N abundance	Low precision, require special apparatus

Table 1 (continued)

Method	Advantages	Disadvantages
BO-azide	Rapid, low N requirement, small sample volumes, ease of batch processing, small ^{15}N dilution by reagents and fractionation	Toxic, potential NO_2^- interferences
BO-NH$_2$OH	Small sample volumes, rapid, ease of batch processing, small ^{15}N dilution by reagents and fractionation	Not for acidic soils, potential NO_2^- interferences
Diffusion-BO-azide	Small sample volumes, matrix versatility, ease of batch processing,	Toxic, time and labor consuming, potential fractionation and ^{15}N dilution by DON and reagents
Diffusion-BO-NH$_2$OH	Small sample volumes, matrix versatility, ease of batch processing, small fractionation	Time and labor consuming, potential fractionation and ^{15}N dilution by DON and reagents
Diffusion-BO-denitrifier	Low N requirement, small sample volumes, toxic-free, matrix versatility, ease of batch processing	Time and labor consuming, long time incubation and special care of bacterial culture, potential fractionation and ^{15}N dilution by DON and reagents
Ion exchange-BO-denitrifier	Low N requirement, non-toxic, small ^{15}N dilution by reagents and fractionation	Time and labor consuming, salinity-sensitive, high cost, long time incubation and special care of bacterial culture, potential fractionation and ^{15}N dilution by DON and reagents
Diffusion-PO-azide	Small sample volumes, matrix versatility, ease of batch processing	Toxic, time and labor consuming, fractionation at low N concentrations, potential ^{15}N dilution by DON and reagents
Diffusion-PO-denitrifier	Small sample volumes, matrix versatility, ease of batch processing	Time and labor consuming, long time incubation and special care of bacterial culture, potential ^{15}N dilution by DON and reagents
AIRTS-HPLC	Small sample volumes, non-toxic, simultaneous determination of N concentration and ^{15}N abundance, minimal sample preparation, small ^{15}N dilution by reagents and fractionation	Require high ^{15}N-enrichment, low precision, time-consuming for large batches, error-prone for refrozen samples

Method	References
Distillation	(Bremner and Edwards 1965; Bremner and Keeney 1965; Keeney and Bremner 1966; Cline and Kaplan 1975; Hauck and Bremner 1976; Hauck 1982; Velinsky et al. 1989; Mulvaney et al. 1994; Mulvaney 1996; Feast and Dennis 1996; Mulvaney and Khan 1999)
Diffusion	(Conway 1947; Brooks et al. 1989; Sørensen and Jensen 1991; Liu and Mulvaney 1992a; Saghir et al. 1993b; Lory and Russelle 1994; Herman et al. 1995; Mulvaney 1996; Stark and Hart 1996; Sigman et al. 1997; Khan et al. 1998; Holmes et al. 1998; Chang et al. 2004; Sebilo et al. 2004; Heiling et al. 2006; Cao et al. 2018)
Ion exchange	(Lehmann et al. 2001)
Hg precipitation	(Fisher and Morrissey 1985)
TPB precipitation	(Stock et al. 2019)
Derivatization	(Selmer and Sörensson 1986; Dudek et al. 1986; Kanda 1995; Preston et al. 1996; Köster and Jüttner 1999; Clark et al. 2006)
OX-MIMS	(Yin et al. 2014)
SPIN-QMS	(Stange et al. 2007)
SPIN-MIMS	(Eshenbach et al. 2017)
BO-azide	(Zhang et al. 2007)
BO-NH$_2$OH	(Liu et al. 2014)
Diffusion-BO-azide	(Zhang et al. 2019)
Diffusion-BO-NH$_2$OH	(Zhang et al. 2015)
Diffusion-BO-denitrifier	(David Felix et al. 2013)
Ion exchange-BO-denitrifier	(Kawashima et al. 2021)

Table 1 (continued)

Method	References
Diffusion-PO-azide	(Lachouani et al. 2010)
Diffusion-PO-denitrifier	(Houlton et al. 2007; Isobe et al. 2009)
AIRTS-HPLC	(Gardner et al. 1991)

sample preparation approaches. The target N species can be distilled as ammonium salts (Bremner and Edwards 1965; Bremner and Keeney 1965; Cline and Kaplan 1975; Hauck and Bremner 1976; Hauck 1982; Bremner 1996; Mulvaney 1996; Feast and Dennis 1996; Mulvaney and Khan 1999), diffused onto acidified filters (Conway 1947; Brooks et al. 1989; Sørensen and Jensen 1991; Liu and Mulvaney 1992b; Saghir et al. 1993b; Lory and Russelle 1994; Herman et al. 1995; Mulvaney 1996; Sparks et al. 1996; Stark and Hart 1996; Sigman et al. 1997; Khan et al. 1998; Holmes et al. 1998; Sebilo et al. 2004; Cao et al. 2018) or into acid solutions (Saghir et al. 1993a, b; Khan et al. 1997; Mulvaney et al. 1997; Mulvaney and Khan 1999), precipitated as salts (Fisher and Morrissey 1985; Huber et al. 2011, 2012; Stock et al. 2019), concentrated into zeolites (Velinsky et al. 1989; Burke et al. 1990; Böhlke et al. 2006; Frey et al. 2014) or ion-exchange resins (Chang et al. 1999; Silva et al. 2000; Lehmann et al. 2001; Xing and Liu 2011; Li et al. 2015), or derivatized as an azo dye (Preston et al. 1996, 1998; Clark et al. 2006, 2007; Ward 2011). The isolated N-containing materials can then be dried and loaded into an EA-IRMS, or oxidized/combusted off-line to produce N_2 (Fiedler and Proksch 1975; Hauck 1982; Velinsky et al. 1989; Kendall and Grim 1998; Knowles and Blackburn 1993; Mulvaney 1993, 1996). In the derivatization method, the isolated azo dye can be further converted into a volatile derivative, which can then be analyzed using a GC–MS (or GC-QMS, gas chromatography–quadrupole mass spectrometry) (Preston et al. 1996, 1998; Clark et al. 2006, 2007). Apart from these isolation methods, it is also possible to directly convert the target N species (e.g., NH_4^+) in water samples into N_2 gas and analyze the ^{15}N abundance directly using a membrane-inlet mass spectrometer (MIMS) (Yin et al. 2014).

If the initial NH_4^+ or NO_3^- concentration is too low for these sample preparation methods, samples may be first concentrated by evaporation, freeze-drying, ion-exchange, or by diffusing or distilling multiple sample aliquots (Fiedler and Proksch 1975; Hauck and Bremner 1976; Hauck 1982; Owens 1988; Mulvaney 1993, 1996; Bremner 1996; Smith and Mullins 2000; Scrimgeour and Robinson 2003; Chang et al. 2004; Horita and Kendall 2004; Michener and Lajtha 2008). Alternatively, samples can be spiked with standards with known N masses and ^{15}N abundances (Hauck 1982; Glibert and Capone 1993; Mulvaney 1993; Højberg et al. 1994; Stephan and Kavanagh 2009; Griesheim and Mulvaney 2019).

Methods producing N_2O as the analyte (N_2O methods)

While classic sample preparation methods rely on the conversion of NH_4^+ or NO_3^- to N_2 for ^{15}N analysis, contemporary methods primarily favor off-line conversion of NH_4^+ or NO_3^- into N_2O and subsequent IRMS/laser analysis. In earlier studies the N_2O is always reduced to N_2 by Dumas combustion for ^{15}N analysis (Bremner 1965a; Fiedler and Proksch 1975; Bremner and Hauck 1982; Kendall and Grim 1998; Mulvaney 1993; Preston 1993; Shearer and Kohl 1993; Chang et al. 2004). However, recent developments in instrumentation and analytical techniques have allowed to enter N_2O directly into the IRMS or the laser spectrometer for ^{15}N analysis, making it possible to obtain both N and O isotope ratios simultaneously (Dore et al. 1998; Sigman et al. 2001; Toyoda and Yoshida 2004; Groot 2004; McIlvin and Altabet 2005; Kendall et al. 2007; Michener and Lajtha 2008; Altabet et al. 2019). The N_2O derived from NO_3^- or NH_4^+ can be extracted and purified off-line (Sigman et al. 2001; Soto et al. 2015; Wassenaar et al. 2018), or be extracted on-line using a purge and trap system (e.g., Finnigan GasBench II system) coupled to a continuous flow-IRMS with gas chromatograph interfaces (PT-CF-IRMS, GC-IRMS, or PT-IRMS). Because N_2O can be easily trapped and concentrated cryogenically into a small volume of helium

Table 2 Methods reported for [15] N analysis of NO_3^- in liquid samples in case studies

Method	Analyte	[15] N abundance	Instrument	Sample matrix	N requirement (N-NO_3^-)
Distillation	N_2	[15] N-enriched; natural abundance	(DI-/EA-) IRMS	Water samples, soil extracts	140–360 µmol
Diffusion	N_2	[15] N-enriched; natural abundance	(DI-/EA-) IRMS; ES	Water samples, soil extracts	4–14 µmol
Ion exchange	N_2	[15] N-enriched; natural abundance	(DI-/EA-) IRMS; LC-Orbitrap HRMS [c]	Freshwater samples	> 6 µmol
Derivatization	N_2	[15] N-enriched (pool dilution)	ES	Water samples	> 1.5 µM; 200–300 nmol
	N_2	[15] N-enriched; natural abundance	EA-IRMS	Water samples	~ 107 µM
	dye derivative	[15] N-enriched (pool dilution)	GC–MS	Water samples	2–8 nmol/kg
Ba(NO_3)$_2$-acetone	N_2	[15] N-enriched; natural abundance	EA-IRMS	Freshwater samples	4–30 µmol
NaOH-acetone	N_2	[15] N-enriched; natural abundance	EA-IRMS	Freshwater samples, soil extracts	4–9 µmol
SPIN-QMS	NO	[15] N-enriched	QMS	Water samples, soil extracts	> 4 µM (20 nmol)
SPIN-MIMS	NO	[15] N-enriched	MIMS	Water samples, soil extracts	> 7 µM (10 nmol)
Denitrifier	N_2O	[15] N-enriched; natural abundance	GC-QMS;PT-IRMS;LS	Water samples, soil extracts	20 nmol or 1–20 µM (IRMS); 175 nmol (LS); 1–150 µM (GC–MS)
Cd-azide	N_2O	[15] N-enriched; natural abundance	PT-IRMS;LS	Water samples, soil extracts	25–30 nmol (0.5–40 µM)
Cd-NH_2OH	N_2O	[15] N-enriched; natural abundance	PT-IRMS	Water samples	20 nmol (2–20 µM)
VCl$_3$-Azide	N_2O	[15] N-enriched; natural abundance	PT-IRMS	Water samples, soil extracts	40 nmol/16.5–100 µM
Ti(III) reduction	N_2O	[15] N-enriched; natural abundance	PT-IRMS; LS	Freshwater samples	20–40 nmol; 3.5–14.3 µM
AIRTS-HPLC	NH_4^+	[15] N-enriched (x([15] N) = 25–75%)	AIRTS-HPLC	Water samples	4 µM

Method	Optimal ample volume [a]	Time	Toxicity	[15] N dilution by chemicals	Fractionation
Distillation	30–500 ml	10 min–2 h/sample	×	Prone to [15] N dilution by DON and reagents	Prone to fractionation
Diffusion	30–500 ml	1–2 weeks/batch	×	Less prone to [15] N dilution by DON and reagents than distillation	Prone to fractionation
Ion exchange	milliliters–liters	3–5 d/batch	×	Prone to DON interferences Small [15] N dilution by reagents	Neglectable at high recovery
Derivatization	60–500 ml	1–2 d/batch	√	Large [15] N dilution by reagents	Small and constant
Ba(NO_3)$_2$-acetone	~ 200–500 ml [d]	1–2 week/batch [b]	×	Neglectable	Small and constant
NaOH-acetone	~ 500 ml [d]	~ 1 week/batch [b]	×	Neglectable	Small and constant
SPIN-QMS	≤ 10 ml	5–15 min/sample	×	Not reported	Not reported
SPIN-MIMS	1.5 ml	15 min/sample	×	Not reported	Not reported

Table 2 (continued)

Method	Optimal ample volume [a]	Time	Toxicity	^{15}N dilution by chemicals	Fractionation
Denitrifier	< 20 mL	2–3 d/batch	×	Small	Small and constant
Cd-Azide	5–70 mL	2–3 d/batch	√	Small ^{15}N dilution by reagents	Small and constant
Cd-NH$_2$OH	10–15 ml	2–3 d/batch	√	Small ^{15}N dilution by reagents	Small and constant
VCl$_3$-Azide	2.5 ml	2–3 d/batch	√	Small ^{15}N dilution by reagents	Large at < 20 μM N
Ti(III) reduction	2–4 ml	2–3 d/batch	×	Small ^{15}N dilution by reagents	Prone to fractionation
AIRTS-HPLC	15 mL	6–7 samples/day	×	Neglectable	Neglectable

Method	Advantages	Disadvantages
Distillation	Simple, low cost, matrix versatility, non-toxic	Time and labor consuming for large batches, high N requirement, prone to ^{15}N dilution by DON and reagents, require specialized apparatus
Diffusion	Simple, low cost, matrix versatility, non-toxic, ease of batch processing	Time and labor consuming, high N requirement, potential fractionation and ^{15}N dilution by DON and reagents
Ion exchange	Simple, non-toxic, ease of transport and storage	High N requirement, time-consuming, salinity-sensitive, high cost, potential fractionation
Derivatization	Stable, rapid, high selectivity, low N requirement when using GC–MS	Complicate procedures, toxic, large N contamination by reagents, potential NO$_2^-$ interferences
Ba(NO$_3$)$_2$-acetone	Simple, robust, non-toxic, ease of batch processing	High N requirement, time-consuming, salinity-sensitive
NaOH-acetone	Simple, robust, non-toxic, removal of DON, ease of batch processing	High N requirement, time-consuming freeze-drying
SPIN-QMS SPIN-MIMS	Simple, rapid, fully on-line, ease of batch processing, simultaneous determination of N concentration and ^{15}N abundance	Low precision, require specialized apparatus
Denitrifier	Simple, rapid, low N requirement, small sample volumes, small N contamination and fractionation, toxic-free, ease of batch processing, ease of sample transport and storage	Strain availability, long time incubation and special care of bacterial cultures, potential interference of NO$_2^-$ and toxicant in samples
Cd-azide	Simple, rapid, low N requirement, small sample volumes, matrix versatility, N contamination and fractionation, ease of sample transport, ease of sample transport and storage	Toxic, potential NO$_2^-$ interferences
Cd-NH$_2$OH	Simple, rapid, low N requirement, small sample volumes, low N contamination by reagents, ease of sample transport and storage	Toxic, potential NO$_2^-$ interferences
VCl$_3$-Azide	Simple, rapid, low N requirement, small sample volume, matrix versatility, small ^{15}N dilution by reagents and fractionation, ease of batch processing, ease of sample transport and storage	Toxic, potential NO$_2^-$ interferences
Ti(III) reduction	Simple, rapid, small N contamination, ease of batch processing, ease of sample transport and storage	Low recovery, potential fractionation, sensitive to variances in sample NO$_3^-$concentration and salinity, potential NO$_2^-$ interferences
AIRTS-HPLC	Small sample volumes, low N requirement, non-toxic, neglectable ^{15}N dilution by reagents and fractionation	Require high ^{15}N-enrichment, time-consuming for large batches

Table 2 (continued)

Method	References
Distillation	(Bremner and Edwards 1965; Bremner and Keeney 1965; Hauck and Bremner 1976; Hauck 1982; Mulvaney 1986, 1993, 1996; Mulvaney et al. 1994; Mulvaney and Khan 1999; Sebilo et al. 2004)
Diffusion	(Liu and Mulvaney 1992a; Mulvaney 1993, 1996, 2008; Herman et al. 1995; Khan et al. 1997, 1998, 2000a; Mulvaney et al. 1997; Sigman et al. 1997; Mulvaney and Khan 1999; Chang et al. 2004; Griesheim and Mulvaney 2019)
Ion exchange	(Chang et al. 1999; Silva et al. 2000; Xing and Liu 2011; Li et al. 2015)
Derivatization	(Kator et al. 1992; Preston et al. 1998; Johnston et al. 1999; Clark et al. 2007)
$Ba(NO_3)_2$-acetone	(Huber et al. 2011; Tanu et al. 2020)
NaOH-acetone	(Huber et al. 2012)
SPIN-QMS	(Stange et al. 2007)
SPIN-MIMS	(Eschenbach et al. 2017)
Denitrifier	(Christensen and Tiedje 1988; Højberg et al. 1994; Sigman et al. 2001)
Cd-azide	(McIlvin and Altabet 2005; Tu et al. 2016; Zhao et al. 2019)
$Cd-NH_2OH$	(Liu et al. 2014)
VCl_3-Azide	(Lachouani et al. 2010)
Ti(III) reduction	(Altabet et al. 2019)
AIRTS-HPLC	(Gardner et al. 1995)

[a]The sample volume refers to the volume conducted at once, depending on sample N concentration. In cases where more N is required, samples may either be concentrated or multiple sample aliquots can be combined

[b]The time doesn't involve any pre-concentration (e.g. freeze-drying)

[c]The recently developed LC-Orbitrap HRMS with ion-exchange and gradient dilution preparation allows for ^{15}N analysis of NO_3^- in diverse environmental matrix (i.e. soil extracts and water samples) although it has not yet been used by the isotopic community

carrier gas without significant contamination from background N_2O, a large fraction of the sample can be entered into the IRMS as opposed to being lost to waste at the open split, yielding a high sensitivity (Stevens et al. 1993; Stevens and Laughlin 1994; Dore et al. 1998; Sigman et al. 2001; Weigand et al. 2016). GC–MS was also reported for quantifying ^{15}N abundance in N_2O, but it is limited to ^{15}N-enriched samples due to low instrument precision (Russow and Förstel 1993; Isobe et al. 2009).

For atmospherically-derived NO_3^- (e.g. in rainwater), using N_2O as the analyte for IRMS analysis may result in a slight overestimation of $\delta^{15}N$ values by $1-2‰$ due to mass-independent [17]O fraction (Knowles and Blackburn 1993; Shearer and Kohl 1993; Sigman et al. 2001; Michalski et al. 2002; Ohte et al. 2013). In the mass spectrometer, measurements of m/z 45 and m/z 44 allow for $\delta^{15}N$ calculation after correcting for the contribution of $^{14}N^{14}N^{17}O$. For most environmental samples, this correction can be performed routinely by most IRMS data software, which calculates [17]O contribution from [18]O

abundance by assuming a mass-dependent relationship (Böhlke et al. 2003; McIlvin and Altabet 2005). However, atmospheric NO_3^- is enriched in [17]O due to large mass-independent [17]O fractionation from ozone formation, resulting in an underestimation of the m/z 45 contribution of [17]O (Michalski et al. 2002, 2003; Böhlke et al. 2003; Soto et al. 2015). This error can be minimized by using a denitrifying strain that primarily produce oxygen in N_2O from H_2O (Hastings et al. 2003; Coplen et al. 2004). Alternatively, the N_2O can be analyzed by coupling PT-IRMS to a thermal decomposition system (Brand 1995; Kaiser et al. 2007; Komatsu et al. 2008; Smirnoff et al. 2012; Hattori et al. 2016) or directly by laser spectroscopy (Soto et al. 2015; Wassenaar et al. 2018; Altabet et al. 2019; Harris et al. 2020) to avoid mass-overlap correction and help discern atmospheric N sources (See 3.1.6 Availability of equipment).

Both microbial and chemical methods have been reported for converting dissolved NO_3^- to N_2O gas for ^{15}N analysis. The "denitrifier method" is a well-established technique, in which NO_3^- is reduced

to NO_2^- and finally to N_2O by denitrifying bacteria (Christensen and Tiedje 1988; Sigman et al. 2001; Casciotti et al. 2002; Hastings et al. 2003; Coplen et al. 2004; Mørkved et al. 2007; Michener and Lajtha 2008; Isobe et al. 2009; McIlvin and Casciotti 2011; Weigand et al. 2016). A number of chemical reducing methods are also gaining ground, using cadmium (Cd) or vanadium chloride (VCl_3) to reduce NO_3^- to NO_2^- and using azide or hydroxylamine (NH_2OH) as catalyst for further reduction of NO_2^- to N_2O (McIlvin and Altabet 2005; Lachouani et al. 2010; Ohte et al. 2013; Tu et al. 2016; Wassenaar et al. 2018; Zhao et al. 2019; Jin et al. 2020). Recently, Altabet et al. (2019) presented an one-step chemical method in which NO_3^- is reduced to N_2O using Ti(III) chloride. In all these microbial and chemical methods, the NO_2^- present in the sample is reduced alongside the NO_3^-, and the measured [15] N abundance of the N_2O is a composition of both the NO_2^- and the NO_3^-. When preexisting NO_2^- concentration is high and only the [15] N abundance of NO_3^- is required, NO_2^- can be removed using sulfamic acid (Norman and Stucki 1981; Ward et al. 1984; Mulvaney 1996; Granger et al. 2006).

Methods for generating N_2O gas from dissolved NH_4^+ usually involve the conversion of NH_4^+ to NO_3^- (or NO_2^-) and the reduction of NO_3^- (or NO_2^-) to N_2O, with or without pre-isolation of NH_4^+ from solutions. The isolation of NH_4^+ by diffusion (Holmes et al. 1998; Koba et al. 2010; Lachouani et al. 2010; Zhang et al. 2015) or ion-exchange resins (Kawashima et al. 2021) removes pre-existing NO_3^-, NO_2^- and other interfering chemicals in the matrix. The conversion of NH_4^+ to NO_3^- or NO_2^- can be achieved by persulfate oxidation (PO) (Houlton et al. 2007; Isobe et al. 2009; Lachouani et al. 2010) or hypobromite oxidation (BO) (Stevens et al. 1993; Stevens and Laughlin 1994; Zhang et al. 2007, 2015; David Felix et al. 2013; Liu et al. 2014), respectively. The final conversion of NO_3^- (or NO_2^-) to N_2O can be achieved using bacterial (Houlton et al. 2007; Isobe et al. 2009; Kawashima et al. 2021), azide (Lachouani et al. 2010), or NH_2OH reduction (Zhang et al. 2018), similar to the [15] N analysis methods for NO_3^-.

On-line gas methods

In all of the foregoing preparation methods, the isolation and/or conversion of the target NH_4^+ or NO_3^- is partially performed off-line. Instead, the SPIN (sample preparation unit for inorganic nitrogen) method allows for complete on-line sample preparation and [15] N measurement in [15] N-enriched samples. In this approach, NO_3^- is reduced to NO or N_2O, while NH_4^+ is oxidized to N_2. All the reactions are automated and regulated in the SPIN reaction vessel. The analytical gases are subsequently transferred to a quadrupole mass spectrometer (QMS) with continuous-flow (CF-QMS) or membrane-inlet (MIMS) mode for simultaneous determination of [15] N abundance and N concentration (Stange et al. 2007; Eschenbach et al. 2017, 2018).

Non-gas methods

For [15] N-enriched samples, the [15] N abundance and the concentration of NH_4^+ or NO_3^- can also be analyzed simultaneously in filtered solutions as dissolved NH_4^+ using the ammonium retention time shift-high performance liquid chromatography (AIRTS-HPLC) (Gardner et al. 1991, 1995; Lu et al. 2019). This is based on the difference in retention time between [15]NH_4^+ and [14]NH_4^+ as they pass through HPLC columns. The NH_4^+ in filtered water samples can be analyzed directly by HPLC, while NO_3^- is first reduced to NH_4^+ by zinc reduction under acidic conditions. To calculate the accurate [15] N of NO_3^-, the [15] N abundance and concentration of initial NH_4^+ must be pre-measured.

Development of the decision support tool (DST)

To assist ecological researchers in finding a suitable method for their specific research needs we developed a Decision Support Tool (Figs. 1 and 2), including all currently available methods, as summarized in the overview in the previous section. The users can arrive at the best solution by systematically selecting preferences based on key criteria. The aim was to provide a streamlined, visualized, and action-oriented decision-supporting tool for preparing liquid environmental samples for [15] N analysis of NH_4^+ and NO_3^-, which is easier to use for a researcher than a review that merely sums up all the details of the various methods.

We organize the DST as well as the descriptions of the methods in the supplementary information by presenting NH_4^+ and NO_3^- methods separately, although

Fig. 1 Decision support tool for ^{15}N analysis of NH_4^+ in liquid samples. The figure is created with Microsoft® PowerPoint® version 2012 and Adobe Illustrator CC 2018 version 22.1

Fig. 2 Decision support tool for ^{15}N analysis of NO_3^- in liquid samples. The figure is created by Microsoft® PowerPoint® version 2012 and Adobe Illustrator CC 2018 version 22.1

there are unavoidable overlaps since many methods can be used for both species. To optimize the procedure we provide only key information in the DST, but present a detailed comparison of all the methods in Tables 1 and 2, where the characteristics, advantages, and disadvantages can be easily found. Furthermore, a comprehensive and up-to-date description of the published approaches for both NH_4^+ and NO_3^- preparation is provided as supplement. When navigating by the DST, users can and should always refer to the original literature before proceeding.

Criteria for method selection

Among the numerous ^{15}N analysis approaches that have been reported, each method employs a set of specific procedures, materials, and instruments to deliver optimal results under certain conditions. In this section, we discuss the criteria that should be generally considered when determining the appropriate method for ^{15}N analysis of NH_4^+ and NO_3^- in liquid samples. Note that these criteria are interrelated and should be considered together when evaluating methods.

Sample matrix

In ecological research, the liquid samples containing the $^{15}NH_4^+$ or $^{15}NO_3^-$ to be determined can stem from a range of environmental sources (e.g., freshwater, seawater and soil extracts), and, as a consequence, can vary strongly in composition. Salts, dissolved organic matter, pH, hazardous chemicals, and other nitrogen-containing substances can all contribute to isotopic fractionation and contamination, reducing the precision and accuracy of ^{15}N measurements.

Sample salinity is one of the most important factors limiting the choice of appropriate methods. Salts (e.g., Mg^{2+}, K^+, SO_4^{2-}, Cl^-) and dissolved organic carbon (DOC) can act as competitive chemicals in exchange methods, reducing recovery of target N components (Chang et al. 1999; Silva et al. 2000; Böhlke et al. 2006; Li et al. 2015). Salts may also reduce recovery by changing the kinetics of certain reactions. The conversion of NO_3^- into N_2O by Ti(III) reduction, for example, is significantly impeded by SO_4^{2-} in the solutions (Altabet et al. 2019). Poor recovery causes isotopic fractionation, resulting in erroneous depletion or enrichment of ^{15}N in the final

product (see 3.1.5 Isotopic fractionation). Because salt removal and calibration by references with an identical matrix are often impractical, such salinity-sensitive methods are most suitable for use of freshwater samples.

Distillation and diffusion are used for a wide range of matrices because they isolate target N species from solutions. They can be used to directly produce N-containing salts for off-line combustion or EA-IRMS analysis (Bremner and Edwards 1965; Keeney and Bremner 1966; Fiedler and Proksch 1975; Hauck 1982; Keeney and Nelson 1983; Brooks et al. 1989; Sørensen and Jensen 1991; Mulvaney 1993, 1996; Bremner 1996; Holmes et al. 1998), or they can be used in front of ion exchange or chemical conversions to pre-isolate N from high salinity solutions (Velinsky et al. 1989; Isobe et al. 2009; Lachouani et al. 2010; Zhang et al. 2015). The two methods, especially distillation, are more appropriate for samples containing low amounts of dissolved organic N (DON), because liberation of NH_4^+ by DON hydrolysis can cause errors to ^{15}N abundance of NH_4^+ and NO_3^- being measured. More detailed discussion of this issue can be found in Sect. 3.1.4 (Contamination by other N-containing chemicals).

Other matrix characteristics can also limit choices of sample preparation methods. Methods involving derivatization, MIMS and HPLC techniques, are appropriate for both freshwater and marine samples from tracer studies, but whether they are appropriate for soil extracts has not yet been examined. Toxic compounds and soil extractants (e.g., KCl) can suppress bacterial activity in the denitrifier method, resulting in low recovery and fractionation (Sigman et al. 2001; Casciotti et al. 2002). The presence of NO_2^- might cause errors in methods such as derivatization, denitrifier and azide conversions where it occurs as an intermediate product in the N transformations (Sigman et al. 2001; McIlvin and Altabet 2005; Zhang et al. 2007; Lachouani et al. 2010; Ward 2011; David Felix et al. 2013; Altabet et al. 2019), but it can be measured using a colorimetric method and removed using sulfamic acid (Granger et al. 2006; Ward 2011).

^{15}N abundances of NH_4^+/NO_3^- in samples

Samples for ^{15}N analysis can be at natural ^{15}N abundance or ^{15}N-enriched level (in case of pool dilution

and ^{15}N-tracing experiments), and researchers must choose a method that is sufficiently precise and accurate to measure the large or small ^{15}N variations in their samples.

Natural abundance studies require excellent precision because the δ^{15}N abundances of most natural ecological samples fall within the narrow range of $-20‰$ to $+30‰$ ($x(^{15}$N$) = 0.362 – 0.377\%$) (Kendall 1998; Robinson 2001; Fry 2006; Mulvaney 2008; Michener and Lajtha 2008). Only IRMS and laser spectroscopy provide this level of precision, and only sample preparation methods employing these two instruments are appropriate for natural δ^{15}N abundances. Modern N_2O methods, in particular, are gaining popularity in natural abundance studies due to their high sensitivity and capability for dual O/N isotope analysis (Sigman et al. 2001; Casciotti et al. 2002; McIlvin and Altabet 2005; Altabet et al. 2019).

The ^{15}N abundance of target N species are typically $> 0.5\%$ ($x(^{15}$N$)$) in tracer experiments. The analysis of such samples also requires precision, but the limiting factors are often the highly variable ^{15}N abundances and the low N concentrations in the samples (Knowles and Blackburn 1993; Fillery and Recous 2001). Researchers need to be aware of these factors at initial planning, and calculate the level of ^{15}N addition based on the system, the frequency of tracer addition, the extent of the change in ^{15}N abundance during incubation, as well as the analytical constraints (Edwards 1978; Fillery and Recous 2001; Lipschultz 2008; Mayer et al. 2013). IRMS is usually used to measure ^{15}N abundances from natural abundance to low enrichment (e.g. $x(^{15}$N$) < 10\%$), and measurements beyond this range become less precise and accurate because of nonlinear amplification of ion currents and memory effects (Mulvaney 1993; Werner and Brand 2001). Samples with higher ^{15}N enrichments can be spiked with natural abundance materials to reach the ideal working range for IRMS analysis, although this introduces analytical errors, which increase with increased ^{15}N enrichment (Mulvaney 1993; Mulholland et al. 2004; Griesheim and Mulvaney 2019). It is also possible to measure ^{15}N enriched samples using ES (Fiedler and Proksch 1975; Craswell and Eskew 1991; Hoult and Preston 1992; Preston 1993), GC–MS (Russow and Förstel 1993; Clark et al. 2006, 2007), QMS (Stange et al. 2007), MIMS (Yin et al. 2014; Eschenbach et al. 2017, 2018), or HPLC (Gardner et al. 1991, 1995)

with acceptable precision (SD $< 0.03\%$, SD: standard error).

Whether samples are at natural abundance or have high ^{15}N enrichments, reference materials that bracket the entire expected ^{15}N abundances should always be processed concurrently with the unknown samples to guarantee accurate and reliable results (Klesta et al. 1996; Werner and Brand 2001; Brand and Coplen 2012; Carter and Fry 2013; Brand et al. 2014; Meier-Augenstein and Schimmelmann 2019; Mohn et al. 2022). If possible, the reference materials should be subjected to the same procedures and have the same matrices as those of the samples (i.e. the identical treatment principle). This strategy minimizes systematic errors, such as the aforementioned fractionation and memory effects in the IRMS system.

Concentration of NH_4^+/NO_3^- in samples

The concentrations of target N in samples should be within the working range of the employed sample preparation and analysis method in order to achieve optimal precision and accuracy. A method can vary in its appropriateness for large samples volumes or smaller sample N amounts to accommodate samples with low N concentrations that require special preparation or analysis to obtain high quality data (Hauck and Bremner 1976; Owens 1988; Velinsky et al. 1989; Knowles and Blackburn 1993; Mulvaney 1993, 1996; Shearer and Kohl 1993; Bremner 1996; Holmes et al. 1998; Smith and Mullins 2000; Robinson 2001; Scrimgeour and Robinson 2003; Chang et al. 2004; Groot 2004; Cook et al. 2017). The NH_4^+ and NO_3^- concentrations (or total amounts) as well as sample volumes required in reported methods are shown in Tables 1 and 2.

Methods that involve transformation into N_2 and its ^{15}N analysis by IRMS require 2–400 µmol of target N, resulting in an ideal concentration of > 50 µM in a typical volume of ~ 50 ml. Lower NH_4^+ and NO_3^- concentrations (e.g. $5 - 10$ µM) can, however, occur in many aquatic environments (Holmes et al. 1998; Teece and Fogel 2004) as well as groundwater-paddy soil agroecosystems (Soldatova et al. 2021). In traditional N_2 methods this is resolved by using large volumes of solutions, and then concentrating the N in some way. This processing increases not only the time and effort for sample preparation, but also errors

of isotopic fractionation and contamination by the large amount of reagent N (See 3.1.4 Contamination by other N-containing chemicals and 3.1.5 Isotopic fractionation). As an alternative, it is possible to add a known quantity of a standard of known ^{15}N content into samples and calibrate the results by an isotope dilution equation, but such an addition introduces analytical errors, which increase with increased ^{15}N enrichment (Hauck 1982; Glibert and Capone 1993; Mulvaney 1993; Stephan and Kavanagh 2009). Consequently, methods developed for small N amounts and volumes are more appropriate alternatives. For example, the N_2O methods and non-gas AIRTS-HPLC methods require nanomole levels of target N and allow for samples containing micromolar amounts of NH_4^+ or NO_3^- in milliliters of solutions. With advances in sample preparation, analysis and calibration (Toyoda et al. 2017; Wassenaar et al. 2018; Yu et al. 2020; Harris et al. 2020; Mohn et al. 2022), chemical and biological N_2O methods are increasingly used not only for low N samples but also for large N samples (e.g. > 50 μM) following dilution. This delivers more accurate and consistent results for natural environmental samples varying largely in their concentrations, as well as facilitates lab compatibility and accelerates progress in large-scale isotopic studies. When diluting samples, however, one must pay attention to the water chemistry of the dilution solution, and an accurate blank correction (see 3.1.4 Contamination by other N-containing chemicals) is necessary (Lachouani et al. 2010; Altabet et al. 2019).

Contamination by other N-containing chemicals

When using certain methods, the isotopic composition of non-target N compounds in solutions can bias the ^{15}N measurement of the target N (Hauck 1982; Jensen 1991; Liu and Mulvaney 1992b; Herman et al. 1995; Mulvaney 1996; Stark and Hart 1996; Sigman et al. 1997; Holmes et al. 1998; Mulvaney and Khan 1999; Robinson 2001; Stephan and Kavanagh 2009). Distillation and diffusion of NO_3^- are particularly prone to such contamination, because reagents such as Devarda's alloy and soil KCl extractants, as well as residual NH_4^+ can all result in erroneously dilution or enrichment of ^{15}N abundances of the target NO_3^-. This is of course affected by the purity and quantity of the contaminates, as well as the ^{15}N differences between the N impurities and the target N.

When diffusing 50 μg NO_3^-, Stephan and Kavanagh (2009) found that reagent N depleted by 10‰ relative to the target δ^{15}N resulted in underestimating the target δ^{15}N by 0.8 to 1.6‰. Such an error increases with an increasing amount of reagent N, is difficult to quantify, and can cause significant inaccuracies in samples with low NO_3^- contents (Liu and Mulvaney 1992b; Herman et al. 1995; Sigman et al. 1997; Robinson 2001; Stephan and Kavanagh 2009). Residual ^{15}N-labeled NH_4^+ can also lead to substantial overestimation in successive ^{15}N analysis of unenriched NO_3^-, even at trace levels. Although an isotope-dilution technique or a cleaning procedure can reduce (but not completely eliminate) such an inaccuracy, it can still be undesirable for samples with low NO_3^- contents (e.g. 50 μg) and/or large volumes (e.g. > 30 ml) (Hauck 1982; Liu and Mulvaney 1992b; Saghir et al. 1993b; Mulvaney et al. 1994; Herman et al. 1995; Mulvaney 2008; Griesheim and Mulvaney 2019).

Non-target N compounds such as DON in samples may be converted to NH_4^+ due to chemical hydrolysis under prolonged diffusion and distillation, contributing to excess N and dilute ^{15}N values in the product (Hauck 1982; Velinsky et al. 1989; Liu and Mulvaney 1992b; Mulvaney 1993; Mulvaney et al. 1994, 1997; Khan et al. 1997; Sigman et al. 1997; Mulvaney and Khan 1999; Chang et al. 2004). This contamination was found to be significant for distillation and diffusion at > 50 ml, and is prompted by a higher temperature, pH and the use of Devarda's alloy reagent (Khan et al. 1997; Mulvaney et al. 1997; Sigman et al. 1997; Holmes et al. 1998; Mulvaney and Khan 1999). For organic-rich samples with low target N (especially NO_3^-) concentrations, such as extracts from organic horizons of forest soils, it is preferable to use methods in which DON is removed. The acetone method, for example, isolates NO_3^- from organic matter in solutions and has been used for soils with organic amendments (Reichel et al. 2018), although the sample N requirement is large (4–9 μmol) (Huber et al. 2011, 2012). In the case of NH_4^+, many N_2 and N_2O methods require isolating NH_4^+ from soil extracts by diffusion and are inherently susceptible to DON contamination (Lachouani et al. 2010; Zhang et al. 2015). This risk can be mitigated by a lower temperature, a shorter diffusion period, no vigorous shaking, and gas-phase trapping (Mulvaney 2008; Cao et al. 2018).

In contrast to traditional N_2 methods, chemical and biological N_2O conversion methods generally report

constant and small contamination (only nanomoles of N) for natural abundance samples with low N amount (Sigman et al. 2001; McIlvin and Altabet 2005; Isobe et al. 2009; Tu et al. 2016; Zhao et al. 2019; Jin et al. 2020). In particular, the interferences by DON to target $\delta^{15}N$ is significantly reduced, because NO_3^- (and sometimes also NH_4^+) in matrix can be converted selectively (Sigman et al. 2001; McIlvin and Altabet 2005; Zhang et al. 2007; Liu et al. 2014). The N contribution of non-target chemicals with the denitrifier and azide methods are reported as 2.5–5 and 0.1–10%, respectively (Sigman et al. 2001; McIlvin and Altabet 2005). Since such "method blanks" are often consistent and reproducible, they can be calibrated by using reference materials in N-free water with a similar matrix as the samples. Both reagents and water used for blank correction and sample dilution should be purified (e.g. by boiling or combusting) and, if necessary, calibrated for their N amount and isotopic composition (Sigman et al. 2001; McIlvin and Altabet 2005; Lachouani et al. 2010; Altabet et al. 2019).

Isotopic fractionation

Isotopic fractionation may arise from any stage of the sample preparation and instrument analysis, lowering the precision and accuracy of ^{15}N measurements. It is the result of incomplete transformation or recovery of the target N, as the rate of such a process usually differs between ^{14}N and ^{15}N. To avoid fractionation, a complete recovery is required, but this can be challenging when processing large volumes of samples by distillation/diffusion (Fiedler and Proksch 1975; Hauck 1982; Velinsky et al. 1989; Liu and Mulvaney 1992b; Mulvaney 1993; Holmes et al. 1998; Chang et al. 2004; Stephan and Kavanagh 2009) and freeze-drying techniques (Hauck 1982; Stock et al. 2019), as well as when there are large sample matrix effects (Chang et al. 1999; Silva et al. 2000; Lehmann et al. 2001; Altabet et al. 2019). For example, Holmes et al. (1998) found that fractionation of NH_4^+ diffusion increased from 0.2 to 10‰ in 200 ml to 3 l samples. Stock et al. (2019), observed a fractionation of up to 10‰ when freeze-drying samples from 500 ml to ≤5 ml to obtain enough N for the use of the TPB precipitation method. If not accounted for, such an error can be problematic for natural abundance studies, as the $\delta^{15}N$ in most ecological samples

varies only by < 20‰ (Robinson 2001). Fractionation can also occur when samples are entered into a mass spectrometer (Mulvaney 1993; Dawson and Brooks 2001).

Fractionation during sample preparation and measurement can be largely calibrated by subjecting reference materials and unknowns to the same sample preparation and analysis pathway (Klesta et al. 1996; Holmes et al. 1998; Werner and Brand 2001; Gröning 2004; Stephan and Kavanagh 2009; Brand and Coplen 2012; Carter and Fry 2013; Altabet et al. 2019; Stock et al. 2019). Ideally, one needs to match samples and standards with respect to chemical matrix and sample N concentration, as well as the range of ^{15}N abundance, however finding ideal references may be a difficult task. If there are substantial interferences by reagents and matrix, such as in distillation and diffusion of soil NO_3^-, the calibration becomes more tedious and inaccurate (Liu and Mulvaney 1992b; Herman et al. 1995; Mulvaney et al. 1997; Holmes et al. 1998; Khan et al. 2000b; Stephan and Kavanagh 2009). All of these issues compromise the ultimately achievable accuracy. Whether this error is acceptable is determined by the accuracy necessary for observable changes among samples, and there may be a trade-off between analytical accuracy and the time and effort required for complete recovery and calibration (Mulvaney 1993; Holmes et al. 1998; Silva et al. 2000; Dawson and Brooks 2001; Benson et al. 2006; Stock et al. 2019).

Given the difficulty of complete recovery and accurate calibration when isolating and freeze-drying low N samples, methods adapted for natural abundance, and small N amounts and volumes, are preferred in such instances. For example, the denitrifier (Christensen and Tiedje 1988; David Felix et al. 2013), azide (McIlvin and Altabet 2005; Zhang et al. 2007; Ryabenko et al. 2009; Tu et al. 2016; Zhao et al. 2019), and NH_2OH procedures (Liu et al. 2014; Zhang et al. 2015; Jin et al. 2020) all reported satisfying recovery and negligible fractionation when producing sub-micromoles of N_2O from milliliters of solutions.

Availability of equipment

The availability of equipment, such as special materials and apparatus for sample preparation, as well as analytical instruments, limits the variety of methods

that can be used. For example, the distillation method requires a, commercially available, distillation apparatus; the ion-exchange method entails resins and reagents that are expensive for large batches; the denitrifier method relies on anaerobic denitrifying bacteria strains that are difficult to obtain and maintain in non-microbiology laboratories; the SPIN method uses a non-commercially available reaction unit that has been custom built in only a few laboratories worldwide (Silva et al. 2000; Sigman et al. 2001; Chang et al. 2004; Stange et al. 2007). When such materials and equipment are not readily available, preparation methods using off-the-shelf chemicals are the only alternatives, such as the diffusion, acetone extraction and chemical-based N_2O methods (Brooks et al. 1989; Holmes et al. 1998; McIlvin and Altabet 2005; Zhang et al. 2007; Lachouani et al. 2010; Huber et al. 2011, 2012; Liu et al. 2014).

The most popular instrument for ^{15}N analysis is IRMS (Brenna et al. 1997; Scrimgeour and Robinson 2003; Meier-Augenstein 2004), but there are also alternatives such as LS (laser spectroscopy) (Mohn et al. 2014; Soto et al. 2015; Wassenaar et al. 2018; Ji and Grundle 2019), QMS (quadrupole mass spectrometry) (Eschenbach et al., 2017, 2018; Stange et al., 2007), ES (emission spectrometry) (Fiedler and Proksch 1975; Knowles and Blackburn 1993; Preston 1993; Heiling et al. 2006), and HPLC (Gardner et al. 1991, 1995; Lu et al. 2019).

Due to their high precision (a RSD of $< \pm 0.2$ ‰ and $< \pm 1$‰, respectively, RSD: relative standard deviation), the IRMS and laser analysis following N_2/N_2O-based sample preparation methods are currently the only candidates for quantifying ^{15}N abundance in natural abundance studies (Mulvaney 1993; Robinson 2001; Scrimgeour and Robinson 2003; Soto et al. 2015; Wassenaar et al. 2018). Off-line Rittenberg oxidation or Dumas combustion and ^{15}N analysis by DI-IRMS was laborious and prone to atmospheric contamination, and have been widely replaced by the commercially available continuous flow-IRMS in routine ecological ^{15}N research (Bremner 1965a; Hauck and Bremner 1976; Hauck 1982; Kendall and Grim 1990; Knowles and Blackburn 1993; Mulvaney 1993, 1996; Preston and Slater 1994; Feast and Dennis 1996; Chang et al. 2004). Such development reduces systematic errors associated with off-line N_2 production steps and significantly improves precision, sensitivity and productivity (Preston and

Owens 1983; Barrie and Workman 1984; Marshall and Whiteway 1985; Barrie et al. 1989; Harris and Paul 1989; Egsgaard et al. 1989; Craswell and Eskew 1991; Jensen 1991; Smith and Mullins 2000; Mulvaney 2008). The EA-IRMS allows for online combustion of samples containing micromoles N (Knowles and Blackburn 1993; Mulvaney 1993; Barrie and Prosser 1996; Brand 1996; Boutton 1996; Horita and Kendall 2004), while the PT-IRMS is capable of ^{15}N analysis in nanomole N_2O gas (Casciotti et al. 2002; Chang et al. 2004; McIlvin and Altabet 2005; Lachouani et al. 2010; Toyoda et al. 2017). In addition, by coupling an on-line decomposition system such as a gold-furnace (Brand 1995; Kaiser et al. 2007; Komatsu et al. 2008; Smirnoff et al. 2012) or a microwave discharge unit (Hattori et al. 2016, 2019) to a modified PT-IRMS, the N_2O can be decomposed to N_2 and O_2 prior to isotopic analysis, preventing the interference from [17]O with δ^{15}N results. More recently, laser spectroscopy is emerging as an alternative technology to IRMS for simultaneous determination of N_2O concentration and isotopic abundance at natural abundance, possessing benefits such as real-time analysis, lower operating costs, and potential field applicability. In addition, laser spectroscopy measurements are not affected by differences in [17]O abundance, thus also eliminating any potential overestimation of ^{15}N in samples containing mass-independent [17]O variations. However, the reliability and accuracy of laser-based analysis substantially depend on the interplay between matrix and trace gas effects, spectral interferences, concentration dependencies of isotopic signals, and challenges in calibrating the instruments, making current applications difficult and limited (Köster et al. 2013; Mohn et al. 2014; Soto et al. 2015; Wassenaar et al. 2018; Ji and Grundle 2019; Yu et al. 2020; Jung et al. 2020; Harris et al. 2020).

[15]N dilution and tracing studies do not necessitate such high precision as can be achieved by IRMS and laser techniques. For such studies equipment may be used that is much less expensive and more readily available, such as ES, GC–MS, MIMS and HPLC. These instruments can serve as alternatives for laboratories where ^{15}N abundances are not routinely determined, requiring much smaller amounts of N but their precision may be 10 to 100 times less than IRMS analysis (See 3.1.9 Precision and accuracy) (Knowles and Blackburn 1993; Russow et al. 1995;

Lu et al. 2019). Although not commonly used, it is possible to connect a QMS or a MIMS to a specialized automated apparatus (e.g., an elemental analyser, or a SPIN unit) to achieve on-line gas production (Russow et al. 1995; Russow and Goetz 1998; Stange et al. 2007; Eschenbach et al. 2017, 2018).

When the instrument needed for the method chosen is not readily available, a final sample that is appropriate for transport and storage is necessary. In such cases, freshwater and marine samples can be extracted into ion-exchange resins and distilled into zeolites, respectively (Velinsky et al. 1989; Lehmann et al. 2001; Li et al. 2015). Alternatively, samples can be prepared using modern N_2O methods, as the produced N_2O gas can be stored in gas-tight containers for years (Sigman et al. 2001; McIlvin and Altabet 2005; Lachouani et al. 2010).

Toxicity of reagents

Several of the methods described involve the use of toxic reagents, and this aspect may also affect the suitability of these methods (Fisher and Morrissey 1985; Dudek et al. 1986; McIlvin and Altabet 2005). The mercury precipitation method has been rarely used since its development, due to its extreme toxicity. Some modern N_2O methods involve the use of an azide buffer to convert NH_4^+ or NO_3^- to N_2O (McIlvin and Altabet 2005; Zhang et al. 2007; Lachouani et al. 2010). For non-microbiology laboratories, these are alternatives to the denitrifier methods. Under the required acidic conditions, the azide reagent is highly toxic, volatile and explosive, necessitating high safety precautions in handling and disposal of the chemicals. Another example is the derivatization method, in which the caustic and toxic phenol is used (Selmer and Sörensson 1986; Dudek et al. 1986; Kator et al. 1992; Preston et al. 1996, 1998; Johnston et al. 1999; Köster and Jüttner 1999; Clark et al. 2006, 2007). Ecologists generally are not trained as chemists to use such dangerous chemicals, so the handling of these chemicals would require experienced technicians.

New developments, especially in chemical N_2O methods, have reduced the environmental and health risks brought by toxic reagents. For example, a modification to the azide method has substantially reduced the dose of the azide reagent used for preparing ^{15}N analysis of NO_3^- (Tu et al. 2016). A safer alternative to the azide buffer is hydroxylamine hydrochloride ($NH_2OH\cdot HCl$), which has been used in recent sample preparation methods (Liu et al. 2014; Zhang et al. 2015; Jin et al. 2020). Another chemical method is the use of Ti(III) chloride to convert NO_3^- directly to N_2O (Altabet et al. 2019). These advances give a comparable precision and accuracy as their predecessors while being more user-friendly, providing practical options for users without assistance from a chemist or microbiologist.

Preparation time

Sample preparation methods for ^{15}N analysis in NH_4^+ and NO_3^- require hours to days for complete recovery, depending on sample N concentrations and the batch size. The N_2 methods with EA-IRMS analysis (e.g., diffusion, ion-exchange, precipitation and acetone extraction) requires processing large volumes of liquids for low N samples (e.g., $< 5\ \mu M$), which considerably increases the preparation time, up to several days. To reduce sample preparation time, contemporary N_2O methods, which were developed for samples with low N concentrations and small volumes, are more appropriate.

Some sample preparation methods are fast for a small number of samples, but inefficient for batch processing of large numbers of samples. For example, the distillation method takes only minutes for one sample, but the procedures to reduce cross-contamination to successive samples and to dry samples for combustion can be very cumbersome (Bremner 1965a, b, 1996; Bremner and Edwards 1965; Hauck 1982; Mulvaney 1986, 1993, 1996; Chang et al. 2004). In contrast, the diffusion and ion-exchange method require days for complete recovery, but a batch of samples can be processed simultaneously (Tables 1 and 2).

Precision and accuracy

The sample preparation and analysis method chosen must offer a precision and accuracy capable of detecting the level of variation among samples. As previously stated, in order to acquire optimal results it is important that the methods and instruments are used under their required matrix, concentration and ^{15}N enrichment, with interferences from fractionation and ^{15}N dilution being minimized. In addition, the samples must be subject to quality control with

the use of reliable RMs (reference materials), and detailed guidelines can be found in literature (Klesta et al. 1996; Dawson and Brooks 2001; Werner and Brand 2001; Brand and Coplen 2012; Carter and Fry 2013; Brand et al. 2014; Skrzypek 2019; Meier-Augenstein and Schimmelmann 2019).

The precision and accuracy of reported methods have been often assessed using reference materials of different $x(^{15}$ N$)$/ δ^{15}N ranges, and have been frequently expressed in different ways (e.g., relatively or absolutely), making comparisons difficult. Traditional distillation and diffusion methods and IRMS analysis were usually assessed at $x(^{15}$ N$) < 10\%$ because most ^{15} N-enriched studies are conducted at this range. Methods with GC–MS, MIMS and HPLC were assessed at a higher ^{15} N enrichment. Natural abundance methods were often evaluated in original papers over a narrow range of δ^{15}N values, but they may be appropriate for wider applications as well (Michener and Lajtha 2008). We present the general range of precision and accuracy of different methods given by case papers, but they have not been included as criteria in the DST. It is worth noting that the DST is intended to be used as a navigation tool and it does not include the plethora of details for each method. Users should consult the original papers to get thorough information and verify the methods under their own scenarios.

Precision and accuracy of instruments The analysis of natural abundance requires considerable precision, which can currently only be achieved by IRMS and laser spectroscopy. Precision of DI-IRMS is usually $< 0.1‰$ δ^{15}N with a N amount of $40 – 400$ μmol N for manual N_2 preparation and ^{15} N analysis. When coupled to an automatic analyzer (i.e. EA-IRMS) or a purge and trap system (i.e. PT-IRMS), precision of CF-IRMS is usually $< 0.2‰$ δ^{15}N from natural abundance to tracer level ($< 5\%$) with a N mass of 2–10 μmol N or $20 – 60$ nmol N_2O, respectively (Bremner and Hauck 1982; Dawson and Brooks 2001; Sigman et al. 2001; McIlvin and Altabet 2005; Michener and Lajtha 2008). Precision may be lower when analyzing samples with sub-micromoles of N or higher ^{15} N enrichment (Mulvaney 1993).The recently emerged laser spectroscopy yields a precision of $< 0.5‰$ with down to 0.5 nmol N_2O at near natural abundances (Mohn et al. 2014; Soto et al. 2015; Wassenaar et al. 2018; Ji and Grundle 2019).

Precision for instruments other than IRMS and laser spectrometers is typically between $1 – 5\%$ RSD in a wide range of ^{15} N-enriched levels, which precludes their use for ^{15} N-depleted or natural abundance samples. Optimal emission spectrometers and GC–MS yield a similar precision and accuracy of $< 3\%$ (RSD and percent error) with down to $1 – 10$ μg N at tracer levels (e.g. $x(^{15}$ N$) > 0.5\%$) (Fiedler and Proksch 1975; Bremner and Hauck 1982; Craswell and Eskew 1991; Hoult and Preston 1992; Preston 1993; Heiling et al. 2006). For a similar enrichment range, GC–MS yields a precision of $1 – 3\%$ RSD for both N_2 (> 30 nmol) and N_2O (> 200 pmol) (Russow and Förstel 1993; Isobe et al. 2009). Precision for HPLC and MIMS is typically $< 5\%$ RSD, but the latter can measure a wider range of $x(^{15}$ N$)$ ($25 – 75\%$ vs. 0.5–100%) (Gardner et al. 1991, 1995; Yin et al. 2014).

Precision and accuracy of sample preparation methods Tracer studies are usually conducted at $x(^{15}$ N$) > 0.5\%$, and sample preparation methods developed for these studies generally showed standard deviations ranging from 0.02 to 5% ($x(^{15}$ N$)$) and accuracies within $\pm 5\%$ of the true values, depending on the ^{15} N abundances, N amounts, and procedures and equipment used. Previous evaluations have found that with optimal concentrations (~ 140–360 μmol and ~ 4–11 μmol N, respectively), distillations and diffusions yield comparable precision (SD $< 0.05\%$) and accuracy (percent error $< 5.3\%$) for tracer samples at $x(^{15}$ N$) < 5\%$ (Liu and Mulvaney 1992b; Lory and Russelle 1994; Mulvaney et al. 1994, 1997; Høgh-Jensen and Schjoerring 1994; Khan et al. 1997, 1998; Sigman et al. 1997; Holmes et al. 1998; Sebilo et al. 2004; Diaconu et al. 2005; Stephan and Kavanagh 2009). However, caution should be given to sequential distillation and diffusion, because cross-contamination by highly ^{15} N-enriched NH_4^+ can cause enormous errors to natural ^{15} N analysis of NO_3^- (Liu and Mulvaney 1992b; Mulvaney et al. 1994; Herman et al. 1995; Griesheim and Mulvaney 2019). At lower N masses and the whole tracer ^{15} N range, the denitrifier methods with GC–MS analysis achieved a precision and accuracy of 0.02–0.08% and 0.02% $x(^{15}$ N$)$, respectively (Christensen and Tiedje 1988; Højberg et al. 1994), better than those of SPIN methods (SD and percent error of 1–3% and 0.6%) (Russow 1999; Stange et al. 2007; Eschenbach et al. 2017) and derivatization methods (SD and percent error of 0.2–0.7%

and 0.7%) (Selmer and Sörensson 1986; Dudek et al. 1986; Kator et al. 1992; Kanda 1995; Clark et al. 2006, 2007).

The analysis of natural ^{15}N variances necessitates considerably higher precision and methods usually offer comparable performance at their working range (Owens 1988; Shearer and Kohl 1993; Fry 2006; Mulvaney 2008). Several modifications have expanded the use of distillation and diffusion methods to natural $\delta^{15}N$ range. For example, the modification to distillation by Velinsky et al. (1989) have enabled analysis of $\delta^{15}N$ of NH_4^+ with an overall precision of $< 0.5\text{‰}$ (SD) and a relative error of within $\pm 4\%$ of the true $\delta^{15}N$ at low (< 5 µM) concentrations. At a similar working range, modifications to diffusion by Sigman et al. (1997) and Holmes et al. (1998) have allowed $\delta^{15}N$ analysis of NO_3^- and NH_4^+ in water samples to be within 0.3‰ and 0.36–0.73‰ $\delta^{15}N$ (after fractionation correction) of the true values, respectively, and with an overall precision of $< 0.3\text{‰}$ for both N species. Stephan and Kavanagh (2009) found that the diffusion method can be applied to KCl extracts at natural ^{15}N abundance if a precision or accuracy of 1.3‰ is not required. Other methods for natural $\delta^{15}N$ analysis, such as ion-change (Chang et al. 1999; Silva et al. 2000; Lehmann et al. 2001; Fukada et al. 2003; Xing and Liu 2011; Gebus-Czupyt et al. 2020), acetone extraction (Huber et al. 2011, 2012), denitrifier (Sigman et al. 2001; David Felix et al. 2013), as well as azide (McIlvin and Altabet 2005; Zhang et al. 2007; Tu et al. 2016), NH_2OH (Liu et al. 2014; Zhang et al. 2015; Jin et al. 2020) and Ti(III) reduction (Altabet et al. 2019) methods, achieved comparable precision ranging from 0.2 to 1‰ $\delta^{15}N$. The $\delta^{15}N$ values from these methods were generally within 5‰ of the true values, which were verified by a multi-point calibration using international or in-house isotopic standards following the identical treatment principle (Werner and Brand 2001; Meier-Augenstein and Schimmelmann 2019). The accurate $\delta^{15}N$ values of the samples were calibrated using the linear regression established by the measured $\delta^{15}N$ values of the standards against their assigned $\delta^{15}N$ values (Klesta et al. 1996; Werner and Brand 2001; Brand and Coplen 2012; Brand et al. 2014; Meier-Augenstein and Schimmelmann 2019).

Integrating ^{15}N analysis methods and criteria into the DST

The criteria discussed are not all given the same importance in the DST. The ^{15}N abundance, the sample N concentration, the instrument availability, and the sample matrix are given priority, because they always limit the preparation approaches available. The ^{15}N abundance and N concentration of samples determine the acceptable levels of ^{15}N dilution and isotopic fractionation. Sometimes the researchers must make trade-offs between the different criteria. The toxicity of reagents and the sample preparation time are of course more malleable, as relaxing the requirements based on these criteria does not compromise the result of the measurement, but simply requires more investments. All criteria are organized in the DST in a way to streamline the decision-making steps and minimize repetitions while matching the most common situations, so that the users and laboratories of diverse interests and disciplines can quickly find the suitable approaches by following the criteria.

In the DST, the "^{15}N abundance" is set as the first criterion, dividing the decision trees into two parts: methods for ^{15}N-enriched samples (the left branch) and methods for natural ^{15}N abundance samples (the right branch) (Figs. 1 and 2). The "Sample N" is organized as the third criterion after the choice of "Instruments", followed by "Sample matrix" (including salinity and DON content), "Toxicity", "Time" and "Fractionation". In the following sections we guide the readers through the tree and explain how we split sample preparation methods into small groups based on these criteria. Because their structures are similar, we discuss the DSTs for NH_4^+ and NO_3^- at the same time.

Non-IRMS/laser methods for ^{15}N-enriched samples in the DST

The ^{15}N-enriched samples can be prepared either by methods suitable for precise IRMS/Laser analysis, or by methods developed for other instruments such as ES, GC–MS, QMS/MIMS, or HPLC. If researchers decide to use the latter, they will be guided to the left branch of the decision tree, where they have to make a choice at the node "Sample N", based on the N amounts and volumes of their samples (Figs. 1 and 2). For ^{15}N-enriched samples of sufficiently

large volumes and containing sufficient N amounts (e.g. > 30 ml and > 4 μmol), the NH_4^+ or NO_3^- can be isolated by diffusion or distillation from various kinds of sample matrices, followed by off-line Dumas combustion and optimal emission analysis (Fiedler and Proksch 1975; Hauck 1982; Mulvaney et al. 1990; Preston 1993; Mulvaney 1996). These methods are easy to set up, but they can be tedious and error-prone for large batches of low-N samples.

In case samples have low N amounts and small volumes (e.g., < 1 μmol and < 20 ml), one should pick the other branch of the node "Sample N", and then make decisions based on the kind of sample matrix, the availability of materials, and concerns about toxicity and time. In terms of sample matrix, both soil extracts and water samples can be prepared using the denitrifier method and then measured using a GC–MS. If NH_4^+ is the target N compound (Fig. 1), a hybrid denitrifier method can be used, which involves isolating NH_4^+ by diffusion, converting NH_4^+ to NO_2^- by hypobromite oxidation, and converting NO_2^- to N_2O (Diffusion-BO-denitrifier). Alternatively, liquid samples of diverse matrices can be prepared using the SPIN-QMS/MIMS method. In that case the liquid samples are entered by means of an autosampler into a special sample preparation unit, in which different reagents are automatically dosed, to convert NH_4^+ and NO_3^- to a gaseous N-product (e.g., N_2, N_2O, or NO). The N-gases are then fed into the coupled mass spectrometer. It is the only fully on-line method to analyze the isotopic composition of NH_4^+ and NO_3^- (and NO_2^-) in liquid samples. However, this specialized SPIN apparatus is probably not available in most laboratories.

Other reported non-IRMS/Laser methods are limited to freshwater and seawater samples, and vary in the level of toxicity and sample preparation time. The Hg precipitation is salinity-sensitive and extremely toxic, and is rarely used today (Fig. 1). The derivatization methods also use hazardous chemicals. However, adaptations to derivatization using GC–MS make it possible to analyze samples containing only nanomoles of target N, which can be of interest in case of pool dilution experiments in oligotrophic ocean environments. Methods without the use of highly toxic reagents include OX (oxidation)-MIMS (only for NH_4^+) (Fig. 1) and AIRTS-HPLC (for both NH_4^+ and NO_3^-) (Figs. 1 and 2), both of which allow for the analysis of small volume samples with low N

concentrations (Tables 1 and 2). In the OX-MIMS method, NH_4^+ in solution is directly converted to N_2 using hypobromite iodine oxidation. This procedure is prone to N contamination and has low precision (< 5%), making it ideal for studying small and highly dynamic natural water NH_4^+ pools, such as those in sediment oxygen-transition zones (Yin et al. 2014). In the AIRTS-HPLC method, the conversion into N_2 gas is not needed, and the isotopic ratio of NH_4^+ is measured based on a retention-time shift of the combined peak of the $^{15}NH_4^+$ and $^{14}NH_4^+$. The shift is relative to the percentage of $^{15}NH_4^+$ and the best relationship occurs between 25–75% $x(^{15}N)$. As a result, the AIRTS-HPLC method is suitable for pool dilution experiments with variations in ^{15}N abundances within this range (Gardner et al. 1991, 1995, 1997; Lu et al. 2019). Because the individual NH_4^+ peak is isolated from organic-N peaks on the HPLC column, it is advantageous for applications such as analyzing the degradation of peptide and amino acid ^{15}N to $^{15}NH_4^+$ (Gardner et al. 1995; Yin et al. 2014).

IRMS/Laser Methods in the DST

Both ^{15}N-enriched and natural ^{15}N abundance samples can be processed using sample preparation methods that produce N_2 or N_2O for IRMS/laser analysis, but only the latter necessitates such highly precise methods and instruments. IRMS and laser methods can be found on the right branch of both decision trees and are split up at the node "Instrument", and the node "Sample N" (Figs. 1 and 2). There are two major categories of methods in this part: samples are prepared in one category as solids for on-line combustion into N_2 and ^{15}N analysis by EA-IRMS (i.e., N_2 methods), and in the other as N_2O gas for ^{15}N analysis by PT-IRMS or laser spectroscopy (i.e., N_2O methods). They vary in their requirements of sample N amounts and volumes. The first category of methods include distillation, diffusion, derivatization, ion-exchange, TPB precipitation (only for NH_4^+) and acetone extraction (only for NO_3^-), all of which are appropriate for samples containing large N amounts (e.g., > 1 μmol). For samples with a low N concentration, the concentration procedure is tedious and prone to errors from fractionation and ^{15}N dilution by reagents. In the second group of methods N_2O is produced as the analyte by means of microbes or chemicals. Because the ^{15}N analysis of N_2O has

a high sensitivity, these methods allow samples containing smaller N amounts (20–60 nmol) and volumes (< 20 ml). Because the ^{15}N analysis of N_2O has a high sensitivity, these methods allow samples containing smaller N amounts (20–60 nmol) and volumes (< 20 ml). Samples with larger N amount can also be prepared using N_2O methods after dilution, yielding more compatible and precise results for natural abundance studies in environmental samples.

N_2 methods for samples with large N amounts (micromoles of N) After choosing a N_2 or N_2O method based on sample N amount and volume, researchers must check the salinity level and DON content of the matrix. Among N_2 methods, the distillation, diffusion, and NaOH-acetone extraction are salinity-insensitive and hence appropriate for both soil extracts and water samples. In particular, the NaOH-acetone method extracts NO_3^- into an acetone solvent, isolating it from other salts and organic matter, which is advantageous for samples containing large quantities of DON (e.g., several times that of NO_3^-). However, the freeze-drying process to obtain large N amounts (4–9 μmol) increases preparation time. The diffusion also requires days of preparation, but many samples can be processed simultaneously. In contrast, the distillation method is efficient when preparing small batches of samples but can be tedious and contamination-prone for large batches. It is used primarily for ^{15}N-enriched studies in contemporary ecological research. Another salinity-insensitive method is derivatization, but the use of toxic chemicals and the need for complex procedures render it impractical.

Methods that are salinity-sensitive are only appropriate for freshwater samples. Both NH_4^+ and NO_3^- can be prepared using the ion-exchange methods, which are simple to set up, and allow for field processing and long-term storage with minimal fractionation. However, the costs of resin and chemicals (e.g., silver oxide for NO_3^- elution) can be an issue for large numbers of samples. The TPB precipitation and the $Ba(NO_3)_2$-acetone techniques, on the other hand, use less expensive materials and are appropriate for preparing NH_4^+ and NO_3^-, respectively. They both use freeze-drying to concentrate solutions, which means that fractionation must be minimized in that process or accounted for.

N_2O methods for samples with small N amounts (nanomoles of N) For samples with low N content

and small volumes, the N_2O methods with IRMS/laser analysis are more appropriate as they eliminate time-consuming concentration processes and reduce potential fractionation and contamination. In addition, they produce N_2O gas in gas-tight vials, which can be easily transported and preserved. These methods are located on the right side of the decision trees (Figs. 1 and 2). In these methods, NH_4^+ is usually first isolated from solution using diffusion or ion-exchange resins, and then converted to NO_3^- or NO_2^- by persulfate oxidation (PO) or hypobromite oxidation (BO), respectively. The NO_3^- or NO_2^- is then converted to N_2O by denitrifying bacteria, azide or NH_2OH techniques (Fig. 1). If NO_3^- is the target N, it can be converted to NO_2^- and then to N_2O by denitrifying bacteria, Cd-azide, VCl_3-azide, Cd-NH_2OH, or Ti(III) reduction methods (Fig. 2).

The sample matrix and the N species being studied determine the time and procedures required for N_2O methods. NH_4^+ in freshwater and seawater samples can usually be prepared within 2–3 days using hypobromite oxidation coupled to denitrifier, azide or hydroxylamine reduction (BO-denitrifier, BO-azide and BO-NH_2OH), although the pre-incubation of denitrifying bacteria may take 10–12 days. On the other hand, NH_4^+ in soil extracts is usually first isolated by diffusion, due to interferences from soil extractants (e.g., KCl), which unavoidably increases the preparation time by 3–5 days (Fig. 1). If NO_3^- is of interest, both soil extracts and water samples can be prepared within a few days using one of the above-mentioned conversions.

The N_2O methods are usually similar in performance, but the chemicals involved differ in toxicity. Many N_2O techniques involve the use of the highly toxic azide reagent, which necessitates safety precautions and chemical guidance (Figs. 1 and 2). As alternatives, less-toxic NH_2OH methods or non-toxic bacterial methods can be used. The denitrifier method is the safest, but the difficulties in getting denitrifying bacteria strains, as well as the need for long-term incubation and particular care of microbial cultures, make it less practical than hydroxylamine methods for most laboratories. Another toxin-free option for NO_3^- preparation is the Ti(III) method, but it needs a strict calibration strategy to account for isotopic fractionation, owing to the fact that the recovery is relatively low and declines significantly with increasing salt concentration (Fig. 2). The other N_2O methods

usually have very small isotopic fractionation and ^{15}N dilution by reagents at their working range (Tables 1 and 2).

Discussion

Application cases

In this section, we present two examples to give a better understanding of the use of the DST.

Case 1: Measuring natural ^{15}N abundance of NH_4^+ and NO_3^- in agricultural soils

The variations in natural ^{15}N abundance ($\delta^{15}N$) of N in soils have been used to identify N contamination sources and transformation processes (Robinson 2001). Agricultural soil extracts typically contain high concentrations of NH_4^+ and NO_3^- (e.g., > 50 μM) and DON due to high nutrient input from fertilizers. We assume that IRMS is available in this case.

To find the ^{15}N analysis method for NH_4^+ and NO_3^-, we first select "Natural abundance" for "^{15}N abundance" in the DST (Figs. 1 and 2). If a PT-IRMS or a laser spectrometer is available, the N_2O methods are recommended, which are more specific and less prone to contamination and fractionation. Therefore, we choose "Small" for "Sample N" and "Soil extracts" for "Sample matrix" for both NH_4^+ (Fig. 1) and NO_3^- (Fig. 2). In the case of NH_4^+ (Fig. 1), alkaline soil extracts can be analyzed using the $BO-NH_2OH$ method within 2–3 days; otherwise, the NH_4^+ must first be isolated using diffusion, resulting in a longer preparation time. Following persulfate oxidation or hypobromite oxidation, the denitrifier techniques (Diffusion-PO/BO denitrifier) enable an efficient and eco-friendly sample preparation. On the other hand, azide or NH_2OH techniques (Diffusion-PO-azide, Diffusion-BO-azide, and Diffusion-BO-NH_2OH) can be used in non-microbiology laboratories, and the latter employs less hazardous reagents. In the case of NO_3^- (Fig. 2), similarly, the final choice depends on toxicity concern and strain availability.

If only a EA-IRMS is available, alternatively, we choose "Large" for "Sample N" and "Soil extracts" for "Sample matrix" in the DST for ^{15}N analysis of NH_4^+, which brings us to the node "Time" (Fig. 1). The distillation method provides field applicability

with a commercially available apparatus, but it may lead to large inaccuracies in natural $\delta^{15}N$ results due to the high risk of fractionation and cross-contamination. In contrast, the diffusion method eliminates cross-contamination, and is more suitable for batch-processing without requiring specialized apparatus. In the DST for NO_3^- (Fig. 2), we choose, similarly, "Natural abundance", "Large sample N", and "Soil extracts". In the current case, the soil extracts are enriched with a high level of DON that is likely to break down and cause large contamination during sample preparation. Therefore, we prefer to use the NaOH-acetone method due to its DON-removal capability.

Case 2: Estimating gross N transformations in a pool dilution experiment in seawater

Gross NH_4^+ and NO_3^- transformation rates in aquatic and terrestrial ecosystems can be estimated using the ^{15}N pool dilution technique, whereby small amounts of $^{15}NH_4^+$ or $^{15}NO_3^-$ are added, and the rates of individual N transformation processes can be estimated from changes in size and ^{15}N abundance of the mineral N pools during incubation (Kirkham and Bartholomew 1954; Tietema and Wessel 1992; Laine et al. 2018). The pool dilution technique has been widely used to measure N dynamics such as NH_4^+ regeneration and nitrification in benthic and pelagic freshwater and marine ecosystems (Gardner et al. 1991; Ward 2011). Here we consider a case of a pool-dilution experiment in seawater. In such a case, samples are generally characterized by low NH_4^+ and NO_3^- concentrations (e.g., around 10 μM NH_4^+ or NO_3^-) and a high salinity. Furthermore, we assume that samples are small in volume (e.g., 20 ml), and that either the IRMS or the LS, and also denitrifier cultures are not readily available. The lack of such instruments is often the case in laboratories where the ^{15}N abundance is not routinely measured.

In the DST for $^{15}NH_4^+$ analysis, we choose "^{15}N enriched" for "^{15}N abundance", "Others" for "Instrument", and "Small" for "Sample N" (Fig. 1). We can then choose both "Water samples only" and "Soil extracts and water samples" for "Sample matrix". Among methods appropriate for both soil extracts and water samples, the SPIN method is rapid but it requires a higher NH_4^+ concentration (> 70 μM) and special reaction apparatus. The

diffusion-BO-denitrifier method, on the other hand, allows for the preparation of samples with lower NH_4^+ concentrations (> 1 μM), but it needs a long preparation time and the use of special denitrifying bacteria. Alternatively, we can choose sample preparation methods that are "Water samples only" and with low "Salinity sensitivity". The derivatization method uses hazardous reagents and necessitates a sample volume of > 50 mL (Table 1), which is inappropriate in this case. As a result, we can choose between "AIRTS-HPLC" and "OX-MIMS", both of which work for small sample N and volumes. The AIRTS-HPLC method is capable of determining the concentration and the ^{15}N abundance of NH_4^+ simultaneously, but it requires more time than the OX-MIMS method (1 h vs. 24 h for a set of ~ 10 samples) (Table 1).

With regard to the $^{15}NO_3^-$ analysis (Fig. 2), we similarly choose "^{15}N enriched" for "^{15}N abundance", "Small" for "Sample N" and "Water samples" for "Sample matrix". Due to the toxicity and large volume requirement of the derivatization method, we prefer other methods such as the "AIRTS-HPLC", "SPIN-QMS/MIMS" and "denitrifier" methods. If it is possible to obtain the SPIN apparatus or the cultures of denitrifying bacteria, the latter two methods are fast and simple choices. On the other hand, the AIRTS-HPLC method has a low throughput while using more readily available reagents and equipment. As NO_3^- is reduced to NH_4^+ as the analyte, the ^{15}N abundance of $^{15}NO_3^-$ should be calculated based on the NH_4^+ results. Therefore, if this method is used, the NH_4^+ should be prepared and measured prior to the NO_3^-.

Pros and cons of the present DST

In this work, we focus on the ^{15}N analysis of NH_4^+ and NO_3^- in liquid samples from various environmental matrices, as they are the two key mineral N species in ecosystems and primarily used in environmental ^{15}N studies. While some simple and cheap methods such as the OX-MIMS and Ti(III) reduction method target only a single N species, most other methods are in principle applicable to both N species following an initial N conversion procedure. Other N-bearing compounds, such as NO_2^- and organic N compounds, are discussed as interfering chemicals in this work. However, the ^{15}N analysis of such species

in environmental samples can also be of great interest to ecological researchers. In neutral or alkaline soils where NH_4^+ or NH_4^+ forming fertilizers are applied, the amount of NO_2^- can be significant (Burns et al., 1995; Russow et al., 2009; Ward, 2011). Many NH_4^+ and NO_3^- methods convert target N to NO_2^- and further to N_2O, and they can be modified to convert only NO_2^- by controlling the reaction procedures (Sigman et al. 2001; McIlvin and Altabet 2005; Zhang et al. 2007; Ward 2011; David Felix et al. 2013; Liu et al. 2014). On the other hand, to prepare organic N for ^{15}N analysis, it is usually first separated from inorganic N via diverse techniques. The isolated organic N is then converted to NH_4^+ or NO_3^-, or is combusted directly (Bronk and Glibert 1991; Feuerstein et al. 1997; Chang et al. 2004; Knapp et al. 2005; Toshihiro et al. 2005; Mulvaney 2008; Tsunogai et al. 2008; Johnson et al. 2013; Lu et al. 2019; Cao et al. 2021). The ^{15}N abundances of particular types of DON, such as free amino acids, can now be measured using GC–MS, CE-MS (capillary electrophoresis-mass spectrometry), GC-C-IRMS (gas chromatography-combustion-isotope ratio mass spectrometry), and high-resolution-MS (Mawhinney et al. 1986; Hofmann et al. 2003; Meier-Augenstein 2004; Wanek et al. 2010; Warren 2012, 2018, 2019; Andresen et al. 2015). Such techniques in combination with ^{15}N pool dilution have been developed recently to quantify gross rates of amino acid transformations, facilitating a better understanding of protein depolymerization and soil N dynamics (Wanek et al. 2010; Andresen et al. 2015). The integration of these important N compounds into the present DST can make it applicable to a wider range of ^{15}N-related studies.

The present DST highlights the knowledge gaps in the application of certain published methods to other matrices or samples. Most non-IRMS/LS methods, such as derivatization, HPLC, and OX/MIMS methods, have been developed for freshwater and marine samples, but may be applied also to soil samples if interfering chemicals are removed by additional procedures (Gardner et al. 1991, 1995; Stephan and Kavanagh 2009; Yin et al. 2014). Such adaptations and developments offer more options to different laboratories and are attractive to those subject to equipment and budget limitations. Likewise, the NH_2OH method has just been reported and tested in a few studies, but with additional purification procedures it may become suitable for

samples with a more complex matrix (Liu et al. 2014; Zhu et al. 2015; Jin et al. 2020). Because NH_2OH is more environmentally friendly than the azide reagent, such techniques may rise in favor over the azide-based methods in the future due to rising environmental and health concerns, as well as risk-control regulations in many laboratories and nations.

On the other hand, some promising [15] N analysis methods, have received less widespread implementation due to the lack of access to specialized equipment, or challenges with compatible and accurate isotopic analysis (Mohn et al. 2014; Harris et al. 2020). For example, the SPIN unit and the PT-IRMS equipped with a thermal decomposition system are only available in specialized laboratories (Stange et al. 2007; Smirnoff et al. 2012; Mohn et al. 2014; Hattori et al. 2016; Eschenbach et al. 2017). The laser-based methods have just been developed for [15] N analysis of NO_3^- following azide and bacterial methods (Soto et al. 2015; Wassenaar et al. 2018) and the accuracy is limited by instrumental precision, drift, matrix effects and spectral interferences (Mohn et al. 2014; Harris et al. 2020). Recent developments in calibration protocols and reference materials (Harris et al. 2020; Mohn et al. 2022) will encourage the routine use of laser- and N_2O-based techniques to a wider range of environmental [15] N studies. Another promising novel approach is to measure diverse isotopologues of environmental NO_3^- by LC-ESI-Orbitrap-MS (liquid chromatography mass spectrometry with electrospray ionization Orbitrap) after sample preparation by ion-exchange and gradual elution, yielding high precision and accuracy at natural abundance level (Hilkert et al. 2021). This technique allows for the calculation of mass-independent O isotope variations, as well as the exploration of non-random isotopic distributions. Advances in sample preparation, analysis, and data interpretation procedures may prompt the application of the ESI-Orbitrap technique, allowing researchers to gain insights into multidimensional isotopic fingerprints in diverse organic and inorganic solutes.

Finally, it is also important to note that the DST is intended to be used as a navigation tool, but it does not offer comprehensive details about all the methods. Users are always advised to check the original papers and evaluate the performance of the method in their own laboratories before routine use is undertaken.

Conclusion

In this paper, we developed a decision support tool based on the criteria that are primarily considered when selecting methods for [15] N analysis of NH_4^+ or NO_3^- in liquid samples. This tool is straightforward, visually appealing, and user-friendly, with the potential to be applied to a broader spectrum of [15] N-related environmental research. Integration of other N species and sample matrices into the current tool, along with advancements in current preparation approaches, can further improve the applicability of the DST in the future.

Data Availability The authors declare that the data are available within the article and its supplementary information files.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Consent for publication All authors consent to publication.

References

Altabet MA, Wassenaar LI, Douence C, Roy R (2019) A Ti(III) reduction method for one-step conversion of seawater and freshwater nitrate into N_2O for stable isotopic analysis of $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{17}O/^{16}O$. Rapid

Commun Mass Spectrom 33:1227–1239. https://doi.org/10.1002/rcm.8454

Andresen LC, Bode S, Tietema A et al (2015) Amino acid and N mineralization dynamics in heathland soil after long-term warming and repetitive drought. SOIL 1:341–349. https://doi.org/10.5194/soil-1-341-2015

Barrie A, Davies JE, Park AJ, Workman CT (1989) Continuous-flow stable isotope analysis for biologists. Spectroscopy 4:42–52

Barrie A, Prosser SJ (1996) Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. Mass Spectrom Soils N Y Marcel Dekker 1–46

Barrie S, Workman CT (1984) An automated analytical system for nutritional investigations using ^{15}N tracers. Spectrosc Int J 3:439–447

Benson S, Lennard C, Maynard P, Roux C (2006) Forensic applications of isotope ratio mass spectrometry—a review. Forensic Sci Int 157:1–22. https://doi.org/10.1016/j.forsciint.2005.03.012

Björsne A-K, Rütting T, Ambus P (2014) Combined climate factors alleviate changes in gross soil nitrogen dynamics in heathlands. Biogeochemistry 120:191–201. https://doi.org/10.1007/s10533-014-9990-1

Böhlke JK, Mroczkowski SJ, Coplen TB (2003) Oxygen isotopes in nitrate: new reference materials for 18O:17O:16O measurements and observations on nitrate-water equilibration. Rapid Commun Mass Spectrom 17:1835–1846. https://doi.org/10.1002/rcm.1123

Böhlke JK, Smith RL, Miller DN (2006) Ammonium transport and reaction in contaminated groundwater: application of isotope tracers and isotope fractionation studies. Water Resour Res. https://doi.org/10.1029/2005WR004349

Boutton T (1996) Mass spectrometry of soils. CRC Press

Brand WA (1995) PreCon: a fully automated interface for the Pre-Gc concentration of trace gases on air for isotopic analysis. Isotopes Environ Health Stud 31:277–284. https://doi.org/10.1080/10256019508036271

Brand WA (1996) High precision isotope ratio monitoring techniques in mass spectrometry. J Mass Spectrom 31:225–235

Brand WA, Coplen TB (2012) Stable isotope deltas: tiny, yet robust signatures in nature. Isotopes Environ Health Stud 48:393–409. https://doi.org/10.1080/10256016.2012.666977

Brand WA, Coplen TB, Vogl J et al (2014) Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report). Pure Appl Chem 86:425–467. https://doi.org/10.1515/pac-2013-1023

Braun J, Mooshammer M, Wanek W et al (2018) Full ^{15}N tracer accounting to revisit major assumptions of ^{15}N isotope pool dilution approaches for gross nitrogen mineralization. Soil Biol Biochem 117:16–26. https://doi.org/10.1016/j.soilbio.2017.11.005

Bremner JM (1965) Isotope-ratio analysis of nitrogen in nitrogen–15 tracer investigations in methods of soil analysis. Wiley

Bremner JM (1965) Inorganic forms of nitrogen methods of soil analysis. Wiley

Bremner JM (1996) Nitrogen-total. Methods Soil Anal Part 3 Chem Methods 5:1085–1121

Bremner JM, Edwards AP (1965) Determination and isotope-ratio analysis of different forms of nitrogen in soils: i apparatus and procedure for distillation and determination of ammonium. Soil Sci Soc Am J 29:504

Bremner JM, Hauck RD (1982) Advances in methodology for research on nitrogen transformations in soils in nitrogen in agricultural soils. Wiley

Bremner JM, Keeney DR (1965) Steam distillation methods for determination of ammonium, nitrate and nitrite. Anal Chim Acta 32:485–495. https://doi.org/10.1016/S0003-2670(00)88973-4

Brenna JT, Corso TN, Tobias HJ, Caimi RJ (1997) High-precision continuous-flow isotope ratio mass spectrometry. Mass Spectrom Rev 16:227–258

Bronk D, Glibert P (1991) A ^{15}N tracer method for the measurement of dissolved organic nitrogen release by phytoplankton. Mar Ecol Prog Ser 77:171–182. https://doi.org/10.3354/meps077171

Brooks PD, Stark JM, McInteer BB, Preston T (1989) Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci Soc Am J 53:1707. https://doi.org/10.2136/sssaj1989.03615995005300060016x

Burke IC, O'Deen LA, Mosier AR, Porter LK (1990) Diffusion of soil extracts for nitrogen and nitrogen-15 analyses by automated combustion/mass spectrometry. Soil Sci Soc Am J 54:1190–1192. https://doi.org/10.2136/sssaj1990.03615995005400040047x

Burns LC, Stevens RJ, Laughlin RJ (1995) Determination of the simultaneous production and consumption of soil nitrite using ^{15}N. Soil Biol Biochem 27:839–844. https://doi.org/10.1016/0038-0717(94)00219-Q

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of earth's nitrogen cycle. Science 330:192–196. https://doi.org/10.1126/science.1186120

Cao HTT, Nakamura T, Toyama T, Nishida K (2021) A protocol for nitrogen isotopic measurement of dissolved organic nitrogen with a combination of oxidation-denitrification and gas phase diffusion methods. Isotopes Environ Health Stud. https://doi.org/10.1080/10256016.2021.1948411

Cao TTH, Nakamura T, Saiki M et al (2018) Effect of dissolved organic nitrogen contamination on $\delta^{15}N$–NH_4^+ determination in water samples by modification of the diffusion method with gas-phase trapping. Rapid Commun Mass Spectrom 32:635–638. https://doi.org/10.1002/rcm.8091

Carol K, Elizabeth G (1990) Combustion tube method for measurement of nitrogen isotope ratios using calcium oxide for total removal of carbon dioxide and water. Anal Chem 62:526–529. https://doi.org/10.1021/ac00204a019

Carter JF, Fry B (2013) Ensuring the reliability of stable isotope ratio data—beyond the principle of identical treatment. Anal Bioanal Chem 405:2799–2814. https://doi.org/10.1007/s00216-012-6551-0

Casciotti KL, Sigman DM, Hastings MG et al (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem 74:4905–4912. https://doi.org/10.1021/ac020113w

Chalk PM, Inácio CT, Craswell ET, Chen D (2015) On the usage of absolute (x) and relative (δ) values of 15N abundance. Soil Biol Biochem 85:51–53. https://doi.org/10.1016/j.soilbio.2015.02.027

Chang CC, Langston J, Riggs M et al (1999) A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations. Can J Fish Aquat Sci 56:1856–1864. https://doi.org/10.1139/f99-126

Chang SSR, Kendall C et al (2004) Chapter 15 - preparation and analysis of nitrogen-bearing compounds in water for stable isotope ratio measurement. In: de Groot PA (ed) Handbook of stable isotope analytical techniques. Elsevier

Christensen S, Tiedje JM (1988) Sub-parts-per-billion nitrate method: use of an N_2O-producing denitrifier to convert NO_3^- or $^{15}NO_3^-$ to N_2O. Appl Environ Microbiol. https://doi.org/10.1128/aem.54.6.1409-1413.1988

Clark DR, Fileman TW, Joint I (2006) Determination of ammonium regeneration rates in the oligotrophic ocean by gas chromatography/mass spectrometry. Mar Chem 98:121–130. https://doi.org/10.1016/j.marchem.2005.08.006

Clark DR, Rees AP, Joint I (2007) A method for the determination of nitrification rates in oligotrophic marine seawater by gas chromatography/mass spectrometry. Mar Chem 103:84–96. https://doi.org/10.1016/j.marchem.2006.06.005

Cline JD, Kaplan IR (1975) Isotopic fractionation of dissolved nitrate during denitrification in the eastern tropical north pacific ocean. Mar Chem 3:271–299. https://doi.org/10.1016/0304-4203(75)90009-2

Conway EJ (1947) Microdiffusion analysis and volumetric error. Microdiffusion Anal Vol Error 35:871

Cook CS, Erkkila BR, Chakraborty S, et al (2017) Stable isotope biogeochemistry and ecology: Laboratory manual. University of Utah

Coplen TB (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results: Guidelines and recommended terms for expressing stable isotope results. Rapid Commun Mass Spectrom 25:2538–2560. https://doi.org/10.1002/rcm.5129

Coplen TB, Böhlke JK, Casciotti KL (2004) Using dual-bacterial denitrification to improve $\delta^{15}N$ determinations of nitrates containing mass-independent ^{17}O. Rapid Commun Mass Spectrom 18:245–250. https://doi.org/10.1002/rcm.1318

Craswell ET, Eskew DL (1991) Nitrogen and nitrogen-15 analysis using automated mass and emission spectrometers. Soil Sci Soc Am J 55:750–756. https://doi.org/10.2136/sssaj1991.03615995005500030019x

David Felix J, Elliott EM, Gish TJ et al (2013) Characterizing the isotopic composition of atmospheric ammonia emission sources using passive samplers and a combined oxidation-bacterial denitrifier approach: improved method for isotope characterization of ammonia in air. Rapid Commun Mass Spectrom 27:2239–2246. https://doi.org/10.1002/rcm.6679

Davidson EA, Hart SC, Shanks CA, Firestone MK (1991) Measuring gross nitrogen mineralization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. J Soil Sci 42:335–349. https://doi.org/10.1111/j.1365-2389.1991.tb00413.x

Dawson TE, Brooks PD (2001) Fundamentals of stable isotope chemistry and measurement. In: Unkovich M, Pate J, McNeill A, Gibbs DJ (eds) Stable isotope techniques in the study of biological processes and functioning of ecosystems. Springer

de Groot PA (2004) Handbook of stable isotope analytical techniques. Elsevier

Denk TRA, Mohn J, Decock C et al (2017) The nitrogen cycle: a review of isotope effects and isotope modeling approaches. Soil Biol Biochem 105:121–137. https://doi.org/10.1016/j.soilbio.2016.11.015

Diaconu C, Brion N, Elskens M, Baeyens W (2005) Validation of a dynamic ammonium extraction technique for the determination of 15N at enriched abundances. Anal Chim Acta 554:113–122. https://doi.org/10.1016/j.aca.2005.08.028

Dore JE, Popp BN, Karl DM, Sansone FJ (1998) A large source of atmospheric nitrous oxide from subtropical North Pacific surface waters. Nature 396:63–66. https://doi.org/10.1038/23921

Dudek N, Brzezinski MA, Wheeler PA (1986) Recovery of ammonium nitrogen by solvent extraction for the determination of relative ^{15}N abundance in regeneration experiments. Mar Chem 18:59–69. https://doi.org/10.1016/0304-4203(86)90076-9

Edwards AP (1978) A Guide to the Use of ^{14}N and ^{15}N in Environmental Research. Cold Regions Research And Engineering Lab Hanover NH

Egsgaard H, Larsen E, Jensen ES (1989) Evaluation of automated determination of nitrogen-15 by on-line combustion. Anal Chim Acta 226:345–349. https://doi.org/10.1016/S0003-2670(89)80017-0

Eschenbach W, Lewicka-Szczebak D, Stange CF et al (2017) Measuring ^{15}N abundance and concentration of aqueous nitrate, nitrite, and ammonium by membrane inlet quadrupole mass spectrometry. Anal Chem 89:6076–6081. https://doi.org/10.1021/acs.analchem.7b00724

Eschenbach W, Well R, Dyckmans J (2018) NO reduction to N_2O improves nitrate ^{15}N abundance analysis by membrane inlet quadrupole mass spectrometry. Anal Chem 90:11216–11218. https://doi.org/10.1021/acs.analchem.8b02956

Feast NA, Dennis PF (1996) A comparison of methods for nitrogen isotope analysis of groundwater. Chem Geol 129:167–171. https://doi.org/10.1016/0009-2541(95)00186-7

Feuerstein TP, Ostrom PH, Ostrom NE (1997) Isotopic biogeochemistry of dissolved organic nitrogen: a new technique and application. Org Geochem 27:363–370. https://doi.org/10.1016/S0146-6380(97)00071-5

Fiedler R, Proksch G (1975) The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: a review. Anal Chim Acta 78:1–62. https://doi.org/10.1016/S0003-2670(01)84751-6

Fillery IRP, Recous S (2001) Use of enriched ^{15}N sources to study soil N transformations. In: Unkovich M, Pate J, McNeill A, Gibbs DJ (eds) Stable isotope techniques in the study of biological processes and functioning of ecosystems. Springer

Fisher TR, Morrissey KM (1985) A new method for the recovery of ammonium from natural waters for measurement of ^{15}N composition in isotope dilution

experiments. Mar Chem 16:11–21. https://doi.org/10.1016/0304-4203(85)90025-8

Frey C, Dippner JW, Voss M (2014) Close coupling of N-cycling processes expressed in stable isotope data at the redoxcline of the Baltic Sea. Glob Biogeochem Cycles 28:974–991. https://doi.org/10.1002/2013gb004642

Fry B (2006) Stable isotope ecology. Springer

Fukada T, Hiscock KM, Dennis PF, Grischek T (2003) A dual isotope approach to identify denitrification in groundwater at a river-bank infiltration site. Water Res 37:3070–3078. https://doi.org/10.1016/S0043-1354(03)00176-3

Galloway JN (2005) The global nitrogen cycle: past, present and future. Sci China C Life Sci 48:669–678. https://doi.org/10.1007/BF03187108

Gardner WS, Bootsma HA, Evans C, John PAST (1995) Improved chromatographic analysis of $^{15}N:^{14}N$ ratios in ammonium or nitrate for isotope addition experiments. Mar Chem 48:271–282. https://doi.org/10.1016/0304-4203(94)00060-Q

Gardner WS, Cavaletto JF, Cotner JB, Johnson JR (1997) Effects of natural light on nitrogen cycling rates in the Mississippi River plume. Limnol Oceanogr 42:273–281. https://doi.org/10.4319/lo.1997.42.2.0273

Gardner WS, Herche LR, St. John PA, Seitzinger SP, (1991) High-performance liquid chromatographic determination of nitrogen-15 to nitrogen-14 plus nitrogen-15 ammonium ion ratios in sea water for isotope dilution experiments. Anal Chem 63:1838–1843. https://doi.org/10.1021/ac00017a031

Gathumbi SM, Cadisch G, Giller KE (2002) ^{15}N natural abundance as a tool for assessing N_2-fixation of herbaceous, shrub and tree legumes in improved fallows. Soil Biol Biochem 34:1059–1071. https://doi.org/10.1016/S0038-0717(02)00038-X

Gebus-Czupyt B, Chmiel S, Trembaczowski A et al (2020) Simultaneous preparation of N_2 and CO_2 from water nitrates for $\delta^{15}N$ and $\delta^{18}O$ analysis on the example of the Zemborzycki Reservoir studies. Chemosphere 248:125754. https://doi.org/10.1016/j.chemosphere.2019.125754

Glibert PM, Capone DG (1993) 9 - Mineralization and assimilation in aquatic, sediment, and wetland systems. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques. Academic Press

Granger J, Sigman DM, Prokopenko MG et al (2006) A method for nitrite removal in nitrate N and O isotope analyses. Limnol Oceanogr Methods 4:205–212. https://doi.org/10.4319/lom.2006.4.205

Granger J, Wankel SD (2016) Isotopic overprinting of nitrification on denitrification as a ubiquitous and unifying feature of environmental nitrogen cycling. Proc Natl Acad Sci 113:E6391–E6400. https://doi.org/10.1073/pnas.1601383113

Griesheim KL, Mulvaney RL (2019) Improving the accuracy of diffusion for inorganic ^{15}N analyses of soil extracts and water. Commun Soil Sci Plant Anal 50:1161–1169. https://doi.org/10.1080/00103624.2019.1604734

Griffiths LA, Hackett AM, Shaw IC, Brown S (1981) Recent developments in mass spectrometry in biochemistry, medicine and environmental research. Elsevier

Gröning M (2004) Chapter 40 - international stable isotope reference materials. In: de Groot PA (ed) Handbook of stable isotope analytical techniques. Elsevier

Harris D, Paul EA (1989) Automated analysis of ^{15}N and ^{14}C in biological samples. Commun Soil Sci Plant Anal 20:935–947

Harris SJ, Liisberg J, Xia L et al (2020) N_2O isotopocule measurements using laser spectroscopy: analyzer characterization and intercomparison. Atmospheric Meas Tech 13:2797–2831. https://doi.org/10.5194/amt-13-2797-2020

Hastings MG, Sigman DM, Lipschultz F (2003) Isotopic evidence for source changes of nitrate in rain at Bermuda. J Geophys Res Atmospheres. https://doi.org/10.1029/2003JD003789

Hattori S, Nuñez Palma Y, Itoh Y et al (2019) Isotopic evidence for seasonality of microbial internal nitrogen cycles in a temperate forested catchment with heavy snowfall. Sci Total Environ 690:290–299. https://doi.org/10.1016/j.scitotenv.2019.06.507

Hattori S, Savarino J, Kamezaki K et al (2016) Automated system measuring triple oxygen and nitrogen isotope ratios in nitrate using the bacterial method and N_2O decomposition by microwave discharge. Rapid Commun Mass Spectrom 30:2635–2644. https://doi.org/10.1002/rcm.7747

Hauck RD (1982) Nitrogen—isotope-ratio analysis methods of soil analysis. Wiley

Hauck RD (1983) Nitrogen—Isotope-ratio analysis. Methods Soil Anal Part 2 Chem Microbiol Prop 9:735–779

Hauck RD, Bremner JM (1976) Use of tracers For soil and fertilizer nitrogen research. In: Brady NC (ed) Advances in agronomy. Academic Press, pp 219–266

Heiling M, Arrillaga J, Hood-Nowotny R, Videla X (2006) Preparation of ammonium- ^{15}N and Nitrate- ^{15}N samples by microdiffusion for isotope ratio analysis by optical emission spectrometry. Commun Soil Sci Plant Anal 37:337–346. https://doi.org/10.1080/00103620500439931

Herman DJ, Brooks PD, Ashraf M et al (1995) Evaluation of methods for nitrogen-15 analysis of inorganic nitrogen in soil extracts. II. diffusion methods. Commun Soil Sci Plant Anal 26:1675–1685. https://doi.org/10.1080/00103629509369400

Hilkert A, Böhlke JK, Mroczkowski SJ et al (2021) Exploring the potential of electrospray-orbitrap for stable isotope analysis using nitrate as a model. Anal Chem 93:9139–9148. https://doi.org/10.1021/acs.analchem.1c00944

Hofmann D, Gehre M, Jung K (2003) Sample preparation techniques for the determination of natural $^{15}N/^{14}N$ variations in amino acids by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Isotopes Environ Health Stud 39:233–244. https://doi.org/10.1080/1025601031000147630

Høgh-Jensen H, Schjoerring JK (1994) Measurement of biological dinitrogen fixation in grassland: Comparison of the enriched ^{15}N dilution and the natural ^{15}N abundance methods at different nitrogen application rates and defoliation frequencies. Plant Soil 166:153–163. https://doi.org/10.1007/BF00008328

Højberg O, Johansen HS, Sørensen J (1994) Determination of ^{15}N abundance in nanogram pools of NO_3^- and NO_2^- by denitrification bioassay and mass spectrometry. Appl Env Microbiol 60:2467–2472

Holmes RM, McClelland JW, Sigman DM et al (1998) Measuring ^{15}N–NH_4^+ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Mar Chem 60:235–243. https://doi.org/10.1016/S0304-4203(97)00099-6

Horita J, Kendall C (2004) Chapter 1 - stable isotope analysis of water and aqueous solutions by conventional dual-inlet mass spectrometry. In: de Groot PA (ed) Handbook of stable isotope analytical techniques. Elsevier

Hoult DI, Preston CM (1992) Inexpensive plasma discharge source for molecular emission spectroscopy with application to ^{15}N analysis. Rev Sci Instrum 63:1927–1931. https://doi.org/10.1063/1.1143306

Houlton BZ, Sigman DM, Schuur EAG, Hedin LO (2007) A climate-driven switch in plant nitrogen acquisition within tropical forest communities. Proc Natl Acad Sci 104:8902–8906. https://doi.org/10.1073/pnas.0609935104

Huber B, Bernasconi SM, Luster J, Pannatier EG (2011) A new isolation procedure of nitrate from freshwater for nitrogen and oxygen isotope analysis: a new isolation procedure of nitrate from freshwater. Rapid Commun Mass Spectrom 25:3056–3062. https://doi.org/10.1002/rcm.5199

Huber B, Bernasconi SM, Pannatier EG, Luster J (2012) A simple method for the removal of dissolved organic matter and δ^{15}N analysis of NO_3^- from freshwater: removal of DOM and δ^{15}N analysis of NO_3^- from freshwater. Rapid Commun Mass Spectrom 26:1475–1480. https://doi.org/10.1002/rcm.6243

Isobe K, Suwa Y, Ikutani J et al (2009) Analytical techniques for quantifying ^{15}N/^{14}N of nitrate, nitrite, total dissolved nitrogen and ammonium in environmental samples using a gas chromatograph equipped with a quadrupole mass spectroscope. Microbes Environ. https://doi.org/10.1264/jsme2.ME10159

Ito M, Newman BD, Hadwen WL et al (2013) The application of isotope techniques in nutrient assessment and management in riverine systems. Present Fut 25:846

Jensen ES (1991) Evaluation of automated analysis of ^{15}N and total N in plant material and soil. Plant Soil 133:83–92. https://doi.org/10.1007/BF00011902

Ji Q, Grundle DS (2019) An automated, laser-based measurement system for nitrous oxide isotope and isotopomer ratios at nanomolar levels. Rapid Commun Mass Spectrom 33:1553–1564. https://doi.org/10.1002/rcm.8502

Jin J, Jiang S, Zhang J (2020) Nitrogen isotopic analysis of nitrate in aquatic environment by cadmium- hydroxylamine hydrochloride reduction. Rapid Commun Mass Spectrom n/a: https://doi.org/10.1002/rcm.8804

Johnson LT, Tank JL Jr et al (2013) Quantifying the production of dissolved organic nitrogen in headwater streams using ^{15}N tracer additions. Limnol Oceanogr 58:1271–1285. https://doi.org/10.4319/lo.2013.58.4.1271

Johnston AM, Scrimgeour CM, Henry MO, Handley LL (1999) Isolation of NO_3^--N as 1-phenylazo-2-naphthol

(Sudan-1) for measurement of δ^{15}N. Rapid Commun Mass Spectrom 13:1531–1534

Jung H, Koh D-C, Kim Y et al (2020) Stable isotopes of water and nitrate for the identification of groundwater flow-paths: a review. Water 12:138. https://doi.org/10.3390/w12010138

Kaiser J, Hastings MG, Houlton BZ et al (2007) Triple oxygen isotope analysis of nitrate using the denitrifier method and thermal decomposition of N_2O. Anal Chem 79:599–607. https://doi.org/10.1021/ac061022s

Kanda J (1995) Determination of ammonium in seawater based on the indophenol reaction with o-phenylphenol (OPP). Water Res 29:2746–2750. https://doi.org/10.1016/0043-1354(95)00149-F

Kator H, Morris LJ, Wetzel RL, Koepfler ET (1992) A rapid chromatographic method for recovery of $^{15}NO_2^-$ and NO_3^- produced by nitrification in aqueous samples. Limnol Oceanogr 37:900–907. https://doi.org/10.4319/lo.1992.37.4.0900

Kawashima H, Yoshida O, Suto N (2021) Ion-exchange resin and denitrification pretreatment for determining δ^{15}N-NH_4^+, δ^{15}N-NO_3^-, and δ^{18}O-NO_3^- values. Rapid Commun Mass Spectrom 35:e9027. https://doi.org/10.1002/rcm.9027

Keeney D, Nelson D (1983) Nitrogen—inorganic forms methods of soil analysis. Wiley

Keeney DR, Bremner JM (1966) Determination and isotope-ratio analysis of different forms of nitrogen in soils: 4. exchangeable ammonium, nitrate, and nitrite by direct-distillation methods1. Soil Sci Soc Am J 30:583. https://doi.org/10.2136/sssaj1966.03615995003000050016x

Kendall C (1998) Tracing nitrogen sources and cycling in catchments isotope tracers in catchment hydrology. Elsevier

Kendall C, Elliott EM, Wankel SD (2007) Tracing anthropogenic inputs of nitrogen to ecosystems stable isotopes in ecology and environmental science. Wiley

Kendall C, McDonnell JJ (2012) Isotope tracers in catchment hydrology. Elsevier

Khan SA, Mulvaney RL, Brooks PD (1998) Diffusion methods for automated nitrogen-15 analysis using acidified disks. Soil Sci Soc Am J 62:406. https://doi.org/10.2136/sssaj1998.03615995006200020017x

Khan SA, Mulvaney RL, Hoeft RG (2000a) Direct-diffusion methods for inorganic-nitrogen analysis of soil. Soil Sci Soc Am J 64:1083–1089. https://doi.org/10.2136/sssaj2000.6431083x

Khan SA, Mulvaney RL, Mulvaney CS (1997) Accelerated diffusion methods for inorganic-nitrogen analysis of soil extracts and water. Soil Sci Soc Am J 61:936–942. https://doi.org/10.2136/sssaj1997.03615995006100030032x

Khan SA, Mulvaney RL, Strle K, Horgan BP (2000b) Evaluation of diffusion for inorganic-nitrogen analysis of natural water and wastewater. J Environ Qual 29:1890–1895. https://doi.org/10.2134/jeq2000.00472425002900060023x

Kiba H, Hyodo F, Asano Y, Maeda M (2016) A Sequential Diffusion Method for ^{15}N Natural Abundance Measurement of Ammonium, Nitrate and Total Dissolved Nitrogen in Water Samples. 4

Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer data1. Soil Sci Soc Am J 18:33. https://doi.org/10.2136/sssaj1954.03615995001800010009x

Klesta EJ, Bartz JK, Sparks D et al (1996) Quality assurance and quality control. Methods Soil Anal Part 3:19–48

Knapp AN, Sigman DM, Lipschultz F (2005) N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda atlantic time-series study site. Glob Biogeochem Cycles. https://doi.org/10.1029/2004GB002320

Knowles R, Blackburn TH (1993) Nitrogen isotope techniques. Isot Tech Plant Soil Aquat Biol Ser 25:6532

Koba K, Inagaki K, Sasaki Y, et al (2010) Earth, Life, and Isotopes. N Ohkouchi Tayasu K Koba Kyoto Univ Press Kyoto 17

Komatsu DD, Ishimura T, Nakagawa F, Tsunogai U (2008) Determination of the $^{15}N/^{14}N$, $^{17}O/^{16}O$, and $^{18}O/^{16}O$ ratios of nitrous oxide by using continuous-flow isotope-ratio mass spectrometry. Rapid Commun Mass Spectrom 22:1587–1596. https://doi.org/10.1002/rcm.3493

Köster JR, Well R, Tuzson B et al (2013) Novel laser spectroscopic technique for continuous analysis of N_2O isotopomers – application and intercomparison with isotope ratio mass spectrometry. Rapid Commun Mass Spectrom 27:216–222. https://doi.org/10.1002/rcm.6434

Köster O, Jüttner F (1999) NH_4^+ utilization and regeneration rates in freshwater lakes determined by GC–MS of derivatised dihydroindophenol. J Microbiol Methods 37:65–76. https://doi.org/10.1016/S0167-7012(99)00042-1

Lachouani P, Frank AH, Wanek W (2010) A suite of sensitive chemical methods to determine the $\delta^{15}N$ of ammonium, nitrate and total dissolved N in soil extracts: preparation of soil N pools for natural ^{15}N abundance measurement. Rapid Commun Mass Spectrom 24:3615–3623. https://doi.org/10.1002/rcm.4798

Laine M, Rutting T, Alakukku L et al (2018) Process rates of nitrogen cycle in uppermost topsoil after harvesting in no-tilled and ploughed agricultural clay soil. Nutr Cycl Agroecosystems 110:39–49. https://doi.org/10.1007/s10705-017-9825-2

Lehmann MF, Bernasconi SM, McKenzie JA (2001) A method for the extraction of ammonium from freshwaters for nitrogen isotope analysis. Anal Chem 73:4717–4721. https://doi.org/10.1021/ac010212u

Li W-B, Song Y-B, Xu H-K et al (2015) Ion-exchange method in the collection of nitrate from freshwater ecosystems for nitrogen and oxygen isotope analysis: a review. Environ Sci Pollut Res 22:9575–9588. https://doi.org/10.1007/s11356-015-4522-7

Lipschultz F (2008) Chapter 31 - isotope tracer methods for studies of the marine nitrogen cycle. In: Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (eds) Nitrogen in the marine environment, 2nd edn. Academic Press

Liu D, Fang Y, Tu Y, Pan Y (2014) Chemical method for nitrogen isotopic analysis of ammonium at natural abundance. Anal Chem 86:3787–3792. https://doi.org/10.1021/ac403756u

Liu YP, Mulvaney RL (1992a) Automated nitrogen-15 analysis of ammonium in diffusion disks by the Rittenberg technique. Soil Sci Soc Am J 56:1185–1188

Liu YP, Mulvaney RL (1992b) Use of diffusion for automated nttrogen-15 analysis of soil extracts. Commun Soil Sci Plant Anal 23:613–629. https://doi.org/10.1080/00103629209368613

Lory JA, Russelle MP (1994) Evaluation of a diffusion method for preparing low-nitrogen samples for nitrogen-15 analysis. Soil Sci Soc Am J 58:1400. https://doi.org/10.2136/sssaj1994.03615995005800050017x

Lu K, Lin X, Gardner WS, Liu Z (2019) A streamlined method to quantify the fates of 15N in seawater samples amended with 15N-labeled organic nitrogen. Limnol Oceanogr Methods 3:10345. https://doi.org/10.1002/lom3.10345

Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303:685–687. https://doi.org/10.1038/303685a0

Marshall RB, Whiteway JN (1985) Automation of an interface between a nitrogen analyser and an isotope ratio mass spectrometer. Analyst 110:867–871

Mawhinney TP, Robinett RR, Atalay A, Madson MA (1986) Analysis of amino acids as their tert.-butyldimethylsilyl derivatives by gas—liquid chromatography and mass spectrometry. J Chromatogr A 358:231–242

Mayer B, Sebilo M, Wassenaar LI (2013) Isotopic assessment of nitrogen cycling in river basins: potential and limitations for nutrient management purposes

McIlvin MR, Altabet MA (2005) Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. Anal Chem 77:5589–5595. https://doi.org/10.1021/ac050528s

McIlvin MR, Casciotti KL (2011) Technical updates to the bacterial method for nitrate isotopic analyses. Anal Chem 83:1850–1856. https://doi.org/10.1021/ac1028984

McInteer BB, BB M, JG M (1981) Automation of a mass spectrometer for nitrogen isotope analysis

McInteer BB, Montoya JG, Stark EE (1984) Automated mass spectrometer grows up. Los Alamos National Lab. (LANL), Los Alamos, NM (United States)

Meier-Augenstein W (2004) Chapter 8 - GC and IRMS technology for ^{13}C and ^{15}N analysis on organic compounds and related gases. In: de Groot PA (ed) Handbook of stable isotope analytical techniques. Elsevier

Meier-Augenstein W, Schimmelmann A (2019) A guide for proper utilisation of stable isotope reference materials. Isotopes Environ Health Stud 55:113–128. https://doi.org/10.1080/10256016.2018.1538137

Michalski G, Savarino J, Böhlke JK, Thiemens M (2002) Determination of the total oxygen isotopic composition of nitrate and the calibration of a Δ17O nitrate reference material. Anal Chem 74:4989–4993. https://doi.org/10.1021/ac0256282

Michalski G, Scott Z, Kabiling M, Thiemens MH (2003) First measurements and modeling of $\Delta^{17}O$ in atmospheric nitrate. Geophys Res Lett. https://doi.org/10.1029/2003GL017015

Michener R, Lajtha K (2008) Stable isotopes in ecology and environmental science. Wiley

Mohn J, Biasi C, Bodé S et al (2022) Isotopically characterised N^2O reference materials for use as community standards. Rapid Commun Mass Spectrom 36:e9296. https://doi.org/10.1002/rcm.9296

Mohn J, Wolf B, Toyoda S et al (2014) Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. Rapid Commun Mass Spectrom 28:1995–2007. https://doi.org/10.1002/rcm.6982

Mørkved PT, Dörsch P, Søvik AK, Bakken LR (2007) Simplified preparation for the δ^{15}N-analysis in soil NO_3^- by the denitrifier method. Soil Biol Biochem 39:1907–1915. https://doi.org/10.1016/j.soilbio.2007.02.004

Mulholland PJ, Helton AM, Poole GC et al (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202–205. https://doi.org/10.1038/nature06686

Mulholland PJ, Tank JL, Sanzone DM et al (2000) Nitrogen cycling in a forest stream determined by a 15N tracer addition. Ecol Monogr 70:471–493. https://doi.org/10.2307/2657212

Mulholland PJ, Valett HM, Webster JR et al (2004) Stream denitrification and total nitrate uptake rates measured using a field 15N tracer addition approach. Limnol Oceanogr 49:809–820. https://doi.org/10.4319/lo.2004.49.3.0809

Mulvaney RL (1991) Some recent advances in the use of ^{15}N for research on nitrogen transformations in soil. Stable Isot Plant Nutr Soil Fertil Environ Stud 3:66

Mulvaney RL (1996) Nitrogen—inorganic forms methods of soil analysis. Wiley

Mulvaney RL (2008) Advances in methodology for research on nitrogen transformations in soils nitrogen in agricultural systems. Wiley

Mulvaney RL (1986) Comparison of procedures for reducing cross-contamination during steam distillations in nitrogen-15 tracer research. Soil Sci Soc Am J 50:92–96. https://doi.org/10.2136/sssaj1986.03615995005000010018x

Mulvaney RL (1993) 2 - Mass spectrometry. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques. Academic Press

Mulvaney RL, Ashraf M, Azam F et al (1994) Evaluation of methods for nitrogen-15 analysis of inorganic nitrogen in soil extracts: I. steam-distillation methods. Commun Soil Sci Plant Anal 25:2187–2200. https://doi.org/10.1080/00103629409369181

Mulvaney RL, Fohringer CL, Bojan VJ et al (1990) A commercial system for automated nitrogen isotope-ratio analysis by the Rittenberg technique. Rev Sci Instrum 61:897–903

Mulvaney RL, Khan SA (1999) Use of diffusion to determine inorganic nitrogen in a complex organic matrix. Soil Sci Soc Am J 63:240–246

Mulvaney RL, Khan SA, Stevens WB, Mulvaney CS (1997) Improved diffusion methods for determination of inorganic nitrogen in soil extracts and water. Biol Fertil Soils 24:413–420. https://doi.org/10.1007/s003740050266

Mulvaney RL, Liu YP (1991) Refinement and evaluation of an automated mass spectrometer for nitrogen isotope analysis by the Rittenberg technique. J Autom Chem 13:273–280. https://doi.org/10.1155/S1463924691000433

Nikolenko O, Jurado A, Borges AV et al (2018) Isotopic composition of nitrogen species in groundwater under agricultural areas: a review. Sci Total Environ 621:1415–1432. https://doi.org/10.1016/j.scitotenv.2017.10.086

Norman RJ, Stucki JW (1981) The determination of nitrate and nitrite in soil extracts by ultraviolet spectrophotometry. Soil Sci Soc Am J 45:347–353

Ohte N, Nagata T, Tayasu I, et al (2013) Simultaneous measurement of nitrogen and oxygen isotopes of nitrate to evaluate nitrate sources and processes in catchments

Owens NJ, Rees A (1989) Determination of nitrogen-15 at sub-microgram levels of nitrogen using automated continuous-flow isotope ratio mass spectrometry. Analyst 114:1655–1657. https://doi.org/10.1039/AN9891401655

Owens NJP (1988) Natural variations in 15N in the marine environment advances in marine biology. Elsevier

Peoples MB, Palmer B, Boddey RM (2001) The use of ^{15}n to study biological nitrogen fixation by perennial legumes. In: Unkovich M, Pate J, McNeill A, Gibbs DJ (eds) Stable isotope techniques in the study of biological processes and functioning of ecosystems. Springer

Pörtl K, Zechmeister-Boltenstern S, Wanek W et al (2007) Natural ^{15}N abundance of soil N pools and N_2O reflect the nitrogen dynamics of forest soils. Plant Soil 295:79–94. https://doi.org/10.1007/s11104-007-9264-y

Preston CM (1993) 3 - Optical emission spectrometry. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques. Academic Press

Preston T, Bury S, Présing M et al (1996) Isotope dilution analysis of combined nitrogen in natural waters: I. Ammonium Rapid Commun Mass Spectrom 10:959–964

Preston T, Owens N (1983) Interfacing an automatic elemental analyzer with an isotope ratio mass-spectrometer - the potential for fully automated total nitrogen and N-15 analysis. Analyst 108:971–977. https://doi.org/10.1039/an9830800971

Preston T, Slater C (1994) Mass spectrometric analysis of stable-isotope-labelled amino acid tracers. Proc Nutr Soc 53:363–372. https://doi.org/10.1079/PNS19940042

Preston T, Zainal K, Anderson S et al (1998) Isotope dilution analysis of combined nitrogen in natural waters. III. nitrate and nitrite. Rapid Commun Mass Spectrom 12:423–428

Reichel R, Wei J, Islam MS et al (2018) Potential of wheat straw, spruce sawdust, and lignin as high organic carbon soil amendments to improve agricultural nitrogen retention capacity: an incubation study. Front Plant Sci 9:900. https://doi.org/10.3389/fpls.2018.00900

Révész K, Böhlke JK, Yoshinari T (1997) Determination of δ^{18}O and δ^{15}N in nitrate. Anal Chem 69:4375–4380. https://doi.org/10.1021/ac9610523

Robinson D (2001) δ^{15}N as an integrator of the nitrogen cycle. Trends Ecol Evol 16:153–162. https://doi.org/10.1016/S0169-5347(00)02098-X

Russow R (1999) Determination of ^{15}N in ^{15}N-enriched nitrite and nitrate in aqueous samples by reaction continuous flow quadrupole mass spectrometry. Rapid Commun Mass Spectrom 13:1334–1338. https://doi.org/10.1002/(SICI)1097-0231(19990715)13:13%3c1334::AID-RCM606%3e3.0.CO;2-C

Russow R, Förstel H (1993) Use of GC-QMS for stable isotope analysis of environmentally relevant main and trace gases in the air. Isot Isot Environ Health Stud 29:327–334. https://doi.org/10.1080/00211919308046700

Russow R, Goetz A (1998) Automatic simultaneous determination of total carbon and ^{13}C as well as total nitrogen and ^{15}N in isotopically enriched samples of soil and plant material using a quadrupole mass spectrometer coupled to an elemental analyser. Arch Agron Soil Sci 43:349–359. https://doi.org/10.1080/03650349809366050

Russow R, Schmidt G, Faust H (1995) Elemental analyzer-quadrupole ms coupling—a low cost equipment for the simultaneous determination of carbon/^{13}C and nitrogen/^{15}N in isotopically enriched soil and plant samples. Isotopes Environ Health Stud 31:211–218. https://doi.org/10.1080/10256019508234019

Russow R, Stange CF, Neue H-U (2009) Role of nitrite and nitric oxide in the processes of nitrification and denitrification in soil: Results from ^{15}N tracer experiments. Soil Biol Biochem 41:785–795. https://doi.org/10.1016/j.soilbio.2009.01.017

Rütting T, Huygens D, Staelens J et al (2011) Advances in ^{15}N-tracing experiments: new labelling and data analysis approaches. Biochem Soc Trans 39:279–283. https://doi.org/10.1042/BST0390279

Rütting T, Müller C (2007) ^{15}N tracing models with a Monte Carlo optimization procedure provide new insights on gross N transformations in soils. Soil Biol Biochem 39:2351–2361. https://doi.org/10.1016/j.soilbio.2007.04.006

Ryabenko E, Altabet MA, Wallace DWR (2009) Effect of chloride on the chemical conversion of nitrate to nitrous oxide for δ^{15}N analysis: effect of chloride on δ^{15}N analysis. Limnol Oceanogr Methods 7:545–552. https://doi.org/10.4319/lom.2009.7.545

Saghir NS, Mulvancy RL, Azam F (1993a) Determination of nitrogen by microdiffusion in mason Jars. I. inorganic nitrogen in soil extracts. Commun Soil Sci Plant Anal 24:1745–1762. https://doi.org/10.1080/00103629309368912

Saghir NS, Mungwari FP, Mulvaney RL, Azam F (1993b) Determination of nitrogen by microdiffusion in mason jars: II. Inorganic nitrogen-15 in soil extracts. Commun Soil Sci Plant Anal 24:2747–2763. https://doi.org/10.1080/00103629309368993

Scrimgeour CM, Robinson D (2003) Stable isotope analysis and applications soil and environmental analysis. CRC Press

Sebilo M, Mayer B, Grably M et al (2004) The use of the "Ammonium diffusion" method for δ^{15}N-NH$_4^+$ and δ^{15}N-NO$_3^-$ measurements: comparison with other techniques. Environ Chem 1:99. https://doi.org/10.1071/EN04037

Selmer J-S, Sörensson F (1986) New procedure for extraction of ammonium from natural waters for ^{15}N isotopic ratio determinations. Appl Environ Microbiol 52:577–579. https://doi.org/10.1128/aem.52.3.577-579.1986

Shearer G, Kohl DH (1993) 4 - Natural abundance of ^{15}N: fractional contribution of two sources to a common sink and use of isotope discrimination. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques. Academic Press

Sigman DM, Altabet MA, Michener R et al (1997) Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Mar Chem 57:227–242. https://doi.org/10.1016/S0304-4203(97)00009-1

Sigman DM, Casciotti KL, Andreani M et al (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem 73:4145–4153. https://doi.org/10.1021/ac010088e

Silva SR, Kendall C, Wilkison DH et al (2000) A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. J Hydrol 228:22–36. https://doi.org/10.1016/S0022-1694(99)00205-X

Skrzypek G, Dunn PJH, Carter JF (2019) Good practice guide for isotope ratio mass spectrometry. Springer

Smirnoff A, Savard MM, Vet R, Simard M-C (2012) Nitrogen and triple oxygen isotopes in near-road air samples using chemical conversion and thermal decomposition. Rapid Commun Mass Spectrom 26:2791–2804. https://doi.org/10.1002/rcm.6406

Smith KA, Mullins CE (2000) Soil and environmental analysis. Marcel Dekker Incorporated

Soldatova E, Dong Y, Li J et al (2021) Nitrogen transformation and pathways in the shallow groundwater–soil system within agricultural landscapes. Environ Geochem Health 43:441–459. https://doi.org/10.1007/s10653-020-00733-w

Sørensen P, Jensen ES (1991) Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Anal Chim Acta 252:201–203. https://doi.org/10.1016/0003-2670(91)87215-S

Soto DX, Koehler G, Hobson KA (2015) Combining denitrifying bacteria and laser spectroscopy for isotopic analyses (δ^{15}N, δ^{18}O) of dissolved nitrate. Anal Chem 87:7000–7005. https://doi.org/10.1021/acs.analchem.5b01119

Sparks DL, Page AL, Helmke PA, Loeppert RH (1996) Methods of soil analysis, part 3: chemical methods. Wiley

Stange CF, Spott O, Apelt B, Russow RWB (2007) Automated and rapid online determination of ^{15}N abundance and concentration of ammonium, nitrite, or nitrate in aqueous samples by the SPINMAS technique. Isotopes Environ Health Stud 43:227–236. https://doi.org/10.1080/10256010701550658

Stark JM, Hart SC (1996) Diffusion technique for preparing salt solutions, kjeldahl digests, and persulfate digests for Nitrogen-15 analysis. Soil Sci Soc Am J 60:1846. https://doi.org/10.2136/sssaj1996.03615995006000060033x

Stephan K, Kavanagh KL (2009) Suitability of the diffusion method for natural abundance nitrogen-15 analysis. Soil Sci Soc Am J 73:293. https://doi.org/10.2136/sssaj2007.0079

Stevens RJ, Laughlin RJ (1994) Determining nitrogen-15 in nitrite or nitrate by producing nitrous oxide. Soil Sci Soc Am J 58:1108–1116. https://doi.org/10.2136/sssaj1994.03615995005800040015x

Stevens RJ, Laughlin RJ, Atkins GJ, Prosser SJ (1993) Automated determination of nitrogen-15-labeled dinitrogen and nitrous oxide by mass spectrometry. Soil Sci Soc Am J 57:981–988. https://doi.org/10.2136/sssaj1993.03615995005700040017x

Stewart GR (2001) What do δ15N signatures tell us about nitrogen relations in natural ecosystems? In: Unkovich M, Pate J, McNeill A, Gibbs DJ (eds) Stable isotope

techniques in the study of biological processes and functioning of ecosystems. Springer

Stock P, Hösch A, Burghardt D (2019) $\delta^{15}N$ analysis of ammonium in freeze-dried natural groundwater samples by precipitation with sodium tetraphenylborate. Rapid Commun Mass Spectrom 33:1730–1738. https://doi.org/10.1002/rcm.8526

Tanu FZ, Miyata T, Sadzuka N et al (2020) An adaptation of the acetone method for $\delta^{15}N\text{-}NO_3^-$ analysis of saline water with a low concentration of nitrate. Rapid Commun Mass Spectrom. https://doi.org/10.1002/rcm.8770

Teece MA, Fogel ML (2004) Preparation of ecological and biochemical samples for isotope analysis handbook of stable isotope analytical techniques. Elsevier

Tietema A, Wessel WW (1992) Gross nitrogen transformations in the organic layer of acid forest ecosystems subjected to increased atmospheric nitrogen input. Soil Biol Biochem 24:943–950. https://doi.org/10.1016/0038-0717(92)90021-O

Toshihiro M, Yasuaki T, Isao K (2005) Determining ^{15}N enrichment of dissolved organic nitrogen in environmental waters by gas chromatography/negative-ion chemical ionization mass spectrometry. Limnol Oceanogr Methods 3:164–173. https://doi.org/10.4319/lom.2005.3.164

Toyoda S, Yoshida N (2004) Site-specific nitrogen isotope analysis in N2O by mass spectrometry handbook of stable isotope analytical techniques. Elsevier

Toyoda S, Yoshida N, Koba K (2017) Isotopocule analysis of biologically produced nitrous oxide in various environments. Mass Spectrom Rev 36:135–160. https://doi.org/10.1002/mas.21459

Tsunogai U, Kido T, Hirota A et al (2008) Sensitive determinations of stable nitrogen isotopic composition of organic nitrogen through chemical conversion into N2O. Rapid Commun Mass Spectrom 22:345–354. https://doi.org/10.1002/rcm.3368

Tu Y, Fang Y, Liu D, Pan Y (2016) Modifications to the azide method for nitrate isotope analysis. Rapid Commun Mass Spectrom 30:1213–1222. https://doi.org/10.1002/rcm.7551

Velinsky DJ, Pennock JR, Sharp JH et al (1989) Determination of the isotopic composition of ammonium-nitrogen at the natural abundance level from estuarine waters. Mar Chem 26:351–361. https://doi.org/10.1016/0304-4203(89)90040-6

Wanek W, Mooshammer M, Blöchl A et al (2010) Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel 15N isotope pool dilution technique. Soil Biol Biochem 42:1293–1302. https://doi.org/10.1016/j.soilbio.2010.04.001

Ward BB (2011) Chapter thirteen - measurement and distribution of nitrification rates in the oceans. In: Klotz MG (ed) Methods in Enzymology. Academic Press, pp 307–323

Ward BB, Talbot MC, Perry MJ (1984) Contributions of phytoplankton and nitrifying bacteria to ammonium and nitrite dynamics in coastal waters. Cont Shelf Res 3:383–398. https://doi.org/10.1016/0278-4343(84)90018-9

Warren CR (2012) Post-uptake metabolism affects quantification of amino acid uptake. New Phytol 193:522–531. https://doi.org/10.1111/j.1469-8137.2011.03933.x

Warren CR (2018) Development of online microdialysis-mass spectrometry for continuous minimally invasive measurement of soil solution dynamics. Soil Biol Biochem 123:266–275. https://doi.org/10.1016/j.soilbio.2018.05.022

Warren CR (2019) Isotope pool dilution reveals rapid turnover of small quaternary ammonium compounds. Soil Biol Biochem 131:90–99. https://doi.org/10.1016/j.soilbio.2019.01.004

Wassenaar LI, Douence C, Altabet MA, Aggarwal PK (2018) N and O isotope ($\delta^{15}N^\alpha$, $\delta^{15}N^\beta$, $\delta^{18}O$, $\delta^{17}O$) analyses of dissolved NO_3^- and NO_2^- by the Cd-azide reduction method and N2O laser spectrometry. Rapid Commun Mass Spectrom 32:184–194. https://doi.org/10.1002/rcm.8029

Weigand MA, Foriel J, Barnett B et al (2016) Updates to instrumentation and protocols for isotopic analysis of nitrate by the denitrifier method. Rapid Commun Mass Spectrom 30:1365–1383. https://doi.org/10.1002/rcm.7570

Werner RA, Brand WA (2001) Referencing strategies and techniques in stable isotope ratio analysis. Rapid Commun Mass Spectrom 15:501–519. https://doi.org/10.1002/rcm.258

Wessel WW, Tietema A (1992) Calculating gross N transformation rates of ^{15}N pool dilution experiments with acid forest litter: Analytical and numerical approaches. Soil Biol Biochem 24:931–942. https://doi.org/10.1016/0038-0717(92)90020-X

Xing M, Liu W (2011) An improved method of ion exchange for nitrogen isotope analysis of water nitrate. Anal Chim Acta 686:107–114. https://doi.org/10.1016/j.aca.2010.11.051

Xue D, Botte J, De Baets B et al (2009) Present limitations and future prospects of stable isotope methods for nitrate source identification in surface- and groundwater. Water Res 43:1159–1170. https://doi.org/10.1016/j.watres.2008.12.048

Yin G, Hou L, Liu M et al (2014) A novel membrane inlet mass spectrometer method to measure $^{15}NH_4^+$ for isotope-enrichment experiments in aquatic ecosystems. Environ Sci Technol 48:9555–9562. https://doi.org/10.1021/es501261s

Yu L, Harris E, Lewicka-Szczebak D et al (2020) What can we learn from N2O isotope data? – Analytics, processes and modelling. Rapid Commun Mass Spectrom 34:e8858. https://doi.org/10.1002/rcm.8858

Zhang J, Cai Z, Müller C (2018) Terrestrial N cycling associated with climate and plant-specific N preferences: a review: Soil N cycle in relation to climate and plant N preferences. Eur J Soil Sci 69:488–501. https://doi.org/10.1111/ejss.12533

Zhang L, Altabet MA, Wu T, Hadas O (2007) Sensitive measurement of NH_4^+ $^{15}N/^{14}N$ ($\delta^{15}NH_4^+$) at natural abundance levels in fresh and saltwaters. Anal Chem 79:5297–5303. https://doi.org/10.1021/ac070106d

Zhang S, Fang Y, Xi D (2015) Adaptation of micro-diffusion method for the analysis of ^{15}N natural abundance of ammonium in samples with small volume. Rapid Commun Mass Spectrom 29:1297–1306. https://doi.org/10.1002/rcm.7224

Zhang S, Zheng Q, Noll L et al (2019) Environmental effects on soil microbial nitrogen use efficiency are controlled by allocation of organic nitrogen to microbial growth and regulate gross N mineralization. Soil Biol Biochem 135:304–315. https://doi.org/10.1016/j.soilbio.2019.05.019

Zhao Z-Y, Cao F, Zhang W-Q et al (2019) Determination of stable nitrogen and oxygen isotope ratios in atmospheric aerosol nitrates. Chin J Anal Chem 47:907–915. https://doi.org/10.1016/S1872-2040(19)61166-7

Zhu X, Zhang W, Chen H, Mo J (2015) Impacts of nitrogen deposition on soil nitrogen cycle in forest ecosystems: A review. Acta Ecol Sin 35:35–43. https://doi.org/10.1016/j.chnaes.2015.04.004