



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

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**Abstract** The stable nitrogen isotope ( $^{15}\text{N}$ ) analysis of ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) 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  $^{15}\text{N}$  abundance of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $^{15}\text{N}$  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

$^{15}\text{N}$  analysis in  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . 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  $^{15}\text{N}$  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  $^{15}\text{N}$  analysis. Potential applications of our decision trees include  $^{15}\text{N}$  studies ranging from natural abundance to tracer level in a wide range of terrestrial, freshwater and marine ecosystems.

**Keywords** Decision support tool ·  $^{15}\text{N}$  analysis · Ammonium · Nitrate · Liquid samples

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## 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 ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) 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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $^{14}\text{N}$  and the rare  $^{15}\text{N}$ . The stable N isotopic composition in a N-compound is usually expressed as either an “absolute value ( $x^{15}\text{N}$ )” or a “relative value ( $\delta^{15}\text{N}$ )” in agricultural and biological research (Fry 2006; Coplen 2011; Mayer et al. 2013; Chalk et al. 2015). The former is preferred in applications where  $^{15}\text{N}$  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  $\text{N}_2$ ) 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  $^{15}\text{N}$  abundance of important N compounds like  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  is commonly involved in  $^{15}\text{N}$ -related ecological investigations such as  $^{15}\text{N}$  pool dilution (Davidson et al. 1991; Wessel and Tietema 1992; Laine et al. 2018),  $^{15}\text{N}$  tracing (Mulholland et al. 2000, 2008; Rütting et al. 2011; Björnsne et al. 2014) and natural abundance studies (Gathumbi et al. 2002; Houlton et al. 2007; Denk et al. 2017). The  $^{15}\text{N}$  pool dilution and tracing assays are both based on  $^{15}\text{N}$  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  $^{15}\text{N}$  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  $^{15}\text{N}$  techniques, the  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  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  $\text{NH}_4^+$  or  $\text{NO}_3^-$  to an isotopic representative gas (e.g.,  $\text{N}_2$  or  $\text{N}_2\text{O}$ ) and then quantifying  $^{15}\text{N}$  abundance of the analyte. The gas conversion is necessary because most  $^{15}\text{N}$  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  $^{15}\text{N}$  ratio of  $\text{NH}_4^+$  in aquatic samples can be analyzed directly using HPLC (Gardner et al. 1991, 1995, 1997).

With so many  $^{15}\text{N}$  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  $^{15}\text{N}$  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  $^{15}\text{N}$  analysis in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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; Hilker 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  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $^{15}\text{N}$  analysis in liquid samples. These criteria concern: the composition of the sample matrix, the  $^{15}\text{N}$  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  $^{15}\text{N}$  in dissolved  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , 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 $\text{N}_2$ as the analyte ( $\text{N}_2$ methods)

In the gas methods, the  $\text{NH}_4^+$  or  $\text{NO}_3^-$  is converted into analytical gases such as  $\text{N}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{NO}$  or volatile derivatives. Most gas methods involve isolating the  $\text{NH}_4^+$  or  $\text{NO}_3^-$  from the liquid sample, converting it into  $\text{N}_2$ , and determining the  $^{15}\text{N}$  abundance of the produced  $\text{N}_2$  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  $\text{N}_2$  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; McIner 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 ( $\text{KNO}_3$ ) into  $\text{CO}_2$ ,  $\text{K}_2\text{CO}_3$  and  $\text{N}_2$  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; McIner 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  $\text{N}_2$  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  $\text{N}_2$  conversion and isotope measurements, the  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in samples can be concentrated and isolated from solutions by a number of

**Table 1** Methods reported for  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  in liquid samples in case studies

| Method                               | Analyte                            | Instrument         | $^{15}\text{N}$ abundance                                          | Sample matrix                                                                | N requirement ( $\text{N-NH}_4^+$ )                 |
|--------------------------------------|------------------------------------|--------------------|--------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------|
| Distillation                         | $\text{N}_2$                       | (DI-/EA-) IRMS; ES | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples, soil extracts                                                 | 4–400 $\mu\text{mol}$                               |
| Diffusion                            | $\text{N}_2$                       | EA-IRMS; ES        | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples, soil extracts                                                 | 2–15 $\mu\text{mol}$                                |
| Ion exchange                         | $\text{N}_2$                       | EA-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | Freshwater samples                                                           | 1–2 $\mu\text{mol}$                                 |
| Hg precipitation                     | $\text{N}_2$                       | ES                 | $^{15}\text{N}$ -enriched (pool dilution samples)                  | Freshwater samples                                                           | ~ 10 $\mu\text{M}$                                  |
| TPB precipitation                    | $\text{N}_2$                       | EA-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | Freshwater samples                                                           | ~ 70 $\mu\text{mol}$                                |
| Derivatization                       | $\text{N}_2$                       | EA-IRMS; ES        | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples                                                                | 2–10 $\mu\text{M}$                                  |
|                                      | dye derivative                     | GC-QMS             | $^{15}\text{N}$ -enriched (pool dilution samples)                  | Water samples                                                                | 10–200 nmol/kg                                      |
| OX-MIMS                              | $\text{N}_2$                       | MIMS               | $^{15}\text{N}$ -enriched                                          | Water samples                                                                | > 0.1 $\mu\text{M}$                                 |
| SPIN-QMS                             | $\text{N}_2$                       | QMS                | $^{15}\text{N}$ -enriched                                          | Water samples, soil extracts                                                 | > 140 $\mu\text{M}$ (700 nmol)                      |
| SPIN-MIMS                            | $\text{N}_2$                       | MIMS               | $^{15}\text{N}$ -enriched                                          | Water samples, soil extracts                                                 | > 70 $\mu\text{M}$ (100 nmol)                       |
| BO-azide                             | $\text{N}_2\text{O}$               | PT-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples                                                                | 0.5–10 $\mu\text{M}$                                |
| BO- $\text{NH}_2\text{OH}$           | $\text{N}_2\text{O}$               | PT-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples, alkaline soil extracts                                        | 10–20 $\mu\text{M}$                                 |
| Diffusion-BO-azide                   | $\text{N}_2\text{O}$               | PT-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples, soil extracts                                                 | 20–50 $\mu\text{M}$                                 |
| Diffusion-BO- $\text{NH}_2\text{OH}$ | $\text{N}_2\text{O}$               | PT-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples, soil extracts                                                 | 15–60 $\mu\text{M}$                                 |
| Diffusion-BO-denitrifier             | $\text{N}_2\text{O}$               | PT-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | $\text{NH}_3$ emissions, water samples, soil extracts                        | 10 $\mu\text{M}$                                    |
| Ion exchange-BO-denitrifier          | $\text{N}_2\text{O}$               | PT-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | $\text{NH}_3$ emissions, freshwater samples                                  | 30 $\mu\text{M}$                                    |
| Diffusion-PO-azide                   | $\text{N}_2\text{O}$               | PT-IRMS            | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples, soil extracts                                                 | 12.4–250 $\mu\text{M}$                              |
| Diffusion-PO-denitrifier             | $\text{N}_2\text{O}$               | PT-IRMS; GC-QMS    | $^{15}\text{N}$ -enriched; natural abundance                       | Water samples, soil extracts                                                 | 1–100 $\mu\text{M}$ (GC-MS)<br>20–60 nmol (PT-IRMS) |
| AIRTS-HPLC                           | $\text{NH}_4^+$                    | HPLC               | $^{15}\text{N}$ -enriched ( $x(^{15}\text{N}) = 25\text{--}75\%$ ) | Water samples                                                                | 2–10 $\mu\text{M}$                                  |
| Method                               | Optimal sample volume <sup>a</sup> | Time <sup>b</sup>  | Toxicity                                                           | $^{15}\text{N}$ dilution by chemicals                                        | Fractionation                                       |
| Distillation                         | 30–500 ml                          | 5–40 min/sample    | ×                                                                  | Prone to $^{15}\text{N}$ dilution by DON and reagents                        | Prone to low recovery and fractionation             |
| Diffusion                            | 10–100 ml                          | 5–10 d/batch       | ×                                                                  | Less prone to $^{15}\text{N}$ 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 $^{15}\text{N}$ dilution by reagents        | Neglectable at high recovery                        |

**Table 1** (continued)

| Method                          | Optimal sample volume <sup>a</sup>                                                                                                  | Time <sup>b</sup> | Toxicity                                                                                                                                                    | <sup>15</sup> N dilution by chemicals              | Fractionation                             |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|-------------------------------------------|
| Hg precipitation                | < 300 ml                                                                                                                            | 1 d/batch         | ✓                                                                                                                                                           | Large <sup>15</sup> N dilution by reagents         | Not reported                              |
| TPB precipitation               | 5 ml after freeze-drying                                                                                                            | 1 d/batch         | ✓                                                                                                                                                           | Small <sup>15</sup> N dilution by reagents         | Significant for freeze-drying of > 100 ml |
| Derivatization                  | 400–500 ml                                                                                                                          | 1–2 d/batch       | ✓                                                                                                                                                           | Large <sup>15</sup> 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 <sup>15</sup> N dilution by reagents         | Small                                     |
| BO-NH <sub>2</sub> OH           | 4 ml                                                                                                                                | 2–3 d/batch       | ×                                                                                                                                                           | Small <sup>15</sup> N dilution by reagents         | Small                                     |
| Diffusion-BO-azide              | 10 ml                                                                                                                               | 5–7 d/batch       | ✓                                                                                                                                                           | Small <sup>15</sup> N dilution by DON and reagents | Not reported                              |
| Diffusion-BO-NH <sub>2</sub> OH | 5 ml                                                                                                                                | 5–7 d/batch       | ×                                                                                                                                                           | Small <sup>15</sup> N dilution by DON and reagents | Small                                     |
| Diffusion-BO-denitrifier        | 20 ml                                                                                                                               | 5–7 d/batch       | ×                                                                                                                                                           | Small <sup>15</sup> 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 <sup>15</sup> N dilution by DON and reagents | Large at < 20 μM N                        |
| Diffusion-PO-denitrifier        | 10–20 ml                                                                                                                            | 5–7 d/batch       | ×                                                                                                                                                           | Small <sup>15</sup> 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 <sup>15</sup> 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 <sup>15</sup> 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 <sup>15</sup> 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 <sup>15</sup> 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 <sup>15</sup> 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 $^{15}\text{N}$ dilution by reagents and fractionation                                                                                                                                                                                          | Toxic, potential $\text{NO}_2^-$ interferences                                                                                                                                                |
| BO- $\text{NH}_2\text{OH}$           | Small sample volumes, rapid, ease of batch processing, small $^{15}\text{N}$ dilution by reagents and fractionation                                                                                                                                                                                                             | Not for acidic soils, potential $\text{NO}_2^-$ interferences                                                                                                                                 |
| Diffusion-BO-azide                   | Small sample volumes, matrix versatility, ease of batch processing,                                                                                                                                                                                                                                                             | Toxic, time and labor consuming, potential fractionation and $^{15}\text{N}$ dilution by DON and reagents                                                                                     |
| Diffusion-BO- $\text{NH}_2\text{OH}$ | Small sample volumes, matrix versatility, ease of batch processing, small fractionation                                                                                                                                                                                                                                         | Time and labor consuming, potential fractionation and $^{15}\text{N}$ 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 $^{15}\text{N}$ dilution by DON and reagents                                |
| Ion exchange-BO-denitrifier          | Low N requirement, non-toxic, small $^{15}\text{N}$ 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 $^{15}\text{N}$ 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 $^{15}\text{N}$ 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 $^{15}\text{N}$ dilution by DON and reagents                                                  |
| AIRTS-HPLC                           | Small sample volumes, non-toxic, simultaneous determination of N concentration and $^{15}\text{N}$ abundance, minimal sample preparation, small $^{15}\text{N}$ dilution by reagents and fractionation                                                                                                                          | Require high $^{15}\text{N}$ -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- $\text{NH}_2\text{OH}$           | (Liu et al. 2014)                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                               |
| Diffusion-BO-azide                   | (Zhang et al. 2019)                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                               |
| Diffusion-BO- $\text{NH}_2\text{OH}$ | (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  $^{15}\text{N}$  analysis of  $\text{NO}_3^-$  in liquid samples in case studies

| Method                              | Analyte                           | $^{15}\text{N}$ abundance                                        | Instrument                                    | Sample matrix                                                                | N requirement ( $\text{N-NO}_3^-$ )                                              |
|-------------------------------------|-----------------------------------|------------------------------------------------------------------|-----------------------------------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Distillation                        | $\text{N}_2$                      | $^{15}\text{N}$ -enriched; natural abundance                     | (DI-/EA-) IRMS                                | Water samples, soil extracts                                                 | 140–360 $\mu\text{mol}$                                                          |
| Diffusion                           | $\text{N}_2$                      | $^{15}\text{N}$ -enriched; natural abundance                     | (DI-/EA-) IRMS; ES                            | Water samples, soil extracts                                                 | 4–14 $\mu\text{mol}$                                                             |
| Ion exchange                        | $\text{N}_2$                      | $^{15}\text{N}$ -enriched; natural abundance                     | (DI-/EA-) IRMS; LC-Orbitrap HRMS <sup>c</sup> | Freshwater samples                                                           | > 6 $\mu\text{mol}$                                                              |
| Derivatization                      | $\text{N}_2$                      | $^{15}\text{N}$ -enriched (pool dilution)                        | ES                                            | Water samples                                                                | > 1.5 $\mu\text{M}$ ; 200–300 nmol                                               |
|                                     | $\text{N}_2$                      | $^{15}\text{N}$ -enriched; natural abundance                     | EA-IRMS                                       | Water samples                                                                | ~ 107 $\mu\text{M}$                                                              |
|                                     | dye derivative                    | $^{15}\text{N}$ -enriched (pool dilution)                        | GC–MS                                         | Water samples                                                                | 2–8 nmol/kg                                                                      |
| $\text{Ba}(\text{NO}_3)_2$ -acetone | $\text{N}_2$                      | $^{15}\text{N}$ -enriched; natural abundance                     | EA-IRMS                                       | Freshwater samples                                                           | 4–30 $\mu\text{mol}$                                                             |
| $\text{NaOH}$ -acetone              | $\text{N}_2$                      | $^{15}\text{N}$ -enriched; natural abundance                     | EA-IRMS                                       | Freshwater samples, soil extracts                                            | 4–9 $\mu\text{mol}$                                                              |
| SPIN-QMS                            | $\text{NO}$                       | $^{15}\text{N}$ -enriched                                        | QMS                                           | Water samples, soil extracts                                                 | > 4 $\mu\text{M}$ (20 nmol)                                                      |
| SPIN-MIMS                           | $\text{NO}$                       | $^{15}\text{N}$ -enriched                                        | MIMS                                          | Water samples, soil extracts                                                 | > 7 $\mu\text{M}$ (10 nmol)                                                      |
| Denitrifier                         | $\text{N}_2\text{O}$              | $^{15}\text{N}$ -enriched; natural abundance                     | GC-QMS;PT-IRMS;LS                             | Water samples, soil extracts                                                 | 20 nmol or 1–20 $\mu\text{M}$ (IRMS); 175 nmol (LS); 1–150 $\mu\text{M}$ (GC–MS) |
| Cd-azide                            | $\text{N}_2\text{O}$              | $^{15}\text{N}$ -enriched; natural abundance                     | PT-IRMS;LS                                    | Water samples, soil extracts                                                 | 25–30 nmol (0.5–40 $\mu\text{M}$ )                                               |
| Cd-NH <sub>2</sub> OH               | $\text{N}_2\text{O}$              | $^{15}\text{N}$ -enriched; natural abundance                     | PT-IRMS                                       | Water samples                                                                | 20 nmol (2–20 $\mu\text{M}$ )                                                    |
| $\text{VCl}_3$ -Azide               | $\text{N}_2\text{O}$              | $^{15}\text{N}$ -enriched; natural abundance                     | PT-IRMS                                       | Water samples, soil extracts                                                 | 40 nmol/16.5–100 $\mu\text{M}$                                                   |
| Ti(III) reduction                   | $\text{N}_2\text{O}$              | $^{15}\text{N}$ -enriched; natural abundance                     | PT-IRMS; LS                                   | Freshwater samples                                                           | 20–40 nmol; 3.5–14.3 $\mu\text{M}$                                               |
| AIRTS-HPLC                          | $\text{NH}_4^+$                   | $^{15}\text{N}$ -enriched ( $x(^{15}\text{N})=25\text{--}75\%$ ) | AIRTS-HPLC                                    | Water samples                                                                | 4 $\mu\text{M}$                                                                  |
| Method                              | Optimal ample volume <sup>a</sup> | Time                                                             | Toxicity                                      | $^{15}\text{N}$ dilution by chemicals                                        | Fractionation                                                                    |
| Distillation                        | 30–500 ml                         | 10 min–2 h/sample                                                | ×                                             | Prone to $^{15}\text{N}$ dilution by DON and reagents                        | Prone to fractionation                                                           |
| Diffusion                           | 30–500 ml                         | 1–2 weeks/batch                                                  | ×                                             | Less prone to $^{15}\text{N}$ dilution by DON and reagents than distillation | Prone to fractionation                                                           |
| Ion exchange                        | milliliters–liters                | 3–5 d/batch                                                      | ×                                             | Prone to DON interferences Small $^{15}\text{N}$ dilution by reagents        | Neglectable at high recovery                                                     |
| Derivatization                      | 60–500 ml                         | 1–2 d/batch                                                      | ✓                                             | Large $^{15}\text{N}$ dilution by reagents                                   | Small and constant                                                               |
| $\text{Ba}(\text{NO}_3)_2$ -acetone | ~ 200–500 ml <sup>d</sup>         | 1–2 week/batch <sup>b</sup>                                      | ×                                             | Neglectable                                                                  | Small and constant                                                               |
| $\text{NaOH}$ -acetone              | ~ 500 ml <sup>d</sup>             | ~ 1 week/batch <sup>b</sup>                                      | ×                                             | 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 <sup>a</sup>                                                                                                                                                                       | Time            | Toxicity                                                                                                                                                                              | <sup>15</sup> 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 <sup>15</sup> N dilution by reagents | Small and constant     |
| Cd-NH <sub>2</sub> OH                      | 10–15 ml                                                                                                                                                                                                | 2–3 d/batch     | ✓                                                                                                                                                                                     | Small <sup>15</sup> N dilution by reagents | Small and constant     |
| VCl <sub>3</sub> -Azide                    | 2.5 ml                                                                                                                                                                                                  | 2–3 d/batch     | ✓                                                                                                                                                                                     | Small <sup>15</sup> N dilution by reagents | Large at < 20 µM N     |
| Ti(III) reduction                          | 2–4 ml                                                                                                                                                                                                  | 2–3 d/batch     | ×                                                                                                                                                                                     | Small <sup>15</sup> 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 <sup>15</sup> 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 <sup>15</sup> 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 <sub>2</sub> <sup>−</sup> interferences                                                                 |                                            |                        |
| Ba(NO <sub>3</sub> ) <sub>2</sub> -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 <sup>15</sup> 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 <sub>2</sub> <sup>−</sup> 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 <sub>2</sub> <sup>−</sup> interferences                                                                                                                           |                                            |                        |
| Cd-NH <sub>2</sub> OH                      | Simple, rapid, low N requirement, small sample volumes, low N contamination by reagents, ease of sample transport and storage                                                                           |                 | Toxic, potential NO <sub>2</sub> <sup>−</sup> interferences                                                                                                                           |                                            |                        |
| VCl <sub>3</sub> -Azide                    | Simple, rapid, low N requirement, small sample volume, matrix versatility, small <sup>15</sup> N dilution by reagents and fractionation, ease of batch processing, ease of sample transport and storage |                 | Toxic, potential NO <sub>2</sub> <sup>−</sup> 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 <sub>3</sub> <sup>−</sup> concentration and salinity, potential NO <sub>2</sub> <sup>−</sup> interferences |                                            |                        |
| AIRTS-HPLC                                 | Small sample volumes, low N requirement, non-toxic, neglectable <sup>15</sup> N dilution by reagents and fractionation                                                                                  |                 | Require high <sup>15</sup> 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 <sub>3</sub> ) <sub>2</sub> -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 <sub>2</sub> OH                      | (Liu et al. 2014)                                                                                                                                                                                                        |
| VCl <sub>3</sub> -Azide                    | (Lachouani et al. 2010)                                                                                                                                                                                                  |
| Ti(III) reduction                          | (Altabet et al. 2019)                                                                                                                                                                                                    |
| AIRTS-HPLC                                 | (Gardner et al. 1995)                                                                                                                                                                                                    |

<sup>a</sup>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

<sup>b</sup>The time doesn't involve any pre-concentration (e.g. freeze-drying)

<sup>c</sup>The recently developed LC-Orbitrap HRMS with ion-exchange and gradient dilution preparation allows for <sup>15</sup>N analysis of NO<sub>3</sub><sup>−</sup> 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<sub>2</sub>O, 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 <sup>15</sup>N abundance in N<sub>2</sub>O, but it is limited to <sup>15</sup>N-enriched samples due to low instrument precision (Russow and Förstel 1993; Isobe et al. 2009).

For atmospherically-derived NO<sub>3</sub><sup>−</sup> (e.g. in rain-water), using N<sub>2</sub>O as the analyte for IRMS analysis may result in a slight overestimation of δ<sup>15</sup>N values by 1–2‰ due to mass-independent <sup>17</sup>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 δ<sup>15</sup>N calculation after correcting for the contribution of <sup>14</sup>N<sup>14</sup>N<sup>17</sup>O. For most environmental samples, this correction can be performed routinely by most IRMS data software, which calculates <sup>17</sup>O contribution from <sup>18</sup>O

abundance by assuming a mass-dependent relationship (Böhlke et al. 2003; McIlvin and Altabet 2005). However, atmospheric NO<sub>3</sub><sup>−</sup> is enriched in <sup>17</sup>O due to large mass-independent <sup>17</sup>O fractionation from ozone formation, resulting in an underestimation of the m/z 45 contribution of <sup>17</sup>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<sub>2</sub>O from H<sub>2</sub>O (Hastings et al. 2003; Coplen et al. 2004). Alternatively, the N<sub>2</sub>O can be analyzed by coupling PT-IRMS to a thermal decomposition system (Brand 1995; Kaiser et al. 2007; Komatsu et al. 2008; Smirnov 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<sub>3</sub><sup>−</sup> to N<sub>2</sub>O gas for <sup>15</sup>N analysis. The “denitrifier method” is a well-established technique, in which NO<sub>3</sub><sup>−</sup> is reduced

to  $\text{NO}_2^-$  and finally to  $\text{N}_2\text{O}$  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 ( $\text{VCl}_3$ ) to reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$  and using azide or hydroxylamine ( $\text{NH}_2\text{OH}$ ) as catalyst for further reduction of  $\text{NO}_2^-$  to  $\text{N}_2\text{O}$  (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  $\text{NO}_3^-$  is reduced to  $\text{N}_2\text{O}$  using Ti(III) chloride. In all these microbial and chemical methods, the  $\text{NO}_2^-$  present in the sample is reduced alongside the  $\text{NO}_3^-$ , and the measured  $^{15}\text{N}$  abundance of the  $\text{N}_2\text{O}$  is a composition of both the  $\text{NO}_2^-$  and the  $\text{NO}_3^-$ . When preexisting  $\text{NO}_2^-$  concentration is high and only the  $^{15}\text{N}$  abundance of  $\text{NO}_3^-$  is required,  $\text{NO}_2^-$  can be removed using sulfamic acid (Norman and Stucki 1981; Ward et al. 1984; Mulvaney 1996; Granger et al. 2006).

Methods for generating  $\text{N}_2\text{O}$  gas from dissolved  $\text{NH}_4^+$  usually involve the conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  (or  $\text{NO}_2^-$ ) and the reduction of  $\text{NO}_3^-$  (or  $\text{NO}_2^-$ ) to  $\text{N}_2\text{O}$ , with or without pre-isolation of  $\text{NH}_4^+$  from solutions. The isolation of  $\text{NH}_4^+$  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  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and other interfering chemicals in the matrix. The conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  or  $\text{NO}_2^-$  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  $\text{NO}_3^-$  (or  $\text{NO}_2^-$ ) to  $\text{N}_2\text{O}$  can be achieved using bacterial (Houlton et al. 2007; Isobe et al. 2009; Kawashima et al. 2021), azide (Lachouani et al. 2010), or  $\text{NH}_2\text{OH}$  reduction (Zhang et al. 2018), similar to the  $^{15}\text{N}$  analysis methods for  $\text{NO}_3^-$ .

#### On-line gas methods

In all of the foregoing preparation methods, the isolation and/or conversion of the target  $\text{NH}_4^+$  or  $\text{NO}_3^-$  is

partially performed off-line. Instead, the SPIN (sample preparation unit for inorganic nitrogen) method allows for complete on-line sample preparation and  $^{15}\text{N}$  measurement in  $^{15}\text{N}$ -enriched samples. In this approach,  $\text{NO}_3^-$  is reduced to  $\text{NO}$  or  $\text{N}_2\text{O}$ , while  $\text{NH}_4^+$  is oxidized to  $\text{N}_2$ . 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  $^{15}\text{N}$  abundance and  $\text{N}$  concentration (Stange et al. 2007; Eschenbach et al. 2017, 2018).

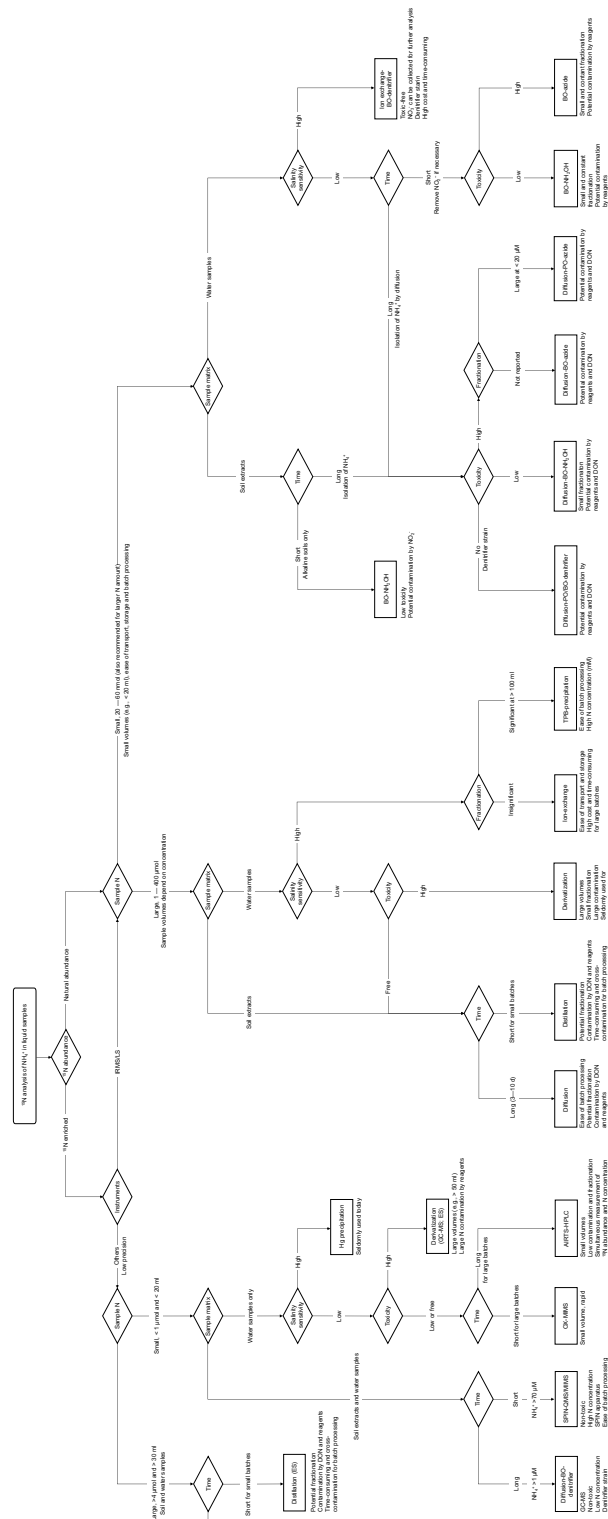
#### Non-gas methods

For  $^{15}\text{N}$ -enriched samples, the  $^{15}\text{N}$  abundance and the concentration of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  can also be analyzed simultaneously in filtered solutions as dissolved  $\text{NH}_4^+$  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  $^{15}\text{NH}_4^+$  and  $^{14}\text{NH}_4^+$  as they pass through HPLC columns. The  $\text{NH}_4^+$  in filtered water samples can be analyzed directly by HPLC, while  $\text{NO}_3^-$  is first reduced to  $\text{NH}_4^+$  by zinc reduction under acidic conditions. To calculate the accurate  $^{15}\text{N}$  of  $\text{NO}_3^-$ , the  $^{15}\text{N}$  abundance and concentration of initial  $\text{NH}_4^+$  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  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , 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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  methods separately, although



**Fig.**  
**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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $^{15}\text{N}$  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  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  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  $^{15}\text{N}$  measurements.

Sample salinity is one of the most important factors limiting the choice of appropriate methods. Salts (e.g.,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ) 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  $\text{NO}_3^-$  into  $\text{N}_2\text{O}$  by Ti(III) reduction, for example, is significantly impeded by  $\text{SO}_4^{2-}$  in the solutions (Altabet et al. 2019). Poor recovery causes isotopic fractionation, resulting in erroneous depletion or enrichment of  $^{15}\text{N}$  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  $\text{NH}_4^+$  by DON hydrolysis can cause errors to  $^{15}\text{N}$  abundance of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $\text{NO}_2^-$  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).

#### $^{15}\text{N}$ abundances of $\text{NH}_4^+/\text{NO}_3^-$ in samples

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

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

Natural abundance studies require excellent precision because the  $\delta^{15}\text{N}$  abundances of most natural ecological samples fall within the narrow range of  $-20\text{‰}$  to  $+30\text{‰}$  ( $x(^{15}\text{N})=0.362 - 0.377\%$ ) (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  $\delta^{15}\text{N}$  abundances. Modern  $\text{N}_2\text{O}$  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  $^{15}\text{N}$  abundance of target N species are typically  $>0.5\%$  ( $x(^{15}\text{N})$ ) in tracer experiments. The analysis of such samples also requires precision, but the limiting factors are often the highly variable  $^{15}\text{N}$  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  $^{15}\text{N}$  addition based on the system, the frequency of tracer addition, the extent of the change in  $^{15}\text{N}$  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  $^{15}\text{N}$  abundances from natural abundance to low enrichment (e.g.  $x(^{15}\text{N}) < 10\%$ ), 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  $^{15}\text{N}$  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  $^{15}\text{N}$  enrichment (Mulvaney 1993; Mulholland et al. 2004; Griesheim and Mulvaney 2019). It is also possible to measure  $^{15}\text{N}$  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 ( $\text{SD} < 0.03\%$ , SD: standard error).

Whether samples are at natural abundance or have high  $^{15}\text{N}$  enrichments, reference materials that bracket the entire expected  $^{15}\text{N}$  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 $\text{NH}_4^+/\text{NO}_3^-$ 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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $\text{N}_2$  and its  $^{15}\text{N}$  analysis by IRMS require 2–400  $\mu\text{mol}$  of target N, resulting in an ideal concentration of  $> 50 \mu\text{M}$  in a typical volume of  $\sim 50 \text{ ml}$ . Lower  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations (e.g. 5–10  $\mu\text{M}$ ) 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  $\text{N}_2$  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  $^{15}\text{N}$  content into samples and calibrate the results by an isotope dilution equation, but such an addition introduces analytical errors, which increase with increased  $^{15}\text{N}$  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  $\text{N}_2\text{O}$  methods and non-gas AIRTS-HPLC methods require nanomole levels of target N and allow for samples containing micromolar amounts of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  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  $\text{N}_2\text{O}$  methods are increasingly used not only for low N samples but also for large N samples (e.g.  $> 50\ \mu\text{M}$ ) 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  $^{15}\text{N}$  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  $\text{NO}_3^-$  are particularly prone to such contamination, because reagents such as Devarda's alloy and soil KCl extractants, as well as residual  $\text{NH}_4^+$  can all result in erroneously dilution or enrichment of  $^{15}\text{N}$  abundances of the target  $\text{NO}_3^-$ . This is of course affected by the purity and quantity of the contaminants, as well as the  $^{15}\text{N}$  differences between the N impurities and the target N.

When diffusing  $50\ \mu\text{g}\ \text{NO}_3^-$ , Stephan and Kavanagh (2009) found that reagent N depleted by 10‰ relative to the target  $\delta^{15}\text{N}$  resulted in underestimating the target  $\delta^{15}\text{N}$  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  $\text{NO}_3^-$  contents (Liu and Mulvaney 1992b; Herman et al. 1995; Sigman et al. 1997; Robinson 2001; Stephan and Kavanagh 2009). Residual  $^{15}\text{N}$ -labeled  $\text{NH}_4^+$  can also lead to substantial overestimation in successive  $^{15}\text{N}$  analysis of unenriched  $\text{NO}_3^-$ , 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  $\text{NO}_3^-$  contents (e.g.  $50\ \mu\text{g}$ ) and/or large volumes (e.g.  $> 30\ \text{ml}$ ) (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  $\text{NH}_4^+$  due to chemical hydrolysis under prolonged diffusion and distillation, contributing to excess N and dilute  $^{15}\text{N}$  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  $> 50\ \text{ml}$ , 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  $\text{NO}_3^-$ ) 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  $\text{NO}_3^-$  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 ( $4\text{--}9\ \mu\text{mol}$ ) (Huber et al. 2011, 2012). In the case of  $\text{NH}_4^+$ , many  $\text{N}_2$  and  $\text{N}_2\text{O}$  methods require isolating  $\text{NH}_4^+$  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  $\text{N}_2$  methods, chemical and biological  $\text{N}_2\text{O}$  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  $\delta^{15}\text{N}$  is significantly reduced, because  $\text{NO}_3^-$  (and sometimes also  $\text{NH}_4^+$ ) 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  $^{15}\text{N}$  measurements. It is the result of incomplete transformation or recovery of the target N, as the rate of such a process usually differs between  $^{14}\text{N}$  and  $^{15}\text{N}$ . 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  $\text{NH}_4^+$  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  $\leq 5$  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  $\delta^{15}\text{N}$  in most ecological samples

varies only by  $< 20\text{‰}$  (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  $^{15}\text{N}$  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  $\text{NO}_3^-$ , 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  $\text{NH}_2\text{OH}$  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  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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  $^{15}\text{N}$  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  $\text{N}_2/\text{N}_2\text{O}$ -based sample preparation methods are currently the only candidates for quantifying  $^{15}\text{N}$  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  $^{15}\text{N}$  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  $^{15}\text{N}$  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  $\text{N}_2$  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  $^{15}\text{N}$  analysis in nanomole  $\text{N}_2\text{O}$  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; Smirnov et al. 2012) or a microwave discharge unit (Hattori et al. 2016, 2019) to a modified PT-IRMS, the  $\text{N}_2\text{O}$  can be decomposed to  $\text{N}_2$  and  $\text{O}_2$  prior to isotopic analysis, preventing the interference from  $^{17}\text{O}$  with  $\delta^{15}\text{N}$  results. More recently, laser spectroscopy is emerging as an alternative technology to IRMS for simultaneous determination of  $\text{N}_2\text{O}$  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  $^{17}\text{O}$  abundance, thus also eliminating any potential overestimation of  $^{15}\text{N}$  in samples containing mass-independent  $^{17}\text{O}$  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).

$^{15}\text{N}$  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  $^{15}\text{N}$  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  $x(^{15}\text{N})/\delta^{15}\text{N}$  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  $x(^{15}\text{N}) < 10\%$  because most  $^{15}\text{N}$ -enriched studies are conducted at this range. Methods with GC–MS, MIMS and HPLC were assessed at a higher  $^{15}\text{N}$  enrichment. Natural abundance methods were often evaluated in original papers over a narrow range of  $\delta^{15}\text{N}$  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  $< 0.1\%$   $\delta^{15}\text{N}$  with a N amount of 40–400  $\mu\text{mol N}$  for manual  $\text{N}_2$  preparation and  $^{15}\text{N}$  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  $< 0.2\%$   $\delta^{15}\text{N}$  from natural abundance to tracer level ( $< 5\%$ ) with a N mass of 2–10  $\mu\text{mol N}$  or 20–60 nmol  $\text{N}_2\text{O}$ , 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  $^{15}\text{N}$  enrichment (Mulvaney 1993). The recently emerged laser spectroscopy yields a precision of  $< 0.5\%$  with down to 0.5 nmol  $\text{N}_2\text{O}$  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  $^{15}\text{N}$ -enriched levels, which precludes their use for  $^{15}\text{N}$ -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  $\mu\text{g N}$  at tracer levels (e.g.  $x(^{15}\text{N}) > 0.5\%$ ) (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  $\text{N}_2$  ( $> 30$  nmol) and  $\text{N}_2\text{O}$  ( $> 200$  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  $x(^{15}\text{N})$  (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  $x(^{15}\text{N}) > 0.5\%$ , and sample preparation methods developed for these studies generally showed standard deviations ranging from 0.02 to 5% ( $x(^{15}\text{N})$ ) and accuracies within  $\pm 5\%$  of the true values, depending on the  $^{15}\text{N}$  abundances, N amounts, and procedures and equipment used. Previous evaluations have found that with optimal concentrations ( $\sim 140$ – $360$   $\mu\text{mol}$  and  $\sim 4$ – $11$   $\mu\text{mol N}$ , respectively), distillations and diffusions yield comparable precision ( $\text{SD} < 0.05\%$ ) and accuracy (percent error  $< 5.3\%$ ) for tracer samples at  $x(^{15}\text{N}) < 5\%$  (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  $^{15}\text{N}$ -enriched  $\text{NH}_4^+$  can cause enormous errors to natural  $^{15}\text{N}$  analysis of  $\text{NO}_3^-$  (Liu and Mulvaney 1992b; Mulvaney et al. 1994; Herman et al. 1995; Griesheim and Mulvaney 2019). At lower N masses and the whole tracer  $^{15}\text{N}$  range, the denitrifier methods with GC–MS analysis achieved a precision and accuracy of 0.02–0.08% and 0.02%  $x(^{15}\text{N})$ , 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  $^{15}\text{N}$  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  $\delta^{15}\text{N}$  range. For example, the modification to distillation by Velinsky et al. (1989) have enabled analysis of  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  with an overall precision of  $<0.5\text{‰}$  (SD) and a relative error of within  $\pm 4\%$  of the true  $\delta^{15}\text{N}$  at low ( $<5\text{ }\mu\text{M}$ ) concentrations. At a similar working range, modifications to diffusion by Sigman et al. (1997) and Holmes et al. (1998) have allowed  $\delta^{15}\text{N}$  analysis of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in water samples to be within  $0.3\text{‰}$  and  $0.36\text{--}0.73\text{‰}$   $\delta^{15}\text{N}$  (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  $^{15}\text{N}$  abundance if a precision or accuracy of  $1.3\text{‰}$  is not required. Other methods for natural  $\delta^{15}\text{N}$  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),  $\text{NH}_2\text{OH}$  (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  $1\text{‰}$   $\delta^{15}\text{N}$ . The  $\delta^{15}\text{N}$  values from these methods were generally within  $5\text{‰}$  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  $\delta^{15}\text{N}$  values of the samples were calibrated using the linear regression established by the measured  $\delta^{15}\text{N}$  values of the standards against their assigned  $\delta^{15}\text{N}$  values (Klesta et al. 1996; Werner and Brand 2001; Brand and Coplen 2012; Brand et al. 2014; Meier-Augenstein and Schimmelmann 2019).

## Integrating $^{15}\text{N}$ analysis methods and criteria into the DST

The criteria discussed are not all given the same importance in the DST. The  $^{15}\text{N}$  abundance, the sample N concentration, the instrument availability, and the sample matrix are given priority, because they always limit the preparation approaches available. The  $^{15}\text{N}$  abundance and N concentration of samples determine the acceptable levels of  $^{15}\text{N}$  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 “ $^{15}\text{N}$  abundance” is set as the first criterion, dividing the decision trees into two parts: methods for  $^{15}\text{N}$ -enriched samples (the left branch) and methods for natural  $^{15}\text{N}$  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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  at the same time.

### *Non-IRMS/laser methods for $^{15}\text{N}$ -enriched samples in the DST*

The  $^{15}\text{N}$ -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  $^{15}\text{N}$ -enriched samples of sufficiently

large volumes and containing sufficient N amounts (e.g. > 30 ml and > 4  $\mu\text{mol}$ ), the  $\text{NH}_4^+$  or  $\text{NO}_3^-$  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  $\mu\text{mol}$  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  $\text{NH}_4^+$  is the target N compound (Fig. 1), a hybrid denitrifier method can be used, which involves isolating  $\text{NH}_4^+$  by diffusion, converting  $\text{NH}_4^+$  to  $\text{NO}_2^-$  by hypobromite oxidation, and converting  $\text{NO}_2^-$  to  $\text{N}_2\text{O}$  (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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  to a gaseous N-product (e.g.,  $\text{N}_2$ ,  $\text{N}_2\text{O}$ , or  $\text{NO}$ ). 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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (and  $\text{NO}_2^-$ ) 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  $\text{NH}_4^+$ ) (Fig. 1) and AIRTS-HPLC (for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) (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,  $\text{NH}_4^+$  in solution is directly converted to  $\text{N}_2$  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  $\text{NH}_4^+$  pools, such as those in sediment oxygen-transition zones (Yin et al. 2014). In the AIRTS-HPLC method, the conversion into  $\text{N}_2$  gas is not needed, and the isotopic ratio of  $\text{NH}_4^+$  is measured based on a retention-time shift of the combined peak of the  $^{15}\text{NH}_4^+$  and  $^{14}\text{NH}_4^+$ . The shift is relative to the percentage of  $^{15}\text{NH}_4^+$  and the best relationship occurs between 25–75%  $x(^{15}\text{N})$ . As a result, the AIRTS-HPLC method is suitable for pool dilution experiments with variations in  $^{15}\text{N}$  abundances within this range (Gardner et al. 1991, 1995, 1997; Lu et al. 2019). Because the individual  $\text{NH}_4^+$  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  $^{15}\text{N}$  to  $^{15}\text{NH}_4^+$  (Gardner et al. 1995; Yin et al. 2014).

#### *IRMS/Laser Methods in the DST*

Both  $^{15}\text{N}$ -enriched and natural  $^{15}\text{N}$  abundance samples can be processed using sample preparation methods that produce  $\text{N}_2$  or  $\text{N}_2\text{O}$  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  $\text{N}_2$  and  $^{15}\text{N}$  analysis by EA-IRMS (i.e.,  $\text{N}_2$  methods), and in the other as  $\text{N}_2\text{O}$  gas for  $^{15}\text{N}$  analysis by PT-IRMS or laser spectroscopy (i.e.,  $\text{N}_2\text{O}$  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  $\text{NH}_4^+$ ) and acetone extraction (only for  $\text{NO}_3^-$ ), all of which are appropriate for samples containing large N amounts (e.g., > 1  $\mu\text{mol}$ ). For samples with a low N concentration, the concentration procedure is tedious and prone to errors from fractionation and  $^{15}\text{N}$  dilution by reagents. In the second group of methods  $\text{N}_2\text{O}$  is produced as the analyte by means of microbes or chemicals. Because the  $^{15}\text{N}$  analysis of  $\text{N}_2\text{O}$  has

a high sensitivity, these methods allow samples containing smaller N amounts (20–60 nmol) and volumes (<20 ml). Because the  $^{15}\text{N}$  analysis of  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  methods after dilution, yielding more compatible and precise results for natural abundance studies in environmental samples.

*$\text{N}_2$  methods for samples with large N amounts (micromoles of N)* After choosing a  $\text{N}_2$  or  $\text{N}_2\text{O}$  method based on sample N amount and volume, researchers must check the salinity level and DON content of the matrix. Among  $\text{N}_2$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$ ). However, the freeze-drying process to obtain large N amounts (4–9  $\mu\text{mol}$ ) 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  $^{15}\text{N}$ -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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $\text{NO}_3^-$  elution) can be an issue for large numbers of samples. The TPB precipitation and the  $\text{Ba}(\text{NO}_3)_2$ -acetone techniques, on the other hand, use less expensive materials and are appropriate for preparing  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively. They both use freeze-drying to concentrate solutions, which means that fractionation must be minimized in that process or accounted for.

*$\text{N}_2\text{O}$  methods for samples with small N amounts (nanomoles of N)* For samples with low N content

and small volumes, the  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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,  $\text{NH}_4^+$  is usually first isolated from solution using diffusion or ion-exchange resins, and then converted to  $\text{NO}_3^-$  or  $\text{NO}_2^-$  by persulfate oxidation (PO) or hypobromite oxidation (BO), respectively. The  $\text{NO}_3^-$  or  $\text{NO}_2^-$  is then converted to  $\text{N}_2\text{O}$  by denitrifying bacteria, azide or  $\text{NH}_2\text{OH}$  techniques (Fig. 1). If  $\text{NO}_3^-$  is the target N, it can be converted to  $\text{NO}_2^-$  and then to  $\text{N}_2\text{O}$  by denitrifying bacteria, Cd-azide,  $\text{VCl}_3$ -azide, Cd- $\text{NH}_2\text{OH}$ , or Ti(III) reduction methods (Fig. 2).

The sample matrix and the N species being studied determine the time and procedures required for  $\text{N}_2\text{O}$  methods.  $\text{NH}_4^+$  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- $\text{NH}_2\text{OH}$ ), although the pre-incubation of denitrifying bacteria may take 10–12 days. On the other hand,  $\text{NH}_4^+$  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  $\text{NO}_3^-$  is of interest, both soil extracts and water samples can be prepared within a few days using one of the above-mentioned conversions.

The  $\text{N}_2\text{O}$  methods are usually similar in performance, but the chemicals involved differ in toxicity. Many  $\text{N}_2\text{O}$  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  $\text{NH}_2\text{OH}$  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  $\text{NO}_3^-$  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  $\text{N}_2\text{O}$  methods

usually have very small isotopic fractionation and  $^{15}\text{N}$  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 $^{15}\text{N}$ abundance of $\text{NH}_4^+$ and $\text{NO}_3^-$ in agricultural soils*

The variations in natural  $^{15}\text{N}$  abundance ( $\delta^{15}\text{N}$ ) 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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (e.g.,  $> 50\ \mu\text{M}$ ) and DON due to high nutrient input from fertilizers. We assume that IRMS is available in this case.

To find the  $^{15}\text{N}$  analysis method for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , we first select “Natural abundance” for “ $^{15}\text{N}$  abundance” in the DST (Figs. 1 and 2). If a PT-IRMS or a laser spectrometer is available, the  $\text{N}_2\text{O}$  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  $\text{NH}_4^+$  (Fig. 1) and  $\text{NO}_3^-$  (Fig. 2). In the case of  $\text{NH}_4^+$  (Fig. 1), alkaline soil extracts can be analyzed using the BO- $\text{NH}_2\text{OH}$  method within 2–3 days; otherwise, the  $\text{NH}_4^+$  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  $\text{NH}_2\text{OH}$  techniques (Diffusion-PO-azide, Diffusion-BO-azide, and Diffusion-BO- $\text{NH}_2\text{OH}$ ) can be used in non-microbiology laboratories, and the latter employs less hazardous reagents. In the case of  $\text{NO}_3^-$  (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  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$ , 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  $\delta^{15}\text{N}$  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  $\text{NO}_3^-$  (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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  transformation rates in aquatic and terrestrial ecosystems can be estimated using the  $^{15}\text{N}$  pool dilution technique, whereby small amounts of  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  are added, and the rates of individual N transformation processes can be estimated from changes in size and  $^{15}\text{N}$  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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations (e.g., around  $10\ \mu\text{M}$   $\text{NH}_4^+$  or  $\text{NO}_3^-$ ) 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  $^{15}\text{N}$  abundance is not routinely measured.

In the DST for  $^{15}\text{NH}_4^+$  analysis, we choose “ $^{15}\text{N}$  enriched” for “ $^{15}\text{N}$  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  $\text{NH}_4^+$  concentration ( $> 70\ \mu\text{M}$ ) and special reaction apparatus. The

diffusion-BO-denitrifier method, on the other hand, allows for the preparation of samples with lower  $\text{NH}_4^+$  concentrations ( $>1 \mu\text{M}$ ), 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  $>50 \text{ mL}$  (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  $^{15}\text{N}$  abundance of  $\text{NH}_4^+$  simultaneously, but it requires more time than the OX-MIMS method (1 h vs. 24 h for a set of  $\sim 10$  samples) (Table 1).

With regard to the  $^{15}\text{NO}_3^-$  analysis (Fig. 2), we similarly choose “ $^{15}\text{N}$  enriched” for “ $^{15}\text{N}$  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  $\text{NO}_3^-$  is reduced to  $\text{NH}_4^+$  as the analyte, the  $^{15}\text{N}$  abundance of  $^{15}\text{NO}_3^-$  should be calculated based on the  $\text{NH}_4^+$  results. Therefore, if this method is used, the  $\text{NH}_4^+$  should be prepared and measured prior to the  $\text{NO}_3^-$ .

#### Pros and cons of the present DST

In this work, we focus on the  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in liquid samples from various environmental matrices, as they are the two key mineral N species in ecosystems and primarily used in environmental  $^{15}\text{N}$  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  $\text{NO}_2^-$  and organic N compounds, are discussed as interfering chemicals in this work. However, the  $^{15}\text{N}$  analysis of such species

in environmental samples can also be of great interest to ecological researchers. In neutral or alkaline soils where  $\text{NH}_4^+$  or  $\text{NH}_4^+$  forming fertilizers are applied, the amount of  $\text{NO}_2^-$  can be significant (Burns et al., 1995; Russow et al., 2009; Ward, 2011). Many  $\text{NH}_4^+$  and  $\text{NO}_3^-$  methods convert target N to  $\text{NO}_2^-$  and further to  $\text{N}_2\text{O}$ , and they can be modified to convert only  $\text{NO}_2^-$  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  $^{15}\text{N}$  analysis, it is usually first separated from inorganic N via diverse techniques. The isolated organic N is then converted to  $\text{NH}_4^+$  or  $\text{NO}_3^-$ , 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  $^{15}\text{N}$  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  $^{15}\text{N}$  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  $^{15}\text{N}$ -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  $\text{NH}_2\text{OH}$  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  $\text{NH}_2\text{OH}$  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  $^{15}\text{N}$  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; Smirnov et al. 2012; Mohn et al. 2014; Hattori et al. 2016; Eschenbach et al. 2017). The laser-based methods have just been developed for  $^{15}\text{N}$  analysis of  $\text{NO}_3^-$  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  $\text{N}_2\text{O}$ -based techniques to a wider range of environmental  $^{15}\text{N}$  studies. Another promising novel approach is to measure diverse isotopologues of environmental  $\text{NO}_3^-$  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  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in liquid samples. This tool is straightforward, visually appealing, and user-friendly, with the potential to be applied to a broader spectrum of  $^{15}\text{N}$ -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.

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**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.

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