This document is the accepted manuscript version of the following article:
Yu, L., Harris, E., Lewicka-Szczebak, D., Barthel, M., Blomberg, M. R. A., Harris, S. J., … Mohn, J. (2020). What can we learn from N2O isotope data? – Analytics, processes and modelling. Rapid Communications in Mass Spectrometry, 34(20), e8858 (14 pp.). https://doi.org/10.1002/rcm.8858

What can we learn from N_2O isotope data? - Analytics, processes and modelling

Longfei Yu[1,2†*], Eliza Harris[3*], Dominika Lewicka-Szczebak[4*], Matti Barthel[5], Margareta R.A. Blomberg[6], Stephen J. Harris[7,8], Matthew S. Johnson[9], Moritz F. Lehmann[10], Jesper Liisberg[11], Christoph Müller[12,13], Nathaniel E. Ostrom[14], Johan Six[5], Sakae Toyoda[15], Naohiro Yoshida[15,16], Joachim Mohn[1]

[1]Laboratory for Air Pollution & Environmental Technology, Empa, Swiss Federal Laboratories for Materials Science and Technology, Überlandstrasse 129, CH-8600 Dübendorf, Switzerland.
[2]Institute of Groundwater and Earth Sciences, Jinan University, 510632 Guangzhou, China.
[3]Department of Ecology, University of Innsbruck, Sternwartestrasse 15, A-6020 Innsbruck, Austria
[4]Centre for Stable Isotope Research and Analysis (KOSI), Büsgen Institute, Georg-August University of Göttingen, Germany
[5]ETH Zürich, Department of Environmental Systems Science, Zürich, Switzerland
[6]Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-10691, Stockholm, Sweden
[7]School of Biological, Earth and Environmental Sciences, UNSW Sydney, NSW, Australia
[8]Australian Nuclear Science and Technology Organisation, Lucas Heights, NSW, Australia
[9]Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark
[10]Department of Environmental Science, University of Basel, Basel, Switzerland
[11]University of Copenhagen, Niels Bohr Institute, Copenhagen, Denmark
[12]Institute of Plant Ecology (IFZ), Justus-Liebig University Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Germany
[13]School of Biology and Environmental Science and Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland
[14]Department of Integrative Biology and DOE Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI, USA
[15]Department of Chemical Science and Engineering, School of Materials and Chemical Technology, Tokyo Institute of Technology, Yokohama 226-8502, Japan
[16]Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo 152-8550, Japan

† Correspondence; L. Yu (yulongfei@jnu.edu.cn)

* These authors contributed equally to this study.

Manuscript type: Perspective at Rapid Communications in Mass Spectrometry

Abstract

The isotopic composition of nitrous oxide (N_2O) provides useful information for evaluating N_2O sources and budgets. Due to the co-occurrence of multiple N_2O transformation pathways, it is, however, challenging to use isotopic information to quantify the contribution of distinct processes across variable spatiotemporal scales. Here, we present an overview of recent progress in N_2O isotopic studies and provide suggestions for future research, mainly focusing on: analytical techniques; production and consumption processes; and interpretation and modelling approaches.

Comparing isotope-ratio mass spectrometry (IRMS) with laser absorption spectroscopy (LAS), we conclude that IRMS is a precise technique for laboratory analysis of N_2O isotopes, while LAS is more suitable for *in situ*/inline studies and offers advantages for site-specific analyses. When reviewing the link between the N_2O isotopic composition and underlying mechanisms/processes, we find that at the molecular scale, the specific enzymes and mechanisms involved determine isotopic fractionation effects. In contrast, at plot-to-global scales, mixing of N_2O derived from different processes and their isotopic variability must be considered. We also find that dual isotope plots are effective for semi-quantitative attribution of co-occurring N_2O production and reduction processes. More recently, process-based N_2O isotopic models have been developed for natural-abundance and ^{15}N-tracing studies, and have been shown to be effective, particularly for data with adequate temporal resolution.

Despite the significant progress made over the last decade, there is still great need and potential for future work, including development of analytical techniques, reference materials and inter-laboratory comparisons, further exploration of N_2O formation and destruction mechanisms, more observations across scales, and design and validation of interpretation and modelling approaches. Synthesizing all these efforts, we are confident that the N_2O isotope community will continue to

advance our understanding of N_2O transformation processes in all spheres of the Earth, and in turn to gain improved constraints on regional and global budgets.

1 Introduction

Given the increasing global concern about climate change, fostering mitigation of greenhouse gas (GHG) emissions to the atmosphere has become a pressing focus of research. Nitrous oxide (N_2O), which is a potent GHG and an important ozone-depleting substance[1,2], has been studied extensively for decades. Past research has revealed that N_2O is produced from a number of biological and chemical processes in soils, sediments and water bodies[3]. At the global scale, the continuing increase of the N_2O mixing ratio in the atmosphere is mainly attributed to fertilizer-induced anthropogenic soil emissions[4]. The largest global N_2O sink is photolysis in the stratosphere[5], while N_2O reduction in soils may play a significant role in reducing regional N_2O emissions[6]. N_2O sources and sinks show strong spatial and temporal heterogeneity[3], making it difficult to create accurate N_2O budgets, especially regarding drivers of seasonal and interannual variability at regional and global scales.

With the development of isotope-specific analytics, numerous studies have applied isotopic approaches to investigate N_2O sources and sinks[7–12]. Isotopic labelling of substrates for N_2O production provides a tracing methodology to differentiate between N_2O production pathways[13]. Although isotope tracing experiments are performed mostly in laboratory incubations[13,14], *in situ* stable isotope tracing has recently been conducted in plot- and ecosystem-scales to investigate N_2O sources[9,15]. Despite the advantage of the isotope tracing approach in quantification of N transformations, it also has clear disadvantages, such as ecosystem perturbation, and limitations in the spatiotemporal scales that can be studied, due to the short-term nature of the approach and the cost of tracers as well as the effort required.

While inherently not as direct as the ^{15}N-labelling approach with regards to the actual pathways of N_2O production, the natural abundance of N_2O isotopic species and other related N-substances

represents a valuable and more integrative tracer of N_2O sources and sinks that has been widely used to constrain N_2O budgets in soil[16,17], water[18,19] and the atmosphere[12,20]. The N_2O molecule has a total of twelve distinct isotopocules[21]; the four most abundant ones are: $^{14}N^{14}N^{16}O$, $^{14}N^{14}N^{18}O$, $^{14}N^{15}N^{16}O$ and $^{15}N^{14}N^{16}O$. The relative abundance of different N_2O isotopocules is usually reported in the conventional δ notation (‰):

$$\delta X = (R_{sample} - R_{standard}) / R_{standard} \quad (1)$$

where "X" refers to the rare isotopocule ($^{14}N^{14}N^{18}O$ (abbreviated as "^{18}O"), $^{14}N^{15}N^{16}O$ ($^{15}N^{\alpha}$, central) and $^{15}N^{14}N^{16}O$ ($^{15}N^{\beta}$, terminal)), and "R" refers to the ratio between the amount fraction of the rare isotopocule and that of the most abundant isotopocule $^{14}N^{14}N^{16}O$ in a "sample" or measurement "standard", respectively. Standards are defined on an international isotope ratio scale: Air-N_2 for $^{15}N/^{14}N$ and Vienna Standard Mean Ocean Water (VSMOW) for $^{18}O/^{16}O$. The average of $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ is usually referred to as $\delta^{15}N^{bulk}$ and the difference between $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ (i.e. $\delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$) is commonly called "site preference (SP)" or denoted as "$\delta^{15}N^{SP}$" in this review[10].

The fast increase in the number of N_2O isotopic studies in the last few decades is directly related to the rapid developments in analytical capacities to measure isotope ratios, using isotope-ratio mass spectrometry (IRMS), and more recently, laser absorption spectroscopy (LAS). Both techniques offer complementary strengths (and weaknesses); for example, while IRMS can achieve impressive analytical precision at very low (discrete) sample volume levels, LAS provides the potential for selective analysis of individual N_2O isotopocules (even with similar or same molecular mass, e.g. $^{15}N^{14}N^{16}O$ vs. $^{14}N^{15}N^{16}O$) and real-time data coverage. Despite the extensive application of N_2O isotopic analyses in environmental studies, there are still issues to be addressed, such as data comparability across laboratories. Mohn et al.[22] compared isotopic measurements of N_2O at tropospheric mole fractions from eleven laboratories with both IRMS and LAS techniques,

and detected large deviations (up to 10‰) in $\delta^{15}N^{SP}$ measurements. This finding raises questions regarding the comparability of results, and in turn the usability of isotope data determined in different laboratories and with different stable-isotope techniques to assess N_2O source partitioning.

The natural abundance of N_2O isotopocules is useful for quantifying N_2O sources and reaction pathways if they are isotopically distinct[23]. A large portion of biological N_2O production occurs as an obligatory intermediate of denitrification, during nitric oxide (NO) reduction by nitric oxide reductase (NOR) and as a by-product of nitrification, during enzymatic oxidation of hydroxylamine (NH_2OH) to nitrite (NO_2^-) catalyzed by hydroxylamine oxidoreductase (HAO)[24]. Besides these well-known pathways, other sources of N_2O have been described, including heterotrophic nitrification of organic N[13], codenitrification[25], nitrifier denitrification[26], dissimilatory nitrate reduction to ammonia (DNRA)[27], and chemodenitrification[28]. In addition, N_2O is formed as a by-product of chemical industry, coal burning and transport, contributing significantly to anthropogenic N_2O emissions[29]. The isotopic composition of product N_2O has been related to isotopic discrimination by the involved enzymes, controlled by the structure of the reaction intermediates such as hyponitrous acid (HONNOH) or its mono-anion ($HONNO^-$)[30]. Consequently, $\delta^{15}N^{SP}$ is a unique indicator that can distinguish between different enzymatic pathways, e.g. hydroxylamine oxidation and denitrification, during N_2O production, regardless of the isotopic signature of the substrate[31,32]. In the final step of the denitrification process, i.e. the reduction of N_2O to N_2, $^{15}N^{\alpha}$ and ^{18}O in the residual N_2O become progressively enriched, as reduction of the $^{15}N^{14}N^{16}O$ and $^{14}N^{14}N^{16}O$ isotopocules is favored[33]. Therefore, the kinetic isotopic fractionation associated with the reduction of N_2O results in elevated $\delta^{15}N^{bulk}$, $\delta^{18}O$ and $\delta^{15}N^{SP}$. Based on the magnitude of isotope fractionation during N_2O reduction, one can gain additional constraints for estimating the N_2 flux[34], which is otherwise not directly measureable[10]. Isotopic

fractionation effects during consumption of N_2O must be considered when evaluating overall microbial N_2O budgets[21], e.g. with the "isotope mapping" approach which employs dual isotope plots to constrain N_2O reduction progress and endmember mixing ratios[10,17,35,36]. Similarly, N_2O destruction in the stratosphere by photolysis results in strong ^{15}N and ^{18}O enrichment, which is the key to 'top down' analysis of N_2O sources based on isotope budgets[37]. Further evidence on photolytic N_2O destruction can be obtained from clumped isotope analysis[38]. It is also noteworthy that field-derived N_2O isotopic signatures may significantly deviate from theoretical predictions or pure enzymatic studies, in response to mixing processes[39], diffusion limitation[40] and reaction kinetics[41].

In order to access the complex source information contained in the isotopic composition of N_2O, a number of mathematical data-analyses and modelling approaches have been utilized[23,42,43]. For example, given the higher $\delta^{15}N^{bulk}$ and $\delta^{18}O$ in marine compared to continental N_2O sources[44], Snider et al.[23] applied a Bayesian isotope mixing model to partition the global contribution of N_2O emitted from these sources to tropospheric N_2O. Isotopocule measurements of N_2O from individual laboratory and field studies are often limited by spatial and temporal coverage, thus requiring upscaling to obtain regional information of N_2O emission sources. To disentangle the complexity of N cycling, a stable isotope model for nutrient cycles (SIMONE), has been coupled with a process-based biogeochemical model (DNDC), to simulate the isotopic composition of N_2O emitted from an intensively managed grassland site[43]. Given the increasing availability of high-frequency N_2O isotope datasets, modelling approaches like this are expected to make use of such datasets to address weaknesses in the model parameterization of the N cycle, and ultimately contribute to the development of model-based strategies for mitigating N pollution. Moreover, at the global scale, ambient atmospheric measurements of N_2O isotopocules are often integrated in

Formatted: English (United States)

Formatted: English (United States)

154 atmospheric transport models to assess global N_2O sources[11,37]. Past studies of long-term trends in
155 $\delta^{15}N$-N_2O in the troposphere suggest that anthropogenic sources releasing ^{15}N-depleted N_2O are
156 mainly responsible for the observed increases in N_2O since the 1940s[45]. However, current studies
157 have not yet managed to apportion anthropogenic N_2O source categories in more details at the
158 global scale or resolve causes of variability in both space and time. This can partly be attributed to
159 restrictions in atmospheric N_2O isotopocule measurements (precision and spatiotemporal
160 coverage)[11] as well as limitations regarding our understanding of the N_2O isotopic signatures of
161 major environmental sources[46].

162 Thus, a major aim of this article is to provide a general overview on the state-of-art in analytics,
163 production and destruction processes, as well as interpretation and modelling techniques as related
164 to natural abundance N_2O isotope research. For each of these three major topics, we will illustrate
165 current research activities and provide recommendations for future work. Ideas and concepts
166 presented here are based on the discussions held at a workshop in October 2019 at Empa
167 (Dübendorf, Switzerland).

168

2 Analytics

N_2O isotopic measurements are mainly performed with IRMS and LAS techniques. Given their specific detection schemes, these two techniques, based on fundamentally different principles, offer different strengths and weaknesses, which makes them particularly suited for certain applications (Fig. 1).

IRMS is based on the separation of ionized and accelerated molecules with different mass-to-charge (m/z) ratios in an electromagnetic field and subsequent detection of the separated ions. It can be used to distinguish between different N_2O isotopologues of different bulk mass ($\delta^{15}N^{bulk}$, $\delta^{18}O$). It can also provide site-specific isotopic information ($\delta^{15}N^{SP}$), based on the combined m/z analysis of the N_2O^+ molecular and the NO^+ fragment ions (^{15}N in the central position only)[47]. However, as $\delta^{15}N^{SP}$ is quantified indirectly by measuring $\delta^{15}N^{bulk}$ (via N_2O^+) and $\delta^{15}N^{\alpha}$ (via NO^+), the analytical error of both propagates to $\delta^{15}N^{SP}$, making it challenging to obtain high accuracy within the compatibility goals between laboratories as suggested by Mohn et al.[22] (see example of Monte Carlo simulation in Supporting Information). Moreover, for accurate analyses, gases with similar mass (e.g. CO_2) have to be removed and because some of the N_2O isotopocules are identical or nearly identical in mass (e.g. $^{15}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$ and $^{14}N^{14}N^{17}O$) overlap must be corrected for assuming a mass dependent relationship between ^{17}O and ^{18}O in the reference and the sample gases. In addition, the rearrangement of N atoms between central and terminal position during ionization in the IRMS ion source, called "^{15}N scrambling" or "rearrangement", has to be quantified and corrected for[47,48]. Thus, obtaining accurate isotope data by IRMS requires that the magnitude of scrambling be determined *apriori* and involves mass overlap corrections that introduce uncertainty[48]. If, however, two or more well characterized isotope standards are analyzed together with samples, then measured isotope values can be directly calibrated against the expected

192 standard isotope values using standard bracketing, without the need of instrument-specific
193 corrections for rearrangement or mass overlap[36]. As international N_2O standards continue to be
194 developed[49] this calibration approach will become more viable.

195 The LAS technique enables selective analysis of N_2O isotopocules based on their characteristic
196 rotational-vibrational spectra. The scanning range of the laser light source can be tailored to cover
197 rotational lines of multiple isotopocules of interest. Its ability to differentiate molecules with the
198 same mass (e.g. $^{15}N^{14}N^{16}O$ vs. $^{14}N^{15}N^{16}O$) gives a more direct measure of $\delta^{15}N^{SP}$ than mass
199 spectrometric techniques. LAS is also suitable for online measurements and thus deployable for *in*
200 *situ* experiments. However, direct analysis of the sample gas without pretreatment can cause
201 deviations in the apparent instrument output, *ergo* incorrect isotopic results. These errors are
202 derived from unresolved spectral lines of other trace gases (so called "spectral interference" from
203 H_2O, CO_2, CO, CH_4, etc.), differences in pressure broadening due to a changing composition of
204 the main gas components (e.g. O_2 / N_2 ratio; termed "matrix effects"[50]) and differences in the N_2O
205 mole fractions ("concentration effect"). In a recent study Harris et al.[51] compared N_2O isotope laser
206 spectrometers with the three most common detection schemes (cavity ringdown spectroscopy, off-
207 axis integrated cavity output spectroscopy and quantum cascade laser absorption spectroscopy)
208 and found that the trace gas and gas matrix effects on N_2O isotopocule measurements were
209 analyzer-specific, and had the potential to produce erroneous results. To avoid these errors, Harris
210 et al.[51] proposed a standardized analytical workflow, aiming to minimize the compositional
211 difference between sample and reference gases following the identical treatment (IT) principle.
212 This workflow includes the implementation of scrubbers when appropriate (e.g. H_2O, CO_2, CO),
213 as well as the use of derived correction functions for interferants which cannot be removed
214 efficiently (e.g. O_2, CH_4) and for N_2O mole fraction dependence. However, for gas mixtures with

highly variable composition, this correction procedure becomes significantly more complicated due to the coexistence and interplay of multiple effects. Thus, for complex and/or highly variable gas mixtures LAS might not be suitable without assimilation of the sample gas composition (e.g. preconcentration).

Although both IRMS and LAS techniques have been applied in a wide range of studies, we attempt here to make recommendations regarding the "most suitable" sampling design and instrumental choice for particular applications at different scales (Fig. 1).

Incubation or process-scale studies include laboratory-based as well as field investigations. During laboratory incubations, N_2O mole fractions are usually high (ppm to ppt levels), and the variability of N_2O isotope ratios is often large (up to 100 ‰)[10,32]. However, gas samples collected from batch incubations, e.g. headspace of closed containers or dissolved gas in water samples, come often in small volumes and likely exhibit strong differences in trace gas concentrations or even the matrix gas. Given that IRMS has much smaller sample requirements than laser-based methods and coupling to gas chromatography (GC) allows effectively normalizing the gas composition, GC-IRMS may be a more practical method for incubation studies in particular when high precision is desired (typically around 0.5 ‰; can be improved to 0.1 ‰ or better with dual inlet analysis[21]). On the other hand, if real-time data is desirable in a flow-through setup with significant net N_2O production, on-line analysis by LAS may be the method of choice, for example in waste water treatment plants where real-time isotopic analysis is strongly necessary to follow process changes over time[52]. Care should be taken, however, to adjust the gas composition (gas matrix, trace gas concentrations) of the standard gases to match those of the sample gas, and to limit changes in the sample gas composition by purification (dehumidification, CO_2 removal), or more rigorously preconcentration. The effects of remaining variation in the sample and standard gas compositions

should be considered and, if necessary, recorded and corrections applied to data using pre-defined algorithms.

In plot-scale studies, e.g. studies of *in situ* soil N_2O emissions, N_2O mole fractions typically change within the range between ambient levels (330 ppb) and up to a few ppm[11,45,53]. The associated changes in N_2O isotope ratios are usually up to several per mille. Depending on the experimental design and research question, the study period might focus on singular events, episodic events or continuous monitoring. Particularly for the latter, online measurement of N_2O isotopocules with LAS is an attractive option. The major advantage of this approach, in combination with automation, is the possibility of delivering high-resolution spatial and temporal sampling with much reduced labor efforts compared to discrete sample collection[54]. Additional concerns may, however, arise during online measurements with LAS. First, dynamic changes in N_2O and trace gas mole fractions may affect analytical results and need to be corrected. This is particularly important in highly dynamic systems, e.g. during chamber measurements of soil emission fluxes, with episodic peaks in N_2O fluxes that are one or two orders of magnitudes higher than the baseline, making it challenging to ensure analytical quality for both baseline and peak scenarios[53,55]. Ibraim et al.[55] implemented a preconcentration unit interfaced to LAS for isotope specific analysis of soil emitted N_2O in static flux chambers. Despite the improvement with regards to measurement precision and circumvention of gas matrix, trace gas and N_2O mole fraction effects, the use of the preconcentration system significantly reduced the maximum sampling frequency. Moreover, fluctuations of the environmental conditions (e.g. mobile lab temperature) can cause significant instrumental drifts during long-term measurements[51], thus requiring temperature stabilization or air conditioning[55].

Long-term monitoring in the unpolluted atmosphere indicates an increase in N_2O mole fractions by approx. 1.0 ppb N_2O yr^{-1}, and seasonal fluctuations around 0.5 ppb, with a maximum in early summer and a minimum in late summer (in the Northern hemisphere)[11,45]. Associated changes in N_2O isotope ratios are around -0.05 ‰ yr^{-1} for $\delta^{15}N^{bulk}$, whereas trends in $\delta^{15}N^{SP}$ and $\delta^{18}O$ are less evident. In order to resolve these subtle changes in N_2O isotope values in the background atmosphere (< 0.1 ‰), measurements over extended time periods are prerequisite; more importantly, it is necessary to utilize isotopic instruments that can achieve analytical precisions for singular measurements better than 0.2 ‰[56] and long-term drifts under 0.5 ‰[11]. Recent work has demonstrated that direct measurements of N_2O isotopes at ambient levels with LAS (e.g. cavity ringdown spectroscopy; Picarro Inc., CA, USA) can generally reach a precision better than 0.5 ‰[51]. Nevertheless, for precise and robust measurements of N_2O isotope ratios in the ambient atmosphere, coupling a preconcentration device to either an IRMS or a LAS instrument is still recommended.

To ensure the accuracy of N_2O isotope results and compatibility between laboratories for both IRMS and LAS techniques, laboratory-standards must be related to the respective international scales, Air-N_2 for $^{15}N/^{14}N$ and VSMOW for $^{18}O/^{16}O$. For $\delta^{15}N^{bulk}$ and $\delta^{18}O$, such a link (i.e. normalization against international standards) can be accomplished by N_2O reduction to N_2 or thermal decomposition into N_2 and O_2 and subsequent IRMS analysis of $\delta^{15}N$ and $\delta^{18}O$ of the product gases, respectively[39]. For $\delta^{15}N^{SP}$, thermal decomposition of isotopically characterized NH_4NO_3, with known $\delta^{15}N\text{-}NH_4^+$ and $\delta^{15}N\text{-}NO_3^-$, enables connections of $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ via $\delta^{15}N\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ to the Air-N_2 scale[57]. The concept of this approach is based on the assumption that the N atom at the central position is derived from the precursor nitrate, while the N atom at the distal position in the N_2O molecule originates from the ammonium. The

decomposition reaction and isotopic assessment, however, is complicated by incomplete conversion and site-specific fractionation and has, therefore, been only implemented in very few laboratories. To avoid the transfer of calibration standards from one lab to another, which is discouraged by Global Atmosphere Watch (GAW-World Meteorological Organization)[58], the recent release of gaseous N_2O reference materials, USGS51 and 52 (U.S. Geological Survey, VA, USA)[49], represents a significant step forward in our ongoing efforts to improve inter-laboratory comparability. Preliminary isotopic compositions of the USGS51 and USGS52 revealed relatively large differences between the two standard materials with regards for $\delta^{15}N^{SP}$, but not so much for $\delta^{15}N^{bulk}$ and $\delta^{18}O$. To implement a two-point isotope calibration also for the bulk parameters, however, additional gases with differences in $\delta^{15}N^{bulk}$ and $\delta^{18}O$ are required, and will likely become available within the frame of the ongoing European metrology project SIRS[59]. These primary N_2O reference materials can then be applied to establish secondary N_2O isotope laboratory standards with similar N_2O and trace gas mole fractions and matrix gases as the sample gas, considering the "identical treatment principle"[22,49]. Ideally, such standards need to bracket the range in isotope values observed in the environment of interest to facilitate improved accuracy.

Despite the progress in measurement techniques and the availability of international reference materials, additional efforts are required to improve the quality of N_2O isotopic data. For individual laboratories using LAS, we strongly recommend developing and applying appropriate calibration and correction algorithms to account for differences in trace gas concentrations / gas matrix between sample and reference gases. As IRMS analysis commonly involves preconcentration, matching sample and standard gas composition is not essential; however, the scrambling and mass overlap corrections used often differ between laboratories and, ideally, a single set of corrections would be used across laboratories. Alternatively, analysis of isotope standards within the batch of

306 samples can be performed to avoid the need for scrambling and mass overlap corrections[36]. For
307 both LAS and IRMS we suggest to include one or more target gases into the analytical routine to
308 evaluate instrumental performance. In addition, more inter-laboratory comparisons would assure
309 accuracy between individual laboratories[60]. The further developments of both high-resolution
310 IRMS and LAS techniques for measuring doubly substituted, or so-called clumped N_2O isotopes
311 (e.g. $^{15}N^{15}N^{16}O$, $^{15}N^{14}N^{18}O$ and $^{14}N^{15}N^{18}O$)[61,62], will present a great opportunity to expand the
312 isotopic dimensions to better understand biogeochemical N_2O cycling, but will also be a great
313 challenge that requires even more complex analytical procedures and calibration strategies.
314

3 Processes

An understanding of processes producing or consuming N_2O can be revealed by the isotopic composition of N_2O and applied across wide spatiotemporal scales (Figure 2). Molecular mechanisms determine the isotopic discrimination for individual reactions, which are integrated based on rate-limiting steps to produce a net isotopic effect for a reaction chain ("pathway"), such as N_2O production during denitrification. Mixing of N_2O derived from multiple sources and isotope effects associated with variable production and consumption pathways determines the N_2O isotopic composition at plot, ecosystem site and biome scales, seasonally and intra-annually. Ultimately, these factors drive changes in the atmospheric N_2O mixing ratio and isotopic composition that are the basis for quantifying regional to global budgets on various timescales. In this section, an overview of the key advances and open questions regarding N_2O processes is presented in the context of isotopic studies.

Molecular mechanisms determine the inherent isotopic discrimination of reaction steps, bringing together the fields of computational and physical chemistry and isotope biogeochemistry. For example, the reduction of NO to N_2O by the membrane-bound nitric oxide reductase enzyme (NOR), which is a key step in microbial denitrification, is responsible for the formation of ^{15}N site preference in the resultant N_2O[63]. Blomberg et al.[64,65] used hybrid density functional theory to support a *cis* mechanism for this reaction. In this model, the hyponitrite intermediate binds with one N atom to the heme iron and both oxygen atoms to the non-heme iron of the NOR enzyme. However, the contrasting *trans* mechanism, in which the hyponitrite intermediate coordinates to each iron center with one N atom, has been thought to be more consistent with the observed low $\delta^{15}N^{SP}$ of N_2O produced from denitrification[66]. Reconciling these results as well as other computational and experimental observations will be key in understanding the enzymatic

mechanism of N_2O formation. Furthermore, this research paves the way for the use of novel tracers, such as clumped isotope signatures (e.g. $^{15}N^{14}N^{18}O$ and $^{14}N^{15}N^{18}O$) to decode formation mechanisms and quantify N_2O production pathways. In fact, this will be a central development as the clumped isotope "fingerprint" will provide know-how on the magnitude of reaction reversibility, an additional source of mechanistic information. Another important N_2O pathway is nitrification, which produces N_2O as a side product during the oxidation of hydroxylamine to NO_2^- under the catalysis of hydroxylamine oxidoreductase (HAO). This pathway results in consistently 32-35 ‰ higher $\delta^{15}N^{SP}$ than associated with denitrification[17,31].

While nitrification and denitrification (heterotrophic, nitrifier, and fungal) are relatively well-described pathways included in most process models, the contributions of pathways such as codenitrification[25] and chemodenitrification[67] are mostly overlooked but have received increased attention in recent years. Codenitrification is a microbial pathway whereby one N from NO_2^- or NO combines with an N atom from another species, particularly organic N, to form N_2O or N_2 by N-nitrosation[25]. The resultant N_2O and N_2 are termed "hybrid" as their N atoms arise from two different substrates, which makes codenitrification particularly suited for investigations using isotopic labelling approaches[68]. Chemodenitrification – the abiotic production of N_2O, particularly from NH_2OH, NO_2^- and soil organic matter – has been identified as a significant source of N_2O, which could contribute vastly to N_2O emissions, particularly in anoxic and acidic environments where NO_2^- can actively participate in a number of abiotic reactions[69]. The $\delta^{15}N^{SP}$ of N_2O resulting from chemodenitrification appears to be highly variable, ranging from -4 to 37‰[28,32,67,69–72] depending on soil pH, redox conditions as well as the specific reaction substrates and pathways. Thus, chemodenitrification presents a significant challenge when trying to assess the partitioning of N_2O production pathways based on $\delta^{15}N^{SP}$ endmember values alone. However, understanding

361 the drivers of the relative fluxes and isotopic variability of both codenitrification and
362 chemodenitrification will facilitate the incorporation of these pathways into process and isotope
363 models to constrain their overall contribution to N_2O budgets, which may be particularly important
364 in systems with elevated NO_2^- concentrations. In addition, the consumption process of N_2O, i.e.
365 reduction to N_2, is mediated by N_2O reductase (N2OR), and it is sensitive to pH[73] and O_2 levels in
366 the environment. The isotope effects during N_2O reduction provide information for quantifying
367 N_2O sink strength while at the same time complicating isotope measurement-based N_2O source
368 partitioning[34].

369 In practice, biogeochemical N_2O emission pathways represent multistep processes, where each
370 reaction step is mediated by a different enzyme and, consequently, associated with individual
371 characteristic isotopic fractionation. Denitrification, for example, consists of a series of steps that
372 involves diffusion of nitrate into the microbial cell followed by the sequential reduction to nitrite,
373 nitric oxide, N_2O and finally N_2. The net isotope effects (η) for such a multistep process is a result
374 of several isotopic effects associated with the successive enzymatic reactions, respectively, as well
375 as physical processes like, e.g., substrate transport, adsorption, and formation of substrate-enzyme
376 complexes. Hence, while the intrinsic isotopic effects may be stable and characteristic for a
377 particular process the net isotope effects integrate over a process chain and therefore may differ
378 due to changes in e.g., environmental conditions, process rates and/or substrate availability and
379 diffusion limitation at various scales[74,75].

380 Moving one step up on the spatiotemporal scale, considering the variety of pathways contributing
381 to N_2O production and consumption at site to regional scales has revealed the importance of
382 spatiotemporal heterogeneity as well as non-linear responses to the drivers at work. For example,
383 soil moisture is a key parameter regulating N_2O emission pathways, and is often used in models

as the primary driver of N-gas emissions[76,77]. Although these simple parameterization methods provide the first step in constraining emission processes, recent results highlight the importance of also considering other drivers in models, such as pH regulation of nitrification[77] and N_2O reduction, microbial biomass and land use history, and substrate mobilization and availability[36,78]. The N_2O emissions during "hot moments" and from "hot spots" in the environment is also increasingly recognized as playing a major role in annual and regional N_2O budgets, but their controls are particularly challenging to understand in full complexity, and thus difficult to model[79–82]. Using natural abundance and isotope labelling approaches to gain a mechanistic understanding of the response of N_2O transformation pathways to the most important drivers will be key to improve models and allow predictions of the N_2O budget in heterogeneous environments, in particular in the context of a changing climate.

Quantification of N_2O fluxes and budgets in less studied regions such as the world's oceans in general, the Arctic, the tropics, and the stratosphere is improving rapidly as instrumental developments facilitate isotopic field studies (e.g. analyses of background air at remote sites, and in low concentration water samples). Toyoda et al.[18] used vertical N_2O isotope ratio profiles to examine the source of the ubiquitous N_2O concentration maxima at 100-800 m water depth across the world's oceans, and demonstrated the importance of *in situ* production by ammonia-oxidizing archaea (AOA) as well as nitrifier denitrification and bacterial nitrification, rather than lateral diffusion or advection of N_2O carrying waters from nearby ocean regions. Similarly, in the Peruvian coastal upwelling system, *in situ* N_2O production was observed and mainly attributed to denitrification, based on the low $\delta^{15}N^{SP}$ values[83]. High N_2O emissions in Arctic peat soils were also linked to nitrification by AOAs, although highly variable $\delta^{15}N^{SP}$ values suggest the contributions of several production pathways with high temporal variability[84,85].

In contrast to the multiple complex formation mechanisms, removal of nitrous oxide from the atmosphere is very straightforward; 90% is photolysed by UV light in the stratosphere[37]. There is thus an effort to apply mass balance arguments to constrain the sources[23]. If the atmospheric composition is known and the strength and isotopic bias of the photolytic sink, a picture of the isotopically distinct sources emerges, especially if multiple isotopocules are considered. Isotopic enrichment in ^{15}N and ^{18}O, increasing $\delta^{15}N^{SP}$, as well as "mass independent" oxygen isotope fractionation ($\Delta^{17}O$) (e.g. Kaiser et al.[86] and references therein) in the stratosphere has played a major role in constraining the dominant N_2O destruction process in the global budget. Recent work by Schmidt and Johnson[38] extends previous studies by including clumped N_2O isotopocules, which provide further constraints on stratospheric destruction by UV photolysis, and potentially lead to more accurate quantification of stratosphere-troposphere exchange and its response to a changing climate.

4 Interpretation and modelling

The interpretation of N_2O isotope data is complex and challenging as numerous processes govern the isotopic signature of N_2O. Although there are twelve isotopocules of N_2O, providing a wealth of interpretation perspectives, three isotopic characteristics representing singly substituted isotopocules ($\delta^{18}O$, $\delta^{15}N^{bulk}$ and $\delta^{15}N^{SP}$) are most commonly analyzed. $\delta^{15}N^{SP}$, is a unique natural isotope tracer, which only depends on the mechanisms and pathways of N_2O formation[31] and isotope effects during N_2O reduction[33,40], but unlike $\delta^{18}O$ and $\delta^{15}N^{bulk}$, is independent of substrate isotopic signature and remains unchanged during N_2O diffusion[33,40] (see Supporting Information for more details on isotopic fractionation during N_2O diffusion). Nevertheless, with only $\delta^{15}N^{SP}$, quantification of the complex N_2O production and consumption processes cannot be fully achieved[10]. Distinguishing between the isotope variations due to mixing of different N_2O production pathways on the one end and N_2O consumption on the other is especially problematic. Precise quantification of both, the single production processes and the extent of N_2O reduction, is challenging due to wide ranges of isotopic signatures reported for individual processes, the overlapping of these isotopic signature ranges, variability of fractionation factors associated with N_2O reduction[39] (Fig. 3 and Supporting Information), and limitations in isotopic analytics (see Section 2).

A common interpretation strategy used to determine the origin of N_2O is to create dual isotope plots, also known as "isotope mapping" approaches, presenting the relationship between two isotopic parameters: $\delta^{18}O$ / $\delta^{15}N^{bulk}$, $\delta^{15}N^{SP}$ / $\delta^{15}N^{bulk}$ or $\delta^{15}N^{SP}$ / $\delta^{18}O$[10,87–90] (Fig. 3). With such plots, we can constrain the probable dominance of specific pathways, or importance of isotope fractionation during N_2O reduction. This approach is dependent on characteristic isotopic signatures associated with particular production pathways obtained from pure culture studies and

443 experimentally determined fractionation factors for N_2O reduction, which result in characteristic
444 reduction slopes between corresponding delta values (see Fig. 3 and Supporting Information for
445 detailed values). The interplay between N_2O production and reduction can occur in a number of
446 different ways including: i) N_2O produced from bacterial denitrification is first partially reduced
447 to N_2, followed by mixing of the residual N_2O with N_2O from other pathways, ii) N_2O produced
448 by various pathways is first mixed and afterwards reduced, or iii) a continuum between these two
449 scenarios occurs depending on environment and microclimate conditions. Recent studies suggest
450 the first scenario to be more realistic[36,55,88], as it is likely that N_2O produced by denitrification in
451 anoxic microsites will be further reduced under these conditions. However, a certain portion of
452 N_2O derived by fungal denitrification or nitrifier denitrification might be subsequently reduced by
453 denitrifying bacteria. Reduction of N_2O from nitrification is less likely as it is produced at domains
454 more enriched in oxygen. Regarding partial N_2O reduction to N_2, it is questionable whether open
455 or closed system dynamics should be applied for modelling its isotope effect[34]. If a steady state is
456 assumed, the N_2O pool is constantly renewed, implying open system dynamics. However, in
457 multiple soil studies, isotopic results revealed logarithmic relationships between $\delta^{15}N^{SP}$ and N_2O
458 concentration, which is typical of closed-system (or Rayleigh-type) dynamics[10]. In fact, both
459 scenarios may coexist depending on the balance between N_2O production and reduction, as well
460 as the soil properties influencing gas diffusion[41].

461 A further challenge for interpretation of N_2O isotope data is the knowledge of the isotopic signature
462 of the N and O precursors. Depending on the production pathway, the primary N precursors might
463 be NO_3^- for denitrification or NH_4^+ for nitrification and nitrifier denitrification. In addition, the
464 bulk isotopic composition of the N precursor might not be representative for the actually utilized
465 N substrate pool. Particularly in soils, where the soil matrix can be markedly heterogeneous and

"hotspots" of denitrification can occur in isolated anoxic soil microsites[82], NO_3^- near and in the reactive zones may be strongly enriched in ^{15}N compared to the bulk soil[41,91] and may also be derived from various soil N pools including organic and mineral N[92]. Similarly, in the case of nitrate consumption at strong redox gradients in the ocean and in lakes, most denitrifying activity is localized where the $\delta^{15}N$ of the nitrate pool has already been elevated. In the case of nearly complete substrate consumption within suboxic zones of the water column and/or sediments, the associated apparent isotope effect may be much lower[93,94].

The O isotopic composition of N_2O mainly depends on: 1) $\delta^{18}O$ of the precursor compounds (e.g. NO_3^-/NO_2^- for denitrification), $\delta^{18}O$ of O_2 incorporated during ammonium/hydroxylamine oxidation, $\delta^{18}O$ of H_2O incorporated during O exchange between denitrification intermediates and H_2O[95], and 2) any given O isotope fractionation effect associated to the respective N_2O formation mode. Based on the large differences in $\delta^{18}O$ of the direct and the indirect precursor compounds observed in natural environments (e.g. the ocean water column: $\delta^{18}O_{O2} \geq 23.5$ ‰, $\delta^{18}O_{H2O} = \sim 0$‰, $\delta^{18}O_{NO3} = 0\text{-}30$ ‰[96], $\delta^{18}O_{NO2} = -50\text{-}20$ ‰), the $\delta^{18}O_{N2O}$ can be used to determine the predominant substrate during N_2O production and in turn provides clues on the formation pathways[97,98]. Moreover, $\delta^{18}O_{N2O}$ is potentially a good tracer for distinguishing bacterial versus fungal denitrification. Although both processes exhibit nearly complete O-exchange with ambient water, fungal N_2O is commonly characterized by significantly higher $\delta^{18}O_{N2O}$ due to a larger branching isotope effect for fractional oxygen loss during reduction of nitrate to N_2O[99]. However, variation in O-exchange rates can complicate the interpretation of $\delta^{18}O_{N2O}$ values. For instance, oxygen exchange between NO_3^- and H_2O during denitrification might not be complete under particular soil conditions that are, for example, conducive to rapid turnover[41,42], and certainly not all bacterial strains show complete O-exchange with water[100].

Natural abundance isotope studies are especially suited for natural systems, as they can readily be applied across broad spatial and temporal scales, which can be prohibitive to alternative methods such as tracer applications. Dual isotope plots are often used to provide quantitative estimates on process contributions and reduction progress[36,55,88], however such estimates are associated with numerous limitations. When applying $\delta^{15}N^{bulk}$ values, the proper normalization to the precursor isotopic signatures is challenging due to multiple possible N sources (NH_4^+, NO_3^-, etc.) and variable fractionation effects. In such graphs the mixing endmember values should be expressed in relation to the respective substrate, and for the actual sample data points, the uncorrected real $\delta^{15}N^{bulk}$ should be presented (Fig. 3)[87,89]. Recent studies show quite stable $\delta^{18}O$ isotopic signatures with respect to bacterial and fungal denitrification[99,100], suggesting that the $\delta^{15}N^{SP}$ / $\delta^{18}O$ plot may offer a more promising and accurate approach for process quantification[10]. On the other hand, a recent compilation of model results integrating archival datasets revealed a relatively large uncertainty of N_2O reduction estimates, when the whole spectrum of available literature ranges of endmember isotopic signatures and fractionation factors for N_2O reduction is considered[42]. The model outcomes can be improved if soil-specific (i.e. more constrained) isotopic effects and end-member N_2O isotopic signatures are employed. Yet, assignment of soil-specific isotope fractionation requires sophisticated laboratory approaches, representative measurements, and is thus time-consuming and challenging, in particular when addressing the spatial and temporal heterogeneity of an individual field site[88]. Uncertainties in revealing N_2O sources and the magnitude of reduction based on dual-isotope mapping are the results of variations in substrate isotopic compositions, variation in the expression of net isotope effects and an inability to fully constrain source isotope signatures (particularly, for example, for N_2O produced via chemodenitrification). Whereas, for these reasons, the approach should not be considered truly

512 quantitative, it can nevertheless reveal integrative insight into the origins and cycling of N_2O over
513 time and spatial scales difficult to obtain by other means.

514 Over small spatial or temporal scales, semi-quantitative estimates of the origins and reduction of
515 N_2O provided by isotope mapping may be strengthened by complementary isotope tracing
516 techniques. For example, the ^{15}N labeling "N-trace" model[13] is applied to investigate the fate of
517 applied ^{15}N-enriched nitrate and ammonium in soil micro-plots. Based on the assumption of
518 various soil nitrite pools, the model can quantify rates of production for four N_2O forming
519 pathways: nitrification, denitrification, codenitrification and heterotrophic nitrification.
520 Application of dual isotope labelling (^{15}N, ^{18}O) may provide additional information on the
521 importance of nitrifier denitrification[24]. Recent tracing studies revealed that N_2O production is
522 associated with organic N turnover in many soils, and heterotrophic nitrification often plays a
523 dominant role in N_2O emission[13]. This process has not been evaluated in natural abundance isotope
524 studies so far. Isotope labelling is also a unique method to distinguish hybrid N_2O and N_2
525 production[25] (see section 3). Ideally, labelling methods can be combined with natural abundance
526 studies of N_2O and its precursors; the latter can provide a first semi-quantitative understanding of
527 N_2O production and reduction over large spatial and temporal scales, which can then be supported
528 by more definitive results of isotope tracer studies applied at small scales.

529 Evaluation of N_2O sources can be obtained by the introduction of natural abundance isotope data
530 into process-based biogeochemical models to reconcile measured and modelled N isotopic
531 compositions[43,101]. First attempts of including N isotope ratios into N cycling models comprised
532 the incorporation of soil $\delta^{15}N$ into the DAYCENT process-based model to determine gaseous
533 nitrogen losses from soil[101]. More recently, also N_2O isotopic signatures have been integrated as
534 additional model parameters, for example into the Landscape DNDC model (SIMONE - Stable

Isotope Model for Nutrient cyclEs), helping to reduce uncertainty in the estimates of ecosystems N fluxes[43]. So far SIMONE/LandscapeDNDC has demonstrated its capacity to constrain the dynamics of the N_2O isotopic composition ($\delta^{15}N^{bulk}$ and $\delta^{15}N^{SP}$) and precursors ($\delta^{15}N_{NO3-}$ and $\delta^{15}N_{NH4+}$) within a few European fertilized grasslands[43]. The model outputs have been interpreted jointly with dual isotope plots that suggested some model shortcomings, e.g. an underestimation of N_2O reduction or N immobilization. For further model development, more comprehensive field data are needed, regarding both, model inputs (e.g. distribution and heterogeneity of precursor isotopic composition in soils), and process parametrization in responses to changes in soil conditions. Also, for oceanic N cycling, complex 3-dimensional isotope models have been developed[102,103], but the N_2O isotopic species are not integrated into these models yet. The successful integration of N_2O isotopic signatures into models requires a comprehensive database of isotope effects with their uncertainty, which is still an ongoing effect[104].

N_2O isotopocule analyses are most valuable when complemented/supported by other techniques, such as ^{15}N tracing studies[105], molecular and microbiological methods[106], or the use of inhibitors to block specific N_2O production pathways in incubation experiments[107]. The combined application of all three isotopic parameters ($\delta^{15}N^{bulk}$, $\delta^{15}N^{SP}$, $\delta^{18}O$) coupled with substrate isotope analysis ($\delta^{15}N_{NO3-}$, $\delta^{15}N_{NH4+}$, $\delta^{18}O_{NO3-}$, $\delta^{18}O_{H2O}$) is encouraged as it provides a substantially stronger basis for data interpretation but has rarely been done (Fig. 3). The informative value of N_2O isotope data, for example in soils, is markedly increased by evaluating the data with a biogeochemical model providing independent process information. Current analytical developments (see Section 2) may enable datasets with better data quality, inter-laboratory comparability and superior spatiotemporal coverage, or may establish additional tracers (e.g. clumped isotopes) increasing our interpretative perspectives.

5 Conclusions and Perspective

Given the complexity of the N cycle, in N_2O isotope studies it is particularly important to tailor the analytical methods and interpretative approaches according to specific research questions and scales of study. IRMS is still the most widely established method to analyze N_2O isotope ratios and offers an impressive precision as low as to 0.01‰[22]. It is particularly suitable for laboratory-based measurements with limited sample size. LAS, on the other hand, is the method of choice for real-time isotopic measurements of N_2O during *in situ* studies and relatively high N_2O concentrations. Nevertheless, despite the increasing popularity of LAS, it is important to emphasize that measurements of N_2O isotopocules by LAS are conducted not more easily than with IRMS, as they require considerable efforts regarding calibration and corrections to guarantee the quality of isotopic results[51].

The interpretations of N_2O isotope data (e.g. source partitioning) depend on our understanding of the underlying N_2O production and consumption processes and associated isotope effects. Based on the empirical ranges of isotope effects associated with specific N_2O processes, many scientists have developed dual isotope plots, i.e. $\delta^{15}N^{SP}$ / $\delta^{15}N^{bulk}$ or $\delta^{15}N^{SP}$ / $\delta^{18}O$, to semi-quantitatively determine the contributions of variable processes[10,16]. Such approaches are based on end-member mixing considerations, and provide a simple method to analyze N_2O isotopic results; however, it is difficult to reach quantitative results, due to the uncertainties related to the complex interplay between co-occurring N_2O production and reduction processes[10,36], as well as the dependency of $\delta^{15}N^{bulk}$ or $\delta^{18}O$ on the isotopic signatures of different reaction substrates[16,97]. Current knowledge gaps regarding isotope effects from different N_2O processes (e.g. chemodenitrification[28]) further impede more robust assessment of N_2O sources and sinks with isotope data, not to mention the

uncertainty brought about by spatiotemporal heterogeneity of N_2O cycling in the natural environment.

Although current studies including natural abundance N_2O isotope measurements are still limited and mostly semi-quantitative, they provide a promising starting point to unravel the partitioning of N_2O production and consumption pathways across multiple scales. At local scales, the interpretation of N_2O isotope data can be significantly improved if supported by process- and location-specific information regarding substrates and isotope fractionation effects[42], as well as complementary use of ^{15}N labelling approaches to reduce the uncertainties in process partitioning through cross-validations between the two approaches. At broader spatiotemporal scales, a combination of natural abundance measurements and modelling approaches[43,105] will allow the spatial extrapolation of N_2O source and sink information obtained from individual studies to the regional or even global perspective. We anticipate that in the future, with the advanced development of analytical methods, a better understanding of processes and mechanisms, and further extension of data-analysis approaches, N_2O isotope techniques will be more and more effective in reliably identifying N_2O sources and sinks, providing important, and most probably more accurate constraints on N_2O budgets for the development of effective mitigation strategies.

Acknowledgements

We acknowledge financial support by the Swiss National Science Foundation within grant IZSEZ0_187601 and by the European Metrology Programme for Innovation and Research (EMPIR) 16ENV06 project “Metrology for Stable Isotope Reference Standards (SIRS)”. The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States. Longfei Yu was supported by supported by the Swiss National Science Foundation (SNSF) within grant 200021_163075 and the EMPAPOSTDOCS-II programme, which received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement number 10754364. Dominika Lewicka-Szczebak was supported by the grant of German Research Foundation (DFG LE 3367/1-1). Eliza Harris is funded by Austrian Science Foundation (FWF) project P31132. Matti Barthel was supported by ETH Zurich core funding provided to Johan Six. This work was funded in part by the U.S. DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-SC0018409) in support of Nathaniel Ostrom. The study was carried out in collaboration with the German science foundation (DFG) research unit FOR 2337 “Denitrification in Agricultural Soils: Integrated Control and Modeling at Various Scales (DASIM)”. In addition, we thank Dr. Reinhard Well for his feedback and suggestion on the manuscript especially regarding section 4.

Author contributions

LY, EH, DL and JM led and conceived the study. LY prepared a first version of the manuscript, with help of EH, DL and JM. LY, EH and DL were mainly responsible for sections 2, 3 and 4 (with Supplement), respectively. All other co-authors contributed during the revision process.

Reference

1. Ravishankara AR, Daniel JS, Portmann RW. Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science*. 2009;326(5949):123-125. doi:10.1126/science.1176985

2. Montzka SA, Dlugokencky EJ, Butler JH. Non-CO_2 greenhouse gases and climate change. *Nature*. 2011;476(7358):43-50. doi:10.1038/nature10322

3. Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos Trans R Soc Lond B Biol Sci*. 2013;368:20130122. doi:10.1098/rstb.2013.0122

4. Reay DS, Davidson EA, Smith KA, et al. Global agriculture and nitrous oxide emissions. *Nat Clim Chang*. 2012;2(6):410-416. doi:10.1038/nclimate1458

5. Cicerone RJ. Analysis of sources and sinks of atmospheric nitrous oxide (N_2O). *J Geophys Res*. 1989;94(D15):265-271. doi:10.1029/jd094id15p18265

6. Chapuis-Lardy L, Wrage N, Metay A, Chotte J-L, Bernoux M. Soils, a sink for N_2O? A review. *Glob Chang Biol*. 2007;13(1):1-17. doi:10.1111/j.1365-2486.2006.01280.x

7. Well R, Eschenbach W, Flessa H, von der Heide C, Weymann D. Are dual isotope and isotopomer ratios of N_2O useful indicators for N_2O turnover during denitrification in nitrate-contaminated aquifers? *Geochim Cosmochim Acta*. 2012;90:265-282. doi:10.1016/j.gca.2012.04.045

8. Duan H, Ye L, Erler D, Ni B-J, Yuan Z. Quantifying nitrous oxide production pathways in wastewater treatment systems using isotope technology – A critical review. *Water Res*. 2017;122:96-113. doi:10.1016/j.watres.2017.05.054

9. Yu L, Kang R, Mulder J, Zhu J, Dörsch P. Distinct fates of atmogenic NH_4^+ and NO_3^- in subtropical, N-saturated forest soils. *Biogeochemistry*. 2017;133(3):279-294. doi:10.1007/s10533-017-0332-y

10. Lewicka-Szczebak D, Augustin J, Giesemann A, Well R. Quantifying N_2O reduction to N_2 based on N_2O isotopocules – validation with independent methods (helium incubation and ^{15}N gas flux method). *Biogeosciences*. 2017;14(3):711-732. doi:10.5194/bg-14-711-2017

11. Toyoda S, Kuroki N, Yoshida N, Ishijima K, Tohjima Y, Machida T. Decadal time series of tropospheric abundance of N_2O isotopomers and isotopologues in the Northern Hemisphere obtained by the long-term observation at Hateruma Island, Japan. *J Geophys Res Atmos*. 2013;118(8):3369-3381. doi:10.1002/jgrd.50221

12. Yoshida N, Toyoda S. Constraining the atmospheric N_2O budget from intramolecular site preference in N_2O isotopomers. *Nature*. 2000;405(6784):330-334. doi:10.1038/35012558

13. Müller C, Laughlin RJ, Spott O, Rütting T. Quantification of N_2O emission pathways via a ^{15}N tracing model. *Soil Biol Biochem*. 2014;72(3):44-54. doi:10.1016/j.soilbio.2014.01.013

14. Wrage N, van Groenigen JW, Oenema O, Baggs EM. A novel dual-isotope labelling method for distinguishing between soil sources of N_2O. *Rapid Commun Mass Spectrom*. 2005;19(22):3298-3306. doi:10.1002/rcm.2191

15. Moser G, Gorenflo A, Brenzinger K, et al. Explaining the doubling of N_2O emissions under elevated CO_2 in the Giessen FACE via in-field ^{15}N tracing. *Glob Chang Biol*. 2018;24(9):3897-3910. doi:10.1111/gcb.14136

16. Koba K, Osaka K, Tobari Y, et al. Biogeochemistry of nitrous oxide in groundwater in a forested

ecosystem elucidated by nitrous oxide isotopomer measurements. *Geochim Cosmochim Acta*. 2009;73(11):3115-3133. doi:10.1016/j.gca.2009.03.022

17. Decock C, Six J. How reliable is the intramolecular distribution of ^{15}N in N_2O to source partition N_2O emitted from soil? *Soil Biol Biochem*. 2013;65(2):114-127. doi:10.1016/j.soilbio.2013.05.012

18. Toyoda S, Yoshida O, Yamagishi H, Fujii A, Yoshida N, Watanabe S. Identifying the origin of nitrous oxide dissolved in deep ocean by concentration and isotopocule analyses. *Sci Rep*. 2019;9(1):1-9. doi:10.1038/s41598-019-44224-0

19. Frame CH, Casciotti KL. Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. *Biogeosciences*. 2010;7(9):2695-2709. doi:10.5194/bg-7-2695-2010

20. Kato T, Toyoda S, Yoshida N, Tang Y, Wada E. Isotopomer and isotopologue signatures of N_2O produced in alpine ecosystems on the Qinghai-Tibetan Plateau. *Rapid Commun Mass Spectrom*. 2013;27(13):1517-1526. doi:10.1002/rcm.6595

21. Coplen TB. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Commun Mass Spectrom*. 2011;25(17):2538-2560. doi:10.1002/rcm.5129

22. Mohn J, Wolf B, Toyoda S, et al. Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: Current status and perspectives. *Rapid Commun Mass Spectrom*. 2014;28(18):1995-2007. doi:10.1002/rcm.6982

23. Snider DM, Venkiteswaran JJ, Schiff SL, Spoelstra J. From the ground up: Global nitrous oxide sources are constrained by stable isotope values. *PLoS One*. 2015;10(3):1-19. doi:10.1371/journal.pone.0118954

24. Wrage-Mönnig N, Horn MA, Well R, Müller C, Velthof G, Oenema O. The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biol Biochem*. 2018;123(April):A3-A16. doi:10.1016/j.soilbio.2018.03.020

25. Spott O, Russow R, Stange CF. Formation of hybrid N_2O and hybrid N_2 due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. *Soil Biol Biochem*. 2011;43(10):1995-2011. doi:10.1016/j.soilbio.2011.06.014

26. Wrage N, Velthof GL, Beusichem ML Van, Oenema O. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol Biochem*. 2001;33:1723-1732.

27. Sun Y, De Vos P, Willems A. Influence of nitrate and nitrite concentration on N_2O production via dissimilatory nitrate/nitrite reduction to ammonium in Bacillus paralicheniformis LMG 6934. *Microbiologyopen*. 2018;7(4). doi:10.1002/mbo3.592

28. Wei J, Ibraim E, Brüggemann N, Vereecken H, Mohn J. First real-time isotopic characterisation of N_2O from chemodenitrification. *Geochim Cosmochim Acta*. 2019;267:17-32. doi:10.1016/j.gca.2019.09.018

29. Davidson EA, Kanter D. Inventories and scenarios of nitrous oxide emissions. *Environ Res Lett*. 2014;9(10). doi:10.1088/1748-9326/9/10/105012

30. Fehling C. Mechanistic Insights from the ^{15}N-Site Preference of nitrous oxide utilizing high resolution near-infrared cw cavity ringdown spectroscopy and density functional theory calculations. *Diss "Dr rer.nat" Kiel Univ*. 2012.

31. Sutka RL, Ostrom NE, Ostrom PH, et al. Distinguishing Nitrous Oxide Production from Nitrification and Denitrification on the Basis of Isotopomer Abundances. *Appl Environ Microbiol*. 2006;72(1):638-644. doi:10.1128/AEM.72.1.638

32. Heil J, Wolf B, Brüggemann N, et al. Site-specific ^{15}N isotopic signatures of abiotically produced N_2O. *Geochim Cosmochim Acta*. 2014;139:72-82. doi:10.1016/j.gca.2014.04.037

33. Lewicka-Szczebak D, Well R, Köster JR, et al. Experimental determinations of isotopic fractionation factors associated with N_2O production and reduction during denitrification in soils. *Geochim Cosmochim Acta*. 2014;134:55-73. doi:10.1016/j.gca.2014.03.010

34. Decock C, Six J. On the potential of $\delta^{18}O$ and $\delta^{15}N$ to assess N_2O reduction to N_2 in soil. *Eur J Soil Sci*. 2013;64(5):610-620. doi:10.1111/ejss.12068

35. Sasaki Y, Koba K, Yamamoto M, et al. Biogeochemistry of nitrous oxide in Lake Kizaki, Japan, elucidated by nitrous oxide isotopomer analysis. *J Geophys Res Biogeosciences*. 2011;116(4):1-10. doi:10.1029/2010JG001589

36. Verhoeven E, Barthel M, Yu L, et al. Early season N_2O emissions under variable water management in rice systems: source-partitioning emissions using isotopocule signatures along a depth profile. *Biogeosciences*. 2019;16:383-408. doi:10.5194/bg-2018-254

37. McLinden CA, Prather MJ, Johnson MS. Global modeling of the isotopic analogues of N_2O: Stratospheric distributions, budgets, and the ^{17}O– ^{18}O mass-independent anomaly. J Geophys Res. 2003;108(D8):1-15. doi:10.1029/2002jd002560

38. Schmidt JA, Johnson MS. Clumped isotope perturbation in tropospheric nitrous oxide from stratospheric photolysis. *Geophys Res Lett*. 2015;42(9):3546-3552. doi:10.1002/2015GL063102

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

40. Well R, Flessa H. Isotope fractionation factors of N_2O diffusion. *Rapid Commun Mass Spectrom*. 2008;22:2621-2628. doi:10.1002/rcm.3656

41. Lewicka-Szczebak D, Well R, Bol R, et al. Isotope fractionation factors controlling isotopocule signatures of soil-emitted N_2O produced by denitrification processes of various rates. *Rapid Commun Mass Spectrom*. 2015;29(3):269-282. doi:10.1002/rcm.7102

42. Wu D, Well R, Cárdenas LM, et al. Quantifying N_2O reduction to N_2 during denitrification in soils via isotopic mapping approach : Model evaluation and uncertainty analysis. *Environ Res J*. 2019;179(August). doi:10.1016/j.envres.2019.108806

43. Denk TRA, Kraus D, Kiese R, Butterbach-Bahl K, Wolf B. Constraining N cycling in the ecosystem model LandscapeDNDC with the stable isotope model SIMONE. *Ecology*. 2019;100(5):c02675. doi:10.1002/ecy.2675

44. Rahn T, Wahlen M. A reassessment of the global isotopic budget of atmospheric nitrous oxide. *Global Biogeochem Cycles*. 2000;14(2):537-543. doi:10.1029/1999GB900070

45. Prokopiou M, Martinerie P, Sapart CJ, et al. Constraining N_2O emissions since 1940 using firn air isotope measurements in both hemispheres. *Atmos Chem Phys*. 2017;2011(June):1-50. doi:10.5194/acp-2016-487

46. Harris E, Ibraim E, Henne S, et al. Tracking nitrous oxide emission processes at a suburban site with semicontinuous , in situ measurements of isotopic composition. *J Geophys Res Atmos*. 2017;122:1850-1870. doi:10.1002/2016JD025906

47. Toyoda S, Yoshida N. Determination of nitrogen isotopomers of nitrous oxide on a modified isotope ratio mass spectrometer. *Anal Chem*. 1999;71(20):4711-4718. doi:10.1021/ac9904563

48. Harris EJ, Nelson DD, Olsewski W, et al. Development of a spectroscopic technique for continuous online monitoring of oxygen and site-specific nitrogen isotopic composition of

atmospheric nitrous oxide. *Anal Chem*. 2014;86(3):1726-1734.

49. Ostrom NE, Gandhi H, Coplen TB, et al. Preliminary assessment of stable nitrogen and oxygen isotopic composition of USGS51 and USGS52 nitrous oxide reference gases and perspectives on calibration needs. *Rapid Commun Mass Spectrom*. 2018;32(15):1207-1214. doi:10.1002/rcm.8157

50. Nara H, Tanimoto H, Tohjima Y, et al. Effect of air composition (N_2, O_2, Ar, and H_2O) on CO_2 and CH_4 measurement by wavelength-scanned cavity ring-down spectroscopy: Calibration and measurement strategy. *Atmos Meas Tech*. 2012;5(11):2689-2701. doi:10.5194/amt-5-2689-2012

51. Harris SJ, Liisberg J, Xia L, et al. N_2O isotopocule measurements using laser spectroscopy : analyzer characterization and intercomparison. *Atmos Meas Tech Discuss*. 2019;(December):1-84. doi:https://doi.org/10.5194/amt-2019-451

52. Harris E, Joss A, Emmenegger L, et al. Isotopic evidence for nitrous oxide production pathways in a partial nitritation-anammox reactor. *Water Res*. 2015;83:258-270. doi:10.1016/j.watres.2015.06.040

53. Wolf B, Merbold L, Decock C, et al. First on-line isotopic characterization of N_2O above intensively managed grassland. *Biogeosciences*. 2015;12(8):2517-2531. doi:10.5194/bg-12-2517-2015

54. Mohn J, Guggenheim C, Tuzson B, et al. A liquid nitrogen-free preconcentration unit for measurements of ambient N_2O isotopomers by QCLAS. *Atmos Meas Tech*. 2010;3(3):609-618. doi:10.5194/amt-3-609-2010

55. Ibraim E, Wolf B, Harris E, et al. Attribution of N_2O sources in a grassland soil with laser spectroscopy based isotopocule analysis. *Biogeosciences*. 2019:1-27. doi:10.5194/bg-2018-426

56. Yu L, Harris E, Henne S, et al. The isotopic composition of atmospheric nitrous oxide observed at the high-altitude research station Jungfraujoch, Switzerland. *Atmos Chem Phys Discuss*. 2019;(October). doi:https://doi.org/10.5194/acp-2019-829

57. Mohn J, Gutjahr W, Toyoda S, et al. Reassessment of the NH_4NO_3 thermal decomposition technique for calibration of the N_2O isotopic composition. *Rapid Commun Mass Spectrom*. 2016;(June):2487-2496. doi:10.1002/rcm.7736

58. JMA, WMO. *World Meteorological Organization - Global Atmosphere Watch - World Data Centre for Greenhouse Gases, Data Summary, No. 42, 101 P.,.*; 2018. https://gaw.kishou.go.jp/static/publications/summary/sum42/sum42.pdf.

59. Brewer PJ, Kim JS, Lee S, et al. Advances in reference materials and measurement techniques for greenhouse gas atmospheric observations. *Metrologia*. 2019;56(3). doi:10.1088/1681-7575/ab1506

60. Ostrom NE, Ostrom PH. Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. *Biogeochemistry*. 2017;132(3):359-372. doi:10.1007/s10533-017-0301-5

61. Kantnerová K, Tuzson B, Emmenegger L, Bernasconi SM, Mohn J. Quantifying isotopic signatures of N_2O using quantum cascade laser absorption spectroscopy. *Chimia (Aarau)*. 2019;73(4):232-238. doi:10.2533/chimia.2019.232

62. Magyar PM, Orphan VJ, Eiler JM. Measurement of rare isotopologues of nitrous oxide by high-resolution multi-collector mass spectrometry. *Rapid Commun Mass Spectrom*. 2016;(June):1923-1940. doi:10.1002/rcm.7671

63. Schmidt HL, Werner RA, Yoshida N, Well R. Is the isotopic composition of nitrous oxide an indicator for its origin from nitrification or denitrification? A theoretical approach from referred data and microbiological and enzyme kinetic aspects. *Rapid Commun Mass Spectrom*.

2004;18(18):2036-2040. doi:10.1002/rcm.1586

64. Blomberg MRA. Can reduction of NO to N_2O in cytochrome c dependent nitric oxide reductase proceed through a trans-mechanism? *Biochemistry*. 2017;56(1):120-131. doi:10.1021/acs.biochem.6b00788

65. Blomberg MRA, Siegbahn PEM. Improved free energy profile for reduction of NO in cytochrome c dependent nitric oxide reductase (cNOR). *J Comput Chem*. 2016;37(19):1810-1818. doi:10.1002/jcc.24396

66. Magyar PM. Insights into pathways of nitrous oxide generation from novel isotopologue measurements. *PhD Thesis Calif Inst Technol*. 2017.

67. Heil J, Liu S, Vereecken H, Brüggemann N. Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. *Soil Biol Biochem*. 2015;84:107-115. doi:10.1016/j.soilbio.2015.02.022

68. Clough TJ, Lanigan GJ, De Klein CAM, et al. Influence of soil moisture on codenitrification fluxes from a urea-affected pasture soil. *Sci Rep*. 2017;7(1):1-12. doi:10.1038/s41598-017-02278-y

69. Wei J, Amelung W, Lehndorff E, Schloter M, Vereecken H, Brüggemann N. N_2O and NO_x emissions by reactions of nitrite with soil organic matter of a Norway spruce forest. *Biogeochemistry*. 2017;132(3):325-342. doi:10.1007/s10533-017-0306-0

70. Wei J, Zhou M, Vereecken H, Brüggemann N. Large variability in CO_2 and N_2O emissions and in ^{15}N site preference of N_2O from reactions of nitrite with lignin and its derivatives at different pH. *Rapid Commun Mass Spectrom*. 2017;31(16):1333-1343. doi:10.1002/rcm.7912

71. Murray AE, Kenig F, Fritsen CH, et al. Microbial life at -13°C in the brine of an ice-sealed Antarctic lake. *Proc Natl Acad Sci U S A*. 2012;109(50):20626-20631. doi:10.1073/pnas.1208607109

72. Stanton CL, Reinhard CT, Kasting JF, et al. Nitrous oxide from chemodenitrification: A possible missing link in the Proterozoic greenhouse and the evolution of aerobic respiration. *Geobiology*. 2018;16(6):597-609. doi:10.1111/gbi.12311

73. Liu B, Mørkved PT, Frostegård A, Bakken LR. Denitrification gene pools, transcription and kinetics of NO, N2O and N_2 production as affected by soil pH. *FEMS Microbiol Ecol*. 2010;72(3):407-417. doi:10.1111/j.1574-6941.2010.00856.x

74. Brandes JA, Devol AH. Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. *Geochim Cosmochim Acta*. 1997;61(9):1793-1801. doi:10.1016/S0016-7037(97)00041-0

75. Lehmann MF, Sigman DM, McCorkle DC, et al. The distribution of nitrate $^{15}N/^{14}N$ in marine sediments and the impact of benthic nitrogen loss on the isotopic composition of oceanic nitrate. *Geochim Cosmochim Acta*. 2007;71(22):5384-5404. doi:10.1016/j.gca.2007.07.025

76. Bai E, Houlton BZ, Wang YP. Isotopic identification of nitrogen hotspots across natural terrestrial ecosystems. *Biogeosciences*. 2012;9(8):3287-3304. doi:10.5194/bg-9-3287-2012

77. Inatomi M, Hajima T, Ito A. Fraction of nitrous oxide production in nitrification and its effect on total soil emission: A meta-analysis and global-scale sensitivity analysis using a process-based model. *PLoS One*. 2019;14(7):e0219159. doi:10.1371/journal.pone.0219159

78. Leininger S, Urich T, Schloter M, et al. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*. 2006;442(August):806-809. doi:10.1038/nature04983

79. Groffman PM, Butterbach-Bahl K, Fulweiler RW, et al. Challenges to incorporating spatially and

temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry*. 2009;93(1-2):49-77. doi:10.1007/s10533-008-9277-5

80. Hall SJ, Reyes L, Huang W, Homyak PM. Wet Spots as Hotspots: Moisture Responses of Nitric and Nitrous Oxide Emissions From Poorly Drained Agricultural Soils. *J Geophys Res Biogeosciences*. 2018;123(12):3589-3602. doi:10.1029/2018JG004629

81. Müller C, Clough TJ. Advances in understanding nitrogen flows and transformations: Gaps and research pathways. *J Agric Sci*. 2014;152(2014):S34-S44. doi:10.1017/S0021859613000610

82. Kravchenko AN, Guber AK, Quigley MY, Koestel J, Gandhi H, Ostrom NE. X-ray computed tomography to predict soil N_2O production via bacterial denitrification and N2O emission in contrasting bioenergy cropping systems. *GCB Bioenergy*. 2018;10(11):894-909. doi:10.1111/gcbb.12552

83. Bourbonnais A, Letscher RT, Bange HW, et al. N_2O production and consumption from stable isotopic and concentration data in the Peruvian coastal upwelling system. *Global Biogeochem Cycles*. 2017;31(4):678-698. doi:10.1002/2016GB005567

84. Gil J, Pérez T, Boering K, Martikainen PJ, Biasi C. Mechanisms responsible for high N_2O emissions from subarctic permafrost peatlands studied via stable isotope techniques. *Global Biogeochem Cycles*. 2017;31(1):172-189. doi:10.1002/2015GB005370

85. Siljanen HMP, Alves RJE, Ronkainen JG, et al. Archaeal nitrification is a key driver of high nitrous oxide emissions from arctic peatlands. *Soil Biol Biochem*. 2019;137(March):107539. doi:10.1016/j.soilbio.2019.107539

86. Kaiser J, Engel A, Borchers R, Röckmann T. Probing stratospheric transport and chemistry with new balloon and aircraft observations of the meridional and vertical N_2O isotope distribution. *Atmos Chem Phys*. 2006;6(11):3535-3556. doi:10.5194/acp-6-3535-2006

87. Toyoda S, Yano M, Nishimura S, et al. Characterization and production and consumption processes of N_2O emitted from temperate agricultural soils determined via isotopomer ratio analysis. *Global Biogeochem Cycles*. 2011;25(2):1-17. doi:10.1029/2009GB003769

88. Buchen C, Lewicka-Szczebak D, Flessa H, Well R. Estimating N_2O processes during grassland renewal and grassland conversion to maize cropping using N_2O isotopocules. *Rapid Commun Mass Spectrom*. 2018;32(13):1053-1067. doi:10.1002/rcm.8132

89. Zou Y, Hirono Y, Yanai Y, Hattori S, Toyoda S, Yoshida N. Isotopomer analysis of nitrous oxide accumulated in soil cultivated with tea (Camellia sinensis) in Shizuoka, central Japan. *Soil Biol Biochem*. 2014;77:276-291. doi:10.1016/j.soilbio.2014.06.016

90. Ostrom NE, Piit A, Sutka R, et al. Isotopologue effects during N_2O reduction in soils and in pure cultures of denitrifiers. *J Geophys Res Biogeosciences*. 2007;112(2):1-12. doi:10.1029/2006JG000287

91. Yu L, Mulder J, Zhu J, Zhang X, Wang Z, Dörsch P. Denitrification as a major regional nitrogen sink in subtropical forest catchments: evidence from multi-site dual nitrate isotopes. *Glob Chang Biol*. 2019;(25):1765-1778. doi:10.1111/gcb.14596

92. Zhang J, Müller C, Cai Z. Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. *Soil Biol Biochem*. 2015;84:199-209. doi:10.1016/j.soilbio.2015.02.028

93. Lehmann MF, Sigman DM, Berelson WM. Coupling the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of nitrate as a constraint on benthic nitrogen cycling. *Mar Chem*. 2004;88(1-2):1-20. doi:10.1016/j.marchem.2004.02.001

94. Deutsch C, Sigman DM, Thunell RC, Meckler AN, Haug GH. Isotopic constraints on

glacial/interglacial changes in the oceanic nitrogen budget. *Global Biogeochem Cycles*. 2004;18(4):1-22. doi:10.1029/2003GB002189

95. Kool DM, Wrage N, Oenema O, Dolfing J, Van Groenigen JW. Oxygen exchange between (de)nitrification intermediates and H_2O and its implications for source determination of NO_3^- and N_2O: A review. *Rapid Commun Mass Spectrom*. 2007;21(22):3569-3578. doi:10.1002/rcm.3249

96. Casciotti KL, Buchwald C, McIlvin M. Implications of nitrate and nitrite isotopic measurements for the mechanisms of nitrogen cycling in the Peru oxygen deficient zone. *Deep Res Part I Oceanogr Res Pap*. 2013;80:78-93. doi:10.1016/j.dsr.2013.05.017

97. Buchwald C, Casciotti KL. Isotopic ratios of nitrite as tracers of the sources and age of oceanic nitrite. *Nat Geosci*. 2013;6(4):308-313. doi:10.1038/ngeo1745

98. Casciotti KL, Forbes M, Vedamati J, Peters BD, Martin TS, Mordy CW. Nitrous oxide cycling in the Eastern Tropical South Pacific as inferred from isotopic and isotopomeric data. *Deep Res Part II Top Stud Oceanogr*. 2018;156(August):155-167. doi:10.1016/j.dsr2.2018.07.014

99. Lewicka-Szczebak D, Dyckmans J, Kaiser J, Marca A, Augustin J, Well R. Oxygen isotope fractionation during N_2O production by soil denitrification. *Biogeosciences*. 2016;13(4):1129-1144. doi:10.5194/bg-13-1129-2016

100. Rohe L, Well R, Lewicka-Szczebak D. Use of oxygen isotopes to differentiate between nitrous oxide produced by fungi or bacteria during denitrification. *Rapid Commun Mass Spectrom*. 2017;31(16):1297-1312. doi:10.1002/rcm.7909

101. Bai E, Houlton BZ. Coupled isotopic and process-based modeling of gaseous nitrogen losses from tropical rain forests. *Global Biogeochem Cycles*. 2009;23(2):1-10. doi:10.1029/2008GB003361

102. Martin TS, Primeau F, Casciotti KL. Modeling oceanic nitrate and nitrite concentrations and isotopes using a 3-D inverse N cycle model. *Biogeosciences*. 2019;16(2):347-367. doi:10.5194/bg-16-347-2019

103. Somes CJ, Schmittner A, Galbraith ED, et al. Simulating the global distribution of nitrogen isotopes in the ocean. *Global Biogeochem Cycles*. 2010;24(4):1-16. doi:10.1029/2009GB003767

104. Denk TRA, Mohn J, Decock C, et al. The nitrogen cycle: A review of isotope effects and isotope modeling approaches. *Soil Biol Biochem*. 2017;105:121-137. doi:10.1016/j.soilbio.2016.11.015

105. Deppe M, Well R, Giesemann A, Spott O, Flessa H. Soil N_2O fluxes and related processes in laboratory incubations simulating ammonium fertilizer depots. *Soil Biol Biochem*. 2017;104:68-80. doi:10.1016/j.soilbio.2016.10.005

106. Snider D, Thompson K, Wagner-Riddle C, Spoelstra J, Dunfield K. Molecular techniques and stable isotope ratios at natural abundance give complementary inferences about N_2O production pathways in an agricultural soil following a rainfall event. *Soil Biol Biochem*. 2015;88:197-213. doi:10.1016/j.soilbio.2015.05.021

107. Wu D, Senbayram M, Well R, et al. Nitrification inhibitors mitigate N_2O emissions more effectively under straw-induced conditions favoring denitrification. *Soil Biol Biochem*. 2017;104:197-207. doi:10.1016/j.soilbio.2016.10.022

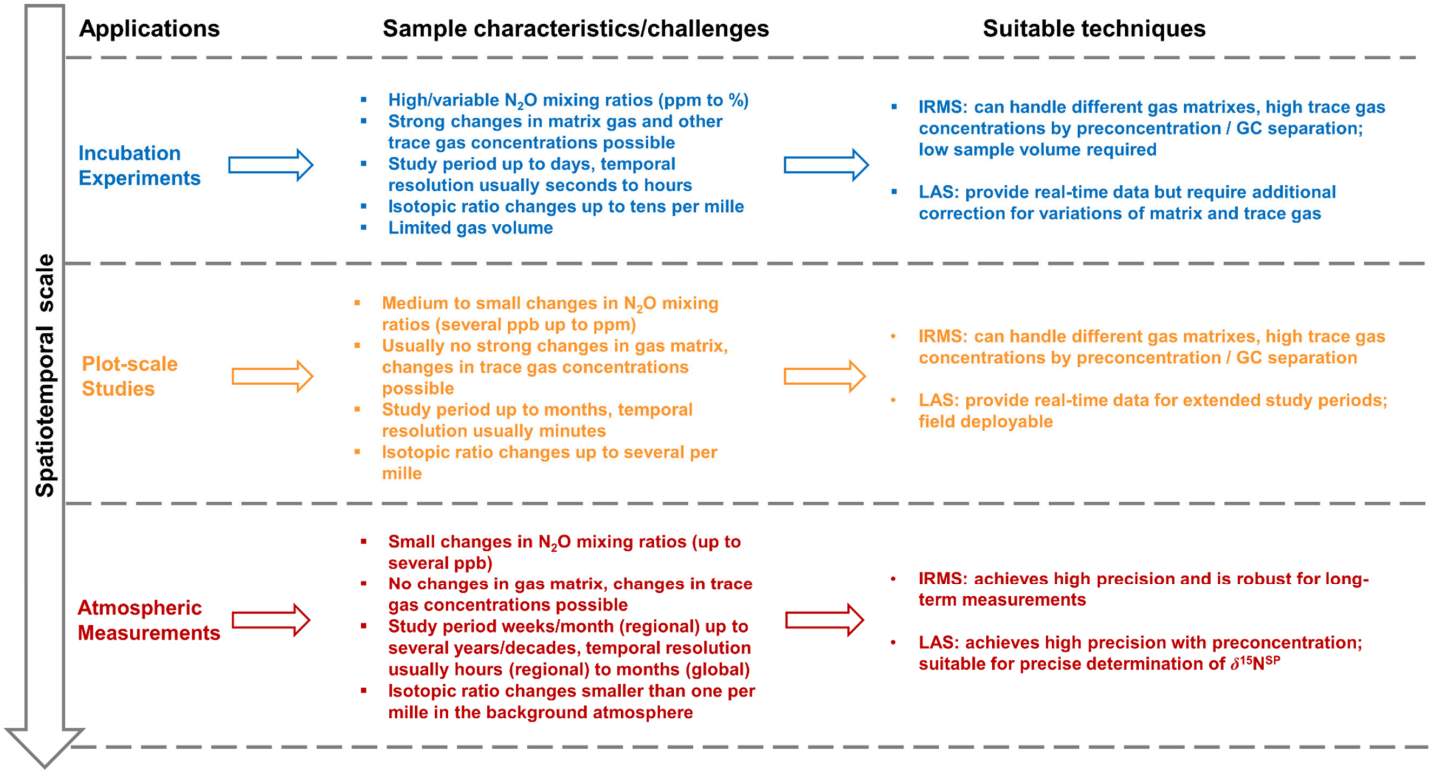
Figure legends

Figure 1 Schematic illustration of analytical challenges/strategies for N_2O isotopic studies at different spatio-temporal scales. While incubation studies vary widely with regards to the gas matrix, trace gas concentrations and N_2O isotopic composition, atmospheric measurements pose a challenge with respect to the "desired" analytical precision. A list of advantages and disadvantages for IRMS and LAS techniques according to particular applications are presented as a recommendation for designing experiments.

Figure 2 Conceptual figure illustrating how N_2O isotopes – particularly $\delta^{15}N^{SP}$ – can link our understanding of N_2O processes (production and consumption pathways) across a wide range of spatiotemporal scales. Experimental and modelling methods suitable for isotopic studies at the different scales are highlighted.

Figure 3 N_2O mixing endmembers (bD - bacterial denitrification, nD – nitrifier denitrification, fD – fungal denitrification, Ni – nitrification) presented in 3D map (A) for $\delta^{15}N^{SP}$ (y-axis), $\delta^{18}O$ (x-axis) and $\delta^{15}N^{bulk}$ (z-axis) and dual isotope plots (B, C and D) with theoretical reduction line (red line) and mixing line between denitrification and nitrification (black solid line) and between bacterial and fungal denitrification. The detailed summary and justification of endmember values used with relevant references is presented in the Supporting Information. Chemical denitrification was not shown on the graphs due to large variations in observed values depending on various environments and substrates[28,32,67,69–71]. The endmember ranges for bD and fD depend on $\delta^{15}N_{NO3}$ whereas those for Ni and nD depend on $\delta^{15}N_{NH4}$. These values as shown are true for $\delta^{15}N_{NO3}$=0‰ and $\delta^{15}N_{NH4}$=0‰, and for particular case study should be related to respective measured substrates. The endmember ranges for bD, fD and nD depend on $\delta^{18}O_{H2O,}$ whereas that for Ni depends on $\delta^{18}O_{O2}$. These values as shown are true for $\delta^{18}O_{H2O}$=0‰ and $\delta^{18}O_{O2}$=23.5‰, and for particular case study should be related to respective measured substrates.

946 **Figure1**



947

948

949

Figure 2

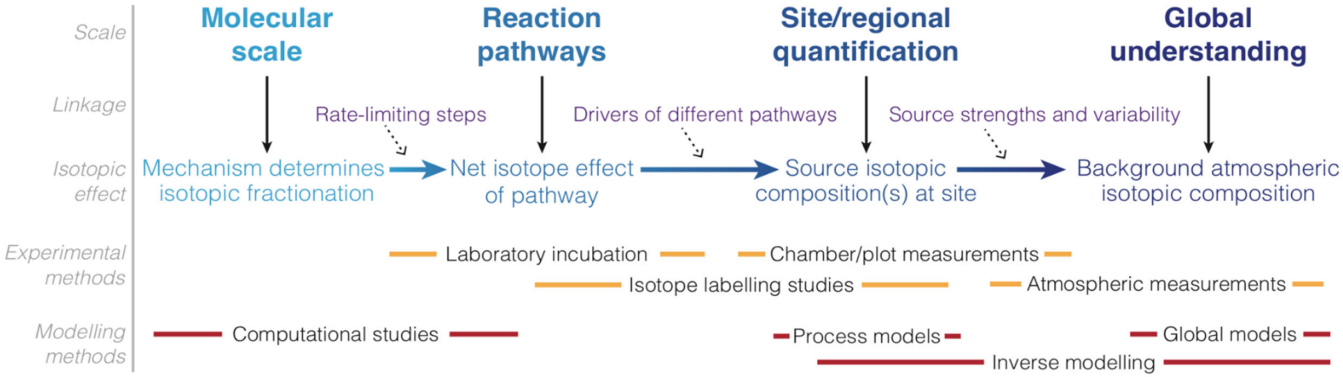
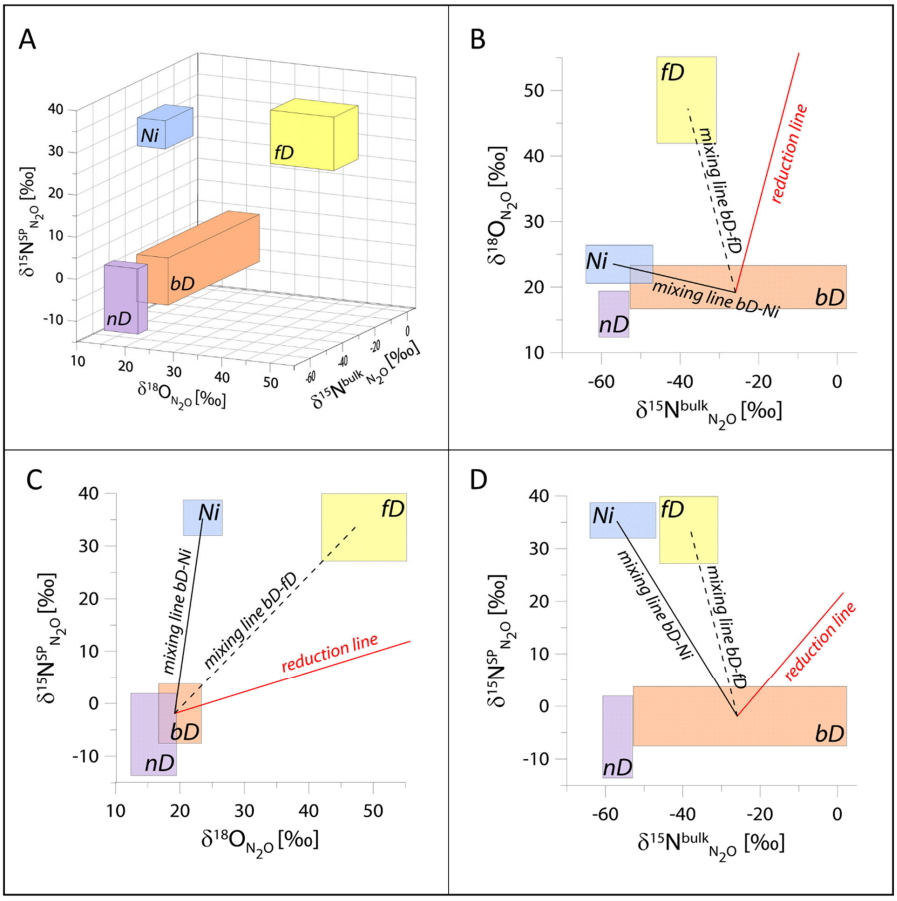

Scale
Linkage
Isotopic effect
Experimental methods
Modelling methods
Molecular scale
Reaction pathways
Site/regional quantification
Global understanding
Rate-limiting steps
Drivers of different pathways
Source strengths and variability
Mechanism determines isotopic fractionation
Net isotope effect of pathway
Source isotopic composition(s) at site
Background atmospheric isotopic composition
Laboratory incubation
Chamber/plot measurements
Isotope labelling studies
Atmospheric measurements
Computational studies
Process models
Global models
Inverse modelling

Figure 3


A
B
C
D
Ni
fD
bD
nD
mixing line bD-fD
mixing line bD-Ni
reduction line
δ15NSP N2O [‰]
δ18ON2O [‰]
δ15Nbulk N2O [‰]