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Measuring denitrification and the N_2O:(N_2O + N_2) emission ratio from terrestrial soils

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Denitrification, a significant pathway of reactive N-loss from terrestrial soils, impacts on agricultural production and the environment. Net production and emission of the denitrification product nitrous oxide (N_2O) is readily quantifiable, but measuring denitrification's final product, dinitrogen (N_2), against a high atmospheric background remains challenging. This review examines methods quantifying both N_2 and N_2O emissions, based on inhibitors, helium/O_2 atmosphere exchange, and isotopes. These methods are evaluated regarding their capability to account for pathways of N_2 and N_2O production and we suggest quality parameters for measuring denitrification from controlled environments to the field scale. Our appraisal shows that method combinations, together with real-time monitoring and soil-gas diffusivity modelling, have the potential to significantly improve our quantitative understanding for denitrification from upland soils. Requirements for instrumentation and experimental setups however highlight the need to develop more mobile and easily accessible field methods to constrain denitrification from terrestrial soils across scales.

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Introduction

Denitrification, the sequential reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to gaseous emissions of nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) is a key process within the nitrogen (N) cycle, directly impacting agricultural production and the environment. Denitrification research usually focusses on N_2 and N_2O, assuming NO to account for only a small fraction of overall denitrification. Advances in measuring N_2O as a trace gas have improved N_2O estimates at both spatial and temporal scales. However, measuring N_2 emissions against the high atmospheric N_2 background remains challenging, making the magnitude of total denitrification losses, defined here as N_2 + N_2O, and N_2:N_2O partitioning a major uncertainty for N-budgets from terrestrial ecosystems. This uncertainty is further aggravated by i) the use of methods associated with bias,ii) low method sensitivity, precluding measurements beyond peak emissions and iii), the use of methods/experimental setups which change substrate availability and soil conditions different from those found in situ [1••]. These shortcomings preclude the use of some of the available methods listed in Table 1 to obtain realistic and unbiased measurements of N_2 and N_2O: For example, the widely used Acetylene Inhibition Technique (AIT) creates a systematic and irreproducible underestimation of denitrification [2–5], resulting in biased estimates of denitrification across scales [6]. The low sensitivity of the N_2/Ar method precludes its use for denitrification measurements from upland soils. The denitrification potential (DP), also acetylene based, is quantified in a soil slurry after the addition of glucose and non-limiting NO_3^-, severely altering substrate availability for denitrification. Even some ^{15}N denitrification methods such as the modified isotope pairing technique (IPT) require soil slurries and anaerobic (pre-) incubations. These approaches have been used to obtain 'potential' denitrification rates or served as a proof of concept. The present choice of methods however enables researchers to move past the quantification of potential denitrification rates if conditions are kept similar to those found in situ, allowing realistic estimates of N_2 and N_2O to be obtained.

The Helium/Oxygen atmosphere method (He/O_2 method) [7,8,9•,10] and the ^{15}N gas flux method (^{15}NGF) [11] avoid most of the shortcomings of the methods mentioned above, and are considered suitable for the direct quantification of N_2 and N_2O from

Table 1

Comparison of different methods for measuring N_2 and N_2O emissions from terrestrial soils. Method development stage, the suitability of the method to quantify actual denitrification rates (N_2 and N_2O) and relative differences between treatments and/or soils as well as instrument requirements are rated from low (*) to high (*****). Italicised methods are in the early development stage and ratings are only indicative due to the small number of published studies using this method

Method	Principle	Method development stage	Soil manipulation/ added Substrate	Field studies	Suitability to quantify		Source partitioning		Instrument requirements	Reference
					Actual denitrification rates	relative differences	N_2	N_2O		
Potential denitrification assay	Inhibition of N_2O reduction to N_2	*****	Slurry, non-limiting C and NO_3^-		*	*			*	[4]
Acetylene inhibition technique	Inhibition of N_2O reduction to N_2	*****	Introduction of Acetylene	✓	*	*			*	[3]
Modified slurry Isotope pairing technique	Isotope pairing	****	Slurry, anoxic preincubation		*	***	✓		****	[58,59]+
N_2/Ar technique	N_2/Ar ratio	**	–	✓	*	*			****	[60]
^{15}N gas flux method	Non-random distribution of $^{15}N_2$ isotopologues	****	Addition of fertiliser and water	✓	*****	*****	✓	✓	****	[37,42]
He/O_2 method	Measuring soil borne N_2 in a He/O_2 atmosphere	****	–		****	*****			****	[10]
Reduced N_2 atmosphere combined with ^{15}N tracer application	Improved detection of $^{15}N_2$ against a reduced N_2 atmosphere	***	Addition of fertiliser and water		****	*****	✓	✓	****	[48]
Improved ^{15}N gas flux method	Improved detection of $^{15}N_2$ against a reduced N_2 atmosphere	**	Addition of fertiliser and water	✓	*****	*****	✓	✓	*****	[36••]
Isotopic mapping approach	Isotopocules of N_2O	*	–	✓	**	***		✓	****	[54•,55•]
Naturally occurring $^{15}N^{15}N$ isotopes	Naturally occurring clumped isotope tracer Δ30	*	–	✓	*****	*****		✓	*****	[56]
Raman multi-gas sensing	Interaction of photons with of NO, N_2O and N_2	*	–		*	*			****	[57]

+ ratings are given for upland soils; the method is originally used for sediments and is therefore better suited to measure denitrification from saturated soils.

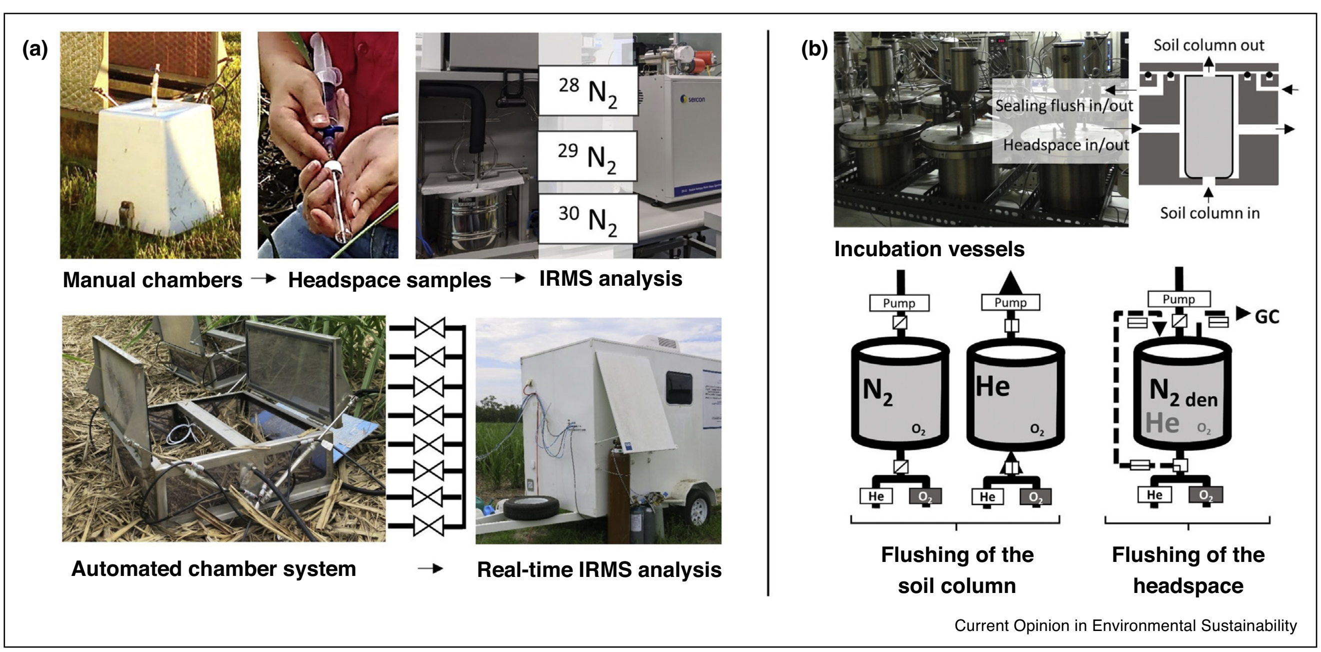
upland soils. Both methods require extensive instrumentation and in-depth knowledge for sound application. '*Methodsfor measuring denitrification*' from 2006 [1••], describes development and application of both methods and a recent meta-analysis discusses their use in comparison to other methods in denitrification studies up to 2015 [12•]. Furthermore, the authors suggest a framework for standardised reporting of denitrification metadata to provide better information for biogeochemical models, limited by the current lack and/or bias of denitrification data. Building on these studies, this review provides a concise technical overview on the He/O_2 method and the ^{15}NGF to guide researchers regarding method choice, method evaluation, and quality assessment of denitrification data. We

- revisit the principles of the He/O_2 method and ^{15}NGF for the direct quantification of N_2 and N_2O emissions from upland soils,
- discuss instrument requirements, applicability, and detection limits (DL),
- investigate their ability to account for different pathways of N_2 and N_2O production,
- highlight recent advances in method development and propose minimum requirements for quality control and reporting for each method,
- and, finally, explore the potential of new approaches to measure N_2 and N_2O emissions highlighting research needs to further advance denitrification studies.

The He/O_2 atmosphere method

This method avoids the problem of the high N_2 background during N_2 measurements by replacing the soil-headspace atmosphere inside the closed incubation system with a He/O_2 mixture. Increases in headspace N_2 concentrations due to soil emissions can be directly measured in the artificial He/O_2 headspace atmosphere by gas chromatography at high precision, and with DLs for N_2 fluxes $<10\ \mu g\ N_2\ m^{-2}\ hour^{-1}$ achievable [1••,13••]. This setup requires extremely gas-tight incubation systems to minimise intrusion of atmospheric N_2 into the incubation vessel and the sampling units. This requires extensive engineering efforts such as double He-flushed O-ring seals, submerging of the incubation vessels and tubing connections underwater (Figure 1), and/or placing the system or its potential leaky components such as tubing connections, valves and sample loops in a He-purged chamber [10,13••]. Despite these efforts, small N_2 leakage rates remain and must be corrected for by measuring empty vessels or vessels with containers of similar form and volume as soil cores, referred to as 'dummies'. The lower the measured N_2 emissions, the higher are the requirements for gas-tightness of the system. To establish an N_2-free atmosphere, the soil columns are purged with a He/O_2 mixture in a dynamic flow-through mode (Figure 1), which may include alternating evacuation cycles to speed up the exchange process [13••]. For quantification of N_2, systems are either run in a static [10,13••], or dynamic chamber

Figure 1



(a) Sampling procedure for the ^{15}N gas flux method using manual static chambers with subsequent IRMS analysis or an automated static chamber system coupled to a mobile 'Field-isotope ratio mass spectrometer' [46••] and **(b)** setup of the incubation vessels used for the Helium/Oxygen atmosphere method, showing the flushing of the soil column and the headspace for subsequent N_2 analysis with a GC [13••].

Table 2

Challenges, solutions and improvements, and proposed quality criteria to be reported in denitrification studies measuring N_2 and N_2O emissions from terrestrial soils using the Helium/Oxygen headspace method or the ^{15}N gas flux method. Further details on reporting are given in the Supplementary Material

	Challenge	Solution/Improvement	Quality criteria to be reported	References
Helium/Oxygen Headspace Method	Measurements compromised by leakage of atmospheric N_2	Improve tightness (see text); Regular quantification of N_2 intrusion with empty system or soil core dummies; Subtract leakage rates from measurements	• Leakage rate for each incubation vessel. Temporal stability of leakage rates during measurement period, Leakage rates << N_2 emission rates	[10,13••]
	Long time needed to replace soil atmosphere with no N_2 measurements possible during that time	Repeated vacuum/purge cycles	• Details on purging approach (flow rate, time under pressure and/or vacuum, actual pressure/vacuum applied)	[10,13••]
	Sufficiently purged to remove N_2 from soil?	Experimental and/or mathematical verification using the mathematical framework of Wu et al. [61] (supplementary material) for a conservative calculation of required flushing time	• Report initial N_2 concentration in the system vs. flushing time (once per soil is enough)	[61]
	Biological production or physical degassing from soil?	Ensure sufficient soil He-purging time (see above) to avoid N_2 gradients	Compare N_2 production at 4°C vs 20°C as indication of biological N_2 production	[13••]
	Insufficient detection limit	Reduced headspace height, improved N_2 detector	• Report SD of 10 calibration gas measurements Report precision of N_2 analysis	
	Potential destruction of anaerobic microsites by soil atmosphere exchange	Allow for reestablishment through soil respiration before start of N_2 measurements – research need for accurate O_2 sensors		
	Including plants	Setup including a light source enabling photosynthesis, controlling CO_2 mixing ratios, irrigation water free of N_2 and enough space for plant growth		
^{15}N gas flux method	IRMS precision for ^{29}R ($^{29}N_2/^{28}N_2$) and ^{30}R ($^{30}N_2/^{28}N_2$)	Improving the leak tightness of the IRMS Removal of O_2 and H_2O in the N_2 sample stream Optimising sample loop size and Ionisation energy Reduction of the N_2 background in the chamber headspace	• Overall SD for ^{29}R and ^{30}R of ambient air samples included as QC in each run (between batch SD) or • SD for ^{29}R and ^{30}R of ambient air samples minimum 10 representative for the time of the respective IRMS analysis • Resulting DL and MDL	[36••,62]
	Estimating the ^{15}N enrichment of the soil NO_3^- pool undergoing denitrification	Calculation of the $^{15}NO_3^-$ enrichment based on N_2 and N_2O Determination of the ^{15}N enrichment of soil NO_3^- following soil extraction via diffusion technique	• Report a comparison of the $^{15}NO_3^-$ enrichment based on N_2 vs. based on N_2O	[45,46••]
	Uniform distribution of ^{15}N in the soil	Application of a high rate of ^{15}N fertiliser as a solution waters the ^{15}N label evenly into the soil. Saturation of soil cores with ^{15}N labelled fertiliser solution. Injection via syringe ensuring even	• Comparison of the $^{15}NO_3^-$ enrichment based on N_2 vs. derived from N_2O over time. Distribution of the $^{15}NO_3^-$ label in the soil	[35,42,63•]

Table 2 (*Continued*)

Challenge	Solution/Improvement	Quality criteria to be reported	References
	distribution across the plot and at depth	• Comparison of the theoretical vs. actual $^{15}NO_3^-$ enrichment via pool mixing.	
Achieving the target ^{15}N enrichment	Determination of soil NO_3^- levels prior fertilisation Tests to account for the dilution of $^{15}NO_3^-$ due to increased nitrification after ^{15}N and water addition	• Report theoretical vs. actual $^{15}NO_3^-$ enrichment using a pool mixing model	[46••,63•]
Subsoil diffusion of N_2 and N_2O in field studies Increased Reduction of N_2O to N_2	Experimental quantification of N_2 and N_2O emitted from soil cores enclosed at the bottom Correction of surface fluxes via soil gas diffusivity modelling	• Minimise chamber closure time (≤ 3 hours) according to expected flux rates. Establish N_2O linearity	[49•]
Linearity of N_2 and N_2O fluxes	Several gas samples in even intervals over time to evaluate the linear increase of both N_2 and N_2O over time	• Report test for linearity, and the coefficient of determination	[21••,46••]
Discarding N_2 measurements vs. zero fluxes	Set of rules how N_2 measurements are handled if ^{29}R and/or ^{30}R are negative and/or below DL.	• Report handling of N_2 measurements below DL and the number of discarded N_2 measurements	[35,63•]

• denotes minimum quality criteria to be reported for the respective method.

mode [9•,14], with no or continuous He/O_2 flow through the chamber, respectively. The size of soil cores can range from small cores (5.6 cm diameter and 4 cm height), incubated in sets in the same incubation vessel to cover spatial variability [15] to relatively large soil columns (12.5 cm diameter and 15 cm height) [10,16]. The key strengths of the method are direct and simultaneous measurements of N_2 and N_2O without chemical perturbation of the soil caused by ^{15}N labelled fertiliser or an inhibitor, without the need for stable isotope analyses of headspace gas.

Major drawbacks and challenges of this method are: (i) the extreme technical effort required to make the experimental system gas-tight against intrusion of atmospheric $N_2$and (ii) the period needed (up to 48 hours) to establish an N_2-free atmosphere, during which quantification of N_2 emissions is not possible. These challenges require specific solutions and adaption of the incubation setup as outlined in Table 2. Further method limitations arising from setup requirements and gas detection include (iii) the inability to operate in the field and therefore disturbance of soil (iv) limited replication and (v) limitation to distinguish between processes generating N_2 and/or N_2O such as nitrification, denitrification or anammox [10,12•,13••]. Notwithstanding this, for studies focusing on ecosystem N balances and total gaseous N losses, the ability of the He/O_2 method to facilitate integrative N_2 flux determination over time, regardless of source, can be regarded as an advantage. To obtain seasonal or annual estimates of soil N_2 emissions at the field scale, field cores can be brought to the laboratory for short measurement periods and immediately reburied in the field [17,18]. Field N_2 emissions can then be constrained by $N_2O/(N_2 + N_2O)$ product ratios obtained from He/O_2 laboratory incubations i) used in combination with high-frequency N_2O measurements and soil data relating to environmental variables [19•,20] or ii) using field measurements of soil O_2 as a proxy for denitrification [21••].

Flushing the soil core with a gas mixture containing 20% O_2 can alter the O_2 concentration profile and may destroy anaerobic micropores (Figure 1). The O_2 molecule regulates denitrification rates and activity of the N_2O reductase enzyme thus varying the soil exposure to O_2 can affect both denitrification rate and the $N_2O/(N_2 + N_2O)$ product ratio [6,21••,22]. Although soil respiration may quickly restore anaerobic soil pores after purging the soil core with a He/O_2 mixture, the effect of flushing on anaerobic micropores and the time required to re-establish original conditions is not known. Some studies have tried to adjust the He/O_2 mixtures used to purge the cores based on soil O_2 levels measured in the field, developing relationships between precipitation and soil O_2 concentrations to extrapolate point measurements of denitrification to seasonal scales [21••,22,23]. Most O_2 probes are however only able to measure O_2 in soil-macropores, but not at the micropore-scale where denitrification preferentially takes place [22]. Owing to the inherent spatial variation of soil O_2 and analytical constraints, the appropriate scale and method to determine O_2 dynamics in the soil profile and adjust O_2 levels in the He/O_2 purge gas remain open research questions.

The importance of plant–soil–microbe interactions and their corresponding effects on denitrification poses another challenge, as available systems usually do not contain active plants. This is expected to result in major bias, as N gases can be directly emitted from plants [24], and plant activities such as root exudation of labile C and competition for NO_3^- are assumed to be a major driver of rhizosphere denitrification [25,26], an effect that has not yet been quantified based on direct N_2 measurements because of the methodological limitations outlined here. Currently, several groups are constructing and testing He/O_2 systems with translucent chambers to include plant effects [9•,27]. Such a setup, however, further increases engineering challenges, due to the need to (i) install light sources to enable realistic levels of photosynthetic active radiation in the incubation vessels(ii) control CO_2 mixing ratios in the headspace and (iii) introduce irrigation-water free of dissolved N_2 to the plants. Furthermore, growing crops of realistic size involves a significant increase in the volume of soil used and a suitable headspace height, which results in trade-offs: these systems require a longer period of He flushing to replace the soil atmosphere resulting in higher He consumption, as well as an increased DL. Nonetheless, such plant–soil incubation systems using the He/O_2 method are expected to provide more realistic measurements of denitrification and N_2:N_2O emission ratios from terrestrial soils.

The He/O_2 atmosphere method is one of the two main approaches considered suitable for the direct quantification of N_2 and N_2O emissions from soils and is especially well suited to laboratory incubations with controlled environmental settings and parameterization studies. Method-inherent limitations and drawbacks demand careful operation to avoid flaws and erroneous N_2 emission measurements. Quality control is challenging, as only customised systems are available, with no universal quality indicators available or in use. Table 2 summarises the discussed challenges of the method, approaches for improving the method, and quality indicators that should be reported.

The ^{15}N gas flux method

The ^{15}NGF is the only method that can be applied under laboratory and field conditions. Highly enriched ^{15}N fertiliser is applied to the soil, and gas samples are taken using a static chamber approach (Figure 1). Gas samples are then analysed for their different isotopologues (i.e. molecules differing in their isotopic composition) of N_2 and N_2O via isotope ratio mass spectrometry (IRMS). As the ^{15}N enriched NO_3^- pool undergoes denitrification, emitted N_2 contains three different isotopologues: $^{28}N_2$ ($^{14}N^{14}N$), $^{29}N_2$ ($^{14}N^{15}N$) and $^{30}N_2$ ($^{15}N^{15}N$), following a binomial, i.e. random distribution [28••]. The mixture of background N_2 and the N_2 produced from the $^{15}NO_3^-$ pool will, however, have a non-random distribution of isotopologues. This deviation from the random distribution permits the ^{15}N abundance in the NO_3^- pool, and subsequently, the N_2 fluxes to be calculated [29•,30•,31]. Based on the isotopologues of N_2O ($^{14}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$ and $^{15}N^{15}N^{16}O$), N_2O production can be attributed to nitrification (N_2O_n) or denitrification (N_2O_d) [32], and allows, in contrast to the He/O_2 method, quantification of the denitrification product ratio ($N_2O_d/(N_2 + N_2O_d)$).

Challenges faced when using the ^{15}NGF include: a) Gas analysis - — accurate measurements of $^{29}N_2$ ($^{14}N^{15}N$) and $^{30}N_2$ ($^{15}N^{15}N$) for estimating the soil $^{15}NO_3^-$ pool enrichment, (b) the uniform distribution of ^{15}N in the soil (c) achieving the target ^{15}N enrichment, and (d) subsoil diffusion of N_2 and N_2O in field experiments.

High precision of the IRMS enables the detection of small changes in $^{29}N_2$ and $^{30}N_2$. The detection of $^{29}N_2$ is quite robust, but the formation of NO ($N^{14}O^{16}$) in the ion source of the IRMS [33] can mask changes in mass $^{30}N_2$. The standard deviation of ^{29}R ($^{29}N_2/^{28}N_2$) and ^{30}R ($^{30}N_2/^{28}N_2$) of ambient air samples, ideally included in each analysis, determines the precision of the IRMS. Despite efforts [34••], this precision has not significantly improved over the last four decades [2,35,36••,37]. This defines the relatively high method DL (MDL) of the ^{15}NGF, which is typically in the range of 10–60 g ha^{-1} day^{-1}, assuming a $^{15}NO_3^-$ pool enrichment of 50%, a headspace closure time of 2 hours, and a headspace-volume to soil area ratio of 10 (see supplementary material). Consequently, the ^{15}NGF is primarily used in fertilised agroecosystems, where denitrification is expected to be a major pathway of N loss.

Estimates of the $^{15}NO_3^-$ pool enrichment are critical for accurate determination of N_2 fluxes. Assuming that N_2 and N_2O are produced from the same NO_3^- pool undergoing denitrification, the isotopologues of N_2O can be used to estimate the $^{15}NO_3^-$ pool enrichment [38]. The fraction of N_2O derived from denitrification in the chamber headspace is usually higher than that of N_2, making this approach more reliable if source pool uniformity can be ensured [39]. Direct measurement of the $^{15}NO_3^-$ following soil extraction [40] is not recommended since this is likely to underestimate the $^{15}NO_3^-$ pool enrichment undergoing denitrification leading to a severe overestimation of N_2 emissions. Estimates of the $^{15}NO_3^-$ pool enrichment based on the isotopologues of N_2 and N_2O can be compared over the time of denitrification studies. This comparison provides an indication of uniform ^{15}N labelling and should be therefore included in denitrification studies.

Uniform ^{15}N labelling of the soil is a basic assumption of the ^{15}NGF. If denitrification occurs in multiple

NO_3^- pools with differing ^{15}N enrichments, N_2 production may be underestimated [41]. To address this problem, ^{15}N fertiliser is usually applied in solution: either sprayed on to the soil and/or mixed in [42], injected into the soil with a needle at different depths [37], using a capillary applicator, or simply watered into the soil [35]. Sieving and mixing is a popular practice in incubation studies, usually reducing the variation between replicates. The disturbance of the soil structure through sieving and mixing, and its effect on N turnover recommend this approach for process studies only, precluding the upscaling of results to the field scale. The quantity of applied ^{15}N is also critical, as accurate estimates of gaseous N losses can be made without uniform distribution of ^{15}N in the soil when large amounts of highly enriched N fertiliser are applied [43,44]. Thus, the resulting unnaturally elevated soil N concentration, together with the application of water, may limit the applicability of the ^{15}NGF in natural ecosystems but not in fertilised and irrigated agroecosystems. Over time, differences in O_2 availability determine nitrifier activity at the microscale, causing lower dilution of $^{15}NO_3^-$ in anoxic, and stronger dilution of $^{15}NO_3^-$ in oxic microsites [45,46••]. Such heterogeneity can be reflected in differences between the $^{15}NO_3^-$ pool enrichment derived from N_2O versus the one from N_2, showing the production of N_2O and N_2 in different microsites according to their different O_2 status. Leaching and lateral flow in the field, or preferential flow and pooling of $^{15}NO_3^-$ in incubation studies can further skew the distribution of $^{15}NO_3^-$ in the soil. Even if uniform ^{15}N labelling is achieved in the beginning of an experiment, this is likely to change over time, demanding close evaluation to reveal potential bias of flux estimates.

The target enrichment of the soil NO_3^- aims to maximise the signal for both $^{29}N_2$ and $^{30}N_2$ and is also critical for the MDL. The relative abundance of the $^{29}N_2$ as a function of $^{15}NO_3^-$ enrichment over the range 0–100%, plots in a quadratic fashion with the maximum relative abundance of $^{29}N_2$ occurring at a $^{15}NO_3^-$ enrichment of 50 atom%, while $^{30}N_2$ increases exponentially over the same range of $^{15}NO_3^-$ enrichment. Thus, the target $^{15}NO_3^-$ enrichment within the uniform soil pool is between 40 and 60 atom% in order to optimise the relative abundance of all isotopologues at detectable levels. This also allows the calculation of N_2 fluxes purely based on $^{29}N_2$ as a fall-back strategy should detection of $^{30}N_2$ fail [29•,30•]. The MDL decreases with decreasing ratio of headspace volume to the area of soil enclosed [47], increasing closure time of the chamber [48], and increasing ^{15}N enrichment of the NO_3^- pool undergoing denitrification. The first two parameters need to be optimised to provide enough headspace atmosphere for sampling, while avoiding increased reduction of N_2O to N_2 due to extended chamber closure times, and limiting subsoil diffusion of N_2 and N_2O. The last parameter, is however, the most difficult one to manage, since the ^{15}N label in the NO_3^- pool is subject to dilution via nitrification and consumption via denitrification and DNRA leading to a gradual decrease of the ^{15}N label in the soil NO_3^- pool over time. In agroecosystems, where N fertiliser is usually applied at the beginning of the cropping season, the use of the ^{15}NGF is limited to a certain time, during which the $^{15}NO_3^-$ label ensures detection of N_2 fluxes above the MDL. In turn, the ^{15}NGF works well in systems with repeated N fertiliser application such as intensively managed pastures [35]. Applying a high ^{15}N label at a low N rate, also termed 'spiking', enables N_2 measurements while assuming no interference with the soil N dynamics of the native soil N pool [37]. Uniform ^{15}N labelling is, however, challenging, as the antecedent soil N pool may not mix uniformly with a small amount of ^{15}N fertiliser. The comparison of theoretical versus actual ^{15}N enrichment of the NO_3^- pool undergoing denitrification can demonstrate whether the observed N_2 and N_2O emissions are representative or show only the so-termed 'fertiliser denitrification' [6].

The accumulation of N_2 and N_2O in the chamber headspace also changes the gas diffusion gradients of N_2 and N_2O and can therefore reduce surface emissions. This may produce an underestimation of denitrification rates in field studies of >30% [49•], as the soil volume undergoing denitrification is not enclosed and N_2 and N_2O may move out of the respective soil volume via subsoil diffusion. This is not the case in incubation studies, but denitrification products can remain entrapped in soil pores [50], in particular at high soil water content. Gas entrapment in soil pores is not necessarily caused but may be increased due to the use of static chambers. While it is relatively straightforward to measure entrapped N_2 and N_2O in incubation studies [51], accounting for diffusive ^{15}N loss via N_2 and N_2O in the field requires gas flux measurements from enclosed soil cores and correction via gas diffusion modelling [49•]. This correction via modelling approaches is however one of the key challenges for future denitrification research to improve denitrification estimates from terrestrial soils.

The ^{15}NGF is a powerful method to quantify both N_2 and N_2O from terrestrial soils, splitting N_2O production into nitrification or denitrification. As such, this method covers some of the key uncertainties of biogeochemical models, recommending its use for model parameterization and validation. Other than classical denitrification, the formation of N_2 and N_2O via hybrid pathways (co-denitrification [52], chemo-denitrification and anammox) can be investigated if $^{15}NO_3^-$ pool uniformity can be ensured. Combining the ^{15}NGF with ^{15}N tracing models [53] enables N transformations to be 'captured' in terrestrial soils, while ^{15}N recoveries in the soil-plant-atmosphere system can reveal the fate of applied ^{15}N fertiliser, and the contribution of denitrification to overall ^{15}N loss from the system. Recent advances regarding temporal resolution [46••] and sensitivity [36••] further extend the capability of the method to measure denitrification from agroecosystems. This shows the ample scope of the method for both basic process research and applied agronomic questions, yet the challenges of the ^{15}NGF demand constant method evaluation to ensure accuracy of denitrification data. To this end, detailed suggestions for quality criteria are given in Table 2.

New developments

Over the recent years, there has been ongoing development of new and improved methods for measuring denitrification from soils. Table 1 summarises the most important approaches, captures their key features and compares them against the more classic methods. All approaches are evaluated regarding their ability to measure actual denitrification rates in upland soils, which excludes for example approaches that require the use of slurries and/or anoxic pre-treatments.

The most important new developments include:

(i) The use of N_2O isotopocule data ($\delta^{15}N^{sp}$ and $\delta^{18}O$) in combination with a numerical mapping approach to indirectly quantify N_2O reduction to N_2 at field or larger spatial scales [54•,55•]. This method has the advantage that it can be applied field based, in real-time using novel quantum cascade laser absorption spectroscopy for the detection of N_2O isotope signatures. However, it still needs independent parameter calibration and at this stage cannot be treated as a precise quantitative tool.

(ii) Determination of N_2 production in soils based on the proportions of naturally occurring $^{15}N^{15}N$ isotopes. Recently developed methods to measure $^{15}N^{15}N$ in N_2 with high precision at natural abundances using a ultra-high resolution mass spectrometer offer a new approach to quantifying N_2 production in situ with DLs <1 N_2 g ha^{-1} day^{-1} reported [56]. The analytical precision of the novel mass spectrometer also has the potential to significantly improve the MDL of the ^{15}NGF, but currently this technique is not commercially available and has not been tested with ^{15}N tracer approaches.

(iii) Direct measurements of N_2 emissions via Raman multi-gas sensing have been used to quantify N_2 fluxes of 78 ± 5 μmol $hour^{-1}$ in a laboratory chamber system based on N fixation [57]. It has been proposed that the same method can also be used to detect N_2 fluxes by denitrification, but it remains to be seen if the necessary precision can be achieved with this analytical approach.

(iv) Quantification of N_2 and N_2O fluxes in real-time at a subdaily resolution using the ^{15}NGF coupled to a fully automated chamber system [46••]. The highly episodic nature of N_2 and N_2O gas emissions severely compromises denitrification estimates if not carried out with adequate frequency. Automated chamber systems are needed to increase sampling frequency and thus accuracy of denitrification estimates.

(v) A combination of different methods can increase the sensitivity of denitrification measurement, overcoming the constraints of using a single method. Well *et al.* [36••] showed that combining the ^{15}NGF with a N_2-depleted He/O_2 atmosphere can increase the sensitivity 80-fold.

These methods are still in the development stage and require expensive instrumentation and specialist knowledge resulting in limited accessibility, and therefore limited adoption by the scientific community. Further development in instrumentation should make new techniques more affordable, while improving and combining these novel approaches will help to produce estimates of denitrification from upland soils at high temporal resolution and better spatial coverage.

Conclusions

Revisiting the challenges of the He/O_2 and the ^{15}NGF method demonstrates the need to meet experimental and analytical requirements and stringent quality criteria to obtain reliable denitrification datasets. Standardised reporting of metadata and quality criteria is therefore critical in enabling the evaluation of denitrification datasets and their further use for calibration and validation of biogeochemical models. Direct, side by side comparisons of the He/O_2 and the ^{15}NGF method are needed to test both methods and enable data comparison across different soils. These comparisons can also help to validate attempts to upscale incubation data to the field scale, improving seasonal estimates for denitrification.

Recent advances in isotopic approaches and analytical methods have shown the potential to significantly improve sensitivity, temporal resolution, and accuracy of denitrification measurements. In particular, the combination of methods (He/O_2 with ^{15}NGF) with soil-gas diffusivity modelling is a promising approach, which could pave the way for an improved quantitative understanding of N-cycling and denitrification in terrestrial agroecosystems. Requirements for instrumentation and experimental setups however highlight the need to develop more mobile and easily accessible field methods to constrain denitrification from terrestrial soils across scales.

Conflict of interest

None declared.

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Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.cosust.2020.08.006.

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