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Measuring denitrification and the N_2:N_2O emission ratio from agricultural systems

Johannes Friedl *[1], Clemens Scheer[2], David Rowlings[1]

[1] Institute for Future Environments, Queensland University of Technology, Brisbane, QLD 4000, Australia
[2] Institute for Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Kreuzeckbahnstrasse 19, 82467 Garmisch-Partenkirchen, Germany.

Corresponding author: johannes.friedl@qut.edu.au

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Extended Abstract

Denitrification is a significant pathway of reactive N-loss from terrestrial environments and has direct impacts on agricultural production and the environment. "Unfortunately, it is a miserable process to measure." [1]. Quantification of denitrification and its final products - N_2O and N_2 - is hindered by the high spatial and temporal variability of these emissions, but foremost by the difficulties of measuring N_2 emissions against a high atmospheric background. Several methods are available to tackle this challenge, including the acetylene inhibition technique (AIT), the Helium/O_2 atmosphere method and the ^{15}N gas flux method (^{15}NGF). The evaluation of denitrification methods regarding analytical accuracy, applicability from the laboratory to the field scale, and instrumentation requirements is critical for (a) method choice in denitrification studies, (b) interpretation of denitrification data and (c) the use of denitrification datasets for biogeochemical models simulating the N cycle. Understanding methods for measuring denitrification is therefore important for a broad spectrum of researchers beyond a purely analytical perspective.

The AIT has been widely used but leads to an irreproducible underestimation of denitrification rates, a bias which continues when denitrification estimates based on biogeochemical models include AIT datasets [2]. This bias demands the use of alternative methods when quantifying denitrification losses from agro-ecosystems. The Helium/O_2 atmosphere method allows the direct quantification of N_2 and N_2O evolved from soil against an N_2 free or reduced atmosphere. This method is non-destructive, no isotopically labelled fertiliser or inhibitor needs to be applied, and method sensitivity for N_2 fluxes is higher compared to the ^{15}N gas flux method [1]. However, the extensive instrument requirements of the Helium/O_2 atmosphere method limit its use to laboratory studies. The ^{15}NGF is the only sound method to quantify both N_2 and N_2O applicable in laboratory and field studies. It requires homogenous ^{15}N soil labelling, usually achieved by applying costly ^{15}N fertiliser with water. The application of large amounts of ^{15}N fertiliser with water does not affect the applicability of the ^{15}NGF in

intensively managed agro-ecosystems but impedes the quantification of denitrification losses in natural ecosystems.

Table 1. Advantages and disadvantages of methods measuring denitrification losses (N_2 and N_2O) from agricultural soils.

Method	Advantage	Disadvantage
Acetylene Inhibition technique (AIT)		
• inhibits the reduction of N_2O to N_2 • indirect estimate of denitrification • applicable in laboratory and field studies	• allows a large number of samples to be processed at a time • simple to conduct	Systematic underestimation of denitrification due to: • short supply of nitrifier NO_3^- • incomplete inhibition due to restricted diffusion in water saturated or fine-textured soil • the utilisation of C_2H_2 as a substrate • Rapid decomposition of C_2H_2 by microbes • Contamination of C_2H_2 with other gases • Scavenging of NO
Helium/O_2 atmosphere method		
• quantifies N_2 emissions in an N_2 free or reduced atmosphere • direct quantification of N_2 • applicable in laboratory studies only	• non-destructive • does not require the application of N fertiliser • low detection limit allows quantification of denitrification rates in natural ecosystems • incorporation of plants in incubation setups to simulate in field conditions and N plant uptake	• limited to laboratory studies • laborious procedure to establish N_2 reduced atmosphere Detection limit depends on: • the sensitivity of the GC • the leak-tightness of the incubation vessel • the density of the soil sample
^{15}N gas flux method (^{15}NGF)		
• direct quantification of N_2 • applicable in laboratory and field studies	• only sound method applicable in laboratory an field studies • Use of ^{15}N fertiliser allows the combination with ^{15}N tracer and pool dilution methods	• expensive equipment (IRMS) • requires the application of expensive ^{15}N enriched fertiliser • high detection limit

The ^{15}NGF uses a static chamber approach, and the detection limit depends on the headspace to area ratio and the closure time, but critically on the ^{15}N enrichment of the soil NO_3^- pool undergoing denitrification. In agro-ecosystems such as sugarcane, where fertiliser N is only applied at the beginning of the growing season, the application of ^{15}N fertiliser enables N_2 and N_2O measurements as long as $^{15}NO_3^-$ enrichment ensures detectable N_2 fluxes. The $^{15}NO_3^-$ enrichment decreases over time, leading to an increase of the method detection limit, which in consequence hinders N_2 and N_2O quantification over the entire growing season. Agro-ecosystems with repeated fertiliser N application such as intensively managed pastures are however ideal for the application of the ^{15}NGF, as the repeated application of ^{15}N fertiliser allows continuous detection of N_2 and N_2O fluxes.

Recent advances in denitrification methods target (a) the sensitivity of N_2 detection (b) the temporal resolution of N_2 measurements and (c) integrate soil gas diffusion into the quantification of denitrification losses from agricultural soils.

The sensitivity of the ^{15}NGF for N_2 fluxes has been improved by reducing the N_2 background in incubation vessels [3]. This approach has been taken to the field scale, improving the method's sensitivity and therefore allowing N_2 detection outside peak emission events [4]. This improved version of the ^{15}NGF requires a rather complex setup to flush the chamber headspace with a Helium/O_2 mixture to reduce the N_2 background, increasing costs for equipment and its maintenance. Nevertheless, the "improved ^{15}NGF" could be applied in agro-ecosystems with a single N fertiliser application, accounting for low N_2 fluxes and extending N_2 measurement periods, and therefore improving estimates of denitrification for these agro-ecosystems.

The development of automated greenhouse gas monitoring systems has generated high-frequency N_2O datasets, accounting for highly episodic N_2O fluxes. In contrast, the sampling frequency for N_2 and N_2O in the field using the ^{15}NGF method is usually restricted to daily measurements. A newly developed, mobile isotope ratio mass spectrometer (Field-IRMS), coupled to an automated chamber system detects N_2 and N_2O fluxes in-situ, in real time and at a sub-daily resolution [5]. Instrument requirements, costs and the challenges of operating the system under harsh weather conditions may limit the broad applicability of this approach. However, the Field-IRMS represents a significant step forward regarding the temporal resolution of N_2 and N_2O flux measurements, and further improvements such as the combination with reduced N_2 atmosphere [4] are promising to further the robustness of denitrification estimates from agricultural soils.

Soil gas diffusivity determines the influx of O_2 into the soil matrix, as well as the efflux of N_2 and N_2O following denitrification. In contrast to water filled pore space, soil gas diffusivity integrates air filled porosity as a function of soil bulk density, allowing establish empirical relationships between soil biological and physical interactions across soils with different bulk densities. Recent studies have suggested diffusivity thresholds for the onset of denitrification [6] and the increased reduction of N_2O to N_2 [7], and linked gas diffusivity to the temporal variation of N_2 surface fluxes from agricultural soils [8].

However, the diffusion of gases also has implications from a method point of view: The efflux of gases from the soil matrix is defined by the diffusion gradient between soil and atmosphere, an equilibrium which is altered by the use of the static chamber method. This can lead to an underestimation of surface fluxes, increased storage of gases in the soil matrix and subsoil diffusion of gases. Recent research [9] has confirmed this effect for N_2 and N_2O fluxes measured with the ^{15}NGF, demonstrating an underestimation of surface fluxes by more than 30%. Further research is needed to establish gas diffusion modelling estimating subsoil diffusion and storage of gases, allowing to correct for the underestimation of surface fluxes in chamber based denitrification methods.

Advances in denitrification methods have delivered an increasing number of datasets investigating N_2 and N_2O emissions across different agro-ecosystems using sound methods. The integration of soil gas diffusion modelling is likely to progress our understanding of drivers on magnitude and N_2:N_2O partitioning of denitrification, while ensuring the analytical accuracy of the measurements. New N_2 and N_2O detection methods, such as cavity-enhanced Raman multi-gas sensing [10] are still at the development stage but have the potential to overcome limitations of available methods. The appraisal of advances regarding temporal resolution and accuracy of denitrification measurements suggests that a combination of methods with soil-gas diffusion modelling is the way forward to improve denitrification estimates from agricultural systems in the future.

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