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Denitrification as a source of nitric oxide emissions from incubated soil cores from a UK grassland soil

Nadine Loick [a], Elizabeth R. Dixon [a], Diego Abalos [b], Antonio Vallejo [b], G. Peter Matthews [c], Karen L. McGeough [d], Reinhard Well [e], Catherine J. Watson [d], Ronnie J. Laughlin [d], Laura M. Cardenas [a, *]

[a] Rothamsted Research, North Wyke, Okehampton, Devon EX20 2SB, UK
[b] Technical University of Madrid, Chemistry and Agricultural Analysis, Madrid, Spain
[c] School of Geography, Earth and Environmental Sciences, University of Plymouth, Davy Building, Drake Circus, Plymouth, Devon PL4 8AA, UK
[d] Agri-Food and Biosciences Institute, Newforge Lane, Belfast BT9 5PX, UK
[e] Thünen-Institut für Agrarklimaschutz, Bundesallee 50, 38116 Braunschweig, Germany

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ABSTRACT

Agricultural soils are a major source of nitric oxide (NO) and nitrous oxide (N_2O), which are produced and consumed by biotic and abiotic soil processes. The dominant sources of NO and N_2O are microbial nitrification and denitrification. While N_2O emissions have been attributed to both processes, depending on the environmental conditions such as substrate availability, pH and water filled pore space (WFPS), NO emissions are thought to predominantly derive from nitrification. Although attributing gaseous emissions to specific processes is still difficult, recent findings challenge the latter of those assumptions. Using the gas-flow-soil-core method, i.e soil cores incubated under a He/O_2 atmosphere at constant surface gas flow, combined with ^{15}N labelled isotopic techniques, the present study investigated the role of denitrification on NO, N_2O and N_2 emissions in a UK grassland soil under high soil moisture and an aerobic headspace atmosphere. With the application of KNO3 and glucose to support denitrification, denitrification was the source of N loss of between 0.61 and 0.67% of the added N via NO emissions, 1.60 −1.68% via N_2O and 0.03−0.05% via N_2 emissions. Overall, our study showed that denitrification has been overlooked as a source of NO emissions.

1. Introduction

Agricultural soils are the dominant source of nitrous oxide (N_2O), a potent greenhouse gas and a major cause of ozone layer depletion (IPCC, 2007; Ravishankara et al., 2009). Other gaseous forms of nitrogen (N) are lost from agricultural soils, such as N_2 which together with N_2O represents less N available for crop growth. Soils also act as a significant source of nitric oxide (NO), which catalyses the formation of ground level ozone, affecting human health and vegetation (Crutzen, 1981), and contributes to the formation of acid rain and the eutrophication of semi-natural ecosystems. Microbial denitrification is often the dominant process generating N_2O, and as such, intense investigations (i.e. >1000 published studies) have led to a good understanding of the abiotic factors regulating N_2O emissions via denitrification (Beaulieu et al., 2011). However, the role of this process on NO emissions remains largely unexplored, apart from a few studies (Wang et al., 2011, 2013), even though NO is an obligatory intermediate of N_2O formation in denitrification (Wolf and Russow, 2000; Russow et al., 2009).

Most experiments suggest that NO emitted from soils is mainly produced through nitrification (Skiba et al., 1997), whereas that produced from denitrification is further reduced to N_2O before it escapes to the soil surface (Skiba et al., 1997). This is attributed to high soil water content (it has been shown that at a WFPS above 70%, N_2O was produced solely by denitrification (Bateman and Baggs, 2005)), soil compaction and fine soil texture (sieved to <2 mm) creating low diffusivity for gases, which increases the residence time and the potential for further reduction when denitrification conditions dominate. Recent findings, however, challenge these assumptions. Using the gas-flow-soil-core technique, which has been proven to be a reliable tool for quantifying

* Corresponding author. Tel.: +44 (0) 1837 883 500.
E-mail address: laura.cardenas@rothamsted.ac.uk (L.M. Cardenas).

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emissions from denitrification, Wang et al. (2013) observed significant NO fluxes from nitrate (NO_3^-) amended soils. Attributing these emissions specifically to denitrification has remained elusive due to methodological constraints to elucidate the underlying microbial production and consumption processes. Previous efforts to identify these processes have mostly relied on acetylene inhibition and isotope labelling techniques (Baggs, 2008).

Isotope analysis has emerged as a way to identify the source and thereby the processes from which N_2O is being produced (Arah, 1997). It is also known that microorganisms discriminate against the heavier molecule (e.g ^{15}N vs. ^{14}N), preferring to use the lighter molecule which requires less energy to break the bonds (Kendall and Caldwell, 1998). This should be considered when applying labelled substrate to investigate microbial processes.

The aim of this study was to explore the potential role of denitrification as a significant source of NO emissions. We hypothesise that denitrification can be a major source of NO emissions in a UK grassland soil under high moisture content. This study uses the gas-flow-soil-core technique (Cárdenas et al., 2003), further developed to include NO measurements, combined with isotopic analyses. A ^{15}N labelled substrate as well as an unlabelled substrate at the same application ratio was used to determine whether there was an effect of the labelled N on the investigated processes at a 5 atom% enrichment. Additionally to adding potassium nitrate (KNO_3) as N source, glucose was added to supply a readily available C source and thereby promote denitrification. During denitrification C is used as electron donor and C availability is one factor controlling denitrification rates and compared to other C-compounds, denitrification tends to be most stimulated after addition of ethanol or glucose (Morley and Baggs, 2010).

2. Materials and methods

2.1. Soil preparation

A clayey pelostagnogley soil of the Hallsworth series (Clayden and Hollis, 1984) (44% clay, 40% silt, 15% sand (w/w), Table 1) was collected on the 4th of November 2013 from a typical grassland in SW England, located at Rothamsted Research, North Wyke, Devon, UK (50°46′10″N, 3° 54′05″W). Spade-squares (20 × 20 cm to a depth of 15 cm) of soil were taken from 12 locations along a 'W' line across a field of 600 m^2 size. After sampling, the soil was air dried to ~30% H_2O (dry basis), roots and plant residue were removed and the soil sieved to <2 mm and stored at 4 °C for 5 days before packing into cores and starting the incubation.

2.2. Experimental setup

The incubation was carried out using the DENItrification System (DENIS), a specialized gas-flow-soil-core incubation system (Cárdenas et al., 2003). Twelve cores were packed with soil to a bulk density of 0.8 g cm^{-3} and a height of 75 mm into stainless steel vessels of 140 mm diameter. To ensure denitrification conditions, the soil moisture was adjusted to 85% WFPS, taking the later amendment into account. This WFPS was similar to those used in previous studies to promote denitrification processes (Meijide et al., 2010; Bergstermann et al., 2011). In order to measure N_2 fluxes the native atmosphere was removed by flushing the soil cores from the bottom with a mixture of $He:O_2$ (80:20) at 30 ml min^{-1} for 14 h Flow rates were then decreased to 12 ml min^{-1} and the flow re-directed over the surface of the soil core for three days before amendment application to measure baseline emissions. O_2 was kept in the gas mixture at atmospheric levels as the objective was to investigate denitrification achieved by high WFPS instead of forcing anaerobic conditions by preventing any O_2 diffusion.

Table 1
Soil characteristics (before amendment application). Mean ± standard error ($n = 3$).

Parameter	Amount
pH water [1:2.5]	5.6 ± 0.27
Available Magnesium (mg kg^{-1} dry soil)	100.4 ± 4.81
Available Phosphorus (mg kg^{-1} dry soil)	10.4 ± 1.10
Available Potassium (mg kg^{-1} dry soil)	97.5 ± 12.83
Available Sulphate (mg kg^{-1} dry soil)	51.7 ± 0.62
Total N (% w/w)	0.5 ± 0.01
Total Oxidised N (mg kg^{-1} dry soil)	15.1 ± 0.07
Ammonium N (mg kg^{-1} dry soil)	9.2 ± 0.09
Organic Matter (% w/w)	11.7 ± 0.29

The following treatments were applied to four replicate vessels: (a) labelled (^{15}N-labelled KNO_3 at 5 atom% and glucose); (b) unlabelled (KNO_3 and glucose); (c) control (water only). The labelled and unlabelled treatments contained nitrogen at a rate equivalent to 75 kg N ha^{-1} (i.e. 121.5 mg N kg^{-1} dry soil) and C as glucose at 400 kg C ha^{-1} (i.e. 648 mg C kg^{-1} dry soil), which is similar to previous studies (Meijide et al., 2010; Bergstermann et al., 2011). The amendment for each core was dissolved in 50 ml distilled water, and the controls also received 50 ml distilled water each. The vessels were kept at 20 °C during the whole incubation period, which lasted for 10 days after amendment application.

2.3. Gas analyses and data manipulation

Gas samples were taken every two hours for each vessel. Fluxes of N_2O and CO_2 were quantified using a Perkin Elmer Clarus 500 gas chromatograph (Perkin Elmer Instruments, Beaconsfield, UK) equipped with an electron capture detector (ECD) for N_2O, and with a flame ionization detector (FID) and a methanizer for CO_2. N_2 emissions were measured by gas chromatography with a helium ionisation detector (VICI AG International, Schenkon, Switzerland) (Cárdenas et al., 2003), while NO concentrations were determined by chemiluminescence (Sievers NOA280i, GE Instruments, Colorado, USA). All gas concentrations were corrected for the surface area and flow rate going through the vessel (measured daily). Fluxes were calculated on a kg N or C ha^{-1} day^{-1} basis.

2.4. Isotopic analyses of N_2O

Gas sampling times for ^{15}N analysis were pre-determined based on data from previous experiments (data not shown). Samples were taken just before (0 h) and 4 h after amendment application, then every 24 h for the first week, followed by a final sample at day 10. This sampling strategy was decided on from previous experimental results to cover changes in isotopic signature before amendment application, as well as during the NO and N_2O peaks (4–5 h and 3–4 d, respectively), and after emissions returned to background levels. Samples were taken from the outlet line of each vessel using 12 ml exetainers (Labco) which had previously been flushed with He and evacuated. ^{15}N enrichment of N_2O was measured using a TG2 trace gas analyser (Europa Scientific, now Sercon, Crewe, UK) and Gilson autosampler, interfaced to a Sercon 20–22 isotope ratio mass spectrometer (IRMS). Solutions of 6.6 and 2.9 atom% ammonium sulphate ($(NH_4)_2SO_4$) were prepared and used to generate 6.6 and 2.9 atom% N_2O (Laughlin et al., 1997) which were used as reference and quality control standards.

The process leading to the formation of the measured N_2O, i.e. whether it is produced by nitrification or denitrification, was determined by calculating how much of the N_2O was derived from NO_3^- as the parent molecule. When ^{15}N labelled NO_3^- is added, it is