



Anaerobic digestates lower N₂O emissions compared to cattle slurry by affecting rate and product stoichiometry of denitrification – An N₂O isotopomer case study

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ABSTRACT

Assessing effects of organic fertilizer applications on N₂O emissions is of great interest because they can cause higher N₂O emissions compared to inorganic fertilizers for a given amount of added nitrogen (N). But there are also reports about enhanced N₂O reduction to climate-neutral elemental N₂ after application of organic manures to soils. Factors controlling the N₂O/(N₂O + N₂) product ratio of denitrification are interrelated, and also the ratio is difficult to study because of limitations in N₂ flux measurements. In this study, we investigated N₂O and N₂ emissions from soil treated with organic fertilizers with different C/N ratios. An N₂O isotopomer approach combined with conventional N₂O and N₂ flux measurements was employed to study underlying microbial pathways.

A grassland soil was amended with anaerobic digestate (AD) from food waste digestion (low C/N ratio) or cattle slurry (CS; high C/N ratio), respectively, adjusted to 90% WFPS, and incubated for 52 days under helium–oxygen atmosphere (10% O₂) using a soil incubation system capable of automated N₂O, N₂, and CO₂ measurements. N₂O isotopomer signatures, i.e. the δ¹⁸O and SP values (site preference between ¹⁵N at the central and the peripheral position in the N₂O molecule), were determined by Isotope Ratio Mass Spectrometry and used to model and subsequently estimate the contribution of bacterial denitrification and autotrophic nitrification to N₂O production. For this approach the direct determination of emitted N₂ is essential to take isotope effects during N₂O reduction to N₂ into account by correcting the measured isotope signatures for isotope effects during N₂O reduction using previously determined fractionation factor ranges.

The addition of both organic fertilizers to soil drastically increased the rate of gaseous N emissions (N₂O + N₂), probably due to the effects of concurrent presence of nitrate and labile C on the denitrification rate. In the initial phase of the experiment (day 1 to ~15), gaseous N emissions were dominated by N₂ fluxes in soils amended with organic manures; meanwhile, N₂O emissions were lower compared to untreated Control soils, but increased after 15–20 days relative to the initial fluxes, especially with CS. Extremely low N₂O, but high N₂ emissions in the initial phase suggest that reduction of N₂O to N₂ via denitrification was triggered when the soil was amended with organic fertilizers. In contrast in the untreated Control, N₂O release was highest during the initial phase. Total N₂O release from AD treated soil was similar to Control, while N₂O from CS treated soil was considerably higher, indicating that denitrification was triggered more by the high labile carbon content in CS, while the cumulative N₂O/(N₂O + N₂) product ratio and thus N₂O reduction were similar with both organic fertilizers.

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The results of the N₂O source partitioning based on the isotopomer data suggest that about 8–25% (AD) and 33–43% (CS) of the cumulated N₂O emission was due to nitrification in organically amended soil, while in the untreated Control nitrification accounted for about 5–16%. The remaining N₂O production was attributed mainly to denitrification, while the poor model fit for other source pathways like fungal denitrification suggested their contribution to be of minor importance. The observed rather distinct phases with predominance first of denitrification and later of nitrification may help developing mitigation measures by addressing N₂O source processes individually with appropriate management options. The observation of relatively large shares of nitrification-derived N₂O is surprising, but may possibly be related to the low soil pH and will require further investigation.

The determination of N₂ production is essential for this isotopomer-based source partitioning approach, but so far only applicable under laboratory conditions. The results of this study indicate that the combination of N₂O $\delta^{18}\text{O}$ and SP values is very useful in obtaining more robust source estimates as compared to using SP values alone.

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1. Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas (GHG; Stocker et al., 2013) and ozone depleting substance affecting the stratospheric ozone layer (Ravishankara et al., 2009). Increasing atmospheric N₂O concentrations are caused by anthropogenic activities, in particular by the intensification of agriculture production and introduction of huge amounts of reactive nitrogen (N) species into the global N cycle (Galloway et al., 2004).

N₂O mainly derives from agricultural soils, where high N fertilizer inputs are directly related to N₂O production (Mosier et al., 1998; Galloway et al., 2004), resulting in 2–2.5% conversion of fertilizer N to N₂O (Davidson, 2009). However, understanding of factors controlling N₂O production and consumption processes in soils has still to be improved to implement more advanced mitigation strategies to reduce undesirable side effects of fertilizer use.

Renewable energy sources like biogas produced from various organic substrates like food waste, animal wastes, and energy crops for generating electricity and heat are explicitly promoted and gained importance in several European countries (Herrmann and Rath, 2012). The residues of the biogas fermentation process, anaerobic digestates (AD), are mostly applied to agricultural land as 'organic' fertilizers. However, AD may, similar to other organic fertilizers (e.g. livestock manures, human waste), increase N₂O emissions from soils compared to mineral N fertilizers (e.g. Senbayram et al., 2009; Köster et al., 2011), because residual organic carbon substrates favor microbial denitrification, the respiratory nitrate reduction, in soils (Robertson and Groffman, 2007).

Various stable isotope techniques have been developed to study N transitions and to differentiate N₂O source processes as reviewed by Baggs (2008). However, they are all afflicted with various shortcomings. Recently, the analysis of isotopomers, the intramolecular ¹⁵N distribution in the linear asymmetric N₂O molecule, has been introduced as a mean of differentiating source processes (Toyoda and Yoshida, 1999; Yoshida and Toyoda, 2000). In addition to bulk isotope signatures, the parameter 'site preference' (SP), defined as the difference of $\delta^{15}\text{N}$ at the central (α) and the peripheral (β) position of the N₂O molecule has been proposed. Distinct N₂O $\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{18}\text{O}$, and SP signatures have been identified to be specific for bacterial denitrification (including nitrifier denitrification), nitrification (i.e. ammonium oxidation via hydroxylamine), or fungal denitrification (Sutka et al., 2003, 2004, 2006, 2008; Toyoda et al., 2005), and the SP is considered to be independent of the isotopic signature of the precursor species (Toyoda et al., 2002). However, this approach may be complicated by some overlap of signatures of autotrophic nitrification and fungal denitrification (Sutka et al., 2006, 2008), and by N₂O reduction to N₂

altering the N₂O SP (Ostrom et al., 2007). Additional information may be obtained by the relationship of N₂O $\delta^{18}\text{O}$ and SP values, which have been shown to be linearly correlated with the SP in N₂O significantly affected by N₂O reduction (Jinuntuya-Nortman et al., 2008; Well and Flessa, 2009a; Köster et al., 2013b), but interpretation of isotopomer results still remains challenging (Decock and Six, 2013). However, this approach can provide valuable information on N₂O releasing soil processes, especially when combined with other techniques like direct measurements of the main denitrification products N₂O and N₂.

Recent developments and application of improved process models promote the understanding of isotope effects during N₂O production and consumption processes and associated fractionation factors, thus allowing better estimations on source processes (Toyoda et al., 2011; Lewicka-Szczebak et al., 2014).

The objectives of this study were to compare organic fertilizers of different C/N ratio for their effect on total N₂O and N₂ losses by denitrification and associated changes in the N₂O/(N₂O + N₂) product ratio. Therefore, an incubation experiment was carried out under conditions favorable for denitrification. The use of an N₂-free helium-oxygen incubation atmosphere in an automated soil incubation system allowed direct simultaneous measurement of N₂O, N₂, and CO₂. Denitrification was expected to be the major microbial pathway contributing to N₂O production, though also nitrification (i.e. ammonium oxidation via hydroxylamine) was expected to play a role following the application of AD and cattle slurry (CS) as ammonium based organic fertilizers. This was to be confirmed by applying the N₂O isotopomer approach, as nitrification-derived N₂O is characterized by higher $\delta^{18}\text{O}$ and SP values. ¹⁸O and site-specific ¹⁵N fractionation factors may vary over relative wide ranges, which thus was taken into account. N₂O source partitioning was conducted independently using N₂O $\delta^{18}\text{O}$ and SP signatures, respectively, to cross-check the N₂O source estimates.

2. Materials and methods

2.1. Sampling and properties of soil and organic fertilizers

The soil was collected in Rowden Moor (50°46'26"N, 03°55'47"W; 162 m above sea level), Okehampton, Devon, UK. This soil is classified as a Hallsworth Series soil, which is a clayey non-calcareous Pelostagnogley in head from clay shale (Harrod and Hogan, 2008). The bulk density was about 0.9–1 g cm⁻³. The sampling site had previously been in grassland use but it has been left fallow for the last ten years. The upper 2 cm of soil and roots

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