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Quantifying the contribution of nitrification and denitrification to the nitrous oxide flux using ^{15}N tracers

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Identifying the mechanisms responsible for N_2O emission in soils could help define potential mitigation methods.

Abstract

Microbial transformations of nitrification and denitrification are the main sources of nitrous oxide (N_2O) from soils. Relative contributions of both processes to N_2O emissions were estimated on an agricultural soil using ^{15}N isotope tracers ($^{15}NH_4^+$ or $^{15}NO_3^-$), for a 10-day batch experiment. Under unsaturated and saturated conditions, both processes were significantly involved in N_2O production. Under unsaturated conditions, 60% of N-N_2O came from nitrification, while denitrification contributed around 85–90% under saturated conditions. Estimated nitrification rates were not significantly different whatever the soil moisture content, whereas the proportion of nitrified N emitted as N_2O changed from 0.13 to 2.32%. In coherence with previous studies, we interpreted this high value as resulting from the decrease in O_2 availability through the increase in soil moisture content. It thus appears that, under limiting aeration conditions, some values for N_2O emissions through nitrification could be underestimated.

Keywords: Nitrification; Denitrification; Nitrous oxide; ^{15}N tracer; Soil moisture content

1. Introduction

Nitrous oxide (N_2O) is a greenhouse gas involved in global warming and stratospheric ozone depletion (Williams et al., 1992). Soils, and particularly agricultural soils, are a dominant source of N_2O (IPCC, 2001). Soil heterogeneity permits the coexistence of aerobic and anaerobic zones which allow organisms in adjacent ecological niches to function simultaneously. Nitrous oxide is an intermediate product formed during the denitrification process (Tiedje, 1988). It is also formed during the nitrification process via the oxidation of ammonia (NH_3) to nitrite (NO_2^-), and through chemical decomposition of intermediates such as hydroxylamine (NH_2OH). Nitrous oxide can also be formed via nitrifier-denitrification, as defined by Wrage et al. (2001), when autotrophic NH_3 oxidisers oxidise NH_3 to NO_2^- and then reduce the NO_2^- to N_2O and N_2.

Identifying the mechanisms responsible for N_2O emission could help define potential mitigation methods. The relative contribution of nitrification and denitrification to the formation of N_2O varies considerably, and depends on the environmental conditions. It is generally admitted that under aerobic conditions, nitrification is the dominant process for N_2O formation, while denitrification generally occurs under anaerobic conditions (Stevens et al., 1997; Wolf and Russow, 2000).

Process-oriented models of N_2O emissions from soils propose different methods for calculating the relative denitrification and nitrification contributions of a specific N_2O emission event. For example, the CENTURY model assumes 2% of nitrified N is lost as N_2O, while in Expert-N (Frolking et al., 1998), this same fraction is set as 0.5%. In the PnET-N-DNDC model, nitrification is only allowed to occur outside

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the anaerobic zone and nitrification-induced N_2O represents 0.06% of the predicted gross nitrification rate (Li, 2000). In the NOE model, the nitrification derived N_2O is based on the individual soil (Hénault et al., 2005). Hence, in simulations, both nitrification-induced N_2O and the relative contribution of nitrification to N_2O simulation vary with the model used.

In order to improve process-orientated models of N_2O emissions we must obtain more accurate and realistic data on N_2O emission mechanisms. Difficulties in obtaining accurate data may be due to methodological techniques not discriminating simultaneously occurring emission mechanisms, i.e. nitrification, denitrification and nitrifier-denitrification. Some authors have suggested that the denitrifier component of the N_2O emissions can be separated out by calibrating in aerobiosis and thus the portion of N_2O emitted during nitrification can be determined (Cheng et al., 2004; Garrido et al., 2002). However, results with this method may be biased by the occurrence of denitrification under aerobic conditions thus making extrapolation of field conditions difficult (Müller et al., 2004a; Renault and Stengel, 1994; Robertson et al., 1995). The acetylene inhibition method has been used to try and discriminate between nitrification and denitrification mechanisms of N_2O production (Garrido et al., 2002; Klemedtsson et al., 1988), but is biased due to potential artefacts (Bollmann and Conrad, 1997a,b). Ingwersen et al. (1999) and Müller et al. (2004b) have proposed estimations based on the Barometric Process Separation (BaPS) method, where the changes in pressure and gas concentration provide information about the contribution of nitrification and denitrification to the N_2O emission from soil. Methods based on the radioisotope ^{13}N are of great interest but they are restricted to a very few laboratories (Speir et al., 1999). Recent developments in the use of the isotopic ^{15}N spike, from pair or single treatment, offer good opportunity to identify N_2O sources and to quantify relative contributions of nitrification (clearly identified as either gross or net nitrification) and denitrification to N_2O production (Baggs et al., 2003; Khalil et al., 2004; Russow et al., 2000; Stevens et al., 1997; Wolf and Russow, 2000).

The aim of this work was to investigate the relative contributions of nitrification and denitrification to N_2O emission using the stable isotope ^{15}N. Soil incubations were performed under soil moisture conditions favouring both nitrification and denitrification processes.

2. Materials and methods

2.1. Soil sampling

The study soil was a cultivated Gleyic luvisol located at Cîteaux (47°08′N, 5°06′E) in the Saône river plain, near Dijon (Eastern France). In the surface (0–20 cm) soil, the pH_{water} was 7.1. The inorganic fraction of the soil contained 13.5% clay, 51.9% silt and 34.6% sand. Mean carbon and nitrogen contents were of 8.0 mg C g^{-1} soil and 0.8 mg N g^{-1} soil, respectively. Soil was collected in May 2004 and partially air-dried for one day until it could be sieved through a 2 mm mesh.

2.2. Experimental design and soil incubation

Soil incubations were performed according to the following soil moisture and ^{15}N labelled inorganic-N treatments. Soil was incubated at either −0.1 or −0.001 MPa soil water potential (ψ), corresponding to unsaturated (75% of the field capacity) and saturated (150% of the field capacity) conditions, respectively, along with an aliquot of inorganic-N. Two N treatments were performed: (i) soil treated with a ^{15}N nitrate spike containing ^{15}N-labelled KNO_3 and natural abundance $(NH_4)_2SO_4$; and (ii) soil treated with a ^{15}N-ammonium spike containing natural abundance KNO_3 and ^{15}N-labelled $(NH_4)_2SO_4$. The concentrations of the solutions were adjusted so that the resulting inorganic-N added to the soil was at a concentration of 0.1 mg N g^{-1} soil. The sieved soil (20 g fresh) was placed in a 120 ml flask along with pipetted inorganic-N solution (2.5 and 5.0 ml of solution for the −0.1 and −0.001 MPa treatments, so that the resulting soil moisture content was 14 and 27% (w/w), respectively). Soil incubations were performed at 20 °C, under dark conditions. During incubation, the partial pressure of oxygen in the surrounding atmosphere was poorly affected during the 10-day period, being always higher than 18% at the end of incubation. Flasks were prepared in this way, three replicates per treatment, for sampling at time zero and 2, 4, 6, 8 and 10 days, giving a total of 72 flasks. Headspace samples were taken at the above-mentioned sampling times in previously purged 3-ml Terumo Vacutainer tubes for N_2O concentrations and 10 ml flasks for $^{15}N_2O$ analyses. At each sampling period, mineral nitrogen was extracted on soil samples used for N_2O sampling with 100 ml of 1 M KCl solution. Extracts were filtered through a N°42 Whatman filter paper and then frozen prior to analysis.

2.3. Soil and gas analysis

Nitrate concentrations were determined from KCl extracts by colorimetric analysis using the sodium salicylate procedure described by Yang et al. (1998). Ammonium concentrations were determined using the colorimetric indophenol method. Measurements were performed on an Anthelie Advanced spectrophotometer (Secomam, France). The ^{15}N enrichment of ammonium, nitrate plus nitrite forms, was determined using a diffusion method modified from Brooks et al. (1989). The diffused gases of NH_4^+, produced by alkalising the KCl extract with around 0.1 g of MgO, were first collected on a GF/D glass fiber disc (6 mm diameter) suspended on a stainless steel wire and impregnated with 10 µl of 2.5 M $KHSO_4$. The samples were diffused on an orbital shaker at 150 rpm for 48 h at room temperature. After 48 h, discs were removed for drying. Further discs were then suspended over the extract solution, along with Devarda's reactant (0.5 g) for $NO_2^- + NO_3^-$ determinations and left a further 48 h before being removed. After removal from the extract solution, discs were dried overnight in an H_2SO_4 desiccator, and placed in tin capsules. The ^{15}N