Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil

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ARTICLE INFO

ABSTRACT

Soils are the major source of the greenhouse gas nitrous oxide (N_2O) to our atmosphere. A thorough understanding of terrestrial N_2O production is therefore essential. N_2O can be produced by nitrifiers, denitrifiers, and by nitrifiers paradoxically denitrifying. The latter pathway, though well-known in pure culture, has only recently been demonstrated in soils. Moreover, nitrifier denitrification appeared to be much less important than classical nitrate-driven denitrification. Here we studied a poor sandy soil, and show that when moisture conditions are sub-optimal for denitrification, nitrifier denitrification can be a major contributor to N_2O emission from this soil. We conclude that the relative importance of classical and nitrifier denitrification in N_2O emitted from soil is a function of the soil moisture content, and likely of other environmental conditions as well. Accordingly, we suggest that nitrifier denitrification should be routinely considered as a major source of N_2O from soil.

1. Introduction

Nitrous oxide has become the third most important anthropogenic greenhouse gas (IPCC, 2007), and is today's single most important ozone-depleting emission (Ravishankara et al., 2009). When aiming to mitigate N_2O emissions, accurate understanding of the biochemical processes responsible for N_2O production is crucial (Baggs, 2008). Although a wide range of processes has the potential to produce N_2O, its production in soil is generally primarily attributed to nitrification and denitrification. Semantics may confuse this apparently simple paradigm, since various nitrifiers are able to denitrify as well. This nitrifier denitrification (ND) by ammonia oxidizing bacteria (AOB) has long been acknowledged in pure cultures (Hooper, 1968; Ritchie and Nicholas, 1972), and it has been suggested that ND could be a universal trait in beta-proteobacterial ammonium oxidizers, which are thought to be the dominant ammonium oxidizing bacteria in soil (Shaw et al., 2006). As it is well established that nitrifying micro-organisms contribute

significantly to N_2O emission from soils (Bremner, 1997), and as soils are the major source of N_2O to the atmosphere (IPCC, 2007), insight in the potential of ND in soils is of global environmental interest. An increasing number of studies suggests that ND may contribute significantly to N_2O production in soil (Webster and Hopkins, 1996; McLain and Martens, 2005; Wrage et al., 2005; Venterea, 2007), but definite proof has remained elusive due to methodological constraints (Wrage et al., 2001, 2005; Kool et al., 2007). Only recently a novel multi-isotope tracing approach was presented (Kool et al., 2010) that accounts for the potential exchange of O between water and intermediate compounds of N_2O production (Kool et al., 2007, 2009a). This enabled further discrimination of nitrifier denitrification (ND) as an N_2O production pathway that is distinct from conventional nitrification (NN) and denitrification (FD and NCD: denitrification of applied -fertilizer-NO_3^- and nitrification-coupled denitrification, respectively) (Fig. 1). It provided best proof to date in soil-based experiments that ND can indeed produce N_2O in soil. However, the relative contribution of ND to total N_2O emission was minor in this set-up, as production was dominated by FD (Kool et al., 2010). This may be explained by experimental conditions, which at 80% water-filled pore space (WFPS) were optimal for denitrification. To study the significance of ND under conditions less optimal for denitrification and more representative soil conditions we here incubated soil under

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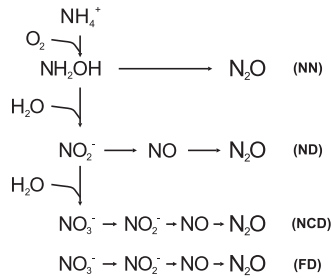 E-mail address: d.kool@science.ru.nl (D.M. Kool).

Fig. 1. Depiction of the major pathways of N_2O formation. We distinguish N_2O production from nitrifiers as by-product of ammonia oxidation, i.e. nitrifier nitrification (NN), and through nitrifier denitrification (ND), and from denitrifiers by reduction of NO_3^- produced from nitrification, i.e. nitrification-coupled denitrification (NCD), and by reduction of applied NO_3^-, i.e. fertilizer denitrification (FD).

a variety of moisture conditions. N_2O production was studied from soil incubated at 50, 70, and 90% WHC using the novel multi-isotope tracing approach (Kool et al., 2010).

2. Materials and methods

2.1. Incubation experiment

Replicate samples of a poor sandy soil (pH 5.4) were incubated in glass jars for 28 h after application of 50 mg NH_4^+–N kg^{-1} and 50 mg NO_3^-–N kg^{-1} soil, with treatment-specific labeled compounds: ^{18}O-labeled H_2O or NO_3^-, or ^{15}N-labeled NO_3^- or NH_4^+ (TR1, TR2, TR3 and TR4 respectively). Three moisture treatments were used, i.e. 50, 70, and 90% WFPS. Lids were kept closed (airtight) during the 28 h incubation period. Analyses on a random selection of gas samples confirmed that O_2 concentrations in the headspace had not notably declined during the incubation. More specifics about the incubation set-up and analyses can be found in previous work (Kool et al., 2009a, 2009b, 2010). The soil used here was not under study in these previous experiments. We here provide a summary of the approach and calculations.

At the end of the incubation, N_2O production, soil mineral N content, and their relevant O and N isotopic signatures were determined (Kool et al., 2009b). From the ^{15}N-enrichment data the relative contributions of NH_4^+–N and NO_3^-–N to total N_2O production were derived (Kool et al., 2009a). Analyses of the ^{15}N–NH_4^+ in the ^{15}N–NO_3^- labeling treatment confirmed that the contribution of DNRA as potential N_2O producing pathway was negligible. Oxygen exchange during production of N_2O from NO_3^- reduction (X_{ERR}) was determined by the ERR method (Kool et al., 2009b)(using the data from the $^{18}O-$ and ^{15}N–NO_3^- labeling treatments) and taken into account with further data evaluation. The potential minimum and maximum contribution of the different pathways to total N_2O production was calculated following the combined O- and N-isotope tracing approach presented by Kool et al. (2010). A summary of the main calculations of this approach is provided below (Section 2.2). The main assumptions underlying the approach are that (i) N_2O produced as by-product of ammonia oxidation (NN) obtains all oxygen from O_2 (no O incorporation from H_2O), (ii) O incorporation from H_2O into N_2O from FD, ND and NCD is respectively zero, 1/2nd and 2/3rd through reaction stoichiometry, and can be increased as an effect of O exchange at the level of X_{ERR} for all these pathways, and that (iii) nitrate (not only nitrite) is an obligatory intermediate for nitrification-coupled denitrification (NCD).

All treatments were replicated 5 times, which yielded standard errors of the means of isotope enrichment data. The variables X_{ERR}, $N_2O(NH_4^+)$, and $N_2O(NO_3^-)$ are defined as ratios of averages of the

replicates, for which standard errors were approximated by a first-order Taylor linearization (Kool et al., 2010). As X_{ERR} is a key parameter in the analyses to derive the relative pathway contributions, we carried out a sensitivity analysis of this parameter. A full data evaluation was additionally carried out using the X_{ERR} plus or minus its standard error in the calculations. Oxygen exchange was set to zero when X_{ERR} was calculated to be negative in the evaluation.

2.2. Data calculations

The actual oxygen incorporation from H_2O into N_2O (AOI) is calculated from the ^{18}O enrichment of the N_2O and H_2O in treatment TR1, $^{18}O(N_2O_{(TR1)})$ and $^{18}O(H_2O_{(TR1)})$ respectively:

$$AOI = 100 \cdot \frac{^{18}O\left(N_2O_{(TR1)}\right)}{^{18}O\left(H_2O_{(TR1)}\right)}$$

The oxygen exchange (between H_2O and intermediates of the N_2O production pathways) during reduction of NO_3^- to N_2O, X_{ERR}, is calculated from the ^{18}O and ^{15}N enrichment of the N_2O in treatment TR2 and TR3 respectively, $^{18}O(N_2O_{(TR2)})$ and $^{15}N(N_2O_{(TR3)})$, and the imposed ^{18}O and ^{15}N enrichment of NO_3^- in those treatments, $^{18}O(NO_3^-_{(TR2)})$ and $^{15}N(NO_3^-_{(TR3)})$:

$$X_{ERR} = 100 \cdot \left(1 - \frac{^{18}O\left(N_2O_{(TR2)}\right)}{^{15}N\left(N_2O_{(TR3)}\right)} \cdot \frac{^{15}N(NO_3^-_{(TR3)})}{^{18}O(NO_3^-_{(TR2)})}\right)$$

The proportions of total N_2O derived from NH_4^+ and NO_3^-, $N_2O_{(NH_4^+)}$ and $N_2O_{(NO_3^-)}$, were calculated from the ^{15}N–N_2O enrichment in treatment TR3 and TR4, $^{15}N(N_2O_{(TR3)})$ and $^{15}N(N_2O_{(TR4)})$ respectively:

$$N_2O_{(NH_4^+)} = 100 \cdot \frac{^{15}N\left(N_2O_{(TR4)}\right)}{^{15}N\left(N_2O_{(TR3)}\right) + {}^{15}N\left(N_2O_{(TR4)}\right)}$$

$$N_2O_{(NO_3^-)} = 100 \cdot \frac{^{15}N\left(N_2O_{(TR3)}\right)}{^{15}N\left(N_2O_{(TR3)}\right) + {}^{15}N\left(N_2O_{(TR4)}\right)}$$

The relative contribution of FD to N_2O production, N_2O_{FD}, is defined as $N_2O_{(NO_3^-)}$:

$$N_2O_{FD} = N_2O_{(NO_3^-)}$$

The maximum proportion of N_2O that could have been derived from NCD, $N_2O_{NCD}^{max}$, was calculated from the ^{15}N enrichment of the N_2O and NO_3^- resulting from treatment TR4, $^{15}N(N_2O_{(TR4)})$ and $^{15}N(NO_3^-_{(TR4)})$:

If $^{15}N\left(N_2O_{(TR4)}\right) \leq {}^{15}N(NO_3^-_{(TR4)})$, then $N_2O_{NCD}^{max} = N_2O_{(NH_4^+)}$;

If $^{15}N\left(N_2O_{(TR4)}\right) > {}^{15}N(NO_3^-_{(TR4)})$, then $N_2O_{NCD}^{max}$

$$= N_2O_{(NH_4^+)} \cdot \left(\frac{^{15}N(NO_3^-_{(TR4)})}{^{15}N(NO_3^-_{(TR4)}) + {}^{15}N(NH_4^+_{(TR4)})}\right)$$

The NH_4^+-derived N_2O comprises the contributions of NN, ND and NCD. The $N_2O_{(NH_4^+)}$ is the sum of either (A) the maximum contribution of NN ($N_2O_{NN}^{max}$), the minimum of ND ($N_2O_{ND}^{min}$), and the maximum of NCD, or (B) the minimum contribution of NN $N_2O_{NN}^{min}$, the maximum of ND ($N_2O_{ND}^{max}$), and the minimum of NCD