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Isotopologue ratios of N_2O emitted from microcosms with NH_4^+ fertilized arable soils under conditions favoring nitrification

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ABSTRACT

Soils represent the major source of the atmospheric greenhouse gas nitrous oxide (N_2O) and there is a need to better constrain the total global flux and the relative contribution of the microbial source processes. The aim of our study was to determine variability and control of the isotopic fingerprint of N_2O fluxes following NH_4^+-fertilization and dominated by nitrification. We conducted a microcosm study with three arable soils fertilized with 0–140 mg NH_4^+–N kg^{-1}. Fractions of N_2O derived from nitrification and denitrification were determined in parallel experiments using the ^{15}N tracer and acetylene inhibition techniques or by comparison with unfertilized treatments. Soils were incubated for 3–10 days at low moisture (30–55% water-filled pore space) in order to establish conditions favoring nitrification. Dual isotope and isotopomer ratios of emitted N_2O were determined by mass spectrometric analysis of $\delta^{18}O$, average $\delta^{15}N$ ($\delta^{15}N^{bulk}$) and ^{15}N site preference (SP = difference in $\delta^{15}N$ between the central and peripheral N positions of the asymmetric N_2O molecule). N_2O originated mainly from nitrification (>80%) in all treatments and the proportion of NH_4^+ nitrified that was lost as N_2O ranged between 0.07 and 0.45%. $\delta^{18}O$ and SP of N_2O fluxes ranged from 15 to 28.4‰ and from 13.9 to 29.8‰, respectively. These ranges overlapped with isotopic signatures of N_2O from denitrification reported previously. There was a negative correlation between SP and $\delta^{18}O$ which is opposite to reported trends in N_2O from denitrification. Variation of average ^{15}N signatures of N_2O ($\delta^{15}N^{bulk}$) did not supply process information, apparently because a strong shift in precursor signatures masked process-specific effects on $\delta^{15}N^{bulk}$. Maximum SP of total N_2O fluxes and of nitrification fluxes was close to reported SP of N_2O from NH_4^+ or NH_2OH conversion by autotrophic nitrifiers, suggesting that SP close to 30‰ is typical for autotrophic nitrification in soils following NH_4^+-fertilization. The results suggest that the $\delta^{18}O$/SP fingerprint of N_2O might be used as a new indicator of the dominant source process of N_2O fluxes in soils.

1. Introduction

N_2O is an atmospheric trace gas contributing to global warming and stratospheric ozone depletion. Its major sources are nitrification and denitrification in soils and aquatic systems. Despite extensive studies on N_2O fluxes and turnover processes in various environments the knowledge on the global N_2O budget is still uncertain. Furthermore, the current knowledge on relative contributions of nitrification and denitrification is still unsatisfactory. Several studies investigated N_2O fluxes from both source processes at the laboratory scale using ^{15}N tracer and/or acetylene inhibition methods (Davidson et al., 1991; Stevens et al., 1997; Wolf and Russow, 2000; Bateman and Baggs, 2005) and were thus able to identify conditions governing the balance between nitrification and denitrification. Nevertheless, the contribution of each process at field or global scales is still not well understood. The study of N_2O fluxes related to microbial production of NO_3^- is further complicated by the various existing pathways (Wrage et al., 2001) including N_2O formation from intermediates of the NH_4^+-to-NO_2^- step of autotrophic and heterotrophic nitrification as well as reduction of NO_2^- from nitrification to N_2O (nitrifier denitrification).

Isotopic signatures of N_2O have been used to study sink and source processes of N_2O in terrestrial and aquatic systems and in the atmosphere (Stein and Yung, 2003) and to improve estimation of the atmospheric N_2O budget (Röckmann et al., 2003). There are several isotopologues differing in isotopic substitution of oxygen and/or the two N atoms within the N_2O molecule. Initially, only isotopologues differing in $\delta^{18}O$ or average $\delta^{15}N$ had been considered (Wada and Ueda, 1996). More recently, techniques to detect isotopomers of N_2O, i.e. isotopologues differing in the terminal and

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central N-positions of the linear molecule, were also developed (Toyoda and Yoshida, 1999; Brenninkmeijer and Röckmann, 1999).

Due to kinetic isotope effects, N_2O production of both nitrification and denitrification yields N_2O which is isotopically light in relation to its precursors whereas reduction during denitrification results in an enrichment of ^{15}N and ^{18}O in the residual N_2O (Barford et al., 1999; Mandernack et al., 2000; Menyailo and Hungate, 2006; Ostrom et al., 2007; Vieten et al., 2007). ^{15}N depleted N_2O found in aerobic aquifers and in oceans has been attributed to nitrification (Ueda et al., 1991; Ostrom et al., 2000). ^{15}N enrichment of N_2O in lakes (Wada and Ueda, 1996; Boontanon et al., 2000), oceans (Naqvi et al., 1998; Popp et al., 2002) and emitted from soils (Mandernack et al., 2000; Wrage et al., 2004; Tilsner et al., 2003) has been explained by N_2O reduction during denitrification.

Isotopomer analysis has recently been used to further refine the isotopic fingerprint of N_2O. In contrast to $\delta^{18}O$ and average $\delta^{15}N$ ($\delta^{15}N^{bulk}$), the difference between central and peripheral ^{15}N enrichment ($\delta^{15}N^{\alpha} - \delta^{15}N^{\beta} =$ site preference (SP)) is considered to be independent of the isotopic signature of the precursor (Popp et al., 2002; Toyoda et al., 2002) and thus supplies process information even if isotopic signatures of additional N species are lacking. Theoretically, N_2O production during nitrification and denitrification can cause ^{15}N accumulation at both N-sites, depending on the type of NO reductase catalyzing this reaction (Schmidt et al., 2004; Stein and Yung, 2003). This was also demonstrated experimentally with pure cultures of N_2O producing microbes, where NO_2^- reduction by *Nitrosomonas multiformis* and by several denitrifiers produced N_2O with negligible site preference (Sutka et al., 2006; Toyoda et al., 2005), NH_4^+ and NH_2OH oxidation of several nitrifiers yielded N_2O with similar high site preference (Sutka et al., 2003, 2004, 2006) and the denitrifier *Pseudomonas fluorescens* exhibited variable results (Toyoda et al., 2005). Because the reduction step of N_2O consists of the cleavage of NO-bonds it is expected to cause ^{15}N accumulation at the central N-position ($^{15}N^{\alpha}$) of the residual N_2O (Yoshida and Toyoda, 2000; Toyoda et al., 2002; Popp et al., 2002; Schmidt et al., 2004). There are only few studies reporting site specific ^{15}N signatures in N_2O emitted from soils (Pérez et al., 2001; Yamulki et al., 2001; Bol et al., 2003, 2004) which exhibited an enrichment of ^{15}N at the central N-position in most cases. However, because the partial processes of N_2O turnover were not determined independently in these studies, the ^{15}N site preference of N_2O originating from nitrification and denitrification, respectively, could not be distinguished. Recently, process-specific isotopomer signatures of N_2O were determined for nitrification and denitrification in tropical forest soils (Pérez et al., 2006) and for denitrification in a temperate arable soil (Well et al., 2006). Until now, there are no isotopomer data for N_2O fluxes from nitrification in arable soils.

How useful is the isotopologue fingerprint of soil emitted N_2O to identify source processes of N_2O in soils? This basic question has still not been sufficiently answered. The specific questions of this study were: are isotopologue signatures of N_2O fluxes from autotrophic nitrification distinct from the signatures of other N_2O forming processes? How variable are the signatures of autotrophic nitrification and what causes variability? To answer this, we investigated N_2O fluxes from arable soils incubated under low moisture and varying NH_4^+ fertilizer level with respect to the contribution from nitrification and denitrification and to the isotopic composition of N_2O ($\delta^{18}O$, $\delta^{15}N$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$).

2. Materials and methods

2.1. Soil properties

Three temperate arable soils were investigated which have been used in earlier N_2O studies (Deurer et al., 2008; Flessa and Beese, 1995; Ruser et al., 2006; Well et al., 2006; Gao, 2004), and which were considerably different in parameters relevant for N_2O turnover, i.e. texture, pH and organic C (Table 1). The soil types were a Haplic Luvisol (HL), a Gleyic Podzol (GP) and a Calcaric Cambisol (CC). Soils were collected from 0 to 10 cm depth, sieved at 4 mm mesh size, adjusted to a water content of approx 0.03 g g^{-1} below the target water content of each treatment and were then pre-incubated for 3 days at room temperature. After pre-incubation, soils contained between 0.2 and 1.6 mg NH_4^+–N kg^{-1} and between 15 and 50 mg NO_3^-–N kg^{-1}

2.2. Experiments on isotopic signatures of N_2O fluxes

We incubated different arable soils at relatively low moisture (30–55% water-filled pore space, WFPS) and varying NH_4^+–N fertilization to determine the isotopic signature of N_2O fluxes from autotrophic nitrification and to determine its variability. The experiments and treatments presented in this study are summarized in Table 2. Treatments are named with abbreviations consisting of the soil type (Haplic Luvisol, HL; Gleyic Podzol, GP; Calcaric Cambisol, CC), a sequential numbering of experiments (1–4), the NH_4^+–N fertilization level (N0–N140, mg N kg^{-1}) and the moisture level in terms of water-filled pore space (WFPS; 30–55%). In experiment 1, the Haplic Luvisol was incubated at 55% WFPS with varying NH_4^+-level. Because there was still some denitrification detectable in experiment 1, the same soil was incubated once more (experiment 2) at a lower moisture range (30–50% WFPS) in order to minimize denitrification. The fertilizer level was augmented to 140 mg NH_4^+–N kg^{-1} to induce longer lasting N_2O fluxes from nitrification, since N_2O fluxes in all fertilizer levels of experiment 1 had rapidly declined. In experiment 3, the Gleyic Podzol was incubated at the same WFPS range as in experiment 2. For the N-level, 40 mg NH_4^+–N kg^{-1} was chosen because this soil had shown relatively slow nitrification in preliminary studies. Finally, a Calcaric Cambisol from China was incubated with 40 mg NH_4^+–N kg^{-1} at 45% WFPS. These settings were chosen to keep conditions comparable to the nitrification treatment of a field experiment with the same soil.

Soils were moistened and fertilized using fertilizer solutions which were homogeneously mixed with the soil. One treatment without ^{15}N labeling (non-labeled treatment) was established to determine the isotopologue fingerprint of the N_2O produced. In experiments 2–4, further treatments with ^{15}N-labeled NO_3^- were conducted to enable quantification of N_2O derived from nitrification and denitrification (Table 2). Both labeling variants were supplemented with unlabeled NH_4SO_4 according to target NH_4-levels of the N-variants (Table 2). In the ^{15}N-labeled variants, 2 mg N kg^{-1} soil were added as KNO_3 at 60 atom % ^{15}N in order to spike the soil NO_3^--pool. Initial ^{15}N enrichments of the NO_3^--pool thus ranged between 3.5 and 15 atom % ^{15}N. The non-labeled treatments received the same amount as unlabeled KNO_3 in order ensure equal NO_3^--levels of the ^{15}N-labeled and non-labeled parallels.

The fertilized soil samples were packed into 400-mL screw-cap jars (8 cm height, 8.2 cm i.d.) to a height of 5 cm with bulk densities of 1.2 g cm^{-3} for the silt loam soils (HL, CC) and 1.5 g cm^{-3} g for the

Table 1
Types and basic properties of experimental soils

Soil type	Location	Sand (%)	Silt (%)	Clay (%)	C_{org} (mg g^{-1})	Total N (mg g^{-1})	pH ($CaCl_2$)
Haplic Luvisol (HL)	Bavaria, Germany	23	55	22	14.8	1.6	6.1
Gleyic Podzol (GP)	Lower Saxony, Germany	96.5	2	2.5	23.0	1.4	5.6
Calcaric Cambisol (CC)	Beijing area, China	32.7	50.2	17.1	13.0	1.0	8.0