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**The N<sub>2</sub>O  
consumption in soils**

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# The fate of N<sub>2</sub>O consumed in soils

**B. Vieten, F. Conen, B. Seth, and C. Alewell**

Institute of Environmental Geosciences, University of Basel, Bernoullistrasse 30, 4056 Basel, Switzerland

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Correspondence to: B. Vieten (b.vieten@unibas.ch)

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## Abstract

Soils are capable to consume  $N_2O$ . It is generally assumed that consumption occurs exclusively via respiratory reduction to  $N_2$  by denitrifying organisms (i.e. complete denitrification). Yet, we are not aware of any verification of this assumption. Some  $N_2O$  may be assimilatorily reduced to  $NH_3$ . Reduction of  $N_2O$  to  $NH_3$  is thermodynamically advantageous compared to the reduction of  $N_2$ . Is this an ecologically relevant process? To find out, we treated four contrasting soil samples in a flow-through incubation experiment with a mixture of labelled (98%)  $^{15}N_2O$  (0.5–4 ppm) and  $O_2$  (0.2–0.4%) in He. We measured  $N_2O$  consumption by GC-ECD continuously and  $\delta^{15}N$  of soil organic matter before and after an 11 to 29 day incubation period. Any  $^{15}N_2O$  assimilatorily reduced would have resulted in the enrichment of soil organic matter with  $^{15}N$ , whereas dissimilatorily reduced  $^{15}N_2O$  would not have left a trace. None of the soils showed a change in  $\delta^{15}N$  that was statistically different from zero. A maximum of 0.27 % (s.e.  $\pm 0.19\%$ ) of consumed  $^{15}N_2O$  may have been retained as  $^{15}N$  in soil organic matter in one sample. On average,  $^{15}N$  enrichment of soil organic matter during the incubation may have corresponded to a retention of 0.019% (s.e.  $\pm 0.14\%$ ;  $n=4$ ) of the  $^{15}N_2O$  consumed by the soils. We conclude that assimilatory reduction of  $N_2O$  plays, if at all, only a negligible role in the consumption of  $N_2O$  in soils.

## 1 Introduction

Nitrous oxide ( $N_2O$ ) is produced in soils during the processes of nitrification and denitrification (Firestone et al., 1980). Since industrialisation the global atmospheric  $N_2O$  concentration increased from about 270 ppb in 1800 to 319 ppb in 2005 (IPCC, 2006). Currently, more than one third of all  $N_2O$  emissions are of anthropogenic origin and primarily due to agriculture (IPCC, 2006). However, soils can also act as a sink for  $N_2O$  (reviewed in Chapuis-Lardy et al., 2007).

What is the possible fate of  $N_2O$  once produced by a soil organism? It can take three

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major routes (Ostrom et al., 2007) (Fig. 1): (1) complete denitrification to  $N_2$  within the cell prior to its escape into the gas phase (reviewed in Zumft, 1997); (2) escape from the cell into the gas phase of soil and potentially to the atmosphere; or (3) reduction to  $N_2$  upon re-entering a cell capable of complete denitrification (e.g. Neftel et al., 2000; Clough et al., 2005). To the best of our knowledge other pathways have not been studied in natural soil so far. One of these (4) could be the assimilatory reduction of  $N_2O$  to  $NH_3$  by soil organisms exhibiting nitrogenase activity (Fig. 1).

The first evidence that  $N_2O$  can be assimilated by nitrogenase was found by Mozen and Burris (1954). Later studies confirmed that  $N_2O$  can be a substrate for nitrogenase which reduces  $N_2O$  to  $N_2$  with subsequent reduction of  $N_2$  to  $NH_3$  (Hoch et al., 1960; Hardy and Knight, 1966; Jensen and Burris, 1986). However, one year later, reduction of  $N_2O$  to  $N_2$  was already questioned by Yamazaki et al. (1987). They concluded from the stable isotope kinetics during  $N_2O$  fixation by *Azotobacter vinelandii* that  $N_2O$  fixation by nitrogenase must be an apparent one-step reaction transforming  $N_2O$  directly into  $NH_3$ , without the intermediary  $N_2$ . This conclusion was supported by a re-interpretation of the original results of Jensen and Burris (1986) by Burgess and Lowe (1996). The re-interpretation suggested there may be an additional pathway from  $N_2O$  to  $NH_3$  which does not involve  $N_2$  as an intermediary. Shestakov and Shilov (2001) concluded from thermodynamical considerations that the reduction of  $N_2O$  would be preferred over that of  $N_2$ , because the dissociation energy for the N-N bond in  $N_2O$  is only half that of the  $N_2$  molecule. The  $N_2O$  fixation via nitrogenase is only one example of how  $N_2O$  may be assimilated in the soil matter. Other, still unknown processes might exist. The objective of this study is therefore to clarify the more general question whether N from  $N_2O$  consumed in soil is partly retained in the soil matrix or whether it is completely lost to the atmosphere.

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## 2 Material and methods

Samples were obtained from the upper 10 cm of soil at three locations in Switzerland and one location in South-West Germany. The first site, A, is located at Zurich-Reckenholz (47°26' N, 8°32' E at 491 m a.m.s.l.), the second site, B, is located in central Switzerland (47°17' N, 7°44' E at 450 m a.m.s.l.; for more details see Flechard et al., 2005). Both sites have soil types classified as cambisol and are experimental grassland sites of the Research Station ART (Agroscope Reckenholz-Tänikon), Switzerland. The third site, C, is located close to Basel (47°28' N, 7°42' E at 476 m a.m.s.l.) in a mixed deciduous forest on pseudogley over limestone; and the fourth site, D, is an open pine (*Pinus spp.*) forest on a peat bog in the Black Forest located 70 km north of Basel (47°52' N, 8°06' E at 975 m a.m.s.l.).

The soils of these sites range in texture from sandy loam to clay loam and organic, in pH from 2.9 to 7.0, in the C:N ratio from 9.4 to 28.8 and in the soil moisture from 23.0 to 93.6% (Table 1). Fresh samples were broken into aggregates of  $\leq 6.2$  mm within 5 h after collection from the field sites and 200 g were placed into an incubation vessel (glass, 415 cm<sup>3</sup>) at 20°C for a 24 h pre-incubation period. To minimise entry of atmospheric N<sub>2</sub> into the sample environment, we placed the incubation vessel during the entire experiment in an aluminium coated foil bag (volume about 2 l), which was continuously flushed with He (200 ml min<sup>-1</sup>). During the incubation, the samples were exposed to a mixture of labelled (98%) <sup>15</sup>N<sub>2</sub>O (0.5–4 ppm) and O<sub>2</sub> (0.2–0.4%) in He (with a purity of 99.9999%). Labelled <sup>15</sup>N<sub>2</sub>O had been produced by the following thermal method. An amount of 0.1 g fully (98%) labelled NH<sub>4</sub>NO<sub>3</sub> (Cambridge Isotope Laboratories, Inc., Andover, USA) was gently heated with 5 ml 6 M HNO<sub>3</sub> and 0.05 g NaCl. The arising <sup>15</sup>N<sub>2</sub>O was collected in a syringe (a nice popular description of the method can be found at: <http://mattson.creighton.edu/N2O/>). Later it was transferred with a stream of He into a 10 l He bottle filled to 1.1 bar pressure and topped up with He to 6 bar. Close to 100% labelling of N<sub>2</sub>O with <sup>15</sup>N was confirmed by mass spectrometry (T. Blunier, personal communication, 2005). In the incubation experiment, a gas stream of

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the labelled  $^{15}\text{N}_2\text{O}$ ,  $\text{O}_2$  and He was mixed together and monitored by mass-flow controllers. It passed a humidifier and was divided into two equal streams with flow rates around  $30\text{ ml min}^{-1}$ . One of the gas streams passed through the incubation vessel containing the soil sample before entering a 6-port selection valve (Valco Instruments Co. Inc.; Houston, Texas, USA). The second gas stream arrived directly at the selection valve. This valve selected alternately one of the gas streams and sent it through a Nafion<sup>®</sup> dryer (MD<sup>TM</sup> Series Gas dryer, Perma Pure LLC.; Toms River, N.J., USA) to a 2 ml sample loop on an injection valve (10-port selection valve, Valco Instruments Co. Inc.; Houston, Texas, USA) for 5 min, while the other gas stream was vented to the atmosphere. Concentrations of  $\text{N}_2\text{O}$  were measured by GC-ECD (SRI8610C Gas Chromatograph; SRI Instruments Inc.; Las Vegas, N.V., USA). By varying the concentration of  $\text{N}_2\text{O}$  in the gas mixture, we were able to determine rates of gross production and gross consumption of  $\text{N}_2\text{O}$  from a linear regression fitted through the measured net  $\text{N}_2\text{O}$  fluxes against  $\text{N}_2\text{O}$  concentrations (Vieten et al., 2007). The  $\delta^{15}\text{N}$  of soil organic matter (SOM) was measured (on three or more sub-samples) before and after an 11 to 29 day incubation period (Table 1) on the Flash Elemental Analyser (Thermo Finnigan; Milano, Italy) connected to a CF-IR-MS (DELTA<sup>plus</sup>XP; Thermo Finnigan MAT; Bremen, Germany).

### 3 Results and discussions

During the incubation period of 11 to 29 days, between 0.81 and 1.86 mg of fully labelled  $^{15}\text{N}_2\text{O}$  were flowing through each soil sample, containing a background of 0.82 mg to 1.91 mg of  $^{15}\text{N}$  in organic matter (Table 1). During this time, 0.18 mg to 0.29 mg of the offered  $^{15}\text{N}_2\text{O}$  was consumed (Table 1). Figure 2 presents the fraction of consumed  $^{15}\text{N}_2\text{O}$ -N that might have been retained as part of the soil organic matter. This fraction was between  $-0.20\%$  ( $\pm 0.11\%$ ) and  $0.27\%$  ( $\pm 0.19\%$ ) with an average across all four soils of  $0.019\%$  ( $\pm 0.12\%$ ). None of the measured values were signifi-

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icantly different from zero ( $p > 0.05$ ). Thus, we can consider them as measurement noise. Still, their presentation is useful insofar as it serves to illustrate the sensitivity of our method. This sensitivity was about one order of magnitude larger than that of the classical assay for  $N_2$  fixation by acetylene reduction, as for example described by Weaver and Danso (1994). This sensitivity was brought about by the long duration of the incubation, the large atom fraction of  $^{15}N$  in the consumed  $N_2O$  ( $> 0.98$ ) and the high rates of  $N_2O$  consumption by the soil samples. During the incubation period, an equivalent of 11.6% to 24.4% of  $^{15}N$  initially present in the soil samples was consumed as  $^{15}N_2O$  (Table 1).

No significant  $N_2O$  production by the soil samples themselves was detected during these experiments. Therefore  $^{15}N_2O$  would have been the principal source for potential  $N_2O$  assimilatory reduction. During our experiments, we incubated the soils with He (purity of 99.9999%) plus small amounts of  $O_2$  and  $^{15}N_2O$ . Since we flushed the aluminium-coated foil bag that was around the incubation vessel continuously with He, we reduced the potential influx of atmospheric  $N_2$  into the sample. We did not measure  $N_2$  concentrations in the sample air but estimate that it has been in the order of ppm rather than per mil. Compared to the natural environment, the competitive advantage of  $N_2O$  (here: 0.5–4 ppm) relative to  $N_2$  to get in contact with nitrogenase had been shifted by orders of magnitude in favour of  $N_2O$ . Thus, the likelihood for bacteria to reduce  $N_2O$  instead of  $N_2$  to  $NH_3$  was substantially increased. The low  $O_2$  concentration (0.2%) will have further enhanced the potential activity of nitrogenase in free-living organisms (e.g. Newton, 2007). Maybe, we should underline here that it was not our objective to measure nitrogenase activity but to find out whether some proportion of  $N_2O$  consumed in soil is assimilated. A mere proof of nitrogenase activity would not have answered this question. Alternatively, a failure of an acetylene reduction assay to prove nitrogenase activity would not have provided a better answer because of the much poorer sensitivity of this assay compared to our  $^{15}N_2O$  labelling study. Weaver and Danso (1994) state that the detection of  $^{15}N$  in tissues of biological systems exposed to labelled N gas is the only direct, unequivocal method for demonstrating that

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its assimilation occurred.

The negative result regarding  $\text{N}_2\text{O}$  assimilation is unexpected, considering the evidence of  $\text{N}_2\text{O}$  fixation by nitrogenase in the cited culture studies. Further, the thermodynamical advantage of reducing  $\text{N}_2\text{O}$  to  $\text{NH}_3$  instead of  $\text{N}_2$  to  $\text{NH}_3$  (Shestakov and Shilov, 2001) supports the expectation to recover  $^{15}\text{N}$  in soil having consumed substantial amounts of  $^{15}\text{N}_2\text{O}$ . The dissociation energy for the  $\text{N}_2$  molecule (9.76 eV) is about twice that for the N-NO bond in the  $\text{N}_2\text{O}$  molecule (4.93 eV, Herzberg, 1966). Thus, one could expect natural selection to have favoured organisms assimilating  $\text{N}_2\text{O}$  rather than  $\text{N}_2$ . We can not completely rule out that  $\text{N}_2\text{O}$  assimilation may have some ecological importance, at least in N limited soils. Still, our results strongly indicate that in environments similar to those studied here,  $\text{N}_2\text{O}$  assimilation is an irrelevant pathway in  $\text{N}_2\text{O}$  consumption by soil. Thus, the general assumption that  $\text{N}_2\text{O}$  is exclusively consumed by respiratory reduction to  $\text{N}_2$  (i.e. complete denitrification) almost certainly applies to these types of environment.

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**Table 1.** Summary of soil sample properties and the results of the incubation experiments with <sup>15</sup>N labelled N<sub>2</sub>O.

Sample	A	B	C	D
Ecosystem	Grassland	Grassland	Forest	Forest
Soil type	Cambisol	Cambisol	Pseudogley	Histosol
Texture	Sandy loam	Clay loam	Loam	Organic
C:N ratio	11.2	9.4	14.6	28.8
pH in 0.01 M CaCl <sub>2</sub>	6.1	5.7	7.0	2.9
Moisture [%]	23.0	33.9	33.3	93.6
dry weight of sample [g]	154	132.2	133.4	12.8
Initial mass of <sup>15</sup> N in sample [mg]	1.02	1.55	1.91	0.82
Duration of incubation [days]	11	20	12	29
<sup>15</sup> N <sub>2</sub> O offered to sample [mg <sup>15</sup> N]	1.23	1.86	0.81	1.84
<sup>15</sup> N <sub>2</sub> O consumed [mg <sup>15</sup> N]	0.21	0.18	0.29	0.20
Shift in δ <sup>15</sup> N during incubation [‰]	0.12	−0.23	−0.09	0.66

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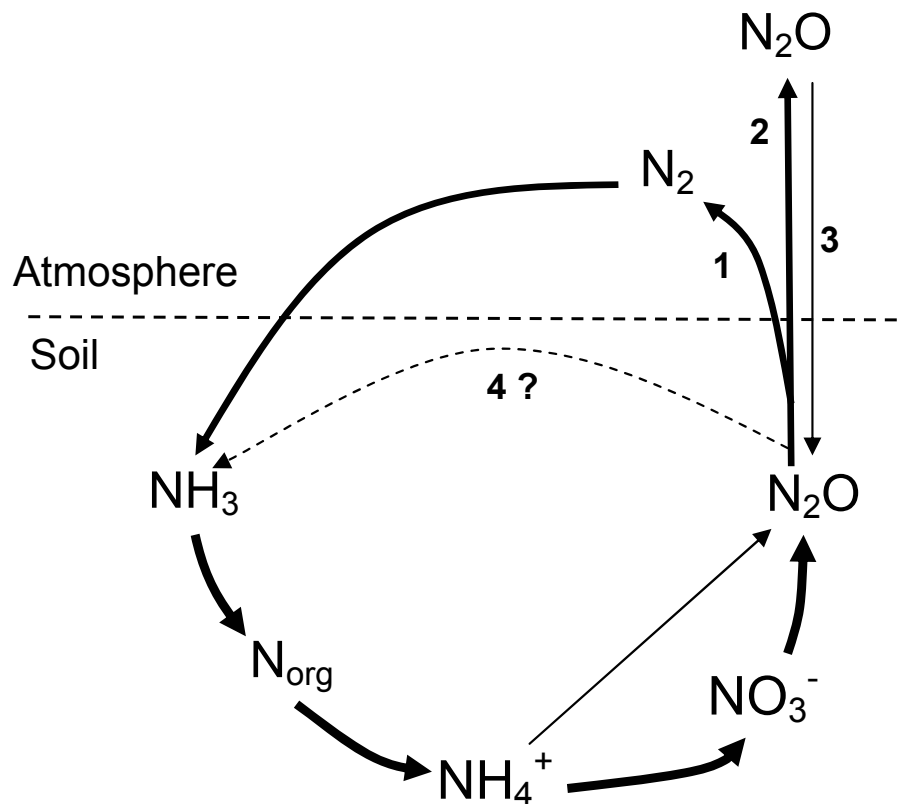
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**Fig. 1.** Origin and possible fate of N<sub>2</sub>O in soil. (1) Complete denitrification to N<sub>2</sub> before escape from the cell; (2) escape from cell; (3) re-entering a cell and subsequent reduction to N<sub>2</sub>, or (4) assimilatory reduction to NH<sub>3</sub>. The ecological relevance of pathway (4) in natural soil is unknown and the focus of this study.

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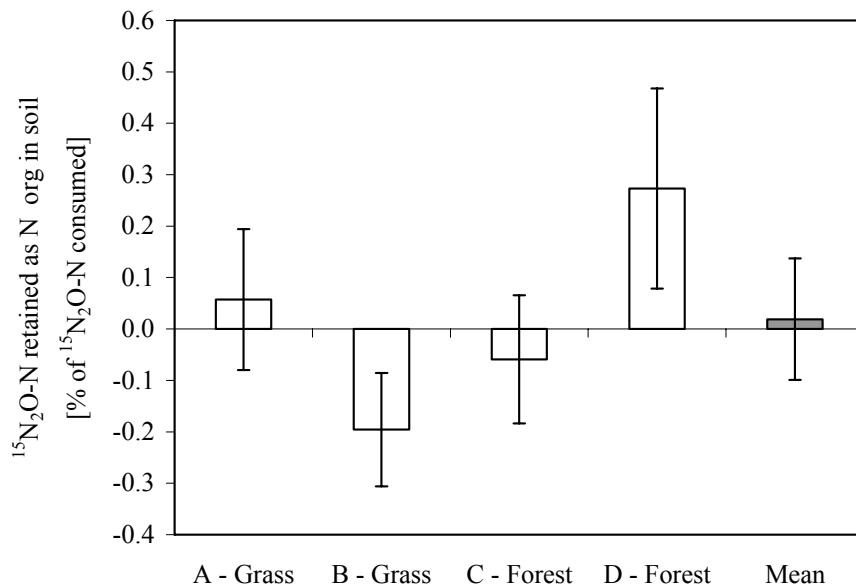
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**Fig. 2.** Proportion of consumed <sup>15</sup>N<sub>2</sub>O-N that might have been retained in four soil samples. Error bars indicate ±1 standard error of the measurement as determined by the measurements of δ<sup>15</sup>N in soil organic matter on replicate sub-samples before and after the incubation period. The proportion retained has been calculated as: Shift in δ<sup>15</sup>N during incubation [‰]/1000[‰] × initial mass of <sup>15</sup>N in sample [mg]/<sup>15</sup>N<sub>2</sub>O consumed [mg] × 100 [%].

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