The fate of $\text{N}_2\text{O}$ consumed in soils

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

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

Received: 8 August 2007 – Published in Biogeosciences Discuss.: 24 September 2007
Revised: 17 December 2007 – Accepted: 5 January 2008 – Published: 1 February 2008

Abstract. Soils are capable to consume $\text{N}_2\text{O}$. It is generally assumed that consumption occurs exclusively via respiratory reduction to $\text{N}_2$ by denitrifying organisms (i.e. complete denitrification). Yet, we are not aware of any verification of this assumption. Some $\text{N}_2\text{O}$ may be assimilatorily reduced to $\text{NH}_3$. Reduction of $\text{N}_2\text{O}$ to $\text{NH}_3$ is thermodynamically advantageous compared to the reduction of $\text{N}_2$. 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%) $\text{N}_2\text{O}$ (0.5–4 ppm) and $\text{O}_2$ (0.2–0.4%) in He. We measured $\text{N}_2\text{O}$ consumption by GC-ECD continuously and $\delta^{15}\text{N}$ of soil organic matter before and after an 11 to 29 day incubation period. Any $\text{N}_2\text{O}$ assimilatorily reduced would have resulted in the enrichment of soil organic matter with $^{15}\text{N}$ that was statistically different from zero. A maximum of 0.27% (s.e. ±0.19%) of consumed $\text{N}_2\text{O}$ may have been retained as $^{15}\text{N}$ in soil organic matter in one sample. On average, $^{15}\text{N}$ enrichment of soil organic matter during the incubation may have corresponded to a retention of 0.019% (s.e. ±0.14%; n=4) of the $\text{N}_2\text{O}$ consumed by the soils. We conclude that assimilatory reduction of $\text{N}_2\text{O}$ plays, if at all, only a negligible role in the consumption of $\text{N}_2\text{O}$ in soils.

1 Introduction

Nitrous oxide ($\text{N}_2\text{O}$) is produced in soils during the processes of nitrification and denitrification (Firestone et al., 1980). Since industrialisation, the global atmospheric $\text{N}_2\text{O}$ concentration increased from about 270 ppb in 1800 to 319 ppb in 2005 (IPCC, 2006). Currently, more than one third of all $\text{N}_2\text{O}$ emissions are of anthropogenic origin and primarily due to agriculture (IPCC, 2006). However, soils can also act as a sink for $\text{N}_2\text{O}$ (reviewed in Chapuis-Lardy et al., 2007). In general, it is implicitly assumed that complete denitrification (reduction of $\text{N}_2\text{O}$ to $\text{N}_2$) is the only process responsible for observed sink activity. Once produced by a soil organism, a molecule of $\text{N}_2\text{O}$ is presumed to take one of the three known routes (Ostrom et al., 2007) (Fig. 1): (1) complete denitrification to $\text{N}_2$ 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) complete denitrification to $\text{N}_2$ upon re-entering a cell capable to reduce $\text{N}_2\text{O}$ (e.g. Neftel et al., 2000; Clough et al., 2005). To the best of our knowledge other pathways have not been considered in natural soil so far. Here, we hypothesise a fourth pathway of assimilatory reduction to $\text{NH}_3$ may be responsible for some of observed $\text{N}_2\text{O}$ consumption in soil (Fig. 1).

The only enzyme known to reduce $\text{N}_2\text{O}$ to $\text{NH}_3$ is nitrogenase. First evidence was provided by Mozen and Burris (1954). Later studies confirmed that $\text{N}_2\text{O}$ can be a substrate for nitrogenase which reduces $\text{N}_2\text{O}$ to $\text{N}_2$ with subsequent reduction of $\text{N}_2$ to $\text{NH}_3$ (Hoch et al., 1960; Hardy and Knight, 1966; Jensen and Burris, 1986). However, one year later, reduction of $\text{N}_2\text{O}$ to $\text{N}_2$ was already questioned by Yamazaki et al. (1987). They concluded from the stable isotope kinetics during $\text{N}_2\text{O}$ fixation by Azotobacter vinelandii that $\text{N}_2\text{O}$ fixation by nitrogenase must be an apparent one-step reaction transforming $\text{N}_2\text{O}$ directly into $\text{NH}_3$, without the intermediary $\text{N}_2$. 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 $\text{N}_2\text{O}$ to $\text{NH}_3$ which does not involve $\text{N}_2$ as an intermediary. Enzyme kinetics indicate a low affinity of $\text{N}_2\text{O}$ to nitrogenase. A $k_m$ value of 24 kPa for purified component proteins from Klebsiella pneumoniae

Published by Copernicus Publications on behalf of the European Geosciences Union.
Reckenholz (47°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 pseudegley 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 ≤ 6.2 mm within a h after collection from the field sites and 200 g were placed into an incubation vessel (glass, 415 cm³) at 20°C for a 24 h pre-incubation period. To minimise entry of atmospheric N₂ 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⁻¹). During the incubation, the samples were exposed to a mixture of labelled (98%) ¹⁵N₂O (0.5–4 ppm) and O₂ (0.2–0.4%) in He (with a purity of 99.9999%). Labelled ¹⁵N₂O had been produced by the following thermal method. An amount of 0.1 g fully (98%) labelled NH₄NO₃ (Cambridge Isotope Laboratories, Inc., Andover, USA) was gently heated with 5 ml 6M HNO₃ and 0.05 g NaCl. The arising ¹⁵N₂O was collected in a syringe. 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 N₂O with ¹⁵N was confirmed by mass spectrometry (T. Blunier, personal communication). In the incubation experiment, a gas stream of the labelled ¹⁵N₂O, O₂ 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 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 alternating one of the gas streams and sent it through a Nafion dryer (MD™ 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 N₂O were measured by GC-ECD (SRI8610C Gas Chromatograph; SRI Instruments Inc., Las Vegas, N.V., USA). By varying the concentration of N₂O in the gas mixture, we were able to determine rates of gross production and gross consumption of N₂O from a linear regression fitted through the measured net N₂O fluxes against N₂O concentrations (Vieten et al., 2007). The ³⁵N 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\(^{\text{plus}}\)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 \(^{15}\text{N}\)N\(_2\)O were flowing through each soil sample, containing a background of 0.82 mg to 1.91 mg of \(^{15}\)N in organic matter (Table 1). During this time, 0.18 mg to 0.29 mg of the offered \(^{15}\)N\(_2\)O was consumed (Table 1). Figure 2 presents the fraction of consumed \(^{15}\)N\(_2\)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 significantly 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 \(\text{N}_2\) 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 \(\text{N}_2\)O (\(>0.98\)) and the high rates of \(\text{N}_2\)O 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\(_2\)O (Table 1).

No significant \(\text{N}_2\)O production by the soil samples themselves was detected during these experiments. Therefore \(^{15}\)N\(_2\)O would have been the principal source for potential \(\text{N}_2\)O assimilation. During our experiments, we incubated the soils with He (purity of 99.9999\%) plus small amounts of \(\text{O}_2\) and \(^{15}\)N\(_2\)O. Since we flushed the aluminium-coated foil bag that was around the incubation vessel continuously with He, we reduced the potential influx of atmospheric \(\text{N}_2\) into the sample. We did not measure \(\text{N}_2\) 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 \(\text{N}_2\O (here: 0.5–4 ppm) relative to \(\text{N}_2\) to be assimilated had been shifted by orders of magnitude in favour of \(\text{N}_2\O. Thus, the likelihood for soil organisms to reduce \(\text{N}_2\O instead of \(\text{N}_2\) to \(\text{NH}_3\) was substantially increased. Maybe, we should underline here that it was not our objective to measure nitrogenase activity but to find out whether some proportion of \(\text{N}_2\O consumed in soil is assimilated, by whatever reduction pathway possible. Our method of choice (\(^{15}\)N\(_2\)O labelling) would be unable to tell us anything specific about the nature of the pathway. We still chose

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ecosystem</th>
<th>Soil type</th>
<th>Texture</th>
<th>C:N ratio</th>
<th>pH in 0.01 M CaCl(_2)</th>
<th>Moisture [%]</th>
<th>dry weight of sample [g]</th>
<th>Initial mass of (^{15})N in sample [mg]</th>
<th>Duration of incubation [days]</th>
<th>(^{15})N(_2)O offered to sample [mg (^{15})N]</th>
<th>(^{15})N(_2)O consumed [mg (^{15})N]</th>
<th>Shift in (\delta^{15})N during incubation []</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Grass</td>
<td>Cambisol</td>
<td>Sandy loam</td>
<td>11.2</td>
<td>6.1</td>
<td>23.0</td>
<td>154</td>
<td>1.02</td>
<td>11</td>
<td>1.23</td>
<td>0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>B</td>
<td>Grass</td>
<td>Cambisol</td>
<td>Clay loam</td>
<td>9.4</td>
<td>5.7</td>
<td>33.9</td>
<td>132.2</td>
<td>1.55</td>
<td>20</td>
<td>1.86</td>
<td>0.18</td>
<td>-0.23</td>
</tr>
<tr>
<td>C</td>
<td>Forest</td>
<td>Pseudogley</td>
<td>Loam</td>
<td>14.6</td>
<td>7.0</td>
<td>33.3</td>
<td>133.4</td>
<td>1.91</td>
<td>12</td>
<td>0.81</td>
<td>0.29</td>
<td>-0.09</td>
</tr>
<tr>
<td>D</td>
<td>Forest</td>
<td>Histosol</td>
<td>Organic</td>
<td>28.8</td>
<td>2.9</td>
<td>93.6</td>
<td>12.8</td>
<td>0.82</td>
<td>29</td>
<td>1.84</td>
<td>0.20</td>
<td>0.66</td>
</tr>
</tbody>
</table>

**Table 1.** Summary of soil sample properties and the results of the incubation experiments with \(^{15}\)N labelled \(\text{N}_2\)O.
this method because detection of $^{15}$N in tissues of biological systems exposed to labelled N gas is the only direct, unequivocal method for demonstrating that its assimilation occurred (Weaver and Danso, 1994).

The negative result regarding N$_2$O assimilation is unexpected, considering the observed large rates of N$_2$O consumption, the thermodynamical advantage of N$_2$O over N$_2$ as a substrate for the production of NH$_3$ (Shestakov and Shilov, 2001), and the evidence of direct N$_2$O to NH$_3$ reduction as a biological process (Yamazaki et al., 1987) albeit only for a low affinity enzyme (Jensen and Burris, 1986) so far. One would expect natural selection to have favoured organisms assimilating N$_2$O rather than N$_2$. We can not completely rule out that N$_2$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, N$_2$O assimilation is an ecologically irrelevant pathway in N$_2$O consumption by soil. Thus, the general assumption that N$_2$O is exclusively consumed by dissimilatory reduction to N$_2$ (i.e. complete denitrification) almost certainly applies in these environments.

**Acknowledgements.** We thank Thomas Blunier from the Institute of Climate and Environmental Physics of the University of Bern for analysing our labelled $^{15}$N$_2$O. This study was supported by the Swiss State Secretariat for Education and Research through grant Nr. C04.0254 to COST Action 856 and the Swiss National Science Foundation.

**References**


Jensen, B. B. and Burris, R. H.: N$_2$O as a substrate and as a competitive inhibitor of nitrogenase, Biochemistry, 25, 1083–1088, 1986.


