Real Time Monitoring of $\text{N}_2\text{O}$ Emissions from Agricultural Soils using FTIR Spectroscopy

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Emissions of $\text{N}_2\text{O}$ from agricultural soils are an important source for this greenhouse gas. The present work examines the potential of Fourier transformed infrared (FTIR) spectroscopy coupled with a long path (LP) infrared (IR) gas cell for on-line measurement of concentration and isotopic signature of $\text{N}_2\text{O}$ emitted from soils. Nitrous Oxide was spectrally monitored during incubations of soil samples in a closed system under different conditions. Its emission from a Grumosol (Vertisol) was measured in presence and absence of acetylene, with various additions of nitrate and glucose, under aerobic and anaerobic conditions, and for two soil thickness layers. For comparison $\text{N}_2\text{O}$ emissions from a Terra Rossa (Cambisol) and a Hamra (Luvisol) were measured in the presence and absence of acetylene. In an additional experimental set the isotopic signature of emitted $\text{N}_2\text{O}$ was quantified after enrichment with $^{15}\text{NO}_3^-$, Acetylene addition led to an increase in $\text{N}_2\text{O}$ emissions in all three soils but at various extents. Under aerobic conditions, $\text{N}_2\text{O}$ emission from the Grumosol became detectable only when running the experiments with a thicker soil layer (10 mm), suggesting the existence of coupled nitrification–denitrification. Nitrate addition to soils enhanced $\text{N}_2\text{O}$ emissions especially when coupled with glucose addition. Addition of $^{15}\text{NO}_3^-$ to the Grumosol resulted in the emission of all four $\text{N}_2\text{O}$ isotopologues: $^{14}\text{N}_2\text{O}$, $^{15}\text{N}_2\text{O}$, $^{14}\text{N}^{15}\text{NO}$, and $^{15}\text{N}^{14}\text{NO}$. The observed slight delay in appearance of the species containing $^{15}\text{N}$ and the relatively lower $^{15}\text{N}$ enrichment of the $\text{N}_2\text{O}$ compared with the soil nitrate, indicate isotopic fractionation during denitrification. Yet, within the accuracy of our isotopic analysis, temporal emission patterns of $^{14}\text{N}^{15}\text{NO}$ and $^{15}\text{N}^{14}\text{NO}$ were similar indicating low possibility for “site preference” under the specific experimental conditions.

Abbreviations: FTIR, Fourier Transform Infrared; IR, infrared; LP, long path;
naturally N₂. The amounts of NO and N₂O that leak out of the denitrification pathway depend on the presence and activities of the enzymes involved in the process, which in turn are influenced by environmental factors, such as the concentrations of O₂, NO₃⁻, pH level, and the availability of C. When the NO₃⁻ source for denitrification is from nitrification, the pathway is denoted nitrification-coupled denitrification (NcD) in contrast to fertilizer denitrification, where the source of NO₃⁻ is fertilization (e.g., Wragge et al., 2001; Kremen et al., 2005).

An increase in N₂O emission rates with increasing N fertilization has been observed both in laboratory and field studies (e.g., Skiba and Smith, 2000). Others reported a positive correlation between soil temperature and N₂O emission rates (e.g., Smith et al., 1998), and between soil moisture and N₂O emission rates (e.g., Klemedtsson et al., 1988). The contribution of nitrification to total N₂O emission has been observed to increase with increasing ammonium concentrations and soil moisture (e.g., Müller et al., 1998), except under completely anaerobic conditions (Klemedtsson et al., 1988), and was observed to decrease at high temperatures (Avrahami et al., 2003). Avrahami and Bohannan (2009) have observed a relationship between N₂O emission rates and shifts in microbial community structure in response to environmental changes. The response of N₂O emission rates to changes in environmental conditions and fertilization is particularly important since temperature, soil moisture, and nitrogen deposition have significantly increased in many regions (e.g., Easterling et al., 2000).

Knowledge regarding the pathways involved in N₂O production is still limited despite efforts to quantify mechanisms and sources of its formation (denitrification, nitrification, nitritifier-denitrification). Such knowledge is essential for developing better management tools and mitigation measures. The most adequate techniques for quantitative investigation of N transformations and N₂O source partitioning in soils are based on isotopic enrichment. The measurements can be further improved by dual-isotope labeling (e.g., Wragge et al., 2005; Baggs, 2008) or by tracking the changes of N₂O isotopologues in the gases emitted from the soil (e.g., Baggs, 2008; Sutka et al., 2006; Well et al., 2006). These require the use of IRMS (isotope ratio mass spectrometry) to get the most accurate results. Yet, IRMS cannot be used on line. Fourier transform infrared spectroscopy, which enables monitoring of N-gases using LP gas cells (e.g., Esler et al., 2000; Griffith et al., 2012, Phillips et al., 2013), offers powerful tools for real-time investigations. In addition, when combining labeling of ¹⁵N/¹⁴N and/or ¹⁸O/¹⁶O, FTIR spectroscopy may allow direct measurements of N₂O isotopologues and isotopomers concentrations (Esler et al., 2000; Griffith et al., 2009, Phillips et al., 2013). Recently, Phillips et al. (2013) showed that high resolution FTIR enables continuous monitoring of N₂O isotopologues emissions under field conditions following fertilization with ¹⁵N-labeled substrate. In the present paper we report the development of a direct method based on low-resolution FTIR spectroscopy for continuous monitoring of either natural ¹⁴N₂O or isotopic N species (including isotopomers) in the gases emitted from agricultural soils during small scale incubations experiments.

METHODS

Three types of soils were used in this study: Acre Grumosol (Vertisols, calcareous clay), Ga’aton Terra Rossa (Cambisols), and Yakum Hamra (sandy loam, Luvisols) (See Table 1 for basic general characteristics). The soil samples were collected from the upper layer (0–30 cm) of agricultural field sites, air-dried, and sieved with a 2-mm sieve.

Experimental. The experimental system (Fig. 1) consisted of an incubation cell located in a temperature controlled water-bath (25 ± 1°C) and connected, via a pumping system, to a LP gas cell with an optical path of 4 m and a volume of 500 cm³ (Model 6V, InfraRed Analysis Inc., Anaheim, CA). The LP cell was coupled to a mid-infrared FTIR spectrometer (Vertex 70, Bruker, Ettlingen, Germany). The total volume of the system including tubing and pump was 690 cm³.

The soils were kept refrigerated and before each experiment samples were conditioned for 24 h in a closed vial at room temperature. In most incubation experiments, 4 g of air-dry soil were placed in the incubation cell (diameter 6.3 cm, height 2.8 cm) af-

Table 1. Selected chemical and physical properties of the studied soils (standard deviation).

<table>
<thead>
<tr>
<th>Soil description: Soil property</th>
<th>Acre Grumosol</th>
<th>Ga’aton Terra Rossa</th>
<th>Hamra Sandy Loam</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.4 (0.2)</td>
<td>7.5 (0.2)</td>
<td>7.4 (0.2)</td>
</tr>
<tr>
<td>Electrical conductivity, ds m⁻¹</td>
<td>0.2 (0.02)</td>
<td>0.3 (0.03)</td>
<td>0.36 (0.03)</td>
</tr>
<tr>
<td>Organic carbon-C(K₂Cr₂O₇), mg g⁻¹</td>
<td>16.5 (0.8)</td>
<td>35.9 (1.7)</td>
<td>8.4 (0.5)</td>
</tr>
<tr>
<td>N-NO₃⁻, mg kg⁻¹</td>
<td>105(5)</td>
<td>110(6)</td>
<td>14(2)</td>
</tr>
<tr>
<td>N-NH₄⁺, mg kg⁻¹</td>
<td>17(3)</td>
<td>45(4)</td>
<td>17(2)</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic description of the experimental system.
ter adding 4 mL of KNO₃ solution (at predetermined concentrations, with or without isotopic labeling of N). The thickness of the resultant soil paste was ~2 mm. When no nitrate enrichment was applied, 4 mL of deionized water (Millipore, 18.2 MΩ) were added. Most denitrification experiments were conducted under an atmosphere of pure N₂ or mixtures of N₂ and acetylene. In some experiments 1 mg glucose g⁻¹ dry soil was added to the soil with the aqueous solution to enhance denitrification.

Immediately after placing the soil in the cell, the desired gas mixture (aerobic or anaerobic) was introduced and the system was sealed. In denitrification experiments, an atmosphere of N₂ and acetylene (0, 10, or 20%) was prepared in a Tedler bag (SKC, Inc., Eighty Four, PA) and then transferred to the pumped-down system via pressure drop. After obtaining the desired atmosphere the gaseous phase was continuously circulated (using a peristaltic pump with flow rate of 150 cm³ min⁻¹) through the LP and incubation cell for the whole duration of the experiment (about 22–24 h for most experiments and in a few experiments with isotopic enrichments longer periods of ~40 or 70 h). During all incubation experiments FTIR spectra of the gaseous phase were recorded every 20 min to monitor the N₂O build up. All spectra were recorded using a deuterated triglycine sulfate (DTGS) detector at 1 cm⁻¹ resolution, averaging 32 scans for each spectrum, and using the spectrum of the initial gas mixture as reference. Each experiment was repeated two to four times and average values are reported, together with deviations from the average for duplicate runs or one standard deviation when more replicates were available.

In the first stage of the study, experiments with the Acre Grumosol focused on determining N₂O emissions under well-defined anaerobic conditions, with and without acetylene addition and at various levels of nitrate addition to the soils. The effect of glucose addition was tested as well. This part was followed by experiments under anaerobic conditions conducted with the Terra Rossa and the Yakum Hamra for testing the effect of acetylene addition on N₂O emission. In addition, the effect of aerobic conditions was tested with the Acre Grumosol using samples with a thickness of 2 mm (4 g soil) compared with 10 mm (20 g soil) in the incubation cell.

The second stage of the study consisted of experiments in which mixtures of labeled ¹⁴NO₃⁻ and ¹⁵NO₃⁻ were added to these soils and the emission of all four nitrous oxide species (¹⁴N₂O, ¹⁵N₂O, the α-isotopomer ¹⁴N¹⁵NO, and the β-isotopomer ¹⁵N¹⁴NO) were estimated using the procedure described below. In these experiments the ¹⁵N enrichment levels were between 46 and 87 atom%.

At the end of each incubation 2.5 g of the soil sample were mixed with 25 mL 1 M KCl solution and put on a shaker for 1 h to extract the inorganic N species. The solution was filtered (Whatman #42 filter) and the extract was analyzed for NO₃⁻, NO₂⁻ - NH₄⁺ using a Quickchem 8500 autoanalyzer (Lachat Instruments, Loveland, CO). Similar analyses were conducted for each soil before incubation so that changes in the inorganic N species could be estimated.

FTIR Analysis. Quantification of the N₂O concentration was based on its asymmetric stretch absorbance band (2080–2260, 2125–2305, 2150–2330 cm⁻¹) for ¹⁴N₂O, ¹⁴N¹⁵NO, and ¹⁵N₂O, respectively), using independent calibration curves for each isotopologue. These calibration curves were obtained by introducing into the LP cell known concentrations of one of either ¹⁴N₂O, ¹⁴N¹⁵NO, or ¹⁵N₂O. Each gas sample was prepared in two steps: first a concentrated mixture of N₂O in N₂ was made using standard gas cylinders of N₂O (¹⁴N₂O Sigma-Aldrich, 99 atom%); ¹⁵N₂O and ¹⁴N¹⁵NO from Cambridge Isotope Laboratories, Tewksbury, MA, 98 atom%) in a vacuum line equipped with a sensitive pressure gauge (Baratron 626, MKS Instruments Inc. Andover, MA), then small volumes of this mixture were injected into Tedler bags (10 L total volume, SKC Inc.) where they were further diluted with N₂ to obtain the desired concentrations. Nitrous oxide concentrations in these samples ranged from 7 to 465 μg N L⁻¹ (i.e., 5.7–407 ppm). Linear calibration curves with r² > 0.96 were obtained for each isotopologue. The detection limit of the system, determined as three times the N₂O concentration calculated based on noise signal in the spectral range of the N₂O absorbance bands (measured with N₂O-free air), was 0.9 μg L⁻¹. This relatively high detection limit is a direct result of the relatively short optical path of the selected LP cell, but is partially compensated by the small volume of this cell, which enables to accumulate high N₂O concentrations during the incubation of small soil volumes.

Figure 2 depicts the absorbance spectra measured in the LP cell for three N₂O isotopologues: ¹⁴N₂O, ¹⁵N₂O, and the α-isotopomer ¹⁴N¹⁵NO. These results show a clear shift in the location of the absorbance bands with no change in their shape, which agrees with previous reports (Esler et al., 2000; Zhang et al., 2000). The specific calibration curves obtained for each of these isotopologues indicated that regardless of the position of the absorbance band, all three species have similar molecular cross-section σ (i.e., same absorbance intensity for same concentration, Fig. 3). Since

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**Fig. 2.** FTIR spectra of the three N₂O isotopologues: ¹⁴N₂O (red solid line), ¹⁴N¹⁵NO (dashed blue line), and ¹⁵N₂O (dotted black line) recorded at 1 cm⁻¹ resolution. The concentrations of the three species were 1.8, 2.6, and 1.7 mmol L⁻¹ (43, 63, and 41 ppmV), respectively.
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the β-isotopomer of N₂O (¹⁵N¹⁴NO) was not available, we assumed that this independency of s on isotopic composition holds for it as well, that is, it has the same correlation between absorbance and concentration as the other isotopologues.

When analyzing the spectra of the incubation experiments, quantification of all four species was achieved by expressing each measured spectrum as a linear combination of known reference spectra: ¹⁴N₂O, ¹⁵N₂O, ¹⁴N¹⁵NO, ¹⁵N¹⁴NO, and, when relevant, acetylene. Mathematically,

\[
S_i = \sum_{j=1}^{4} \alpha_{i,j} R_j
\]

where \( S_1, S_2, \ldots, S_n \) are the spectra recorded during the incubation, \( R_j \) denotes the reference spectrum of compound \( j \), and \( \alpha_{i,j} \) is the contribution of compound \( j \) to spectrum \( i \). For ¹⁴N₂O, ¹⁵N₂O, and ¹⁴N¹⁵NO, the average of the respective single-component spectra collected during the calibration procedure was used as reference spectrum. As a reference spectrum for ¹⁵N¹⁴NO was not available to us during these experiments, this spectrum was estimated directly from the spectra recorded during the incubation of Grumosol enriched with 100 mg g⁻¹ ¹⁵NO₃ under anoxic atmosphere (N₂, 10% acetylene). As observed for the other two isotopologues (¹⁵N₂O and ¹⁴N¹⁵NO), we assumed that the spectrum of ¹⁵N¹⁴NO was identical to that of ¹⁴N₂O, but shifted by an unknown amount \( \Delta \) cm⁻¹. The amplitude of the spectral shift \( \Delta \) was determined by minimizing the sum squared differences between the measured \( (S_1, S_2, \ldots, S_n) \) and reconstructed \( (\sum \alpha_{i,j} R_j) \) spectra. The spectral shift obtained via this procedure \( (\Delta = 20 \text{ cm}^{-1}) \) agrees with the values reported for the β-isotopomer ¹⁵N¹⁴NO by Esler et al. (2000).

RESULTS AND DISCUSSION

Real-Time Monitoring of N₂O Emissions and the Effect of Acetylene Addition

As mentioned above, N₂O can be formed in soil as a by-product during nitrification and as an intermediate product during denitrification. The amount of N₂O that reaches the LP headspace depends on its rates of formation, its diffusion rate through the soil and the rate of its enzymatic reduction in the soil. Figure 4 shows the accumulation of N₂O in the LP cell during the incubation of a 2-mm thick Acre Grumosol layer under anaerobic atmosphere, in the absence and presence of acetylene. Even for such a thin layer the presence of acetylene (10% by volume) was found to increase significantly the concentrations of N₂O in the cell headspace due to inhibition of N₂O conversion to N₂ (e.g., Balderston et al., 1976; Wrage et al., 2004b; Well and Flessa, 2009). This trend was observed with soil initial nitrate concentration ranging from ~105 to 185 mg N-NO₃⁻ g⁻¹ dry soil. Further increase in acetylene concentration (to 20%) did not result in additional increase of N₂O.

At all three tested acetylene concentrations (0, 10, and 20%), the observed N₂O build up in the cell headspace followed a sigmoidal pattern, with a time delay of about 7 h from the beginning of incubation till its concentration reached our detection level (~0.9 mg N L⁻¹) and leveling off after approximately 22 h. Measurements of the nitrate and nitrite concentrations in the soil before and after the incubation indicated that although their concentrations decreased, these species were not exhausted during those 22 h, suggesting that the available organic matter was most likely the factor limiting denitrification.
Comparison between the amount of N accumulated in the cell headspace as N$_2$O and that consumed in the soil during the incubation (calculated following Eq. [1]), shows that in the presence of 10% acetylene the N-mass balance is almost complete (104.5 vs. 106.5 μg N, respectively). By comparison, in absence of acetylene, N$_2$O accumulation accounts for >80% of the consumed nitrate and nitrite (76.5 vs. 108.5 mg N).

\[ T_N = (M_{Ni} - M_{Nf}) \times M_s \]

where \( T_N \), total N mass (μg N) consumed in the soil (as nitrate and nitrite) during the incubation; \( M_{Ni} \) and \( M_{Nf} \) N concentration (i.e., NO$_3^-$+NO$_2^-$) in the soil before and after the incubation, respectively (μg N g$^{-1}$ dry soil), and \( M_s \), the mass of the dry soil (g).

As expected, increasing the initial NO$_3^-$ concentration in this soil (between ~105 to 185 μg N g$^{-1}$ dry soil) resulted in an increase in N$_2$O emissions, although to a relatively small extent (Fig. 5). Increasing the nitrate load in the soil also increased the mass balance discrepancy between the N$_2$O detected in the cell headspace and the N-consumption in the soil (the latter are specified in Fig. 5 caption). It is worth noting that such nitrate loads are common in N-fertilized soils.

Interestingly, the initial delay time for N$_2$O emission in these experiments was independent of initial NO$_3^-$ load (Fig. 5). The observed lag time probably corresponds to the time needed for the microbiological system to recover the disturbance caused by the addition of the aqueous solution (e.g., Krause and Nealson, 1997; Kewei et al., 2008), but it may also partially reflects the time required for the accumulating N$_2$O to reach the detection limit of our system.

As mentioned above, soil analysis revealed incomplete nitrate consumption and accumulation of NO$_2^-$ in the soil during incubation, even when N$_2$O emissions almost ceased (after >20 h). To check the hypothesis that this was a result of insufficient electron donor concentration in the soil, additional incubations were conducted with added glucose to the Acre Grumosol. Indeed, glucose addition (1mg g$^{-1}$ dry soil) led to a sharp increase in N$_2$O emission, both in terms of formation rate and in absolute amount, and to an enhanced consumption of the inorganic NO$_2^-$ and NO$_3^-$ in the soil (Fig. 5). The later was much more intense, such that accumulated N$_2$O accounted for only ~40% of the N consumed in the soil. This enhancement in N$_2$O emission after glucose addition differs from the results reported by Kewei et al. (2008). This may be because Kewei et al. used soil samples that were much thicker than those used here, so that N$_2$O generated in their system had a longer residence time in soil thus being susceptible to biological reduction, which may also have been enhanced by glucose addition. To check the potential of using real-time FTIR monitoring to study the relationship between soil type and N$_2$O emissions, incubation experiments similar to the ones reported above were conducted using Yakum Hamra and Ga’aton Terra Rossa soils. Figure 6 compares the N$_2$O emissions from all three soils and shows significant differences, both in terms of emission kinetics and extent, under the anaerobic environment and 10% acetylene (dashed lines). The natural nitrate concentration in the soil was (105 ± 5) mg N g$^{-1}$ dry soil. The solid red line shows N$_2$O emission after addition of glucose (1 mg g$^{-1}$ dry soil) to soil with no nitrate enrichment. The N-consumptions in the soil, based on nitrite and nitrate concentrations, were (30 ± 4), (35 ± 1), and (47 ± 2) mg N g$^{-1}$ dry soil for nitrate enrichments of 0, 40, and 80 mg N g$^{-1}$ dry soil, respectively. Glucose addition increased N consumption (in the absence of nitrate enrichment) to 110 mg N g$^{-1}$ dry soil.
similar environmental conditions. The emission differences correlate with differences in denitrification activity in these soils, as indicated by the changes in nitrite and nitrate soil concentrations (i.e., N-consumption; Table 2). The differences in soil reactivity (denitrification and N2O emission) cannot be accounted for only by differences in initial NO3− or organic matter loads in the soil. For example, the Terra Rossa and Grumosol have similar initial NO3− concentrations and yet very different N2O emissions (Table 2). These differences in the amount of emitted N2O as well as in the temporal emission patterns may be a result of different microbiological communities and their activities in these soils. The relative effect of acetylene addition was also very different between the soils. While in the Grumosol the addition caused a final N2O increase of ~35%, in the Terra Rossa it was increased by a factor larger than four. In the lighter soil (Hamra) the effect was even more dramatic, and practically no N2O was detected without acetylene, whereas 14 mg N g−1 dry soil were lost when acetylene was added. Also worth noting is the fact that in the clay soils (Terra Rossa and Grumosol) ammonium concentration increased during the incubation, indicating contribution from anaerobic mineralization.

High and constant N2O emission rate (i.e., linear accumulation) was observed during the incubation of Terra Rossa in the presence of acetylene (Fig. 6) until almost complete consumption of nitrate and nitrite initially present in the soil. This linearity suggests that even at the very high N2O levels reached here (up to 80 μg N g−1 soil, equivalent to 360 ppm N2O), there was no significant negative feedback on its emission from the soil. Nitrous Oxide fluxes from all three soils over the time interval corresponding to linear accumulation (6-16 h) were calculated using Eq. [2] and are depicted in Table 3.

\[
F(\text{N}_2\text{O}) = \frac{\Delta[N_2\text{O}]}{dt} \times \frac{V}{A}
\]  

[2]

where \( \frac{\Delta[N_2\text{O}]}{dt} \) is the measured accumulation rate of gaseous N2O (g m\(^{-3}\) s\(^{-1}\)), \( V \) is the volume of the reactor (m\(^3\)), and \( A \) the geometric surface area of the soil sample (m\(^2\)).

In the absence of acetylene and without nitrate enrichment, fluxes varied from 17 to 470 ng N m\(^{-2}\) s\(^{-1}\). These fluxes obtained with the clay soils are of the same order as those measured by Phillips et al. (2013) under real field conditions after rain events when the percentage of soil water-filled pore space exceeded 50% (~ 400 ng N m\(^{-2}\) s\(^{-1}\)). However, considering that fluxes are much lower at lower soil water content (e.g., 5–50 ng N m\(^{-2}\) s\(^{-1}\), Phillips et al., 2013), it is clear that the emission rates measured in our experiments do not represent typical N2O fluxes under ambient conditions.

### Nitrous Emission in the Presence of Aerobic Atmosphere

When incubation experiments were conducted with similar soil samples (i.e., 4 g, 2 mm thick Acre Grumosol) under aerobic atmosphere, no N2O was detected in the cell headspace. This is not surprising considering that under such conditions, oxygen supply via the 2-mm saturated layer may be unlimited and enhance nitrification rather than denitrification, and that under unlimited oxygen supply the potential of N2O formation via nitrification may be negligible. However, when soil thickness was

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Acetylene (replicates)</th>
<th>Pre/Post incubation</th>
<th>Total N2O ²</th>
<th>NO3− + NO2− consumption</th>
<th>NH4⁺†</th>
<th>NO2− ‡</th>
<th>NO3− ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gahaton Terra Rossa</td>
<td>Without(3)</td>
<td>Before</td>
<td>112±2</td>
<td>&lt; DL³</td>
<td>47±4</td>
<td>85±7</td>
<td>21±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>26±6</td>
<td>&lt; DL</td>
<td>85±2</td>
<td>85±2</td>
<td>26±6</td>
</tr>
<tr>
<td></td>
<td>With †(2)</td>
<td>Before</td>
<td>111±3</td>
<td>&lt; DL</td>
<td>42±1</td>
<td>42±1</td>
<td>86±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>29±19</td>
<td>&lt; DL</td>
<td>77±2</td>
<td>82±22</td>
<td>86±6</td>
</tr>
<tr>
<td>Yakum Hamra</td>
<td>Without(2)</td>
<td>Before</td>
<td>15±1</td>
<td>&lt; DL</td>
<td>17±2</td>
<td>17±2</td>
<td>17±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>3±3</td>
<td>&lt; DL</td>
<td>20±2</td>
<td>13±6</td>
<td>&lt; DL⁵</td>
</tr>
<tr>
<td></td>
<td>With †(2)</td>
<td>Before</td>
<td>13±0.5</td>
<td>&lt; DL</td>
<td>19±2</td>
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<td></td>
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<td>&lt; DL</td>
<td>17±0.2</td>
<td>10±3</td>
<td>14±2</td>
</tr>
<tr>
<td>Acre Grumosol</td>
<td>Without(2)</td>
<td>Before</td>
<td>108±2</td>
<td>&lt; DL</td>
<td>20±2</td>
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<td>29±0.3</td>
<td>42±3</td>
<td>32±5</td>
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<td>With †(2)</td>
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<td>&lt; DL</td>
<td>15±1</td>
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<td></td>
<td></td>
<td>After</td>
<td>52±8</td>
<td>24±4</td>
<td>31±0.3</td>
<td>29±3</td>
<td>29±2</td>
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</table>

† acetylene concentration was 10% by volume.
‡ All concentrations are in μg N g−1 dry soil.
§ Detection limit (DL) for N2O was 0.9 μg N L−1 and for nitrite 0.1 μg N g−1 dry soil.

### Table 3. Calculated N2O emission fluxes (ng N m\(^{-2}\) s\(^{-1}\)) during 6 to 16 h of soils incubation.

<table>
<thead>
<tr>
<th>Grumosol</th>
<th>Yakum Hamra</th>
<th>Gahaton Terra Rossa</th>
</tr>
</thead>
<tbody>
<tr>
<td>No acetylene</td>
<td>365</td>
<td>17</td>
</tr>
<tr>
<td>10% acetylene</td>
<td>505</td>
<td>296</td>
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<tr>
<td>10% acetylene and glucose addition (1mg/g soil)</td>
<td>1158</td>
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</tbody>
</table>
increased to 10 mm (20 g soil), a clear accumulation of $\text{N}_2\text{O}$ was detected, although at a somewhat lower extent than under anaerobic conditions: total $\text{N}_2\text{O}$ production of 16.7 mg N g$^{-1}$ dry soil vs. 22.4 mg N g$^{-1}$ dry soil, respectively (both in absence of acetylene and glucose). This suggests that the 10-mm thickness of the Acre Grumosol may be sufficient to generate an oxygen gradient that enables coupled nitrification–denitrification processes. Such coupling may occur in soil aggregates or "microsites" (e.g., Kremen et al., 2005) where oxygen diffusion into the soil is limited relative to its consumption rate. Under such conditions, $\text{NO}_3^-$ is generated (via $\text{NH}_4^+$ nitrification) in the outer oxygenated layer and is reduced via denitrification in the deeper oxygen-poor soil layer. Accordingly, $\text{N}_2\text{O}$ may be generated via both denitrification and nitrification (Kremen et al., 2005; Master et al., 2004, 2005) depending on the degree rate of oxygen supply limitation. Changes in $\text{NO}_3^-$ and $\text{NH}_4^+$ concentrations during these incubations ($t = 22$ h) do support the assumption that nitrification dominated in the 2-mm thick soil layer ($\Delta\text{NO}_3^- = +10.2$ mg N g$^{-1}$ and $\Delta\text{NH}_4^+ = -8.4$ mg N g$^{-1}$). In the 10-mm layer a net nitrate decrease ($\Delta\text{NO}_3^- = -28.9$ mg N g$^{-1}$) and net ammonium increase ($\Delta\text{NH}_4^+ = +15.5$ mg N g$^{-1}$) were observed despite the evidence that in the 2-mm layer aerobic conditions dominated. This was attributed to the coupled nitrification–denitrification occurring concomitantly with mineralization ($\Delta\text{NH}_4^+ = +15.5$ mg N g$^{-1}$) both in the aerobic and in the nonaerobic layers. Although no glucose was added in any of these experiments, no nitrite accumulation was observed. This suggests that, unlike in the experiments under anoxic atmosphere, denitrification in the 10-mm soil layer was not limited by availability of electron donors (organic matter). It is worth noting that the existence of oxygen-limited microenvironments in soil and the resulting combined nitrification–denitrification processes may promote significant $\text{N}_2\text{O}$ emission even under the ambient oxidative atmosphere (e.g., Kremen et al., 2005; Master et al., 2004).

The Use of Isotopic Enrichment for Spectroscopic Monitoring of Denitrification

The mathematical procedure for estimating the concentrations of the various isotopic $\text{N}_2\text{O}$ species (see FTIR analysis in Methods above) was first tested with a gas mixture of $^{14}\text{N}_2\text{O}$, $^{15}\text{N}_2\text{O}$, and $^{14}\text{N}^{15}\text{NO}$ of known concentration. The discrepancy between the actual and calculated relative fraction of each species was $<10\%$. This procedure was then applied to the spectra recorded during the incubation of Grumosol, which contained $^{14}\text{NO}_3^-$ (natural nitrate) and $^{15}\text{NO}_3^-$ (artificial addition) at a ratio close to 1:1 (Fig. 7a). The measured spectra indicate the emission of all four isotopic species of $\text{N}_2\text{O}$. The calculated concentrations of the various species during the incubation are shown in Fig. 8. Within measurement accuracy, temporal pattern for the two isotopomers $^{14}\text{N}^{15}\text{NO}$ and $^{15}\text{N}^{14}\text{NO}$ were similar, suggesting no strong site preference during denitrification under enrichment levels used. Similar observation was reported by Sutka et al. (2006) and Phillips et al. (2013) who used, respectively, IRMS and FTIR for isotopic analysis of $\text{N}_2\text{O}$. Furthermore, there appears to be a time lag in the emission of the heavier isotopologue $^{15}\text{N}_2\text{O}$, which suggests that fractionation occurs during the $\text{N}_2\text{O}$ formation. However, the low signal/noise ratio in the $\text{N}_2\text{O}$ spectra during the first hours of incubation makes it hard to reach a definite conclusion and additional work would be required to confirm this suggestion. Additional support for such fractionation is obtained by comparing the relative distribution of each of the $\text{N}_2\text{O}$ isotopic species after 22-h incubation relative to the initial $^{15}\text{NO}_3^-$/$^{15}\text{NO}_3^-$ ratio in the soil (Table 4). These results show $^{14}\text{N}$ enrichment in the generated $\text{N}_2\text{O}$, especially in the Terra Rossa and the Hamra soils. The isotopic fractionation suggested here under high $^{15}\text{N}$ en-
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richment (>40% enrichment) is commonly observed in studies tracking changes in natural abundance of N and O isotopic species in various soils and sediments (e.g., Mandernack et al., 2009; Well and Flessa, 2009).

Noteworthy, in all tested soils no difference was observed between the two isotopomers concentrations (14N15NO and 15N14NO) throughout the whole incubation period.

CONCLUSIONS

The present work demonstrates the possibility to use low resolution FTIR spectroscopy for real time monitoring emissions of N2O and its isotopologues from soils under controlled laboratory conditions. Spectral analysis of the gas phase above a thin soil layer showed a clear buildup of gaseous N2O during the soil incubation.

Good agreement was obtained between the amount of N emitted as N2O and that consumed as NO3− and NO2− in the soil when the atmosphere contained 10% (v/v) acetylene. This agreement was lower when high nitrate enrichment was applied to the soils. In general, N2O emission rates were shown to be a function of soil type and acetylene addition, oxygen availability, initial nitrate concentration in the soil, and electron donor (i.e., organic matter) availability. The continuous monitoring used here clearly showed that N2O emissions were very low during the initial few hour of incubation, most likely representing the time needed for recovery of microbial activity.

Under aerobic conditions N2O emission in the 2-mm Grumosol layer was negligible. However, increasing soil thickness from 2 to 10 mm induced a gradient of oxygen depletion, resulting in N2O production from what appears to be coupled nitrification–denitrification. Such a finding should raise attention to results obtained from incubation experiments with bioactive soils under varying conditions such as soil depth and soil moisture content, which are likely to induce changes of oxygen concentration with soil depth and also affect the residence time of N2O in the tested soil.

Despite the very thin (2 mm) soil layer used in the incubation, which should enhance the release of the generated N2O from the soil, N2O emission rates in oxygen-free environments were still sensitive to the presence of acetylene in the system headspace.

When soil samples were enriched with 15NO3− (enrichments ranging from 46 to 87 atom%), all four N2O isotopologues were observed in the LP cell headspace. Within the error of our isotopic analysis, similar temporal pattern were observed for the two isotopomers 14N15NO and 15N14NO, suggesting no site preference during denitrification. The relatively lower 15N-enrichment observed in gaseous N2O at the end of the incubation compared with the initial soil enrichment, as well as the delayed formation of gaseous N2O during the soil incubation, most likely representing the time needed for recovery of microbial activity.

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Fig. 8. Concentrations of the N2O isotopic species (calculated using the mathematical procedure described in the text) during incubation of Grumosol after it was spiked with (a) 100 μg 14NO3− or (b) 15NO3− as well as with 1 mg glucose g−1 dry soil. The natural NO3− concentration in the soil was about 106 μg g−1 dry soil.

Table 4. Initial 15N atom% in soil nitrate compared with 15N atom% in gaseous N2O, and the relative distribution of its four isotopologues. All values are in atom% and represent averages of two to three replicates. 15N recovery in N2O (relative to initial NO3−) was calculated as: 15N(N2O/15N(NO3−). Note: the natural nitrate concentrations in Grumosol, Terra Rossa, and Hamra samples were 107, 111, and 14.5 mg N g−1 soil, respectively.

<table>
<thead>
<tr>
<th>Soil</th>
<th>15N fraction in</th>
<th>15N fraction in</th>
<th>15N fraction in</th>
<th>15N fraction in</th>
<th>15N fraction in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial soil NO3−</td>
<td>14N2O</td>
<td>14N15NO</td>
<td>15N14NO</td>
<td>15N2O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grumosol</td>
<td>47</td>
<td>28±1</td>
<td>25±0.5</td>
<td>25±0.5</td>
<td>22±1</td>
</tr>
<tr>
<td>Terra Rossa</td>
<td>46</td>
<td>46±4</td>
<td>23±0.3</td>
<td>23±2</td>
<td>8±2</td>
</tr>
<tr>
<td>Hamra</td>
<td>56</td>
<td>40±6</td>
<td>25±1</td>
<td>25±1</td>
<td>10±4</td>
</tr>
<tr>
<td>Hamra†</td>
<td>87</td>
<td>5±4</td>
<td>18±2</td>
<td>18±3</td>
<td>59±10</td>
</tr>
</tbody>
</table>

† In the Hamra experiment, only 20 μg 15N g−1 were added; 100 μg 15N g−1 dry soil were added to all other soils. In all experiments 1 mg (glucose) g−1 dry soil was added.
soil moisture conditions) for investigating N2O fluxes under condi-
path and larger or thicker soil samples under various oxidation and
in various soils under well-defined conditions. Furthermore, this
method could be adjusted (i.e., using a gas cell with longer optical
path and larger or thicker soil samples under various oxidation and
soil moisture conditions) for investigating N2O fluxes under condi-
tions more relevant to ambient environment.

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