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Nitrous oxide (N\textsubscript{2}O) contributes significantly to global warming and climate change (e.g., IPCC 2007)\textsuperscript{[1]} and it is an efficient ozone-depleting substance\textsuperscript{[2]} with agricultural soils being the dominant sector of anthropogenic N\textsubscript{2}O emission.\textsuperscript{[1]}

Advanced N\textsubscript{2}O mitigation strategies for agricultural production systems rely on improved understanding of N\textsubscript{2}O formation in soils and partitioning to the main N\textsubscript{2}O source processes, i.e. nitrification and denitrification. The microbial enzymatic pathways associated with N\textsubscript{2}O production from nitrification and denitrification induce 15\textsubscript{N} depletion in the emitted N\textsubscript{2}O which is considerably higher for nitrifying bacteria than for denitrifying bacteria.\textsuperscript{[3,4]} Therefore, measurement of the 15\textsubscript{N} content in N\textsubscript{2}O (\(\delta^{15}\text{N}_{\text{bulk}}\) value) is an excellent tool to study these processes, although it has to be considered that its \(\delta^{15}\text{N}_{\text{bulk}}\) value also depends on the precursor signature, fractionation during N\textsubscript{2}O to N\textsubscript{2} reduction,\textsuperscript{[5]} and transport limitations as well as physiological controls.\textsuperscript{[6,7]} In addition to the bulk 15\textsubscript{N} isotopic composition of N\textsubscript{2}O, the site preference (SP = \(\delta^{15}\text{N}_{\text{bulk}} - \delta^{15}\text{N}_{\text{source}}\)) of N\textsubscript{2}O (\(\delta^{15}\text{N}_{\text{source}}\)) which specifies the intramolecular 15\textsubscript{N} distribution on the central (\(\alpha\)) and the end (\(\beta\)) positions of the linear asymmetric N\textsubscript{2}O molecule, has been shown to differ significantly between different microbial N\textsubscript{2}O-releasing
processes in soil. SP values for nitrification (i.e. NH₃ oxidation via hydroxylamine) were found to be between 31 and 37 %, and in the range of -10 to 0 % for denitrification (heterotrophic as well as nitrifier denitrification).[8–11] Therefore, analysis of the N₂O site-specific isotopic composition to allocate N₂O production processes in soil studies is of increasing interest.[12–16] However, N₂O isotopic source signatures for distinct microbial processes are still based on a limited number of pure culture studies. Furthermore, a simple two source mixing model might not always be adequate as, for example, N₂O production by fungal denitrification (ca. 37 %)[10] and N₂O to N₂ reduction by heterotrophic denitrifiers (εSP = 2.9 – 6.8 %)[12,17] significantly increase the N₂O site preference and might result in an overestimation of nitrification-derived N₂O.

Most reported studies analyzing N₂O isotopomers are based on mass spectrometric determination of molecular (N₂O⁰) and fragment (NO⁻) ions of N₂O, allowing the calculation of δ¹⁵Nbulk and SP values.[18,19] In contrast, novel spectroscopic techniques such as Fourier transform infrared (FTIR) spectroscopy,[20] or quantum cascade laser absorption spectroscopy (QCLAS),[21–23] enable the direct quantification of N₂O isotopomers based on their characteristic rotational-vibrational absorption spectra, and hold advantages over isotope ratio mass spectrometry (IRMS) in terms of field applicability.

The aim of the present study was to demonstrate the feasibility of continuous N₂O isotopomer analysis by laser spectroscopy for source identification of soil-derived N₂O and its validation by intercomparison with IRMS as standard technique.

EXPERIMENTAL

Setup

An arable soil, which had been used in previous studies,[24,25] taken from the top horizon of a Luvisol at the Hohenschulen experimental farm of Kiel University, Germany, was sieved and ca. 3 dm³ soil was repacked into 4.25 L glass jars to a bulk density of 1.4 g cm⁻³. Potassium nitrate and sucrose solution was applied on top of the soil at rates equivalent to 0.21 g K nitrate and 0.025 g nitrate-N kg⁻¹ soil dry matter (DM) (equivalent to 1200 kg sucrose ha⁻¹ and 60 kg nitrate-N ha⁻¹, respectively) to foster N₂O production by heterotrophic denitrification. The soil moisture was adjusted to 80 % water-filled pore space. A control treatment was amended with nitrate only. Both treatments were set up in triplicate.

Pressurized air (Messerschweiz AG, Lenzburg, Switzerland) was passed through the headspace of each incubation vessel at a flow rate of 20 mL min⁻¹ (Fig. 1). To assess the variability between different soil cores and to perform an offline intercomparison between QCLAS and IRMS on N₂O isotopomer concentrations, the outlet air of individual soil cores was sampled in Tedlar® bags deploying a peristaltic pump (Ecoline VC-MS/CA 8–6 with Tygon LFL tubing i.d. 0.63 or 0.89 mm; Ismatec, IDEX Health & Science SA, Glattbrugg, Switzerland) at 3.5 mL min⁻¹ (nitrate sucrose treatment) and 6 mL min⁻¹ (control treatment). The remaining outflow gas from the replicates of each treatment was combined and directed to a FTIR spectrometer for trace gas analysis (N₂O, CO₂). For the nitrate-sucrose treatment a FTIR spectrometer (Avatar 370, Thermo Fisher Scientific, Waltham, MA, USA) with a low-volume (50 mL) flow-through gas cell with a 1 m optical path length (model LFT-210; Axiom Analytical Inc., Tustin, CA, USA) and InSb detector was applied.[26] For the control cores, a FTIR spectrometer (CX4000; Gasmet Technologies Oy, Helsinki, Finland), with a 9.8 m optical path cell and MCT detector was deployed. Continuous trace gas analysis was initiated 8 h prior to fertilizer addition and continued until the N₂O mixing ratios decreased to background concentrations.

Prior to online N₂O isotopomer analysis by QCLAS, H₂O and CO₂ were quantitatively removed from the gas flow of the nitrate sucrose-treated soil cores, by means of a permeation drier (MD-070-24S; Perma Pure Inc., Toms River, NY, USA) and a chemical trap filled with Ascarite (20 g, 10–35 mesh; Sigma Aldrich, Buchs, Switzerland) bracketed by Mg(ClO₄)₂ (2 × 8 g; Sigma Aldrich). For N₂O concentrations above 100 ppm, the dried and CO₂-scrubbed sample gas was dynamically diluted with synthetic air (Messerschweiz AG) to a constant N₂O mixing ratio (100 ppm) using a LabVIEW™ controlled mass flow controller (MFC, Red-y Smart series; Vögtlin Instruments AG, Aesch, Switzerland), based on the N₂O concentrations determined by FTIR spectroscopy. This experimental setup greatly reduced the need for non-linearity corrections of the QCLAS results and allowed optimal accuracy.

Laser spectroscopy

The laser spectrometer consisted of a single-mode, pulsed QCL (Alpes Lasers SA, Neuchâtel, Switzerland) emitting at 2188 cm⁻¹, a multipass absorption cell (AMAC-56; optical path length 56 m, volume 500 mL; Aerodyne Research Inc., Billerica, MA, USA) and a detection scheme with pulse normalization.[22] Laser control, data acquisition and simultaneous quantification of the three main N₂O isotopic species (¹⁴N¹⁴N¹⁶O, ¹⁵N¹⁴N¹⁶O, ¹⁴N¹⁵N¹⁶O) were accomplished by TDLWintel software (Aerodyne Research Inc.) taking into account the path length, gas temperature (∼ 305 K), pressure (8 kPa) and laser line width (0.0068 cm⁻¹). The laser spectrometer was operated in a continuous flow through mode with a back pressure regulator (GSK-A3TA-FP22; Vögtlin Instruments AG) mounted upstream of the cell to maintain a constant cell pressure and a scroll pump (TriScroll 300; Agilent Technologies, Santa Clara, CA, USA) with a manual flow adjustment valve downstream.

The relative differences of the isotopic ratios δ¹⁵N² and δ¹⁵N⁰ were determined by deploying a set of laboratory calibration gases produced from pure medical N₂O (Messerschweiz AG) supplemented with distinct amounts of isotopically pure (>98 % ) ¹⁵N¹⁴N¹⁶O and ¹⁴N¹⁵N¹⁶O (Cambridge Isotope Laboratories, Andover, MA, USA).[23] Primary laboratory standards were analyzed for their δ¹⁵N², δ¹⁵N⁰ and δ¹⁵Nbulk values by IRMS at the Tokyo Institute of Technology.[19] Secondary working standards applied in the presented project were measured against primary standards by QCLAS: standard 1: δ¹⁵N² = 2.1 ± 0.1 %o, δ¹⁵N⁰ = 2.0 ± 0.2 %o, 246.9 ± 0.1 ppm N₂O; standard 2: δ¹⁵N² = 25.0 ± 0.1 %o, δ¹⁵N⁰ = 24.8 ± 0.2 %o, 249.1 ± 0.1 ppm N₂O (the precision indicated is the standard error of the mean) and diluted to 100 ppm with synthetic air prior to QCLAS analysis. To account for drift effects, standard 1 was analyzed once per hour. For N₂O concentrations between 60 and 100 ppm, the δ¹⁵N² and δ¹⁵N⁰ values were corrected for dependency on
the N2O mixing ratio. The Tedlar® bag samples were subsequently analyzed for their δ15N² and δ18O² values by QCLAS; for concentrations above 10 ppm N₂O in a continuous flow through mode, for lower concentrations after preconcentration applying a liquid nitrogen-free preconcentration device. During preconcentration N₂O is adsorbed on a porous polymer adsorption trap (HayeSep D 100–120 mesh; Hayes Separations Inc., Bandera, TX, USA) at −150 °C. Desorption is accomplished by resistive heating of the trap to +10 °C and purging the released N₂O with 10 mL min⁻¹ of synthetic air into the evacuated multipass cell of the laser spectrometer.[21,22] To confirm the accuracy of our measurements, N₂O isotopomer concentrations in the pressurized air were measured by QCLAS after preconcentration. The observed N₂O mixing ratios (329.8 ± 0.2 ppb) as well as the N₂O SP value of 17.7 ± 0.3 % (δ15N² = 15.2 ± 0.1 % and δ18O² = −2.5 ± 0.1 %) are consistent with background air (SP of 18.7 ± 2.2 %)[27] with minor contributions of a 15 N-depleted N₂O emission source.

Mass spectrometry

The gas samples collected in the Tedlar® bags were analyzed for their δ15N², δ15N³, and δ18O² values by IRMS as a direct intercomparison between the two techniques at the von Thünen Institute in Braunschweig, Germany. Isotopologue signatures of N₂O were determined by analyzing m/z 44, 45, and 46 of intact N₂O⁺ molecular ions as well as m/z 30, 31 of NO⁺ fragment ions.[19] A modified preconcentration unit consisting of a set of automated cryo-traps (PreCon; ThermoFinnigan, Bremen, Germany) equipped with an autosampler (Combi-PAL; CTC-Analytics, Zwingen, Switzerland) was coupled to a gas chromatograph (Trace GC Ultra; Thermo Fisher Scientific, Bremen, Germany) which was connected via a Conflo IV interface to a Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific). Simultaneous detection of m/z 30, 31, 44, 45, and 46 was hence possible. N-exchange between N₂O⁺ and NO⁺ in the ion source of the mass spectrometer, the so-called scrambling factor, was determined by analyzing defined mixtures of non-labeled N₂O with a N₂O standard labeled at the β-N position (98 atom %; CK Gas Products Ltd., Hook, UK) as described by Röckmann et al.[28] giving a scrambling factor of 0.08 (a scrambling factor of 0.5 would mask the site preference entirely). The isotopologue ratios of 15Rbulk, 15R and 15R¹⁸O were determined, and 15R¹⁸O was obtained by the relationship of 15Rbulk = (15R¹⁸O + 15R¹⁸O)/2, where 15R² = [15N¹⁸O¹⁴N¹⁴O]/[14N¹⁵O¹⁴N¹⁵O], 15R³ = [15N¹⁵O¹⁶O]/[14N¹⁴N¹⁵O¹⁶O], 15R⁴ = [14N¹⁵N¹⁵O]/[14N¹⁴N¹⁵O¹⁶O]. The isotopologue ratios of a sample (Rsample) were expressed as % deviation from the 15 N/14 N and 18 O/16 O ratios of the standard materials (Rstd; i.e. atmospheric N₂ and standard mean ocean water (SMOW)), respectively: δX = (Rsample / Rstd - 1) × 1000, where X = 15Nbulk, 15N², 15N³, or 18O. The typical analytical precision was 0.2, 0.4, and 0.3 % for δ15Nbulk, δ15N³, and δ18O² values, respectively. The detection limit for N₂O-N was 1.5 nM. Pure N₂O (purity >99.995; Linde, Munich, Germany) was used as reference gas which was analyzed for isotopologue signatures in the laboratory of the Tokyo Institute of Technology using the calibration procedures developed earlier.[19] This reference signature was used to correct the raw δ15N² value determined by our IRMS instrumentation. The linear regression between the δ15N² value and m/z 30 peak areas, as determined by analysis of reference gas standards with concentrations between 200 and 10000 ppb, was used to correct for non-linearity of the NO⁺ isotope ratios. The m/z 30 and m/z 44 peak areas were used to determine N₂O concentrations. The correction for 18O for the δ15N-N₂O value was made according to the method described by Brand.[29]

RESULTS AND DISCUSSION

Continuous analysis of trace gas concentrations and N₂O isotope ratios by infrared spectroscopy

Figure 2 displays the N₂O and CO₂ concentration profiles as analyzed by FTIR spectroscopy. Microbial activity in the nitrate sucrose-treated soil cores was considerably enhanced, as indicated by the N₂O and CO₂ mixing ratios in the offgas reaching up to 360 and 3300 ppm, respectively, while the control treatment revealed lower mixing ratios. The site-specific
isotopic composition ($\delta^{15}$N$_{a}$ and $\delta^{15}$N$_{b}$) of N$_2$O emitted from the nitrate sucrose-treated soil cores was analyzed online by QCLAS over 3 days at 1 Hz temporal resolution (Figs. 3(a) and 3(b) show 1-min average values). To our knowledge this study constitutes the first published example of a real-time analysis of N$_2$O isotopomers. During incubation the $^{15}$N content of the emitted N$_2$O ($\delta^{15}$N$_{\text{bulk}}$) changed considerably. Initially, the $\delta^{15}$N$_{\text{bulk}}$ values were around $-35\%$, but they then increased by more than 50\% in an almost linear way, reaching $+16\%$ after 3 days (Fig. 3(a)). Similar results were reported by Meijide et al.$[^{30}]$ who observed an increase in $\delta^{15}$N$_{\text{bulk}}$ values by almost 40\% within 4 days. The observed N$_2$O $\delta^{15}$N$_{\text{bulk}}$ values (relative to the applied nitrate $\delta^{15}$N value of $-3.8 \pm 0.1\%$) are within the range reported for denitrification-derived N$_2$O as summarized by Baggs.$[^{4}]$

Although the emphasis of this study is on the implementation of a novel analytical technique and intercomparison measurements and the detailed discussion of the involved microbial source processes is beyond its scope, it should be pointed out that $\delta^{15}$N$_{\text{bulk}}$ value observed in this study is in agreement with typical values reported for microbial N$_2$O production processes.

The $^{15}$N site preference (SP, Fig. 3(b)) of the N$_2$O released from the nitrate sucrose treatment was $-1\%$ at the beginning of the incubation experiment and declined to around $-2$ to $-3\%$ within the first day after onset. Two short-term shifts in SP and N$_2$O mixing ratios within this period (around 20 and 55 h after onset) are due to pressure fluctuations in the headspace caused by replacement of the Ascarite/Mg(ClO$_4$)$_2$ trap. The SP reached a maximum value of $+5\%$ around 40 h after fertilizer addition, which coincided with the highest N$_2$O emissions (Fig. 2). Subsequently, the SP decreased to around $+3\%$ before it leveled out at $+5\%$. The observed range of SP values is consistent with the dominance of heterotrophic denitrification as the main N$_2$O source process for the nitrate sucrose-amended soil cores. The predominance of

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**Figure 2.** N$_2$O (a) and CO$_2$ (b) concentrations from nitrate sucrose-treated soil and the control treatment (nitrate only) during 4 days of incubation.

**Figure 3.** Continuous laser spectroscopic analysis of soil-emitted N$_2$O for $\delta^{15}$N$_{\text{bulk}}$ values (a) and $^{15}$N site preference (SP; b) after nitrate sucrose treatment. Individual data points are 1-min average values. Analysis of gas samples (Tedlar® bags) integrating over 12 h (nitrate sucrose treatment) for QCLAS-IRMS intercomparison of N$_2$O $\delta^{15}$N$_{\text{bulk}}$ values (c) and $^{15}$N site preference (SP; d).
denitrification-derived N\textsubscript{2}O is congruent with other soil studies under similar conditions\cite{16,31}. While SP values around 0 \% or slightly negative have been reported for N\textsubscript{2}O production by denitrification (heterotrophic as well as nitrifier denitrification)\cite{8-11}, it has been shown that fractionation during partial N\textsubscript{2}O reduction favors \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) reduction relative to \(^{14}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) reduction, resulting in increasing SP\cite{12,17,31}. The increase in SP in the nitrate sucrose-addition treatment, therefore, could be explained by an increasing importance of N\textsubscript{2}O reduction with rising N\textsubscript{2}O emissions. However, as nitrification and fungal denitrification have been reported to produce N\textsubscript{2}O with SP values of 31 to 37 \% or 37 \%, respectively, we cannot exclude a contribution of these processes to the observed SP shift\cite{17,24}.

For the control treatment, no continuous N\textsubscript{2}O isotopic analysis was conducted, but Tedlar® bag gas samples were analyzed by IRMS and QCLAS. The \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) values of the emitted N\textsubscript{2}O displayed only a minor, but still significant increase from −38.7 to −34.2 \% (QCLAS) from day 1 to day 3 (data not shown), while the N\textsubscript{2}O SP increased from 4.3 to 7.7 \% (QCLAS). These results are included in the following section on the method intercomparison without detailed discussion of the underlying microbial production processes.

**Intercomparison of QCLAS and IRMS**

In addition to real-time \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) and SP analysis by QCLAS performed on N\textsubscript{2}O from the nitrate sucrose-treated soil cores, N\textsubscript{2}O isotopomers were determined in time-integrating bag samples by laser spectroscopy and IRMS. Figures 3(a)–3(d) indicate a considerable agreement between online N\textsubscript{2}O SP isotopic composition and offline analysis of Tedlar® bag gas samples by laser spectroscopy and IRMS. The results of both methods follow a similar trend and exhibit an excellent correlation, with R\(^2\) = 0.99 and p < 0.0001 (Fig. 4(a)). However, the \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) values determined by QCLAS show a systematic offset of 1.2 ± 0.1 \% (p < 0.0001) compared with those for the Tedlar® bag samples analyzed by IRMS. The source of this disagreement has not yet been identified, and it might be due to one (or both) of the involved methods. As similar \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) values were obtained with both techniques for N\textsubscript{2}O calibration gases, the discrepancy might be due to differences in the gas matrix (e.g. CO\textsubscript{2}), transportation, or gas conditioning prior to analysis, and this will be the subject of an upcoming research project. For SP the level of agreement is clearly lower (Fig. 4(b), R\(^2\) = 0.76; p < 0.0001). However, the SP values from the two techniques were not significantly different. Both may be explained to some extent by the considerably higher uncertainty of IRMS for SP (1 \%, 2\(\sigma\)) than for \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) (0.4 \%, 2\(\sigma\)) as SP includes the uncertainties of the \(^{15}\text{N}^2\) and \(^{15}\text{N}\)\(^{15}\text{N}\)\(^{16}\text{O}\) values\cite{32}. In contrast, the analytical precision (2\(\sigma\)) of the laser spectrometer at current elevated N\textsubscript{2}O mixing ratios (100 ppm) is higher, around 0.3 \% for both \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) and SP, for 1-min average values.

**CONCLUSIONS**

This study demonstrates the performance of QCLAS in terms of precision and temporal resolution when measuring N\textsubscript{2}O isotopomers. Laser spectroscopy was applied for the first time for the continuous analysis of the site-specific \(^{15}\text{N}\) isotopic composition of soil-derived N\textsubscript{2}O at high temporal resolution. In our intercomparison study using time-integrating bag samples, excellent agreement was observed for the N\textsubscript{2}O \(^{15}\text{N}\)\(^{14}\text{N}\)\(^{16}\text{O}\) value between the QCLAS results and the IRMS analysis. For the \(^{15}\text{N}\) site preference, the correlation suffered from the lower precision of IRMS for SP. These results confirm that laser spectroscopy is a feasible alternative technique to IRMS that will facilitate a large range of new process studies based on its capability for real-time N\textsubscript{2}O isotopic analysis. Moreover, the higher precision of QCLAS than of IRMS will enable more accurate analysis of isotope ratios of soil-derived N\textsubscript{2}O which will improve the investigation of N\textsubscript{2}O processes using the isotopomer approach. Currently, the amount of sample needed for QCLAS is significantly larger than for IRMS. However, this will soon be significantly improved as more sensitive laser spectrometers become available. In addition, we expect that laser spectrometers will be capable of providing data on N\textsubscript{2}O \(^{18}\text{O}\) values in addition to \(^{15}\text{N}^2\) and \(^{15}\text{N}\)\(^{15}\text{N}\)\(^{16}\text{O}\) values in the near future. This may allow the investigation of further processes, such as N\textsubscript{2}O reduction, based on additional isotopic discrimination patterns. Finally, robust field instruments will enable extended field studies with the additional advantage of immediate data availability.
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