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Nitrous oxide (N₂O), a highly climate-relevant trace gas, is mainly derived from microbial denitrification and nitrification processes in soils. Apportioning N₂O to these source processes is a challenging task, but better understanding of the processes is required to improve mitigation strategies. The N₂O site-specific ¹⁵N signatures from denitrification and nitrification have been shown to be clearly different, making this signature a potential tool for N₂O source identification. We have applied for the first time quantum cascade laser absorption spectroscopy (QCLAS) for the continuous analysis of the intramolecular ¹⁵N distribution of soil-derived N₂O and compared this with state-of-the-art isotope ratio mass spectrometry (IRMS).

METHODS: Soil was amended with nitrate and sucrose and incubated in a laboratory setup. The N₂O release was quantified by FTIR spectroscopy, while the N₂O intramolecular ¹⁵N distribution was continuously analyzed by online QCLAS at 1 Hz resolution. The QCLAS results on time-integrating flask samples were compared with those from the IRMS analysis.

RESULTS: The analytical precision (2σ) of QCLAS was around 0.3 ‰ for the ¹⁵Nbulk and the ¹⁵N site preference (SP) for 1-min average values. Comparing the two techniques on flask samples, excellent agreement (R² = 0.99; offset of 1.2 ‰) was observed for the ¹⁵Nbulk values while for the SP values the correlation was less good (R² = 0.76; offset of 0.9 ‰), presumably due to the lower precision of the IRMS SP measurements.

CONCLUSIONS: These findings validate QCLAS as a viable alternative technique with even higher precision than state-of-the-art IRMS. Thus, laser spectroscopy has the potential to contribute significantly to a better understanding of N turnover in soils, which is crucial for advancing strategies to mitigate emissions of this efficient greenhouse gas.
Nitrate was passed through the headspace of each incubation vessel. Both treatments were set up in triplicate.

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 dm² soil was repacked into 4.25 L glass jars to a bulk density of 1.4 g cm⁻³. Potassium nitrate and sucrose solution were applied on top of the soil at rates equivalent to 0.21 g succrose 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 (Messer Schweiz 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 dynamically diluted with synthetic air (Messer Schweiz 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-FF22; 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 (Messer Schweiz 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 δ¹⁵N¹⁵N¹⁶O 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 ‰, δ¹⁵N⁰ = 2.0 ± 0.2 ‰, 246.9 ± 0.1 ppm N₂O; standard 2: δ¹⁵N² = 25.0 ± 0.1 ‰, δ¹⁵N⁰ = 24.8 ± 0.2 ‰, 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 N$_2$O mixing ratio. The Tedlar® bag samples were subsequently analyzed for their $\delta^{15}$N$^\alpha$ and $\delta^{15}$N$^\beta$ values by QCLAS; for concentrations above 10 ppm N$_2$O in a continuous flow through mode, for lower concentrations after preconcentration applying a liquid nitrogen-free preconcentration device. During preconcentration N$_2$O is adsorbed on a porous polymer adsorption trap (HayeSep D 100–120 mesh; Hayes Separations Inc., Bandera, TX, USA) at $-15^\circ$C. Desorption is accomplished by resistive heating of the trap to $+10^\circ$C and purging the released N$_2$O 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, N$_2$O isotope concentrations in the pressurized air were measured by QCLAS after preconcentration. The observed N$_2$O mixing ratios (329.8 ± 0.2 ppb) as well as the N$_2$O SP value of 17.7 ± 0.3 % ($\delta^{15}$N$^\alpha$ = 15.2 ± 0.1 % and $\delta^{15}$N$^\beta$ = -2.5 ± 0.1 %) are consistent with background air (SP of 18.7 ± 2.2 %)[27] with minor contributions of a 15 N-depleted N$_2$O emission source.

Mass spectrometry

The gas samples collected in the Tedlar® bags were analyzed for their $\delta^{15}$N$^\alpha$, $\delta^{15}$N$^\beta$, and $\delta^{18}$O value by IRMS as a direct intercomparison between the two techniques at the von Thünen Institute in Braunschweig, Germany. Isotopologue signatures of N$_2$O were determined by analyzing m/z 44, 45, and 46 of intact N$_2$O$^+$ molecular ions as well as m/z 30, 31 of NO$^+$ fragment ions.[19] A modified preconcentration unit consisting of a set of automated cryotrap (PreCon; ThermoFinnigan, Bremen, Germany) equipped with an autosampler (Combi-PAL; CTC-Analytik, 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 N$_2$O$^+$ and NO$^+$ in the ion source of the mass spectrometer, the so-called scrambling factor, was determined by analyzing defined mixtures of non-labeled N$_2$O with a N$_2$O 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 $^{15}$R$^\alpha$ and $^{15}$R$^\beta$ were determined, and $^{15}$R was obtained by the relationship of $^{15}$R$^\alpha$ = ($^{15}$R$^\alpha$ + $^{15}$R$^\beta$)/2, where $^{15}$R$^\alpha$ = [14N$^{15}$N$^{16}$O]/[14N$^{14}$N$^{16}$O], $^{15}$R$^\beta$ = [15N$^{14}$N$^{16}$O]/[14N$^{14}$N$^{16}$O], $^{18}$R$^\alpha$ = [14N$^{14}$N$^{18}$O]/[14N$^{14}$N$^{16}$O]. The isotopologue ratios of a sample (R$_{sample}$) were expressed as % deviation from the $^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O ratios of the standard materials (R$_{std}$; i.e. atmospheric N$_2$ and standard mean ocean water (SMOW)), respectively: δX = (R$_{sample}$ / R$_{std}$ - 1) × 1000, where X = $^{15}$N$^\alpha$, $^{15}$N$^\beta$, $^{15}$N, or $^{18}$O. The typical analytical precision was 0.2, 0.4, and 0.3 % for $^{15}$N$^\alpha$, $^{15}$N$^\beta$, and $^{18}$O values, respectively. The detection limit for N$_2$O-N was 1.5 nM. Pure N$_2$O (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 $^{15}$N$^\alpha$ value determined by our IRMS instrumentation. The linear regression between the $\delta^{15}$N$^\alpha$ 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 N$_2$O concentrations. The correction for $^{18}$O for the $^{15}$N-N$_2$O value was made according to the method described by Brand.[28]

RESULTS AND DISCUSSION

Continuous analysis of trace gas concentrations and N$_2$O isotope ratios by infrared spectroscopy

Figure 2 displays the N$_2$O and CO$_2$ concentration profile as analyzed by FTIR spectroscopy. Microbial activity in the nitrate sucrose-treated soil cores was considerably enhanced, as indicated by the N$_2$O and CO$_2$ 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.\cite{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.\cite{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
denitrification-derived N2O is congruent with other soil studies under similar conditions.16,31 While SP values around 0 % or slightly negative have been reported for N2O production by denitrification (heterotrophic as well as nitrifier denitrification), it has been shown that fractionation during partial N2O reduction favors 15N14N16O reduction relative to 14N15N16O reduction, resulting in increasing SP.12,17,31 The increase in SP in the nitrate sucrose-addition treatment, therefore, could be explained by an increasing importance of N2O reduction with rising N2O emissions. However, as nitrification and fungal denitrification have been reported to produce N2O with SP values of 31 to 37 %, or 37 %, respectively, we cannot exclude a contribution of these processes to the observed SP shift.7,24

For the control treatment, no continuous N2O isotopic analysis was conducted, but Tedlar® bag gas samples were analyzed by IRMS and QCLAS. The 15Nbulk values of the emitted N2O 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 N2O 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 15Nbulk and SP analysis by QCLAS performed on N2O from the nitrate sucrose-treated soil cores, N2O isotope values were determined in time-integrating bag samples by laser spectroscopy and IRMS. Figures 3(a)-3(d) indicate a considerable agreement between online N2O 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 R² = 0.99 and p < 0.0001 (Fig. 4(a)). However, the 15Nbulk 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 any one (or both) of the involved methods. As similar 15Nbulk values were obtained with both techniques for N2O calibration gases, the discrepancy might be due to differences in the gas matrix (e.g. CO2), 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), R² = 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 %, 2σ) than for 15Nbulk (0.4 %, 2σ) as SP includes the uncertainties of the 15N² and 15N³ values.32 In contrast, the analytical precision (2σ) of the laser spectrometer at current elevated N2O mixing ratios (100 ppm) is higher, around 0.3 % for both 15Nbulk and SP, for 1-min average values.

**CONCLUSIONS**

This study demonstrates the performance of QCLAS in terms of precision and temporal resolution when measuring N2O isotopomers. Laser spectroscopy was applied for the first time for the continuous analysis of the site-specific 15N isotopic composition of soil-derived N2O at high temporal resolution. In our intercomparison study using time-integrating bag samples, excellent agreement was observed for the N2O 15Nbulk value between the QCLAS results and the IRMS analysis. For the 15N 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 N2O isotopic analysis. Moreover, the higher precision of QCLAS than of IRMS will enable more accurate analysis of isotope ratios of soil-derived N2O which will improve the investigation of N2O 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 N2O δ18O values in addition to δ15N² and δ15N³ values in the near future. This may allow the investigation of further processes, such as N2O 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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