First real-time isotopic characterisation of N_2O from chemodenitrification

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Abstract

Chemodenitrification can be a substantial abiotic source of nitrous oxide (N_2O) in soil. The isotopic signature of N_2O from this process could support source partitioning, but it is currently unknown in sufficient detail. In this study, we determined the isotopic composition of N_2O, produced by the reaction of nitrite (NO_2^-) with lignin, four lignin derivatives, and three types of soils, online with a quantum cascade laser absorption spectrometer (QCLAS). We present the first dataset of continuous measurements of $\delta^{15}N^{bulk}$ ($\delta^{15}N^{bulk} \equiv (\delta^{15}N^{\alpha} + \delta^{15}N^{\beta})/2$), $\delta^{18}O$, and site preference (SP_{N2O}, $SP_{N2O} \equiv \delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$) of N_2O from chemodenitrification in both chemical assays and soils. Considerable amounts of N_2O were produced by chemical reduction of NO_2^-, indicating that chemodenitrification could dominate N_2O emission in NO_2^--rich environments. The values of SP_{N2O} varied by more than 20‰ in the reactions of sodium nitrite with organic substances. Contrary to the common assumption that SP_{N2O} values are constant for a distinct N_2O source process, our results reveal a considerable shift in SP_{N2O} over time for most experiments. The large SP_{N2O} variability might be explained by the multiple pathways with hyponitrous acid or nitramide as N_2O precursors. These findings provide important new information to improve our understanding about the dependency of N_2O isotopic signatures on N_2O production processes.

Keywords: Chemodenitrification; Nitrous oxide; Site preference; Nitrite; Lignin derivatives

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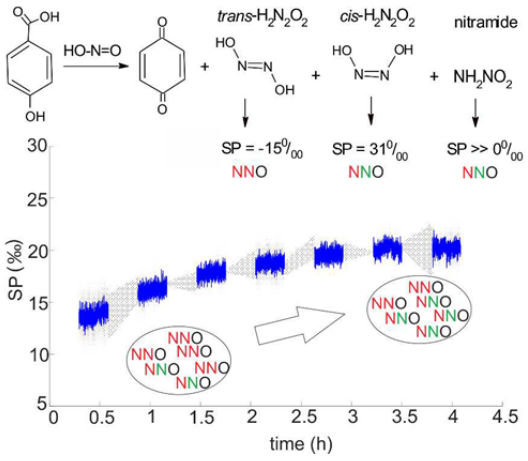


1. INTRODUCTION

Nitrous oxide (N_2O), whose radiative efficiency in the troposphere is almost 300-fold higher than that of carbon dioxide, is the third most important long-lived greenhouse gas contributing about 6% to total anthropogenic global warming (IPCC, 2013). Furthermore, N_2O can decompose to nitric oxide (NO), which further reduces ozone to oxygen, through photolysis in the stratosphere, hence N_2O is also contributing to the depletion of the ozone layer (Ravishankara et al., 2009). After successful emission reduction of chlorine- and bromine-containing halocarbons supported by the Montreal protocol, N_2O currently represents the single most important ozone-depleting substance and it is likely to remain the largest one in the 21st century (Ravishankara et al., 2009). However, the N_2O global mixing ratio has increased by 21% up until 2015 (328 ppb) compared with the pre-industrial level and is still increasing by around 0.89 ppb yr^{-1}, mainly due to the use of nitrogen fertiliser in agriculture (WMO, 2016). Therefore, it is currently imperative to mitigate global N_2O emissions, which would require a better understanding of relevant N_2O production pathways.

For a long period of time, microbial nitrification and denitrification conducted by bacteria, fungi and archaea have been considered as the main N_2O source processes. During nitrification, ammonium (NH_4^+) is first oxidised to hydroxylamine (NH_2OH) by ammonium oxidisers, which is afterwards oxidised to nitrite (NO_2^-) under the catalyst of hydroxylamine oxidoreductase (HAO), while N_2O is released as a byproduct of either biotic or abiotic NH_2OH oxidation (Caranto and Lancaster, 2017; Liu et al., 2017; Stein, 2011). In the case of denitrification including bacterial, fungal, and nitrifier denitrification, N_2O is considered as an obligatory intermediate or end product of biotic reduction of NO_2^- (Stein, 2011; Sutka et al., 2008). However, NO_2^- can be released through both nitrification and denitrification into the soil and rapidly chemically reduced to N_2O by soil organic matter (SOM) or transition metals, which is termed as chemodenitrification (Chalk and Smith, 1983). Acidic conditions largely promote chemodenitrification processes because the chemical reactivity of nitrous acid (HNO_2), formed through the protonation of NO_2^-, is much higher than that of the NO_2^- ion itself (Chalk and Smith, 1983). Increasing evidence demonstrates that N_2O emission from

chemodenitrification can be substantial in various ecosystems, including extreme arid and cold lakes, ponds, and soils (Ostrom et al., 2016; Peters et al., 2014; Samarkin et al., 2010), temperate forests and arable lands (Venterea, 2007; Wei et al., 2017a), and sediments (Wankel et al., 2017). Therefore, the contribution of chemodenitrification to global N_2O emissions could have been largely underestimated.

Stable isotope techniques offer a powerful tool to disentangle N_2O production pathways in the environment. Since the two nitrogen atoms are located asymmetrically in the linear N_2O molecule ($N^{\beta}N^{\alpha}O$), the difference of $\delta^{15}N$ between the α and β position is defined as site preference ($SP_{N2O} \equiv \delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$) (Toyoda and Yoshida, 1999). Independent of the isotopic composition of the substrates, SP_{N2O} has been thought to be very specific for a certain N_2O production pathway and represents the transient state of the N_2O formation and reduction (Toyoda and Yoshida, 1999; Toyoda et al., 2002). Microbial N_2O sources of nitrification and fungal denitrification, as well as chemical oxidisation of NH_2OH, feature relatively high SP_{N2O} values of 27 - 40‰ (Heil et al., 2014; Heil et al., 2015; Rohe et al., 2014; Sutka et al., 2003; Sutka et al., 2006; Sutka et al., 2008; Toyoda et al., 2005), while bacterial denitrification and nitrifier denitrification are characterised by low SP_{N2O} values of -11 to 1.4‰ (Frame and Casciotti, 2010; Sutka et al., 2003; Sutka et al., 2006; Toyoda et al., 2005). The reduction of N_2O to N_2 by heterotrophic denitrifiers is characterised by an isotopic enrichment factor for SP_{N2O} of -5.0 to -6.8‰ (Lewicka-Szczebak et al., 2017; Ostrom et al., 2007).

The $\delta^{15}N^{bulk}$ values of N_2O are highly influenced by the isotope effects of both N_2O production and reduction. During nitrification, the ^{15}N in N_2O is enriched by -0.3–5.7‰ for NH_2OH oxidation (Sutka et al., 2003; Sutka et al., 2006), but depleted by 18.6–68‰ and 12.3–34.3‰ for NH_4^+ oxidation by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA), respectively (Frame and Casciotti, 2010; Jung et al., 2014; Mandernack et al., 2009; Yamazaki et al., 2014). In contrast, N_2O is ^{15}N-depleted by 10–37‰ and 30.9–45.6‰ during nitrate (NO_3^-) reduction by denitrifying bacteria and fungi, respectively (Rohe et al., 2014; Sutka et al., 2006; Toyoda et al., 2005). When NO_2^- is reduced to N_2O, N_2O is ^{15}N-depleted by 31.4–39.5‰ for nitrifier denitrification and by

8.5–29.0‰ for fungal denitrification (Rohe et al., 2014; Sutka et al., 2003; Sutka et al., 2008; Yoshida, 1988). In addition, the reduction of N_2O to N_2 by heterotrophic denitrifiers is accompanied by a 4.1–6.6‰ decrease in $\delta^{15}N$ due to the isotope effect of N-O bond cleavage (Lewicka-Szczebak et al., 2017; Ostrom et al., 2007).

Despite the depletion of ^{15}N, ^{18}O is generally enriched in N_2O by 3–12.6‰ for nitrification (Jung et al., 2014; Santoro et al., 2011), and by 4–23‰ and 13.2–33.3‰ for NO_3^- reduction to N_2O by denitrifying bacteria and fungi, respectively (Rohe et al., 2014; Toyoda et al., 2005). ^{18}O enrichment of N_2O ranges from 34.2 to 60.8‰ for NO_2^- reduction during fungal denitrification and was found to be 8.4‰ during nitrifier denitrification (Frame and Casciotti, 2010; Rohe et al., 2014). The O exchange of reaction intermediates with water is a key factor affecting the fractionation effect of ^{18}O (Kool et al., 2007; Kool et al., 2009; Kool et al., 2011). Significant differences in ^{18}O exchange were observed between bacterial and fungal denitrification (Lewicka-Szczebak et al., 2016), varying from 17 to 94% depending on the N_2O production pathways and environmental conditions (Rohe et al., 2017). Based on the above-mentioned results, N_2O isotopic signatures have been widely used to partition N_2O sources for wastewater treatment systems (Harris et al., 2015; Wunderlin et al., 2013) and natural ecosystems from local (Bol et al., 2003; Toyoda et al., 2009; Toyoda et al., 2011; Wolf et al., 2015) to global scales (Röckmann and Levin, 2005; Snider et al., 2015; Toyoda et al., 2013).

In spite of the fact that chemodenitrification accounts for substantial N_2O emissions in certain ecosystems, its N_2O isotopic signature has not been investigated in sufficient detail (Ostrom et al., 2016; Zhu-Barker et al., 2015). Moreover, the currently reported N_2O isotopic signatures of chemodenitrification are discordant with each other for varying reaction conditions. The reported SP_{N2O} values include: 10 to 22‰ for the abiotic reduction of NO_2^- by ferrous iron (Fe^{2+}) at neutral pH when oxygen is limiting (Jones et al., 2015), 20 to 26‰ for the chemical reaction of NO_2^- with SOM at pH 3.8 (Wei et al., 2017a), around 30‰ for the reaction of NO_2^- with trimethylamine-borane at pH 1 (Toyoda et al., 2005), and 34 to 35‰ for the chemical reaction of NH_2OH with NO_2^- at pH 3–6 (Heil et

al., 2014). Thus, more sophisticated studies are needed to explain this large variability, as well as to evaluate its use for N_2O source attribution.

Lignin is a phenolic polymer accounting for 20% of organic carbon in the terrestrial biosphere, and its degradation in soil produces various phenolic compounds which are chemically highly reactive to NO_2^- (Thevenot et al., 2010). It has been reported that the NO_2^- related N_2O emission is positively correlated to the lignin content in acidic organic-rich forest soils (Wei et al., 2017a). Therefore, the reaction of lignin and its derivatives with NO_2^- might play a key role in chemodenitrification. A previous study showed that the SP_{N2O} of abiotic reactions of NO_2^- with lignin derivatives varied largely depending on pH and lignin-derived reactants (Wei et al., 2017b). The question of whether SP_{N2O} remain constant during NO_2^- reduction by SOM is vital for a process-level understanding and interpretation of potential reaction mechanisms. To answer this question, we investigated the N_2O isotopic signatures from reactions of NO_2^- with five lignin-derived compounds in real time, as well as NO_2^--related N_2O emissions in three types of soils for the first time by laser spectroscopy. In addition to N_2O, other by-products of the reaction, i.e. NO and phenols, were investigated with chemiluminescence and gas chromatography-mass spectrometry, respectively, to further elucidate the reaction mechanisms.

2. MATERIALS AND METHODS

2.1. Laser spectroscopic analysis of N_2O isotopic composition

The analyser used in this study was a commercially available quantum cascade laser absorption spectrometer (QCLAS, CW-QC-TILDAS-76-CS; Aerodyne Research Inc., Billerica, USA) that has been customised for simultaneous analysis of the four most abundant N_2O isotopic species, i.e. $^{14}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, and $^{14}N^{14}N^{18}O$ (Ibraim et al., 2018). The QCLAS comprises a continuous-wave mid-infrared quantum cascade laser source (Alpes Lasers SA, Switzerland) emitting at 2203 cm^{-1} and an astigmatic multi-pass absorption cell with a path length of 76 m and a cell volume of 0.62 L. Laser control, data acquisition, and quantification of the N_2O isotopic species are performed using TDL

Wintel (Aerodyne Research Inc., Billerica, USA). The QCLAS was operated in a flow-through mode with a temporal resolution of 1 Hz for data acquisition. The Allan variance precision of the spectrometer for ratios of isotopic species R^{α}, R^{β}, and R^{18_O} with 1250 s spectral averaging was less than 0.1‰ (1 σ standard deviation) (Werle et al., 1993). The gas flow through the QCLAS gas cell was adjusted to 12 mL min^{-1} and the cell pressure was kept at 26.67 ± 0.01 hPa using a pressure controller (MKS Instruments, Andover, MA, USA).

The isotope ratios of samples were corrected using a two-point calibration approach with calibration gas 1 (Cal1, $\delta^{15}N^{\alpha}$ = -22.21 ± 0.39‰ vs. air-N_2, $\delta^{15}N^{\beta}$ = -49.28 ± 0.40‰ vs. air-N_2, $\delta^{18}O$ = 26.94 ± 0.23‰ vs. VSMOW) and calibration gas 2 (Cal2, $\delta^{15}N^{\alpha}$ = -0.13 ± 0.28‰ vs. air-N_2, $\delta^{15}N^{\beta}$ = 1.35 ± 0.29‰ vs. air-N_2, $\delta^{18}O$ = 38.46 ± 0.15‰ vs. VSMOW) at 36 ppm. Both Cal1 and Cal2 were prepared in synthetic air (20.5% of O_2, 79.5% of N_2, 99.999% purity, Messer Schweiz AG, Switzerland) as described in Waechter et al. (2008) and calibrated against standard gases which were primarily analysed by Sakae Toyoda at Tokyo Institute of Technology (Mohn et al., 2014; Toyoda and Yoshida, 1999). To account for drift effects of the QCLAS, Cal1, diluted to 35 ppm N_2O, was analysed at least every 35 min for 10 min. Nonlinearity effects of N_2O concentration on isotope ratios were determined, before and after each experiment, analysing Cal1 at 12, 25, 36, 45, 54, 68, 82 ppm N_2O and corrected as described below. The selection of sample versus calibration gas was conducted by two 3-way solenoid valves (series 9, Parker Hannifin, USA).

2.2. Experimental setup

The experimental setup for online measurement of N_2O isotopocules is illustrated in Fig. 1. A quartz glass chamber with a PTFE cover plate was used as the reaction chamber. To decrease the delay time and increase the real-time response of the system, the volume of the reaction chamber was reduced to 300 mL by inserting a hollow polypropylene cylinder. In addition, a fan was installed at the top of the chamber to ensure that the headspace gas phase was homogenously mixed. The absence of significant leaks (pressure drop < 100 Pa min^{-1} at 0.1 MPa overpressure) was assured with

a leak test before every experiment. Gas flows in the setup were controlled using mass flow controllers (MFCs, Redy Smart series, Vögtlin Instruments, Switzerland) and a number of two- and three-position solenoid valves (Parker Hannifin Corp., USA). The experimental setup was controlled via a custom-written LabVIEW code (National Instruments Corp., USA), and devices were connected via a 16-port serial-to-ethernet network connector (Etherlite 160, Digi International Inc., USA).

The reactor was continuously purged with 14–16 mL min^{-1} of synthetic air (20.5% of O_2, 79.5% of N_2, 99.999% purity, Messer Schweiz AG, Switzerland) to deliver the liberated process gases into the spectrometer gas cell. The sample gas was dehumidified using a Nafion permeation dryer (MD-050-72S-1, Perma Pure, USA) and the overflow which was not subjected to laser spectroscopic analysis was exhausted into a fume hood, thereby keeping the pressure of the reaction chamber constant at ambient pressure. Carbon monoxide (CO) was removed with a Sofnocat oxidation catalyst (Sofnocat 423, Molecular Products Ltd., United Kingdom), as CO would otherwise induce spectral interferences during QCLAS analysis. Thereafter, carbon dioxide (CO_2) was removed from the process gas with a trap containing 13.8 g of ascarite (10–35 mesh, Fluka, Switzerland) bracketed with magnesium perchlorate ($Mg(ClO_4)_2$, 2 × 3.5 g, Fluka, Switzerland) and separated with glass wool (BGB Analytics AG, Switzerland). If N_2O concentrations in the sample gas were above 82 ppm, the gas was diluted by adding an additional flow of synthetic air through MFC1 and the N_2O concentration in the undiluted process gas was back-calculated by multiplying the measured N_2O concentration with the dilution factor. Since co-production of nitric oxide (NO) was expected together with N_2O, the absence of spectral interferences by NO was assured by a pre-test, where 50 ppm NO was added to a N_2O calibration gas and no significant change in the analysed N_2O isotopic composition was observed.

2.3. Experimental procedure

Sodium nitrite ($NaNO_2$, VWR, Germany) was used as the NO_2^- source, and its $\delta^{15}N$ (-18.00 ± 0.08‰ vs. air-N_2) and $\delta^{18}O$ (10.09 ± 0.05‰ vs. VSMOW) were measured using an elemental analyser coupled to an isotope ratio mass spectrometer (EA-IRMS, Flash EA 2000 and Delta V Plus; Thermo Fisher

Scientific, Bremen, Germany). Common lignin-derived compounds, organosolv lignin (Chemical Point UG, Oberhaching, Germany), 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4-hydroxy-3,5-dimethoxybenzoic acid, and 4-hydroxy-3,5-dimethoxybenzaldehyde (VWR, Germany) (Wei et al., 2017b), were chosen as model substances in this study. All chemicals were reagent grade or better. Forest and grassland soils were sampled from the O_a layer of the Wüstebach catchment (50°30′15″N, 6°18′15″E) and the Rollesbroich grassland (50°37′0″N, 6°26′0″E), respectively, in the northern Eifel region, Germany. The sites are part of the German environmental monitoring programme, named TERENO (Zacharias et al., 2011). Agricultural soil was sampled from the top layer (0–20 cm) of the Hohenschulen experimental farm (54°19'05"N, 9°58'38"E) at the University of Kiel, Germany. The characteristics of all soils are listed in Table 1.

A 100-mL beaker containing 5–26 g of carbon substances or soil together with a stir bar was placed into the chamber. Then, the whole setup was flushed using 18 mL min^{-1} of synthetic air until the N_2O concentration declined and stabilised at < 0.1 ppm. Afterwards, 50 mL of $NaNO_2$ solution in ultrapure water (18.2 MΩ cm) was injected into the beaker through a rubber septum on top of the chamber. A magnetic stirrer was used to mix the reactants for 5 min. Experimental details for each treatment including the amount of carbon substances/soils and $NaNO_2$ and flow rate of the carrier gas are listed in Table 2. Different substrate-to-nitrite ratios were chosen to reach the calibrated N_2O concentration range (12−82 ppm), and higher N_2O mixing ratios (>80 ppm) were diluted by manually adding a dilution flow of synthetic air. For sterilisation treatments, soils were distributed in portions of 10 g each into 50 mL glass bottles with screw caps (VWR, Germany) and autoclaved for 30 min at 1.2 bar and 121°C, and $NaNO_2$ solution and carrier gas were passed through a 0.2 µm PTFE filter. The online measurements of N_2O isotopic signatures lasted for 4-6 h, and all experiments were conducted in triplicate. pH was measured at an interval of 25-90 min using a pH meter (model 720, Orion Research, Inc., Jacksonville, Florida, USA).

2.4. Analysis of N_2O and NO_x emissions and organic reaction products

In a separate set of experiments, N_2O and nitrogen oxide (NO_x) emissions were analysed by a combination of a dual quantum cascade laser absorption spectrometer (QCLAS, DUAL CW RT-QC-TILDAS-76, Aerodyne Research, Inc., Billerica, MA, USA) and a chemiluminescence based analyser (CLD, AC32M, Ansyco GmbH, Karlsruhe, Germany). In each experiment, 0.5 g of lignin or 0.3 mmol of lignin derivatives was placed into a 100 mL beaker in a reaction chamber of 2 L volume. The system was purged with 3 L min^{-1} of synthetic air (20.5% oxygen and 79.5% nitrogen) until N_2O and NO_x signals decreased to below 0.1 and 0.01 ppb, respectively. Then, 100 mL of 1 mM sodium nitrite (VWR, Germany) solution was injected into the reaction beaker and the mixing ratios of NO_x and N_2O were recorded with temporal resolution of 5 s and 1 s, respectively.

The organic products of the reactions of NO_2^- with 4-hydroxy-3-methoxybenzoic acid, 4-hydroxy-3,5-dimethoxybenzoic acid, and lignin were determined using a gas chromatography-mass spectrometry (GC-MS). In detail, 0.06 mmol of lignin derivative (4-hydroxy-3-methoxybenzoic acid or 4-hydroxy-3,5-dimethoxybenzoic acid) or 0.1 g of lignin was placed into a 22.5 mL gas chromatography vial containing 5 mL of 0.1 mM $NaNO_2$ solution and the mixture was incubated for 24 h at room temperature. Afterwards, the products were extracted with 2 mL of methanol through solid phase extraction using a C18-bonded monolithic silica column (Bond Elut C18, 500 mg, 3 mL; VWR, Germany) and afterwards measured with a GC-MS (5973N, Agilent, Santa Clara, California , USA) (Kaiser and Benner, 2012).

2.5. Data analysis

N_2O isotope ratios (R^{α}, R^{β} and R^{18O}) were calculated based on the concentrations of $^{14}N^{14}N^{16}O$, $^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, and $^{14}N^{14}N^{18}O$ obtained from QCLAS (section 2.1):

$$R^{\alpha} = \frac{^{14}N^{15}N^{16}O}{^{14}N^{14}N^{16}O} \quad (1)$$

$$R^{\beta} = \frac{^{15}N^{14}N^{16}O}{^{14}N^{14}N^{16}O} \quad (2)$$

$$R^{18O} = \frac{^{14}N^{14}N^{18}O}{^{14}N^{14}N^{16}O} \quad (3)$$

A drift correction was applied to all isotope ratios based on regular measurements of Cal1 at 36 ppm N_2O. Thereafter, isotope ratios were converted to the δ-notation in per mille by applying the following formula:

$$\delta_{A/B} = \left(\frac{R_A}{R_B} - 1\right) \times 1000‰ \quad (4)$$

2-point calculation was applied using Cal1 as the working standard according to Gröning (2018):

$$r\delta_{sam/std} = \delta_{sam/cal1} \times \frac{\delta_{cal2/std} - \delta_{cal1/std}}{\delta_{cal2/cal1}} + \delta_{cal1/std} \quad (5)$$

In Eq. (5), sam, std, cal1, and cal2 denote sample gas, international standard (air-N_2 for $^{15}N/^{14}N$, and VSMOW for $^{18}O/^{16}O$), and calibration gas 1 and 2, respectively, $r\delta_{sam/std}$ is the raw δ value of sample without a correction of N_2O concentration dependency. Third-order polynomial regressions were applied to correct for the dependency of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and $\delta^{18}O$ on N_2O concentration (Figure S1). Two regression equations were defined: f_{low} for the N_2O range of 12–45 ppm, and f_{high} for the N_2O range of 36–83 ppm. The R^2 values were higher than 0.99 for both f_{low} and f_{high}, and the differences of δ values calculated by f_{low} and f_{high} at intermediate N_2O concentrations from 36 to 45 ppm were lower than 0.5‰ (Figure S2), which indicates that both f_{low} and f_{high} were well fitted. The N_2O concentration dependency was corrected according to the following equation:

$$\delta_{sam/std} = r\delta_{sam/std} + f(C_{cal1}) - f(C_{sam}) \quad (6)$$

In Eq. (6), $r\delta_{sam/std}$ is the raw δ value of the sample calculated based on Eq. (5), C_{cal1} is the average concentration of all the Cal1 measurements at around 36 ppm, C_{sam} denotes the N_2O mixing ratio of the sample, and f denotes f_{low} when C_{sam} is equal to or lower than 36 ppm, but f_{high} when C_{sam} is higher than 36 ppm. Finally, $\delta^{15}N^{bulk}$ was calculated as the average of $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$:

$$\delta^{15}N^{bulk} \equiv (\delta^{15}N^{\alpha} + \delta^{15}N^{\beta}) / 2 \quad (7)$$

while SP_{N2O} was determined using:

$$SP_{N2O} \equiv \delta^{15}N^{\alpha} - \delta^{15}N^{\beta} \quad (8)$$

Net isotope effects $\Delta^{15}N(N_2O/NO_2^-)$ and $\Delta^{18}O(N_2O/NO_2^-)$ were calculated as the difference of δ values of N_2O from that of NO_2^-, and then normalised based on the N_2O concentration:

$$\Delta^{15}N(N_2O/NO_2^-) = \sum((\delta^{15}N_{N2O}^{bulk} - \delta^{15}N_{NO_2^-}) \times C_{N_2O})/\sum C_{N_2O} \quad (9)$$

$$\Delta^{18}O(N_2O/NO_2^-) = \sum((\delta^{18}O_{N2O} - \delta^{18}O_{NO_2^-}) \times C_{N_2O})/\sum C_{N_2O} \quad (10)$$

The relative N_2O emission rate X_{N2O} (μmol N_2O mol^{-1} NO_2^- mol^{-1} s^{-1} for lignin compounds and μmol N_2O mol^{-1} NO_2^- kg^{-1} s^{-1} for soil samples), was normalised for the reactants as follows:

$$X_{N2O} = \frac{C_{N2O} \times P \times F}{M_{NO_2^-} \times R \times T \times m} \quad (11)$$

C_{N2O} is the mixing ratio of N_2O (ppm) measured in real time at 1 Hz, *P* denotes the pressure in the reaction chamber (101325 Pa), *F* represents the flow rate of the carrier gas (ml s^1), *R* is the ideal gas constant (8.314 m^3 Pa mol^{-1} K^{-1}), *T* represents the temperature of the headspace in the reaction chamber (293.15 K), M_{NO2-} is the total applied NO_2^- (mol), and m is the amount of lignin compounds or soil (mol for lignin compounds and kg for soil). The manual dilution of N_2O mixing ratio with synthetic air was corrected with either a dilution factor or polynomial curve fitting. All of the calculations were conducted using MATLAB R2014b (MathWorks, Inc., USA).

3. RESULTS

3.1. N_2O isotopic composition from reactions of NO_2^- with lignin derivatives

In the reaction of NO_2^- with organosolv lignin, approximately 8% of NO_2^- was converted to N_2O within 4 h, accounting for the highest relative N_2O emission rate (Fig. 2a). Compared with organosolv lignin, the reactions of NO_2^- with lignin-derived acids and aldehyde were much slower, with relative N_2O emission rates of less than 900 μmol N_2O mol^{-1} NO_2^- mol^{-1} s^{-1}. The average relative N_2O emission rate decreased in the order: 4-hydroxy-3,5-dimethoxybenzoic acid > 4-hydroxybenzoic acid > 4-hydroxy-3-methoxybenzoic acid > 4-hydroxy-3,5-dimethoxybenzaldehyde (Table 3). In addition, the temporal trend of N_2O emission differed between lignin derivatives: the relative N_2O emission rate peaked within 1.2 h and decreased quickly afterwards in the treatment of 4-hydroxy-3,5-dimethoxybenzoic acid (Fig. 2m), while it took about 3 h to reach the maximum in the experiment

285 with 4-hydroxybenzoic acid (Fig. 2e). By contrast, the reaction rate changed gradually in the
286 experiments with 4-hydroxy-3-methoxybenzoic acid and 4-hydroxy-3,5-dimethoxybenzaldehyde (Figs.
287 2i and 2q). pH increased by one unit in the first 4 h of the experiments with 4-hydroxybenzoic acid, 4-
288 hydroxy-3-methoxybenzoic acid, and 4-hydroxy-3,5-dimethoxybenzoic acid, while it remained
289 between approximately 2.7 and 6.1 in the experiments with organosolv lignin and 4-hydroxy-3,5-
290 dimethoxybenzaldehyde, respectively.

291 A large variability of SP_{N2O} values (from 4.6 ± 1.7‰ to 28.9 ± 8.8‰) was observed in reactions of
292 NO_2^- with lignin and lignin-derived acids as well as aldehyde (Table 1). Most interestingly, SP_{N2O}
293 values of the same reaction also changed substantially over time. In the first 4 h, SP_{N2O} values
294 increased by approximately 5‰ in the treatment of 4-hydroxy-3,5-dimethoxybenzaldehyde (Fig. 2r),
295 while they increased by more than 10‰ in the treatment of organosolv lignin (Fig. 2b). By contrast,
296 no clear temporal trend was observed for the reaction of NO_2^- with 4-hydroxy-3-methoxybenzoic
297 acid with SP_{N2O} values ranging from 10 to 15‰ during the whole experiment (Fig. 2j). The SP_{N2O}
298 fluctuated largely by approximate 10‰ over time in the reaction of NO_2^- with 4-hydroxybenzoic acid
299 and 4-hydroxy-3,5-dimethoxybenzoic acid (Figs. 2f and 2n).

300 The temporal trends of $\delta^{18}O$ and $\delta^{15}N^{bulk}$ of N_2O also differed in the reactions of NO_2^- with lignin
301 and the four lignin-related compounds: both $\delta^{18}O$ and $\delta^{15}N^{bulk}$ of N_2O remained relatively stable in
302 the experiments with 4-hydroxy-3-dimethoxybenzoic acid and 4-hydroxy-3,5-
303 dimethoxybenzaldehyde, however, they varied by more than 12‰ in the reactions of NO_2^- with
304 organosolv lignin and 4-hydroxybenzoic acid (Fig. 2). Correspondingly, the concentration-weighted
305 net isotope effects $\Delta^{15}N(N_2O/NO_2^-)$ and $\Delta^{18}O(N_2O/NO_2^-)$ varied from -20.1 to -10.0‰ and from 10.2 to
306 19.5‰, respectively, in the reactions of NO_2^- with lignin derivatives (Table 3). No significant Rayleigh
307 effects could be observed due to the low conversion ratio of NO_2^- to N_2O.
308

3.2. NOx emission and organic products from the reactions of NO_2^- with lignin derivatives

Considerable NO_x emission was observed in the reactions of NO_2^- with lignin derivatives, and NO_x emission increased to the maximum within 20 min, which was 1–2 h before N_2O reached its maximum (Fig. 3). In the reaction of NO_2^- with lignin, GC-MS analysis revealed the existence of various phenolic products including phenol, 3-methylphenol, 2-methoxyphenol, 4-ethylphenol, 2-methoxy-4-methylphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-vinylphenol, 2,6-dimethoxyphenol, 2,6-dimethoxy-4-methylphenol, 2-methoxy-4-(1-propenyl)phenol, and 2,6-demethoxy-4-[(1E)-prop-1-en-1-yl]phenol (Fig. 4a). While 2-methoxyphenol, 4-hydroxy-2-methoxybenzaldehyde, and 4-hydroxy-3-methoxybenzoic acid methyl ester were formed in the reaction of NO_2^- with 4-hydroxy-3-methoxybenzoic acid (Fig. 4b), and 2,6-dimethoxyphenol and 4-hydroxy-3,5-dimethoxybenzohydrazide were formed in the reaction of NO_2^- with 4-hydroxy-3,5-dimethoxybenzoic acid (Fig. 4c).

3.3. N_2O isotopic signatures from NO_2^- reactions in soils

N_2O emissions from soil samples varied from approximately 0.73 to 5.64 μmol N_2O mol^{-1} NO_2^- kg^{-1} soil s^{-1} in the order: forest soil > grassland soil > agriculture soil, and sterilisation did not inhibit the N_2O emissions (Table 3). The concentration-weighted SP_{N2O} values ranged from 17.9 ± 3.2‰ to 25.7 ± 2.3‰ in soils, which was within the SP_{N2O} range of NO_2^- reactions with lignin derivatives (Table 3). Similar to the treatments of lignin and its derivatives, temporal changes in SP_{N2O} by more than 6‰ were observed in forest, grassland, and agricultural soils (Fig. 5). In contrast, the variability of $\delta^{18}O$ and $\delta^{15}N^{bulk}$ between soil types was much smaller compared with SP_{N2O} (Fig. 5). The real-time $\Delta^{15}N(N_2O/NO_2^-)$ varied from -30 to -20‰ and from -27.7 to -21.9‰ in non-sterilised and sterilised soils, while $\Delta^{18}O(N_2O/NO_2^-)$ changed from 14.4 to 22.5‰ and from 14.8 to 21.4‰ in non-sterilised and sterilised soils, respectively (Table 3).

4. DISCUSSIONS

4.1 N_2O production pathways

Two different pathways, a hyponitrous acid pathway or a nitramide pathway (Fig. 6), have been discussed in relation to NO and N_2O formation from the reaction of NO_2^- with lignin derivatives (Austin, 1961; Kainz and Huber, 1959), which might therefore explain the different N_2O production rates and shifting SP_{N2O} values. Taking 4-hydroxybenzoic acid as an example, NO_2^- is first protonated to HNO_2, and the carboxyl group (-COOH) is subsequently substituted by a nitroso group (-NO) from HNO_2 to form CO_2 and 4-nitrosophenol (compound 1). The elimination of –COOH should account for the increase of pH in the treatments of 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, and 4-hydroxy-3,5-dimethoxybenzoic acid (Fig. 2). As 4-nitrosophenol is unstable at acidic pH, it partly decomposes to phenol (compound 4) and NO, while the other part is transformed to *p*-benzoquinone 4-oxime (compound 2) through rearrangement (Austin, 1961; Stevenson and Swaby, 1964). The formation of NO was confirmed in our experiments by high NO emission at the onset of the reactions measured with a chemiluminescence based analyser (Fig. 3). Similarly, 2-methoxyphenol and 2,6-dimethoxyphenol were detected as a product in the treatment of 4-hydroxy-3-methoxybenzoic acid (Fig. 4b) and 4-hydroxy-3,5-dimethoxybenzoic acid (Fig. 4c), respectively, which further confirmed the decarboxylation and the following removal of the nitroso group. Subsequently, the phenol can be attacked by another HNO_2 molecule through electrophilic substitution, resulting in the formation of 2-nitrosophenol (compound 5), which can rearrange to compound 6.

In the hyponitrous acid pathway, the C=N double bond of oximes can be subject to electrophilic attack of HNO_2 with a nitroso group being attached to the nitrogen atom and a hydroxyl group binding to the carbon atom, leading to the formation of hydroxyl-nitroso compounds (compounds 3 and 7) (Austin, 1961). Hydroxyl-nitroso compounds are very unstable and decompose quickly to hyponitrous acid (compound 8 and 9) and benzoquinones, with hyponitrous acid decomposing to N_2O (Austin, 1961). In the nitramide pathway, electrophilic substitution, instead of electrophilic

addition, occurs at the nitrogen atom of the oximes with the production of nitramines (compounds 10 and 11), which decompose quickly to benzoquinones and nitramide (compound 12), and nitramide further decomposes to N_2O (Kainz and Huber, 1959). With the consumption of NO_2^- and 4-hydroxybenzoic acid, as well as the decomposition of nitroso compounds, pH slowly increased and the formation rate of N_2O decreased (Fig. 2).

The number and position of electrophilic functional groups attached to the aromatic ring can affect the reaction kinetics through mesomeric (+/-M) and inductive (+/-I) effects, as well as steric effects. On the one hand, a methoxy group ($-OCH_3$) enhances the electron density of the aromatic ring by its +M effect, facilitating the electrophilic attack by HNO_2 (or more precisely by the resulting nitrosonium ion NO^+) especially in the ortho and para positions relative to the methoxy group, thereby accelerating the reaction. On the other hand, $-OCH_3$ also hinders the formation of oximes to some extent through a steric effect, thus inhibiting N_2O production. In addition, acidic pH favours the protonation of NO_2^-, thereby promoting the reaction rate of NO_2^- with lignin compounds. Therefore, we hypothesise that the mesomeric and inductive effects can explain the much higher N_2O emission rate in the 4-hydroxy-3,5-dimethoxybenzoic acid treatment than in the 4-hydroxybenzoic acid treatment in the first hour of the reaction, while the higher pH could explain the lower N_2O production rate 2 h later (Figs. 2e and 2m). Even though the methoxy group in 4-hydroxy-3-methoxybenzoic acid increases the aromatic electron density, the increase in electron density is less pronounced as in 4-hydroxy-3,5-dimethoxybenzoic acid. This, in combination with the inhibitory steric effect of the methoxy group and the higher pH, could be the reason why the N_2O emission rate did not increase in the 4-hydroxy-3-methoxybenzoic acid treatment compared with 4-hydroxybenzoic acid in the first 3 h (Figs. 2e and 2i). In the case of 4-hydroxy-3,5-dimethoxybenzaldehyde, the aldehyde group first has to be oxidized to a carboxyl group before decarboxylation can occur on the one hand, and the pH higher than 6.0 limits the protonation of NO_2^- on the other hand. Therefore, the N_2O emission rate was much lower in the experiment with 4-hydroxy-3,5-dimethoxybenzaldehyde compared with 4-hydroxy-3,5-dimethoxybenzoic acid (Figs. 2m

386 and 2q). Since organosolv lignin is extracted with an organic solvent as one of the alternative
387 chemical wood pulping techniques (Wei et al., 2017b), it has a higher content of reactive functional
388 groups compared with other lignin derivatives (Fig. 4). The various reactive functional groups as well
389 as the very low pH in the experiment with organosolv lignin helps to explain the drastically higher
390 N_2O emission rate (Fig. 2a).
391
392 **4.2. N_2O isotopic signatures depending on reaction conditions**
393 *4.2.1 SP_{N2O}*
394 Real-time measurements of SP_{N2O} from the reactions between NO_2^- and lignin derivatives over
395 4–6 hrs resulted in concentration-weighted average SP_{N2O} values between 13.4 ± 3.8‰ and 24.4 ± 1.0‰
396 (Table 3). This is comparable to an earlier off-line study, where a large variability of SP_{N2O} values
397 (11.9–30‰) has been observed (Wei et al., 2017b). Remaining differences in SP_{N2O} might be
398 explained by a substantial longer incubation period in previous off-line experiments (24 h) (Wei et al.,
399 2017b) in comparison to measurements conducted in the present study (4–6 h). SP_{N2O} values for the
400 reaction of NO_2^- with lignin derivatives observed in both studies (Wei et al., 2017b) also coincide with
401 the ones collected from reactions of NO_2^- with SOM fractions at pH 3 (20–26‰) (Wei et al., 2017a),
402 the chemical reduction of NO_2^- by ferrous iron at pH 7 under anaerobic conditions (10–22‰) (Jones
403 et al., 2015), and the reaction of NO_2^- with trimethylamine borane at pH 1 (~30‰) (Toyoda et al.,
404 2005). The significant progress in the present study, to the best of our knowledge, provides the first
405 real-time dataset of SP_{N2O} values for a number of exemplary reactions, which revealed a shift in SP_{N2O}
406 during the reactions. In the following, these temporal trends are discussed with respect to N_2O
407 production pathways. Isotope effects of N_2O reduction are not considered, since it is very unlikely
408 that N_2O is further reduced to N_2 during chemodenitrification under oxic conditions.
409 According to the N_2O reaction mechanisms proposed above (Fig. 6), hyponitrous acid and
410 nitramide are supposed to be the direct precursors of N_2O formation from the reaction of NO_2^- with
411 lignin derivatives. For the symmetric hyponitrous acid molecule, isotope effects during its

412 decomposition are probably the key factor controlling SP_{N2O} values. Theoretical calculations using
413 density functional theory (DFT) calculations by Fehling and Friedrichs (2011) predict the formation of
414 both *cis*- and *trans*-hyponitrous acid, and substantially different SP_{N2O} values for N_2O formation via
415 both intermediates. While a low SP_{N2O} value is predicted for the reaction via *trans*-hyponitrous acid (-
416 15‰), a much higher value is assumed for *cis*-hyponitrous acid decomposition (31‰) (Fehling, 2012).
417 In principle, the formation of *trans*-hyponitrous acid from hydroxyl-nitroso compounds is favoured
418 due to steric effects, however, *trans*-hyponitrous acid could be isomerised to *cis*-hyponitrous acid
419 through a fast acid-base equilibrium which generally dominates under strongly acidic conditions
420 (Bringas et al., 2016; Fehling and Friedrichs, 2011). Hyponitrous acid is also proposed as the key
421 precursor in microbial N_2O production. During the oxidation of NH_2OH to N_2O by HAO, a NH_2OH
422 molecule is first bound to the enzyme, then a second NH_2OH molecule attacks the N atom of the first,
423 leading to the formation of a *cis*-hyponitrous acid-like N-N bond (Yamazaki et al., 2014). Finally, N_2O
424 with a SP_{N2O} value of 30–36‰ is generated through the cleavage of the OH group of the first NH_2OH
425 molecule (Yamazaki et al., 2014). The NO reduction by fungal NO reductase (NOR) to N_2O follows a
426 mechanism that is similar with the NH_2OH oxidation by HAO, as two NO molecules are sequentially
427 bound to NOR and form a *cis*-hyponitrite-like compound which decomposes quickly to N_2O with a
428 SP_{N2O} of 35–37‰ (Obayashi et al., 1998; Rohe et al., 2014; Sutka et al., 2008). By contrast, during
429 bacterial denitrification, two NO molecules are simultaneously bound to the enzyme and a *trans*-
430 hyponitrite-like compound is formed (Watmough et al., 2009), which further decomposes to N_2O
431 with a SP_{N2O} of around zero.

432 In the nitramide pathway, the N atoms of the first- and second-bound nitroso group act as the
433 terminal (N^{β}) and central (N^{α}) nitrogen atoms of the N_2O molecule, respectively. Nitramide formation
434 is the rate-determining step, hence kinetic isotope effects during nitramide formation are supposed
435 to dominate the N_2O isotopic composition. Since the cleavage of the N=C bond favours $^{15}N^{\beta}$ depletion
436 but the formation of the N-N bond of nitramines favours $^{15}N^{\alpha}$ enrichment, we speculate that the
437 SP_{N2O} value via the nitramide pathway should be significantly higher than zero. Consequently, both

438 the nitramide and *cis*-hyponitrous acid pathways favour higher SP_{N2O}, while the *trans*-hyponitrous
439 acid pathway favors lower SP_{N2O}. The temporal changes of SP_{N2O} values could result from the varying
440 ratio of N_2O being produced via the *trans*-hyponitrous acid with respect to the sum of N_2O produced
441 via nitramide and *cis*-hyponitrous acid.

442 The electrophilic attack and the formation of hyponitrous acid can be facilitated by acidic pH. By
443 contrast, the formation of nitramide is favoured at higher pH. Therefore, the increase in pH could
444 account for the dramatic drop of N_2O production rate, accompanied by an increase of SP_{N2O}, at the
445 end of the experiment with 4-hydroxybenzoic acid and 4-hydroxy-3,5-dimethoxybenzoic acid (Fig. 2).

446

447 *4.2.2 Net isotope effects*

448 The net ^{15}N isotope effect, $\Delta^{15}N$ (N_2O/ NO_2^-), observed in this study is within the range of values
449 reported in previous studies on chemodenitrification (Grabb et al., 2017; Jones et al., 2015; Wei et al.,
450 2017b), but slightly higher than that of nitrifier denitrification (Sutka et al., 2006). Isotope effects are
451 affected by the reaction kinetics and they are generally determined by the rate-limiting step (Snider
452 et al., 2009), such as the formation of hyponitrous acid and nitramide in the reactions of lignin
453 derivatives with NO_2^-. The cleavage of ^{14}N-C to form hyponitrous acid and ^{14}N=C to form nitramide
454 are preferred over the cleavage of ^{15}N-C and ^{15}N=C bonds. Therefore ^{15}N depletion was observed in
455 the chemical reduction of NO_2^- to N_2O by lignin derivatives (Table 3). Even though N_2O reduction
456 leads to an increase of $\delta^{15}N^{bulk}$ of the remaining N_2O pool by 2-7‰ (Ostrom et al., 2007), this process
457 is not relevant during chemodenitrification, hence the variation of $\Delta^{15}N$ (N_2O/ NO_2^-) during the
458 reactions of this study (Fig. 2) are likely resulting from the dynamic change of hyponitrous acid and
459 nitramide formation rates, dependent on substrate availability and pH.

460 The concentration-weighted net ^{18}O isotope effect, $\Delta^{18}O$ (N_2O/ NO_2^-), ranged from 10.2 to
461 20.2‰ (Table 3), which is in a similar range of previously reported values (Grabb et al., 2017; Jones et
462 al., 2015; Wei et al., 2017b). The value of $\Delta^{18}O$ (N_2O/ NO_2^-) found in the present study is much higher
463 than that of nitrifier denitrification (Sutka et al., 2006), but much lower than that of fungal

464 denitrification (Rohe et al., 2014; Sutka et al., 2008). Apart from the kinetic isotope effect of N_2O
465 production, $\Delta^{18}O$ (N_2O/ NO_2^-) is also strongly influenced by O exchange with water. Depending on
466 different N_2O production mechanisms, O exchange of substrates with water can vary from
467 approximately 2% to more than 90% (Kool et al., 2007). In the reactions of lignin derivatives with
468 NO_2^-, O exchange could occur both between nitrite itself and water and between the hydroxyl
469 groups of hyponitrous acid and nitramide. However, the rate and degree of O exchange during
470 formation of hyponitrous acid and nitramide are currently unclear and should be explored in future
471 studies.
472
473 **4.3 Chemodenitrification and N_2O source partitioning**
474 *4.3.1 Chemodenitrification in soil*
475 Recently, increasing evidence is provided for the central role of NO_2^- in the soil N cycle and
476 particularly in N_2O emission (Maharjan and Venterea, 2013; Venterea, 2007). In the present study,
477 we also found that NO_2^- led to N_2O emission in all of the three types of soils (Fig. 3), equivalent to
478 43.2–483.8 μmol N_2O mol^{-1} NO_2^- kg^{-1} soil d^{-1}, which is in a similar range as in previous studies where
479 chemodenitrification dominated (Ostrom et al., 2016; Venterea, 2007; Wei et al., 2017a). As NO_2^- -
480 related N_2O production in unsterilised soils may include microbial pathways, e.g. denitrification and
481 nitrifier denitrification, and chemodenitrification (Wankel et al., 2017), sterilised soil samples were
482 also analysed in the present study. Instead of decreasing N_2O production, autoclaving increased the
483 N_2O emission rate dramatically, which could be explained by an increased solubility of SOM after
484 autoclaving, accelerating the reactivity of the soil to NO_2^- (Berns et al., 2008). In addition, the values
485 of SP_{N2O}, $\delta^{18}O$, and $\delta^{15}N^{bulk}$ were within the range of NO_2^--lignin reactions in chemical assays and they
486 changed in a similar trend in sterilised and unsterilised soil samples (Fig. 3), indicating that
487 chemodenitrification was the main N_2O source in soils after NO_2^- application and accounted for the
488 shifts of N_2O isotopic composition.
489

4.3.2 N_2O source partitioning

Even though SP_{N2O} is widely used for N_2O source partitioning, it still remains an open question of whether it is sufficiently conclusive or not (Decock and Six, 2013; Wei et al., 2017b). The reliability of using isotopic signatures in N_2O source partitioning highly depends on the robustness of N_2O isotopic signatures of certain N_2O production and reduction processes. The ranges of SP_{N2O}, $\delta^{15}N^{bulk}$, and $\delta^{18}O$ have been observed to be 6.0 to 37.4‰, -41.0 to -3.9‰, and 14 to 44.6‰ for chemodenitrification (Jones et al., 2015; Toyoda et al., 2005; Wei et al., 2017b), -11 to 1.4‰, -34.8 to -8.8‰, and 10 to 20‰ for bacterial denitrification (Toyoda et al., 2005; Zou et al., 2014), 34 to 40‰, -34 to -21.9‰, and 30 to 40‰ for fungal denitrification (Sutka et al., 2008), and 27 to 37‰, -61.4 to -40.3‰, and 40 to 50‰ for nitrification (Sutka et al., 2006), respectively (Fig. 7a). Net isotope effects of $\Delta^{15}N(N_2O/NO_2^-)$ and $\Delta^{18}O(N_2O/NO_2^-)$, have been observed in ranges of -28.1 to 9.6‰ and 3 to 24.2‰ for chemodenitrification (Grabb et al., 2017; Jones et al., 2015; Wei et al., 2017b), -39.5 to -31.4‰ and 7.0 to 9.8‰ for nitrifier denitrification (Sutka et al., 2006), and -29.0 to -8.8‰ and 55.8 to 60.8‰ for fungal denitrification (Rohe et al., 2014; Sutka et al., 2008), respectively (Fig. 7b).

Due to the distinct ^{15}N and ^{18}O isotopic effects, $\Delta^{15}N(N_2O/NO_2^-)$ and $\Delta^{18}O(N_2O/NO_2^-)$ of chemodenitrification are clearly separated from the corresponding values of fungal denitrification and nitrifier denitrification (Fig. 7b). Even though $\delta^{15}N^{bulk}$ and $\delta^{18}O$ of N_2O of chemodenitrification are also clearly distinct from bacterial denitrification, they strongly overlap with fungal denitrification and nitrification in the end-member map (Fig. 7a). To date, only few data of $\Delta^{15}N(N_2O/NO_2^-)$ and $\Delta^{18}O(N_2O/NO_2^-)$ from NO_2^--related N_2O production have been published (Rohe et al., 2014; Sutka et al., 2006; Sutka et al., 2008), and further studies are needed to explore the isotope effects of N_2O production under various conditions.

Since the range of SP_{N2O} values of chemodenitrification is in the intermediate zone between fungal denitrification and nitrifier denitrification, a classical two-end-member mixing model (Zhang et al., 2016) fails to quantify N_2O sources unambiguously. Therefore, a more sophisticated model involving both SP_{N2O} and isotope effects is needed for more reliable N_2O source partitioning. Similarly,

516 SP_{N2O} values encompassed the ranges of microbial nitrification (33 to 37‰) and denitrification (-10 to
517 0‰) in the McMurdo Dry Valleys, Antarctica, even though chemodenitrification accounted for most
518 of the N_2O emission (Peters et al., 2014). Further, the application of SP_{N2O} also failed to attribute
519 source processes of N_2O in Lake Vida, Victoria Valley, Antarctica (Ostrom et al., 2016; Zhu-Barker et
520 al., 2015) and in the Don Juan Pond, Antarctica (Samarkin et al., 2010), where chemodenitrification
521 with distinguishable N_2O isotope signatures acted as the main N_2O source.
522
523 **5. CONCLUSION**
524 Biotic ammonium oxidisation and nitrate reduction provide NO_2^- sources for
525 chemodenitrification. On the other hand, chemodenitrification and biotic N_2O production processes,
526 including nitrification and denitrification, compete for NO_2^-. In agreement with a previous study (Wei
527 et al., 2017b), we found that SP_{N2O} values of chemodenitrification varied largely in experiments with
528 different lignin derivatives, and end-member maps of N_2O isotopic signatures become highly limited
529 for N_2O source partitioning when chemodenitrification is taken into account. Furthermore, this
530 laboratory study demonstrated that the N_2O isotopic signatures of chemodenitrification also changed
531 over time during the reaction, which contributes to the temporary variation of N_2O isotopic
532 composition in soils. Two different N_2O production mechanisms with hyponitrous acid or nitramide
533 as intermediate would very likely explain the temporal changes of SP_{N2O} during the reactions studied,
534 which deserve to be further explored. Due to the large variability and uncertainty of N_2O isotopic
535 signatures from chemodenitrification, additional constraints, such as a combination of N_2O isotopic
536 composition with N and O isotope effects, novel clumped-isotopes or triple oxygen isotopic
537 information, are needed for making N_2O source partitioning unambiguous.
538

539 **Table 1**. Characteristics of the soil samples. The indicated precision is the standard deviation for

540 replicate sample measurements (n = 3).

Soil sample	Soil pH	Total N ($mg\ g^{-1}$)	Total C (%)	C/N	WHC [a] (%)	Fe ($mg\ g^{-1}$)	Mn ($mg\ g^{-1}$)
Forest soil	3.5 ± 0.0	14.6 ± 0.0	30.1 ± 0.8	20.6 ± 0.6	137.0 ± 0.1	29.0 ± 3.0	2.20 ± 0.12
Grassland soil	5.1 ± 0.0	12.8 ± 0.1	4.62 ± 0.02	3.6 ± 0.1	80.0 ± 1.2	33.5 ± 1.5	1.40 ± 0.40
Agricultural soil	6.0 ± 0.2	1.4 ± 0.1	1.28 ± 0.01	9.1 ± 0.2	35.0 ± 4.2	11.7 ± 0.2	0.46 ± 0.03

541 Note:
542 [a] WHC, water holding capacity.

543 **Table 2**. Experimental details for individual treatment.

Lignin derivative/soil sample	Lignin derivative (mmol)/soil (g)	Nitrite (mmol)	Water (mL)	Flow rate ($mL\ min^{-1}$)
organosolv lignin[a]	3.3	0.1 – 0.2	50	16 – 32
4-hydroxybenzoic acid	57.9	20.0	50	16
4-hydroxy-3-methoxybenzoic acid	59.5	23.5	50	16 – 32
4-hydroxy-3,5-dimethoxybenzoic acid	40.4	27.9	50	16 – 32
4-hydroxy-3,5-dimethoxybenzaldehyde	34.0	58.8	50	16
non-sterilised forest soil	10.0	18.8 – 31.8	50	16 – 32
non-sterilised grassland soil	10.0	17.6 – 29.4	50	16
non-sterilised agricultural soil	10.0	17.6 – 29.4	50	16
sterilised forest soil	10.0	29.4 – 35.3	50	12.8 – 16
sterilised grassland soil	10.0	6.5 – 9.2	50	16 – 20
sterilised agricultural soil	10.0	11.8 – 13.5	50	16 – 18.4

544 Note:
545 [a] organosolv lignin is estimated as $C_{81}H_{92}O_{28}$ with molecular weight of 1513.6 g mol^{-1} according to
546 PubChem.

547 **Table 3** Net isotope effect of ^{15}N and ^{18}O, N_2O site preference (SP_{N2O}), and N_2O production rate of the reaction of nitrite with different organic

548 compounds and soil samples. Results are shown as the average values ± standard deviation of three replicates.

	$\Delta^{15}N(N_2O/NO_2^-)$ (‰ vs. air-N_2)			$\Delta^{18}O(N_2O/NO_2^-)$ (‰ vs. VSMOW)			SP_{N2O} (‰)			Relative N_2O production rate [c]		
	Max. [a]	Min. [a]	Avg. [b]	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.
Organosolv lignin	-3.1 ± 4.8	-17.8 ± 4.0	-10.0 ± 4.1	21.4 ± 1.2	16.0 ± 0.9	19.5 ± 1.7	28.8 ± 0.2	12.1 ± 1.7	24.4 ± 1.0	940 ± 37	475 ± 156	810 ± 33
4-hydroxybenzoic acid	-7.1 ± 4.5	-22.8 ± 0.8	-15.4 ± 1.2	17.0 ± 2.2	4.7 ± 3.7	10.2 ± 3.4	25.6 ± 1.4	7.7 ± 2.8	19.5 ± 0.5	0.28 ± 0.0	0.10 ± 0.02	0.19 ± 0.02
4-hydroxy-3-methoxybenzoic acid	-14.8 ± 3.9	-20.9 ± 4.2	-17.7 ± 3.9	19.4 ± 4.7	10.3 ± 0.9	13.3 ± 1.6	20.5 ± 6.4	7.3 ± 5.4	13.4 ± 3.8	0.20 ± 0.02	0.08 ± 0.02	0.15 ± 0.01
4-hydroxy-3,5-dimethoxybenzoic acid	-9.9 ± 0.3	-18.3 ± 0.4	-13.0 ± 0.5	20.2 ± 2.9	7.2 ± 2.1	12.2 ± 0.4	28.9 ± 8.8	4.6 ± 1.7	15.4 ± 1.7	0.90 ± 0.11	0.00 ± 0.13	0.33 ± 0.02
4-hydroxy-3,5-dimethoxybenzaldehyde	-17.7 ± 3.8	-22.6 ± 1.9	-20.1 ± 2.8	20.6 ± 3.2	16.5 ± 2.7	18.9 ± 2.8	23.2 ± 3.4	13.7 ± 4.0	19.2 ± 2.6	0.12 ± 0.02	0.09 ± 0.02	0.11 ± 0.02
non-sterilised agricultural soil	-20.0 ± 5.2	-26.6 ± 2.4	-22.7 ± 4.8	22.5 ± 2.7	17.3 ± 3.3	20.2 ± 2.4	31.1 ± 3.3	14.8 ± 0.9	22.1 ± 0.6	0.84 ± 0.12	0.42 ± 0.12	0.55 ± 0.02
sterilised agricultural soil	-24.0 ± 2.8	-27.7 ± 2.2	-25.8 ± 2.6	21.2 ± 2.3	17.6 ± 0.7	19.4 ± 1.3	29.2 ± 4.1	21.3 ± 0.8	25.7 ± 2.3	1.81 ± 1.21	1.03 ± 0.13	1.22 ± 0.27
non-sterilised forest soil	-20.8 ± 5.8	-24.0 ± 5.5	-22.8 ± 5.8	20.6 ± 0.9	16.4 ± 1.3	17.8 ± 1.6	21.2 ± 1.3	15.9 ± 0.8	18.6 ± 1.1	2.51 ± 0.07	1.21 ± 0.48	2.23 ± 0.08
sterilised forest soil	-22.3 ± 0.8	-26.6 ± 0.7	-23.9 ± 0.8	20.3 ± 0.9	14.8 ± 2.4	16.8 ± 1.7	25.9 ± 1.5	17.4 ± 1.4	20.1 ± 0.9	6.06 ± 0.35	3.70 ± 0.38	5.64 ± 0.38
non-sterilised grassland soil	-21.6 ± 4.3	-30.0 ± 2.8	-25.2 ± 3.1	20.9 ± 4.0	14.4 ± 3.4	17.9 ± 3.3	24.8 ± 2.2	12.2 ± 5.1	17.9 ± 3.2	0.92 ± 0.13	0.56 ± 0.20	0.73 ± 0.20
sterilised grassland soil	-21.9 ± 1.6	-25.9 ± 2.6	-23.9 ± 2.1	21.4 ± 1.3	17.4 ± 1.1	19.5 ± 1.2	29.1 ± 1.0	19.2 ± 3.9	23.2 ± 2.7	2.75 ± 0.60	1.99 ± 0.16	2.18 ± 0.18

549 Note:

550 [a] Max. and Min. values are the real-time maximum and minimum values, respectively.

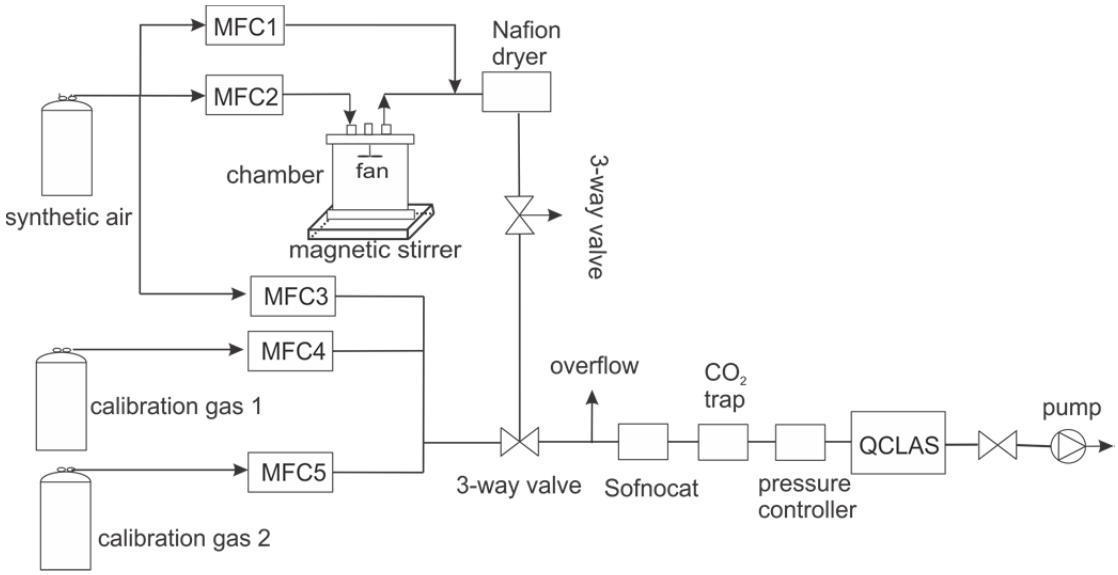
551 [b] Avg. values were concentration-weighted over the complete experimental run.

552 [c] The unit of relative N_2O production rate is µmol N_2O mol^{-1} NO_2^- mol^{-1} compound s^{-1} for lignin and lignin derivatives, and µmol N_2O mol^{-1} NO_2^- kg^{-1} soil s^{-1} for soil samples

553

554 **Figures**

555



556

557 **Fig. 1.** Schematic diagram of the setup used for real-time measurement of N_2O isotopic signatures.

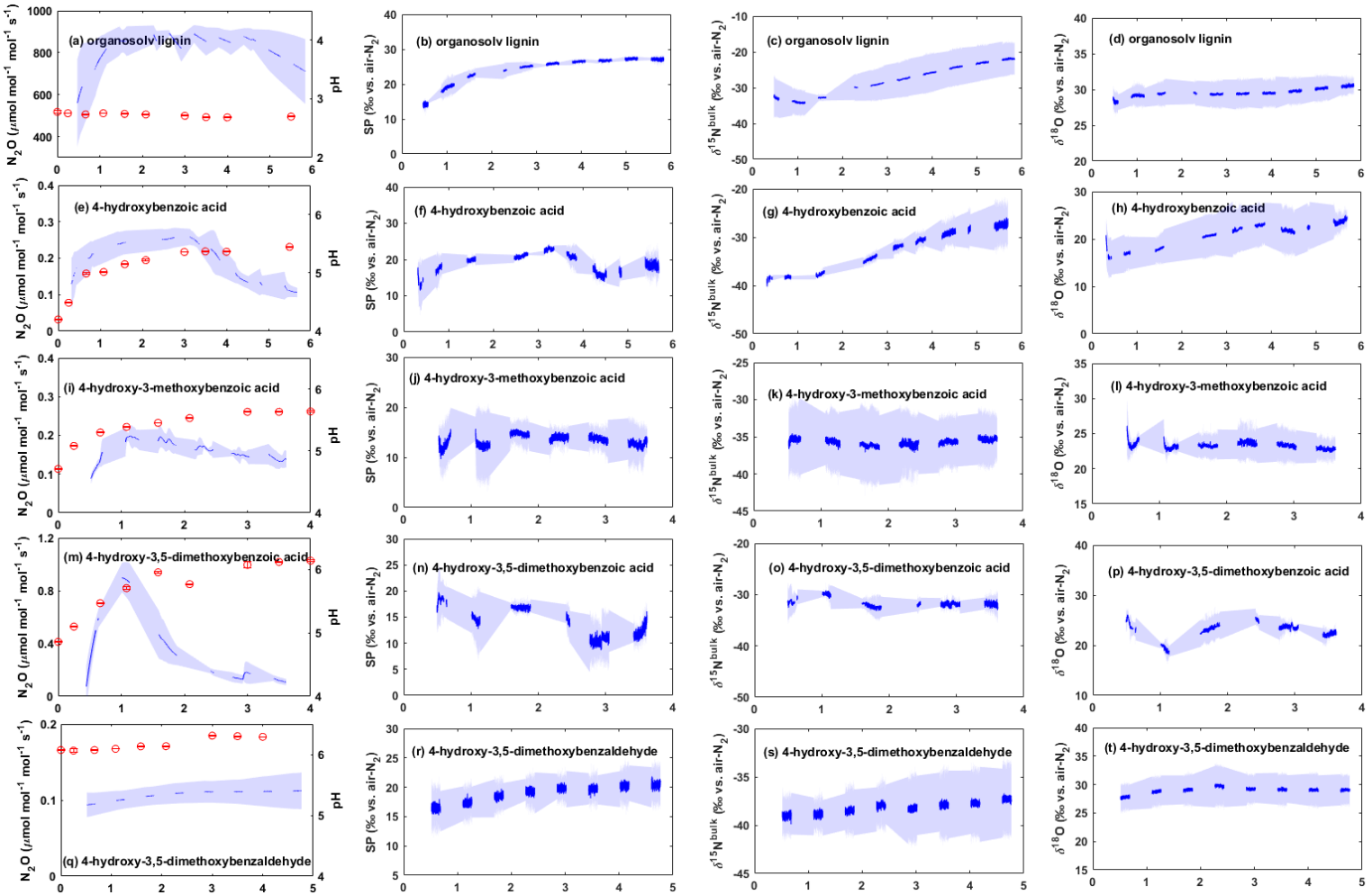


Fig. 2. Reaction of NO_2^- with organosolv lignin and lignin derivatives. Blue dots are the mean N_2O production rates and isotopic signatures of triplicates, the shaded areas indicate the standard deviation (SD), periods without sample measurement are linearly interpolated. Red dots represent mean pH $\pm$ SD of triplicates.

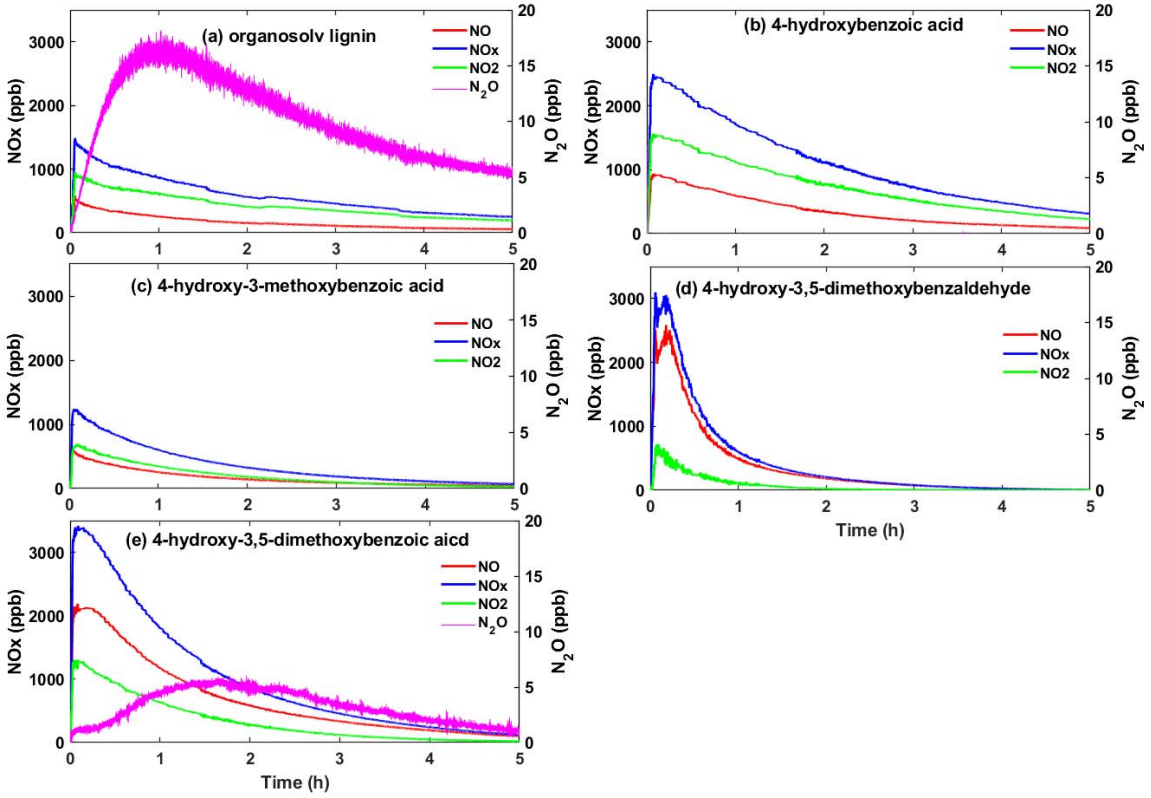


Fig. 3. Simultaneous N_2O and NO_x (NO + NO_2) emissions from the reactions of nitrite with organosolv lignin (a), 4-hydroxybenzoic acid (b), 4-hydroxy-3-methoxybenzoic acid (c), 4-hydroxy-3,5-dimethoxybenzaldehyde (d), 4-hydroxy-3,5-dimethoxybenzoic acid (e). The N_2O mixing ratio in the treatments of 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, and 4-hydroxy-3,5-dimethoxybenzaldehyde was lower than the detection limit.

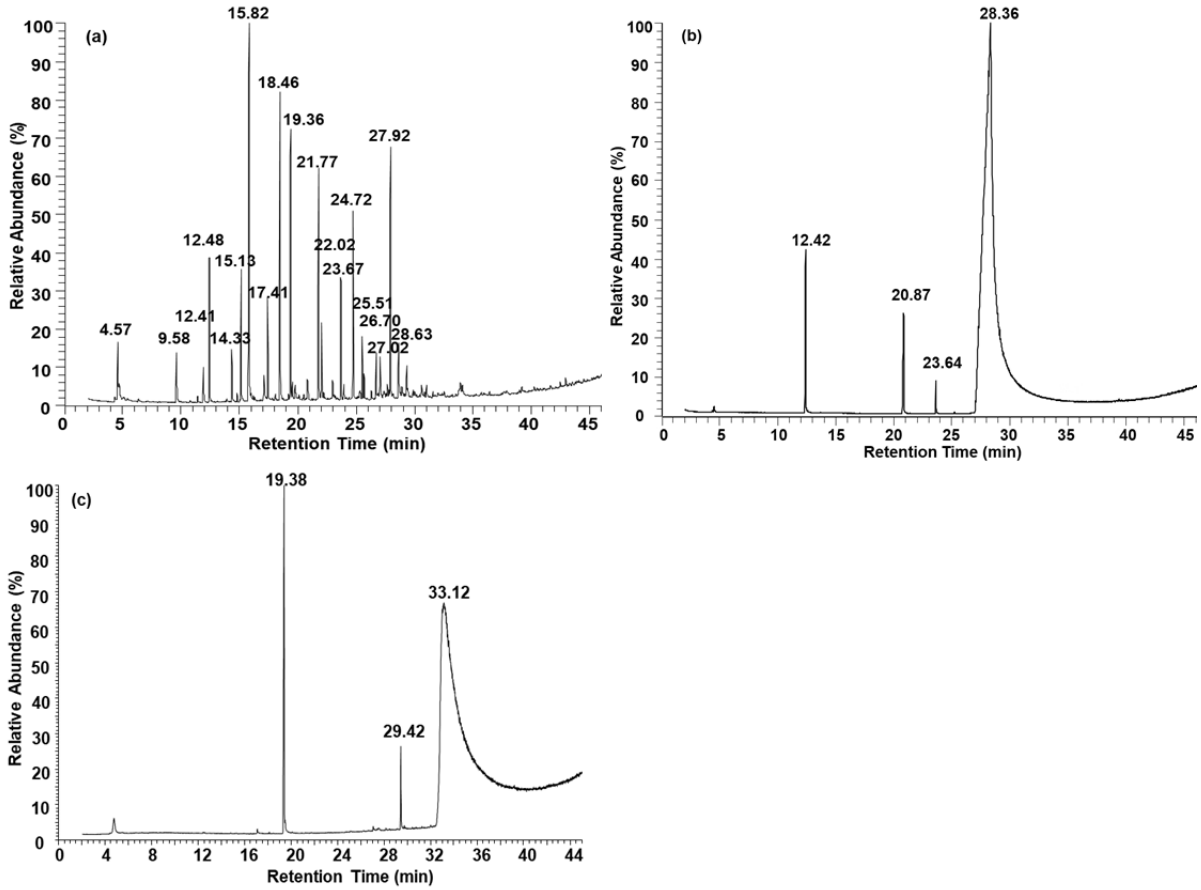
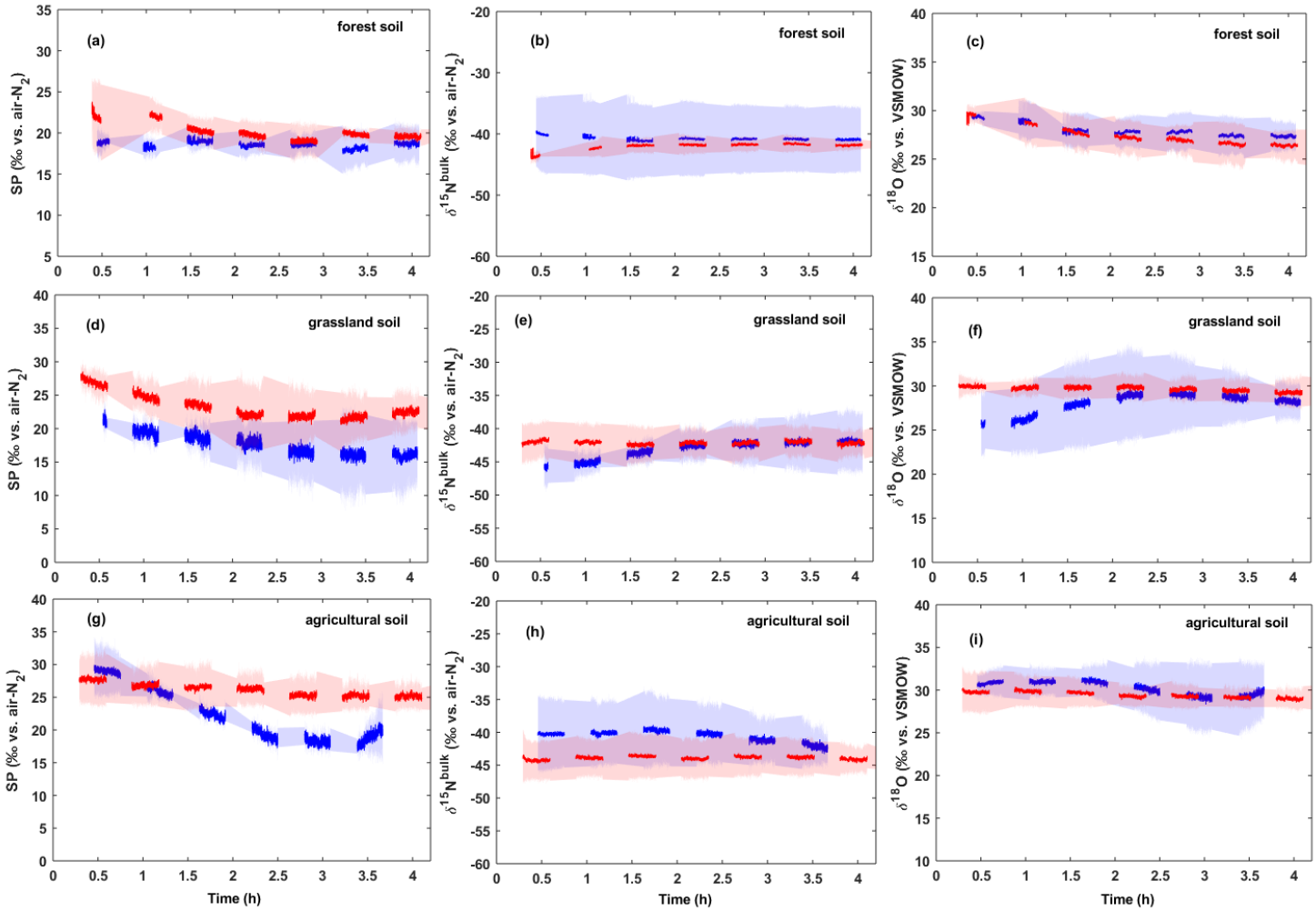


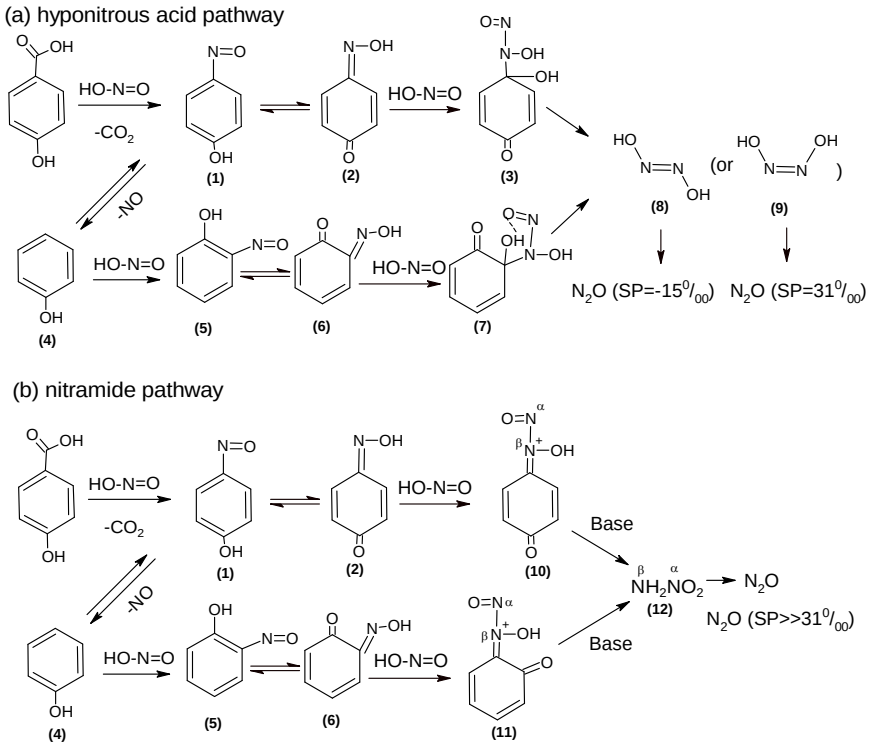
Fig. 4. Gas chromatography–mass spectrometry spectrum of products from the reaction of nitrite with organosolv lignin (a), 4-hydroxy-3-methoxybenzoic acid (b), and 4-hydroxy-3,5-dimethoxybenzoic acid (c). Compounds in (a) represent (2-aziridinylethyl)aminephenol, 3-methylphenol, 2-methoxyphenol, 4-ethylphenol, 2-methoxy-4-methylphenol, 4-methylbenzaldehyde, 4-ethyl-2-methoxyphenol, 2-methoxyl-4-vinylphenol, 2,6-dimethoxyphenol, 2,6-dimethoxy-4-methylphenol, 2-methoxy-4-(1-propenyl)phenol, 1,2,3-trimethoxy-5-methylbenzene, 2-(3,4-dimethoxyphenyl)-6-methyl-3,4-chromanediol, 2,6-demethoxy-4-(1E)-1-propen-1-yl-phenol, 2,6-dimethoxyl-4-(1E)-1-propen-1-yl-phenol, 4-hydroxy-3,5-dimethoxybenzaldehyde, 2,6-dimethoxy-4-(1E)-1-propen-1-yl-phenol, 1-(4-hydroxyl-3,5-dimethoxyphenyl)ethanone at retention time (t_R) of 4.57, 9.58, 12.41, 12.48, 14.33, 15.13, 15.82, 17.41, 18.46, 19.36, 21.77, 22.02, 23.67, 24.72, 25.51, 26.70, 27.02, 27.92, 28.63 min, respectively. Compounds in (b) stand for 2-methoxyphenol, 4-hydroxy-2-methoxybenaldehyde, 4-hydroxy-3-methoxybenzoic acid methyl ester, 4-hydroxy-3-methoxybenzoic acid at t_R of 12.42, 20.87, 23.64, 28.36 min, respectively. Compounds in (c) are 2,6-dimethoxyphenol at t_R of 19.38 min, 4-hydroxy-3,5-dimethoxybenzohydrazide at t_R of 29.42 min, and 4-hydroxy-3,5-dimethoxybenzoic acid at t_R of 33.12 min.



588

589 **Fig. 5**. Isotopic signatures of NO_2^- related N_2O emission in forest, agricultural, and grassland soils. Shown are mean values of three replicates in unsterilized
590 (blue dots) and sterilized (red dots) soils. The shaded areas indicate the standard deviation (SD) of triplicated measurements, periods without measurement are
591 linearly interpolated.

592



593

594 **Fig. 6**. Proposed hyponitrous acid pathway (a) and nitramide pathway (b) of N_2O formation in the
595 reactions of NO_2^- with lignin derivatives. The reaction of NO_2^- with 4-hydroxybenzoic acid is taken as
596 an example.

597

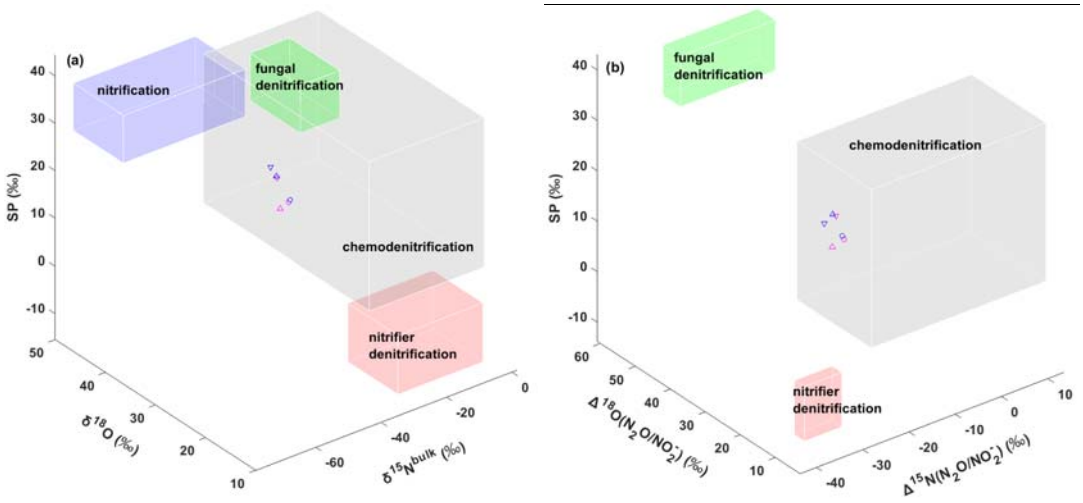


Fig. 7. Three dimensional end-member maps of N_2O isotopic signatures (a), and SP_{N2O} and net ^{15}N and ^{18}O isotopic effects (b). Pink and blue symbols represent mean values for unsterilized and sterilized soil samples, respectively. Downward triangles, agricultural soil samples; upward triangles, grassland soil samples; circles, forest soil samples.

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616

Supporting Information Available

Supporting Information. Stepwise regression of N_2O isotopocule ratios vs N_2O concentration is included.

Research Data. Wei, Jing (2019), "Data for: First real-time isotopic characterization of N2O from chemodenitrification", Mendeley Data, V1, doi: 10.17632/ntt24tgh2b.1.

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