

Accumulation of NO₂⁻ during periods of drying stimulates soil N₂O emissions during subsequent rewetting

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Running title: Nitrite stimulates N₂O emissions during rewetting

Summary

Rewetting of soil might contribute considerably to the annual production of nitrous oxide (N_2O) in ecosystems subjected to long dry periods. Therefore, it is crucial to elucidate the most important factors responsible for large pulses of N_2O with rewetting. In this study, we did a series of rewetting experiments with soil samples collected from upland and riparian forest, grassland and arable land. We analysed the dynamics of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and dissolved organic matter (DOM) of air-dried soil samples after rewetting. We also analysed the effects of sterilization of soil samples by γ -irradiation on N_2O production with rewetting. Furthermore, we explored the effects of rewetting and sterilization on the isotopic composition of N_2O in the different soil samples. The grassland soil produced the largest amount of N_2O ($64.1 \mu\text{g N kg}^{-1}$) in one hour on rewetting, followed by upland forest soil, whereas it was least for soils from riparian forest and arable land. Gamma irradiation, however, decreased soil N_2O production from forest soil samples by 30–90% after rewetting, but increased N_2O production in grassland and arable land soils threefold and twofold, respectively. Correlation analysis revealed that NO_2^- concentration in the soil samples at the time of rewetting was the most relevant factor that explained soil N_2O production after rewetting. Furthermore, the addition of NO_2^- before rewetting increased N_2O production during rewetting more than with additions of NO_3^- and NH_4^+ in all soil samples. The ^{15}N site preference values of N_2O produced after rewetting were close to 0‰, indicating a denitrification-related production process according to the classical view. However, additional abiotic processes responsible for soil N_2O production during rewetting cannot be excluded.

Keywords: nitrification, nitrous oxide, abiotic process, γ -irradiation, air-dried soil, nitrite

41 **Highlights**

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- 43 • Mechanisms responsible for large N₂O production during rewetting of soil are not well
- 44 understood.
- 45 • Nitrite content in dry soil was strongly correlated to N₂O production after rewetting.
- 46 • The ¹⁵N site preference of the N₂O produced was close to 0‰ after rewetting.
- 47 • Additional abiotic processes could have contributed to N₂O formation from NO₂⁻.

Introduction

Emissions of nitrous oxide (N_2O) from soils of various ecosystems under different environmental conditions have been widely studied because it is an important greenhouse gas. Rewetting of soil after long dry periods can lead to accelerated soil C and N mineralization ('Birch effect') and N_2O emissions (Rudaz *et al.*, 1991; Ruser *et al.*, 2006). A single wetting event might be responsible for a large fraction of the annual N_2O emissions for certain ecosystems (Priemé & Christensen, 2001). Recently, several studies have focused on the mechanisms of large soil N_2O emissions on rewetting (Beare *et al.*, 2009; Harrison-Kirk *et al.*, 2013; Snider *et al.*, 2015). Three reasons have been considered responsible for the increased N_2O flux following rewetting: (i) enhanced microbial metabolism including nitrification and denitrification, (ii) abiotic reactions because of the availability of accumulated soluble substrates and (iii) physical mechanisms involving infiltration, reduced diffusivity and gas displacement. Soluble substances accumulated in the soil during the drying process play an important role in the sudden emissions of N_2O . To survive drought, microbes must accumulate large concentrations of solutes to retain osmotic pressure and prevent dehydration (Schimel *et al.*, 2007). However, the accumulated solutes inside the cell might be released during cell rupture after sudden rewetting (Halverson *et al.*, 2000; Fierer & Schimel, 2003). In addition, drought will shrink soil aggregates, but rapid rewetting can rupture them (Fierer & Schimel, 2003). These processes can expose large amounts of soluble substances in the soil to subsequent microbial uptake and turnover, as well as fast chemical reactions.

The resilience of microorganisms to the drying–rewetting process depends largely on soil type and a history of drought (Placella & Firestone, 2013; Thion & Prosser, 2014). In a drought-adapted upland soil, an increase in the abundance of bacterial ammonia monooxygenase (*amoA*) transcripts was detectable within one hour after rewetting and continued until the ammonium (NH_4^+) pool started to decrease (Placella & Firestone, 2013). There was also a rapid increase in denitrifying enzyme activity following rewetting of air-dried soil in

laboratory incubations (Rudaz *et al.*, 1991). However, in a grassland soil without a history of drought, Thion & Prosser (2014) found little evidence for the adaptation of bacterial and archaeal ammonia oxidizers. This accorded with an arable land field experiment in Canada where there was no increase in the transcription of genes catalysing major steps of the inorganic nitrogen cycle during the rewetting process (Snider *et al.*, 2015).

Abiotic reactions, together with biotic processes, might also play an important role in triggering soil N₂O pulses in the wake of rewetting. Hydroxylamine (NH₂OH) and nitrite (NO₂⁻) are the most important reactive N intermediates involved in abiotic N₂O production (Heil *et al.*, 2016). It is unlikely that NH₂OH would accumulate during the soil drying process because of its very reactive nature, especially in dry conditions. Nitrite does not usually accumulate in soil under moist or wet conditions (Robertson & Groffman, 2007) because the oxidation of NO₂⁻ to nitrate (NO₃⁻) proceeds faster than the conversion of ammonia (NH₃) to NO₂⁻. However, NO₂⁻ has considerable potential to accumulate during soil drying. Davidson (1992) reported that accumulation of soil NO₂⁻ during drought probably contributes to pulses of NO and N₂O production following rewetting. The accumulation of NO₂⁻ in soil is probably caused by a time delay between the turnover of NH₄⁺ and NO₂⁻ because of differences in tolerance towards and recovery from soil environmental change between ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), e.g. after an increase in pH at large NH₃ concentrations and during drought stress (Shen *et al.*, 2003; Gelfand & Yakir, 2008; Placella & Firestone, 2013). Shen *et al.* (2003) reported that more NO₂⁻ accumulated at alkaline pH in soil than under acidic conditions with the addition of urea in an incubation experiment. Gelfand & Yakir (2008) also observed an unexpected rapid increase in NO₂⁻ concentration in a forest soil after rewetting by the first winter rains, accompanied by a decrease in NH₄⁺ and only a slight increase in NO₃⁻ concentrations.

Accumulation of NO₂⁻ in soil not only provides substrate for biological processes such as denitrification, nitrification and dissimilatory nitrate reduction to ammonium (DNRA) (Silver

et al., 2001), but also plays a major role in chemodenitrification in which NO_2^- reacts with humic substances or phenolic compounds to form nitroso and nitro compounds (Thorn & Mikita, 2000), which can decompose to nitric oxide (NO) or be reduced by Fe(II) to N_2O (Van Cleemput & Samater, 1995; Samarkin *et al.*, 2010). Another important pathway for N_2O production by chemodenitrification is the direct reaction between NO_2^- and Fe(II), which has been studied recently by analysing the ^{15}N site preference (SP), i.e. the intramolecular distribution of ^{15}N within the linear NNO molecule. It is considered an effective tool to assign the source of N_2O formation by biological (i.e. nitrification, nitrifier denitrification, bacterial denitrification and fungal denitrification) and abiotic reactions (chemodenitrification and NH_2OH oxidation) (Jones *et al.*, 2015; Grabb *et al.*, 2017).

To investigate the processes involved in pulses of N_2O emission after rewetting in more detail and to assess the importance of biotic and abiotic processes in different soils, we designed a series of rewetting experiments with samples from various ecosystems (upland and riparian forest, grassland and arable land). We sterilized part of each soil sample with γ -irradiation and analysed the ^{15}N SP of N_2O . The aims of the experiments were to identify the relevant factors controlling pulses of N_2O emissions caused by rewetting soil, and to quantify the contributions of abiotic and biotic reactions to the pulse. We hypothesized that (i) more N_2O will be produced on rewetting from soil samples with larger NO_2^- accumulation and (ii) abiotic reactions play an important role in N_2O produced on rewetting.

Materials and methods

Soil collection

Fresh samples of soil were taken from three field sites of the Eifel/Lower Rhine Valley Observatory of the network of Terrestrial Environmental Observatories (TERENO) (www.tereno.net): coniferous forest (Wüstebach; 50° 30' 10" N, 6° 19' 50" E), arable land

(Selhausen; 50° 52' 10" N, 6° 27' 4" E) and grassland (Rollesbroich; 50° 37' 18" N, 6° 18' 15" E). The coniferous forest site was in the low mountain ranges of the Eifel National Park, with a tributary of the River Rur flowing through it. The site was dominated by Norway spruce (*Picea abies* (L.) H. Karst). The soil at this site is silty clay loam and is dominated by Cambisol and Planosol in the upland forest, and Gleysol and Histosol in the riparian zone. The mean annual precipitation of the coniferous forest is about 1400 mm. The height above sea level (a.s.l.) of the forest site is 630 m and the mean annual temperature is around 7°C. The agricultural site was planted with sugar beet (*Beta vulgaris* L.) and wheat (*Triticum aestivum* L.) in rotation. The soil is dominated by a (gleyic) Cambisol and (gleyic) Luvisol with a silt loam texture, and the altitude ranges between 102–110 m a.s.l.. Mean annual temperature is 9.8°C, and the average precipitation is 690 mm per year. The grassland site was in the Northern Eifel region and planted with smooth meadow-grass. Dominant soil types at this site are (gleyic) Cambisol, Stagnosol and Cambisol–Stagnosol with a silt loam texture, covering an area of 27 ha with altitude ranging between 474 and 518 m a.s.l.. Mean annual temperature and precipitation are 7.7°C and 1033 mm, respectively (Rötzer *et al.*, 2014). Eight forest soil samples (~ 2 kg each) including those from the riparian zone were taken in July 2015. For the forest site, Liu *et al.* (2016) showed that the spatial variation in N₂O production was large because of the topographic conditions, vegetation and the tributary flowing through the sampling area. ‘Hotspots’ of soil N₂O production occurred in several areas where soil properties, water conditions and vegetation status were different from the rest of the area. Therefore, we collected eight soil samples including one fermented litter sample (For), six humus-rich (Oa horizon) samples (F1, F2, F3, F4, F5 and F6) and one riparian sample (FR) from an area of approximately 27 ha in the Wüstebach forested catchment. Fresh soil samples were transferred to the laboratory on the same day. At the grassland (G) and arable (A) sites, five soil samples (~ 1.5 kg each) were taken from the top 15-cm soil depth of each of the two sites (about 0.5 hectare) in January 2016. The spatial variation of the

grassland and arable sites was less than for the forest site, therefore we mixed the samples from the grassland and arable land sites to form one composite soil sample to represent each site. The fresh soil samples were mixed directly in a large plastic bag after sampling, and were transferred to the laboratory on the same day. In the laboratory, fresh samples (except for the FR sample) were passed through a 2-mm sieve, and coarse plant residues (including roots) and stones were removed manually to homogenize the soil for analysis. The presence of plant material would have biased the effect of the soil and might have limited the results to other soils with the same plant species composition. Soil samples were then put into open plastic bags and stored at 4°C until the start of the experiment.

Experimental set-up

Soil pre-treatment. Fresh soil samples from the fridge were spread out on aluminum foil to form a thin layer of 0.5–1 cm, and kept at room temperature ($21\pm1^{\circ}\text{C}$) for about one month. After that, the air-dried soil samples were put into zipped bags and stored at room temperature. To explore the effects of air-drying on soil mineral N dynamics, mineral N was measured in both fresh and dry soil samples.

Soil γ -irradiation. Half of the air-dried soil samples were sterilized with a dose of 11 kGy γ -irradiation by a Gamma Cell Irradiator 4000 (Best Theratronics, Ottawa, Canada). Plating of the sterilized soil slurries directly after γ -irradiation revealed no microbial growth (R2A agar medium, 24-hour incubation; 25°C ; data not shown). To prevent rapid recovery of microorganisms after γ -irradiation, soil samples were incubated for up to 7 hours only after rewetting.

Rewetting experiments. Rewetting experiments were done with both non-irradiated and γ -irradiated air-dried soil. The experiments with γ -irradiated samples were done on a clean bench with all solutions filtered through 0.2- μ m filters. We placed 1.4 g of air-dried soil (0.7 g for F_{OF}) into 22-ml gas chromatography (GC) vials (VWR international, Darmstadt, Germany), followed by the addition of either H_2O , or NO_2^- , NO_3^- or NH_4^+ solutions to reach around 40% water-holding capacity (WHC), and 1 μ g N g⁻¹ dry soil (for NO_2^-) and 100 μ g N g⁻¹ dry soil (for NH_4^+ and NO_3^-). The vials were closed with butyl septa and aluminum crimp caps (VWR International) immediately after the addition of water or solution. Half of the vials were incubated at room temperature for 1 hour and the others were incubated for 7 hours. Each treatment was carried out in triplicate. The gas sample in the headspace of the sample vials was analysed with a gas chromatograph (Clarus 580, PerkinElmer, Rodgau, Germany) equipped with an electron capture detector (ECD) and flame ionization detector (FID) for N_2O and CO_2 , respectively (Liu *et al.*, 2014). The instrument was calibrated using five different standard gases with 0.25, 0.50, 0.75, 1.00 and 5.00 μ l l⁻¹ N_2O balanced with N_2 (99.5% purity, Linde, Munich, Germany).

Analysis of ^{15}N site preference of N_2O . To determine N_2O SP values, 1.4–2.8 g of soil were weighed into 120-ml headspace bottles, and only water was added to reach about 40% WHC. The bottles were closed immediately after the addition of water and transferred to an autosampler that was programmed so that sample bottles were incubated for 0.5–6.5 hours before analysis. The autosampler was coupled to a pre-concentration unit (TraceGas, Elementar Analysensysteme, Langenselbold, Germany) for real-time separation and purification of N_2O , which in turn was connected to an isotope ratio mass spectrometer (IRMS, IsoPrime 100, Elementar Analysensysteme, Langenselbold, Germany). Molecular ions (N_2O^+) and fragment ions (NO^+) were monitored simultaneously with the IRMS at isotope ratios, m/z (mass-to-charge ratio), of 44, 45, 46, and 30, 31, respectively. The sample

values of $\delta^{15}\text{N}^{\text{bulk}}$ ($\delta^{15}\text{N}$ of total nitrogen) and $\delta^{18}\text{O}$ were calculated according to the isotope ratios of m/z 45 to 44, and 46 to 44, respectively, against a working reference gas. The ^{17}O was corrected according to the mass-dependent fractionation of ^{17}O and ^{18}O , described by the formula (Kaiser *et al.*, 2003)

$$^{17}\text{R} = 0.00937035 \times (^{18}\text{R})^{0.516}, \quad (1)$$

where ^{17}R and ^{18}R are the isotope ratios of $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$, respectively.

Site preference is defined as

$$\text{SP} = \delta^{15}\text{N}^{\alpha} - \delta^{15}\text{N}^{\beta}, \quad (2)$$

where $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$ are the $\delta^{15}\text{N}$ at the central and terminal position of the N_2O molecule, respectively. The $\delta^{15}\text{N}^{\alpha}$ was calculated from the isotope ratio m/z 30 and 31. The $\delta^{15}\text{N}^{\beta}$ was calculated according to the following formula:

$$\delta^{15}\text{N}^{\beta} = 2 \times \delta^{15}\text{N}^{\text{bulk}} - \delta^{15}\text{N}^{\alpha}. \quad (3)$$

Scrambling effects, i.e. the random mixing of isotopes in molecule ions in the ion source of the mass spectrometer, were corrected for by assuming isotopic scrambling of the terminal and central nitrogen atom of about 8% following Kaiser *et al.* (2004). Pure N_2O (99.99%, Linde, Munich, Germany) was used as the working standard (values = mean, standard deviation: $\delta^{15}\text{N}^{\alpha}$ relative to air- N_2 = 3.18, 0.23‰, $\delta^{15}\text{N}^{\beta}$ relative to air- N_2 = 1.42, 0.21‰, $\delta^{18}\text{O}$ relative to Vienna Standard Mean Ocean Water (VSMOW) = 39.35, 0.27‰) for isotope analysis, and the $\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{15}\text{N}^{\alpha}$, $\delta^{15}\text{N}^{\beta}$ and $\delta^{18}\text{O}$ were calibrated against two reference (R) gases (R1: $\delta^{15}\text{N}^{\alpha}$ relative to air- N_2 = 15.70, 0.31‰, $\delta^{15}\text{N}^{\beta}$ relative to air- N_2 = -3.21, 0.37‰, $\delta^{18}\text{O}$ relative to VSMOW = 35.16, 0.35‰; R2: $\delta^{15}\text{N}^{\alpha}$ relative to air- N_2 = 5.55, 0.21‰, $\delta^{15}\text{N}^{\beta}$ relative to air- N_2 = -12.87, 0.32‰, $\delta^{18}\text{O}$ relative to VSMOW = 32.73, 0.21‰) provided by EMPA (Dübendorf, Switzerland) and described in Mohn *et al.* (2014). In addition, different amounts of reference N_2O were added to the 120-ml bottles and isotope signatures were

measured. Strong quadratic relations were observed between N₂O peak height and $\delta^{45}\text{N}_2\text{O}$, $\delta^{46}\text{N}_2\text{O}$ and $\delta^{31}\text{NO}$ relative to the reference gas, with polynomial equations of

$$y = ax^2 + bx + c, \quad (4)$$

where y is N₂O peak height (2.7 to 72 nanoampere, nA), x is $\delta^{45}\text{N}_2\text{O}$ relative to reference ($a = 0.0032$, $b = -0.1689$, $c = 0.5516$), $\delta^{46}\text{N}_2\text{O}$ relative to reference ($a = 0.0054$, $b = -0.2643$, $c = 39.3$) and $\delta^{31}\text{NO}$ relative to reference ($a = 0.0014$, $b = 0.4489$, $c = -0.6767$).

Therefore, all $\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{18}\text{O}$ and SP values in this study were calculated according to the corrected $\delta^{45}\text{N}_2\text{O}$, $\delta^{46}\text{N}_2\text{O}$ and $\delta^{31}\text{NO}$ relative to the reference gas values by the polynomial equations. For the peak area correction and calibration, we did no technical replication because the standard deviation for the isotope analysis was very small, i.e. 0.2, 0.4, 0.3, 0.4, 0.7 and 0.6‰ for $\delta^{15}\text{N}^{\text{bulk}}$ relative to air-N₂, $\delta^{18}\text{O}$ relative to VSMOW, $\delta^{31}\text{N}$ relative to the reference gas, $\delta^{15}\text{N}^{\alpha}$ relative to air-N₂, $\delta^{15}\text{N}^{\beta}$ relative to air-N₂ and SP for a long measurement period, respectively.

Soil chemical analyses

Total carbon (C) and N contents were determined with an elemental analyser (vario EL Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). The element composition of the soil samples was analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). Briefly, 100 mg of sample material were mixed with 3 ml HNO₃ and 2 ml H₂O₂, and heated in a microwave oven at 800 W for 30 minutes. The mixtures were subsequently filled to 14 ml and diluted 10-fold with deionized water followed by the ICP-OES measurement.

Mineral N (NH₄⁺, NO₂⁻ and NO₃⁻) contents were analysed by ion chromatography (ICS-3000 for NO₂⁻ and NO₃⁻, DX-500 for NH₄⁺; both analysers were from Dionex, Sunnyvale, CA, USA). The NH₄⁺ and NO₃⁻ were extracted with 1 M KCl (dry soil: solution = 1:10 w/w) and

shaken for 24 hours. Soil pH was measured by shaking soil with 1 M KCl (dry soil: solution = 1:10 w/w). Nitrite was extracted with water during magnetic stirring for 15 minutes, and 0.2 M NaOH was used to keep the pH around 6 during extraction (Homyak *et al.*, 2015). Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were extracted with deionized water (dry soil: solution = 1:2.5 w/w for grassland and cropland soils, and 1:5 w/w for forest and riparian soils) by shaking for 1 hour at 3.3 revolutions per second. Dissolved organic carbon and DTN were then analysed with a TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). Aromatic substances in the extracted DOC were determined by UV spectrometry (Beckman Coulter DU 800, Beckman Coulter, Inc., Brea, CA, United States) at a wavelength of 254 nm with a path length of 1 cm. The absorbance at 254 nm (A_{254}) was assumed to be specific for aromatic substances.

Data analyses

Nitrous oxide emission was calculated according to the following equation:

$$E = 2 \times C' \times V \times M / (W_{ds} \times V_m), \quad (5)$$

where E is the N_2O emission ($ng\ N\ g^{-1}$ dry soil), C' is the N_2O mixing ratio in the vial headspace ($nl\ l^{-1}$), V is the volume of vial headspace (l), V_m is the molar volume of N_2O at standard pressure and room temperature ($l\ mol^{-1}$), M is molar mass of nitrogen ($g\ mol^{-1}$) and W_{ds} is the mass of the dry soil (g).

Isotope signatures ($\delta^{15}N^{bulk}$, $\delta^{18}O$ and SP values) of soil-emitted N_2O were calculated from the total isotope signature of the gas samples and of ambient air using a two-component mixing model:

$$\delta_1 = (\delta_2 \times Q_2 - \delta_0 \times Q_0) / (Q_2 - Q_0), \quad (6)$$

where δ_1 , δ_2 and δ_0 are the mean isotope signatures of soil-emitted N_2O , the sample bottles, and ambient air, respectively, Q_2 and Q_0 represent the N_2O concentration in the sample bottles and in ambient air, respectively.

The analysis of variance (ANOVA) was used to analyse the main factors, i.e. soil type, N addition and gamma irradiation, and their interactions for the significance ($P < 0.05$) of their effect on N_2O production during rewetting in the R software package (version 3.4.3). Box–Cox transformation of N_2O data was performed before the ANOVA due to the unequal distribution of residuals of ANOVA test from the residual plots. Fisher’s least significant difference test was used to analyse means of the effects for significant differences ($P < 0.05$). Pearson’s correlation coefficients were computed among the variables N_2O after water rewetting, Fe, Mn, C, N, C/N, pH, NO_2^- , NH_4^+ , NO_3^- , DOC, DTN and A_{254} with Origin Pro version 2015. Variable NH_4^+ was ln-transformed before Pearson’s correlation analysis because it was not normally distributed.

Results

Basic soil properties

Basic soil properties, e.g. C and Mn contents and pH, varied considerably between the soil samples from the different ecosystems (Table 1). Soil organic C content ranged from around 10 to 46% in the forest samples, including F_{of} and F_{R} , whereas it was only ~5 and ~1% for the grassland and arable soil, respectively. The forest soil was more acidic with a pH around 3, whereas the pH of grassland and arable soils was much higher (between 5 and 6). Compared to grassland and arable soil samples, the Mn content of forest soil of around 0.02% was relatively small, except for soil samples F5 and F6 which had the largest Mn content of all forest soil samples. There was no distinct difference in Fe content between the soil samples,

only the fermented layer (F_{Of}) and riparian soil (FR) had a smaller Fe content than the other soil samples.

Mineral N and dissolved organic matter (DOM) content before and after drying

The mineral N content (including NH₄⁺ and NO₃⁻) of the fresh soil differed strongly between the soil samples (Figure 1). Before air-drying, forest soil samples F_{Of} and F4 had the largest NH₄⁺ and NO₃⁻ contents, while samples F5 and F6 had smaller NH₄⁺, but larger NO₃⁻ contents than the other forest soil samples. Samples from the riparian zone and arable land had the smallest NH₄⁺ and NO₃⁻ contents of all soil samples, and grassland soil had intermediate contents of NH₄⁺ and NO₃⁻. After air-drying, the NH₄⁺ content decreased in all soil samples. Almost no NH₄⁺ was detectable in the riparian and arable soil samples after air-drying.

In contrast to NH₄⁺, NO₃⁻ increased with drying for most soil samples, except for F_{Of}, F6, grassland and arable land samples. Forest soil sample F_{Of} had the largest NO₃⁻ content, followed by F4 and F6. The grassland soil had an intermediate NO₃⁻ content compared to the forest samples, whereas the riparian and arable land samples were characterized by the smallest NO₃⁻ content.

Before air-drying, NO₂⁻ concentrations were below the detection limit for the fresh soil samples. However, small amounts of NO₂⁻ were detectable in several soil samples after drying (Table 2). Forest soil samples F_{Of}, F3 and F6 had the largest NO₂⁻ content (0.3 mg kg⁻¹), followed by grassland and forest soil F1 (0.2 mg kg⁻¹), whereas no NO₂⁻ was detectable in samples F4, F2 and FR.

The trend in the dynamics of soil DOC and DTN after air-drying was similar to that of soil C content. The largest DOC and DTN contents were in F_{Of} and the smallest was in the arable soil, except for F5 with a relatively large C content but the smallest DOC content of all forest soil samples (Table 2). The DOC and DTN contents in the grassland soil were also relatively

large. Although soil sample F6 had the second largest total N content, it contained a relatively small amount of DTN. The dynamics of A_{254} (i.e. content of aromatic substances) followed a similar trend to DOC, with the largest value for F_{Of} and the smallest for arable soil.

Rewetting effects on soil N_2O emissions

Soil types, the water and different additions of N had significant ($P < 0.05$) effects on the rewetting responses of soil N_2O emissions (Figure 2a, Table S1, Supporting Information).

After rewetting with water only, N_2O emission from grassland soil was large, especially in the first hour after rewetting, with an emission of $64 \mu\text{g } N_2O\text{-N kg}^{-1}$ dry soil. After 7 hours with rewetting, the N_2O emissions from the grassland soil were significantly ($P < 0.05$) larger than most of soils except for forest F_{Of} and F3. Forest soil samples showed different responses to rewetting with water only; samples F_{Of} and F3 had the largest N_2O emissions, whereas they were smaller for F2, F4, F5 and FR. Unlike grassland soil, N_2O emissions from forest soil did not increase substantially in the first hour, but increased between 1 and 7 hours. In contrast, there was almost no rewetting effect on soil N_2O emissions from arable soil.

Nitrite addition increased the rewetting effect significantly ($P < 0.05$) for all soil samples (Figure 2b, Table S1, Supporting Information). It increased N_2O emissions the most for forest soil sample F_{Of} and grassland soil, followed by forest soil sample F3. The effects of NO_2^- on N_2O production in the forest soil samples F4, F5 and FR were not significant. The effects of NO_2^- on N_2O production in the arable soil was significantly ($P < 0.05$) smaller than other soils. The rate of total $NO_2^-:N_2O$ turnover after 7 hours was about 20% for grassland soil, but between only 5–10% for most upland forest, riparian and arable land samples.

Compared to NO_2^- , NO_3^- and NH_4^+ had a significantly ($P < 0.05$) smaller effect on the production of soil N_2O with rewetting (Figure 2c,d), even though the amount of $NH_4^+\text{-N}$ and

NO₃⁻-N added was 100-fold larger than that of NO₂⁻. For most of soil samples, the difference between the effects of NO₃⁻ and NH₄⁺ was not significant ($P < 0.05$).

Effect of γ -irradiation on soil N₂O and CO₂ emissions after rewetting

The effect of γ -irradiation on soil N₂O emissions with rewetting depended on soil type. In general, γ -irradiation affected the emission of N₂O on rewetting significantly ($P < 0.05$). It decreased N₂O emission on rewetting with water only by about 30–90% in most forest soil samples compared to the non-irradiated soil samples, whereas it unexpectedly stimulated N₂O emissions from grassland and arable soils three- and two-fold, respectively, after 7 hours of incubation (Figure 3a, Table 3). Forest soil samples had a large variance after γ -irradiation. It inhibited N₂O production from F₀ the most, followed by the riparian sample and F5, whereas N₂O production was least from samples F1 and F2 during the incubation.

In the NO₂⁻ rewetting treatment, γ -irradiation also increased production of N₂O in grassland and arable soils, but decreased it in forest soil (Figure 3b, Table 3). Samples F1 and F2 were inhibited the least by γ -irradiation.

Compared to the effects on soil N₂O emissions, γ -irradiation decreased production of CO₂ the most in grassland soil by about 50% after rewetting with water only, but had an inhibitory effect of only zero to 20% in forest, arable land and riparian soil samples (Table 3). In sample F1, CO₂ production was stimulated by γ -irradiation.

Control variables of soil N₂O emission on rewetting

Basic soil properties play an important role in biotic and abiotic reactions, and might have contributed to the pulse of N₂O emissions after rewetting. Nitrous oxide production was significantly ($P < 0.05$) and positively correlated with NO₂⁻ ($r = 0.85$) and NH₄⁺ ($r = 0.71$) content (Table 4, Figure S1, Supporting Information), but had no statistically significant

correlations with other basic soil properties such as soil C and NO_3^- content (Table 4). Soil NO_2^- was only significantly ($P < 0.05$) correlated with total soil N content ($r = 0.80$) and N_2O production, but not with mineral N and DTN.

Isotope ratio analyses of N_2O produced during rewetting

The $\delta^{15}\text{N}^{\text{bulk}}$ and $\delta^{18}\text{O}$ values varied from -42.4 to -24.0‰ and from 9.7 to 32.0‰ , respectively, for all the soil samples during rewetting, except for sample F3 where $\delta^{18}\text{O}$ was very large with a value of 110.6‰ (Table 5). Both $\delta^{15}\text{N}^{\text{bulk}}$ and $\delta^{18}\text{O}$ decreased with increasing incubation time for the grassland soil, regardless of whether the soil had been γ -irradiated in advance or not. Ranges of $\delta^{15}\text{N}^{\text{bulk}}$ values for grassland and forest soils were similar, whereas $\delta^{18}\text{O}$ of N_2O were larger for forest than grassland soil. The SP values of N_2O formed after rewetting were close to zero for most of the soil samples (except for F5), independent of the amount of N_2O produced, as indicated by the peak height (Table 5) of incubation time and sterilization treatment. For the forest soil samples, the SP values ranged between -15.9 and 9.9‰ . The SP values for the grassland soil samples ranged from -2.1 to 1.3‰ for both γ -irradiated and non-irradiated samples, even though N_2O production increased largely with incubation time.

Discussion

Soil rewetting-induced N_2O production has received more attention recently because of the potentially large contribution of this fraction of N_2O to the annual N_2O flux (Priemé & Christensen, 2001; Berger *et al.*, 2013). We showed that the rewetting effect was very variable in different ecosystems. Seasonal variation, e.g. winter and summer, might have an effect on the N_2O produced in different ecosystems. The forest soils examined could have been affected more by dry summer conditions, leading to more accumulated substrate and certain microorganisms that are resistant to the drying conditions. Therefore, N_2O produced

during rewetting could have been overestimated in the forest samples compared to the arable and grassland soils. Overall, however, our findings accorded with those of Priemé & Christensen (2001) that more N₂O was emitted with rewetting of grassland soil than arable and forest soils in Germany, Sweden and Finland.

Although knowledge about the exact mechanisms and factors that cause large amounts of N₂O to form on rewetting are still limited, some basic properties such as C content, pH and inorganic N content, together with soil texture and microbial composition were shown to play important roles in the production of an N₂O pulse with rewetting (Ruser *et al.*, 2006; Harrison-Kirk *et al.*, 2013). Our samples showed considerable variation in soil pH and C, N, metal elements and inorganic N (NO₃⁻ and NH₄⁺) contents. In general, forest samples showed the largest soil C content (19.8–45.7%) and the smallest soil pH (2.85–3.92) compared to riparian, grassland and arable soils. Harrison-Kirk *et al.* (2013) reported that more N₂O was produced in soil samples with large soil organic C content. In our study, however, the amount of N₂O from grassland (with less soil C) was larger than from most forest soils (with larger soil C content). Ruser *et al.* (2006) reported that soil compaction and large NO₃⁻ content were two important factors responsible for the rewetting-induced production of N₂O in an arable soil because more anoxic sites could develop when water was added to compacted soil. In our study, air-dried grassland soil had a much larger bulk density (1.09 g cm⁻¹) than forest soil (0.83 g cm⁻¹) according to former research at these sites (Baatz *et al.*, 2014), which might be one reason for the immediate and large N₂O emissions on rewetting for grassland soil.

Large soil NO₃⁻ content has been considered an important factor in rewetting-induced production of N₂O because NO₃⁻ would favour the production of N₂O from denitrification (Ruser *et al.*, 2006). During drying, soil NO₃⁻ might accumulate because of the greater resistance of nitrifier activity to water limitation than denitrifiers (Szukics *et al.*, 2010). In our study, we also observed an increase in soil NO₃⁻ content with air-drying for most of forest soil samples, but not for the grassland and arable land samples (Figure 1). Moreover, there was no

significant correlation between the NO_3^- content of air-dried soil and N_2O production on rewetting (Table 4). These results indicate that NO_3^- accumulation was not the main contributor to the production of large amounts of N_2O on rewetting to around 40% WHC, as in our study. We assumed that this relatively small water content might favour the production of N_2O from nitrification, but the addition of NH_4^+ increased N_2O production from one forest soil sample only (F6), and had no stimulatory effects on the other soil samples.

Soil NO_2^- accumulation has been considered as another important factor for a pulse of N_2O after rewetting (Davidson, 1992; Venterea, 2007), although NO_2^- was often not detected after air-drying in previous studies. In our study, we used a new method of NO_2^- extraction developed by Homyak *et al.* (2015) to extract NO_2^- at a higher pH around 6, and found detectable NO_2^- concentrations in the air-dried samples of F_{Of}, F3 and F6, but none was detectable in the samples F4, F2 and FR.

Despite the small amount of accumulated NO_2^- , there was a close correlation between NO_2^- in air-dried soil and amount of N_2O produced after rewetting (Table 4, Figure S1, Supporting Information). Addition of NO_2^- also increased soil N_2O production largely within the first hour after rewetting in all soil samples (Figure 2b). The reason for the variation in NO_2^- content between different samples remains unclear, but NO_2^- content was positively correlated with total N content (Table 4), but was not correlated with NO_3^- and NH_4^+ content.

There are mainly two sources involved in the release of soil C and N during the rewetting: (i) disruption of soil aggregates by rapid water addition and (ii) the proportion of microorganisms that died during drying or by dehydration or cell lysis, and the associated release of labile intracellular substrates with rewetting. A previous study showed that NO_2^- produced from organic N is an important NO_2^- pool in grassland soil (Müller *et al.*, 2006). Therefore, NO_2^- could originate from aggregate (< 2 mm in this study) disruption or the release of labile intracellular substrates during microbial cell lysis.

There are mainly two pathways responsible for the NO_2^- -mediated production of N_2O : (i) biological nitrifier-denitrification and denitrification and (ii) chemical reactions with organic matter and metal ions (e.g. Fe^{2+}). Stevenson & Swaby (1964) showed that N_2O is produced chemically following the addition of NO_2^- to acidic soil organic matter fractions. Samarkin *et al.* (2010) identified abiotic reactions between NO_2^- and Fe^{2+} -containing minerals derived from the surrounding igneous Ferrar Dolerite, contributing to N_2O emission from the hypersaline Don Juan Pond in Antarctica. We also explored the contribution of abiotic reactions to NO_2^- -mediated N_2O production during rewetting by sterilizing the soil with 11 kGy of γ -irradiation. Our results showed considerable differences between soil samples from the effect of γ -irradiation on soil N_2O production (Table 3). In general, it inhibited N_2O production from the forest and riparian samples; the largest inhibition was in the sample with the fermented organic layer (F_{of}, 91.1%) and the smallest was in soil sample F2 (F2, 28%). The range of inhibition by γ -irradiation was consistent with that reported by Venterea (2007), who also found that production of N_2O in γ -irradiated soils ranged from 31 to 75%.

The small effects of γ -irradiation on soil CO_2 emissions from forest soils were unexpected because we assumed that negligible CO_2 would be produced in the γ -irradiated soils. One reason could be the limited effect of γ -irradiation on certain soil microorganisms, mainly spore forming fungi, even though γ -irradiation is considered very effective and preferable to other methods of sterilization because of its smaller effect on soil chemical and physical properties (Stroetmann *et al.*, 1994). Therefore, it might have changed microbial community structure towards a strong fungal dominance, which partially contributed to N_2O production after rewetting in certain forest soil samples (e.g. F1 and F2). However, chemical reactions such as nitrosative decarboxylation reactions could also produce CO_2 chemically (Thorn & Mikita, 2000) because no microbial growth was detected by plating the γ -irradiated soil slurries in this study. In contrast, in the grassland and arable land samples γ -irradiation increased N_2O production three- and two-fold, respectively, even though CO_2 emission was reduced by about

50% after γ -irradiation (Table 3). The stimulatory effect of γ -irradiation on N_2O production in the grassland samples was surprising, but could indicate an increased contribution from an abiotic mechanism of N_2O production from NO_2^- . It is possible that γ -irradiation might have strongly inhibited the activity of nitrite oxidizers, leaving more NO_2^- available for abiotic N_2O production. This might explain the larger amount of N_2O produced from grassland soil after γ -irradiation, but this assumption remains speculative. In addition, the contribution of abiotic processes to soil N_2O production in the grassland soil could also have been enhanced by γ -irradiation through an alteration in organic matter structure or functional groups involved in nitrosation reactions, which could promote abiotic N_2O production (Venterea, 2007). But this contrasts with reduced N_2O formation in γ -irradiated forest samples. Therefore, further research is needed to elucidate the mechanisms behind stimulation and inhibition of N_2O production from nitrite after γ -irradiation of different types of soil.

Finally, we measured the isotopic signatures ($\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{18}\text{O}$ and SP values) of N_2O formed during rewetting because they are thought to reflect the relative contribution of different sources of N_2O to some extent. There have been several recent studies that examined N_2O SP from chemodenitrification (Heil *et al.*, 2014; Jones *et al.*, 2015; Grabb *et al.*, 2017). The $\delta^{15}\text{N}^{\text{bulk}}$ measured in this study fell within the range of denitrification (-40 to -19‰) in pure cultures (Toyoda *et al.*, 2005), whereas $\delta^{18}\text{O}$ values were in the range of N_2O produced by nitrification in soil (Snider *et al.*, 2012). The SP values have been considered a more useful tool for partitioning sources of N_2O than $\delta^{15}\text{N}^{\text{bulk}}$ and $\delta^{18}\text{O}$ because SP values were relatively stable for the production of N_2O from different soil processes, although there was still some overlap between aerobic nitrification and abiotic NH_2OH oxidation (Sutka *et al.*, 2006; Heil *et al.*, 2014), and denitrification and nitrifier denitrification (Sutka *et al.*, 2006). In our study, the SP values were close to 0‰ for most of the soil samples after rewetting, except for F5, whether or not the samples were sterilized by γ -irradiation (Table 5), which falls within the SP range (-10‰ ... 0‰) reported for bacterial denitrification including nitrifier denitrification

(Sutka *et al.*, 2006). Snider *et al.* (2015) reported that nitrifier denitrification became a more dominant source of N₂O following rain in arable soil by using the $\delta^{15}\text{N}$ of N₂O. In our study, addition of NO₃⁻ did not increase N₂O production significantly and there was no significant correlation between NO₃⁻ and N₂O, therefore, it was more likely that denitrification by nitrifiers was the dominant contributor to the production of N₂O during rewetting. We observed a similar SP for sterile and nonsterile soil samples, but previous studies showed that SP values of N₂O production from NO₂⁻-mediated chemodenitrification varied widely from -45 to 26.5‰ from chemical reactions or soil samples (Samarkin *et al.*, 2010; Jones *et al.*, 2015). Therefore, it is likely that abiotic reactions contributed substantially to soil N₂O production after soil rewetting.

Conclusions

Grassland soil had the largest N₂O emissions after rewetting, whereas arable and riparian soils were characterized by much smaller N₂O emissions. Among the different soil properties, soil NO₂⁻ content was the most relevant factor correlated with soil N₂O production. Addition of NO₂⁻ increased N₂O emissions the most, compared to NH₄⁺ and NO₃⁻. Our results demonstrated that, although biological reactions played an important role in N₂O production in the different soils, the role of abiotic processes in N₂O formation during the rewetting event must also be considered. Further research is required to reveal the conditions under which biotic or abiotic processes contribute most to the formation of N₂O.

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References

- Baatz, R., Bogen, H.R., Hendricks Franssen, H.J., Huisman, J.A., Qu, W., Montzka, C. *et al.* 2014. Calibration of a catchment scale cosmic-ray probe network: A comparison of three parameterization methods. *Journal of Hydrology*, **516**, 231–244.
- Beare, M., Gregorich, E. & St-Georges, P. 2009. Compaction effects on CO₂ and N₂O production during drying and rewetting of soil. *Soil Biology & Biochemistry*, **41**, 611–621.
- Berger, S., Jung, E., Köpp, J., Kang, H. & Gebauer, G. 2013. Monsoon rains, drought periods and soil texture as drivers of soil N₂O fluxes—Soil drought turns East Asian temperate deciduous forest soils into temporary and unexpectedly persistent N₂O sinks. *Soil Biology & Biochemistry*, **57**, 273–281.
- Davidson, E.A. 1992. Pulses of nitric oxide and nitrous oxide flux following wetting of dry soil: An assessment of probable sources and importance relative to annual fluxes. *Ecological Bulletins*, **42**, 149–155.
- Fierer, N. & Schimel, J.P. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal*, **67**, 798–805.
- Gelfand, I. & Yakir, D. 2008. Influence of nitrite accumulation in association with seasonal patterns and mineralization of soil nitrogen in a semi-arid pine forest. *Soil Biology & Biochemistry*, **40**, 415–424.
- Grabb, K.C., Buchwald, C., Hansel, C.M. & Wankel, S.D. 2017. A dual nitrite isotopic investigation of chemodenitrification by mineral-associated Fe(II) and its production of nitrous oxide. *Geochimica et Cosmochimica Acta*, **196**, 388–402.
- Halverson, L.J., Jones, T.M. & Firestone, M.K. 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Science Society of America Journal*, **64**, 1630–1637.
- Harrison-Kirk, T., Beare, M., Meenken, E. & Condon, L. 2013. Soil organic matter and texture affect responses to dry/wet cycles: Effects on carbon dioxide and nitrous oxide emissions. *Soil Biology & Biochemistry*, **57**, 43–55.
- Heil, J., Vereecken, H. & Brüggemann, N. 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. *European Journal of Soil Science*, **67**, 23–39.
- Heil, J., Wolf, B., Brüggemann, N., Emmenegger, L., Tuzson, B., Vereecken, H. *et al.* 2014. Site-specific ¹⁵N isotopic signatures of abiotically produced N₂O. *Geochimica et Cosmochimica Acta*, **139**, 72–82.
- Homyak, P.M., Vasquez, K.T., Sickman, J.O., Parker, D.R. & Schimel, J.P. 2015. Improving nitrite analysis in soils: Drawbacks of the conventional 2 M KCl extraction. *Soil Science Society of America Journal*, **79**, 1237–1242.
- Jones, L.C., Peters, B., Lezama Pacheco, J.S., Casciotti, K.L. & Fendorf, S. 2015. Stable isotopes and iron oxide mineral products as markers of chemodenitrification. *Environmental Science & Technology*, **49**, 3444–3452.
- Kaiser, J., Park, S., Boering, K.A., Brenninkmeijer, C.A.M., Hilker, A. & Röckmann, T. 2004. Mass spectrometric method for the absolute calibration of the intramolecular nitrogen isotope distribution in nitrous oxide. *Analytical and Bioanalytical Chemistry*, **378**, 256–269.
- Kaiser, J., Röckmann, T. & Brenninkmeijer, C.A. 2003. Complete and accurate mass spectrometric isotope analysis of tropospheric nitrous oxide. *Journal of Geophysical Research: Atmospheres*, **108**, 4476.
- Liu, S., Herbst, M., Bol, R., Gottselig, N., Pütz, T., Weymann, D. *et al.* 2016. The contribution of hydroxylamine content to spatial variability of N₂O formation in soil of a Norway spruce forest. *Geochimica et Cosmochimica Acta*, **178**, 76–86.

- Liu, S., Vereecken, H. & Brüggemann, N. 2014. A highly sensitive method for the determination of hydroxylamine in soils. *Geoderma*, **232**, 117–122.
- Mohn, J., Wolf, B., Toyoda, S., Lin, C.T., Liang, M.C., Brüggemann, N. *et al.* 2014. Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. *Rapid Communications in Mass Spectrometry*, **28**, 1995–2007.
- Müller, C., Stevens, R. & Laughlin, R. 2006. Sources of nitrite in a permanent grassland soil. *European Journal of Soil Science*, **57**, 337–343.
- Placella, S.A. & Firestone, M.K. 2013. Transcriptional response of nitrifying communities to wetting of dry soil. *Applied and Environmental Microbiology*, **79**, 3294–3302.
- Priemé, A. & Christensen, S. 2001. Natural perturbations, drying–wetting and freezing–thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soils. *Soil Biology & Biochemistry*, **33**, 2083–2091.
- Robertson, G. & Groffman, P. 2007. Nitrogen transformations. *Soil microbiology, Ecology, and Biochemistry*, **3**, 341–364.
- Rötzer, K., Montzka, C., Bogen, H., Wagner, W., Kerr, Y.H., Kidd, R. *et al.* 2014. Catchment scale validation of SMOS and ASCAT soil moisture products using hydrological modeling and temporal stability analysis. *Journal of Hydrology*, **519**, Part A, 934–946.
- Rudaz, A.O., Davidson, E.A. & Firestone, M.K. 1991. Sources of nitrous oxide production following wetting of dry soil. *FEMS Microbiology Letters*, **85**, 117–124.
- Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F. & Munch, J. 2006. Emission of N₂O, N₂ and CO₂ from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. *Soil Biology & Biochemistry*, **38**, 263–274.
- Samarkin, V.A., Madigan, M.T., Bowles, M.W., Casciotti, K.L., Priscu, J.C., McKay, C.P. *et al.* 2010. Abiotic nitrous oxide emission from the hypersaline Don Juan Pond in Antarctica. *Nature Geoscience*, **3**, 341.
- Schimel, J., Balser, T.C. & Wallenstein, M. 2007. Microbial stress–response physiology and its implications for ecosystem function. *Ecology*, **88**, 1386–1394.
- Shen, Q., Ran, W. & Cao, Z. 2003. Mechanisms of nitrite accumulation occurring in soil nitrification. *Chemosphere*, **50**, 747–753.
- Silver, W.L., Herman, D.J. & Firestone, M.K. 2001. Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology*, **82**, 2410–2416.
- Snider, D., Thompson, K., Wagner-Riddle, C., Spoelstra, J. & Dunfield, K. 2015. Molecular techniques and stable isotope ratios at natural abundance give complementary inferences about N₂O production pathways in an agricultural soil following a rainfall event. *Soil Biology & Biochemistry*, **88**, 197–213.
- Snider, D.M., Venkiteswaran, J.J., Schiff, S.L. & Spoelstra, J. 2012. Deciphering the oxygen isotope composition of nitrous oxide produced by nitrification. *Global Change Biology*, **18**, 356–370.
- Stevenson, F. & Swaby, R. 1964. Nitrosation of soil organic matter: I. Nature of gases evolved during nitrous acid treatment of lignins and humic substances. *Soil Science Society of America Journal*, **28**, 773–778.
- Stroetmann, I., Kämpfer, P. & Dott, W. 1994. [The efficiency of sterilization methods for different soils]. *Zentralblatt für Hygiene und Umweltmedizin = International journal of hygiene and environmental medicine*, **195**, 111–120.
- Sutka, R.L., Ostrom, N., Ostrom, P., Breznak, J., Gandhi, H., Pitt, A. *et al.* 2006. Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. *Applied and Environmental Microbiology*, **72**, 638–644.

- Szukics, U., Abell, G.C., Hödl, V., Mitter, B., Sessitsch, A., Hackl, E. *et al.* 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. *FEMS Microbiology Ecology*, **72**, 395–406.
- Thion, C. & Prosser, J.I. 2014. Differential response of nonadapted ammonia-oxidising archaea and bacteria to drying–rewetting stress. *FEMS Microbiology Ecology*, **90**, 380–389.
- Thorn, K. & Mikita, M. 2000. Nitrite fixation by humic substances nitrogen-15 nuclear magnetic resonance evidence for potential intermediates in chemodenitrification. *Soil Science Society of America Journal*, **64**, 568–582.
- Toyoda, S., Mutoke, H., Yamagishi, H., Yoshida, N. & Tanji, Y. 2005. Fractionation of N₂O isotopomers during production by denitrifier. *Soil Biology & Biochemistry*, **37**, 1535–1545.
- Van Cleemput, O. & Samater, A.H. 1995. Nitrite in soils: Accumulation and role in the formation of gaseous N compounds. *Fertilizer Research*, **45**, 81–89.
- Venterea, R.T. 2007. Nitrite-driven nitrous oxide production under aerobic soil conditions: Kinetics and biochemical controls. *Global Change Biology*, **13**, 1798–1809.

Tables

Table 1 Means of basic properties of air-dried soil samples.

	C	N	C/N	pH	Fe	Mn	Ca	K	Mg
	/%	/%			%	%	/%	%	%
F _{Of}	45.72	1.93	23.7	2.85	0.35	0.03	0.33	0.13	0.05
F1	28.70	1.47	19.5	3.05	1.72	0.01	0.10	0.73	0.15
F2	19.80	1.08	18.4	3.27	2.55	0.02	0.20	1.05	0.25
F3	25.87	1.47	17.6	3.35	2.20	0.01	0.13	0.77	0.16
F4	24.57	1.32	18.5	3.03	1.87	0.02	0.14	0.96	0.16
F5	21.38	0.88	24.4	3.92	3.30	0.21	0.19	1.28	0.21
F6	22.23	1.51	14.7	3.78	3.50	0.07	0.09	1.12	0.17
FR	9.65	0.53	18.1	4.23	1.57	0.02	0.13	1.75	0.31
G	5.29	0.53	9.9	5.25	2.39	0.10	0.28	1.65	0.29
A	1.29	0.14	9.2	5.82	2.10	0.07	0.36	1.46	0.32

F_{Of}, F1, F2, F3, F4, F5, F6 and FR are soil samples collected from fermented litter (F_{Of}), Oa horizon (F1, F2, F3, F4, F5 and F6) and riparian area (FR); G and A are soil samples collected from grassland (G) and arable land (A).

Table 2 Soil NO₂⁻-N (mg kg⁻¹ dry soil), dissolved organic carbon (DOC, mg kg⁻¹ dry soil), dissolved total nitrogen (DTN, mg kg⁻¹ dry soil) and A₂₅₄ (cm⁻¹ g⁻¹ dry soil) after air-drying for forest, grassland and arable soil samples.

Soil samples	NO ₂ ⁻ /mg kg ⁻¹	DOC /mg kg ⁻¹	DTN /mg kg ⁻¹	A ₂₅₄ /cm ⁻¹ g ⁻¹
F _{Of}	0.3	2420	358	1.40
F1	0.2	2110	161	1.27
F2	n.d.	1680	123	1.00
F3	0.3	1825	183	0.78
F4	n.d.	1885	221	1.01
F5	0.1	555	118	0.41
F6	0.3	890	84	0.24
FR	n.d.	575	74	0.42
G	0.2	636	105	0.41
A	0.1	177	21	0.22

The standard deviation of the NO₂⁻ assay is about 20% of the values (n.d., not detectable). There was only one extraction to determine soil DOC, DTN and A₂₅₄.

654 **Table 3** The inhibitory effect (%) of γ -irradiation on soil N₂O and CO₂ emissions after 7
655 hours of incubation after rewetting of air-dried soil.

Additions/%	F _{0f}	F1	F2	F3	F5	FR	G	A
H ₂ O addition								
N ₂ O	91.1	30.4	28.0	60.8	73.3	73.4	-304.2	-210.0
CO ₂	13.2	-28.2	-0.8	-12.2	25.8	31.0	53.9	26.0
NO ₂ ⁻ addition								
N ₂ O	85.7	26.5	24.5	49.0	71.0	63.5	-121.3	-48.9
CO ₂	21.7	-25.5	1.1	4.9	28.0	24.6	53.2	21.2

656 Negative values represent a stimulating effect of γ -irradiation. The data of F4 after γ -irradiation treatment was missing
657 due to shortage of material.

Table 4 Pearson's correlation coefficients between soil N₂O emission after 7 hours of incubation after water rewetting and basic soil properties of air-dried soil samples (excluding Ca, Mg and K) across all soil samples ($n = 10$).

	N ₂ O	Fe	Mn	C	N	C/N	pH	NO ₂ ⁻	lnNH ₄ ⁺	NO ₃ ⁻	DOC	DTN	A ₂₅₄
N ₂ O	1.00												
Fe	-0.31	1.00											
Mn	-0.24	0.57	1.00										
C	0.53	-0.41	-0.53	1.00									
N	0.62	-0.26	-0.35	0.95	1.00								
C/N	0.08	-0.24	-0.11	0.77	0.61	1.00							
pH	-0.32	0.26	0.40	-0.88	-0.89	-0.79	1.00						
NO ₂ ⁻	0.85	-0.28	-0.64	0.62	0.80	0.16	-0.6	1.00					
lnNH ₄ ⁺	0.71	-0.28	-0.21	0.81	0.84	0.47	-0.73	0.72	1.00				
NO ₃ ⁻	0.42	-0.42	-0.15	0.79	0.75	0.50	-0.62	0.65	0.71	1.00			
DOC	0.54	-0.54	-0.60	0.86	0.86	0.55	-0.87	0.67	0.73	0.56	1.00		
DTN	0.58	-0.65	-0.30	0.89	0.80	0.63	-0.74	0.59	0.93	0.88	0.85	1.00	
A ₂₅₄	0.40	-0.66	-0.54	0.79	0.71	0.58	-0.79	0.42	0.72	0.56	0.95	0.83	1.00

Bold values indicate significance of the respective correlation coefficient at $P < 0.05$.

663 **Table 5** The ^{15}N site preference (SP) values of N_2O production (peak height) on rewetting
664 with water for different soil samples and incubation times. The peak height of ambient air and
665 standard N_2O gas (400 nl l^{-1}) was about 1.9 and 2.4 nA, respectively.

Samples	Soil	Incubation time	Peak height	$\delta^{15}\text{N}^{\text{bulk}}$	$\delta^{18}\text{O}$	SP
	/g	/hours	/nA	/‰ vs. air- N_2	/‰ vs. VSMOW	/‰
F _{Of}	1.4	6.0	3.8	-24.0	29.3	1.6
F1	1.4	6.0	2.6	-24.8	32.0	-15.4
F3	1.4	6.0	4.3	-35.6	110.6	2.4
F4	2.8	6.0	3.2	-28.6	24.8	2.4
F5	2.8	6.0	2.3	-42.4	16.0	9.9
F6	2.8	6.0	5.1	-35.7	13.5	-1.0
G	2.8	0.5	11.1	-28.4	12.7	-0.3
G	2.8	3.5	25.7	-33.8	10.4	-1.6
G	2.8	6.5	31.1	-35.1	9.7	-2.1
G (Sterilized)	2.8	0.5	8.5	-24.6	11.7	1.3
G (Sterilized)	2.8	3.5	51.5	-27.0	9.9	-0.3
G (Sterilized)	2.8	6.5	64.9	-29.0	10.9	-0.7

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Figure captions

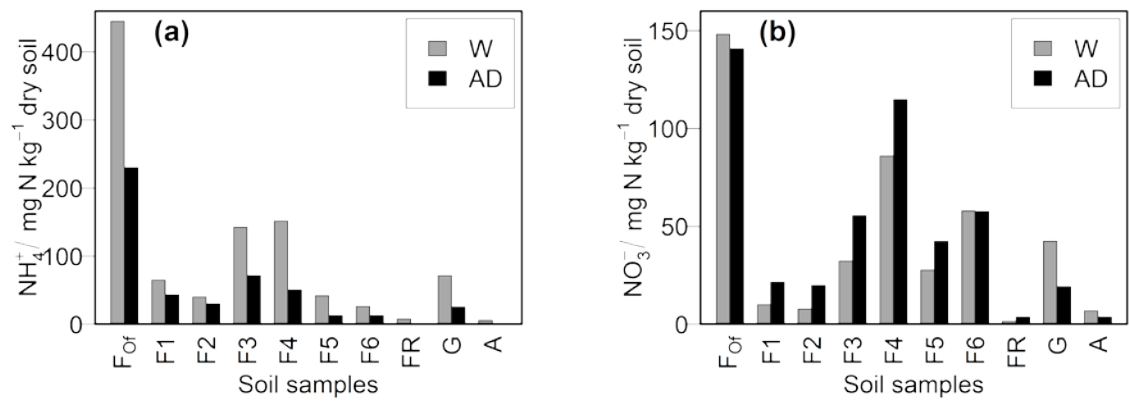
Figure 1 Soil (a) NH_4^+ and (b) NO_3^- contents before (W, grey) and after air-drying (AD, black) for forest (F_{Of} , F1, F2, F3, F4, F5, F6 and FR), grassland (G) and arable (A) soil samples. Only one extraction was done to determine soil NH_4^+ and NO_3^- contents.

Figure 2 Rewetting effects by the addition of (a) water, (b) aqueous solutions of NO_2^- , (c) NO_3^- and (d) NH_4^+ on soil N_2O production (ng N g^{-1} dry soil) for forest (F_{Of} , F1, F2, F3, F4, F5, F6 and FR), grassland (G) and arable (A) soil samples for different incubation times (1 hour and 7 hours) before γ -irradiation. The values are presented as mean \pm standard error (SE).

Figure 3 Rewetting effects by the addition of (a) water and (b) aqueous NO_2^- solution on soil N_2O production (ng N g^{-1} dry soil) of forest (F_{Of} , F1, F2, F3, F4, F5, F6 and FR), grassland (G) and arable (A) soil samples for different incubation times (1 hour and 7 hours) after γ -irradiation. The data of F4 after γ -irradiation treatment was missing due to shortage of material. The values are presented as mean \pm standard error (SE).

684 Figures

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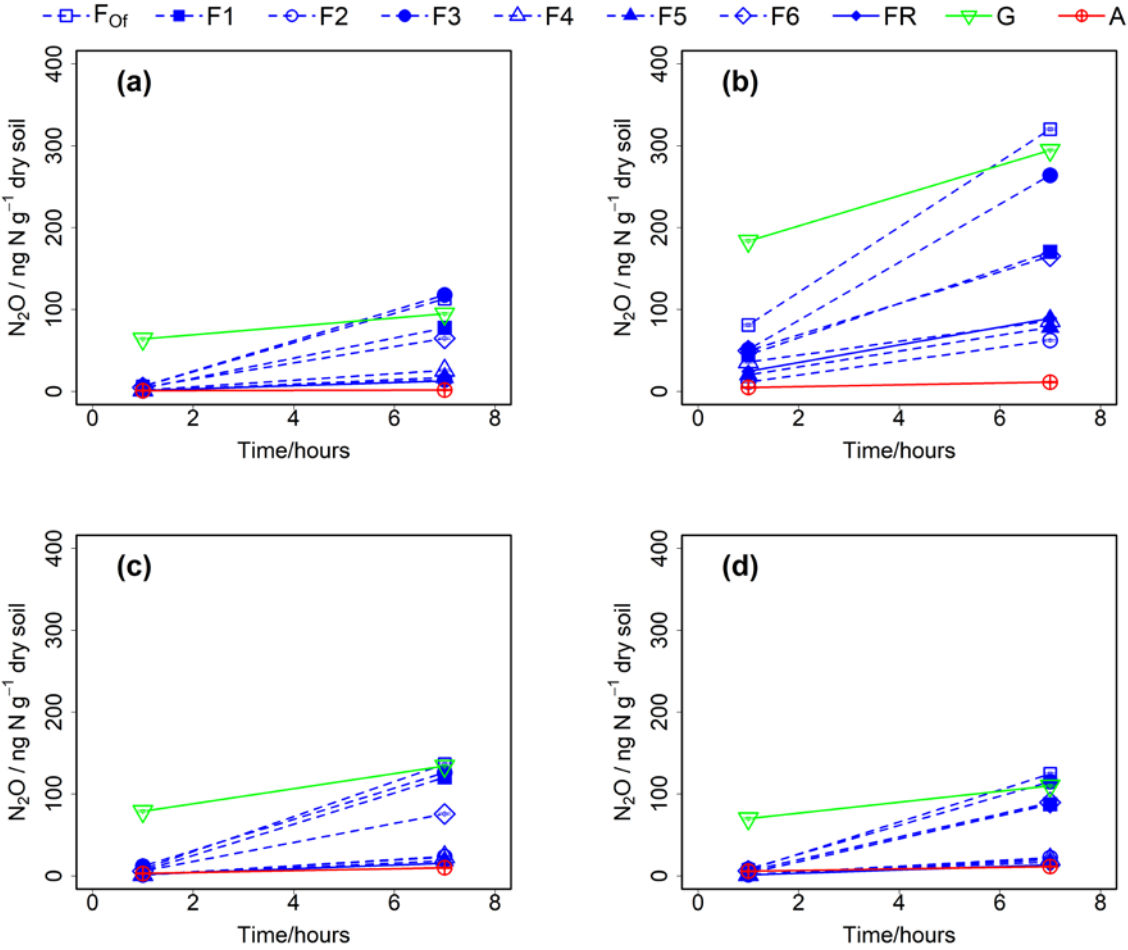
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687 Figure1

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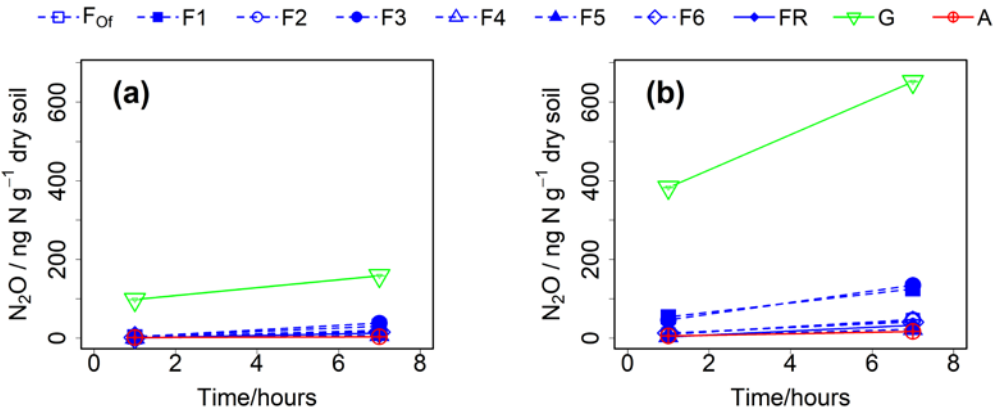
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692 Figure 2

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697 Figure 3