Accumulation of NO$_2^-$ during periods of drying stimulates soil N$_2$O emissions during subsequent rewetting

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Running title: Nitrite stimulates N$_2$O emissions during rewetting
Summary

Rewetting of soil might contribute considerably to the annual production of nitrous oxide (N$_2$O) in ecosystems subjected to long dry periods. Therefore, it is crucial to elucidate the most important factors responsible for large pulses of N$_2$O 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$_2$O production with rewetting. Furthermore, we explored the effects of rewetting and sterilization on the isotopic composition of N$_2$O in the different soil samples. The grassland soil produced the largest amount of N$_2$O (64.1 µg 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$_2$O production from forest soil samples by 30–90% after rewetting, but increased N$_2$O 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$_2$O production after rewetting. Furthermore, the addition of NO$_2^-$ before rewetting increased N$_2$O 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$_2$O 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$_2$O production during rewetting cannot be excluded.

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

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

Emissions of nitrous oxide (N\textsubscript{2}O) 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\textsubscript{2}O emissions (Rudaz et al., 1991; Ruser et al., 2006). A single wetting event might be responsible for a large fraction of the annual N\textsubscript{2}O emissions for certain ecosystems (Priemé & Christensen, 2001). Recently, several studies have focused on the mechanisms of large soil N\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O. 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 (\textit{amoA}) transcripts was detectable within one hour after rewetting and continued until the ammonium (NH\textsubscript{4}\textsuperscript{+}) 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$_2$O (Van Cleemput & Samater, 1995; Samarkin et al., 2010). Another important pathway for N$_2$O 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$_2$O formation by biological (i.e. nitrification, nitrifier denitrification, bacterial denitrification and fungal denitrification) and abiotic reactions (chemodenitrification and NH$_2$OH oxidation) (Jones et al., 2015; Grabb et al., 2017).

To investigate the processes involved in pulses of N$_2$O 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 $\gamma$-irradiation and analysed the $^{15}$N SP of N$_2$O. The aims of the experiments were to identify the relevant factors controlling pulses of N$_2$O emissions caused by rewetting soil, and to quantify the contributions of abiotic and biotic reactions to the pulse. We hypothesized that (i) more N$_2$O will be produced on rewetting from soil samples with larger NO$_2^-$ accumulation and (ii) abiotic reactions play an important role in N$_2$O 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±1°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-µm filters. We placed 1.4 g of air-dried soil (0.7 g for F0d) into 22-ml gas chromatography (GC) vials (VWR international, Darmstadt, Germany), followed by the addition of either H2O, or NO2−, NO3− or NH4+ solutions to reach around 40% water-holding capacity (WHC), and 1 µg N g⁻¹ dry soil (for NO2−) and 100 µg N g⁻¹ dry soil (for NH4+ and NO3−). 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 N2O and CO2, 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 µl l⁻¹ N2O balanced with N2 (99.5% purity, Linde, Munich, Germany).

Analysis of $^{15}$N site preference of N₂O. To determine N₂O 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 N₂O, which in turn was connected to an isotope ratio mass spectrometer (IRMS, IsoPrime 100, Elementar Analysensysteme, Langenselbold, Germany). Molecular ions (N₂O⁺) 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 $\delta^{15}$N$_{\text{bulk}}$ ($\delta^{15}$N of total nitrogen) and $\delta^{18}$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)

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

(1)

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

Site preference is defined as

$$\text{SP} = \delta^{15}$N$^\alpha - \delta^{15}$N$^\beta,$$$

(2)

where $\delta^{15}$N$^\alpha$ and $\delta^{15}$N$^\beta$ are the $\delta^{15}$N at the central and terminal position of the N$_2$O molecule, respectively. The $\delta^{15}$N$^\alpha$ was calculated from the isotope ratio m/z 30 and 31. The $\delta^{15}$N$^\beta$ was calculated according to the following formula:

$$\delta^{15}$N$^\beta = 2 \times \delta^{15}$N$_{\text{bulk}} - \delta^{15}$N$^\alpha,$$$

(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$_2$O (99.99%, Linde, Munich, Germany) was used as the working standard (values = mean, standard deviation: $\delta^{15}$N$^\alpha$ relative to air-N$_2$ = 3.18, 0.23‰, $\delta^{15}$N$^\beta$ relative to air-N$_2$ = 1.42, 0.21‰, $\delta^{18}$O relative to Vienna Standard Mean Ocean Water (VSMOW) = 39.35, 0.27‰) for isotope analysis, and the $\delta^{15}$N$_{\text{bulk}}$, $\delta^{15}$N$^\alpha$, $\delta^{15}$N$^\beta$ and $\delta^{18}$O were calibrated against two reference (R) gases (R1: $\delta^{15}$N$^\alpha$ relative to air-N$_2$ = 15.70, 0.31‰, $\delta^{15}$N$^\beta$ relative to air-N$_2$ = -3.21, 0.37‰, $\delta^{18}$O relative to VSMOW = 35.16, 0.35‰; R2: $\delta^{15}$N$^\alpha$ relative to air-N$_2$ = 5.55, 0.21‰, $\delta^{15}$N$^\beta$ relative to air-N$_2$ = -12.87, 0.32‰, $\delta^{18}$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$_2$O were added to the 120-ml bottles and isotope signatures were
measured. Strong quadratic relations were observed between N$_2$O peak height and $\delta^{15}$N$_2$O, $\delta^{16}$N$_2$O and $\delta^{31}$NO relative to the reference gas, with polynomial equations of

$$y = ax^2 + bx + c,$$

where $y$ is N$_2$O peak height (2.7 to 72 nanoampere, nA), $x$ is $\delta^{15}$N$_2$O relative to reference ($a = 0.0032$, $b = -0.1689$, $c = 0.5516$), $\delta^{16}$N$_2$O relative to reference ($a = 0.0054$, $b = -0.2643$, $c = 39.3$) and $\delta^{31}$NO relative to reference ($a = 0.0014$, $b = 0.4489$, $c = -0.6767$).

Therefore, all $\delta^{15}$N$_{\text{bulk}}$, $\delta^{18}$O and SP values in this study were calculated according to the corrected $\delta^{15}$N$_2$O, $\delta^{16}$N$_2$O and $\delta^{31}$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 $\delta^{15}$N$_{\text{bulk}}$ relative to air-N$_2$, $\delta^{18}$O relative to VSMOW, $\delta^{31}$N relative to the reference gas, $\delta^{15}$N$_{\text{a}}$ relative to air-N$_2$, $\delta^{15}$N$_{\beta}$ relative to air-N$_2$ 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 HNO$_3$ and 2 ml H$_2$O$_2$, 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 (NH$_4^+$, NO$_2^-$ and NO$_3^-$) contents were analysed by ion chromatography (ICS-3000 for NO$_2^-$ and NO$_3^-$, DX-500 for NH$_4^+$; both analysers were from Dionex, Sunnyvale, CA, USA). The NH$_4^+$ and NO$_3^-$ 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:

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

(5)

where $E$ is the N$_2$O emission (ng N g$^{-1}$ dry soil), $C'$ is the N$_2$O 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$_2$O 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$^{\text{bulk}}$, $\delta^{18}$O and SP values) of soil-emitted N$_2$O were calculated from the total isotope signature of the gas samples and of ambient air using a two-component mixing model:

$$\delta_1 = \left( \delta_2 \times Q_2 - \delta_0 \times Q_0 \right) / (Q_2 - Q_0),$$

(6)
where $\delta_1$, $\delta_2$ and $\delta_0$ are the mean isotope signatures of soil-emitted N$_2$O, the sample bottles, and ambient air, respectively, $Q_2$ and $Q_0$ represent the N$_2$O 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$_2$O production during rewetting in the R software package (version 3.4.3). Box–Cox transformation of N$_2$O 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$_2$O 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 FoF and FR, 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 (FOf) 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 NH$_4^+$ and NO$_3^-$) of the fresh soil differed strongly between the soil samples (Figure 1). Before air-drying, forest soil samples FOf and F4 had the largest NH$_4^+$ and NO$_3^-$ contents, while samples F5 and F6 had smaller NH$_4^+$, but larger NO$_3^-$ contents than the other forest soil samples. Samples from the riparian zone and arable land had the smallest NH$_4^+$ and NO$_3^-$ contents of all soil samples, and grassland soil had intermediate contents of NH$_4^+$ and NO$_3^-$. After air-drying, the NH$_4^+$ content decreased in all soil samples. Almost no NH$_4^+$ was detectable in the riparian and arable soil samples after air-drying.

In contrast to NH$_4^+$, NO$_3^-$ increased with drying for most soil samples, except for FOf, F6, grassland and arable land samples. Forest soil sample FOf had the largest NO$_3^-$ content, followed by F4 and F6. The grassland soil had an intermediate NO$_3^-$ content compared to the forest samples, whereas the riparian and arable land samples were characterized by the smallest NO$_3^-$ content.

Before air-drying, NO$_2^-$ concentrations were below the detection limit for the fresh soil samples. However, small amounts of NO$_2^-$ were detectable in several soil samples after drying (Table 2). Forest soil samples FOf, F3 and F6 had the largest NO$_2^-$ content (0.3 mg kg$^{-1}$), followed by grassland and forest soil F1 (0.2 mg kg$^{-1}$), whereas no NO$_2^-$ 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 FOf 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$_2$O emissions
Soil types, the water and different additions of N had significant ($P<0.05$) effects on the
rewetting responses of soil N$_2$O emissions (Figure 2a, Table S1, Supporting Information).
After rewetting with water only, N$_2$O emission from grassland soil was large, especially in the
first hour after rewetting, with an emission of 64 µg N$_2$O-N kg$^{-1}$ dry soil. After 7 hours with
rewetting, the N$_2$O 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$_2$O emissions, whereas they
were smaller for F2, F4, F5 and FR. Unlike grassland soil, N$_2$O 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$_2$O 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$_2$O 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$_2$O production in the forest soil samples F4, F5 and FR were not significant. The effects of
NO$_2^-$ on N$_2$O production in the arable soil was significantly ($P<0.05$) smaller than other soils.
The rate of total NO$_2^-$:N$_2$O 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$_2$O with rewetting (Figure 2c,d), even though the mount of NH$_4^+$-N and
NO$_3^-$-N added was 100-fold larger than that of NO$_2^-$. For most of soil samples, the difference between the effects of NO$_3^-$ and NH$_4^+$ was not significant ($P < 0.05$).

Effect of $\gamma$-irradiation on soil N$_2$O and CO$_2$ emissions after rewetting

The effect of $\gamma$-irradiation on soil N$_2$O emissions with rewetting depended on soil type. In general, $\gamma$-irradiation affected the emission of N$_2$O on rewetting significantly ($P < 0.05$). It decreased N$_2$O 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 N$_2$O 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 $\gamma$-irradiation. It inhibited N$_2$O production from the most, followed by the riparian sample and F5, whereas N$_2$O production was least from samples F1 and F2 during the incubation.

In the NO$_2^-$ rewetting treatment, $\gamma$-irradiation also increased production of N$_2$O in grassland and arable soils, but decreased it in forest soil (Figure 3b, Table 3). Samples F1 and F2 were inhibited the least by $\gamma$-irradiation.

Compared to the effects on soil N$_2$O emissions, $\gamma$-irradiation decreased production of CO$_2$ 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, CO$_2$ production was stimulated by $\gamma$-irradiation.

Control variables of soil N$_2$O emission on rewetting

Basic soil properties play an important role in biotic and abiotic reactions, and might have contributed to the pulse of N$_2$O emissions after rewetting. Nitrous oxide production was significantly ($P < 0.05$) and positively correlated with NO$_2^-$ ($r = 0.85$) and NH$_4^+$ ($r = 0.71$) 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$_2$O
production, but not with mineral N and DTN.

**Isotope ratio analyses of N$_2$O produced during rewetting**

The $\delta^{15}$N$^{\text{bulk}}$ and $\delta^{18}$O values varied from $−42.4$ to $−24.0\%o$ and from $9.7$ to $32.0\%o$, respectively, for all the soil samples during rewetting, except for sample F3 where $\delta^{18}$O was very large with a value of $110.6\%o$ (Table 5). Both $\delta^{15}$N$^{\text{bulk}}$ and $\delta^{18}$O decreased with increasing incubation time for the grassland soil, regardless of whether the soil had been $\gamma$-irradiated in advance or not. Ranges of $\delta^{15}$N$^{\text{bulk}}$ values for grassland and forest soils were similar, whereas $\delta^{18}$O of N$_2$O were larger for forest than grassland soil. The SP values of N$_2$O formed after rewetting were close to zero for most of the soil samples (except for F5), independent of the amount of N$_2$O 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 $9.9\%o$. The SP values for the grassland soil samples ranged from $−2.1$ to $1.3\%o$ for both $\gamma$-irradiated and non-irradiated samples, even though N$_2$O production increased largely with incubation time.

**Discussion**

Soil rewetting-induced N$_2$O production has received more attention recently because of the potentially large contribution of this fraction of N$_2$O to the annual N$_2$O 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$_2$O 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$_2$O 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$_2$O 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$_2$O on rewetting to around 40% WHC, as
in our study. We assumed that this relatively small water content might favour the production
of N$_2$O from nitrification, but the addition of NH$_4^+$ increased N$_2$O 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$_2$O
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 F06, 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$_2$O produced after rewetting (Table 4, Figure S1, Supporting
Information). Addition of NO$_2^-$ also increased soil N$_2$O 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$_2$O: (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$_2$O 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$_2$O emission from the hypersaline Don Juan Pond in Antarctica. We also explored the contribution of abiotic reactions to NO$_2^-$-mediated N$_2$O production during rewetting by sterilizing the soil with 11 kGy of $\gamma$-irradiation. Our results showed considerable differences between soil samples from the effect of $\gamma$-irradiation on soil N$_2$O production (Table 3). In general, it inhibited N$_2$O production from the forest and riparian samples; the largest inhibition was in the sample with the fermented organic layer (FOf, 91.1%) and the smallest was in soil sample F2 (F2, 28%). The range of inhibition by $\gamma$-irradiation was consistent with that reported by Venterea (2007), who also found that production of N$_2$O in $\gamma$-irradiated soils ranged from 31 to 75%.

The small effects of $\gamma$-irradiation on soil CO$_2$ emissions from forest soils were unexpected because we assumed that negligible CO$_2$ would be produced in the $\gamma$-irradiated soils. One reason could be the limited effect of $\gamma$-irradiation on certain soil microorganisms, mainly spore forming fungi, even though $\gamma$-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$_2$O 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 $\gamma$-irradiated soil slurries in this study. In contrast, in the grassland and arable land samples $\gamma$-irradiation increased N$_2$O 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 N₂O production in the grassland samples was surprising, but could indicate an increased contribution from an abiotic mechanism of N₂O production from NO₂⁻. It is possible that γ-irradiation might have strongly inhibited the activity of nitrite oxidizers, leaving more NO₂⁻ available for abiotic N₂O production. This might explain the larger amount of N₂O produced from grassland soil after γ-irradiation, but this assumption remains speculative. In addition, the contribution of abiotic processes to soil N₂O 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 N₂O production (Venterea, 2007). But this contrasts with reduced N₂O formation in γ-irradiated forest samples. Therefore, further research is needed to elucidate the mechanisms behind stimulation and inhibition of N₂O production from nitrite after γ-irradiation of different types of soil.

Finally, we measured the isotopic signatures (δ¹⁵Nbulk, δ¹⁸O and SP values) of N₂O formed during rewetting because they are thought to reflect the relative contribution of different sources of N₂O to some extent. There have been several recent studies that examined N₂O SP from chemodenitrification (Heil et al., 2014; Jones et al., 2015; Grabb et al., 2017). The δ¹⁵Nbulk measured in this study fell within the range of denitrification (−40 to −19‰) in pure cultures (Toyoda et al., 2005), whereas δ¹⁸O values were in the range of N₂O produced by nitrification in soil (Snider et al., 2012). The SP values have been considered a more useful tool for partitioning sources of N₂O than δ¹⁵Nbulk and δ¹⁸O because SP values were relatively stable for the production of N₂O from different soil processes, although there was still some overlap between aerobic nitrification and abiotic NH₂OH 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 δ¹⁵N 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.

Acknowledgements

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Kubica for their assistance in the TOC and TN analysis, and Dr. Jinsong Zhao for the assistance of statistical analysis. This study was supported by the Chinese Scholarship Council (scholarship no 201206760007).
References


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

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>N</th>
<th>C/N</th>
<th>pH</th>
<th>Fe</th>
<th>Mn</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0F</td>
<td>45.72</td>
<td>1.93</td>
<td>23.7</td>
<td>2.85</td>
<td>0.35</td>
<td>0.03</td>
<td>0.33</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>F1</td>
<td>28.70</td>
<td>1.47</td>
<td>19.5</td>
<td>3.05</td>
<td>1.72</td>
<td>0.01</td>
<td>0.10</td>
<td>0.73</td>
<td>0.15</td>
</tr>
<tr>
<td>F2</td>
<td>19.80</td>
<td>1.08</td>
<td>18.4</td>
<td>3.27</td>
<td>2.55</td>
<td>0.02</td>
<td>0.20</td>
<td>1.05</td>
<td>0.25</td>
</tr>
<tr>
<td>F3</td>
<td>25.87</td>
<td>1.47</td>
<td>17.6</td>
<td>3.35</td>
<td>2.20</td>
<td>0.01</td>
<td>0.13</td>
<td>0.77</td>
<td>0.16</td>
</tr>
<tr>
<td>F4</td>
<td>24.57</td>
<td>1.32</td>
<td>18.5</td>
<td>3.03</td>
<td>1.87</td>
<td>0.02</td>
<td>0.14</td>
<td>0.96</td>
<td>0.16</td>
</tr>
<tr>
<td>F5</td>
<td>21.38</td>
<td>0.88</td>
<td>24.4</td>
<td>3.92</td>
<td>3.30</td>
<td>0.21</td>
<td>0.19</td>
<td>1.28</td>
<td>0.21</td>
</tr>
<tr>
<td>F6</td>
<td>22.23</td>
<td>1.51</td>
<td>14.7</td>
<td>3.78</td>
<td>3.50</td>
<td>0.07</td>
<td>0.09</td>
<td>1.12</td>
<td>0.17</td>
</tr>
<tr>
<td>FR</td>
<td>9.65</td>
<td>0.53</td>
<td>18.1</td>
<td>4.23</td>
<td>1.57</td>
<td>0.02</td>
<td>0.13</td>
<td>1.75</td>
<td>0.31</td>
</tr>
<tr>
<td>G</td>
<td>5.29</td>
<td>0.53</td>
<td>9.9</td>
<td>5.25</td>
<td>2.39</td>
<td>0.10</td>
<td>0.28</td>
<td>1.65</td>
<td>0.29</td>
</tr>
<tr>
<td>A</td>
<td>1.29</td>
<td>0.14</td>
<td>9.2</td>
<td>5.82</td>
<td>2.10</td>
<td>0.07</td>
<td>0.36</td>
<td>1.46</td>
<td>0.32</td>
</tr>
</tbody>
</table>

F0F, F1, F2, F3, F4, F5, F6 and FR are soil samples collected from fermented litter (F0F), 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 NO$_2^-$-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 A$_{254}$ (cm$^{-1}$ g$^{-1}$ dry soil) after air-drying for forest, grassland and arable soil samples.

<table>
<thead>
<tr>
<th>Soil samples</th>
<th>NO$_2^-$ /mg kg$^{-1}$</th>
<th>DOC /mg kg$^{-1}$</th>
<th>DTN /mg kg$^{-1}$</th>
<th>A$_{254}$ /cm$^{-1}$ g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_{Or}$</td>
<td>0.3</td>
<td>2420</td>
<td>358</td>
<td>1.40</td>
</tr>
<tr>
<td>F1</td>
<td>0.2</td>
<td>2110</td>
<td>161</td>
<td>1.27</td>
</tr>
<tr>
<td>F2</td>
<td>n.d.</td>
<td>1680</td>
<td>123</td>
<td>1.00</td>
</tr>
<tr>
<td>F3</td>
<td>0.3</td>
<td>1825</td>
<td>183</td>
<td>0.78</td>
</tr>
<tr>
<td>F4</td>
<td>n.d.</td>
<td>1885</td>
<td>221</td>
<td>1.01</td>
</tr>
<tr>
<td>F5</td>
<td>0.1</td>
<td>555</td>
<td>118</td>
<td>0.41</td>
</tr>
<tr>
<td>F6</td>
<td>0.3</td>
<td>890</td>
<td>84</td>
<td>0.24</td>
</tr>
<tr>
<td>FR</td>
<td>n.d.</td>
<td>575</td>
<td>74</td>
<td>0.42</td>
</tr>
<tr>
<td>G</td>
<td>0.2</td>
<td>636</td>
<td>105</td>
<td>0.41</td>
</tr>
<tr>
<td>A</td>
<td>0.1</td>
<td>177</td>
<td>21</td>
<td>0.22</td>
</tr>
</tbody>
</table>

The standard deviation of the NO$_2^-$ assay is about 20% of the values (n.d., not detectable). There was only one extraction to determine soil DOC, DTN and A$_{254}$. 
Table 3 The inhibitory effect (%) of γ-irradiation on soil N₂O and CO₂ emissions after 7 hours of incubation after rewetting of air-dried soil.

<table>
<thead>
<tr>
<th>Additions/%</th>
<th>F₀f</th>
<th>F₁</th>
<th>F₂</th>
<th>F₃</th>
<th>F₅</th>
<th>FR</th>
<th>G</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>N₂O</td>
<td>91.1</td>
<td>30.4</td>
<td>28.0</td>
<td>60.8</td>
<td>73.3</td>
<td>73.4</td>
<td>-304.2</td>
<td>-210.0</td>
</tr>
<tr>
<td>CO₂</td>
<td>13.2</td>
<td>-28.2</td>
<td>-0.8</td>
<td>-12.2</td>
<td>25.8</td>
<td>31.0</td>
<td>53.9</td>
<td>26.0</td>
</tr>
<tr>
<td>NO₂⁻ addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₂O</td>
<td>85.7</td>
<td>26.5</td>
<td>24.5</td>
<td>49.0</td>
<td>71.0</td>
<td>63.5</td>
<td>-121.3</td>
<td>-48.9</td>
</tr>
<tr>
<td>CO₂</td>
<td>21.7</td>
<td>-25.5</td>
<td>1.1</td>
<td>4.9</td>
<td>28.0</td>
<td>24.6</td>
<td>53.2</td>
<td>21.2</td>
</tr>
</tbody>
</table>

Negative values represent a stimulating effect of γ-irradiation. The data of F₄ after γ-irradiation treatment was missing due to shortage of material.
Table 4 Pearson’s correlation coefficients between soil $\text{N}_2\text{O}$ 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 ($n = 10$).

|        | $\text{N}_2\text{O}$ | Fe  | Mn  | C   | N   | C/N | pH  | NO$_2^-$ | lnNH$_4^+$ | NO$_3^-$ | DOC  | DTN | $A_{254}$ |
|--------|------------------------|-----|-----|-----|-----|-----|-----|---------|-----------|----------|-------|------|------|-----------|
| $\text{N}_2\text{O}$ | 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                | −0.21| 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 $P < 0.05$.  

30
Table 5 The $^{15}$N site preference (SP) values of N$_2$O production (peak height) on rewetting with water for different soil samples and incubation times. The peak height of ambient air and standard N$_2$O gas (400 nl l$^{-1}$) was about 1.9 and 2.4 nA, respectively.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Soil /g</th>
<th>Incubation time /hours</th>
<th>Peak height /nA</th>
<th>$\delta^{15}$N$^{bulk}$ /‰ vs. air-N$_2$</th>
<th>$\delta^{18}$O /‰ vs. VSMOW</th>
<th>SP /‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_{Of}$</td>
<td>1.4</td>
<td>6.0</td>
<td>3.8</td>
<td>−24.0</td>
<td>29.3</td>
<td>1.6</td>
</tr>
<tr>
<td>F1</td>
<td>1.4</td>
<td>6.0</td>
<td>2.6</td>
<td>−24.8</td>
<td>32.0</td>
<td>−15.4</td>
</tr>
<tr>
<td>F3</td>
<td>1.4</td>
<td>6.0</td>
<td>4.3</td>
<td>−35.6</td>
<td>110.6</td>
<td>2.4</td>
</tr>
<tr>
<td>F4</td>
<td>2.8</td>
<td>6.0</td>
<td>3.2</td>
<td>−28.6</td>
<td>24.8</td>
<td>2.4</td>
</tr>
<tr>
<td>F5</td>
<td>2.8</td>
<td>6.0</td>
<td>2.3</td>
<td>−42.4</td>
<td>16.0</td>
<td>9.9</td>
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<td>F6</td>
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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 (FOf, 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$_2$O production (ng N g$^{-1}$ dry soil) for forest (FOf, 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 $\gamma$-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$_2$O production (ng N g$^{-1}$ dry soil) of forest (FOf, 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 $\gamma$-irradiation. The data of F4 after $\gamma$-irradiation treatment was missing due to shortage of material. The values are presented as mean $\pm$ standard error (SE).
Figures

Figure 1
Figure 2
Figure 3