Accumulation of NO_2^- during periods of drying stimulates soil N_2O emissions during subsequent rewetting

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Running title: Nitrite stimulates N_2O 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 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_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**

42

43 • Mechanisms responsible for large N_2O production during rewetting of soil are not well
44 understood.
45 • Nitrite content in dry soil was strongly correlated to N_2O production after rewetting.
46 • The ^{15}N site preference of the N_2O produced was close to 0‰ after rewetting.
47 • Additional abiotic processes could have contributed to N_2O formation from NO_2^-.

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 N_2O pulses in the wake of rewetting. Hydroxylamine (NH_2OH) and nitrite (NO_2^-) are the most important reactive N intermediates involved in abiotic N_2O production (Heil *et al.*, 2016). It is unlikely that NH_2OH 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 NO_2^- to nitrate (NO_3^-) proceeds faster than the conversion of ammonia (NH_3) to NO_2^-. However, NO_2^- has considerable potential to accumulate during soil drying. Davidson (1992) reported that accumulation of soil NO_2^- during drought probably contributes to pulses of NO and N_2O production following rewetting. The accumulation of NO_2^- in soil is probably caused by a time delay between the turnover of NH_4^+ and NO_2^- 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 NH_3 concentrations and during drought stress (Shen *et al.*, 2003; Gelfand & Yakir, 2008; Placella & Firestone, 2013). Shen *et al*. (2003) reported that more NO_2^- 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 NO_2^- concentration in a forest soil after rewetting by the first winter rains, accompanied by a decrease in NH_4^+ and only a slight increase in NO_3^- concentrations.

Accumulation of NO_2^- in soil not only provides substrate for biological processes such as denitrification, nitrification and dissimilatory nitrate reduction to ammonium (DNRA) (Silver

100 *et al.*, 2001), but also plays a major role in chemodenitrification in which NO_2^- reacts with
101 humic substances or phenolic compounds to form nitroso and nitro compounds (Thorn &
102 Mikita, 2000), which can decompose to nitric oxide (NO) or be reduced by Fe(II) to N_2O
103 (Van Cleemput & Samater, 1995; Samarkin *et al.*, 2010). Another important pathway for N_2O
104 production by chemodenitrification is the direct reaction between NO_2^- and Fe(II), which has
105 been studied recently by analysing the ^{15}N site preference (SP), i.e. the intramolecular
106 distribution of ^{15}N within the linear NNO molecule. It is considered an effective tool to assign
107 the source of N_2O formation by biological (i.e. nitrification, nitrifier denitrification, bacterial
108 denitrification and fungal denitrification) and abiotic reactions (chemodenitrification and
109 NH_2OH oxidation) (Jones *et al.*, 2015; Grabb *et al.*, 2017).
110 To investigate the processes involved in pulses of N_2O emission after rewetting in more detail
111 and to assess the importance of biotic and abiotic processes in different soils, we designed a
112 series of rewetting experiments with samples from various ecosystems (upland and riparian
113 forest, grassland and arable land). We sterilized part of each soil sample with γ-irradiation and
114 analysed the ^{15}N SP of N_2O. The aims of the experiments were to identify the relevant factors
115 controlling pulses of N_2O emissions caused by rewetting soil, and to quantify the
116 contributions of abiotic and biotic reactions to the pulse. We hypothesized that (i) more N_2O
117 will be produced on rewetting from soil samples with larger NO_2^- accumulation and (ii)
118 abiotic reactions play an important role in N_2O produced on rewetting.

119 **Materials and methods**

120 *Soil collection*
121 Fresh samples of soil were taken from three field sites of the Eifel/Lower Rhine Valley
122 Observatory of the network of Terrestrial Environmental Observatories (TERENO)
123 (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 N_2O production was large because of the topographic conditions, vegetation and the tributary flowing through the sampling area. 'Hotspots' of soil N_2O 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 (F_{Of}), 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 4ºC 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; 25ºC; data not shown). To prevent rapid recovery of microorganisms after γ-irradiation, soil samples were incubated for up to 7 hours only after rewetting.

174 *Rewetting experiments.* Rewetting experiments were done with both non-irradiated and γ-
175 irradiated air-dried soil. The experiments with γ-irradiated samples were done on a clean
176 bench with all solutions filtered through 0.2-µm filters. We placed 1.4 g of air-dried soil (0.7
177 g for F_{Of}) into 22-ml gas chromatography (GC) vials (VWR international, Darmstadt,
178 Germany), followed by the addition of either H_2O, or NO_2^-, NO_3^- or NH_4^+ solutions to reach
179 around 40% water-holding capacity (WHC), and 1 µg N g^{-1} dry soil (for NO_2^-) and 100 µg N
180 g^{-1} dry soil (for NH_4^+ and NO_3^-). The vials were closed with butyl septa and aluminum crimp
181 caps (VWR International) immediately after the addition of water or solution. Half of the vials
182 were incubated at room temperature for 1 hour and the others were incubated for7 hours. Each
183 treatment was carried out in triplicate. The gas sample in the headspace of the sample vials
184 was analysed with a gas chromatograph (Clarus 580, PerkinElmer, Rodgau, Germany)
185 equipped with an electron capture detector (ECD) and flame ionization detector (FID) for
186 N_2O and CO_2, respectively (Liu *et al.*, 2014). The instrument was calibrated using five
187 different standard gases with 0.25, 0.50, 0.75, 1.00 and 5.00 µl l^{-1} N_2O balanced with N_2 (99.5%
188 purity, Linde, Munich, Germany).
189
190 *Analysis of ^{15}N site preference of N_2O.* To determine N_2O SP values, 1.4–2.8 g of soil were
191 weighed into 120-ml headspace bottles, and only water was added to reach about 40% WHC.
192 The bottles were closed immediately after the addition of water and transferred to an
193 autosampler that was programmed so that sample bottles were incubated for 0.5–6.5 hours
194 before analysis. The autosampler was coupled to a pre-concentration unit (TraceGas,
195 Elementar Analysensysteme, Langenselbold, Germany) for real-time separation and
196 purification of N_2O, which in turn was connected to an isotope ratio mass spectrometer
197 (IRMS, IsoPrime 100, Elementar Analysensysteme, Langenselbold, Germany). Molecular
198 ions (N_2O^+) and fragment ions (NO^+) were monitored simultaneously with the IRMS at
199 isotope ratios, *m/z* (mass-to-charge ratio), of 44, 45, 46, and 30, 31, respectively. The sample

values of $\delta^{15}N^{bulk}$ ($\delta^{15}N$ 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)

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

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

Site preference is defined as

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

where $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ are the $\delta^{15}N$ at the central and terminal position of the N_2O 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:

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

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: $\delta^{15}N^{\alpha}$ 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 $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ 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_2O were added to the 120-ml bottles and isotope signatures were

226 measured. Strong quadratic relations were observed between N_2O peak height and $\delta^{45}N_2O$,
227 $\delta^{46}N_2O$ and $\delta^{31}NO$ relative to the reference gas, with polynomial equations of

228 $$y = ax^2 + bx + c, \quad (4)$$

229 where y is N_2O peak height (2.7 to 72 nanoampere, nA), x is $\delta^{45}N_2O$ relative to reference (a =
230 0.0032, b = –0.1689, c = 0.5516), $\delta^{46}N_2O$ relative to reference (a = 0.0054, b = –0.2643, c =
231 39.3) and $\delta^{31}NO$ relative to reference (a = 0.0014, b = 0.4489, c = –0.6767).
232 Therefore, all $\delta^{15}N^{bulk}$, $\delta^{18}O$ and SP values in this study were calculated according to the
233 corrected $\delta^{45}N_2O$, $\delta^{46}N_2O$ and $\delta^{31}NO$ relative to the reference gas values by the polynomial
234 equations. For the peak area correction and calibration, we did no technical replication
235 because the standard deviation for the isotope analysis was very small, i.e. 0.2, 0.4, 0.3, 0.4,
236 0.7 and 0.6‰ for $\delta^{15}N^{bulk}$ relative to air-N_2, $\delta^{18}O$ relative to VSMOW, $\delta^{31}N$ relative to the
237 reference gas, $\delta^{15}N^{\alpha}$ relative to air-N_2, $\delta^{15}N^{\beta}$ relative to air-N_2 and SP for a long measurement
238 period, respectively.
239

240 *Soil chemical analyses*
241 Total carbon (C) and N contents were determined with an elemental analyser (vario EL Cube,
242 Elementar Analysensysteme GmbH, Langenselbold, Germany). The element composition of
243 the soil samples was analysed by inductively coupled plasma optical emission spectrometry
244 (ICP-OES). Briefly, 100 mg of sample material were mixed with 3 ml HNO_3 and 2 ml H_2O_2,
245 and heated in a microwave oven at 800 W for 30 minutes. The mixtures were subsequently
246 filled to 14 ml and diluted 10-fold with deionized water followed by the ICP-OES
247 measurement.
248 Mineral N (NH_4^+, NO_2^- and NO_3^-) contents were analysed by ion chromatography (ICS-3000
249 for NO_2^- and NO_3^-, DX-500 for NH_4^+; both analysers were from Dionex, Sunnyvale, CA,
250 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:

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

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 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,

297 only the fermented layer (F_{Of}) and riparian soil (FR) had a smaller Fe content than the other
298 soil samples.
299
300 *Mineral N and dissolved organic matter (DOM) content before and after drying*
301 The mineral N content (including NH_4^+ and NO_3^-) of the fresh soil differed strongly between
302 the soil samples (Figure 1). Before air-drying, forest soil samples F_{Of} and F4 had the largest
303 NH_4^+ and NO_3^- contents, while samples F5 and F6 had smaller NH_4^+, but larger NO_3^- contents
304 than the other forest soil samples. Samples from the riparian zone and arable land had the
305 smallest NH_4^+ and NO_3^- contents of all soil samples, and grassland soil had intermediate
306 contents of NH_4^+ and NO_3^-. After air-drying, the NH_4^+ content decreased in all soil samples.
307 Almost no NH_4^+ was detectable in the riparian and arable soil samples after air-drying.
308 In contrast to NH_4^+, NO_3^- increased with drying for most soil samples, except for F_{Of}, F6,
309 grassland and arable land samples. Forest soil sample F_{Of} had the largest NO_3^- content,
310 followed by F4 and F6. The grassland soil had an intermediate NO_3^- content compared to the
311 forest samples, whereas the riparian and arable land samples were characterized by the
312 smallest NO_3^- content.
313 Before air-drying, NO_2^- concentrations were below the detection limit for the fresh soil
314 samples. However, small amounts of NO_2^- were detectable in several soil samples after drying
315 (Table 2). Forest soil samples F_{Of}, F3 and F6 had the largest NO_2^- content (0.3 mg kg^{-1}),
316 followed by grassland and forest soil F1 (0.2 mg kg^{-1}), whereas no NO_2^- was detectable in
317 samples F4, F2 and FR.
318 The trend in the dynamics of soil DOC and DTN after air-drying was similar to that of soil C
319 content. The largest DOC and DTN contents were in F_{Of} and the smallest was in the arable
320 soil, except for F5 with a relatively large C content but the smallest DOC content of all forest
321 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 μg N_2O-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 mount of NH_4^+-N and

346 NO_3^--N added was 100-fold larger than that of NO_2^-. For most of soil samples, the difference
347 between the effects of NO_3^- and NH_4^+ was not significant ($P < 0.05$).
348
349 *Effect of γ-irradiation on soil N_2O and CO_2 emissions after rewetting*
350 The effect of γ-irradiation on soil N_2O emissions with rewetting depended on soil type. In
351 general, γ-irradiation affected the emission of N_2O on rewetting significantly ($P < 0.05$). It
352 decreased N_2O emission on rewetting with water only by about 30–90% in most forest soil
353 samples compared to the non-irradiated soil samples, whereas it unexpectedly stimulated N_2O
354 emissions from grassland and arable soils three- and two-fold, respectively, after 7 hours of
355 incubation (Figure 3a, Table 3). Forest soil samples had a large variance after γ-irradiation. It
356 inhibited N_2O production from F_{Of} the most, followed by the riparian sample and F5, whereas
357 N_2O production was least from samples F1 and F2 during the incubation.
358 In the NO_2^- rewetting treatment, γ-irradiation also increased production of N_2O in grassland
359 and arable soils, but decreased it in forest soil (Figure 3b, Table 3). Samples F1 and F2 were
360 inhibited the least by γ-irradiation.
361 Compared to the effects on soil N_2O emissions, γ-irradiation decreased production of CO_2 the
362 most in grassland soil by about 50% after rewetting with water only, but had an inhibitory
363 effect of only zero to 20% in forest, arable land and riparian soil samples (Table 3). In sample
364 F1, CO_2 production was stimulated by γ-irradiation.
365
366 *Control variables of soil N_2O emission on rewetting*
367 Basic soil properties play an important role in biotic and abiotic reactions, and might have
368 contributed to the pulse of N_2O emissions after rewetting. Nitrous oxide production was
369 significantly ($P < 0.05$) and positively correlated with NO_2^- ($r = 0.85$) and NH_4^+ ($r = 0.71$)
370 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 $\delta^{15}N^{bulk}$ and $\delta^{18}O$ 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 $\delta^{18}O$ was very large with a value of 110.6 ‰ (Table 5). Both $\delta^{15}N^{bulk}$ and $\delta^{18}O$ decreased with increasing incubation time for the grassland soil, regardless of whether the soil had been γ-irradiated in advance or not. Ranges of $\delta^{15}N^{bulk}$ values for grassland and forest soils were similar, whereas $\delta^{18}O$ 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

395 during rewetting could have been overestimated in the forest samples compared to the arable
396 and grassland soils. Overall, however, our findings accorded with those of Priemé &
397 Christensen (2001) that more N_2O was emitted with rewetting of grassland soil than arable
398 and forest soils in Germany, Sweden and Finland.
399 Although knowledge about the exact mechanisms and factors that cause large amounts of N_2O
400 to form on rewetting are still limited, some basic properties such as C content, pH and
401 inorganic N content, together with soil texture and microbial composition were shown to play
402 important roles in the production of an N_2O pulse with rewetting (Ruser *et al.*, 2006;
403 Harrison-Kirk *et al.*, 2013). Our samples showed considerable variation in soil pH and C, N,
404 metal elements and inorganic N (NO_3^- and NH_4^+) contents. In general, forest samples showed
405 the largest soil C content (19.8−45.7%) and the smallest soil pH (2.85−3.92) compared to
406 riparian, grassland and arable soils. Harrison-Kirk *et al.* (2013) reported that more N_2O was
407 produced in soil samples with large soil organic C content. In our study, however, the amount
408 of N_2O from grassland (with less soil C) was larger than from most forest soils (with larger
409 soil C content). Ruser *et al.* (2006) reported that soil compaction and large NO_3^- content were
410 two important factors responsible for the rewetting-induced production of N_2O in an arable
411 soil because more anoxic sites could develop when water was added to compacted soil. In our
412 study, air-dried grassland soil had a much larger bulk density (1.09 g cm^{-1}) than forest soil
413 (0.83 g cm^{-1}) according to former research at these sites (Baatz *et al.*, 2014), which might be
414 one reason for the immediate and large N_2O emissions on rewetting for grassland soil.
415 Large soil NO_3^- content has been considered an important factor in rewetting-induced
416 production of N_2O because NO_3^- would favour the production of N_2O from denitrification
417 (Ruser *et al.*, 2006). During drying, soil NO_3^- might accumulate because of the greater
418 resistance of nitrifier activity to water limitation than denitrifiers (Szukics *et al.*, 2010). In our
419 study, we also observed an increase in soil NO_3^- content with air-drying for most of forest soil
420 samples, but not for the grassland and arable land samples (Figure 1). Moreover, there was no

421 significant correlation between the NO_3^- content of air-dried soil and N_2O production on
422 rewetting (Table 4). These results indicate that NO_3^- accumulation was not the main
423 contributor to the production of large amounts of N_2O on rewetting to around 40% WHC, as
424 in our study. We assumed that this relatively small water content might favour the production
425 of N_2O from nitrification, but the addition of NH_4^+ increased N_2O production from one forest
426 soil sample only (F6), and had no stimulatory effects on the other soil samples.
427 Soil NO_2^- accumulation has been considered as another important factor for a pulse of N_2O
428 after rewetting (Davidson, 1992; Venterea, 2007), although NO_2^- was often not detected after
429 air-drying in previous studies. In our study, we used a new method of NO_2^- extraction
430 developed by Homyak *et al.* (2015) to extract NO_2^- at a higher pH around 6, and found
431 detectable NO_2^- concentrations in the air-dried samples of F_{Of}, F3 and F6, but none was
432 detectable in the samples F4, F2 and FR.
433 Despite the small amount of accumulated NO_2^-, there was a close correlation between NO_2^- in
434 air-dried soil and amount of N_2O produced after rewetting (Table 4, Figure S1, Supporting
435 Information). Addition of NO_2^- also increased soil N_2O production largely within the first
436 hour after rewetting in all soil samples (Figure 2b). The reason for the variation in NO_2^-
437 content between different samples remains unclear, but NO_2^- content was positively correlated
438 with total N content (Table 4), but was not correlated with NO_3^- and NH_4^+ content.
439 There are mainly two sources involved in the release of soil C and N during the rewetting: (i)
440 disruption of soil aggregates by rapid water addition and (ii) the proportion of microorganisms
441 that died during drying or by dehydration or cell lysis, and the associated release of labile
442 intracellular substrates with rewetting. A previous study showed that NO_2^- produced from
443 organic N is an important NO_2^- pool in grassland soil (Müller *et al.*, 2006). Therefore, NO_2^-
444 could originate from aggregate (< 2 mm in this study) disruption or the release of labile
445 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 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 ($\delta^{15}N^{bulk}$, $\delta^{18}O$ 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 $\delta^{15}N^{bulk}$ measured in this study fell within the range of denitrification (−40 to −19‰) in pure cultures (Toyoda *et al.*, 2005), whereas $\delta^{18}O$ 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 $\delta^{15}N^{bulk}$ and $\delta^{18}O$ 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

498 (Sutka *et al.*, 2006). Snider *et al.* (2015) reported that nitrifier denitrification became a more
499 dominant source of N_2O following rain in arable soil by using the $\delta^{15}N$ of N_2O. In our study,
500 addition of NO_3^- did not increase N_2O production significantly and there was no significant
501 correlation between NO_3^- and N_2O, therefore, it was more likely that denitrification by
502 nitrifiers was the dominant contributor to the production of N_2O during rewetting. We
503 observed a similar SP for sterile and nonsterile soil samples, but previous studies showed that
504 SP values of N_2O production from NO_2^--mediated chemodenitrification varied widely from
505 −45 to 26.5‰ from chemical reactions or soil samples (Samarkin *et al.*, 2010; Jones *et al.*,
506 2015). Therefore, it is likely that abiotic reactions contributed substantially to soil N_2O
507 production after soil rewetting.

508 **Conclusions**

509 Grassland soil had the largest N_2O emissions after rewetting, whereas arable and riparian soils
510 were characterized by much smaller N_2O emissions. Among the different soil properties, soil
511 NO_2^- content was the most relevant factor correlated with soil N_2O production. Addition of
512 NO_2^- increased N_2O emissions the most, compared to NH_4^+ and NO_3^-. Our results
513 demonstrated that, although biological reactions played an important role in N_2O production
514 in the different soils, the role of abiotic processes in N_2O formation during the rewetting event
515 must also be considered. Further research is required to reveal the conditions under which
516 biotic or abiotic processes contribute most to the formation of N_2O.
517

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638

639

640 **Tables**

641 **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

642

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

645

646 **Table 2** Soil NO_2^--N (mg kg^{-1} dry soil), dissolved organic carbon (DOC, mg kg^{-1} dry soil),
647 dissolved total nitrogen (DTN, mg kg^{-1} dry soil) and A_{254} (cm^{-1} g^{-1} dry soil) after air-drying
648 for forest, grassland and arable soil samples.

649

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

650

651 The standard deviation of the NO_2^- assay is about 20% of the values (n.d., not detectable). There was only one
652 extraction to determine soil DOC, DTN and A_{254}.

653

654 **Table 3** The inhibitory effect (%) of γ-irradiation on soil N_2O and CO_2 emissions after 7

655 hours of incubation after rewetting of air-dried soil.

Additions/%	F_{Of}	F1	F2	F3	F5	FR	G	A
H_2O addition								
N_2O	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_2O	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.

658 **Table 4** Pearson's correlation coefficients between soil N_2O emission after 7 hours of
659 incubation after water rewetting and basic soil properties of air-dried soil samples (excluding
660 Ca, Mg and K) across all soil samples (n = 10).

	N_2O	Fe	Mn	C	N	C/N	pH	NO_2^-	$lnNH_4^+$	NO_3^-	DOC	DTN	A_{254}
N_2O	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

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

662

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	$\delta^{15}N^{bulk}$	$\delta^{18}O$	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

666

667 **Figure captions**

668

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

672

673 **Figure 2** Rewetting effects by the addition of (a) water, (b) aqueous solutions of NO_2^-, (c)
674 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,
675 F5, F6 and FR), grassland (G) and arable (A) soil samples for different incubation times (1
676 hour and 7 hours) before γ-irradiation. The values are presented as mean ± standard error (SE).

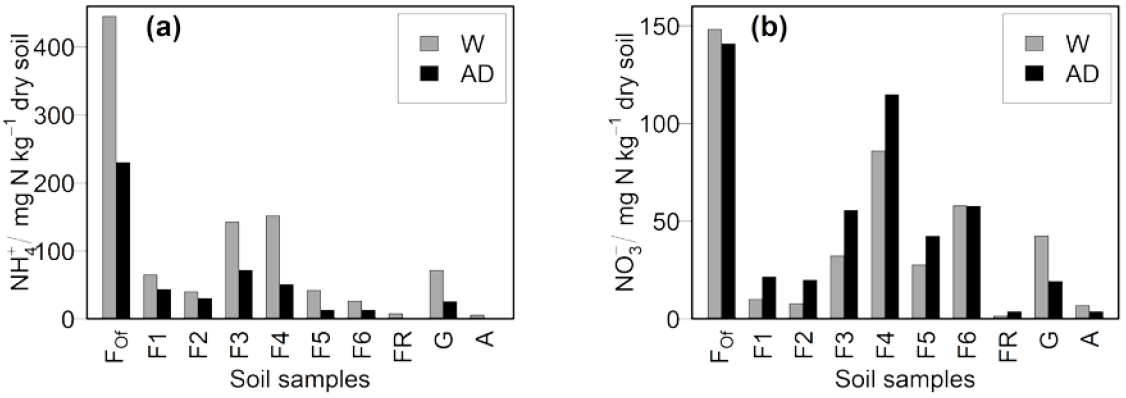
677

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

683

684 Figures

685



686

687 Figure1

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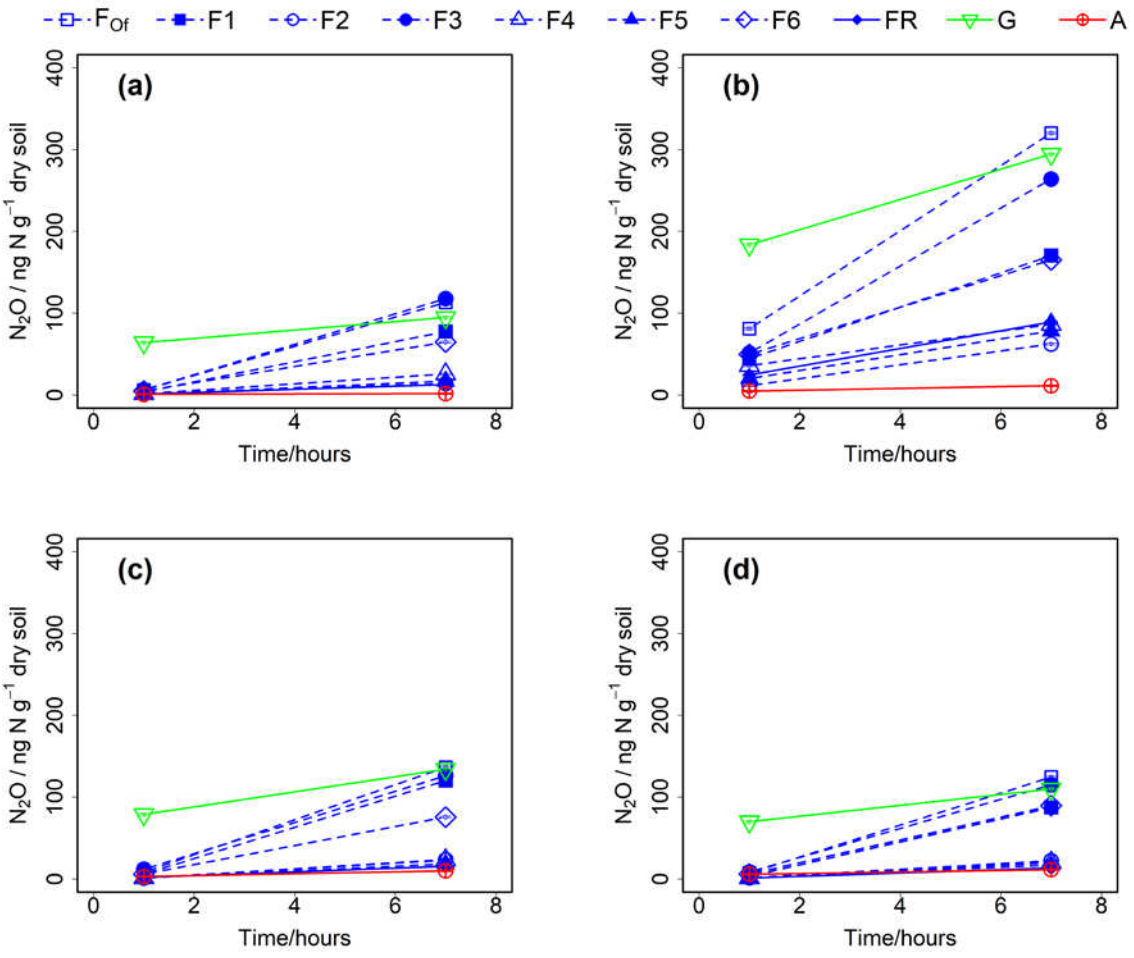

691

692 Figure 2

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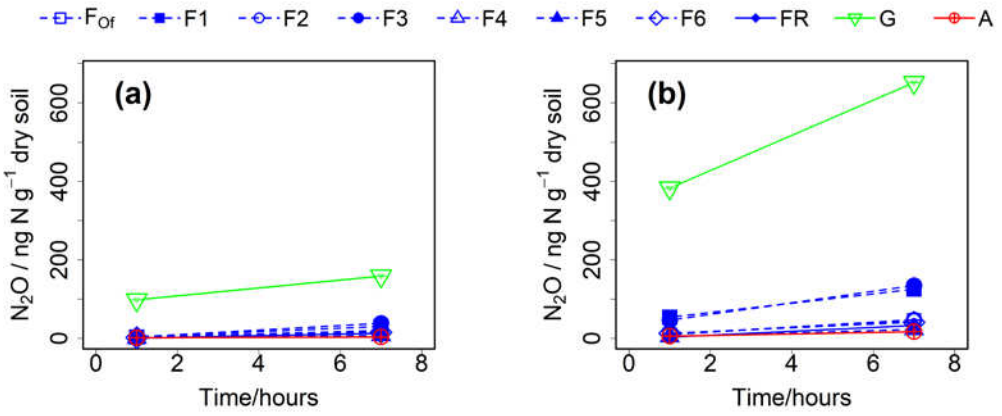

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697 Figure 3