frontiers in Environmental Science

ORIGINAL RESEARCH
published: 17 April 2019
doi: 10.3389/fenvs.2019.00047

Hydroxylamine Contributes More to Abiotic N_2O Production in Soils Than Nitrite

Shurong Liu[1†], Michael Schloter[2], Ronggui Hu[3], Harry Vereecken[1] and Nicolas Brüggemann[1]*

[1] Institute of Bio- and Geosciences–Agrosphere (IBG-3), Forschungszentrum Jülich GmbH, Jülich, Germany, [2] Research Unit for Comparative Microbiome Analysis, Helmholtz Zentrum München, Neuherberg, Germany, [3] Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River), Ministry of Agriculture, College of Resources and Environment, Huazhong Agricultural University, Wuhan, China

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***Correspondence:**
Shurong Liu
shurongliu.edu@gmail.com

†Present Address:
Shurong Liu, Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Centre for Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria

Specialty section:
This article was submitted to Biogeochemical Dynamics, a section of the journal Frontiers in Environmental Science

Received: *18 January 2019*
Accepted: *27 March 2019*
Published: *17 April 2019*

Citation:
Liu S, Schloter M, Hu R, Vereecken H and Brüggemann N (2019) Hydroxylamine Contributes More to Abiotic N_2O Production in Soils Than Nitrite. Front. Environ. Sci. 7:47. doi: 10.3389/fenvs.2019.00047

Nitrite (NO_2^-) and hydroxylamine (NH_2OH) are important intermediates of the nitrogen (N) cycle in soils. They play a crucial role in the loss of nitrous oxide (N_2O) and nitric oxide (NO) from soil due to their high reactivity. In this study, we collected soil samples from three ecosystems (grassland, arable land, and forest with a riparian zone) and explored the contribution of NO_2^- and NH_2OH to N_2O formation in the different soils after exposure to oxic or anoxic pre-treatment. In addition, the importance of abiotic processes on the N_2O formation from the two intermediates was studied by irradiating the soil samples with γ-irradiation. Our results demonstrate that NO_2^- addition induced the largest N_2O production in the grassland soil, followed by the forest and arable soils. Only 9–39% of the produced N_2O after NO_2^- addition came from abiotic processes. NH_2OH addition increased N_2O emissions the most from the arable soil, followed by the grassland and forest soils. The conversion of NH_2OH to N_2O was mostly (73–93%) abiotic. Anoxic pre-treatment decreased N_2O production from NH_2OH remarkably, especially for the grassland soil, while it increased N_2O production from NO_2^- for most of the soils. Correlation analysis showed that NO_2^- effects on N_2O production were strongly correlated to NH_4^+ content in soils with anoxic pre-treatment, while NH_2OH effects on N_2O production were strongly correlated to soil Mn and C content in soils with oxic pre-treatment. Our results indicate that NH_2OH plays an important role for abiotic N_2O formation in soils with low C and high Mn content, while the effect of NO_2^- was important mainly during biotic N_2O production. Anoxic periods prior to N addition may increase the contribution of NO_2^-, but reduce the contribution of NH_2OH, to soil N_2O formation.

Keywords: nitrification intermediate, reactive N, abiotic process, chemodenitrification, anoxic, γ-irradiation

INTRODUCTION

Nitrous oxide (N_2O) is an important greenhouse gas that also contributes to the depletion of the ozone layer. Soils are the most important source of N_2O, but the exact mechanisms responsible for N_2O production in soils are not fully clarified yet. In general, nitrification, and denitrification are the two main source processes of N_2O (Hu et al., 2015). These two pathways not only utilize enzymes that catalyze N_2O production, but also provide substrates, e.g., hydroxylamine (NH_2OH),

nitrite (NO_2^-), and nitric oxide (NO), which can be released to the environment and form N_2O chemically, i.e., so-called coupled biotic-abiotic N_2O production (Liu et al., 2017a). Therefore, studying the biotic pathways and abiotic processes of N_2O production based on NH_2OH and NO_2^- are necessary for the understanding of N_2O production mechanisms.

NO_2^- and NH_2OH are important nitrification intermediates responsible for soil N_2O production. Both are very reactive with relatively high self-decomposition rates dependent on soil pH, metals and organic matter. In oxic soils without fertilizer application and drought, NO_2^- is rarely accumulated due to the faster oxidation of NO_2^- to nitrate (NO_3^-) than oxidation of ammonia (NH_3) to NO_2^- during nitrification (Robertson and Groffman, 2007). However, high NO_2^- concentrations can be found after fertilizer application and drought (Ma et al., 2015; Homyak et al., 2016; Liu et al., 2018). The other reactive nitrification intermediate, NH_2OH, is even more reactive and unstable in its natural environment (Butler and Gordon, 1986). At neutral or slightly alkaline pH, about 30% of NH_2OH degrades within 3 h at room temperature in seawater samples at micromolar concentrations (Butler and Gordon, 1986). Nevertheless, NH_2OH has been detected in cultures of ammonia oxidizers (Liu et al., 2017a) and heterotrophic nitrifiers (Daum et al., 1998), and acid forest soils (Liu et al., 2014).

For a long time, NO_2^- had been considered as a common compound for N_2O production either via biological or abiotic processes. NO_2^- can be reduced biologically to N_2O either by NO_2^- reductase through either "classical" denitrification or a pathway called "nitrifier denitrification" (Wrage et al., 2001), as well as biologically or chemically by Fe^{2+} with the help of iron oxidizers and other microorganisms (Kampschreur et al., 2011). Moreover, soil organic matter (SOM) can also react chemically with NO_2^- to form N_2O (Stevenson and Swaby, 1964). However, the contribution of NH_2OH to N_2O formation has been neglected until recently, although it was shown that N_2O can be formed both biologically by the enzyme NH_2OH oxidoreductase (Ritchie and Nicholas, 1972) and chemically by O_2 and several other soil oxidants (e.g., MnO_2 and Fe^{3+}) (Bremner, 1997; Heil et al., 2016).

Different soil preconditions may have a strong impact on biotic and abiotic N_2O formation from NO_2^- and NH_2OH in soil. For example, quality and quantity of dissolved organic matter (DOM) may have strong effects on N_2O formation from NH_2OH and NO_2^-. Soils rich in DOM, especially in phenolic lignin derivatives, may favor N_2O formation from NO_2^- (Stevenson and Swaby, 1964; Wrage et al., 2001; Wei et al., 2017), but may decrease N_2O formation from NH_2OH, as NH_2OH binds readily to carbonyl groups of organic matter to form oximes (Thorn et al., 1992; Liu et al., 2017b). Moreover, the content and oxidation state of transition metals may also affect the formation of N_2O from NO_2^- and NH_2OH. In soil samples with high Fe and Mn content, the oxidized form will promote the conversion of NH_2OH to N_2O, whereas under reduced conditions the formation of N_2O from NO_2^- will be favored (Heil et al., 2016).

Therefore, in this paper, we compared the contribution of NO_2^- and NH_2OH to N_2O formation in the same soils. We collected soil samples from forest, grassland, and arable land with large ranges of C and Mn contents as well as pH. Oxic or anoxic pre-incubations were carried out. The effect of sterilization with γ-irradiation was scrutinized to quantify the relevance of abiotic processes. We hypothesized that (1) NH_2OH plays a more important role in N_2O production in soils with higher Mn and lower SOM content, whereas NO_2^- contributes more to N_2O formation in soils with higher SOM and Fe content; (2) anoxic pre-incubation increases the contribution of NO_2^- to soil N_2O formation, but decreases the contribution of NH_2OH to soil N_2O formation; (3) the contribution of NH_2OH to N_2O formation is mainly from abiotic processes, while there is a mixed contribution of biotic pathways and abiotic processes to N_2O formation from NO_2^-.

MATERIALS AND METHODS

Soil Collection

Soils were collected from three field sites of the Terrestrial Environmental Observatory (TERENO) (www.tereno.net) from the Eifel/Lower Rhine Valley, Germany, including a coniferous forest (Wüstebach; 50° 30′ 10″ N, 6° 19′ 50″ E), an extensive grassland (Rollesbroich; 50° 37′ 18″ N, 6° 18′ 15″ E) and an arable land (Selhausen; 50° 52′ 10″ N, 6° 27′ 4″ E). The coniferous forest site is located in the low mountain ranges of the Eifel National Park, 630 m above mean sea level, with a tributary of the river Rur basin flowing through it. The site is dominated by Norway spruce (*Picea abies* (L.) H. Karst). The hillslopes are characterized by Cambisol and Planosol, whereas the riparian zone is dominated by Gleysol and Histosol. The texture of soil in this forest is silty clay loam. The mean annual rainfall is about 1,400 mm, and the mean annual temperature is around 7°C. The vast majority of the precipitation occurred in the form of rain. The grassland site is located in the Northern Eifel region with smooth meadow grassland. Dominant soils at this site are (gleyic) Cambisol, Stagnosol, and Cambisol-Stagnosol with a silt loam texture. The climate is temperate maritime with a mean annual temperature and rainfall at the grassland site of 7.7°C and 1,033 mm, respectively, for the period from 1981 to 2000. The agricultural site is dominated also by (gleyic) Cambisol and (gleyic) Luvisol with a silt loam texture, and regularly cultivated with sugar beet, wheat, and oilseed rape, depending on the year. Mean annual temperature and rainfall at the cropland site are 9.8°C and 690 mm, respectively.

For grassland and arable land, mixed soil samples from five points for each site (∼1.5 kg each) were collected from an area of 0.5 hectare from the top 15 cm in January 2016. Due to the strong spatial heterogeneity of soil properties at the forest site (Liu et al., 2016), six soil samples (∼3 kg) were collected in January 2016 from the humus-rich layer (Oa horizon, depth 3–5 cm) of five sampling points (F1, F2, F3, F4, and F5) of the forest upland area and in addition from one sampling point of the forest riparian zone (FR) within an area of ∼27 hectare of the forested Wüstebach catchment. The riparian zone represented only a small part of the forest, therefore we did not consider spatial heterogeneity of the riparian zone in contrast to the whole forest, and sampled only one point in the riparian zone area. Soil samples were transferred to the laboratory at the day of sampling.

In the laboratory, fresh samples (except for the riparian zone sample) were passed through a 2-mm sieve, and coarse plant residues (including roots) and stones were manually removed.

Oxic and Anoxic Pre-treatment of Soil

For the anoxic pre-treatment, about 600 g fresh soil from each sampling site, stored at 4°C for 3 days after sampling in open plastic bags, was put into a 1 liter glass bottle and sealed with a rubber plug within a plastic lid. The gravimetric water content (*w/w*, water/dry soil) of the fresh soils was around 59–108% for the forest, 22% for the grassland, and 10% for arable land. The bottles were then evacuated for 10 min and refilled with helium to 0.4 bar overpressure. This procedure was repeated three times. Then the bottles were incubated with helium as headspace gas at ambient pressure at room temperature for 1 week. For the oxic pre-treatment, another ∼600 g fresh soil was put in large open plastic bags and kept under oxic conditions at room temperature for 1 week. All plastic bags were stored in a large plastic box to reduce air flow and further reduce soil water evaporation. All soil samples were freeze-dried immediately after the oxic/anoxic pre-incubations to preserve the chemical status of the soil samples until further treatment. One side effect of freeze-drying could have been that this process led to a disruption of soil aggregates, which would have made more of the substrates soluble when the solution was added, and would have led to an overestimation of the N_2O production from NO_2^- (via both biotic and abiotic pathways), but an underestimation of the N_2O production from NH_2OH (via abiotic processes). After freeze-drying, half of each oxic and anoxic pre-incubated soil was transferred to 50-ml falcon tubes and sterilized with γ-irradiation (Best Theratronics, Canada) for 14 h (total dose: 11 kGy), and the other half of each soil was kept in ziplock bags at room temperature (21 ± 1°C). The success of the sterilization process was checked by plating soil slurries after the sterilization on R2A medium and incubated for 24 h at 25°C. No growth of bacteria or fungi was observed (data not shown).

Addition of NH_2OH and NO_2^- to Freeze-Dried Soils

About 1.4 g of freeze-dried soil with and without γ-irradiation were weighed into 22-ml gas chromatography (GC) vials (VWR International, Darmstadt, Germany), followed by the addition of either H_2O, or NO_2^- and NH_2OH solutions to reach around 40% water-holding capacity (WHC) and 1 μg N g^{-1} dry soil (for NO_2^- and NH_2OH). The water was added to reactivate microbial activity (Morillas et al., 2015). The NO_2^- concentration in the freeze-dried soil was around 0–0.3 mg kg^{-1} dry soil. The added N amount corresponded to NO_2^- content in soil with fertilizer application (Shen et al., 2003; Venterea et al., 2003), and was assumed also realistic for NH_2OH in soils with fertilizer application, as concentrations of 0.3–34.8 μg N kg^{-1} dry soil had been observed at this forest site (Liu et al., 2014). Solid MnO_2 (Merck, Darmstadt, Germany) was added to the soil with NH_2OH addition to explore the effect of MnO_2 on NH_2OH-to-N_2O conversion in soil with both oxic and anoxic pre-treatment. The added Mn amount amounted to 0.1% (*w/w*) of soil dry weight, while the natural Mn content of the soil samples of this study ranged between 0.015 and 0.194% (*w/w)* (**Table 1**). Each treatment was carried out in triplicate. In total, there were 96 vials used for each soil. All vials were closed gas-tight immediately after addition of the N solution and MnO_2 with butyl septa and aluminum crimp caps (VWR International). Half (48) of the vials were incubated aerobically at room temperature (21 ± 1°C) for 1 h, and the other half (48) were incubated aerobically for 7 h.

N_2O Analysis

The gas in the headspace of the sample vials was analyzed for N_2O using a gas chromatograph (Clarus 580, PerkinElmer, Rodgau, Germany) equipped with an electron capture detector (ECD)

TABLE 1 | Summary of soil properties in this study.

	C (%)	N (%)	C/N	Fe (%)	Mn (%)	pH		DOC (mg kg^{-1} dry soil)		DTN (mg kg^{-1} dry soil)		A_{254} (cm^{-1} g^{-1} dry soil)		NH_4^+ (mg kg^{-1} dry soil)		NO_3^- (mg kg^{-1} dry soil)	
						Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
F1	27.4[§]	1.4	19.3	1.62	0.015	2.88	2.92	2865	3650	155	207	1.42	2.00	15.2	49	14.4	n.d.
F2	26.8	1.5	18.0	2.02	0.027	3.13	3.12	2215	3090	145	168	1.24	1.61	26.4	60.8	26.8	n.d.
F3	21.0	1.1	20.0	2.44	0.026	3.26	3.23	3175	3720	253	220	1.16	1.68	39.6	71.4	64.8	n.d.
F4	25.7	1.3	19.2	1.92	0.018	2.99	3.05	2390	3350	126	158	1.24	1.64	4.8	39.2	12.8	n.d.
F5	23.7	1.1	21.2	2.81	0.194	3.67	3.71	1510	1590	135	121	0.61	0.80	12.6	77.8	34.6	3.2
FR	9.7	0.5	18.1	1.57	0.024	4.14	4.13	–[‡]	930	–	86	–	0.52	7.6	n.d.[†]	n.d.	n.d.
Grassland	5.3	0.5	9.9	2.39	0.097	5.45	5.82	720	1023	133	126	0.41	0.62	32.0	195.0	39.0	n.d.
Arable land	1.3	0.1	9.2	2.10	0.074	5.87	6.19	226	236	24	19	0.29	0.37	4.4	9.0	6.2	n.d.

For the determination of total C, N, Fe, and Mn content, soils with oxic pre-incubation were used.

[§]*Values in this table are presented as mean of three replicates. The coefficient of variation of all data was smaller than 10% and is therefore not shown. For the determination of pH, DOC, DTN, A_{254}, NH_4^+, and NO_3^-, soils with both oxic and anoxic pre-incubation were used, and only one extraction was carried out.*

[‡]*-, value is missing due to shortage of material.*

[†]*n.d., not detectable.*

and flame ionization detector (FID) for N_2O and CO_2 detection, respectively. The N_2O emission rate was calculated according to the following equation:

$$E = \frac{2 \cdot C' \cdot V \cdot M}{W_{ds} \cdot V_m} \quad (1)$$

where E is the N_2O emission (ng N g^{-1} dry soil); the factor two is used as a constant to the ratio of N_2O-N to N_2O; 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}); W_{ds} is the mass of the dry soil (g). The instrument was calibrated each day using five different standard gases with 0.25, 0.50, 0.75, 1.00, and 5.00 μL L^{-1} N_2O, balanced with N_2 (99.999% purity, Linde, Munich, Germany).

Soil Chemical Analyses

Chemical properties of the soils with oxic and anoxic pre-treatment were analyzed before the incubation. The elemental composition of the organic materials was analyzed by using 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_2O_2, heated in the microwave at 800 W for 30 min. The mixtures were subsequently filled up to 14 ml and diluted 10-fold with deionized water followed by the ICP-OES measurement. Total C and N contents were determined with an elemental analyzer (vario EL Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany).

In addition, mineral N and the quality and quantity of soil DOM were analyzed to determine the effects of anoxic pre-treatment on the DOM dynamics. The mineral N (NH_4^+ and NO_3^-) contents were analyzed with ion chromatography (ICS-3000 for NO_3^-, DX-500 for NH_4^+; Dionex, Sunnyvale, CA, USA). NH_4^+ and NO_3^- were extracted with 1 M KCl (dry soil: solution = 1:10 *w/w*) and shaken for 24 h. Soil pH was measured by shaking soil with 1 M KCl (dry soil: solution = 1:10 w/w). NO_2^- was extracted according to the method of Homyak et al. (2015) and measured by ion chromatography. NH_2OH was extracted and measured according to the method of Liu et al. (2014). Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were extracted with deionized water (dry soil: water = 1:2.5 for grassland and arable land soils, and 1:5 for forest and riparian soils) by shaking for 1 h at 200 rpm. DOC and DTN were then analyzed with a TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). In addition, for characterization of aromatic substances the absorbance of the DOC extract at 254 nm (A_{254}) was determined with UV-VIS spectrometry (DU 800, Beckman Coulter, Inc., United States) and a path length of 1 cm.

Data Analyses

The effects of NO_2^- and NH_2OH on N_2O emission were calculated by subtracting N_2O emission after water addition only (as control) from the N_2O emission in response to NO_2^- and NH_2OH addition. Contribution of abiotic processes to formation of N_2O from NH_2OH and NO_2^- was calculated as follows:

$$R = \frac{E_1' - E_0'}{E_1 - E_0} \cdot 100 \quad (2)$$

where R means the contribution (%) of abiotic conversion of NH_2OH to N_2O and of NO_2^- to N_2O; E_1' (ng g^{-1}) represents the N_2O production after NH_2OH or NO_2- addition to soil after γ-irradiation; E_0' (ng g^{-1}) represents the N_2O production after H_2O addition to soil after γ-irradiation; E_1 (ng g^{-1}) represents the N_2O production after NH_2OH or NO_2- addition to soil without γ-irradiation; E_0 (ng g^{-1}) represents the N_2O production after H_2O addition to soil without γ-irradiation.

Analysis of variance (ANOVA) was used to test the main factors, i.e., soil type, oxic, and anoxic pre-treatment, N addition and γ-irradiation, and their interactions for significance ($P < 0.05$) of their effect on N_2O production using the R software package (version 3.4.3). Box-Cox transformation of N_2O data was performed before the ANOVA test. Fisher's Least Significant ($P < 0.05$) Difference test was used to test means of the effects for significant ($P < 0.05$) differences. Spearman's rank correlation analysis was performed between variables C, N, C/N, Fe, Mn, pH, DOC, DTN, A_{254}, NH_4^+ and NO_3^- for the oxic and anoxic pre-treatment using Origin Pro V. 2015.

RESULTS

Effect of NH_2OH and NO_2^- Addition on N_2O Production in Different Oxic Pre-treated Soils

NO_2^- addition increased N_2O production in the grassland soil significantly ($P < 0.05$), whereas it had only minor effect in the other soil samples (**Figure 1A**). In the grassland soil with oxic pre-treatment, 30.4% of the NO_2^- had been converted to soil N_2O within 7 h, assuming that all the N_2O came from the added NO_2^-. Even with water addition only, grassland soil which was pretreated under oxic conditions showed a strong rewetting effect, with a large N_2O production of 512.1 μg N kg^1 dry soil after 7 h (**Table S1**, supplementary information). For the forest soils, the N_2O formation after NO_2^- addition amounted to about 40 μg N kg^{-1} dry soil after 7 h, which was 13% of the grassland soil N_2O production. In general, the N_2O production after NO_2^- addition from the riparian and arable soils after oxic pre-treatment was significantly ($P < 0.05$) lower than from the other soil samples.

In contrast, NH_2OH addition induced the highest N_2O production in the arable soil, followed by the grassland soil and the forest soil from sampling point F5 for soil samples with oxic pre-treatment. The conversion of NH_2OH to N_2O ranged from 12 to 47% for the arable, grassland and F5 forest soils within 7 h, assuming that all the N_2O came from the added NH_2OH. NH_2OH addition had only a minor effect on the other forest soil samples during the whole incubation period (**Figure 1B**). Compared to the effect of NO_2^-, NH_2OH had a larger effect on N_2O production in the arable soil and in forest soil F5. Moreover, N_2O was produced very quickly in the first hour after NH_2OH addition.

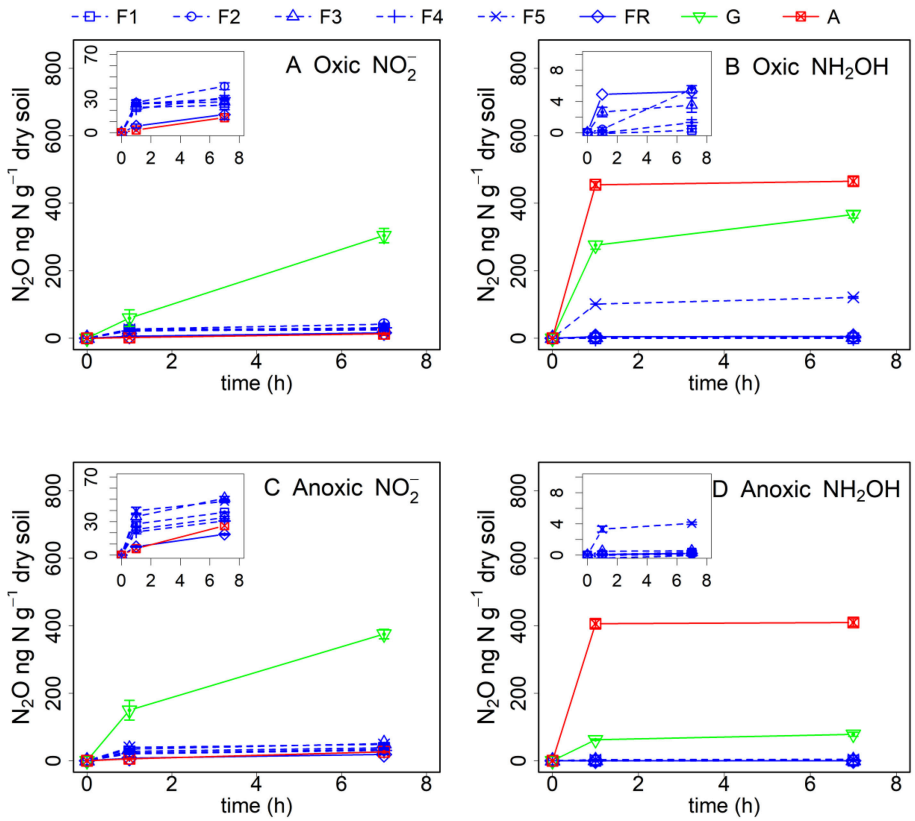

FIGURE 1 | Net N_2O (ng N g^{-1} dry soil) production in forest (F1, F2, F3, F4, F5, and FR), grassland (G) and arable land (A) soils without γ-irradiation after NO_2^- (**A**: oxic, **C**: anoxic pre-incubation) and NH_2OH (**B**: oxic, **D**: anoxic pre-incubation) addition. Net N_2O production was calculated by subtracting mean N_2O emission values after addition of pure water (as control) from the N_2O emission values after addition of NO_2^- or NH_2OH solution. The values are presented as mean ± standard deviation (SD, $n = 3$).

Effect of Anoxic Pre-incubation on N_2O Production From NH_2OH and NO_2^- Addition

Anoxic pre-incubation increased soil NH_4^+ concentration up to 7-fold, with the largest NH_4^+ concentration (195 mg N kg^{-1} dry soil) in the grassland soil (**Table 1**), and decreased NO_3^- concentration in most of the soil samples (except forest sample F5) to nearly zero. The quality (reflected in the A_{254} value) and quantity of DOM (reflected in the concentrations of DOC and DTN) varied substantially after anoxic pre-incubation between the different soil samples, with 5–42% higher DOC content compared to soil samples with oxic pre-incubation. The A_{254} value followed a trend very similar to DOC, indicating that more aromatic substances were available in dissolved form after anoxic pre-incubation. The difference in DTN between the different treatments was not as pronounced as for DOC and A_{254}.

Anoxic pre-treatment of the soil samples had a significant ($P < 0.05$) effect on soil N_2O emission after the addition of NH_2OH and NO_2^-. For the grassland and arable soil, anoxic pre-incubation resulted in a significant ($P < 0.05$) increase of N_2O production during the first hour after NO_2^- addition (**Figure 1C**, **Table 2**). For the forest soil, anoxic pre-incubation increased N_2O production from samples F1, F3, F5, and FR after NO_2^- addition compared to the oxic pre-treatment, but decreased the effect of NO_2^- on N_2O production in F2 and F4. In terms of NH_2OH, anoxic pre-treatment had a negative effect on the N_2O production after NH_2OH addition in most of soil samples, especially in those with a large NH_2OH effect after oxic pre-incubation, i.e., grassland and F5 forest soils, but had a relatively small, but significant ($P < 0.05$) effect on the N_2O production after NH_2OH addition in arable soil (**Table 2**). Anoxic pre-incubation decreased N_2O production only by 12% in arable soil, but by 79% in grassland soil after NH_2OH addition after 7 h of incubation (**Figure 1D**, **Table 2**).

Contribution of Abiotic Pathways to N_2O Production From NH_2OH and NO_2^- Addition

Abiotic pathways contributed to 9–39% of soil N_2O production within 7 h after NO_2^- addition from the different soils after oxic pre-incubation, but contributed to 73–93% of soil N_2O production within 7 h after NH_2OH addition in the arable land, grassland and the F5 soil (**Figure 2**, **Table 3**). For the soil samples with anoxic pre-incubation, abiotic pathways contributed to 14–49% of NO_2^--induced N_2O production after 7 h, but contributed to 67–99% of N_2O production only after NH_2OH addition in the grassland, arable, and F5 soil in the same time period. In general, abiotic pathways played a more important role in N_2O

TABLE 2 | Effect (%, $n = 3$) of anoxic pre-treatment on soil N_2O emissions after 1 and 7 h of incubation of soils with NO_2^- and NH_2OH additions.

	F1	F2	F3	F4	F5	FR	Grassland	Arable land
NO_2^-								
1 h	23.4[§]	−14.8[‡]	34.2	−1.9	51.5	27.2	151.3	146.7
7 h	56.5	−19.1	85.0	−2.2	61.7	15.5	23.3	96.9
NH_2OH								
1 h	–[†]	−100.3	−81.3	–	−96.7	−98.3	−77.5	−10.7
7 h	–	−98.7	−84.4	−104.5	−96.7	−95.8	−78.6	−12.0

[§]*Effect values in this table indicate the relative increase (%) in N_2O emission in anoxic vs. oxic pre-treatment.*
[†]*Relative increase could not be calculated correctly due to negligible N_2O emission after NH_2OH addition.*
[‡]*Negative values indicate a decrease.*

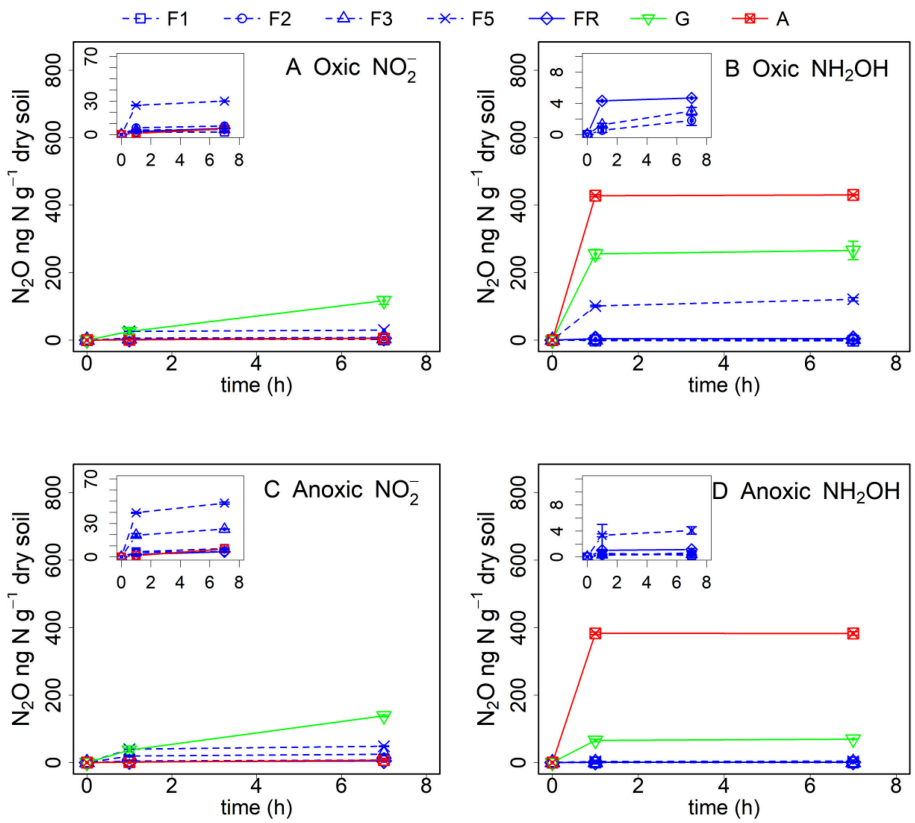

FIGURE 2 | Net N_2O (ng N g^{-1} dry soil) production in forest (F1, F2, F3, F5, and FR), grassland (G) and arable land (A) soils after γ-irradiation and NO_2^- (**A**: oxic, **C**: anoxic pre-incubation) and NH_2OH (**B**: oxic, **D**: anoxic pre-incubation) addition. Net N_2O production was calculated by subtracting mean N_2O emission values after addition of pure water (as control) from the N_2O emission values after addition of NO_2^- or NH_2OH solution. The values are presented as mean ± standard deviation (SD, $n = 3$).

production after NH_2OH addition compared to NO_2^- addition in the tested soils.

Controlling Factors of N_2O Production in Response to NH_2OH and NO_2^- Addition to Soils After Oxic and Anoxic Pre-incubation

Correlation analysis showed that soil Mn, C, N, DOC, and A_{254} content as well as pH were important variables responsible for soil N_2O formation from NH_2OH in the tested soils (**Table 4**). The NH_2OH-to-N_2O conversion was positively and significantly correlated with soil Mn content and pH, but negatively and significantly correlated with soil C, N, and DOC content, and A_{254}. Soil N_2O production after NO_2^- addition was found to be significantly ($r = 0.93$, $P < 0.05$) correlated with NH_4^+ content, and only marginally ($r = 0.69$, $P = 0.06$) correlated with soil Fe content after anoxic pre-treatment. No significant correlation was observed between the NO_2^--to-N_2O conversion and any soil properties after oxic pre-treatment.

TABLE 3 | Contribution (%, $n = 3$) of abiotic pathways to soil N_2O emissions after 7 h incubation of soils with addition of aqueous solutions of NH_2OH or NO_2^- to soil samples with oxic or anoxic pre-incubation and freeze-drying treatment.

		F1	F2	F3	F5	FR	Grassland	Arable land
Oxic	NO_2^-	9.1[§]	18.9	16.4	16.5	34.7	38.6	37.4
	NH_2OH	–[‡]	–	85.3	84.2	88.9	72.5	92.5
Anoxic	NO_2^-	19.6	14.4	48.9	18.3	25.1	36.9	27.5
	NH_2OH	84.5	–	67.2	98.7	–	88.5	93.4

[§]*The data of F4 in this table after γ-irradiation treatment is missing due to shortage of material.*

[‡]*–Contribution of abiotic processes could not be calculated correctly due to negligible N_2O emission after NH_2OH addition.*

TABLE 4 | Spearman's correlation coefficients between soil N_2O emissions and soil properties after 7 h of incubation.

	NO_2^- addition		NH_2OH addition	
	Oxic	Anoxic	Oxic	Anoxic
C	0.26	0.07	**−0.73**	−0.69($P = 0.06$)
N	0.42	0.14	**−0.65**	**−0.71**
C/N	0	0.33	−0.57	−0.23
Fe	0.31	0.69($P = 0.06$)	0.5	0.59
Mn	0.29	0.38	**0.88**	0.67
pH	−0.21	−0.07	**0.88**	**0.74**
DOC	−0.11	0.45	**−0.89**	**–**0.45
DTN	−0.04	0.52	−0.57	−0.40
A_{254}	0.05	0.40	**−0.94**	−0.47
NH_4^+	0.52	**0.93**	−0.14	0.21
NO_3^-	0.43	–[§]	0	–

Bold values indicate a significant correlation ($n = 8$, $P < 0.05$).

[§]*-value is missing due to values under detection limit.*

The addition of MnO_2 increased the NH_2OH-to-N_2O conversion in all soil samples after oxic or anoxic pre-incubation (**Figure 3**). However, addition of MnO_2 increased the NH_2OH-to-N_2O conversion more in the soil with oxic pre-incubation (especially during the first hour after NH_2OH addition) compared to the soil with anoxic pre-incubation. Only N_2O emission from soil F3 with anoxic pre-incubation was largely affected by the addition of MnO_2 (as high as 2.5 mg kg^{-1} N after 7 h), which disappeared completely after γ-irradiation (data not shown). The NH_2OH-to-N_2O conversion of the grassland soil, F5 and other forest soil samples after anoxic pre-incubation also increased after MnO_2 addition, but was still much lower than with NH_2OH addition only after oxic pre-incubation.

DISCUSSION

The Importance of NO_2^- and NH_2OH on Biotic and Abiotic N_2O Formation in Different Soils

Our results showed that the role of NO_2^- and NH_2OH in N_2O production was strongly dependent on soil properties. The by far largest amount of N_2O was produced in non-γ-irradiated grassland soil after NO_2^- addition, much higher than in all other soils (**Figures 1A,C**). About 30% of the added NO_2^- was converted to N_2O in the grassland soil with oxic pre-incubation after 7 h of incubation, assuming that all the N_2O produced came from the added NO_2^-. This large and quick N_2O pulse could easily lead to the assumption of abiotic processes, e.g., chemodenitrification, being responsible for the N_2O production upon addition of NO_2^-. However, γ-irradiation decreased the N_2O production after NO_2^- addition to soil with oxic pre-incubation by 72% (**Figure 2**, **Table 3**), indicating that biotic pathways played a more important role in N_2O production after NO_2^- addition compared to abiotic processes. It was reported that nitrifier denitrification involving biological NO_2^- reduction can play an important role in soil N_2O emissions, especially in grassland and arable soil with large nitrifier activity (Wrage et al., 2001, 2004). Thus, the large pulse of N_2O production with NO_2^- addition in the grassland soil could be due to nitrifier denitrification. In contrast, the smaller effect of NO_2^- addition on N_2O production in forest soils was at first unexpected, as there was more carbon available in the forest soils than in the grassland soil for biotic (denitrification) and abiotic (chemodenitrification) pathways leading to N_2O production. One possible reason responsible for the smaller N_2O production in the forest soils could be that more NO instead of N_2O was produced as it mostly decomposes to NO and NO_2 at low pH (Davidson, 1992; Venterea et al., 2005). This assumption is supported by the fact that in our study the forest soil was acidic with pH values lower than 3.5 for most of the samples and more NO instead of N_2O was produced in this forest site during our former research (Wei et al., 2017).

In contrast to NO_2^-, large N_2O production was observed after NH_2OH addition to the arable land, grassland, and the forest soil sample F5, and only negligible amounts of N_2O were produced in the other forest soils (**Figures 1B,D**). Abiotic reactions played a much larger role in the case of NH_2OH addition compared to NO_2^- addition. Comparison of the results from the experiments with γ-irradiated and non-irradiated soils revealed that most of the N_2O from NH_2OH was chemically produced, contributing 73–93% to the total conversion of NH_2OH to N_2O for the arable, F5 and grassland soil. We found larger Mn content in soils of the grassland, the arable land and the F5 forest sub-sample (**Table 1**), and a positive and significant correlation was observed between soil N_2O production in response to NH_2OH addition and Mn content (**Table 4**). This is in accordance with previous findings, which identified the chemical reaction between MnO_2 and NH_2OH as important factor for abiotic N_2O production in soil (Bremner, 1997; Heil et al., 2015). The contradictory observation that the forest soil with the largest Mn content (F5) had a lower N_2O production upon NH_2OH addition than the grassland and cropland soils can be explained with the inhibitory effect of SOM on the abiotic conversion of NH_2OH to N_2O, as the effect of NH_2OH on soil N_2O emissions had been found earlier to be related to SOM quantity, quality and Mn content, with larger NH_2OH-to-N_2O conversion ratios in soils with higher Mn content and lower soil organic C content or, more specifically,

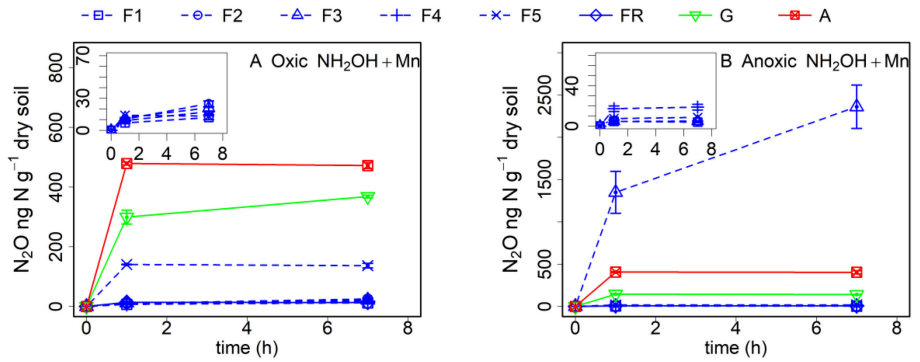

FIGURE 3 | Soil N_2O production (ng N g^{-1} dry soil) after MnO_2 and NH_2OH addition after oxic **(A)** and anoxic **(B)** pre-incubation for samples from forest (F1, F2, F3, F4, F5, and FR), grassland (G) and arable land (A) without γ-irradiation. The values are presented as mean ± standard deviation (SD, $n = 3$).

lower content of carbonyl groups to which NH_2OH could bind chemically (Liu et al., 2017b).

Effect of Soil Redox History on N_2O Formation From NO_2^- and NH_2OH

Despite their reactivity, the two N intermediates NH_2OH and NO_2^- may accumulate in soils under anoxic conditions. NH_2OH accumulation in anoxic sediment slurries has been observed in a preliminary experiment (**Figure S1**, supporting information), while transient NO_2^- accumulation as well as the absence of NO_3^- have been reported in soil slurries during anaerobic incubation (Clément et al., 2005). In the present study, the NH_4^+ concentration, especially of the grassland soil, increased largely with anoxic pre-incubation (**Table 1**). Soil organic matter quality, transition metal redox state, and pH may change remarkably at this low redox potential (Dassonville and Renault, 2002). According to thermodynamic theory, the following sequential reduction of electron acceptors is observed with decreasing redox potential: O_2, NO_3^-, MnO_2, Fe_2O_3, SO_4^{2-}, and CO_2 reduction (Froelich et al., 1979) during respiratory or other dissimilatory processes. Thus, Fe^{2+}, Mn^{2+}, and fermented organic matter would accumulate during anoxic pre-incubation. Furthermore, the transient occurrence of reactive C substances could have reversed the effects of NH_2OH and NO_2^- addition, as a transient increase in reactive C rich in carbonyl groups would preferentially react with NH_2OH and decrease N_2O production from abiotic conversion of NH_2OH, while a transient increase in reactive phenolic compounds would lead to preferential reaction with NO_2^- to produce N_2O chemically.

We hypothesized that anoxic pre-incubation would lead to higher N_2O release after NO_2^- addition and less N_2O release after NH_2OH addition due to the accumulation of more reduced substances. Our results suggest that anoxic pre-incubation increased N_2O production after NO_2^- addition in most of the soils. The stimulatory effect of anoxic pre-incubation on N_2O production could be due to the increased contribution of N_2O production via chemodenitrification, as more reduced transition metal ions, e.g., Fe^{2+}, may accumulate after anoxic pre-incubation, which could have explained the observed very fast abiotic N_2O production. However, since most of the N_2O produced after NO_2^- addition came from biotic pathways, anoxic pre-incubation may have increased denitrification activity by stimulating denitrifier growth during the anoxic phase, leading to a positive effect of anoxic pre-incubation on N_2O production via NO_2^- in soils.

Anoxic pre-incubation had an even more pronounced effect on N_2O production after NH_2OH addition, with a significant reduction of N_2O production in the forest soil F5 and the grassland soil (97 and 79%, respectively), in accordance with our hypothesis, but with only a small effect (12%) on the arable soil after 7 h. As a strong oxidant, most of the MnO_2 should have been reduced to Mn^{2+} during the anoxic pre-incubation period according to the large increase in NH_4^+ (**Table 1**), which may indicate a soil redox potential that was lower than +200 mV (Froelich et al., 1979). In those soil samples with high C content, the organic carbon can be used by microorganisms that reduce Fe^{3+} or Mn^{4+} instead of oxygen (Lovley et al., 2004). The lower effect of the anoxic pre-treatment on the conversion of NH_2OH to N_2O in the arable soil could be attributed to the lower C content in this soil, where less Mn^{4+} would be reduced to Mn^{2+}. To further explore the effect of MnO_2 on the NH_2OH-to-N_2O conversion ratio, we added 0.1% (*w/w*) MnO_2-Mn (equal to the Mn content of the grassland soil) to the soil samples with both oxic and anoxic pre-treatment. We hypothesized that this amount of MnO_2 addition would increase the NH_2OH-to-N_2O conversion ratio of the soil samples pre-incubated under anoxic conditions. However, only the grassland soil and forest soils FR and F5 showed a larger Mn effect after anoxic pre-treatment (by comparing **Figures 1D**, **3B**), but the added Mn amount could not make up the reduction in N_2O production caused by the anoxic pre-incubation, despite the large increase of N_2O production from F3 with anoxic pre-incubation (comparing **Figures 1B**, **3B**).

It was reported that large amounts of fermented substances could accumulate during anoxic incubation (Dassonville and Renault, 2002). In the present study, we found more DOC and dissolved aromatic substances (represented as A_{254}) in the soil samples with anoxic pre-incubation than with oxic pre-incubation (**Table 1**). The change of soil DOC quality and quantity could be responsible for the difference in N_2O production after NH_2OH addition to soils with different redox

history, as the increase in DOC and aromatic substances after anoxic pre-incubation would increase the likelihood of fast binding of NH_2OH to organic compounds once added to the soil, and lead to a lower availability of NH_2OH for the reaction with MnO_2 to produce N_2O. Therefore, the absence of a MnO_2 addition effect on the NH_2OH-to-N_2O conversion ratio could be due to the accumulation of fermented substances that can quickly react with NH_2OH.

CONCLUSIONS

In summary, we show that the response of soil N_2O production to the addition of the reactive intermediates NH_2OH and NO_2^- of microbial N metabolism depends on the soil precondition, i.e., oxic vs. anoxic. The addition of NO_2^- increased N_2O emissions mainly from biotic pathways, while the addition of NH_2OH increased N_2O from abiotic processes. Anoxic pre-incubation decreased N_2O emissions in the NH_2OH treatment, while it increased N_2O emissions after NO_2^- addition. Soil properties, especially the DOM, Fe, and Mn content, have strong effects on the contribution of NH_2OH and NO_2^- to N_2O formation. This study emphasizes the higher importance of NH_2OH for abiotic N_2O production compared to NO_2^- in soils with high Mn content and less C content under oxic conditions, which may give useful information for the understanding of N_2O formation mechanisms and prediction of N_2O emission in such soils.

AUTHOR CONTRIBUTIONS

NB and SL designed the experiment. SL and MS carried out the experiment and analyzed the data. NB, SL, MS, RH, and HV wrote the paper.

ACKNOWLEDGMENTS

The authors wish to thank Daniela Gesekus and Volker Nischwitz for the analysis of soil basic properties and mineral N, and Diana Hofmann, Martina Krause, and Andrea Kubica for their assistance in the TOC/TN analysis. This study was supported by the Chinese Scholarship Council (scholarship no. 201206760007). This manuscript is based on the chapter six of a PhD thesis published in the online library of University of Bonn (Liu, 2017).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2019.00047/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.