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Role of Chemodenitrification for N_2O Emissions from Nitrate Reduction in Rice Paddy Soils

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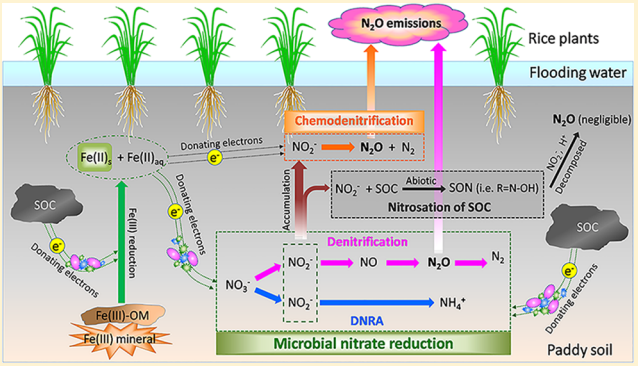
Supporting Information

ABSTRACT: Atmospheric nitrous oxide (N_2O) causes global warming and ozone depletion. Nitrate and nitrite reduction are the main sources for N_2O emission in anoxic environments including both microbial (denitrification) and abiotic reactions (chemodenitrification), besides nitrification in oxic habitats. In flooded paddy soils, substantial concentrations of Fe(II) and nitrite are available, potentially triggering chemodenitrification. It is currently unknown to what extent chemodenitrification contributes to N_2O emissions in such environments. We conducted anoxic microcosm experiments with two paddy soils that differ in natural Fe(II) and organic carbon content. We amended them with nitrite or nitrate and quantified N_2O emissions. In sterilized soils, nitrite and not nitrate was abiotically reduced, pointing toward chemodenitrification. In microbially active soils, nitrate reduction was accompanied by nitrite accumulation, ammonium production, and N_2O emission, implying the co-occurrence of denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and chemodenitrification. N_2O emissions from chemodenitrification accounted for 6.8−67.6% of the total N_2O emissions, depending on the concentrations of Fe(II), nitrite, nitrate, and organic carbon, and the N_2O emission rate from abiotic reactions was up to 2.4 mg N kg^{-1} d^{-1}. Elevated Fe(II) levels in soils facilitated nitrite accumulation, chemodenitrification, and high abiotic N_2O emission (up to 42.9%). In low organic carbon soil, more N_2O was emitted by chemodenitrification in nitrite-amended setups (20.5% of total N_2O emission) compared to nitrate-amended setups (6.8%). High organic carbon content in soils indirectly enhanced the proportion of abiotic N_2O production (up to 67.6%), potentially favoring DNRA over denitrification, which decreased the biotic contribution to N_2O formation. Our results suggest that chemodenitrification could be a significant contributor for N_2O emissions in paddy soils via a complex network of biotic and abiotic processes involving C, Fe, and N biogeochemical cycling.



KEYWORDS: *laughing gas, Fe(II) oxidation, abiotic nitrite reduction, denitrification, paddy soils*

INTRODUCTION

Nitrous oxide (N_2O) is a potent greenhouse gas and plays a key role in ozone depletion.[1,2] Agricultural soils constitute the largest source of global N_2O emissions.[3,4] Rice paddies represent classical agricultural soils with specific features.[5] In flooded paddy soils, microbial nitrate (NO_3^-) reduction (denitrification) is expected to be the main source for N_2O emissions.[6] Denitrification is a stepwise reductive process, during which N_2O gets released to the atmosphere as an intermediate product. Each reduction step is controlled by particular genes and partly by different microorganisms.[7−9] Thus, microbial reactions carry the potential to regulate N_2O emissions during denitrification.[4,10−13] Additionally, the abiotic reaction of nitrite (NO_2^-, the denitrification intermediate) with ferrous iron [Fe(II)] can also cause N_2O production, that is, via chemodenitrification.[14−17]

Most environmental research focused on N_2O emission from nitrification[18−20] and denitrification,[4,11,12,21−23] and only a few studies considered N_2O production via chemodenitrification in nature.[16,17,24] However, it has been suggested that chemodenitrification can account for 31−75% of the total N_2O production in agricultural soils,[18] which indicates the important role of chemodenitrification in soil N_2O emissions. Microbially mediated nitrate-reducing Fe(II) oxidation (NRFO) is an important process in soils, including both microbial (denitrification) and abiotic (chemodenitrification) reactions that lead to N_2 and N_2O formation. During NRFO,

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nitrate gets reduced microbially and the produced intermediate NO_2^- gets reduced to N_2O by Fe(II) via a chemical pathway.[25−32]

The chemical reaction between NO_2^- and Fe(II) proceeds very fast,[33] and the accumulation of NO_2^- during microbial nitrate reduction will favor the occurrence of chemodenitrification and concomitant abiotic N_2O emission. NO_2^- accumulation occasionally appears in soils,[14] depending on the relative rates of NO_2^- and NO_3^- reduction,[34] which was caused by the competition between NO_2^- and NO_3^- reductases for common electron donors.[35−37] In addition, mineral precipitation on outer membrane proteins resulting from Fe(II) oxidation by NO_2^- or NO_3^- could also inhibit the activity of NO_2^- reductases and lead to NO_2^- accumulation.[28,30,38] In the presence of NO_2^-, substantial amounts of Fe(II), which are produced by microbial Fe(III) reduction in anoxic paddy soils,[39] also favor chemodenitrification and abiotic N_2O emission. Therefore, chemodenitrification is likely to happen in anoxic paddy soils, which together with denitrification contributes to soil N_2O emissions.

Although it has been recognized that both chemodenitrification and denitrification produce N_2O during NRFO, less is known about the extent chemodenitrification contributes to N_2O production. Chemodenitrification and denitrification co-occur and are coupled to each other during NRFO, which makes it difficult to distinguish N_2O from abiotic and biotic sources. As an intermediate of microbial nitrate reduction, NO_2^- formation and consumption occur simultaneously.[14] It is challenging to determine the exact amount and rates of biotic and abiotic NO_2^- consumption [in the presence of Fe(II)] during microbial nitrate reduction. Moreover, Fe(II) is not only oxidized chemically by NO_2^- but also enzymatically by (autotrophic) nitrate-reducing microorganisms,[25−28,33] which interferes with the quantification of abiotic Fe(II) oxidation by NO_2^-. Furthermore, Fe(II) and organic carbon (C) as electron donors for nitrate reduction[10,27,28,40] coexist in paddy soils, which obscure N_2O emissions from heterotrophic/autotrophic denitrification and chemodenitrification.

To evaluate the contribution of abiotic and biotic pathways during NRFO, so far, only two studies have estimated the contribution of chemodenitrification to Fe(II) oxidation in bacterial cultures by modeling.[32,41] Few studies have quantified the N_2O emissions from abiotic and biotic sources during NRFO.[42,43] In the present study, we therefore attempt to unravel the contribution of abiotic and biotic reactions to N_2O emissions during nitrate reduction in two rice paddy soils. Specifically, the objectives of this study were (i) to quantify N_2O emissions from abiotic and biotic sources during nitrate reduction in two paddy soils, (ii) to evaluate the role of chemodenitrification in N_2O emissions, and (iii) to explore the effect of Fe(II) oxidation on N_2O emissions during nitrate reduction in anoxic paddy soils differing in natural Fe(II) concentrations.

MATERIALS AND METHODS

Soil Characteristics. Paddy soils were collected from Hubei province (30°1.1′ N, 114°22.1′ E, referred to as soil S1) and Hunan province (28°42.9′ N, 112°55.6′ E, soil S2) in China. The land-use practices of the two paddy fields were the double rice-winter fallow rotation pattern (early rice grown in late April to mid-July, late rice grown in mid-July to late October, and final fallow in late October to late April in the next year). The parent materials of both the paddy soils were quaternary red earth. Soil samples were taken from 0 to 20 cm depth of paddy fields in triplicates, air-dried, and sieved to <1 mm. The pH values were 5.3 and 5.2 in soils S1 and S2, respectively. The concentrations of extractable Fe and organic C in soil S2 were higher than those in soil S1 (the geochemical properties are shown in the Supporting Information, Table S1).

Experimental Design. Air-dried soils (S1 and S2) were activated at a soil moisture of 20% (w/w) at 25 °C for 3 days and stored at 4 °C in dark. One part of the activated soil was sent for gamma sterilization (Synergy Health, Allershausen, Germany; radiation 50 kGy) for abiotic control experiments, and the other was stored for biotic experiments. Before microcosm experiments, a 12 day anoxic preincubation was performed to deplete the remaining NO_3^- and NO_2^- and to generate a natural source of Fe(II) from microbial Fe(III) reduction. For the preincubation, 5 g of activated soil was amended with 50 mL of bicarbonate buffer (22 mM $NaHCO_3$, pH 7.0 ± 0.2) and stored in serum bottles for 12 days at 25 °C. Serum bottles were closed with airtight butyl stoppers, and the headspace of serum bottles was flushed with N_2/CO_2 (90/10 v/v). For abiotic control experiments, serum bottles with gamma-sterilized soils were setup and preincubated under the same conditions. During the preincubation, Fe(II) concentrations in the sterilized soils did not increase, while in the nonsterilized soils, they reached a plateau on day 12 of the preincubation (Supporting Information, Figures S1 and 3b,d). To keep the same amount of available Fe(II) in the abiotic and biotic setups of microcosm experiments, Fe(II) concentration in the abiotic setups was adjusted to the same levels as in the biotic setups that contained native nonsterilized soils. Based on the measured extractable Fe(II) concentration in the biotic setups on day 12 of the preincubation (3.2 ± 0.0 and 4.0 ± 0.1 g Fe kg^{-1} in soils S1 and S2, respectively), the same amount of Fe(II) (as $FeCl_2$ solution) was added to the sterilized setups. Before $FeCl_2$ addition, the headspace of the soil microcosm bottles was flushed with N_2/CO_2 to remove gaseous compounds generated during the anoxic preincubation. After preincubation, the microcosms were split into three treatments: (1) control without N addition, (2) KNO_3 addition, and (3) KNO_2 addition at a fertilization rate of 100 mg N kg^{-1} dry soil (corresponding to 225 kg N ha^{-1} $year^{-1}$). The aim of KNO_2 addition in this study was not to refer to a potential nitrite fertilization but to quantify the contribution of chemodenitrification during denitrification. Because NO_2^- as the intermediate of denitrification is produced and consumed simultaneously, it is difficult to determine the exact amount and rates of abiotic and biotic NO_2^- consumption during denitrification. Additionally, in natural environments, there are anoxic microniches with high NO_3^- concentration, such as soil aggregates, which could favor denitrification and accumulate high concentrations of NO_2^-. Therefore, we added nitrite to account for such processes. Each treatment of the microcosm experiments was performed in triplicate setups under anoxic conditions in dark at 25 °C for 9 days. Two parallel setups were included in the abiotic and biotic experiments: one for soil slurry analysis and the other for gas sampling. During the anoxic preincubation and microcosm experiments, all the soil microcosm setups stood in the incubator without any disturbance.

Sampling and Analysis. For N_2O analysis, 1 mL of headspace gas was taken from the bottles and replaced by 1 mL

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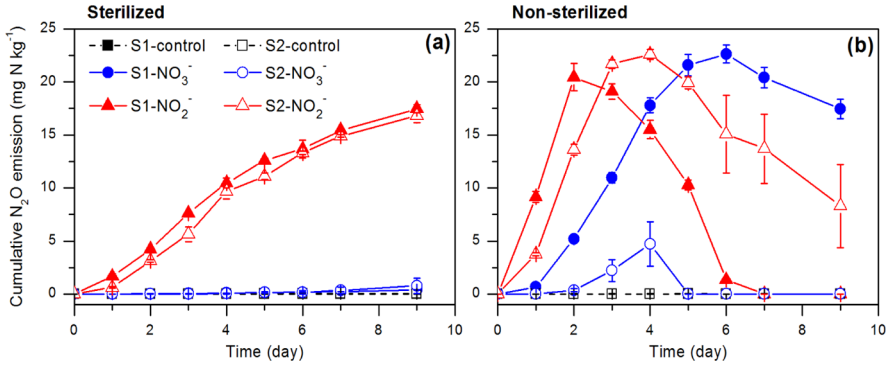

Figure 1. N_2O emissions from two paddy soils (S1 and S2) under sterilized (a) and nonsterilized (i.e., microbially active) (b) anoxic conditions amended with nitrate (S1-NO_3^- and S2-NO_3^-) or nitrite (S1-NO_2^- and S2-NO_2^-) compared to nonamended soils (S1-control and S2-control). The values represent the mean of triplicate setups; the error bars represent standard errors.

N_2/CO_2 (90/10 v/v). The sample was injected into gas vials (22.5 mL) filled with pure N_2 for N_2O analysis by an automated gas chromatography system with a ^{63}Ni electron capture detector (Hewlett Packard 5890 Series II, USA). Soil samples for Fe and inorganic N species analyses were taken at the same time point as gas sampling in an anoxic glovebox (100% N_2). Before soil sampling, each serum bottle was shaken for homogenization. An aliquot of the soil slurry (0.5 mL) was sampled for Fe and N analyses. The slurry sample (100 μL) was added to 1 mL of 40 mM sulfamic acid prepared in 1 M HCl to extract for 1 h for the quantification of extractable Fe(II).[44] The slurry (200 μL) was added to 1 mL of 2 M KCl for 1 h extraction and consecutive NO_3^-, NO_2^-, and NH_4^+ analyses. The remaining soil sample was centrifuged (13 400*g*, 5 min), and 100 μL of the supernatant was stored in 500 μL of 40 mM sulfamic acid prepared in 1 M HCl for dissolved Fe(II) quantification. Fe(II) concentrations were determined by a modified ferrozine method at 562 nm with a microplate reader (Thermo Scientific).[44] Inorganic N was analyzed by flow injection analysis, with a device that contains a dialysis membrane to eliminate the interferences of Fe and organic matter (Seal Analytical AA3, Norderstedt, Germany).

Data Calculation and Analysis. The cumulative N_2O emission was calculated as described in the Supporting Information. Assuming that the organic N pool in the soils was constant during the incubation (regardless of the instability and negligible amount of gas products such as NO and N_2O_4), the main products of nitrate reduction could be NO_2^-, N_2O, N_2, and NH_4^+. Based on the stoichiometry of electron transfer between NO_x^- (NO_3^- and NO_2^-) reduction and Fe(II) oxidation, the equations of the half reactions are as follows

$$Fe(II) - e^- \rightarrow Fe(III) \quad (1)$$

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \quad (2)$$

$$NO_2^- + 2e^- + 3H^+ \rightarrow \frac{1}{2}N_2O + \frac{3}{2}H_2O \quad (3)$$

$$NO_2^- + 3e^- + 4H^+ \rightarrow \frac{1}{2}N_2 + 2H_2O \quad (4)$$

$$NO_2^- + 6e^- + 8H^+ \rightarrow NO_4^+ + 2H_2O \quad (5)$$

The electron contribution of Fe(II) oxidation to NO_x^- reduction was

$$\varphi_{Fe(II)/NO_3^-} = \frac{1 \times [Fe(II)]}{2 \times [NO_2^-] + 4 \times [N_2O] + 5 \times [N_2] + 8 \times [NH_4^+]} \quad (6)$$

$$\varphi_{Fe(II)/NO_2^-} = \frac{1 \times [Fe(II)]}{2 \times [N_2O] + 3 \times [N_2] + 6 \times [NH_4^+]} \quad (7)$$

where φ is the electron contribution of Fe(II) oxidation to NO_x^- reduction (%); [Fe(II)] is the consumption of extractable Fe(II) (mmol Fe kg^{-1}); $[NO_2^-]$, $[N_2O]$, $[N_2]$, and $[NH_4^+]$ are the N products of nitrate or nitrite reduction (mmol N kg^{-1}); and the numbers in front of Fe(II) and N products represent the electrons donated by Fe(II) and accepted by NO_3^- or NO_2^- to produce NO_2^-, N_2O, N_2, and NH_4^+.

The proportion (P) of the chemical reaction pathways in NRFO process is

$$P = \frac{[Fe(II)]_{abiotic}}{[Fe(II)]_{abiotic} + [Fe(II)]_{biotic}} \quad (8)$$

The contribution of chemodenitrification (F) to N_2O emissions in paddy soils is

$$F = \frac{[N_2O]_{abiotic}}{[N_2O]_{total}} \quad (9)$$

where $[Fe(II)]_{abiotic}$ and $[Fe(II)]_{biotic}$ are the consumption of extractable Fe(II) in chemical and biological reactions during NRFO, $[N_2O]_{abiotic}$ is the N_2O produced from chemical nitrite reduction, and $[N_2O]_{total}$ is the total N_2O production from both chemical and biological reactions during NO_3^- or NO_2^- reduction.

RESULTS

N_2O Emissions from Anoxic Paddy Soils. In sterilized soils, N_2O steadily increased in nitrite amendments but neither in nitrate amendments nor in the control soils (Figure 1a). After 9 days, cumulative N_2O emissions reached 17.5 ± 0.4 and 16.8 ± 0.7 mg N kg^{-1} in sterilized soils S1 and S2, respectively. In nonsterilized soils, N_2O emissions in both nitrate- and nitrite-amended setups first increased and then decreased during incubation (Figure 1b). N_2O emissions from the nonsterilized soil S1 peaked within 2 days (20.5 ± 1.3 mg N kg^{-1}) and within 6 days (22.7 ± 0.9 mg N kg^{-1}) in the

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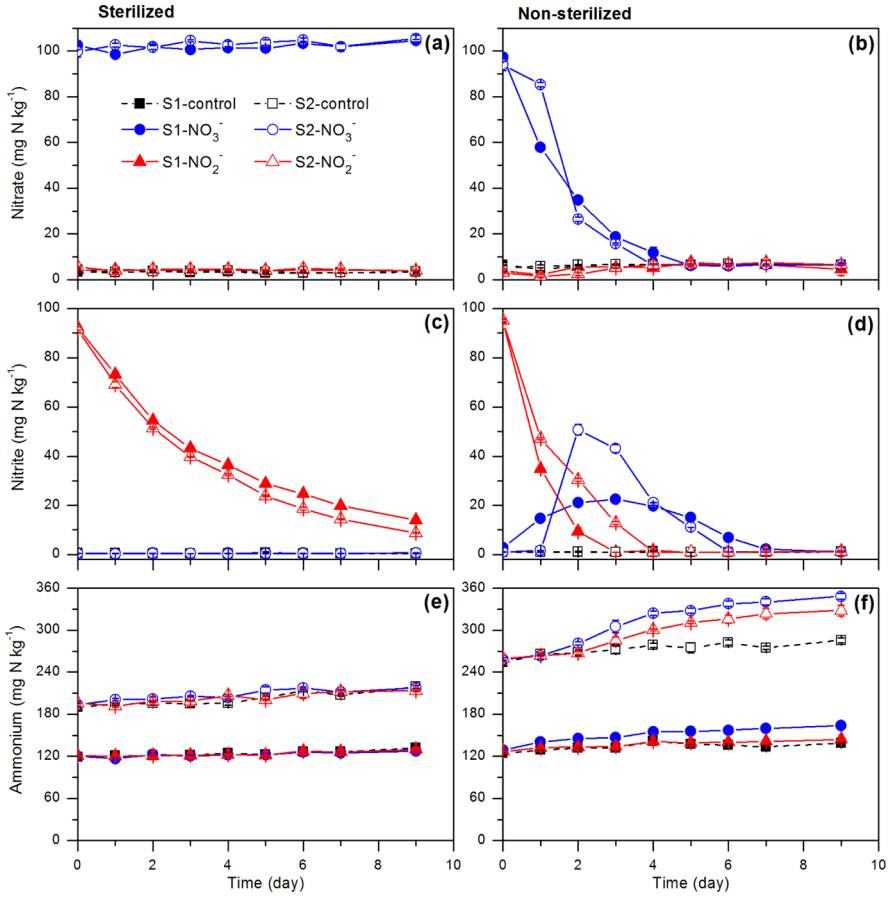

Figure 2. Nitrate, nitrite, and ammonium concentrations in two paddy soils (S1 and S2) under sterilized (a,c,e) and microbially active (b,d,f) anoxic conditions amended with nitrate (S1-NO_3^- and S2-NO_3^-) or nitrite (S1-NO_2^- and S2-NO_2^-) compared to nonamended soils (S1-control and S2-control). The values represent the mean of triplicate setups; the error bars represent standard errors.

nitrite and nitrate treatments, while in the nonsterilized soil S2, the maximum N_2O emissions in nitrite and nitrate treatments both appeared on day 4 (22.6 ± 0.5 and 4.7 ± 2.1 mg N kg^{-1}, Figure 1b).

N_2O emission rates in the sterilized nitrite-amended soils (2.1 and 2.4 mg N kg^{-1} d^{-1} in soils S1 and S2, respectively) were lower than in the microbially active nitrite-amended soils (11.2 and 7.2 mg N kg^{-1} d^{-1} in soils S1 and S2, respectively) (Figure 1). The maximum N_2O emission from the microbially active soil S2 with nitrate amendment was 21% of that from the microbially active soil S1 (Figure 1b).

Inorganic N Speciation Changes during Anoxic Incubation of Paddy Soils. NO_3^- concentrations remained constant in the sterilized nitrate-amended soils (Figure 2a). In contrast, in the microbially active nitrate-amended soils, NO_3^- concentrations immediately decreased by 40 and 9% on day 1 in soils S1 and S2, respectively and were then gradually depleted during further anoxic incubation (Figure 2b). This indicates that nitrate reduction is triggered by microbial activity and not by chemical reactions.

In contrast to NO_3^-, NO_2^- concentrations decreased in both the sterilized and nonsterilized nitrite-amended soils (Figure 2). The consumption rates of NO_2^- in the two nonsterilized soils were 31.4 mg N kg^{-1} d^{-1} in soil S1 within 3 days and 23.5 mg N kg^{-1} d^{-1} in soil S2 within 4 days, respectively, which were larger than the rates in the sterilized soils (16.3 and 14.8 mg N kg^{-1} d^{-1} in soils S1 and S2 within 3 and 4 days, respectively) (Figure 2c,d), demonstrating the co-occurrence of biological and chemical reduction of nitrite. The abiotic removal rates of NO_2^- in the two sterilized soils were similar, while in the microbially active setups, the NO_2^- reduction rates in soil S2 were slower than in soil S1 (Figure 2c,d), indicating a lower microbial NO_2^- reduction rate in soil S2 than in soil S1. In the microbially active nitrate-amended soils, NO_2^- accumulated as the intermediate of microbial NO_3^- reduction (Figure 2d). The maximum amount of NO_2^- accumulation in soil S2 on day 2 (50.8 ± 2.1 mg N kg^{-1}) was higher than in soil S1 on day 3 (22.5 ± 0.4 mg N kg^{-1}). In both microbially active soils, the NO_2^- which accumulated over the first 3 days was depleted after further 6 days of incubation.

Ammonium concentrations in the sterilized soils did not change during anoxic incubation (Figure 2e). In contrast, both nitrate and nitrite addition enhanced ammonium concentrations over the 9 days of incubation in the microbially active soils; the amount of ammonium was higher in soil S2 (62.8 and 42.5 mg N kg^{-1} deriving from NO_3^- and NO_2^- reduction, respectively) than in soil S1 (24.9 and 5.2 mg N kg^{-1}, respectively) (Figure 2f), implying the occurrence of dissimilatory nitrate/nitrite reduction to ammonium (DNRA). The initial ammonium concentrations in microbially active soils (124.0 ± 1.7 and 253.7 ± 3.9 mg N kg^{-1} in soils S1 and S2, respectively) were higher than those in sterilized soils (118.9 ± 3.4 and 190.3 ± 4.3 mg N kg^{-1} in soils S1 and S2, respectively), potentially because of ammonium release from the decomposition of soil organic matter during preincubation.

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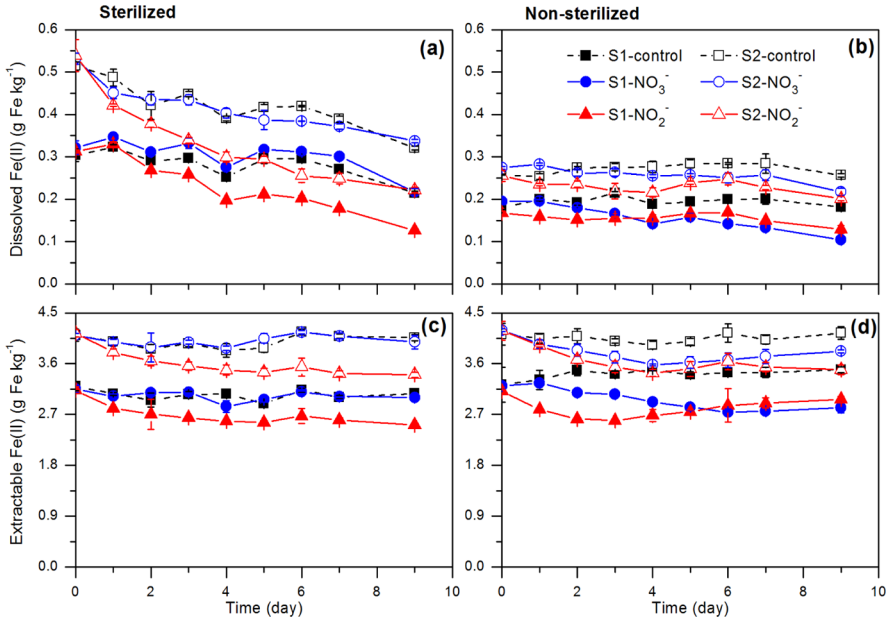

Figure 3. Concentrations of dissolved Fe(II) and 1 M HCl-extractable Fe(II) in two paddy soils (S1 and S2) under sterilized (a,c) and microbially active (b,d) anoxic conditions amended with nitrate (S1-NO_3^- and S2-NO_3^-) or nitrite (S1-NO_2^- and S2-NO_2^-) compared to nonamended soils (S1-control and S2-control). The values represent the mean of triplicate setups; the error bars represent standard errors.

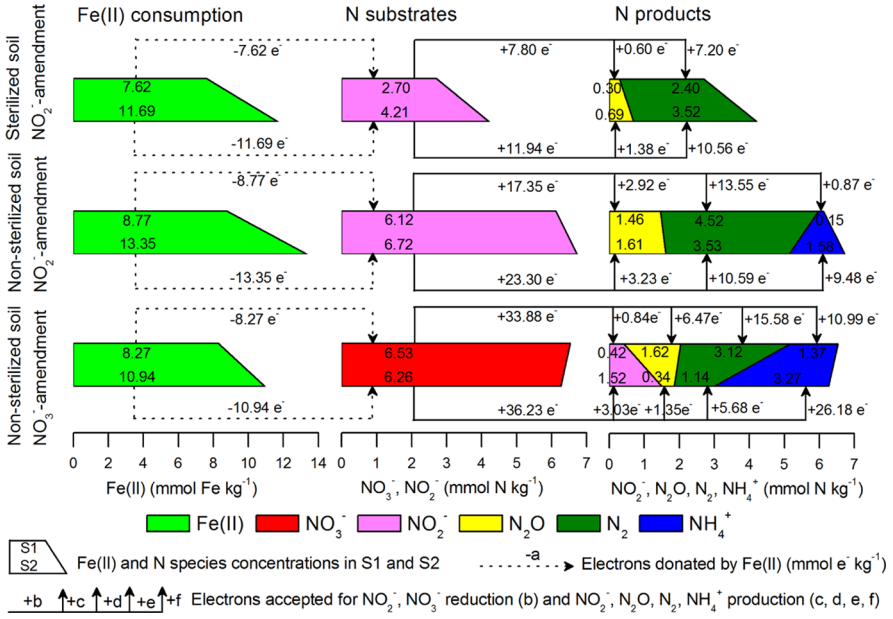

Figure 4. Reaction stoichiometry and electron transfer between NO_3^-, NO_2^-, and Fe(II) in two paddy soils (S1 and S2) under sterilized and nonsterilized anoxic conditions with nitrite and nitrate amendment. The stoichiometry was calculated at the time points of maximum N_2O emissions in the nonsterilized soils during anoxic incubation. The three rows indicate the stoichiometric calculations in the sterilized soils with NO_2^- amendment (top), the nonsterilized soils with NO_2^- amendment (middle), and the nonsterilized soils with NO_3^- amendment. No data are shown for the sterilized soils with NO_3^- amendment because no chemical reaction between NO_3^- and Fe(II) was observed. The light green trapezoid presents Fe(II) consumption; the red trapezoid presents NO_3^- consumption; the pink trapezoid presents the consumption or production of NO_2^-; the yellow, dark green, and blue trapezoids present N_2O, N_2, and NH_4^+ production, respectively. The upper and bottom lines of the trapezoids display the variations of Fe(II) or N content (NO_3^-, NO_2^-, N_2O, N_2, and NH_4^+) in soils S1 and S2, respectively. The solid arrows show the electrons accepted by NO_3^- or NO_2^-, and dotted arrows show the electrons donated by Fe(II).

Fe(II) Oxidation during Anoxic Incubation of Paddy Soils. In sterilized soils, nitrite addition lowered both the dissolved and extractable Fe(II) concentrations, while nitrate addition did not impact the Fe(II) concentrations (Figure 3a,c). However, in the microbially active nitrate- and nitrite-amended soils, both dissolved and extractable Fe(II) decreased (Figure 3). At the beginning of the experiment (after 12 days of preincubation), dissolved Fe(II) concentrations in the microbially active soils (0.2 ± 0.0 and 0.3 ± 0.0 g Fe kg^{-1} in soils S1 and S2, respectively) were much lower than extractable Fe(II) (3.2 ± 0.3 and 4.2 ± 0.2 g Fe kg^{-1} in soils S1 and S2, respectively) (Figure 3b,d). Additionally, at the beginning of the microcosm experiments (after 12 days of preincubation), both dissolved and extractable Fe(II) in the two natural soils

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accounted for over 95% of the total dissolved and extractable Fe (Supporting Information, Figure S2). This proportion of Fe(II) concentration in total Fe(II) content indicates that Fe(II) is the main species of bioavailable Fe present in anoxic paddy soils.

The initial dissolved Fe(II) concentrations in the sterilized soils in the microcosm experiments (after 12 days of preincubation) were higher than in the nonsterilized soils (Figure 3a,b), which is caused by the addition of $FeCl_2$ to the sterilized soils to reach the same amount of extractable Fe(II) as in the nonsterilized soils. The dissolved Fe(II) in sterilized soils decreased over time (Figure 3a) and can be attributed to sorption of dissolved Fe(II) to minerals or precipitation as Fe(II) carbonate minerals [e.g., siderite ($FeCO_3$)] because of the bicarbonate buffer that was used for pH maintenance in the soil microcosms.

Reaction Stoichiometry between NO_3^-, NO_2^-, and Fe(II). Stoichiometric calculations of redox reactions, that is, electron transfer between NO_3^-, NO_2^-, and Fe(II), were done at the time points of maximum N_2O emissions in the microbially active soils during anoxic incubations (Figure 4). N_2 concentrations were calculated via NO_3^- or NO_2^- consumption subtracting the total amount of produced NO_2^-, N_2O, and NH_4^+.

In the sterilized soils, the reaction products of abiotic NO_2^- reduction were N_2O and potentially N_2 (not measured), while in nonsterilized soils, the products were N_2O, NH_4^+, and potentially N_2. Stoichiometric calculation for the sterilized nitrite-amended soils revealed that the electrons stemming from Fe(II) oxidation (7.6 and 11.7 mmol e^- kg^{-1}) were almost equal to the electrons accepted by NO_2^- reduction (7.8 and 11.9 mmol e^- kg^{-1}) in soils S1 and S2, respectively (Figure 4). This calculation confirmed that only chemical reactions between NO_2^- and Fe(II) occurred in the sterilized soils. The higher NO_2^- consumption in the microbially active soils (Figure 4) was attributed to microbial nitrite reduction in addition to chemodenitrification. In these cases, Fe(II) oxidation contributed 50.5% (8.8 mmol e^- kg^{-1}/17.4 mmol e^- kg^{-1}) and 57.3% (13.4 mmol e^- kg^{-1}/23.3 mmol e^- kg^{-1}) of electrons to the observed NO_2^- reduction in the microbially active soils S1 and S2, respectively.

A comparison of Fe(II) consumption in the sterilized and microbially active nitrite-amended soils showed that abiotic Fe(II) oxidation accounted for 86.9 and 87.6% of total Fe(II) oxidation in soils S1 and S2. The obtained data indicate that chemical reactions represent the dominant pathway for Fe(II) oxidation by NO_2^-. Additionally, N_2O emissions caused by chemodenitrification were 20.5% (0.3 mmol N kg^{-1}/1.5 mmol N kg^{-1}) and 42.9% (0.7 mmol N kg^{-1}/1.6 mmol N kg^{-1}) of the total N_2O emissions from NO_2^- reduction in soils S1 and S2, respectively, suggesting a considerable role of chemodenitrification in N_2O emissions from the paddy soils.

In the sterilized soils, NO_3^- did not chemically react with Fe(II), but in nonsterilized soils, microbially driven NO_3^- reduction with Fe(II) oxidation produced NO_2^-, N_2O, NH_4^+, and potentially N_2 (Figure 4). The calculation of electron transfer between NO_3^- and Fe(II) indicated that microbial plus abiotic Fe(II) oxidation contributed 24.4% (8.3 mmol e^- kg^{-1}/33.9 mmol e^- kg^{-1}) and 30.2% (10.9 mmol e^- kg^{-1}/36.2 mmol e^- kg^{-1}) of the electrons for NO_3^- reduction in soils S1 and S2. In nonsterilized nitrate-amended soils, N_2O emission from soil S1 was 3.8 times higher than that from soil S2, while NH_4^+ production in S1 was 0.58 folds lower than in S2 (Figure 4).

DISCUSSION

Abiotic and Biotic N_2O Emissions from NO_2^- and NO_3^- Reduction in Paddy Soils. Chemodenitrification typically results in the formation of N_2O, while biological reduction of NO_2^- usually produces N_2,[38] unless the N_2O reductase is inhibited by O_2, low pH, or sulfide.[45−47] For the studied paddy soils, we discovered a different pattern, showing that the biotic process is the dominant pathway for N_2O production during NO_2^- reduction. Biotic N_2O emissions accounted for over 57% of total N_2O emissions during NO_2^- reduction, as chemodenitrification only contributed 20.5 and 42.9% to N_2O emissions in soils S1 and S2, respectively. The main biotic processes in regulating N_2O emissions during NO_2^- reduction are the reduction of NO_2^- to N_2O but then in particular the microbial reduction of N_2O. Nearly 40% of the denitrifiers possessing genes that encode NO_2^- reductases lack the *nosZ* gene (N_2O reductase) to reduce N_2O to N_2.[48] Moreover, microorganisms that are capable of complete denitrification may downregulate the *nosZ* gene expression when NO_3^- or NO_2^- is sufficiently available, but other N sources are limiting.[49] The toxicity of NO_2^-[14,50] might also downregulate *nosZ* gene expression and thus cause increasing N_2O emission, and NO_2^- might also be toxic to other microorganisms which contribute to N_2O production.

N_2O emissions from abiotic and biotic sources during the reduction of NO_3^- cannot be directly quantified with the data in our study because the extent of the abiotic reaction of NO_2^- with Fe(II) during NO_3^- reduction is unknown (please note that no reactions between NO_3^- and Fe(II) were observed in the nonsterilized nitrate-amended soils). However, taking into account the quantification of chemodenitrification from the study of Jamieson et al.,[41] we can estimate the abiotic Fe(II) oxidation during NO_3^- reduction and deduce how much N_2O is produced by chemodenitrification with this amount of Fe(II) oxidation, based on the stoichiometric calculation in the abiotic setups with nitrite amendment. Jamieson et al. have reported that the contribution of chemodenitrification to Fe(II) oxidation was 25−40% with an average value of 35.2% in five NRFO bacterial cultures.[41] The extent of chemodenitrification was similar across these NRFO bacterial strains,[41] potentially indicative of the biological process that is common in most denitrifiers,[25] and all the denitrifiers studied could drive the NRFO process.[51] Assuming that the extents of abiotic and biotic Fe(II) oxidation for nitrate-reducing bacteria in the microbially active paddy soils were similar to the results reported by Jamieson et al.,[41] we estimated the abiotic Fe(II) oxidation during NO_3^- reduction to be 2.9 and 3.9 mmol Fe kg^{-1} in soils S1 and S2, respectively. Based on the molar ratio in the reaction between NO_2^- and Fe(II) in the sterilized soils, the estimated amount of Fe(II) oxidation would chemically reduce 1.03 and 1.39 mmol N kg^{-1} NO_2^- concomitantly generating 0.11 and 0.23 mmol N kg^{-1} N_2O in soils S1 and S2, respectively. Therefore, the contribution of chemodenitrification to the total N_2O emission during NO_3^- reduction would be 6.8 and 67.6% in microbially active soils S1 and S2, respectively (Supporting Information, Figure S3).

Lower biotic N_2O emission in soil S2 (57.1 and 33.4% for nitrite and nitrate treatments, respectively) compared to soil S1 (79.5 and 93.2%, respectively) can be attributed to the high

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organic C content in soil S2 (Supporting Information, Figure S4), which favors DNRA over denitrification, thus leading to less N_2O emission.[52−54] NH_4^+ production in soil S2 (23.5 and 52.2% for nitrite and nitrate treatments, respectively) was higher than those in soil S1 (2.4 and 21.0%, respectively) (Supporting Information, Figure S4), suggesting that higher organic C in soil S2 facilitated NO_x^- reduction to ammonium. Additionally, microbial NO_3^- reduction coupled to Fe(II) oxidation could also produce NH_4^+.[27,55,56] High Fe(II) levels also facilitate DNRA along with the decrease of denitrification rates.[57] Higher abiotic N_2O emission in soil S2 (42.9 and 67.6% for nitrite and nitrate treatments, respectively) relative to soil S1 (20.5 and 6.8%) is due to the elevated Fe(II) concentration in soil S2 (Figure 3), which stimulates N_2O emission via chemodenitrification. Similar results were obtained from a dual nitrite isotopic study, showing that high levels of Fe(II) (present as green rust) increased N_2O production in chemodenitrification.[17] These data suggest that chemodenitrification can contribute significantly to N_2O emissions, especially in Fe-rich systems when substantial NO_2^- is present.[16]

Apart from the N_2O produced by the chemical reactions of Fe(II) with NO_2^- (chemodenitrification), abiotic reactions between organic carbon and NO_2^- (i.e., nitrosation) could also take place.[58,59] Although nitrosation of organic matter was predicted to occur in particular in acidic environments with high organic carbon content,[60] which was not the case in our soil microcosms [pH value of 7, less than 2% soil organic carbon (SOC)], it could still occur depending on the organic carbon composition (if containing, e.g., phenols).[58] Nevertheless, the recovery of nitrite added to the soils within 30 min in our study was 91.4−95.1%, implying that the abiotic immobilization of NO_2^- to organic carbon in our soil microcosms played only a minor role, if at all. Additionally, the electrons donated by Fe(II) were almost equal to the electrons accepted by NO_2^- in the sterilized soils, which also indirectly suggested a minor role of the abiotic reaction of organic carbon with NO_2^-.

Effect of Fe(II) on NO_2^- Accumulation and Chemodenitrification in Paddy Soils. NO_2^- accumulates during microbial NO_3^- reduction because of the lower reduction rates of NO_2^- compared to the reduction of NO_3^-.[34,35] When enzymatic NO_2^- reduction is lowered or even inhibited, microorganisms release NO_2^- to the environment in order to decrease the nitrite toxicity for the cells.[61] The abiotic Fe(II) oxidation by NO_2^- outside the cells could consume NO_2^- and lower NO_2^- concentrations. However, NO_2^- still accumulates in the studied soils even in the presence of considerable high Fe(II) concentrations (Figure 3). Soil S2 with a higher Fe(II) concentration showed more NO_2^- accumulation compared to soil S1 (Figures 2 and 3). NO_2^- accumulation in the studied paddy soils is attributed to the inhibition of microbial reduction of NO_2^- but not due to the differences in chemical NO_2^- reduction because the rates of abiotic NO_2^- reduction in soils S1 and S2 were similar (Figure 2c). However, the rate of microbial NO_2^- reduction in soil S2 was lower than that in soil S1 (Figure 2d). Microbial NO_2^- reduction is affected by dissolved (aqueous) Fe(II) concentrations in the environment. Aqueous Fe(II) entering the periplasm of cells is oxidized coupled to enzymatic reduction of NO_3^- or NO_2^- (by nitrate-/nitrite-reducing Fe(II)-oxidizing bacteria) forming poorly soluble Fe(III) that can precipitate as Fe(III) mineral directly at the cellular NO_2^- reductase, which would slow down or even inhibit the enzymatic NO_2^- reduction activity, reducing NO_2^- consumption and leading to NO_2^- accumulation.[25,28,38,62] Compared to soil S1, the higher extent of NO_2^- accumulation in soil S2 results from the lower microbial NO_2^- reduction rate (Figure 2d), which is potentially attributed to the stronger extent of intracellular mineralization[28,38] and extracellular encrustation[30,63] caused by the high Fe(II) concentration in soil S2 (Figure 3b).

Fe(II) highly impacts the stability of NO_2^- in the environment.[14,64] The fast rate of chemical NO_2^- reduction by Fe(II)[33,65] leads to a competition between chemodenitrification and microbial NO_2^- reduction. Usually, high Fe(II) concentrations increase the rates of chemodenitrification. However, the rates of chemical NO_2^- reduction in the two sterilized paddy soils were similar (Figure 2c), in spite of the higher Fe(II) concentration in soil S2 relative to soil S1. This is ascribed to the excess amount of Fe(II) (56 and 72 mmol Fe kg^{-1} in soils S1 and S2) for NO_2^- reduction (7 mmol N kg^{-1}) because the molar ratios of Fe(II) to NO_2^- leading to the production of either N_2O, N_2, or NH_4^+ are 2, 3, and 6, respectively.[33] Thus, the NO_2^- concentration controls the rate of chemodenitrification, and the chemical NO_2^- reduction rate shows a linear dependency on NO_2^- concentration.[15,66] The kinetics of abiotic nitrite consumption (Figure 2c) appears to be first-order, and we calculated the rate constants of nitrite reduction as 0.22 day^{-1} (equal to 1.5×10^{-4} min^{-1}) in both sterilized soils. Compared with rate constants in sterilized peat (1.6×10^{-2} min^{-1} at pH 5.09 at 25 °C),[67] the chemical reduction of NO_2^- at pH 7 in our soil microcosms was much slower. Although the high Fe(II) concentration in soil S2 did not directly accelerate the rate of chemodenitrification, it could have enhanced the extent of chemodenitrification by suppressing enzymatic NO_2^- reduction.

Abiotic and Biotic Oxidation of Fe(II) by NO_x^- in Paddy Soils. Although the thermodynamics of Fe(II) oxidation coupled to NO_3^- reduction are favorable, the kinetics of abiotic Fe(II) oxidation by NO_3^- is slow, except in the presence of catalysts,[33,68] which might explain the absence of the reaction between Fe(II) and NO_3^- in the sterilized soils (Figures 2 and 3). In contrast, the abiotic Fe(II) oxidation by NO_2^- proceeds fast in the environment.[33] The significant decrease of NO_2^- and Fe(II) (Figures 2c and 3) in the sterilized soils is related to the chemical NO_2^- reduction by Fe(II) concomitantly producing N_2O (Figure 1a) and potentially N_2, which is consistent with a previous study that showed no NH_4^+ was produced in sterilized soils.[64] The contributions of electrons from Fe(II) oxidation to NO_2^- reduction (50.5 and 57.3% in soils S1 and S2, respectively) emphasize the important role of Fe(II) in NO_2^- reduction in paddy soils. Moreover, Fe(II) oxidation by NO_2^- is mainly caused by the abiotic reaction (as 86.9 and 87.6% of total Fe(II) oxidation in soils S1 and S2, respectively), suggesting that chemodenitrification outcompetes microbial Fe(II) oxidation. The important role of Fe(II) in abiotic reactions with NO_2^- implies a high potential for chemodenitrification in paddy soils when substantial NO_2^- is accumulated during NO_3^- reduction.

NO_2^- accumulation in nonsterilized nitrate-amended soils (Figure 2d) suggests the potential of chemical Fe(II) oxidation by biogenically formed NO_2^- during NRFO.[25,28] The contribution of electrons from Fe(II) oxidation to NO_3^- reduction (24.4 and 30.2% in soils S1 and S2, respectively) agrees with previous results that high Fe(II) concentration in

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paddy soils contributes a large percentage of electrons to NO_3^- reduction.[69] In our study, the abiotic Fe(II) oxidation during microbial NO_3^- reduction (via the formed NO_2^-) only accounts for about 35% of the total Fe(II) oxidation (2.9 and 3.9 mmol Fe kg^{-1} in soils S1 and S2, respectively). Even though the chemical Fe(II) oxidation by NO_2^- is rapid,[33] the major amount of Fe(II) is oxidized by enzymatic reactions during NRFO. The possible reasons could be as follows: (i) NO_2^- release from NO_3^- bioreduction[25,26,28,30] is a slow process which retards the reaction of abiotic Fe(II) oxidation and (ii) NO_2^- accumulation during microbial NO_3^- reduction only occurs at two circumstances, in the presence of aqueous Fe(II) or with limited bioavailable organic C, not in the case of Fe(II)−organic matter complexes and abundant organic source,[33,35,36,38] implying that enzymatic NO_2^- reduction limits the abiotic Fe(II) oxidation by NO_2^-.

Both aqueous and solid-phase Fe(II) participate in the reaction with NO_2^- or NO_3^- in our study, which is in agreement with previous results.[15,33,70] Extractable Fe(II) was more than 8-fold higher than dissolved Fe(II), and the decrease of extractable Fe(II) in the microbially active nitrite- and nitrate-amend soils was larger than that of dissolved Fe(II) (Figure 3), implying that solid-phase Fe(II) is the dominant form reacting with NO_x^-. Aqueous Fe(II) only accounts for less than 20% of the total Fe(II) production and oxidation in Fe redox cycling.[71] Solid Fe(II) or Fe(II) complexes show a higher reactivity than aqueous Fe(II) in the reaction with NO_x^- because solid-phase Fe(II) serves not only as a source of Fe(II) but also as a catalyst for Fe(II) oxidation.[17,33,66,70,72]

Implications for Fe(II) Oxidation on N_2O Emission in Anoxic Paddy Soils. As the Fe content in paddy soils can be as high as 3.6%,[73] and Fe redox reactions are coupled to soil N transformation during the alternative flooding and drainage management,[5] Fe plays a crucial role in N_2O emissions from paddy soils. Both biotic and abiotic N_2O emissions during NO_3^- reduction in paddy soils are affected by Fe(II) oxidation. As the high bioavailability of both organic carbon and Fe(II) favors DNRA over denitrification,[52,54,57] N_2O emissions decrease under the anoxic conditions where high amounts of organic carbon are available and Fe(II) is abundant. Generally, higher SOC is expected to contribute to higher (incomplete) denitrification (in particular in microoxic soils) and thus higher N_2O emission. However, in anoxic soils such as flooded paddy soils, the higher SOC could create a lower redox potential, which would lead to complete denitrification producing more N_2 and less N_2O, or even favoring further reduction of nitrate to ammonium (DNRA). In this case, N_2O emissions decrease in the anoxic soils with higher SOC content. Based on the contribution of electrons from Fe(II) oxidation to NO_3^- reduction, the contribution of Fe(II) oxidation to biotic N_2O emissions seems to be lower than that of organic carbon. Because organic carbon frequently serves as the dominant electron donor for NO_3^- reduction,[74,75] organic carbon might be responsible for the main part of biotic N_2O emission during NO_3^- reduction in paddy soils.

However, Fe(II) oxidation could enhance the abiotic N_2O emission from paddy soils by facilitating NO_2^- accumulation and chemodenitrification during NO_3^- reduction. NO_2^- accumulation usually appears in neutral and alkaline soils,[14] and NO_2^- concentration even increases with elevated pH.[50] In paddy fields, flooding irrigation neutralizes the soil pH and ameliorates the acidification effect of chemical fertility,[5,76] which supports the accumulation of NO_2^-. Additionally, in flooded paddy soils, microbial reduction of Fe(III) minerals forms substantial amounts of Fe(II), including the release of dissolved Fe(II).[5,77] The flooding irrigation in paddy soils not only facilitates NO_2^- accumulation during NO_3^- reduction[38] but also creates a favorable environment (the presence of Fe(II) and NO_2^-) for chemodenitrification.[33,78] Fe(II) oxidation can therefore play a dominant role in abiotic N_2O emissions during NO_3^- reduction, particularly in Fe-rich paddy soils under flooding conditions.

Our results imply that N_2O emission from paddy soils is influenced by N speciation (NO_3^- and NO_2^-), Fe(II) concentration, and organic C content and is regulated by the complex network of abiotic and biotic C, Fe, and N biogeochemical processes. Our quantification of abiotic and biotic N_2O emissions from paddy soils has implications for potential future N_2O management and suggests to (i) avoid nitrate application in Fe-rich soils to minimize the contribution of chemodenitrification to N_2O emissions and (ii) increase the use of organic fertilizers in low organic soils to lower N_2O emissions from biological denitrification.

Challenges in Evaluation of Abiotic and Biotic Contributions to N_2O Formation in Soils. Paddy soil is a complex system, where several biogeochemical processes (i.e., biotic denitrification, chemodenitrification, and microbial Fe(III) reduction) co-occur and even compete with each other.[5] These coexisting processes can cause the uncertainties in the quantitative evaluation of abiotic and biotic N_2O emissions in paddy soils, for example, (i) chemical and microbial reactions are two coupled pathways for electron transfer during NO_x^- reduction (i.e., biotic and abiotic denitrification), and more electrons would be transferred through the chemical pathway when the microbial pathway was inhibited by gamma radiation, which could lead to an overestimation of the contribution of chemodenitrification to the total N_2O emissions from the studied paddy soils; and (ii) Fe(II) oxidation coupled to NO_x^- reduction co-occurs with microbial Fe(III) reduction fueled by organic matter in the anoxic paddy soils, and a part of the Fe(II) consumption (oxidation) caused by NO_x^- reduction was compensated by the production of Fe(II) from microbial Fe(III) reduction, which could lead to an underestimation of the total Fe(II) consumption by NO_x^- reduction and thus an overestimation of the proportion of abiotic Fe(II) oxidation in the total Fe(II) oxidation.

Additionally, some N species such as N_2 and NO were not measured in our study, and N_2 production was calculated as the difference of NO_3^- or NO_2^- consumption minus the content of N products (i.e., NO_2^-, N_2O, and NH_4^+), regardless of the instability and minor content of the N products, such as NO and N_2O_4. This calculation could slightly overestimate the amount of electrons accepted by NO_x^- reduction, thus underestimating the electron contribution of Fe(II) oxidation to NO_x^- reduction. Moreover, we estimated the extent of the NRFO process in paddy soils, which was driven by the denitrifiers similar to that in the NRFO cultures, based on the results from previous studies: the relevant biological processes are common to most denitrifiers,[25] all denitrifiers studied could drive the NRFO process,[51] and the extent of chemodenitrification during microbial nitrate reduction was similar across different NRFO bacteria.[41] This comparison (pure cultures vs soil microbial communities) could also cause the uncertainties in the evaluation of abiotic and biotic contributions in paddy soils.

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For the complexity of soil environments and the lack of available experimental data on abiotic and biotic reactions in soil systems, the uncertainties of the estimation of abiotic and biotic N_2O emissions from the anoxic paddy soils are inescapable at present. Nevertheless, our study makes an attempt to quantitatively assess the relative contributions of N_2O formation via chemodenitrification versus denitrification and therefore provides a valuable contribution to raise our attention on the role of chemodenitrification in paddy soils. It has been proposed that Fe and N stable isotope fractionation and elementary kinetic modeling approaches could be a potentially useful methodology for quantitatively evaluating the abiotic and biotic contributions in NRFO processes.[79] In the future, the combination of the isotope technique and modeling approach may resolve some of the uncertainties in the quantitative estimations of abiotic and biotic reactions in soil systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsearthspacechem.9b00296.

Geochemical properties, Fe concentrations, calculation of cumulative N_2O emissions, contributions of abiotic and biotic reactions, and DOC contents (PDF)

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Notes

The authors declare no competing financial interest.

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