

Article

Effects of Increasing pH on Nitrous Oxide and Dinitrogen Emissions from Denitrification in Sterilized and Unsterilized Forest Soils

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Abstract: Denitrification, as an important part of the soil nitrogen cycle, is widely considered to be a major source of nitrous oxide (N₂O). Both biotic and abiotic denitrification processes contribute significantly to soil N₂O emission, especially under acidic conditions. Increasing soil pH was found to suppress N₂O emissions from denitrification, while the underlying mechanism remains uncertain. In this study, we incubated fresh forest soil anaerobically after increasing soil pH and adding nitrate (NO₃⁻) under both sterilized and unsterilized conditions. The dynamic changes of NO₃⁻, nitrite (NO₂⁻), N₂O and dinitrogen (N₂) were monitored continuously during the 15 days of incubation. The results showed that nitrate reduction rates increased with soil pH in both sterilized and unsterilized soils, with the former having higher rates. The obvious production and consumption of nitrite were found at pH 7.1, rather than at pH 5.5, especially in sterilized soils. In both sterilized and unsterilized soils, accumulative emission of N₂O and N₂O-N/(N₂O+N₂)-N product ratios decreased significantly with increasing pH, while N₂ showed the opposite trend. In sterilized soils, N₂O was the dominant end gas product, accounting for 40.88% and 29.42% of the added nitrate at pH 5.5 and 7.1, respectively. In unsterilized soils, N₂ was the only final gas product at pH 7.1 (59.34% of the added nitrate), whereas N₂O dominated at pH 5.5 (26.67% of the added nitrate). Our results here showed that increasing soil pH promoted the conversion of N₂O to N₂ under both sterilized and unsterilized conditions, and highlighted the potential importance of abiotic denitrification on N₂O emission.

Keywords: nitrous oxide; N₂O/(N₂O+N₂) product ratio; abiotic denitrification; soil pH; nitrate reduction



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1. Introduction

Nitrous oxide (N₂O) is well known as the third important greenhouse gas [1–3] and the largest anthropogenic contributor to stratospheric ozone decomposition for the remainder of this century [4,5]. Its atmospheric concentration at present has increased by 20% compared to pre-industrial levels [1]. Soil has been identified as the main source of atmospheric N₂O, contributing 50%–70% of the increase [6,7]. Biotic and abiotic denitrification, by which nitrate (NO₃⁻) can be reduced to nitrite (NO₂⁻), nitric oxide (NO), nitrous oxide and dinitrogen (N₂), are thought to play a key role in soil N₂O emissions [8–13]. Biotic denitrification is performed primarily by a wide variety of bacteria, archaea and fungi, and requires different enzymes (i.e., nitrate reductase (Nar/Nap), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos) [10,14,15]. Abiotic denitrification is considered as a series of redox reactions between nitrogen and ferrous iron (Fe^{II}) or Fe^{II}-bearing compounds or zero-valent Fe [16–21]. So far, the relative contributions of biotic and abiotic denitrification to N₂O emissions are still unclear and may vary due

to differences in environmental conditions (e.g., temperature and precipitation) and soil properties (e.g., pH, organic carbon, total nitrogen, bulk density, texture) [16,18,19,22,23].

Soil pH, as the chief modifier for regional nitrous oxide emission, regulates both biotic and abiotic denitrification. In general, high soil pH increases both biotic and abiotic denitrification rates, but simultaneously decreases the contribution of N_2O to total nitrogenous gas (i.e., $N_2O/(N_2O+N_2)$ product ratio) [23–27]. However, the mechanisms of pH affecting biotic and abiotic denitrification are quite different. So far, the possible mechanisms for the effects of pH on biotic denitrification rate and N_2O emissions have been attributed to its effect on the structure of the denitrifying community and the activity of four denitrification reductases, especially Nos [28,29]. In acidic soils, the higher sensitivity of Nos to that of the other reductases, or the lack of functional *nosZ* gene (encoding Nos) in some de-nitrifiers (e.g., fungi), blocks N_2O reduction to N_2 , and, thus, N_2O is released as the end product [24,30]. The effects of pH on abiotic denitrification may mainly depend on the role of the protons (H^+), which were involved as a final product of abiotic denitrification [22,31,32]. Higher pHs are more conducive to nitrate reduction to N_2 and NH_4^+ , while lower pHs are more conducive to reduction to NO and N_2O [22,23,32,33]. The responses of biotic and abiotic denitrification to pH imply that both processes play important roles in N_2O emission from acidic soils.

From 1980 to 2019, China's forest soils have been acidified significantly, with an average decrease of 0.56 pH units [34]. Since forest soils are also being simultaneously affected by high nitrogen deposition, their contribution to N_2O emission may be non-negligible. Increasing soil pH can reduce N_2O emission and $N_2O/(N_2O+N_2)$ product ratio, which has potential significance for alleviating the greenhouse effect. Here, we incubated sterilized and unsterilized acidic forest soils anaerobically after NO_3^- addition and soil pH increase. Dynamic changes of N_2O and N_2 were monitored at an interval of 8 h during the 15 days of incubation, and the concentrations of NO_3^- and NO_2^- were analyzed at 8, 16, 24, 36, 48, 72, 96, 144, 168, 264, and 360 h, respectively. The primary objectives of this work were to: (i) investigate the effects of pH increase on the reduction of NO_3^- and emissions of N_2O and N_2 from biotic and abiotic denitrification; (ii) elucidate the potential importance of abiotic denitrification to N_2O emission; (iii) clarify the different responses of biotic and abiotic denitrification to soil pH.

2. Materials and Methods

2.1. Site Description and Soil Sample Collection

Soil used in this study was collected from a mixed broad-leaved/Korean pine forest in Changbai Mountain, located in Jilin Province of northeastern China ($42^{\circ}99' N$, $127^{\circ}06' E$). This region has a subtropical continental monsoon climate with long, cold winters and warm, rainy summers. The annual mean temperature is 2.9–4.8 °C and annual mean precipitation is 700–800 mm. The atmospheric wet N deposition in this region was about 10–15 kg N ha⁻¹ yr⁻¹ [35]. The dominant tree species is the Korean pine (*Pinus koraiensis*), mixed with other species such as Amur linden (*Tilia amurensis*), Mongolian oak (*Quercus mongolica*), and mono maple (*Acer mono*). The soil is an upland, mountain dark brown forest soil classified as cambisols, according to the World Reference Base for Soil Resources (WRB), and developed from volcanic ashes (mainly basalt). It is rich in organic matter and iron providing an optimal environment for denitrification. Soil samples were collected from the upper 15 cm soil layer after removing the litter fall. Fresh soil was sieved through 2.0 mm meshes to remove stones and visible roots, and then stored at 4 °C until used. The initial soil samples had a pH (H_2O) of 5.5, 6.8% of soil organic carbon, and 0.6% of total N. The original contents of soil moisture, NO_3^- -N, NO_2^- -N and total Fe are shown in Table 1.

Table 1. Related properties of the original soil and soil samples after pre-incubation.

Parameter	Original Soil pH 5.5	After Pre-Incubation			
		Sterilized		Unsterilized	
		pH 5.5	pH 7.1	pH 5.5	pH 7.1
Moisture (%)	29.6 ± 0.1	29.3 ± 0.1	29.1 ± 0.1	29.3 ± 0.1	29.1 ± 0.1
NO ₃ ⁻ (mg N kg ⁻¹)	19.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NO ₂ ⁻ (mg N kg ⁻¹)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Total Fe (mg kg ⁻¹)	639.3 ± 29.3	779.8 ± 15.4	704.6 ± 9.4	811.3 ± 11.6	751.4 ± 15.6

2.2. Soil Pretreatment

To remove the indigenous NO_x⁻, fresh soil was washed with deionized water thoroughly 3–4 times. The washed soil was air dried to a moisture content close to the initial value, then sieved (2 mm) and homogenized again. The content of NO₃⁻-N and NO₂⁻-N in the soil were found to be less than 0.01 mg kg⁻¹.

2.3. Experimental Set-Up

The washed fresh soil was divided into two sub-samples. One (6.0 kg) was restored by spraying with 180 mL Na₂CO₃ solution with concentration of 0.64 mmol mL⁻¹ to adjust soil pH to 7.1. The other (6.0 kg) was maintained at the initial pH of 5.5 by spraying with an equal volume of deionized water. After thoroughly mixing, two sub-samples were stored at 4 °C for 30 days until soil pH was stable [36].

Nine replicates of 30 g fresh weight of each sub-sample were wrapped in aluminum foil and then sterilized by ⁶⁰Co-γ irradiation. Sterilized soil samples were transferred to 120 mL serum flasks in an aseptic preparation chamber, and then capped immediately with a bromobutyl stopper and screw cap (Macherey-Nagel, Düren, Germany). At the same time, 30 g of fresh soil (nine replicates of each sub-sample) were transferred directly to 120 mL serum flasks and sealed. The sealed flasks with both sterilized and unsterilized soils were vacuumed and replenished with ultrapure helium (He, 99.999%), and this procedure was repeated three times to create an anaerobic condition. Then, the flasks were pre-incubated anaerobically in an artificial climate box at 20 °C for 5 days to deplete NO_x⁻ produced during ⁶⁰Co-γ sterilization. After pre-incubation, three flasks of each pH gradient were opened to analyze NO_x⁻. The content of NO_x⁻-N was found to be less than 0.01 mg kg⁻¹ (Table 1). Three replicates of the six remaining flasks were sprayed with 3 mL sterile deionized water using a disposable sterile syringe and the other three were sprayed with 3 mL potassium nitrate (KNO₃) solution with a concentration of 450 mg N L⁻¹. Two NO₃⁻-N gradients were 0 and 64 mg kg⁻¹, recorded as N0 and N64, respectively. All flasks were flushed again using ultrapure He through 3 vacuuming-replenishing cycles. The pressure in the headspace was then equilibrated with atmosphere using a syringe filled with sterilized water. Finally, all sample flasks were incubated for 15 days at 20 °C, using a robotized online incubation-monitoring system similar to that described by Molstad et al. (2007) [37]. N₂O and N₂ in the headspace were monitored at an interval of 8 h during the whole incubation.

To elucidate the dynamic changes of NO₃⁻ and NO₂⁻ after nitrate addition, the above soil samples in N64 treatments were incubated in the artificial climate box at 20 °C for 15 days a total of eleven times. The concentrations of NO₃⁻ and NO₂⁻ in the flasks were analyzed at 8, 16, 24, 36, 48, 72, 96, 144, 168, 264, 360 h, respectively.

2.4. Sterilization

For sterilized incubation, soil samples were sterilized by ⁶⁰Co-γ irradiation with a dose of 25 kGy for 72 h, which has been widely used as an effective method to sterilize soil samples [38–40]. Sterilization of media and other solutions was achieved by autoclaving for 30 min at 121 °C.

2.5. Nitrogenous Gas and Aqueous Phase Analysis

The analysis methods of nitrogenous gas in the head space and soil physiochemical characteristics were described in detail in our earlier work [22]. Briefly, N_2O and N_2 in the headspace were analyzed simultaneously by the robotized incubation system. It consists of a temperature adjustable thermostatic water bath, a headspace gas auto-sampler, and a gas chromatograph (model 7890A, Agilent Technologies, Santa Clara, CA, USA). Soil physiochemical characteristics were determined using frequently used methods. For example, soil pH was determined potentiometrically in deionized water (1:2.5 w:v) using a combination pH electrode (PHS-3E, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). Soil moisture content was measured from weight loss after drying at 105 °C for 8 h. Soil organic carbon (SOC) and total nitrogen (TN) were monitored using an elemental analyzer (vario MACRO cube, Elementar, Langensfeld, Germany). NO_x^- were extracted from soil samples using 1.0 mol L⁻¹ KCl (1:2.5 w:v) and determined using the sulphanilamide method. Total iron was extracted from soil samples using 0.5 mol L⁻¹ HCl (1:5 w:v) and analyzed using the ferrozine method. In this paper, all reported results are based on dry soil weight.

2.6. Statistical Analyses

The results were analyzed using Student's *t*-test by SPSS software (version 24.0). Statistical significance was denoted at $p < 0.01$ unless otherwise noted. All figures were made using Origin 8.5.

3. Results

3.1. Dynamic Changes in Nitrate Concentration

Since NO_x^- -N concentrations in soil samples without nitrate addition (N0 treatments) were negligible (Table 1), only the results from nitrate addition treatments (N64) are presented in this paper. As shown in Figure 1a, clear nitrate decreases were found in both sterilized and unsterilized soils. In sterilized soils, nitrate concentrations decreased almost linearly in the first 72 h at pH 5.5 and 7.1, with the latter having a greater rate. Subsequently, the rate of nitrate decrease slowed down gradually until nitrate concentrations were negligible. Nitrate was almost depleted (less than 1.00 mg N kg⁻¹) at 144 h and 96 h at pH 5.5 and 7.1, respectively. This suggested that increasing soil pH accelerated abiotic nitrate reduction. Similar results were found in unsterilized soils, but with slightly lower rates. In unsterilized soils, nitrate was reduced thoroughly (less than 1.00 mg N kg⁻¹) at 264 h and 144 h at pH 5.5 and 7.1, respectively. The comparable nitrate reduction in sterilized and unsterilized soils demonstrated that nitrate reduction in acidic and neutral forest soils can be achieved by both abiotic and biotic processes.

3.2. Dynamic Changes in Nitrite Concentration

As shown in Figure 1b, nitrite was observed as a transient intermediate during anaerobic incubation after nitrate addition in both sterilized and unsterilized soils. In sterilized soils, the accumulation and consumption of nitrites varied significantly with soil pH. At pH 7.1, nitrite concentration increased sharply during the first 36 h, decreased rapidly afterward, and had virtually disappeared (0.07 mg N kg⁻¹) at 96h. The maximum cumulative of nitrite was 5.34 mg N kg⁻¹. At pH 5.5, nitrite concentration reached a peak of 0.54 mg N kg⁻¹ at 16 h, and then decreased slowly to 0.06 mg N kg⁻¹ at 144 h. The fast formation and consumption of nitrite at pH 7.1 was primarily due to the rapid reduction of nitrate (Figure 1a), reflecting that elevating pH promoted the abiotic reduction of nitrate to nitrite and the further reduction of nitrite. Despite the low nitrite concentrations (less than 0.15 mg N kg⁻¹) in unsterilized soils, clear accumulation and consumption of nitrite was still found at pH 7.1, similar to that in sterilized soils.

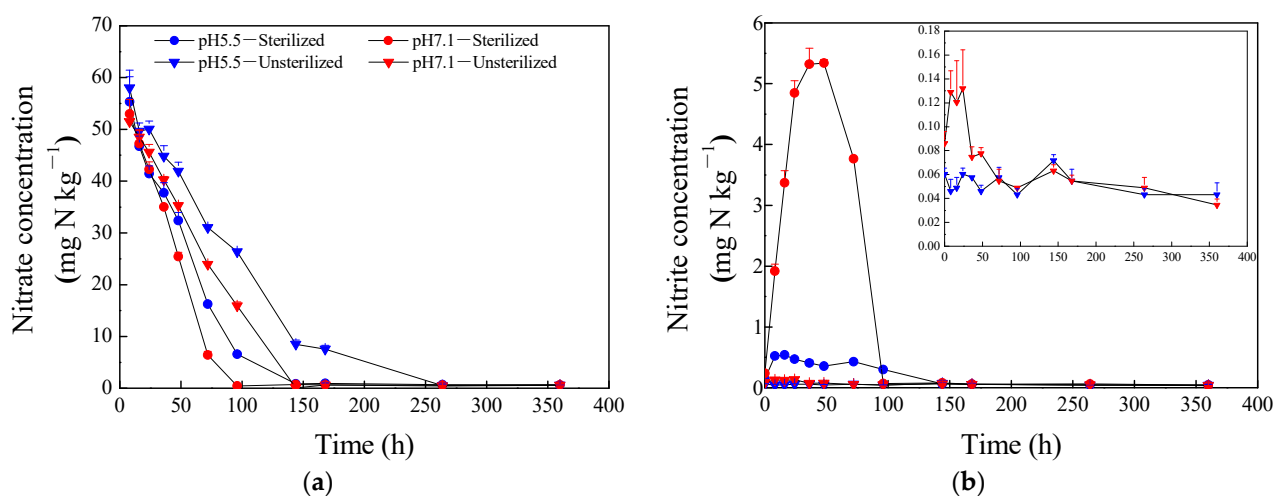


Figure 1. Dynamic changes in nitrate (a) and nitrite (b) concentration in N64 treatments during the incubation of sterilized and unsterilized soils. Error bars represent standard deviation ($n = 3$).

3.3. Dynamic Changes in N_2O and N_2

Due to the negligible nitrate content, the maximum accumulations of N_2O and N_2 were less than 0.01 and 0.25 $mg\ N\ kg^{-1}$ in sterilized and unsterilized soils in N0 treatments, respectively. As shown in Figure 2, there were obvious accumulations of N_2O and N_2 in N64 treatments. In sterilized soils (Figure 2a), it was visible that there were inflection points in both N_2O and N_2 at pH 5.5 and 7.1. The corresponding inflection points occurred earlier at high soil pHs. At pH 7.1, N_2O increased almost linearly in the first 120 h to a maximum of 35.19 $mg\ N\ kg^{-1}$, and then decreased linearly to 18.83 $mg\ N\ kg^{-1}$ at the end of incubation. The final concentration of N_2O represented 29.42% of the added nitrate. N_2 increased slowly to 0.98 $mg\ N\ kg^{-1}$ at 120 h, and then increased sharply to 9.49 $mg\ N\ kg^{-1}$ at the end of incubation. The accumulative emission of N_2 accounted for 14.83% of the added nitrate. At the end of incubation, N_2O was the dominant gas product instead of N_2 . The dynamic changes in N_2O and N_2 at pH 5.5 followed the same pattern as that at pH 7.1, but at a slightly slower rate. The maximum and final concentrations of N_2O were 34.77 $mg\ N\ kg^{-1}$ at 168 h and 26.16 $mg\ N\ kg^{-1}$ at the end of incubation, respectively. The final concentrations of N_2 were 1.91 $mg\ N\ kg^{-1}$, representing 2.98% of the added nitrate. Comparison of the results at pH 7.1 and 5.5 indicated that increasing soil pH accelerated the production of N_2O and N_2 , and the conversion of N_2O to N_2 .

In unsterilized soils (Figure 2b), the inflection point was only found at pH 7.1. Within the first 240 h, N_2O at pH 7.1 increased gradually at a decreasing rate to a maximum of 9.00 $mg\ N\ kg^{-1}$, and then decreased rapidly to less than 0.01 $mg\ N\ kg^{-1}$ at 312 h. Concomitantly, N_2 increased continuously to 37.82 $mg\ N\ kg^{-1}$ at 312 h at an increasing rate, and then remained almost constant until the end of incubation. The accumulative emission of N_2 represented 59.34% of the added nitrate. At the end of incubation, N_2 was the only gaseous product. At pH 5.5, N_2O increased at a decreasing rate and N_2 increased at an increasing rate throughout the incubation. At the end of soil incubation, the total N_2O emission was 17.07 $mg\ N\ kg^{-1}$, accounting for 26.67% of the added nitrate. The accumulative emission of N_2 was 14.12 $mg\ N\ kg^{-1}$, representing 22.06% of the added nitrate. This suggested that N_2O was the main gas product at pH 5.5. Clear decrease of N_2O and simultaneous rapid increase of N_2 at pH 7.1, rather than at pH 5.5, suggested that increasing soil pH promoted the conversion of N_2O to N_2 . Moreover, at the same pHs, the inflection points were achieved earlier in sterilized soils (Figure 2a) than those in unsterilized soils (Figure 2b). The final N_2O emissions in sterilized soils were significantly higher than those in unsterilized soils, while N_2 accumulations had an opposite trend.

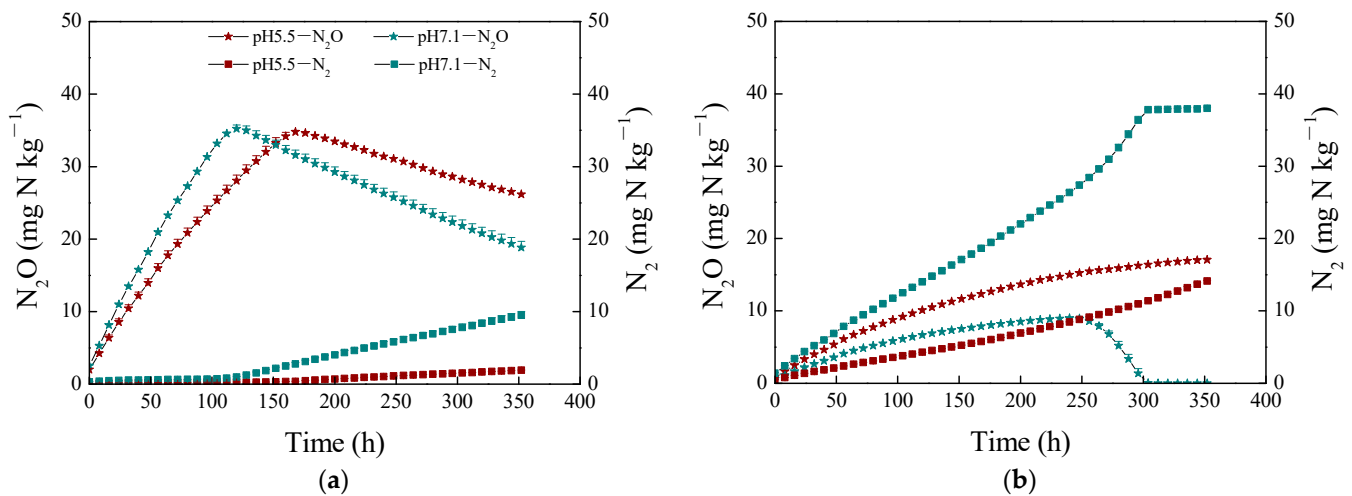


Figure 2. Dynamic changes in N_2O and N_2 in N64 treatments during the incubation of sterilized (a) and unsterilized (b) soils. Error bars represent standard deviation ($n = 3$).

3.4. Dynamic Changes in (N_2O+N_2) and $N_2O-N/(N_2O+N_2)-N$ Product Ratio

As shown in Figure 3a, the total gas emissions (i.e., N_2O+N_2) varied significantly with incubation conditions. In sterilized soils, the emission rates of total gas increased significantly with pH before the inflection points. Subsequently, the total gas decreased slightly at almost similar rates at pH 5.5 and 7.1. At the end of incubation, the accumulative total gas emissions were unaffected by soil pH. Total gas emissions accounted for 43.86% and 44.25% of the added nitrate at pH 5.5 and 7.1, respectively. In unsterilized soils, the total gas production rates increased with pH throughout the incubation. Gas emissions at the end of incubation were 31.19 and 37.99 $mg\ N\ kg^{-1}$, accounting for 48.73% and 59.36% of the added nitrate at pH 5.5 and 7.1, respectively. At the same pH conditions, in the early stage of incubation, total gas emission rates were found to be significantly higher in sterilized soils than in unsterilized soils. However, at the later stage, the total gas emissions increased continuously in unsterilized soils and decreased slightly in sterilized soils. This led to slightly higher total gas emissions in unsterilized soils than those in sterilized soils at the end of incubation.

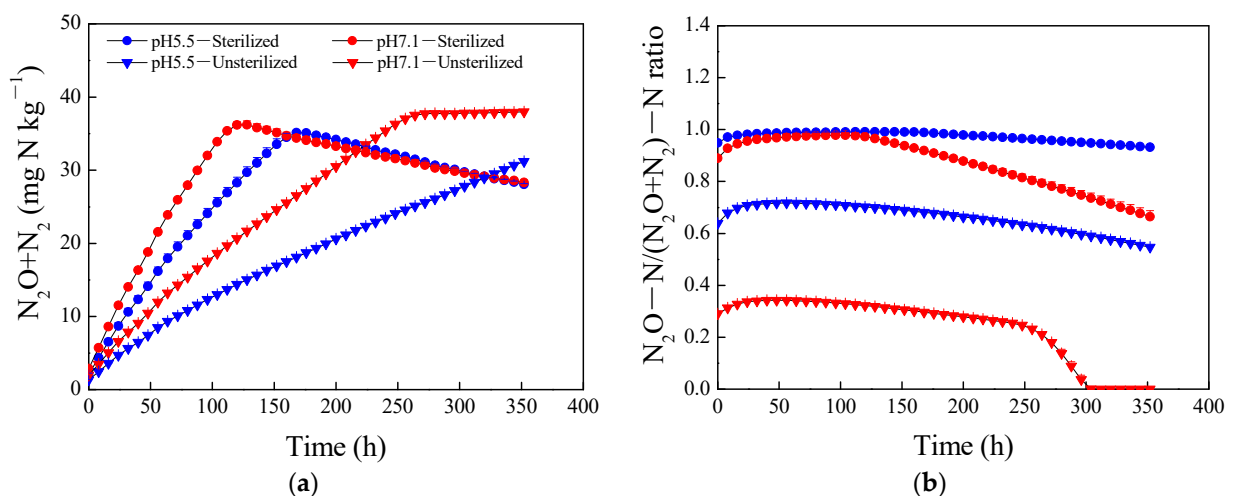


Figure 3. Dynamic changes in N_2O+N_2 (a) and $N_2O/(N_2O+N_2)$ ratio (b) in N64 treatments during the incubation of sterilized and unsterilized soils. Error bars represent standard deviation ($n = 3$).

As shown in Figure 3b, $N_2O/(N_2O+N_2)$ product ratios increased during the first 48 h and then decreased slowly during the incubation in both sterilized and unsterilized soils,

which further indicated the reduction of N_2O to N_2 . It was found that $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratios in sterilized soils were significantly ($p < 0.01$) higher than those in unsterilized soils. In sterilized and unsterilized soils, $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratios ranged from 0.66 to 0.99 and 0.00 to 0.72, respectively. In sterilized soils, $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratios were lower at higher pH, especially at the end of incubation, which was mainly due to the decrease in absolute N_2O emissions (Figure 2a). A similar result was observed in unsterilized soils, but it was probably due to both the decrease in absolute N_2O emissions (Figure 2b) and increase in total gas emissions (Figure 3a).

4. Discussion

4.1. The Potential Importance of Abiotic Denitrification to N_2O Emission

Our study suggested that there was a substantial N_2O emission through abiotic nitrate reduction in forest soils. The accumulative emissions of N_2O through abiotic nitrate reduction ranged from 29.42% to 40.88% of the added nitrate, which were significantly higher than those under unsterilized conditions (up to 26.67% of the added nitrate). However, most previous research conceived that N_2O released from upland soils after nitrate addition was predominantly by microbial denitrification [41,42]. Parkin et al. (1985) found no N_2O emissions from sterilized (achieved by autoclaving) neutral soil after nitrate addition [41]. Cayuela et al. (2013) suggested that sterilization (achieved by autoclaving) of agricultural soils completely eliminated N_2O production from nitrate [42]. Several studies also demonstrated the emission of N_2O from sterilized soils after NO_2^- addition, but the release was not sufficient to compare with the biological process [41,43,44]. Nägele and Conrad (1990) found that abiotic emission of N_2O from an acidic forest soil accounted for only 6% of the nitrite loss [43]. Venterea (2007) estimated that 31%–75% of total N_2O production came from abiotic NO_2^- reduction in an agricultural soil [44]. The common explanation for the different contributions of abiotic nitrate and nitrite reduction to N_2O emissions is that the process of nitrate reduction to nitrite is mainly mediated by microorganisms. This may be plausible, since previous works undertaken in aqueous and slurry systems suggested that the abiotic nitrate reduction to nitrite was the rate-limiting step of abiotic denitrification [32,45–47]. However, our study suggested that nitrate was rapidly reduced by abiotic process and nitrite was found as a transient intermediate. Our results illustrated that abiotic nitrate reduction is an alternative and potentially important pathway for nitrate loss and N_2O emission from acidic and neutral forest soil after sterilization. However, the results from sterilized soils could not clarify the absolute contribution of abiotic denitrification to N_2O emission under unsterilized conditions, since the total N_2O emission was the result of a combination of abiotic and biotic processes.

4.2. The Effect of pH on Denitrification Rate

Our results showed that the rates of denitrification (i.e., the rates of nitrate loss) at pH 7.1 were significantly higher than those at pH 5.5, in both sterilized and unsterilized soils. The direct effect of pH on nitrate reduction in unsterilized soils was commonly due to its effect on the bacterial community composition and the activity of nitrate reductase (Nar/Nap) [48,49]. Early studies have suggested that pH was an important driver of de-nitrifier community composition and the optimum pH for cultures of de-nitrifiers was in the neutral area [48,50]. In addition, the activity of nitrate reductase was also closely related to pH. Richardson et al. (2009) suggested that nitrate reductase was generally more active at $\text{pH} < 7$ [49]. Townsend (1970) showed that less nitrate reductase activity was found at pH 4.0 than that at pH 6.0 [51]. Generally, the optimum pH for bacteria strains and nitrate reductase activity seems to be neutral, which strongly supports our results. Furthermore, the influence of pH on the availability of organic carbon to de-nitrifiers might also indirectly affect N_2O emissions from denitrification [52,53]. The direct effect of pH on abiotic nitrate reduction was usually attributed to the role of H^+ . Previous studies have suggested that higher pH was more conducive to the reduction of NO_3^- by Fe^{II} since H^+ was involved as the final product [22,32,54]. In addition, the indirect influence of pH on

abiotic denitrification rate might be attributed to its effect on the availability of organic carbon and electron donors (especially Fe^{II}). Iron involved in abiotic denitrification is usually associated with minerals and soil organic matter and is predominantly in the form of Fe^{II} and Fe^{III} [55]. The Fe^{II} - Fe^{III} system could be used as both a reducing agent and oxidizing agent, which depended mainly on the reaction system of pE-pH conditions [56]. Compared with our previous study in agricultural soils [22], we found that at the same pH (7.1) nitrate reduction rates under sterilized and unsterilized conditions were significantly higher in this study. This was mainly due to the significantly higher contents of SOC and iron in this study.

4.3. The Effect of pH on N_2O Emission and Gaseous Product Stoichiometry

Our results showed that the cumulative emissions of N_2O and $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratios substantially decreased with increasing soil pH under unsterilized conditions, which were in accordance with general results in previous studies [24,26,52,57]. Thus, increasing soil pH by lime, biochar and dolomite application has been studied as a means to mitigate soil N_2O emissions [24,26,39,42,58,59]. Studies from long-term liming experiments showed decreases in N_2O emission and $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio with increase in soil pH [24]. Under laboratory conditions, similar conclusions were obtained during microbial denitrification from short-term liming experiments [26]. Recently, Obia et al. (2015) suggested that increase of soil pH by biochar can also suppress N_2O emissions while increasing N_2 emissions [58]. The underlying biological mechanism for the above conclusions is that high soil pH enhances the activity of N_2O reductase, thus facilitating the reduction N_2O to N_2 [24,28]. Liu et al. (2010) implied that the detrimental effect of low pH on N_2O reductase occurred at a post-transcriptional level, either by interfering with translation, protein assembly, or by directly affecting the activity of the functional enzymes [24]. Bergaust et al. (2010) demonstrated that the translation or protein assembly of N_2O reductase was positively affected by high pH [28]. However, we were unable to find published data that analyzed the effects of pH increase on N_2O and N_2 emissions during abiotic denitrification incubation in acidic forest soil. Our results under sterilized conditions demonstrated a negative control of pH on both the cumulative emissions of N_2O and $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio as well as under unsterilized conditions, but the mechanisms may differ. Low N_2O cumulative emissions and low $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio at high pH under sterilized conditions was probably due to the rapid reduction of N_2O by Fe^{II} at high pH. Moraghan and Buresh (1977) showed that N_2O was stable in the Fe^{II} medium in the presence of Cu^{2+} at pH 6, while it was rapidly reduced to N_2 at pH 8 [60].

4.4. Differences in the Responses of Abiotic and Biotic Denitrification to Soil pH

Under both sterilized and unsterilized conditions, our results suggest that high pH favored nitrate reduction, nitrite transient accumulation, N_2 emission and conversion of N_2O to N_2 . However, the responses of total gas emissions (i.e., $\text{N}_2\text{O}+\text{N}_2$) under sterilized and unsterilized conditions to soil pH were different. Under unsterilized conditions, total gas emissions increased with soil pH. It was obvious that oxidized N compounds, principally NO_3^- , NO_2^- and N_2O , were completely reduced to N_2 at pH 7.1, while N_2O dominated at pH 5.5. However, total gas emissions at the end of incubation in sterilized soils were substantially unaffected by soil pH. This might be attributed to the fact that abiotic nitrate reductions were incomplete at both pH 5.5 and 7.1. In addition, total gas emissions showed a decreasing trend in sterilized soils at both pH 5.5 and 7.1. This suggested that there might still be other nitrogen products, such as NO , NH_2OH and NH_4^+ , that were not measured. Previous studies reported that NO_3^- was reduced stoichiometrically to NH_4^+ by green rusts under neutral and alkaline conditions and by wüstite under slightly acidic conditions [31,33,61]. Van Hecke et al. (1990) found NH_2OH was produced as an intermediate during the reduction of NO_3^- to NH_4^+ in the presence of Fe^{II} and Cu^{II} in the

pH range 7.5 to 8.1 [62]. Homyak et al. (2017) suggested that acidity promoted abiotic NO production in drying soils with pH ranging from 4.4 to 6.2 [63].

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