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Chenxia Su and Ronghua Kang are contributed equally to this work.

Key Points:
- The δ15N-NO from soil (−62‰ to −23‰) is different from fuel combustion (0‰ to 20‰), allowing effective source partitioning
- The δ15N-NO produced under aerobic condition (favor nitrification) was more negative than under anaerobic condition (favor denitrification)
- The δ15N for NO production under aerobic condition (61 ± 3‰) was significantly higher than under anaerobic condition (35 ± 6‰)

Supporting Information:
- Supporting Information S1

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δ15N of Nitric Oxide Produced Under Aerobic or Anaerobic Conditions From Seven Soils and Their Associated N Isotope Fractionations

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Abstract Measuring the nitrogen isotope compositions (δ15N) of nitric oxide (NO) from different sources helps to quantify the relative contributions of atmospheric NOx. Soil is one of the most important sources of atmospheric NOx, but only limited measurements on the δ15N of soil-emitted NO exist, hampering our ability to partition sources to air pollution. Here we conducted soil incubations to measure the δ15N-NO under defined aerobic or anaerobic conditions, favoring either nitrification or denitrification. Soils were collected from seven sites spanning three ecosystems in northern China (two agricultural, two forest, and three grassland sites). We found that the δ15N-NO and their associated N isotope fractionations were significantly different between aerobic and anaerobic conditions in seven soils. Under aerobic condition, the δ15N-NO ranged from −62‰ to −50‰ (averaged −56 ± 4‰), being significantly more negative (by 23‰) than those under anaerobic condition (−45‰ to −23‰, averaged −33 ± 7‰). The apparent N isotope fractionation for NO production under aerobic condition (εaerobic = 61 ± 3‰) was significantly higher (by 26‰) than under anaerobic condition (εanaerobic = 35 ± 6‰), with a small variability among ecosystem types. Our study demonstrates that the δ15N-NO from different soils are very different from fuel combustions (mainly from 0‰ to +20‰), supporting that measuring 15N is a useful tool to partition the contributions of soil NO to atmospheric NOx. Our results also imply δ15N-NO produced by nitrification and denitrification distinctly different, as these two processes are dominant processes producing NO under aerobic and anaerobic conditions, respectively.

Plain Language Summary Nitric oxide (NO) affects the atmosphere chemistry and NO itself a key component of air pollution and a precursor to other air pollutants like particulate matter and ozone. To reduce the air pollution, it is crucial to identify the atmospheric NO sources. Measuring 15N natural abundance has been considered as a promising tool to identify different sources, but the measurement on δ15N for soil-emitted NO is limited, adding to the large uncertainties on NO partitioning. Here we measured the δ15N-NO emitted from three ecosystem types across seven sites (two agriculture, two forest, and three grassland sites) under defined anaerobic or aerobic conditions. We found that soil-emitted NO was 15N-depleted (−62‰ to −23‰) related to the source from fossil fuel combustion (0–20‰), suggesting that δ15N can be used to separate these two sources. We also found that the δ15N-NO under aerobic condition (nitrification prevailing, −62‰ to −50‰) was much lower than under anaerobic condition (denitrification prevailing, −45‰ to −23‰), which provide a useful tool to better understand the relative contributions of different sources of NO. In sum, soil-emitted NO has their own different isotope fingerprint, depending on the soil oxygen conditions, and can be used to partition sources.

1. Introduction

Atmospheric haze pollution is a major environmental problem in many regions of the world, which severely impacts visibility and human health (Lelieveld et al., 2019; Li et al., 2018; Pan et al., 2016). Nitrate (NO3−)
represents a major component of haze, and in China, accounts for 7% to 14% of fine particulate matter (PM$_{2.5}$) in the atmosphere (Huang et al., 2014). Nitric oxide (NO) is oxidized to NO$_2$ by hydroxyl (OH) and ozone (O$_3$), and further formed to NO$_3^-$, thus inducing the formation of haze (Wang et al., 2016). In addition, NO is highly reactive and plays an important role in atmospheric chemistry by influencing the production and destruction of ozone and thereby the oxidizing capacity of the atmosphere (Crutzen, 1979; Jacob et al., 1996). To reduce the atmospheric pollution, it is crucial to identify the sources of NO for a given region at a given time.

Globally, fossil fuel combustion and biomass burning are major sources of NO (IPCC, 2013), but soil is also an important source (Kang et al., 2017; Medinets et al., 2015). The global NO emitted from soils has been estimated between 4 and 21 Tg N year$^{-1}$ (Holland et al., 1999; Pilegaard, 2013; Yan et al., 2005), which is comparable to that from fossil fuel combustion (IPCC, 2013). In some regions with large rural agriculture, soil emission is much greater than combustion-related emission (Almaraz et al., 2018; Jaeglé et al., 2005; Miyazaki et al., 2017; Vinken et al., 2014; Williams et al., 1992). However, identifying the sources of atmospheric NO is challenging because of multiple anthropogenic and natural origins.

Stable nitrogen (N) isotope composition ($\delta^{15}$N) has been used as a promising tool to identify different sources of atmospheric NO (Elliott et al., 2019). For example, for anthropogenic sources including vehicle exhaust, coal-fired power-plant emission, and biomass burning, their $\delta^{15}$N values of NO range from $-19\%$ to $27\%$ (Felix & Elliott, 2014; Fibiger & Hastings, 2016; Heaton, 1990; Walters et al., 2015). Soil-emitted NO is typically $^{15}$N-depleted related to fuel combustion, as NO is predominantly produced by microbial processes, which exert a large discrimination against $^{15}$N. So far, there are limited measurements on $^{15}$N natural abundance of soil-emitted NO, and these studies show a wide range from $-60\%$ to $-12\%$ (Felix & Elliott, 2014; Homyak et al., 2016; Li & Wang, 2008; Miller et al., 2018; Yu & Elliott, 2017), yet the underlying mechanisms remain unknown. These results support the notion that soil-emitted NO is $^{15}$N-depleted. But these studies also call for more research on N isotope composition of NO produced from soil, particularly to link microbial processes associated with the NO production.

Microbial denitrification and nitrification are the dominant processes for the production of NO from soils under anaerobic and aerobic conditions, respectively (Conrad, 1996; Davidson et al., 1991; Firestone & Davidson, 1989), although other processes, including chemodenitrification and nitrifier denitrification (Medinets et al., 2015; Venterea et al., 2005; Wrage et al., 2001), can also contribute to NO flux in some soils. Denitrification is the stepwise anaerobic reduction of nitrate (NO$_3^-$) to nitrite (NO$_2^-$), NO, nitrous oxide (N$_2$O), and dinitrogen (N$_2$), while nitrification is the aerobic oxidation of ammonium (NH$_4^+$) via hydroxylamine (NH$_2$OH) to NO$_2^-$ and further on to NO$_3^-$ (Firestone & Davidson, 1989). Following the conceptual model of Hole-In-The-Pipe (HIP), NO is the intermediate product of denitrification and the byproduct of nitrification leaking from the denitrification and nitrification “process pipe” (Davidson et al., 2000; Firestone & Davidson, 1989).

Nitrogen isotope fractionation ($\epsilon$, defined as $^{14}$k/$^{15}$k $-$ 1, reported in $\%\epsilon$, with k being the rate constant) is used to quantify the contribution of particular pathways in the N cycle, and can be considered as an integrated signal of microbial processes for testing biogeochemical models (Chien et al., 1977; Denk et al., 2017; Mariotti et al., 1981). It is based on the fact that organisms transform compounds containing lighter isotopes ($^{14}$N) at a slightly higher rate than compounds containing heavier isotopes ($^{15}$N), leave the residual substrate pool enriched in $^{15}$N and the product depleted in $^{15}$N relative to the substrate (Robinson, 2001). Each of the microbial enzymatic step involved in nitrification and denitrification is likely to affect the isotope compositions of both product and substrate as the process progresses (Denk et al., 2017; Ostrom & Ostrom, 2012). N isotope fractionation has been used to apportion production of N$_2$O (another important trace gas with significant soil origin) to denitrification and nitrification; both bacteria pure culture studies and soil incubation experiments have shown greater N isotope fractionation of N$_2$O by nitrification ($\epsilon = 35\%\epsilon$ to $111\%\epsilon$) than by denitrification ($\epsilon = 9\%\epsilon$ to $30\%\epsilon$) (Barford et al., 1999; Denk et al., 2017; Mariotti et al., 1981; Menyailo & Hungate, 2006; Pérez et al., 2006; Yoshida, 1988). Because of the similar microbial processes involved for both NO and N$_2$O production, we hypothesized that the $\delta^{15}$N values of NO derived from nitrification also might be more negative than that by denitrification, and that such difference might be able to explain the large range of $\delta^{15}$N values observed in previous NO studies (Felix & Elliott, 2014; Homyak et al., 2016; Li & Wang, 2008; Miller et al., 2018; Yu & Elliott, 2017). Consequently, it will be possible to evaluate the
relative contribution of denitrification and nitrification to soil-emitted NO by measuring the isotope composition of NO. Up to now, however, there have been no N isotope fractionation being reported for soil-emitted NO from denitrification and nitrification processes.

In this study, we conducted laboratory incubation experiments with soils from three ecosystem types across seven sites, including two temperate forest, two agricultural, and three grassland soils. Soils were incubated under defined conditions favoring either denitrification (headspace filled with N₂ and added nitrification inhibitor DCD, hereafter called anaerobic incubation) or nitrification (headspace filled with ambient air, hereafter called aerobic incubation). We used passive samplers to collect NO released from soils during the anaerobic and aerobic incubations and measured N isotope compositions in the emitted NO as well as the remaining soil NO₃⁻ and NH₄⁺. The main objectives of this study are the following: (1) to characterize the δ¹⁵N values of NO produced under the anaerobic condition (favoring denitrification) and under the aerobic condition (favoring nitrification); and (2) to estimate N isotope fractionation associated under these two conditions; (3) to explore the impact of different ecosystem types and soils on the δ¹⁵N-NO under these two conditions and their associated N isotope fractionations. Based on this study, we expect to better constrain the δ¹⁵N values of NO values produced from soil, to explore the mechanism responsible for the wide range of δ¹⁵N values of soil-produced NO observed in previous studies, and thereby to provide a potential tool to evaluate the relative contribution of denitrification and nitrification to soil NO production.

2. Materials and Methods
2.1. Experimental Sites and Soil Sampling
Soils were collected from three ecosystem types across seven sites in northern China, including two temperate forest, two agricultural, and three grassland soils (Shenyang [SY-A], Jilin [JL-A], Qingyuan larch forest [QY-LF], Qingyuan mixed forest [QY-MF], Erguna [EG-G], Haibei [HB-G], Duolun [DL-G]), spanning a large geographic area (Figure 1, suffix “A, F, and G” indicate “agriculture, forest, and grassland,” respectively). Soil texture ranges from sandy to clay loam (Table 1). At each site, about 60 soil cores (5 cm diameter) of the surface 10 cm soils were collected, mixed, and air-dried; dried soils were sieved through 2 mm to remove coarse fragments, and refrigerated at 4°C. Detailed soil properties are listed in Table 1.

2.2. Soil Incubation Experiment
We conducted laboratory incubation experiments under anaerobic (headspace filled with pure N₂, to facilitate denitrification) or aerobic (headspace filled with ambient air, to facilitate nitrification) conditions. Before the incubation, all soils were preincubated at 40% WHC (water holding capacity) by adding deionized water for 7 days. The purpose of the preincubation was to activate microbial processes and avoid the pulse of respiration associated with wetting dry soils (Kieft et al., 1987; Zhu et al., 2013). After the preincubation, the NH₄⁺ and NO₃⁻ concentration of forest soils changed dramatically (the concentration of NH₄⁺ increased to 150 mg N kg⁻¹ and the concentration of NO₃⁻ increased to 40 mg N kg⁻¹), but agricultural and grassland soils did not. Thus, we adjusted both NH₄⁺ and NO₃⁻ concentrations to 150 mg N kg⁻¹ for the forest soils but kept those for agricultural soils at 50 mg N kg⁻¹. For the three grassland soils, we first incubated HB-G soils, and adjusted the concentration to 50 mg N kg⁻¹ for both NH₄⁺ and NO₃⁻; but noticed under anaerobic condition, all NO₃⁻ was consumed within 2 days. So for other two grassland soils (EG and DL), we adjusted initial concentration to 100 mg N kg⁻¹ NH₄⁺ and 100 mg N kg⁻¹ NO₃⁻. Thereafter, soil moisture in all soils were adjusted to 60% WHC which is optimal for microbial activity and not affecting gas diffusion (Davidson et al., 2000; Franzluebbers, 1999; Linn & Doran, 1984).

After adjusting N concentration and water content, 120 g fresh-weight soils (about 87–98 g dry-weight equivalent) were transported into individual 0.5 L incubation jars (diameter = 8.6 cm, height = 18 cm) to start formal incubation. To establish condition favoring nitrification, the soil samples were incubated in ambient air and were aerated by removing the stoppers for 30 min every 2 days throughout the experiment to maintain aerobic condition. To favor denitrification, each jar was vacuumed and then flushed with pure N₂ (99.999%) at ca. 500 ml min⁻¹ for 5 min, this procedure was repeated three times. We added nitrification inhibitor DCD (C₂H₄N₄, at the concentration of 10% of applied NH₄⁺) into soils to inhibit nitrification under anaerobic condition, while we did not carry out the inhibition of denitrification under aerobic condition due to the lack of technique currently. All jars were incubated at 25°C in the dark. We set five sampling time,
which is on days 0, 1, 3, 5, and 7 after the N addition (except for the soil collected from Shenyang, which was not sampled at day 5), with four replicates at each sampling time. For each soil, the total number of incubation jars was 40 (five sample times × 4 replicates × 2 treatments). Jars were destructively sampled at each sample time to determine the concentration and isotope compositions of cumulative NO produced and the remaining soil KCl-extractable NH$_4^+$ and NO$_3^−$. At the beginning of incubation (day 0), no NO was collected.

2.3. Soil Extract and Isotopic Analysis

At each sampling time, 10 g soil from each jar was extracted by 50 ml of 2 M KCl and shaken for 1 hr at 200 rpm before being filtered (20 μm pore size, Whatman, UK). The NO$_3^−$ and NH$_4^+$ concentrations in the filtrates were measured by a SmartChem instrument 200 discrete chemistry analyzer (Westco Scientific Instruments, Inc., Italy). The $\delta^{15}$N values of NO$_3^−$ were determined by the denitrifier method (Sigman et al., 2001), which converts NO$_3^−$ and NO$_2^−$ to N$_2$O by a denitrifying bacterium (Pseudomonas).

Table 1

| Site   | Land use     | Climate                | MAT (°C) (mm) | Soil texture | pH (H$_2$O) | Bulk density (g cm$^{-3}$) | Total C (%) | Total N (%) | NH$_4^+$ (mg N kg$^{-1}$) | NO$_3^−$ (mg N kg$^{-1}$) |
|--------|--------------|------------------------|---------------|--------------|-------------|---------------------------|--------------|--------------|--------------------------|---------------------------|
| SY-A   | Maize cropland | Temperate monsoon      | 7.5           | Silt loam    | 5.6 ± 0.03  | 1.2 ± 0.02                | 1.1 ± 0.04   | 0.13 ± 0.01 | 5.0 ± 0.5            | 6.7 ± 0.6                  |
| JL-A   | Maize cropland | Temperate monsoon      | 5.2           | Clay loam    | 6.5 ± 0.01  | 1.1 ± 0.02                | 1.8 ± 0.01   | 0.19 ± 0.01 | 0.9 ± 0.2             | 17.6 ± 0.5                 |
| QY-LF  | Larch forest  | Temperate monsoon      | 4.7           | Silt loam    | 5.4 ± 0.03  | 0.9 ± 0.02                | 6.0 ± 0.04   | 0.56 ± 0.01 | 8.7 ± 0.0             | 17.7 ± 0.2                 |
| QY-MF  | Mixed forest  | Temperate monsoon      | 4.7           | Silt loam    | 5.9 ± 0.01  | 0.6 ± 0.01                | 5.1 ± 0.18   | 0.43 ± 0.01 | 8.0 ± 0.2             | 25.2 ± 0.2                 |
| EG-G   | Meadow grassland | Temperate continental | −2.4          | Sandy clay loam | 6.9 ± 0.02  | 1.3 ± 0.05                | 2.3 ± 0.01   | 0.21 ± 0.01 | 2.1 ± 0.1             | 0.7 ± 0.3                  |
| HB-G   | Alpine grassland | Temperate continental | −1.7          | Silt loam    | 6.5 ± 0.03  | 0.7 ± 0.02                | 6.7 ± 0.01   | 0.62 ± 0.01 | 6.1 ± 1.7             | 6.0 ± 0.1                  |
| DL-G   | Typical grassland | Temperate monsoon      | 2.1           | Sandy loam   | 6.7 ± 0.01  | 1.4 ± 0.02                | 2.6 ± 0.01   | 0.26 ± 0.01 | 2.3 ± 0.4             | 7.3 ± 0.0                  |

Note. Data shown are means ± standard deviations (n = 4). MAT is mean annual temperature; MAP is mean annual precipitation. A = agriculture; F = forest; G = grassland.
aurofaciens) that lacks the N₂O reductase. Four international standards (USGS-32, USGS-34, USGS-35, and IAEA-N3) were included in each batch to calibrate δ¹⁵N – NO₃⁻ samples. The δ¹⁵N values of the produced N₂O were determined by an IsoPrime100 automated continuous flow isotope ratio mass spectrometer (IsoPrime Ltd, Stockport, United Kingdom) coupled with an autosampler (Gilson, Inc., Middleton, WI) and a Trace Gas Pre-concentrator cryogenic unit (IsoPrime Ltd). Detailed instrumental information can be found in Liu et al. (2014) and Zhang et al. (2015). Isotopic data are reported as δ values, where δ = ((R_sample/R_standard) – 1) × 1,000, R = ¹⁵N/¹⁴N. The δ¹⁵N values of NH₄⁺ were determined by the method of microdiffusion followed by hypobromite oxidation and subsequent hydroxylamine reduction (Zhang et al., 2015). Three international standards (USGS-25, USGS-26, and IAEA-N1) were included in each batch to calibrate δ¹⁵N – NH₄⁺ of samples. Contents of soil total carbon (TC) and total nitrogen (TN) were determined by an elemental analyzer (Elementar Analysen systeme GmbH, Germany). Soil pH was determined in a 1:2.5 soil-water suspension.

2.4. NO Collection

We used NOx pads (Ogawa & Co., United States) to capture the NO released from the soil incubation. NOx pads have been routinely used to monitor air pollution and recently adopted to measure emitted NO₂ using a chemiluminescence NOx analyzer (42i-TL, Thermo Electron Corporation, Waltham, MA) in the field, and observed negligible NO₂ concentration (data not shown), suggesting that NO₂⁻ adsorbed by the NOx pads primarily originated from NO. Therefore, the NO amount was assumed equal to the amount of NO₂⁻ extracted from the Ogawa filter in this study. The δ¹⁵N values in NO captured as NO₂⁻ were analyzed using the denitrifier method as mentioned above (Sigman et al., 2001).

2.5. Isotope Fractionation Calculation and Statistical Analysis

We used Equations 1 and 2 to calculate the NO₃⁻ consumption rates and NH₄⁺ consumption rates in the experiment:

\[
\text{Nitrate consumption rates (mg N kg}^{-1} \text{ day}^{-1}) = \frac{(C_0 - C_t)}{t},
\]

\[
\text{Ammonium consumption rates (mg N kg}^{-1} \text{ day}^{-1}) = \frac{(C^*_0 - C^*_t)}{t},
\]

where \(t\) is the sampling time in days, \(C_0\) and \(C_t\) are the concentration of NO₃⁻ on day 0 (the starting time point) and day 7 (the terminating time point) of incubation, \(C^*_0\) and \(C^*_t\) are the concentration of NH₄⁺ on day 0 and day 7 of incubation.

The incubation experiment here is in a closed system and the isotope dynamics can be assumed modeled as a Rayleigh distillation process (Mariotti et al., 1981; Rayleigh, 1896). Thus, we can calculate isotope fractionation factors according to the dynamics of δ¹⁵N values and concentrations of product and/or substrate. The kinetic isotope fractionation factor is defined as
\[ \alpha P/S = R_{Pi}/R_{St}, \]  

where \( R_{Pi} \) and \( R_{St} \) represent the isotope ratios of the instantaneous products and substrates at time \( t \), respectively (Chien et al., 1977; Mariotti et al., 1981). On the basis of the isotope composition (3) definition and approximation \( \delta/1,000 \ll 1 \), Equation 3 can be rewritten as

\[ \varepsilon = \delta_{St} - \delta_{Pi}, \]  

where \( \varepsilon \) is the fractionation effect and equals to \( 1,000 (\alpha - 1) \) (Mariotti et al., 1981).

In the initial phase of the reaction, isotope composition of substrate is almost constant, and \( \delta_{Pi} \) is nearly equal to the isotope ratios of accumulated product \( \delta_{Pt} \). \( \varepsilon \) can be calculated as the equation

\[ \varepsilon = \delta_{S0} - \delta_{Pt}, \]  

where \( \delta_{S0} \) denotes the isotope compositions of the initial substrate.

As the reaction proceeds, substantial amount of substrate was consumed, and the isotope composition of substrate changed, so Equation 5 does not hold. The \( \varepsilon \) can be calculated based on the changes in the isotope compositions of accumulated product NO (\( \delta_{Pt} \)) and the progress of the reaction (\( f \)), using the following equation:

\[ \delta_{Pt} = \delta_{S0} - \varepsilon \ln f/(1 - f), \]  

where \( f \) is expressed by the fraction of remaining substrate relative to the initial substrate amount.

We can also calculate the \( \varepsilon \) according to the changes in isotope compositions of the remaining substrate as the reaction proceeds, using the following equation:

\[ \delta_{St} = \delta_{S0} + \varepsilon \ln f. \]

In our study, there are some uncertainties on the calculated \( ^{15}\varepsilon \) values based on Equations 6 and 7, shown in the supporting information. Therefore, the calculation of N isotope fractionation for NO production under aerobic or anaerobic conditions were mainly based on Equation 5.

All reported values were expressed on a soil dry weight basis. Statistical analyses were performed using SPSS software (version 22.0; SPSS Inc., Chicago, IL), including analysis of variance and Pearson correlation analysis. Kruskal-Wallis test was utilized to determine the difference of physiochemical properties, \( \delta^{15}\text{N}-\text{NO} \), isotope fractionation factors among ecosystems under anaerobic and aerobic conditions, respectively. Statistically significant differences were set at the \( p \) value of 0.05.

3. Results

3.1. Soil Properties

The soil properties determined in this study are summarized in Table 1 and varied widely among soil types and sites collected. The pH values ranged from 5.4 to 6.9; the pH of three grassland soils were significantly higher than those of the forest soils (\( p < 0.05 \), Table 1). Total C and N contents in agricultural soils were significantly lower than those from forest and grassland sites (\( p < 0.05 \)). Soil texture ranged from sandy to clay loam. The amount of inorganic N (\( \text{NH}_4^+ \) and \( \text{NO}_3^- \)) extracted from forest soils was the highest (Table 1).

3.2. Anaerobic Incubation

Under the anaerobic condition, which favor denitrification to occur, \( \text{NO}_3^- \) was consumed in all soils during the incubation. The \( \text{NO}_3^- \) consumption rates were highest in HB-G soil (23.6 mg N kg\(^{-1}\) day\(^{-1} \), Table 2), with its initial 50 mg N kg\(^{-1}\) \( \text{NO}_3^- \) completely consumed within just 2 days (Figure 2o). The \( \text{NO}_3^- \) concentration also decreased quickly in the two forest soils, from the initial 150 mg N kg\(^{-1}\) to around 75 mg N kg\(^{-1}\) on day 3, and was approaching zero on day 7 (Figure 2n). In other two grassland soils, \( \text{NO}_3^- \) consumption was relatively slow, 13.6 mg N kg\(^{-1}\) day\(^{-1}\) for EG-G and 9.1 mg N kg\(^{-1}\) day\(^{-1}\) for DL-G, respectively.
Table 2

| NO₃⁻ and NH₄⁺ Consumption Rates Under Anaerobic or Aerobic Conditions | Anaerobic condition | Aerobic condition |
|---------------------------------------------------------------|---------------------|------------------|
| Consumption rates (mg N kg⁻¹ day⁻¹)                          | NO₃⁻  | NH₄⁺  | NH₄⁺  | NO₃⁻  |
| SY-A                                                          | −1.0 ± 0.6 | 0.4 ± 0.1 | 0.0 ± 0.2 | 1.7 ± 0.6 |
| JL-A                                                          | −2.0 ± 0.2 | −0.2 ± 0.05 | −1.0 ± 0.5 | 6.3 ± 0.8 |
| QY-LF                                                         | −21.5 ± 0.1 | 7.5 ± 0.6 | 5.9 ± 0.3 | 0.7 ± 2.4 |
| QY-MF                                                         | −22.9 ± 0.4 | 5.7 ± 0.4 | 1.2 ± 0.3 | 3.1 ± 0.2 |
| EG-G                                                          | −13.6 ± 0.3 | 3.7 ± 0.3 | −6.6 ± 0.5 | 3.9 ± 0.2 |
| HB-G                                                          | −23.6 ± 0.1 b | 3.8 ± 0.4 | −6.0 ± 0.1 | 2.0 ± 0.2 |
| DL-G                                                          | −9.1 ± 0.7 | 0.7 ± 0.3 | −5.7 ± 0.3 | 2.0 ± 0.4 |

Note. The consumption rates based on 7 days data; positive data means net production and negative means net consumption. Data shown are means ± standard deviations (n = 4).

aNH₄⁺ consumed after 3 days in JL-A soil, so it is based on first 3 days data.

bNO₃⁻ consumed after 2 days in HB-G soil, so it is based on first 2 days data.

The δ¹⁵N values of NO₃⁻ increased in all seven soils during the incubation (Figure 2p–2r), while in HB-G soil, the δ¹⁵N values of NO₃⁻ increased from −1% to 17% on day 1, afterward decreased when NO₃⁻ was mostly consumed. For the two forest soils, the δ¹⁵N-NO₃⁻ values increased from around 0% on day 0 to 84% and 99% on day 7 (Figure 2q). In two agricultural soils, with only small consumption of NO₃⁻, the δ¹⁵N-NO₃⁻ values showed only slight increase over 7 days (Figure 2p). In the EG-G and DL-G soils, the δ¹⁵N values increased from −3% to 62% and from 1% to 25% throughout the 7-day incubation, respectively (Figure 2r). The δ¹⁵N values of soil NH₄⁺ remained constant in all seven soils over the entire incubation period (Figures 2j–2l).

The amount of NO produced accounted for 0.2% to 2.6% of the initial available NO₃⁻, being largest in the HB-G soil and smallest in the SY-A soil. Nitrogen isotopes in NO were depleted at the beginning of incubation in all soils and gradually became more enriched throughout the incubation, along with the soil δ¹⁵N-NO₃⁻. For the two forest soils, the δ¹⁵N values of NO increased from −26% and −23% on day 1 to −9% and 40% on day 7 (Figure 2e). Compare to the forest soil, the NO produced in the two agricultural and three grassland soils was enriched in δ¹⁵N much slowly (Figures 2d and 2f). The δ¹⁵N values of NO were highly depleted relative to the δ¹⁵N-NO₃⁻ (Figures 2d–2f and 2p–2r).

Equation 5 was used to calculate the N isotope fractionation factors (ε) of NO produced by soil denitrification. The ε ranged from 31% to 47% (averaged 35 ± 6%), among seven soils, being largest in the DL-G soil and smallest in the QY-LF soil (Table 3). There were significant differences of ε among ecosystems (Figure 5a), while no significant correlation between ε and soil properties (soil pH, C/N, and BD, Figure S1) was obtained. We also used Equations 6 and 7 to calculate the ε according to the changes in δ¹⁵N values of product or substrate (Figure 3). Based on the product NO, the ε ranged greatly from 7% to 79% (Figures 3a–3c; Table S1). Based on the substrate NO₃⁻, the ε ranged from 21% to 32% (Figures 3d–3f; Table S1). Significant and linear regressions between changes in N isotope composition and the remaining fraction of substrate were observed in most cases (Figure 3).

### 3.3. Aerobic Incubation

Under the aerobic condition, which favor nitrification to occur, ammonium concentrations of two agricultural and three grassland soils all decreased throughout the incubation, with the NO₃⁻ concentrations increased (Figure 4; Table 2). The NH₄⁺ was mostly consumed on day 3 in the JL-A soil and its NO₃⁻ concentration increased in the first 3 days and then stayed constant (Figures 4g and 4m). In contrast, NH₄⁺ concentration in the two forest soils increased during the incubation (Figure 4h), and the NO₃⁻ production rates of QY-MF and QY-LF were 3.1 and 0.7 mg N kg⁻¹ day⁻¹, respectively (Table 2).

The δ¹⁵N values of NH₄⁺ increased in three grassland and SY-A agricultural soils throughout the 7-day incubation, while in JL-A soil, the δ¹⁵N-NH₄⁺ values increased from 3% to 11% on day 1, afterward decreased when NH₄⁺ was mostly consumed (Figures 4j and 4l). In two forest soils, with increase of NH₄⁺ concentration (Figure 4h), the δ¹⁵N-NH₄⁺ values showed varying patterns (Figure 4k). The δ¹⁵N values of NO₃⁻ in the agricultural and grassland soils decreased significantly over the incubation period (Figures 4p and 4r), while in the two forest soils remained unchanged (Figure 4q).

The cumulative amount of NO collected was significantly lower than that in the anaerobic condition (Figures 2a–2c and 4a–4c). The amount of NO produced accounted for 0.09% to 1.1% of the initial
available NH$_4^+$, being largest in the DL-G soil and smallest in the QY-LF soil. For all soils, the $\delta^{15}$N values of NO produced were much more negative under aerobic condition than under anaerobic condition (Figures 2d–2f and 4d–4f). The range of $\delta^{15}$N-NO over the 7-day aerobic incubation (from $-62$‰ to $-6$‰).

**Table 3**

|                | SY-A     | JI-A     | QY-LF    | QY-MF    | EG-G     | HB-G     | DL-G     |
|----------------|----------|----------|----------|----------|----------|----------|----------|
| **Anaerobic Condition** | 32 ± 0.9 | 38 ± 0.8 | 31 ± 0.8 | 31 ± 0.4 | 36 ± 1.8 | 32 ± 2.9 | 47 ± 0.6 |
| **Aerobic Condition**   | 60 ± 2.1 | 62 ± 1.4 | 58 ± 1.7 | 57 ± 1.1 | 64 ± 1.4 | 64 ± 2.3 | 65 ± 0.7 |

*Note. Data shown are means ± standard deviations (n = 4).*

*In this equation, the $\delta_{50}$ is the average of $\delta^{15}$N values of NO$_3^-$ on day 0 and day 1 of the incubation, and $\delta_{pt}$ is the $\delta^{15}$N values of NO in day 1 of the incubation.*
−50‰) were much smaller than those from the anaerobic condition (from −45‰ to 40‰) (Figures 2d–2f). With the substrate (NH₄⁺) consumed, the δ¹⁵N values of both substrate (NH₄⁺) and product (NO) were enriched, but no clear patterns for JL-A and two forest soils (Figure 4). The δ¹⁵N values of NO were highly depleted relative to the δ¹⁵N-NH₄⁺ (Figure 4).

We used Equation 5 to calculate the N isotope fractionation factors (¹⁵ε) of NO production under aerobic condition for all seven soils, and obtained a range from 57‰ to 65‰ with a mean of 61 ± 3‰ (Table 3). There were significant differences among ecosystems (Figure 5b), being highest in grassland soils (averaged 64‰, Table 3). There was no significant correlation between ¹⁵ε and soil properties (soil pH, C/N, and BD, Figure S1). The ¹⁵ε of NO production under aerobic condition were significantly higher than those under anaerobic condition (Table 3). We also used Equations 6 and 7 to calculate ¹⁵ε associated, according to the changes in δ¹⁵N values of product or substrate (Figure 6; Table 3). Based on the product NO, the ¹⁵ε ranged from 12‰ to 24‰ (Figures 6a and 6c). Based on the substrate NH₄⁺, the calculated ¹⁵ε ranged from 25‰ to 45‰ (Figures 6d and 6f).

4. Discussion

4.1. Dominant Processes Under Anaerobic or Aerobic Conditions

Generally, nitrification is the main process under aerobic condition, while denitrification dominates under anaerobic condition (Bollmann & Conrad, 1998; Davidson et al., 2000; Medinets et al., 2015; Pilegaard, 2013). However, attributing soil-emitted NO to particular microbial processes is difficult, because different processes can occur simultaneously in close proximity (Davidson, 1992; Zhu et al., 2013). In our study, we strictly controlled the conditions to favor either denitrification or nitrification. To establish
condition favoring denitrification, the soil samples were incubated in a N₂ atmosphere, and added DCD to inhibit nitrification, which has been used widely to inhibit nitrification without influence on denitrification (Bremner & Yeomans, 1986; Müller et al., 2002; Wang et al., 2018). In this study, to favor nitrification occurring, we adjusted soil moisture to 60% WHC and aerated the incubation jars every 2 days to reduce amounts of anerobic microsites. This aeration treatment is widely used for supporting nitrification in most previous studies (Lan et al., 2014; Tilsner et al., 2003; Zhang et al., 2011).

Under the anaerobic condition, NO was produced dominantly by denitrification, for which the reasons are presenting as follows. First, nitrate concentration, which is the substrate for denitrification, decreased quickly in forest and grassland soils and ^15^N in NO₃⁻ was progressively enriched (Figure 2), suggesting that denitrification was strongly favored as expected (Mariotti et al., 1988). Second, the temporal pattern of δ^15^N-NO (product) was consistent with those of δ^15^N-NO₃⁻ (substrate) (Figure 2), suggesting that NO was formed from the reduction of NO₃⁻. Under anaerobic condition, only denitrification and chemodenitrification can

Figure 4. Changes in the concentrations and ^15^N abundances of NO (a–c and d–f), NH₄⁺ (g–i and j–l), and NO₃⁻ (m–o and p–r) with time during the aerobic incubation. Shown are mean ± standard deviation (n = 4).
Figure 6. Changes in the isotope composition of product NO (a–c) and substrate NH$_4^+$ (d–f) as a function of reaction during aerobic incubation. The absolute values of the linear slopes represent the isotope fractionation factors ($^{15}$ε). Shown are mean ± standard deviation ($n = 4$).

Figure 5. The difference of N isotope fractionation for NO production among different ecosystems under aerobic and anaerobic conditions. Letters indicating statistical significance: ns = $p > 0.05$; * = $0.01 < p < 0.05$; ** = $p < 0.01$. 

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occur as the soils were also treated with nitrification inhibitor. Chemodenitrification, the chemical decomposition of NO$_3^-$, mainly occurs in acidic soils (pH < 5, Medinets et al., 2015; Van Cleemput & Baert, 1984; Venterea et al., 2005). If we assumed that chemodenitrification dominated in our study, soils incubated under both anaerobic and aerobic conditions will produce the same amount of NO, but the results showed that the amount of NO produced under aerobic condition accounted for less than 10% of the NO produced under anaerobic condition (except DL-G soil, Figures 2a–2c and 4a–4c). In other words, the chemodenitrification under anaerobic accounts for less than 10%. Thus, denitrification was the dominant process under anaerobic condition.

Under the aerobic condition, the decreasing NH$_4^+$ concentration (except in forest soils) accompanied by NO$_3^-$ increase suggests that nitrification is the dominant process (Figure 4). In addition, the increase of $\delta^{15}$N-NH$_4^+$ and decrease of $\delta^{15}$N-NO$_3^-$ support that nitrification was prevailing in the study soils (Figure 4). However, anaerobic microsites might exist within soil aggregates, so denitrification, chemodenitrification, and nitrifier denitrification (the pathway of nitrification in which NH$_4^+$ is oxidized to NO$_2^-$, followed by the reduction of NO$_2^-$ to NO, N$_2$O, and N$_2$ under low O$_2$ content) could occur. Consequently, our estimates of the $\varepsilon$ of NO attributed only to nitrification are likely underestimated and therefore conservative (i.e., the $\varepsilon$ of NO from nitrification should be even higher). Previous studies have found that with sufficient O$_2$ and NH$_4^+$ supply, nitrification is the predominant process for soil NO production (Ludwig et al., 2001; Robertson, 1989). A recent study that incubated soil at 60% WPFS under ambient air showed that soil anoxic volume was less than 3% within the first few days of study (Keiluweit et al., 2018). Zhu et al. (2013) suggested that at high O$_2$ level (21%), nitrification was the main process responsible for NO formation. Kool et al. (2011) reported that nitrifier denitrification could be inhibited by certain levels of NH$_4^+$. The high NH$_4^+$ concentrations, ambient air in headspace, and 60% WHC in our experiment would most likely inhibit nitrifier denitrification and denitrification to occur, especially in the early stage of the incubation.

In all, our experimental design and the results imply that nitrification is the dominant process and denitrification is the predominant processes producing NO under aerobic and anaerobic conditions, respectively.

### 4.2. $\delta^{15}$N of NO Produced Under Aerobic or Anaerobic Conditions and Their Associated N Isotope Fractionations

We found much more negative $\delta^{15}$N-NO values from aerobic incubation than from anaerobic incubation (Figures 2d–2f and 4d–4f) in all seven soils from three ecosystem types collected from a wide geographic area that differed in texture, pH, and N status (Table 1). Since the $\delta^{15}$N values of produced NO were related to the substrate concentrations over time during the incubation, we suggest that $\delta^{15}$N-NO values in the initial phase (sampled on day 1) best represent the $\delta^{15}$N values of NO produced from either anaerobic or aerobic incubation. In the first day, the $\delta^{15}$N-NO produced under anaerobic and aerobic conditions ranged from $-45\%_\varepsilon$ to $-23\%_\varepsilon$ (averaged $-33 \pm 7\%_\varepsilon$) and $-62\%_\varepsilon$ to $-50\%_\varepsilon$ (averaged $-56 \pm 4\%_\varepsilon$), respectively (Figure 7). Our results agree with the findings in previous studies that soil-emitted NO can be readily separated from industrial sources due to their different $\delta^{15}$N values (Figure 8).

According to the first way to calculate isotope fractionation factors (Equation 5, the $^{15}$N-enrichment of substrate is minor), we found consistently higher $^{15}$N produced by aerobic incubation ($61 \pm 3\%_\varepsilon$) than by anaerobic incubation ($35 \pm 6\%_\varepsilon$) under our strictly controlled experimental conditions, independent of soil types (Table 3). Even under varying soil conditions, including initially adjusted NH$_4^+$ and NO$_3^-$ concentrations, we still obtained consistent isotope fractionation for NO production. The observed lower fractionation factors under anaerobic condition is most likely due to the intermediate role of NO in denitrification, which could be further reduced to N$_2$O and N$_2$. Lighter $^{14}$N in NO reacts faster than $^{15}$N, resulting in $^{15}$N enrichment in the remaining NO (Fry, 2006). The significant isotope effect between denitrification and nitrification may also represent different intrinsic enzymatic isotope effects involved in the two processes (Granger & Wankel, 2016). There are many research found that the N$_2$O production by denitrification (small fractionation) and nitrification (large fractionation) can be distinct by the N isotope fractionation (Denk et al., 2017), as mentioned in section 1. Our study showed that, similar to N$_2$O production, the $\varepsilon$ for NO production was larger during nitrification than denitrification, as these two processes are dominant under aerobic and anaerobic conditions, respectively. These results well support our expectations.
Figure 7. The $\delta^{15}$N values of product NO and their substrate (NO$_3^-$ and NH$_4^+$) over the first 24 hr incubation. Shown are mean ± standard deviation ($n = 4$).

Figure 8. The $\delta^{15}$N values of the NO produced from different sources. $a =$ Yu and Elliott (2017). $b =$ Felix and Elliott (2014). $c =$ Li and Wang (2008). $d =$ Miller et al. (2018). $e =$ Homyak et al. (2016). $f =$ Fibiger and Hastings (2016). $g =$ Heaton (1990). $h =$ Ammann et al. (1999). $i =$ Miller et al. (2017). $j =$ Walters et al. (2015). $k =$ Walters et al. (2015). $l =$ Felix et al. (2012).
4.3. Ecosystem Impact on $^{15}\varepsilon$ of NO Production Under Aerobic or Anaerobic Conditions

Under the anaerobic condition, the $^{15}\varepsilon$ of NO production from forest soils was significantly smaller than agriculture and grassland soils (Table 3). Previous studies reported that $^{15}\varepsilon$ was highly correlated with denitrification rates for the studied soils, with $^{15}\varepsilon$ exponentially decreasing with an increasing NO$_3^-$ consumption rates (expressed as rate constant $k_1$) (Mariotti et al., 1988; Wang et al., 2018). Similar to these findings, across forest and grassland soils in the present study, $^{15}\varepsilon$ was also found to exponentially decrease with the denitrification rates (Figure 9). However, the NO$_3^-$ consumption rates in two agricultural soils were much lower than the forest and grassland soils (Table 3), probably due to the limitation of available organic carbon (Table 1) required for denitrification (as an electron donor, Burgin et al., 2011). The $^{15}\varepsilon$ of NO produced from DL-G was much higher (47‰) than other soils (range from 31‰ to 38‰), probably because the soil sand content (62.8%) in DL-G was much higher than other soils, which is favorable to the escape of lighter $^{14}$NO before being further reduced. Pérez et al. (2000) found that the bulk $^{15}$N isotopic composition of N$_2$O showed large differences associated with soil texture. Another uncertain factor that we cannot assess in the present study is that different microbial community is likely responsible for the differences of $^{15}\varepsilon$ for NO production in soils from different ecosystem types. It is worth to make an effort to include some characterization of the microbial communities in incubated soils in future studies.

Under the aerobic condition, the $^{15}\varepsilon$ of NO production had a smaller variability with ecosystem types (by 8‰, range from 57‰ to 65‰) compared to the anaerobic condition (by 16‰, range from 31‰ to 47‰, Table 3). Therefore, we speculated that the microbial communities involved in aerobic incubations in different ecosystem types follow similar enzymatic pathways to produce NO, leading to similar $^{15}$N fractionation. The microbial groups involved in nitrification were relatively simple, while those involved in other processes were complex (Bernhard & Bollmann, 2010; Philippot et al., 2007), indicating nitrification dominant under aerobic condition. The NH$_4^+$ consumption rates in agricultural and grassland soils were higher than the forest soils (Table 2), a possible reason for the higher NH$_4^+$ consumption in agricultural soils is that nitrifier communities are more abundant and have adapted to high N contents from intensive fertilization, plus the aerobic condition due to plowing (Liu et al., 2017). In forest soils, the consumption of NH$_4^+$ in nitrification can be compensated by NH$_4^+$ production through N mineralization. The lowest NO$_3^-$ production rates of QY-LF may be also affected by the soil pH (pH = 5.4); several studies indicate that low pH can depress the kinetics of nitrification (Cheng et al., 2004; Ste-Marie & Paré, 1999).

Figure 9. Relationship between N isotopic effect ($^{15}\varepsilon$) and denitrification rates (first order) under anaerobic conditions in our study in comparison with previous studies.
5. Implications and Conclusions

Our detailed understanding of process-based and condition-dependent N isotope fractionations (Table 3) can help to evaluate relative contributions from different microbial processes to soil-emitted NO. For example, Li and Wang (2008) measured $\delta^{15}N$-NO from a vegetable field fertilized with ammonium bicarbonate and urea, and speculated that NO emitted 2 days after fertilization was mainly produced by nitrification; their $\delta^{15}N$ value of NO near $\sim 50\%$ is in line with our laboratory result. Yu and Elliott (2017) reported in a laboratory study that the initial pulse NO (when soil was wet at 100% WHC) had less negative $\delta^{15}N$ value ($\sim 37\%$) than the NO emitted after 1 day of rewetting when soil moisture declined to 40% WHC ($\sim 54\%$). Their interpretation of a shift from initial denitrification to later nitrification is supported by the N isotope fractionation we reported in this study. Homyak et al. (2016) reported a study on the dynamic process of NO emissions from natural grassland soils and the interactive controls of aridity and plant uptake. The $\delta^{15}N$ values of NO released from their rewetting soils were the highest within 15 min postwetting ($\sim 12\%$), likely from abiotic transformations of NO$_2^\cdot$ to NO through chemodenitrification (quick reaction), and gradually decreased after 24 hr ($\sim 43\%$), when nitrification contribution increased coincident with the decline of soil moisture. Our measurement and observed N isotope fractionation under different incubation conditions well support their interpretations.

In sum, our results provide solid evidence for that soil-emitted $\delta^{15}N$‐NO have a large range from $\sim 61\%$ to $\sim 23\%$. These values were very different from other sources, e.g., biomass and fossil fuel burning, which have values of 0% to $+20\%$. Our results confirm that soil NO can be readily separated from industrial sources by measuring $^{15}$N natural abundance. In addition, for the first time, we have separately quantified the N isotope fractionation of NO production under anaerobic condition (denitrification prevailing) and aerobic condition (nitrification prevailing). That, in combination with more intensive measurement of the $\delta^{15}N$ values of soil-emitted NO along with their substrate NH$_4^+$ and NO$_3^-$, plus better understanding of soil characteristics and soil management, can greatly aid our understanding of NO biogeochemistry. The fact that we obtained distinctly different N isotope fractionation under different oxygen conditions in all seven soils across three ecosystem types (agriculture, forests, and grasslands) further adds to the robustness of our finding and can well explain the large range of $\delta^{15}N$-NO observed in previous studies.

Data Availability Statement

Data sets for this research are available in these in-text data citation references: Su et al. (2020) (https://datadryad.org/stash/share/qLjdR-vRL5TdoNelTf_mZG_JYeRVEffNu4Cp5wy99nc).

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