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Key Points:

- N addition increased soil N_2O emission in the valley across the 3 years
- High N addition stimulated N_2O emission on the slope at the early stage
- Moderate N stimulated greater N_2O emission in the valley than on the slope

Supporting Information:

Supporting Information may be found in the online version of this article.

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Responses of Soil Nitrous Oxide Emission to Nitrogen Addition at Two Topographic Positions of a Subtropical Forest

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Abstract Topography can influence nitrous oxide (N_2O) emission via its influences on soil nutrient availability, moisture, and microbial communities. Nevertheless, it is still unclear whether topography modulates the responses of soil N_2O emissions to elevated N deposition. Here the N addition experiment was conducted in the valley and on the slope of a subtropical karst forest in southwest China. Nitrogen was applied as NH_4NO_3 in two levels, that is, 50 (moderate N) and 100 (high N) kg N ha^{-1} yr^{-1} with no N addition plots as the control. Nitrogen addition consistently increased N_2O emission in the valley, but only high N addition significantly increased N_2O emission on the slope in 2017. The cumulative N_2O fluxes across the 3 years were 1.16 ± 0.24 kg N ha^{-1} in the valley and 1.50 ± 0.06 kg N ha^{-1} on the slope under the control. Nitrogen addition stimulated N_2O emission by 88.7%–113.3% in the valley due to increased ammonium, nitrate and dissolved organic carbon availabilities and ammonia-oxidizing bacteria (AOB) *amoA* abundance. High N addition stimulated N_2O emission by 84.3% on the slope owing to increased nitrate and carbon availabilities, AOB *amoA,* and *nirK* abundances. The stimulation of N_2O emission by moderate N addition was more pronounced in the valley than on the slope largely owing to the lower N status in the valley. This work highlights the importance of N status in regulating the responses of soil N_2O emissions to elevated N deposition.

Plain Language Summary Atmospheric N deposition is identified as one of the strongest driving factors responsible for the increase of forest soil N_2O emission, and topography can influence N_2O emission via its influences on soil nutrient availability, moisture, and microbial communities. However, there is uncertainty about whether topography modulates the responses of soil N_2O emissions to elevated N deposition. We investigated soil N_2O emission and related microbial functional gene abundances at two topographic positions under three N addition levels in a subtropical region of China. We find that the N addition consistently increased N_2O emission in the valley, but only stimulated N_2O emission at the early stage on the slope. Stimulation of soil N_2O emission by moderate N addition was more pronounced in the valley than on the slope. Our findings highlight the importance of N status in regulating the response of soil N_2O emissions to elevated N deposition.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas in the troposphere with an atmospheric lifetime of ~116 years and contributes to ozone depletion in the stratosphere (Oikawa et al., 2015; Ravishankara et al., 2009). Forest soils are a significant source of atmospheric N_2O with a mean flux of 3.62 ± 0.16 Tg N yr^{-1} (1 Tg = 10^{12} g) and an increment rate of about 9.9×10^6 g N yr^{-1} from 1992 to 2015 (Zhang et al., 2019). Atmospheric nitrogen (N) deposition is identified as one of the strongest driving factors responsible for the increase of soil N_2O emissions (H. Tian et al., 2019). However, the contribution of elevated N deposition to the increase of soil N_2O emission from forests is poorly constrained with a range from 92% to 739%, largely due to uncertainties in the understanding of mechanisms responsible for soil N_2O emission under elevated N deposition (Deng et al., 2020; Liu & Greaver, 2009). Considering that global N deposition is projected to be doubled in 2050 relative to the level of 100–115 Tg yr^{-1} in 2000 (Galloway et al., 2004), it is hence imperative to better understand the mechanisms underlying the impacts of elevated atmospheric N deposition on soil N_2O emission.

Multiple mechanisms, including alteration of N and phosphorus (P) status, an increase of ammonium (NH_4^+) and nitrate (NO_3^-) availability, and soil acidification, have been proposed to explain the responses of soil N_2O emission to elevated N deposition (Deng et al., 2020; Hall & Matson, 1999; Koehler et al., 2009; Martinson et al., 2013; Redding et al., 2016; Rowlings et al., 2012). However, the results from individual studies in the subtropical/tropical region are inconsistent. For instance, Han et al. (2019) found that experimental N addition (N addition hereafter) increased N_2O emission under N poor conditions via stimulating the abundances of nitrifiers and denitrifiers, but decreased N_2O emission in N rich conditions by depressing the abundances of denitrifiers in a subtropical forest. M. H. Zheng et al. (2016) observed significant increases in soil N_2O emission under N addition in the N rich but not in the N limited tropical forests. Müller et al. (2015) also reported that N addition only increased N_2O fluxes during the periods with high soil moisture content. Corre et al. (2014) also reported that the response of soil N_2O emissions from tropical forests to N addition was more pronounced in wet years than in dry years. It seems that initial N status and moisture contents affect the response of soil N_2O emission to N addition in the subtropical/tropical forests.

Soil N_2O is mainly produced in nitrification and denitrification processes (Butterbach-Bahl et al., 2013; Duan, Zhou, et al., 2019). During nitrification, N_2O production is regulated by *amoA* gene encoding the ammonia monooxygenase enzyme of ammonia-oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB; Prosser et al. [2020]). During denitrification, a key step, in which nitrite is reduced to nitric oxide (NO), is catalyzed by nitrite reductases encoded by *nirK* and/or *nirS* genes (Clark et al., 2012). Another important denitrification step for N_2O emission is the reduction of N_2O to N_2, which is catalyzed by nitrous oxide reductase encoded by the *nosZI* and/or *nosZII* genes, representing the only known microbial sink for N_2O (Hallin et al., 2018). Since soil N_2O emission is almost entirely controlled by microbial activities, the quantification and characterization of specific functional genes involved in nitrification and denitrification pathways help predict N_2O emission (Hu et al., 2015; Levy-Booth et al., 2014). For example, high N_2O emission from arctic peatlands is primarily fueled by nitrification mediated by AOA. Higher *nirK* than *nosZ* abundance in a subtropical forest induced more N_2O production than N_2O reduction, resulting in a high contribution of denitrification to N_2O emission (Han et al., 2018). Although microbial regulation of N_2O emission has been widely investigated (Banerjee et al., 2016; Duan, Zhou, et al., 2019; Srikanthasamy et al., 2018), limited studies have been conducted in terms of microbial regulation of soil N_2O emission in responses to N addition.

Topography controls soil N status, soil N transformation rates, soil organic carbon level, soil microbial community composition, and abundance, soil moisture, etc., which may subsequently affect soil N_2O emission (Arias-Navarro et al., 2017; Enanga et al., 2016; Stewart et al., 2014). For example, it has been reported that the lower slope positions are hot spots of N_2O emission due to high denitrification (Stewart et al., 2014; Yu et al., 2019). Topography can also influence N availability and the fate of atmospherically deposited N via its influences on soil nutrient availability, moisture, and microbial communities (Enanga et al., 2017). Since N status likely affects the response of soil N_2O emission to N addition as aforementioned, it is possible that the responses of soil N_2O emission to N addition would vary among topographic positions.

In the current study, a field N manipulation experiment was conducted at two topographic positions, that is, in the valley and on the slope of a subtropical karst forest with calcareous soil in southwest China (Wang et al., 2019). Calcareous soils cover more than 30% of the Earth's surface (Bertrand et al., 2007). These soils are usually characterized by high Ca and Mg contents, and would therefore exhibit high soil buffering capacity to N fertilization (Wang et al., 2019; L. Zheng et al., 2020). Moreover, elevated N addition can promote nitrification in calcareous soil without acidification (Hao et al., 2020). We, therefore, hypothesized that N addition would increase soil N_2O emission at both topographic positions by increasing the abundance of nitrifiers because of the rapid utilization of N substrate from N addition (**H1**). In a previous study, we reported that asymbiotic N_2 fixation in soil was inhibited by N addition in the valley but not on the slope due to the difference in soil N status, which is higher on the slope than in the valley as indicated by NO_3^- leaching (Wang et al., 2019). Since soil N status is often found to affect the response of soil N_2O emission to N addition (Koehler et al., 2009), we hypothesized that topography would modulate the responses of soil N_2O emission to N addition (**H2**). Accordingly, the major questions we aimed to address include: (a) are the responses of soil N_2O emission to N addition in the valley and on the slope controlled by the same set of variables and (b) how does topography modulates the responses of soil N_2O emission to N addition?

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2. Materials and Methods

2.1. Site Description and Experimental Design

The N addition experiment was conducted in a subtropical karst forest in Mulun National Nature Reserve of Huanjiang County, southwest China (Wang et al., 2019). This forest was developed from extensively disturbed (biomass harvesting and grazing) shrubland 35 years ago. The area has a humid subtropical climate with mean annual precipitation and air temperature being 1,389 mm and 19°C, respectively. *Cryptocar yachinensis* (Hance) Hemsl., *Cinnamomum saxatile* H.W.Li, *Koelreuteria minor* Hemsl., *Pittosporum tobira* (Thunb.) Ait., *Bridelia tomentosa* Bl., and *Murraya exotica* L. Mant are the dominant tree species. The soil in the karst forest is classified as leptosols (limestone soil) according to the World Reference Base classification system. Soil depth in the valley and on the slope varies from 0.0 to 0.8 m and 0.0–0.3 m, respectively. Atmospheric N deposition rate was approximately 37 kg N ha^{-1} yr^{-1} according to Zhu et al. (2015).

The experiment was carried out in the valley and on the slope, respectively (Wang et al., 2019; Figure S1). For each topographic position, a randomized complete block design was adopted with three blocks and three N addition levels, that is, control (N0, 0 kg N ha^{-1} yr^{-1}), moderate N addition (N50, 50 kg N ha^{-1} yr^{-1}) and high N addition (N100, 100 kg N ha^{-1} yr^{-1}). Therefore, there are nine plots (10 × 10 m each) at each topographic position. Each plot was surrounded by a 10 m wide buffering zone to avoid potential influence from each other (Figure S1). Nitrogen was applied in the form of NH_4NO_3 at the beginning of each month since April 2016. On each N addition event, NH_4NO_3 was dissolved in 10 L of water (equivalent to 1.2 mm precipitation per year) and sprayed with a back–pack sprayer. The control plots received an equal amount of water.

2.2. Determination of N_2O Fluxes

At each plot, two static PVC chambers were deployed for the determination of N_2O fluxes twice a month from November 2016 to October 2019. Each chamber consisted of an anchor ring (25 cm in inner diameter and 10 cm in height, inserted 3 cm into the soil) and a removable cover chamber (25 cm in inner diameter and 35 cm in height). Sampling was conducted between 8:00 and 11:00 in the morning at the mid and end of each month. It should be noted that the sampling scheme was chosen to avoid measuring N_2O fluxes immediately after N addition when transitory peaks were expected. Gas samples were taken at 0, 10, 20, and 30 min after chamber closure using a 60 mL plastic syringe and transferred immediately into a 12–ml pre-evacuated glass vial (Labco Exetainer, Labco Limited, UK). N_2O was measured using a gas chromatograph equipped with an FID detector (Agilent GC 7890A, Agilent, USA) within 1 week after collection. We considered the linearity and nonlinearity of flux calculation when designing the sampling (Levy et al., 2011). First, we conducted a preliminary experiment to determine the time range in which N_2O concentration in the chamber increased linearly. We found that N_2O concentration in the chamber increased linearly within 60 min. Second, we assessed each group of data to judge whether N_2O concentration increased linearly within 30 min when calculating N_2O flux. We found that linearity was applicable to all the data sets, so the N_2O fluxes were calculated from the linear increase of N_2O concentration in the chamber versus time and were adjusted for the field-measured air temperature and atmospheric pressure (Koehler et al., 2009). Cumulative N_2O emissions were calculated assuming constant flux rates between two sampling dates (Duan, Zhang, et al., 2019).

2.3. Auxiliary Analysis

Soil temperature and volumetric moisture at 5 cm depth were simultaneously measured using a digital thermometer (JM 624, Jinming Instrument Co. Ltd., Tianjin, China) and a portable soil moisture meter (Mode TZS–1K, Zhejiang Top Instrument Corporation Ltd., China), respectively, along with gas sample collection. Volumetric soil moisture content was further converted to a percentage of water-filled pore space (%WFPS) based on soil bulk density and volumetric water content.

Soil samples to a depth of 0–10 cm were collected once every 3 months after litter removal. At each plot, three soil samples were collected using a soil corer of 2 cm in diameter and mixed thoroughly to form a composite soil sample. The soils were sieved through a 2–mm mesh sieve and were separated into two parts. Samples were extracted within 24 hr after field sampling. Soil NH_4^+ and NO_3^- were extracted with 2 M KCl (w/v of 1/5) and analyzed by an auto-analyzer (FIAstar 5000, FOSS, Sweden). DOC was extracted with 0.5 M K_2SO_4 (w/v of 1/5) and analyzed by the wet–oxidation method (Carter & Gregorich, 2007). Soil NH_4^+, NO_3^-, and DOC intensities (mg N or C g^{-1} d^{-1}) were calculated using trapezoidal integration of the content versus time. Nitrogen or C intensity is similar to a time-weighted average of N or C content but includes the duration of exposure, and may reflect longer-term impacts of C or N availability on microbial processes (Maharjan & Venterea, 2013).

Soil samples were collected from each plot on 24 September 2019 for DNA extraction. Soil DNA was extracted using a FastDNA Spin kit for soil (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The purified DNA was stored at −20°C until further analysis. Quantitative PCR (qPCR) was used to amplify the targets of N_2O–producers (AOB and archaea (AOA) ammonia monooxygenase genes (*amoA*), bacterial nitrite reductase genes (*nirS* and *nirK*) and fungal nitrite reductase gene (fungal *nirK*)) and N_2O–reducers (nitrous oxide reductase genes (*nosZI* and *nosZII*)) using a Bio–Rad CFX96 Real–Time PCR Detection System (Bio–Rad Laboratories, Inc.). Briefly, PCR reactions were performed in a 20 μl solution including 10.0 μl of SYBR Premix Ex Taq (TaKaRa Biotechnology Co. Ltd., Dalian, China), 2 μl of soil DNA, and 0.25 μM of each primer. The thermal conditions of the PCR reactions were similar to those previously reported for the functional genes (Harter et al., 2014; Jones et al., 2013; Long et al., 2015). The amplification resulted in single peaks with efficiencies of 90.9%–101.2% and R^2 values of 0.991–0.997. Each qPCR run included soil DNA samples, corresponding standards, and no-template control reactions. Melting curve analysis was performed at the end of each run to confirm the reaction specificity. The gene-specific primers are presented in Table S1.

2.4. Statistical Analysis

In order to assess whether the responses of N_2O fluxes to the same level of N addition differed between the two topographic positions, N–induced change of soil N_2O emission was calculated by Equation 1:

$$Change\,(\%) = \frac{X_t - X_c}{X_c} \times 100 \tag{1}$$

where X_t and X_c represent cumulative N_2O emission from a N addition plot and the control, respectively, in the same block. One way analysis of variance (ANOVA) was used to examine the effects of N addition on cumulative N_2O emission, intensities of NH_4^+, NO_3^-, DOC, and DOC:NO_3^- ratio, and functional gene abundances for each topographic position. Independent samples *t*–test was used to examine whether the difference is significant between the two topographic positions for the same N addition treatment. Regression analysis was used to identify significant correlations of cumulative N_2O emission with substrate concentrations or gene abundances. The effect was considered significant at $p < 0.05$ level. Linear mixed effect models (LMEM) were used to examine the effects of N addition, topography, sampling date, and their interactions on soil N_2O flux and N–induced change of N_2O flux and with a plot nested in time as a random effect. The above analyses were performed in SPSS 25.0 (IBM Co., Armonk, NY, USA).

3. Results

3.1. Soil Temperature, WFPS, and N_2O Emission

Soil temperature and water content varied from 4 to 38°C (Figures 1a and 1b) and 15%–90% WFPS (Figures 1c and 1d), respectively, over the measurement period. The averaged N_2O fluxes for the control were 4.43 ± 5.49 μg N m^{-2} h^{-1} in the valley and 6.12 ± 5.89 μg N m^{-2} h^{-1} on the slope (Figures 1e and 1f). Soil N_2O emission was significantly influenced by N addition in 2017, 2018, and 2019, and topography in 2018 (Table S3). Although there was no significant effect of topography on N_2O flux in 2017 and 2019, the significant interactive effect of topography × N addition × sampling date and topography × N addition was found in 2017 and 2019, respectively (Table S3).

Cumulative N_2O emission was significantly influenced by N addition ($p < 0.05$; Figure 2). The cumulative N_2O emissions in 2017, 2018, and 2019 were 0.59 ± 0.07 kg N ha^{-1}, 0.35 ± 0.03 kg N ha^{-1}, and 0.22 ± 0.18 kg N ha^{-1} in the valley, and were 0.46 ± 0.08 kg N ha^{-1}, 0.56 ± 0.14 kg N ha^{-1}, and 0.49 ± 0.13 kg N ha^{-1} on the slope, respectively, under the control (Figures 2a–2c). Moderate N addition significantly ($p < 0.05$) increased cumulative N_2O emission by 94.68% in 2018% and 251.17% in 2019 in the valley. High N addition significantly ($p < 0.05$) increased cumulative N_2O emission by 53.31% in 2017%, 124.30% in 2018%, 252.92% in 2019 in

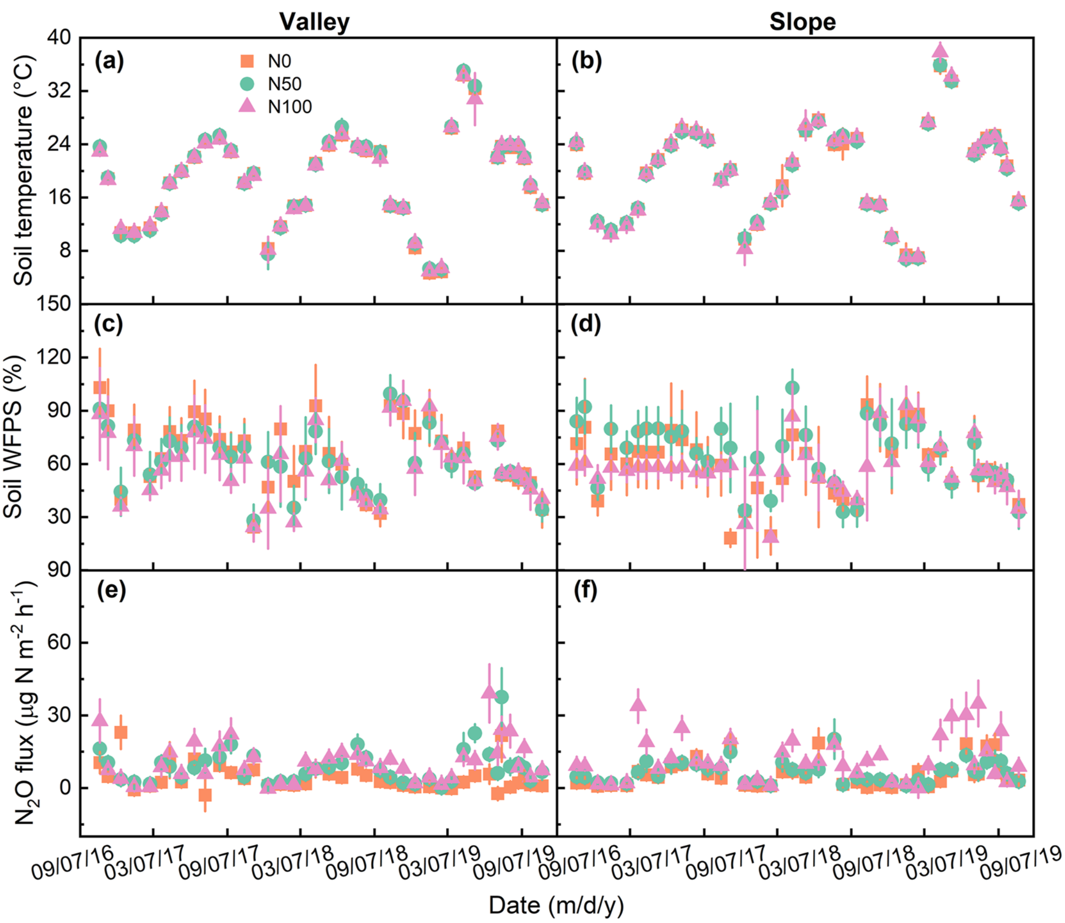

Figure 1. Temporal variations of soil temperature (a–b), water-filled pore space (c–d), and N_2O fluxes (g–h) in the valley and on the slope, respectively. N0, N50, and N100 indicate control (0 kg N ha^{-1} yr^{-1}), moderate N (50 kg N ha^{-1} yr^{-1}), and high N (100 kg N ha^{-1} yr^{-1}) treatments, respectively. Values are presented as means with standard deviations ($n = 3$).

the valley, and by 117.54% in 2017 on the slope. Cumulative N_2O emissions for the control were 0.93 ± 0.07 kg N ha^{-1} and 1.02 ± 0.15 kg N ha^{-1}, respectively, in the valley and on the slope across 2017 and 2018, and were 1.16 ± 0.24 kg N ha^{-1} and 1.50 ± 0.06 kg N ha^{-1}, respectively, in the valley and on the slope across the 3 years (Figures 2d and 2e). Across the 3 years, moderate and high N addition significantly increased soil N_2O emission by 88.7%–113.3% in the valley, and high N addition significantly increased soil N_2O emission by 84.27% on the slope. Cumulative N_2O emission across the 3 years was higher in the valley than on the slope only under N50 ($p < 0.05$; Figure 2e). There was a significant effect of topography on N–induced change of N_2O fluxes across 2017 and 2018, and there were significant interactive effects of topography × sampling date on N–induced change of N_2O fluxes in 2017, 2018, and 2019 (Table S3). Accordingly, N–induced change of cumulative N_2O emission across the 3 years was significantly higher ($p < 0.05$) in the valley than on the slope under N50 (Figure 2f).

3.2. Soil Abiotic and Biotic Variables

High N addition significantly increased the intensities of soil NH_4^+ and NO_3^- across the 3 years in the valley (Figures 3a–3j). High N addition significantly increased soil DOC intensity in 2019 in the valley (Figure 3m). High N addition significantly decreased soil DOC:NO_3^- ratio across 2017 and 2018 in the valley (Figures 3p and 3q). On the slope, high N addition significantly increased soil NH_4^+ and NO_3^-intensities (Figures 3a and 3f), but decreased soil DOC:NO_3^- ratio in 2017 (Figure 3p). Compared to the control, N addition significantly increased the abundances of AOB *amoA* (Figure 4b) and *nirK* (Figure 4d) on the slope, but decreased ($p < 0.05$) AOA:AOB *amoA* ratio in the valley (Figure 4c).

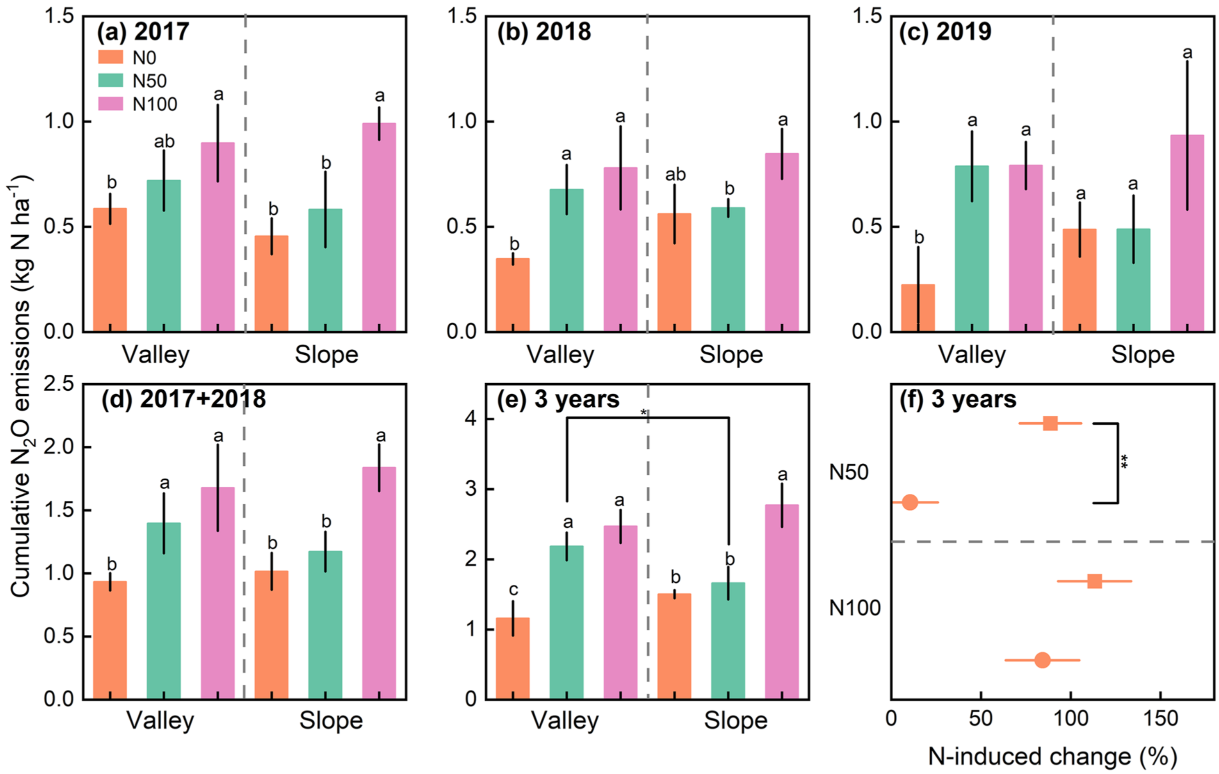

Figure 2. Impacts of N addition on cumulative N_2O emission in 2017, 2018, 2019, 2018 + 2019, and across 3 years (a–e), and N–induced change of cumulative N_2O emission (f) in the valley and on the slope. Different letters denote significant differences at $p < 0.05$ among N addition treatments in the valley or on the slope. The asterisk (*) indicates significant difference at $p < 0.05$ between the valley and slope for the same N addition level. Values are presented as means with standard deviations ($n = 3$).

3.3. Relationships Between Cumulative N_2O Emission and Soil Properties

Cumulative N_2O emission across the 3 years was positively ($p < 0.05$) correlated with the soil NH_4^+, NO_3^-, DOC intensities, and AOB *amoA* abundance (Figures 5a–5c, e), but negatively ($p < 0.05$) correlated with the AOA:AOB *amoA* and DOC:NO_3^- ratios in the valley (Figures 5d and 5g). There was a significant positive ($p < 0.05$) correlation of cumulative N_2O emission with the NO_3^- intensity, AOB *amoA* or *nirK* abundance (Figures 5b, 5f and 5h), and negative ($p < 0.05$) correlation with the DOC:NO_3^- ratio (Figure 5d) on the slope.

4. Discussion

4.1. Soil N_2O Emission in the Control Plots

The annual N_2O emissions (0.39 ± 0.08–0.50 ± 0.02 kg N ha^{-1} yr^{-1}) in the control plots of the current study are comparable to some previously reported values (0.39–0.52 kg N ha^{-1} yr^{-1}) observed in some subtropical/tropical forests (Fan et al., 2017; Rowlings et al., 2012; Soper et al., 2018), but are much lower than those from other subtropical/tropical forests (0.70–3.50 kg N ha^{-1} yr^{-1}; Han et al., 2019; Wieder et al., 2011; Xie et al., 2018; M. H. Zheng et al., 2016). Several potential mechanisms are responsible for the relatively low N_2O emission in the current study. First, soil water content, with an average of 61.4% WFPS, was high in most of the sampling period (Figure 1). High WFPS may hinder the diffusion of N_2O from the soil profile to the atmosphere (Butterbach-Bahl et al., 2013). Second, complete denitrification (from N_2O to N_2) may dominate the denitrification processes in the forests of the current study, since soil pH (Table S2) is within the suitable range (7.1–7.5) benefiting complete denitrification (Jan-Michael et al., 2018), which ultimately decreases soil N_2O production.

Our results show that there was no significant difference in N_2O emission between the two topographic positions (Figure 2). This is inconsistent with some previous studies which found that topography affects soil N_2O fluxes in forests (Almaraz et al., 2019; Fang et al., 2009; Yu et al., 2021). In these studies, soil moisture (Fang et al., 2009;

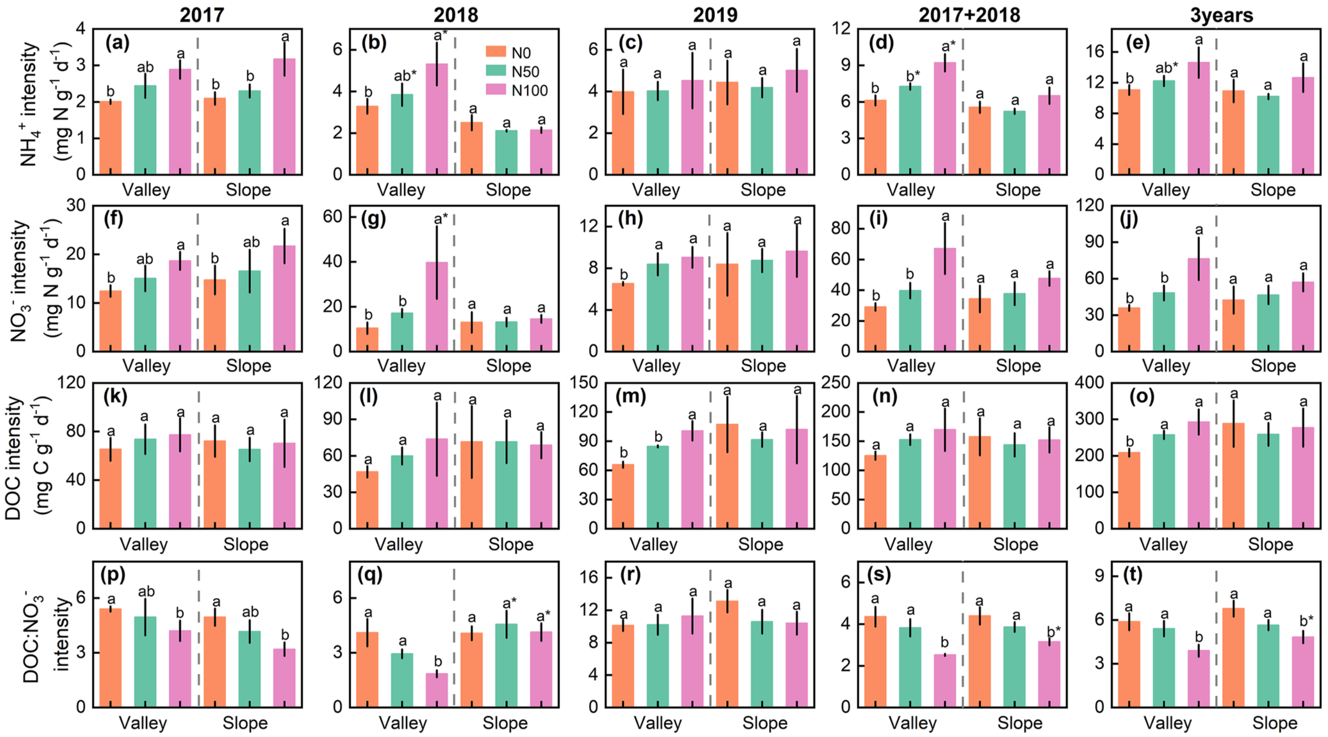

Figure 3. Impacts of N addition on the intensities of soil NH_4^+ (a–e), NO_3^- (f–j), DOC (k–o), and DOC:NO_3^- ratio (p–t) in the valley and on the slope. The asterisk (*) indicates significant difference at $p < 0.05$ between the valley and slope for the same N addition level. Values are presented as means with standard deviations ($n = 3$).

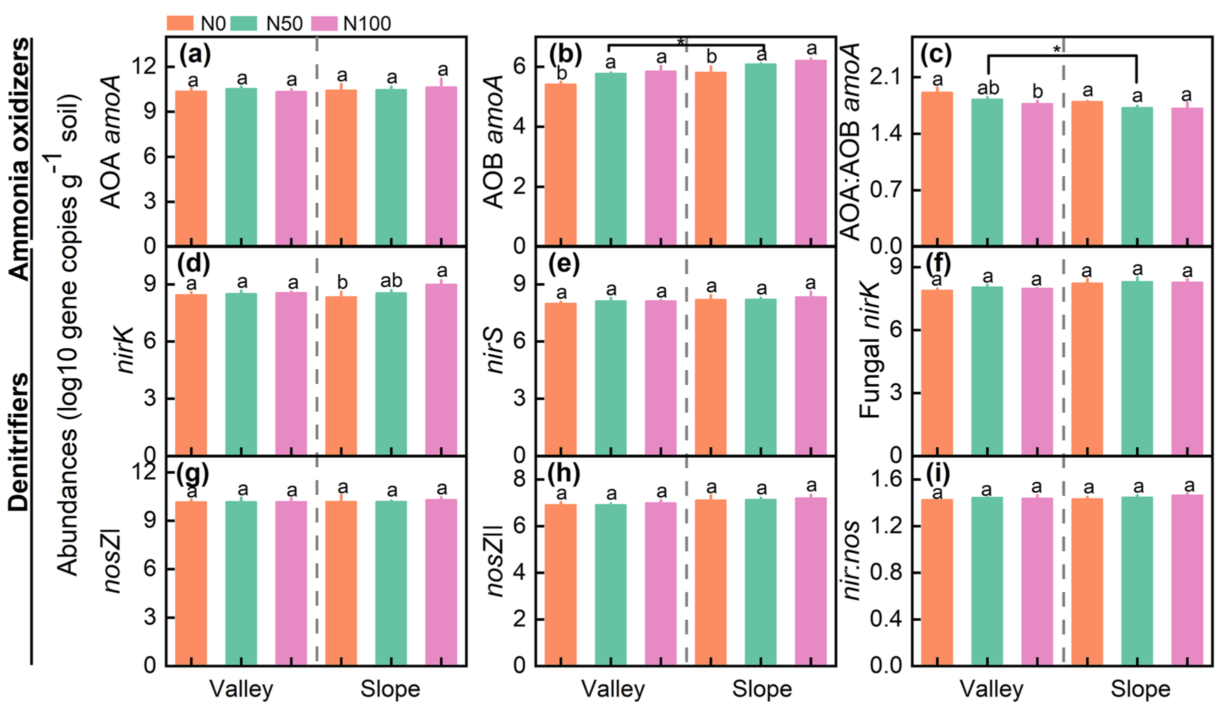

Figure 4. Impacts of N addition on ammonia oxidizers (a–c) and denitrifiers (d–i) gene abundances in the valley and on the slope, respectively. Different letters denote significant differences at $p < 0.05$ among N addition treatments in the valley or on the slope. Values are presented as means with standard deviations ($n = 3$).

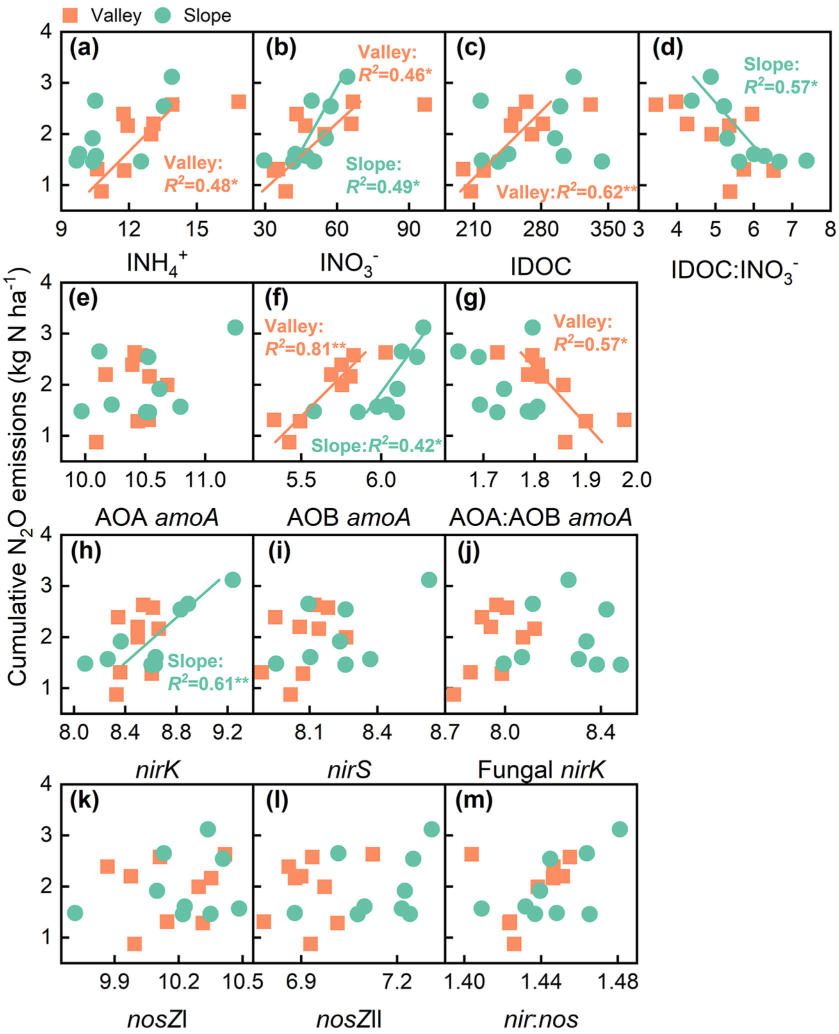

Figure 5. Relationships of cumulative N_2O emission with soil substrate intensities (a–d), and functional genes abundances (e–m) in the valley and on the slope. The asterisks * and ** represent significance levels at $p < 0.05$ and $p < 0.01$, respectively.

Yu et al., 2021) and substrate availability (Almaraz et al., 2019) have been used to explain the difference in N_2O emission among topographic positions. However, levels of these variables were similar between the two topographic positions in the current study (Figure 3, Table S2).

4.2. Factors Responsible for the Stimulation of Soil N_2O Emission by Nitrogen Addition Are Topography-Dependent

We find that N addition consistently stimulated soil N_2O emission in the valley across the 3 years. Some studies show that the stimulation of N_2O emission is caused by N addition–induced soil acidification (Tian & Niu, 2015), since acidification promotes AOA–driven nitrification (Shi et al., 2018), reduces the consumption of N_2O by denitrification (Koehler et al., 2009) and stimulates fungal driven denitrification (Chen et al., 2015). However, soil pH was not significantly altered by N addition in the current study (Table S2), so the stimulation of soil N_2O emission by N addition should not be caused by soil acidification. An increase in soil N availability has often been used to explain the enhancement of N_2O emission under N addition (Redding et al., 2016). In the current study, two more reasonable mechanisms underlying the increase in soil N_2O emission in responses to N addition are proposed. First, N addition may promote N_2O emission via its positive impacts on soil N availability. As indicated by soil NH_4^+ and NO_3^- intensities, soil N availability was increased by N addition in the valley (Figure 3). Soil

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nitrification in the karst forest is fast (Li et al., 2017, 2018), so the deposited NH_4^+ would be rapidly transformed to NO_3^- upon entering into the soil. Therefore, soil NH_4^+ and NO_3^- intensities were found to be simulated by N addition (Figure 3). Enhanced soil NH_4^+ and NO_3^- availabilities resulted in high nitrification and denitrification (Butterbach-Bahl et al., 2013). In the current study, both soil NH_4^+ and NO_3^- intensities had direct positive effects on N_2O emission in the valley (Figure 5, S4), supporting the critical role of N availability in promoting N_2O emission under N addition. Second, N_2O emission may also be promoted by N addition via increased DOC availability. Previous studies reported that high DOC concentrations are indicative of high C availability for denitrification and N_2O emissions (Levy-Booth et al., 2014; Yin et al., 2017). We found that soil NO_3^- and DOC intensities had a positive correlation with the N_2O emission in the valley in 2019 (Figure S4 j, k), indicating that N_2O emission should be more closely related to the denitrification process this year.

In contrast to the valley, N addition only stimulated soil N_2O emission at the early stage on the slope in the current study. This is consistent with the findings of some previous studies (L. Song et al., 2017; X. Song et al., 2020). Similar to the valley, increased N_2O emission by N addition may be attributed to stimulated N availability (Figure 3, S4). Additionally, decreased DOC:NO_3^- ratio as a result of limiting DOC availability was reported to inhibit N_2O reduction so N_2O emission increased (Assémien et al., 2019; Grave et al., 2018). Therefore, a decrease in the soil DOC:NO_3^- ratio was likely another mechanism underlying the increased N_2O emission on the slope in 2017 in the current study. As aforementioned, the N status and soil NO_3^- leaching were higher on the slope than in the valley according to our previous study (Chen et al., 2021). N saturation would have occurred on the slope after 2017. Due to the high soil nitrification in the karst forest, more deposited NH_4^+ would be transformed into NO_3^- and more input N would be leached on the slope than that in the valley (Wang et al., 2019). This explained why soil NH_4^+ and NO_3^- intensities, and N_2O emission as well were not significantly altered on the slope after 2017.

The stimulation of soil N_2O emission ultimately results from the increase in the activities of nitrifiers and denitrifiers due to the enhanced N substrates under N addition (Tang et al., 2016). AOB is favored by relatively high NH_4^+ supply (Prosser et al., 2020). In the current study, AOB *amoA* abundance was positively and significantly related to N_2O emission, supporting that AOB was mainly responsible for N_2O production at both topographic positions. AOB has been found to survive under anoxic conditions and contain the *nirK* gene, which is involved in nitrifier denitrification (Wrage-Mönnig et al., 2018). In the current study, *nirK* abundance was positively correlated with AOB *amoA* abundance on the slope (Figure S5), implying that the observed *nirK* gene mainly belonged to AOB. Moreover, positive relationships were observed between N_2O emission and AOB *amoA* or *nirK* abundance (Figures 4e and 4g), indicating the possible contribution of nitrifier denitrification to N_2O emission. High NO_3^- accumulation in soil often increases soil N_2O emission via stimulating nitrite reductase rather than reducing N_2O reductase (Giles et al., 2012). Thus, we propose that the increased N_2O emission on the slope in 2017 may be attributed to NO_3^- accumulation which accelerates the AOB-driven denitrification. Nitrifier denitrification is prevalent in soils with high ammonia oxidization rates (Butterbach-Bahl et al., 2013; Duan, Zhang, et al., 2019), but this needs to be further confirmed by using double isotopes method.

4.3. Higher Stimulation of Soil N_2O Emission by Nitrogen Addition in the Valley Than on the Slope

Our results show that N addition–induced stimulation of N_2O emission may be modulated by topography with higher stimulation in the valley than on the slope under N50. The topography dependent responses of N_2O emission to N addition are likely caused by soil N status. There is evidence that N addition increased N availability more pronouncedly in N limited than in N rich ecosystems so N addition would lead to greater stimulation of soil N_2O emission in more N limited ecosystems (Fu et al., 2015). According to a meta-analysis, N addition significantly increases soil N_2O emissions in N limited forests but has no significant effect in N rich forests (Deng et al., 2020). With regard to the current study, soil N status is higher on the slope than in the valley as indicated by soil NO_3^- leaching, which was higher on the slope (2.14 ± 0.06 kg N ha^{-1} year^{-1}) than in the valley (1.91 ± 0.04 kg N ha^{-1} year^{-1}) according to the observation in a neighboring forest (Wang et al., 2019). The soil N:P ratio is often used as an indicator of soil N or P status with a greater ratio implying a higher N status (Tian et al., 2010). The higher soil N:P ratio on the slope than in the valley for the control (Table S2) hence corroborates a higher soil N status on the slope. Due to the lower N status, soil NH_4^+ intensity was enhanced more pronouncedly in the valley than on the slope under N50 (Figures 3b, 3d and 3e). Nevertheless, functional gene abundances were not significantly different between the valley and slope (Figure S5b, c). Accordingly, the greater stimulation of soil N_2O emission in the valley should be primarily ascribed to the dynamics of soil N availability under N50. In contrast, high N addition could have increased soil N status in the valley to a level comparable to that on the slope. Due to the relatively high N status, soil N availability was insensitive to N addition both in the valley and on the slope under N100. Subsequently, the degrees of soil N_2O emission responses to high N addition were comparable at the two topographic positions.

5. Conclusions

Our results show that N addition consistently increased N_2O emission in the valley, but only stimulated N_2O emission at the early stage on the slope. Additionally, we find that the stimulation of soil N_2O emission by moderate N addition was more pronounced in the valley than on the slope mostly due to the lower N status in the valley. However, we are not sure if the patterns observed in the current study are applicable to other climate conditions. Since the difference in soil N status among topographic positions is common (Arias-Navarro et al., 2017; Enanga et al., 2016; Stewart et al., 2014), we guess that the topography dependent responses of soil N_2O emission to N addition should be widespread. Our findings highlight that topography should be included in Earth system models in order to better predict the responses of soil N_2O emission to atmospheric N deposition.

Data Availability Statement

The data presented in this work can be found in Dataset S1, and can also be accessed at Science Data Bank (http://www.doi.org/10.11922/sciencedb.01328).

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