# The simulated N deposition accelerates net N mineralization and nitrification in a tropical forest soil

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**Abstract.** Elevated nitrogen (N) deposition affects soil N transformations in the N-rich soil of tropical forests. However, the change in soil functional microorganisms responsible for soil N cycling remains largely unknown. Here, we investigated the variation in soil inorganic N content, net N mineralization ($R_m$), net nitrification ($R_n$), inorganic N leaching ($R_l$), $N_2O$ efflux and N-related functional gene abundance in atropical forest soil over a two-year period with four levels of N addition. The responses of soil net N transformations (*in situ* $R_m$ and $R_n$) and $R_l$ to N additions were negligible during the first year of N inputs. The $R_m$, $R_n$, and $R_l$ increased with the medium nitrogen (MN) and high nitrogen (HN) treatments relative to the control treatments in the second year of N additions. Furthermore, the $R_m$, $R_n$, and $R_l$ were higher in the wet season than in the dry season. The $R_m$ and $R_n$ were mainly associated with the N addition-induced lower C:N ratio in the dry season but with higher microbial biomass in the wet season. Throughout the study period, high N additions increased the annual $N_2O$ emissions by 78%. Overall, N additions significantly facilitated $R_m$, $R_n$, $R_l$ and $N_2O$ emission. In addition, the MN and HN treatments increased the ammonia-oxidizing archaea (AOA) abundance by 17.3% and 7.5%, respectively. Meanwhile, the HN addition significantly increased the abundance of *nirK*-denitrifiers but significantly decreased the abundance of ammonia-oxidizing bacteria (AOB) and *nosZ*-containing $N_2O$ reducers. To some extent, the variation in functional gene abundance was related to the corresponding N transformation processes. Partial least squares path modelling (PLS-PM) indicated that inorganic N contents had significantly negative direct effects on the abundances of N-related functional genes in the wet season, implying that chronic N deposition would have a negative effect on the N-cycling-related microbes and the function of N transformation. Our results provide evidence that elevated N deposition may impose consistent stimulatory effects on soil N transformation rates but differentiated impacts on related microbial functional genes. Long-term experimentation or observation are needed to decipher the interrelations between the rate of soil N transformation processes and the abundance/expression of related functional genes.

## 1 Introduction

Due to anthropogenic activity in recent decades, the increased atmospheric reactive nitrogen (N) deposition in terrestrial ecosystems has altered the N status and dynamics (Galloway et al., 2008). Excessive N inputs to forest ecosystems will certainly influence soil N cycling and ecosystem function. In the last three decades, several studies have focused on the impacts of N deposition on soil N cycling in northern and temperate forests (Aber et al., 1989;1998;Gundersen et al., 1998;Nave et al., 2009;Tian et al., 2018). However, in recent years, tropical forests have received the most dramatic increases in N deposition and are considered as N-rich areas (Hietz et al., 2011;Liu et al., 2013). In southern China, forest ecosystems, such as the hotspots of N deposition receiving 13.8-113 kg N $ha^{-1}$ $year^{-1}$ through precipitation, have reached N saturation status (Fang et al., 2008;Chen et al., 2016;Yu et al., 2018). Little is known about the hazards of constant N inputs on N-saturated forest ecosystem functioning. More attention should be focused on examining the effects of N addition on soil N transformations in N-rich tropical forests.

Soil N availability and turnover are quantified by the N transformation rates in the forest soil (Gao et al., 2016;Patel and Fernandez, 2018). Few previous studies reported the alteration of N transformation rates after N additions have received inconsistent results for tropical forest ecosystems. For example, in Hawaiian Islands, N addition to a P-limited tropical forest (4.1-million-year-old) did not change the rate of net N mineralization ($R_m$), but significantly increased the net nitrification rate ($R_n$) and N-oxide (i.e., NO and $N_2O$) emission (Lohse and Matson, 2005). In contrast, N addition to a younger N-limited forest (300-year-old) significantly increased soil $R_m$, $R_n$ and nitrate leaching (Hall and Matson, 1999, 2003), and the differentiated responses between the two forests were mainly determined by soil age and nutrient status (Hall and Matson, 1999, 2003;Lohse and Matson, 2005). The N addition effects on gross N mineralization and nitrification rates were apparent after chronic (9-year) N additions in a lowland tropical forest but was obvious with short-term (1-year) N additions in a montane tropical forest in Panama; the difference was mainly due to the different soil types and whether there existed an organic layer (Koehler et al., 2009;Corre et al., 2010). In southern China, a 6-year N addition significantly increased $N_2O$ emission and nitrate leaching but decreased $R_m$ and $R_n$ in a tropical broadleaf forest, possibly due to the alteration of the soil microbial community composition and reduction of enzyme activity with N addition (Chen et al., 2016). In contrast, significant increases in $R_m$, $R_n$ and $R_l$ were observed with a 3-year N addition in an adjacent broadleaf forest (Zhang et al., 2008a;Fang et al., 2009b;Fang et al., 2011). These previous studies suggest that the responses of soil N transformations to N addition in the tropical forests may vary with soil type, nutrient status (e.g., N-limited, N-saturated, or P-limited), duration of N addition, and the alteration of soil microbial communities. Until now, only a small number of studies have directly quantified soil N transformation rates in tropical forests, and the mechanisms of their conflicting responses to N additions are still unclear (Cheng et al., 2019).

A global meta-analysis showed that N deposition had a negative effect on soil microbial growth, diversity, composition, and function (Zhang et al., 2018c;Wang et al., 2018a), but soil N mineralization was mainly driven by soil microorganisms (Ollivier et al., 2011;Li et al., 2019b). Ammonia oxidation, the first and rate-limiting step of autotrophic nitrification, is performed by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) harbouring AOA *amoA* and AOB *amoA* genes, respectively, which are valuable indices for predicting soil potential nitrification rates (Petersen et al., 2012). AOA play a dominant role in ammonia oxidation in acidic forest soil and have a positive correlation with gross nitrification rates (Isobe et al., 2012). In addition, elevated N deposition enhances N loss by nitrate leaching and denitrification in tropical forest soil (Chen et al., 2016). The second step in denitrification of reducing $NO_2^-$ to nitric oxide is catalysed by copper-containing reductase (encoded by the *nirK* gene) or cytochrome cd1-containing reductase (encoded by the *nirS* gene) (Braker et al., 2000). Previous studies have shown that *nirK* denitrifiers are more sensitive to environmental changes than are *nirS* denitrifiers (Chen et al., 2010;Li et al., 2019a). Furthermore, the abundances of the *nirK* gene are positively related to potential denitrification rates in an acidic forest soil (Zhang et al., 2018b). The reduction of $N_2O$ to $N_2$ catalyzed by nitrous oxide reductase (encoded by the *nosZ* gene) plays a vital role in mitigating $N_2O$ emissions (Liu et al., 2014;Nie et al., 2016). Therefore, the combination of soil N transformation processes and functional gene abundances is essential to better explain the response mechanism of the soil N cycle to N additions, to explore the relationships between the abundances of soil N-related functional genes and N transformation rates, and to assess the effects of N addition on soil N-related functional microbes.

Soil net N transformation rates are thought to be primarily controlled by environmental factors, including temperature, precipitation, carbon to nitrogen (C:N) ratio, soil organic matter (SOM) content, soil texture and pH (Templer et al., 2005;Chen et al., 2017;Song et al., 2018). Importantly, the contents of soil organic carbon (SOC) and carbon to nitrogen ratio (C:N) are the key factors that determine soil net N dynamics in terrestrial ecosystems (Li et al., 2014;Liu et al., 2017). On the other hand, N inputs to forests could alter soil properties. For instance, elevated N deposition can result in soil acidification (Lu et al., 2014;Mao et al., 2017) and a relatively lower soil C:N ratio and lower available P in the forest soil (Shi et al., 2018). The tropical forest soil itself is P-limited and acidic; thus, it is also essential to assess the complex interactions between soil physiochemical characteristics and net N transformation rates under N deposition.

Here, we investigated the effects of N addition on $R_m$, $R_n$, $R_l$, $N_2O$ emission and N-related functional gene abundance within two years using the *in situ* intact soil core incubation method in an acidic tropical forest. The main aims were to investigate (1) the effects of elevated N deposition on field-measured net N transformation rates ; (2) the effects of N deposition on the abundance of microbial functional genes associated with the N transformation processes and (3) the relationships among functional gene abundance, N transformation rates, and environmental factors under elevated N deposition.

## 2 Materials and methods

## 2.1 Study sites

The study was carried out in the Dinghushan Biosphere Reserve (DHSBR) (112°10′ E, 23°10′ N) in Guangdong Province of southern China. An experiment using a gradient of nitrogen addition was used to simulate N deposition in an evergreen broad-leaved forest with the age of about 110 years. The climate of this forest is considered a humid monsoon with an annual average temperature of 21 °C and a mean annual precipitation of 1927 mm (Mo et al., 2006;Zhao et al., 2011). The minimum monthly mean temperature in this study area is 12.6 °C in January, and the maximum monthly mean temperature is 28.0°C in July (Mo et al., 2006). The elevation of this site ranges from 300 to 355 m above sea level. The major tree species of the study site are *Castanopsis chinensis*, *Schima superba*, *Cryptocarya chinensis,* and *Randia canthioides*. In this site, the wet season is concentrated from April to September (approximately 80% of the annual rainfalls), and the dry season extends from October to March (approximately 20% of the annual rainfalls). Wet N deposition measured as dissolved inorganic N in bulk precipitation was 34.6 and 31.6 kg N $ha^{-1}$ $year^{-1}$ in 2004 and 2005, respectively (Fang et al., 2008). In addition, the soil type in this region is classified as strongly acidic lateritic red earth formed from sandstone with a pH below 4.0 (Mo et al., 2006;Zhang et al., 2008a).

## 2.2 Experimental design

Four concentrations of $NH_4NO_3$ were applied: control (0), low N (LN, 35 kg N $ha^{-1}$ $year^{-1}$), medium N (MN, 70 kg N $ha^{-1}$ $year^{-1}$), and high N (HN, 105 kg N $ha^{-1}$ $year^{-1}$), which were 1, 2, and 3 times of the ambient wet N deposition rate, respectively. Twelve (4 treatments × 3 replicates) experimental plots (15 m × 15 m per plot) were randomly scattered in the study area and established in October 2013; the plots were surrounded by buffer strips (> 10 m wide) to avoid the disturbance of surface runoff and flow diffusion between adjacent plots. The corresponding dose of N ($NH_4NO_3$) solution (30 L) and an equal amount of water (without $NH_4NO_3$) were evenly sprayed over the N-treated and control plots, respectively, below the canopy using a knapsack sprayer (i.e., a low rate of 0.1 L $m^{-2}$ was applied to avoid liquid effects) at the end of each month starting in September 2014.

## 2.3 Soil N transformations

Soil net mineralization, net nitrification and inorganic N leaching rates were determined nine times from September 2014 to October 2016 using the *in situ* resin-core incubation method (Reichmann et al., 2013;Chen et al., 2017). The installation of the incubation PVC tubes were done a couple of days before 24th of the month when N addition was applied. The nine times of incubations were scattered in September 2014, December 2014, March 2015, June 2015, September 2015, December 2015, March 2016, June 2016, and September 2016, and each incubation lasted for 30 days.

In each plot, six soil incubation sites were evenly distributed in uphill and downhill areas. At each

incubation site, a pair of PVC tubes (5 cm in diameter and 17 cm in length) were inserted into the soil surface layer (10 cm depth) after the surface litter was removed. A resin bag containing 30 g ion exchange resin (cation exchange resin: anion resin = 1:2) was placed in the bottom of one PVC tube (accounting for approximately 2 cm of the PVC tube) under a 10 cm soil layer. The resin cores in the PVC tubes were incubated *in situ* for 30 days in the field prior to the collection of the soil samples and resin bags to measure the concentrations of soil $NH_4^+$-N and $NO_3^-$-N. The other PVC tube with a 10 cm soil core was taken immediately, and then the soils in the PVC tubes were mixed thoroughly (six total soil cores in each plot) into a composite soil sample for further analysis. Soil samples were divided into two parts. One part was passed through a 2-mm sieve and used to analyse the initial concentration of soil $NH_4^+$-N and $NO_3^-$-N, and a small part of the fresh soil was kept at -80 °C to extract soil DNA for quantifying the functional microorganisms. The other part was air-dried at room temperature, and then it was passed through a 100-mesh sieve to estimate the basic soil physicochemical properties. Soil net mineralization ($R_m$), net nitrification ($R_n$) and inorganic N leaching ($R_l$) rates were calculated according to the following formulas:

$$R_m = \frac{(NH_4^+ - N_{i+1} - NH_4^+ - N_i) + (NO_3^- - N_{i+1} - NO_3^- - N_i)}{t_{i+1} - t_i} \quad (1)$$

$$R_n = \frac{NO_3^- - N_{i+1} - NO_3^- - N_i}{t_{i+1} - t_i} \quad (2)$$

$$R_l = \frac{(NH_4^+ - N_{i+1} - l) + (NO_3^- - N_{i+1} - l)}{t_{i+1} - t_i} \quad (3)$$

where $t_i$ and $t_{i+1}$ are the beginning and end dates of each incubation period, respectively; $NH_4^+$-$N_i$ and $NH_4^+$-$N_{i+1}$ are the contents of soil $NH_4^+$-N before and after incubation, respectively, and $NO_3^-$-$N_i$ and $NO_3^-$-$N_{i+1}$ are the concentrations of soil $NO_3^-$-N before and after incubation, respectively (Li et al., 2018a). $NH_4^+$-$N_{i+1}$-$l$ and $NO_3^-$-$N_{i+1}$-$l$ are the contents of $NH_4^+$-N and $NO_3^-$-N in the resin after 30 days of incubation, respectively.

In addition, the concentrations of $NH_4^+$-N and $NO_3^-$-N in the resin were used to calculate the ammonium and nitrate leaching rates respectively. Soil $N_2O$ emissions were monitored using the closed chamber method, and $N_2O$ gas samples were taken twice in the middle and the end of each month across October 2014 to September 2016. The $N_2O$ concentrations was analyzed with a gas chromatograph (Agilent 7890A, Agilent Technologies, USA) as previously described (Chen et al., 2017). The $N_2O$ efflux rate was calculated using the following equation:

$$F = \rho \times \frac{V}{A} \times \frac{P}{P_0} \times \frac{T_0}{T} \times \frac{dC_1}{dt} \quad (4)$$

where F represents the $N_2O$ flux (μg N $m^{-2}$ $h^{-1}$); $\rho$ the density of $N_2O$ under standard conditions (mg $L^{-1}$),, $V$ gas volume in the chamber ($m^3$), $A$ chamber coverage area ($m^2$), $P$ atmosphere pressure at the sampling time (Pa), $P_0$ standard atmosphere pressure (Pa), $T$ absolute temperature (K) at the sampling time, absolute temperature (K) under standard conditions, and $dC_1/dt$ the liner slope of gas concentration changes within

the sampling time period. The annual rates of $N_2O$ emission (kg N $ha^{-1}$ $yr^{-1}$) after N addition were calculated by linear interpolation between sampling dates in the two observation years: October 2014 to September 2015 and October 2015 to September 2016.

## 2.4 Soil physiochemical properties

The soil organic carbon (SOC) was estimated using the external heating method with potassium dichromate ($K_2Cr_2O_7$). To obtain the total nitrogen (TN) and total phosphorus (TP), semi-micro Kjeldahl digestion and molybdenum antimony colorimetric approaches were performed, respectively. The contents of soil $NH_4^+$-N and $NO_3^-$-N were detected with 1 M KCl extraction by indophenol-blue colorimetry and double wavelength (220 nm and 275 nm), respectively, using a spectrophotometer (UV-6000, China). Soil pH was measured by a pH meter with a glass electrode (Horiba F-71S, Japan) (soil: water ratio, 1:2.5 dry wt/v). Soil microbial carbon (MBC) and soil microbial nitrogen (MBN) were determined on a TOC analyser (Shimadzu TOC-VCSH Analyser) by the fumigation-extraction method (Vance et al., 1987) and calculated using the conversion factors of 0.45 and 0.54, respectively (Brookes et al., 1985;Joergensen et al., 2011).

2.5 Quantification of the abundances of soil functional genes

Soil DNA was extracted using a PowerSoil®DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA). DNA concentrations were quantified on a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). Subsequently, quantitative PCR was performed on an ABI 7500 CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories, Inc., Hercules, CA) to quantify the abundances of N-cycling functional genes, including AOA *amoA* and AOB *amoA* genes in nitrification and *nirK* and *nosZ* genes in denitrification. The pair primes of these functional genes are shown in Table S1. The total volume (20 μl) of the reaction systems contained 10 μl SYBR® Premix Ex Taq™ (TaKaRa Biotech, Japan), 0.4 μl forward and 0.4 μl reverse primer, 0.4 μl Rox Reference Dye II (TaKaRa Biotech, Japan), 1 μl amplification template (genomic DNA) and 7.8 μl sterile $ddH_2O$. The preparation of standard curves and the details of the amplification conditions were conducted as described in Table S2. The amplification efficiencies of qPCR ranged from 95.3% to 103.0%, and the $R^2$ values of the calibration curves were ≥ 0.98.

## 2.6 Statistics

One-way analysis of variance (ANOVA) was used to compare the differences in inorganic N concentrations, soil N transformations, and soil functional gene abundances between control and N-treated plots at each sampling time with the least significant difference (LSD) test for multiple comparisons and two-way repeated measures ANOVA was performed to examine the effects of N additions on these variables over time using SPSS (SPSS 18.0, SPSS Inc., Chicago, USA). Redundancy

analysis (RDA) was conducted to determine the comprehensive relationships among soil physiochemical properties, functional gene abundance and N transformations using Canoco 5.0 (Wageningen UR, Netherlands). The correlation coefficients of soil properties, soil N transformations and functional genes were calculated using PAST (version 2.16). The Partial least squares path modelling (PLS-PM) was carried out to test the effects of inorganic N, soil conditions, microbial biomass, and functional gene abundance on soil N transformation rates ($R_m$, $R_n$, $R_l$ and $N_2O$ emission) using the "plspm" package in R (version 3.3.3).

## 3 Results

### 3.1 Soil properties and inorganic N contents

The soil C:N ratio in this study site ranged from 11.3 to 18.5, and the pH was between 3.7 and 3.9 (Table S3). The HN addition decreased the SOC, C:N ratio and pH by 14.1%, 9.3% and 1.4%, respectively. The soil TN showed no significant difference between the control and N-treated plots after N addition (Table S3). The concentrations of MBC and MBN decreased obviously by 15.1% and 14.5% respectively in the HN treatment plots in the dry season (Table S3). The contents of soil $NH_4^+$-N and $NO_3^-$-N significantly increased with N addition ($P < 0.05$, Fig. 1). Our results showed that the amounts of soil $NH_4^+$-N and $NO_3^-$-N in the MN and HN plots were significantly higher than those in the control plots. The mean value of $NH_4^+$-N accounted for 25.1% of the mean total inorganic N, and the $NH_4^+$-N /$NO_3^-$-N ratio ranged from 0.05 to 0.97. Over the entire study period, the mean soil $NH_4^+$-N contents in the LN, MN, and HN treatment plots increased by 27.5%, 38.3%, and 38.6%, respectively. Similarly, the mean concentrations of $NO_3^-$-N in these three plots increased by 0.4%, 29.3% and 37.2%, respectively.

### 3.2 Soil net N mineralization and nitrification rates

The results showed that *in situ* $R_m$ and $R_n$ significantly increased after one year of N addition in the MN and HN plots ($P < 0.05$, Fig. 2a and b). However, there were no significant differences in both N transformation rates between the control and N-treated plots during the first N-treated year ($P > 0.05$). The range of *in situ* $R_m$ (from 4.9 to 44.9 mg N $kg^{-1}$ $month^{-1}$) over the first year of N addition was obviously lower by approximately 50% than the range (from 10.0 to 108.6 mg N $kg^{-1}$ $month^{-1}$) over the second year of N addition. In addition, the responses of $R_m$ and $R_n$ to N addition exhibited different seasonal patterns. The mean values of $R_m$ in the LN, MN and HN plots in the wet season were 60.3%, 18.5%, and 50.2% higher than those in the dry season over the second year of N addition, respectively. Similarly, the mean value of $R_n$ in the wet season in these three N-treated plots was 1.5-, 1.2-, and 1.3-fold higher than those in the dry season within the same period of N addition. A repeated measures ANOVA indicated that N additions had significant effects on $R_m$ and $R_n$, which also exhibited the significant time effects. However, there were no significant interaction effects between N and time in these two N processes (Table S4).

### 3.3 Inorganic N leaching and $N_2O$ emission

The HN addition significantly increased the ammonium-leaching rates (Fig. 3a), but the ammonium leaching rates accounted for only a small proportion (less than 20%) in the total of $R_l$ and were found to range from 0.08 to 10 mg N $kg^{-1}$ $month^{-1}$. After a one-year period of N additions, the nitrate leaching rates significantly increased in the MN and HN treatment plots ($P < 0.05$, Fig. 3b). The $R_l$ was significantly correlated with the nitrate leaching rate (Fig. 3d, $R$ =0.939, $P < 0.001$), indicating that inorganic N leaching was predominantly determined by nitrate leaching. The mean values of $R_l$ in the LN, MN, and HN treated plots in the wet season were 1.22, 0.56, and 1.11 times greater than those in the dry season, respectively (Fig. 3e). The addition of N significantly increased the annual $N_2O$ emission (Fig. 3f, $P <$ 0.05), showing increases of 18.3%, 18.4% and 77.7% in the LN, MN and HN, respectively, in comparison to the control plots. In addition, a strong positive correlation was observed between the soil $NO_3^-$-N concentration and nitrate leaching rate in the wet season ($R$ = 0.63, $P < 0.001$) (Table 1b). This finding suggested that the accumulation of $NO_3^-$-N content with N addition might accelerate N loss from the acidic forest soil. Repeated measures ANOVA showed that N additions had significant effects on $N_2O$ emission. However, the interaction effects between N and time are indistinctive (Table S4).

### 3.4 Soil microbial functional genes

As shown in Fig. 4a, the copy numbers of the archaeal AOA *amoA* gene ranged from $1.7 \times 10^8$ to $5.2 \times 10^8$ $g^{-1}$ dry soil. Although AOA abundance showed no significant difference in all treatments, its mean value increased by 17.3% and 7.5% in the MN and HN plots, respectively, compared with the value in the control plots. AOA abundance showed a significantly negative correlation with soil pH ($R$ = -0.64, $P < 0.01$) and a positive correlation with $NO_3^-$-N content ($R$ = 0.47, $P < 0.05$) in the dry season (Table 1a). However, the MN and HN additions significantly decreased the copy numbers of the AOB *amoA* gene ($P < 0.05$ and $P < 0.01$, respectively, Fig. 4b). In addition, AOA were more abundant than AOB in the acidic forest soils. The ratio of AOA:AOB abundance ranged from 9.5 to 191.2. However, the abundance of *nirK* genes significantly increased in the second year of HN addition ($P < 0.01$, Fig. 4c). Initially, the abundance of *nosZ* genes decreased in the HN-treated plots compared with that in the control plots in January 2015 and January 2016 ($P$ = 0.057, Fig.4d). However, the differences between both were weakened with the duration of N addition. Repeated measures ANOVA also indicated the interaction effects of N and time on soil functional gene abundance are not apparent except for *nir*K genes (Table S5).

### 3.5 Interactions among N transformation rates, soil physicochemical properties and functional gene abundance

RDA was carried out to separately determine the relationship between the soil biotic/abiotic factors and N transformation rates for the dry season and wet seasons. RDA in the dry season was confirmed as

unreliable because the *P* value of the RDA was >0.05 (data not shown). Linear correlation analysis showed that the C:N ratio had significant negative correlations with both the $R_m$ and $R_n$ ($R$ = -0.45, $P$ < 0.05, Table 1a) but had positive relationships with the abundance of AOA *amoA*, AOB *amoA* and *nosZ* genes ($R$ = 0.44, $P$ < 0.05; $R$ = 0.58, $P$ < 0.01; $R$ = 0.48, $P$ < 0.05, respectively). In addition, no significant correlations were found between N transformation rates and biotic factors in the dry season (Table 1a). In the wet season, the first two axes of the RDA explained 65.3% of the total variance in all determined biotic and abiotic parameters and N transformation rates of the soil samples (Fig. 5). The $R_m$, $R_n$ and $R_l$ had significantly positive correlations with the soil $NO_3^-$-N contents, MBN, MBC, SWC, SOC and TN. In contrast, the above N transformation rates had significantly negative relationships with soil pH and $NH_4^+$-N contents. Similarly, $N_2O$ emission was significantly positively correlated with the MBN, MBC, SWC, SOC and TN but significantly negatively correlated with the soil $NH_4^+$-N content. According to the above analysis, we found more complex relationships among the biotic and abiotic factors and N transformations in the wet season than in the dry season.

The PLS-PM was constructed to integrate the complex interrelationships among environmental factors, microbial biomass and soil N transformations in the wet season (Fig. 6). The results showed that inorganic N had positive direct effects on soil conditions (path coefficient = 0.78, $P$ < 0.001), microbial biomass (path coefficient = 0.11, $P$ > 0.05) and N transformations (path coefficient = 0.18, $P$ > 0.05). However, inorganic N had a negative direct effect on N-related functional gene abundance (path coefficient = -0.7, $P$ < 0.01). Soil conditions had a positive direct effect on microbial biomass (path coefficient = 0.75, $P$ < 0.001). The positive direct contributors to N transformations were inorganic N (path coefficient = 0.18, $P$ > 0.05) and microbial biomass (path coefficient = 0.44, $P$ > 0.05). In contrast, the negative direct effects on N transformations were soil conditions (path coefficient = -0.07, $P$ > 0.05) and N-related functional gene abundance (path coefficient = -0.37, $P$ = 0.09).

# 4 Discussion

## 4.1 Effects of N addition on N transformation rates

In contrast to N-limited temperate forests, the N-rich tropical broadleaved forest soil in the DHSBR was considered to be N saturated (Fang et al., 2008). In our study, no significant differences of $R_m$, $R_n$ and $R_l$ were found in the control and N-treated plots during the first-year of N addition, which could possibly be ascribed to plant uptake of mineral N and soil N retention (Fang et al., 2011;Gurmesa et al., 2016). However, $R_m$, $R_n$ and $R_l$ significantly increased in the MN and HN treatment plots in the second year of N addition (Fig. 2a, b and 3c). This result is in agreement with the hypothesis early proposed that once N input exceeds the total biotic demands, it will form a status of N saturation and subsequently promote N mineralization, nitrification, N loss through nitrate ($NO_3^-$-N) leaching and $N_2O$ emissions in boreal and temperate forest ecosystems (Aber et al., 1989;1998). The strong increments of $R_m$, $R_n$ and $R_l$ under N additions lasted from October 2015 to July 2016 (Fig. 2a, b and 3c). This data provides evidence of the

stimulating effects of N inputs on net N transformation processes (i.e., *in situ* $R_m$ and $R_n$) in tropical forests. In a recent laboratory incubation study using the $^{15}N$ dilution method with the soil samples collected from the same experimental plots, Han et al. (2018) found that the N additions stimulated the gross N mineralization rate but decreased the gross N immobilization rate in the second-year soil samples. Therefore, the increased net N mineralization rate observed in the second year of this study might be due to the simulative N effect on gross N mineralization and the suppressive effect on gross immobilization rate. However, the filed-measured net N mineralization rate in this study cannot be directly and quantitatively linked to the laboratory-measured gross N mineralization and immobilization rates in Han et al.'s study since they are inherently different measures of N transformation rates (Cheng et al., 2019). Further studies combining field-measured net N transformation rates, laboratory-incubation measured net N transformation rates (as in Lovett et al., 2004), and $^{15}N$ dilution-measured gross N transformation rates may provide a more mechanistic understanding to the impacts of N addition on soil N transformation processes.

The significant increases in $R_m$ and $R_n$ in the second year of N addition are consistent with the result of the previous study showing significant increases of the two N transformation processes after a 3-year N addition (Fang et al., 2011). However, significant decreases in $R_m$ and $R_n$ after a 6-year N addition were previously demonstrated in the adjacent tropical forest (Chen et al., 2016). The different effects of short-term and long-term N addition on $R_m$ and $R_n$ are possibly caused by the reasons below. First, long-term N additions could lead to high amounts of $NO_3^-$-N accumulation relative to short-term N additions, which may form high osmotic potential and ion toxicity and directly affect soil microorganisms (Wang et al., 2018a). Second, long-term N addition results in a lower soil pH (Lu et al., 2014) and an increase in $Al^{3+}$ content which is toxic to soil microorganisms (He et al., 2012). Third, long-term N deposition has negative impacts on protein depolymerization (Chen et al., 2018), which is considered a rate-limiting step of organic N mineralization (Jan et al., 2009;Mooshammer et al., 2014).

Although the rates of nitrate leaching measured under the 10 cm soil layer might overestimate the N loss attributed to plant uptake below this layer, the result is in agreement with the previous studies of substantial nitrate leaching under N deposition (Fang et al., 2009a;Chen et al., 2016). The inorganic N leaching ($R_l$) mainly resulted from nitrate leaching (Fig. 3a, b, c, and d), because the negatively charged $NO_3^-$-N is easier to lose from the soil while $NH_4^+$-N tends to be taken by plants in acidic forest soils (Fang et al., 2011;Chen et al., 2017). Hall and Matson (1999) found that $N_2O$ emissions were higher in the P-limited tropical forest than in the N-limited forest. In this study, the mean rates of soil $N_2O$ emissions in the control plots were $40.4 \pm 6.5$ and $45.1 \pm 5.7$ μg $N_2O$–N $m^{-2}$ $h^{-1}$ in the first two years after N additions, respectively (Table S6), which were obviously higher than the results of $29.3 \pm 1.6$ μg $N_2O$–N $m^{-2}$ $h^{-1}$ reported by Zhang et al. (2008a), indicating that $N_2O$ emission rates had increased over the past 10 years in the forests of DHSBR. In addition, the rates of $N_2O$ emissions ($95.0 \pm 9.0$ μg $N_2O$–N $m^{-2}$ $h^{-1}$) in the HN treatment plots were significantly higher than those in the LN, MN and control plots, indicating that the soil $N_2O$ emission flux was dependent on the N-addition gradients (Zhang et al., 2008a;Fang et al.,

2011;Chen et al., 2016).

### 4.2 Responses of the abundances of microbial functional genes to N additions

AOA play a more important role than do AOB in ammonia oxidation of acidic soils (Zhang et al., 2012;Tang et al., 2016). Similar to the results of a previous study performed in the old-growth broadleaf forest at DHSBR (Isobe et al., 2012), AOA were more abundant than AOB in our younger broadleaf forest as well. The two ammonia oxidizers showed different response patterns to N additions; the abundance of AOA did not differ statistically significantly among the four N treatments but showed an increasing trend as N addition level increased (Fig. 4a); contrastingly the abundance of AOB were significantly decreased by the MN- and HN-treatments (Fig. 4b). These results are similar to what have been found in a previous study where a 6-year N input increased AOA abundance but decreased AOB abundance in an acidic subtropical forest soil (Shi et al., 2018). The reason for the increased AOA but decreased AOB abundance under N addition might be ascribed to: (1) the decreasing soil pH in the tropical forest soil with elevated N deposition (Lu et al., 2014). AOA could adapt well to strongly acidic soil conditions but AOB tended to be higher in neutral or slightly alkaline soils over all terrestrial ecosystems (Nicol et al., 2008;Hu et al., 2013;Wang et al., 2019) and were more sensitive to N enrichment (Ning et al., 2015). In addition, some previous studies reported that the AOA and AOB ratio increased with decreasing soil pH (He et al., 2007;Shen et al., 2008;Yao et al., 2011;Tang et al., 2019), and (2) the lower ammonia ($NH_3$) availability with decreasing soil pH, which may have limited the direct substrate for nitrifiers, and AOA are more competitive than are AOB (He et al., 2012;Shi et al., 2018). The abundances of *nirK*-denitrifiers have been found positively related to potential denitrification (Zhang et al., 2018b;Tang et al., 2019). Here, we found that HN additions initially decreased *nirK* gene abundance in the first year of N addition but significantly increased *nirK* gene abundance in the second year of N addition (Fig. 2b), which is possibly ascribed to the accumulation of soil $NO_3^-$-N and the subsequent acceleration of denitrification. The reduction of $N_2O$ to $N_2$ was reported to be regulated by the abundance of *nosZ*-harbouring dentrifiers (Levy-Booth et al., 2014). In our study, the HN addition decreased *nosZ* gene abundance in the earlier stage of N addition, but a decrease in the difference was also observed with the duration of N addition. The decrease in the difference in *nosZ* gene abundance between the control and N-treated plots with time was possibly attributed to the tendency of microbial adaption to N addition. Furthermore, $N_2O$ emissions had significantly negative correlation with *nos*Z gene abundance in the wet season (Table 1b). It supports the previous findings that decreased *nos*Z gene abundance with N addition is a major factor causing low $N_2O$ consumption and high $N_2O$ emissions in the acidic forest soil (Zhang et al., 2008a). These variations in *nirK* and *nosZ* gene abundance and the greater abundance of *nirK* than the *nosZ* gene (Fig. 4c and d) could explain the significant increase in $N_2O$ emissions with N additions in the tropical forest soil (Han et al., 2018). In contrast, an opposite pattern with a higher *nosZ* gene abundance but a lower total *nirK* and *nirS* gene abundance was found in a Masson pine forest soil with low $N_2O$ emissions with a mean annual temperature of 17 °C and a mean annual precipitation of 1200–1400 mm (Li et al., 2019a),

suggesting that *nirS*-, *nirK*- and *nosZ*-denitrifiers are critical in regulating $N_2O$ emission in forest ecosystems. Therefore, the *nirS* gene abundance is also very important in mediating $N_2O$ emission (Chen et al., 2019). In addition, soil water contents exhibited a significantly negative relationship with the abundance of *nosZ* gene in the wet season (Table 1b), which explains the higher $N_2O$ emission in the wet season than in the dry season (Fu et al., 2015).

### 4.3 Seasonal variations in N transformations under N additions

Seasonal patterns were more obvious for the N transformations in the second year of N additions. The $R_m$, $R_n$ and $R_l$ were apparently higher in the wet season than in the dry season (Fig. 2c, d and Fig. 3e), suggesting that soil temperature and moisture were the critical environmental factors affecting N transformations (Chen et al., 2017; Li et al., 2018a). Similar seasonal patterns have been documented in previous studies (Zhang et al., 2008b;Contosta et al., 2011;Li et al., 2014). In the dry season, the HN addition decreased the MBC and MBN by 15.1% and 14.5 respectively (Table S3), and the low temperature and precipitation suppressed the microbial biomass and activity and then depressed the N mineralization (Contosta et al., 2011;Chen et al., 2017). Our results indicated that the lower soil C:N ratio with N enrichment was the dominant factor that increased $R_m$ and $R_n$, and subsequently led to higher N losses ($N_2O$ emission and nitrate leaching) in the dry season (Table 1a). In contrast, the factors controlling the processes of N transformation in the wet season were more complicated, with microbial biomass and SWC being the most important ones (Fig. 5) over soil pH, inorganic N content, TN, SOC and N-related functional gene abundance (Table 1b). The higher soil microbial biomass and SWC in the wet season could facilitate N mineralization and nitrification and then cause larger nitrate leaching.

### 4.4 The interactions between soil N transformations and abiotic/biotic conditions

In the dry season, the variations in $R_m$, $R_n$ and $R_l$ exhibited significant negative correlations with the soil C:N ratio (Table 1a), suggesting that the C:N ratio was a dominant factor determining soil N dynamics (Fang et al., 2011;Liu et al., 2017). In this study, the HN addition decreased the soil C:N ratio, which was consistent with the results of a previous study in an acidic forest soil (Shi et al., 2018). Significant positive correlations between the C:N ratios and the abundances of AOA, AOB, and *nos*Z-$N_2O$ reducers were also observed (Table 1a), indicating that a low C:N ratio had a negative effect on N-related functional microbes. AOA abundance was positively correlated with soil $NO_3^-$-N concentration ($R = 0.47$, $P < 0.05$), which was in accordance with the previous studies (Hu et al., 2013;Tang et al., 2016). In addition, AOA abundance was negatively correlated with soil pH (Li et al., 2019a), indicating that AOA could adapt to the strong acidic tropical forest soil.

In the wet season, AOB *amoA* and *nos*Z gene abundances were positively related to soil $NH_4^+$-N contents and pH but negatively related to soil $NO_3^-$-N contents (Fig. 5 and Table 1b), indicating that the lower pH and accumulation of soil $NO_3^-$-N with N addition might result in decreases in the AOB *amoA*

and *nosZ* gene abundances. However, we found no significant relationship between AOA abundance and $R_n$ during the 2-year study period (Table 1a and b), which is inconsistent with the results of Isobe et al. (2012) where they found a significant correlation between AOA *amoA* abundance and gross nitrification rate in an adjacent tropical forest soil, possibly because of the different N transformation rates measured in the two studies: net N nitrification rate measured using the *in situ* incubation method in our study and gross N nitrification rate measured using the $^{15}N$ dilution method in Isobe et al.'s study. In addition, the findings also indicated that $R_m$, $R_n$ and $R_l$ were significantly and negatively correlated with soil $NH_4^+$-N content (Fig. 5), and the possible explanation was that $NH_4^+$-N could have a negative feedback on soil N mineralization (Geisseler et al., 2010;Zhang et al., 2018b). Interestingly, $R_m$, $R_n$ and $R_l$ were significantly and negatively correlated with soil pH, which contrasted with the results of previous studies (Fu et al., 1987;Kemmitt et al., 2006). The most reasonable explanation is that soil pH has a negative correlation with soil N transformation in strongly acidic soils (pH < 4.0), which is likely due to the highest nitrification rates existing in the soils with lower pH (Booth et al., 2005).

The PLS-PM showed that the inorganic N had significantly negative direct effects on the N-related functional gene abundance (Fig. 6), suggesting that the functional microorganisms were more sensitive to N addition, and ongoing N deposition had significant negative effects on soil functional microbes (Zhang et al., 2018c). However, these negative effects of N addition on microbial gene abundance did not cause significant negative effects on net N transformations in the study period, which was possibly explained by the microbial function redundancy or buffer capacity of the acidic forest soil. In addition, we found that the microbial biomass was the dominant factor driving net N transformations in the wet season (Fig. 6), suggesting that microbes played a critical role in driving the processes of N transformation (Li et al., 2019b). However, it was previously found that a 13-year N addition significantly decreased the MBC and MBN in adjacent forest soil (Wang et al., 2018b), implying that chronic N deposition would have a negative effect on soil N transformations.

## 5 Conclusions

The addition of N increased the *in situ* net mineralization, net nitrification, inorganic N leaching rate, and $N_2O$ emission during the short term, which supported the traditional N saturation hypothesis. To some extent, the alterations of functional gene abundance with N additions were related to the corresponding processes of N transformation. The variations in $R_m$, $R_n$ and $R_l$ exhibited different seasonal patterns. They were higher in the wet season than in the dry season. The C:N ratio was the dominant driving factor of N transformations in the dry season, while the biotic factors (microbial biomass) played an important role in accelerating N transformations in the wet season. According to the PLS-PM analysis, N additions had negative effects on the abundance of N-related functional genes in the dry season, which implies that

chronic N deposition poses a potential risk to forest ecosystem functions. More attention should be focused on the combination of soil nitrogen cycling processes and functional microorganisms to long-term continuous N inputs in forest ecosystems and the strategies to alleviate the negative effects of elevated N deposition on soil microbial function.

*Data availability.* All the relevant data are presented in the paper and supplementary materials.

*Author contributions.* WS designed the study, planned the field experiments and obtained research funding. YN carried out the experiment and analyzed the data. YN, WS and MW wrote the manuscript. XH provided the $N_2O$ observations and guidance on their interpretation. JC helped in the field experiments of N transformation (*in situ* $R_m$, $R_n$ and $R_l$) and provided part of the data. All the authors provided feedback and gave constructive suggestions on the manuscript.

*Competing interests.* The authors declare that they have no conflict of interest.

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Table 1 The linear correlation *R* and *P* value among soil properties, N-related functional genes and N transformation processes in the dry (a) and wet (b) seasons. The gray region represents the *R* value, and the white region represents the *P* value.

**(a)**

| **Dry season** | C/N | PH | $NH_4^+$-N | $NO_3^-$-N | MBC | MBN | TN | TP | SWC | SOC | AOA | AOB | *nirK* | *nosZ* | $R_m$ | $R_n$ | $N_2O$ | $R_I$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C/N | | 0.61 | 0.03 | 0.36 | 0.91 | 0.34 | 0.02 | 0.14 | 0.06 | 0.00 | 0.03 | 0.00 | 0.30 | 0.02 | 0.03 | 0.03 | 0.57 | 0.42 |
| PH | -0.11 | | 0.83 | 0.02 | 0.37 | 0.72 | 0.02 | 0.97 | 0.18 | 0.11 | 0.00 | 0.58 | 0.41 | 0.71 | 0.94 | 0.94 | 0.83 | 0.44 |
| $NH_4^+$-N | -0.44 | -0.05 | | 0.50 | 0.47 | 0.15 | 0.66 | 0.23 | 0.20 | 0.19 | 0.40 | 0.23 | 0.76 | 0.19 | 0.22 | 0.22 | 0.21 | 0.55 |
| $NO_3^-$-N | -0.20 | -0.47 | 0.15 | | 0.10 | 0.17 | 0.19 | 0.60 | 0.20 | 0.76 | 0.02 | 0.30 | 0.52 | 0.27 | 0.22 | 0.22 | 0.38 | 0.17 |
| MBC | -0.03 | -0.19 | -0.16 | 0.34 | | 0.00 | 0.07 | 0.86 | 0.21 | 0.25 | 0.26 | 0.36 | 0.08 | 0.27 | 0.56 | 0.56 | 0.98 | 0.74 |
| MBN | -0.20 | -0.08 | -0.31 | 0.29 | 0.69 | | 0.92 | 0.32 | 0.07 | 0.64 | 0.94 | 0.89 | 0.20 | 0.57 | 0.77 | 0.77 | 0.62 | 0.88 |
| TN | 0.47 | -0.48 | -0.09 | 0.27 | 0.37 | 0.02 | | 0.02 | 0.60 | 0.00 | 0.00 | 0.01 | 0.15 | 0.02 | 0.28 | 0.28 | 0.79 | 0.94 |
| TP | 0.31 | -0.01 | -0.25 | -0.11 | -0.04 | -0.21 | 0.49 | | 1.00 | 0.02 | 0.43 | 0.26 | 0.75 | 0.41 | 0.15 | 0.15 | 0.68 | 0.60 |
| SWC | -0.39 | -0.29 | -0.27 | 0.27 | 0.26 | 0.38 | -0.11 | 0.00 | | 0.16 | 0.99 | 0.09 | 0.27 | 0.12 | 0.33 | 0.33 | 0.12 | 0.39 |
| SOC | 0.83 | -0.34 | -0.28 | 0.06 | 0.24 | -0.10 | 0.88 | 0.46 | -0.30 | | 0.00 | 0.00 | 0.13 | 0.00 | 0.07 | 0.07 | 0.61 | 0.61 |
| AOA | 0.44 | -0.64 | -0.18 | 0.47 | 0.24 | 0.02 | 0.56 | 0.17 | 0.00 | 0.59 | | 0.01 | 0.29 | 0.04 | 0.61 | 0.61 | 0.97 | 0.34 |
| AOB | 0.58 | -0.12 | -0.25 | -0.22 | 0.20 | 0.03 | 0.51 | 0.24 | -0.36 | 0.65 | 0.49 | | 0.01 | 0.00 | 0.18 | 0.18 | 0.71 | 0.68 |
| *nirK* | 0.22 | -0.18 | -0.07 | -0.14 | 0.36 | 0.27 | 0.30 | -0.07 | -0.23 | 0.32 | 0.23 | 0.51 | | 0.00 | 0.50 | 0.50 | 0.57 | 0.36 |
| *nosZ* | 0.48 | -0.08 | -0.28 | -0.24 | 0.23 | 0.12 | 0.47 | 0.17 | -0.33 | 0.57 | 0.43 | 0.89 | 0.67 | | 0.38 | 0.38 | 0.57 | 0.97 |
| $R_m$ | -0.45 | -0.02 | 0.26 | 0.26 | 0.13 | 0.06 | -0.23 | -0.30 | 0.21 | -0.38 | -0.11 | -0.28 | -0.14 | -0.19 | | 0.00 | 0.84 | 0.00 |
| $R_n$ | -0.45 | -0.02 | 0.26 | 0.26 | 0.13 | 0.06 | -0.23 | -0.30 | 0.21 | -0.38 | -0.11 | -0.28 | -0.14 | -0.19 | 1.00 | | 0.84 | 0.00 |
| $N_2O$ | -0.12 | -0.05 | -0.27 | -0.19 | 0.01 | 0.11 | -0.06 | -0.09 | 0.32 | -0.11 | 0.01 | 0.08 | -0.12 | 0.12 | -0.04 | -0.04 | | 0.82 |
| $R_I$ | -0.17 | -0.16 | 0.13 | 0.29 | 0.07 | 0.03 | -0.02 | -0.11 | 0.18 | -0.11 | 0.20 | -0.09 | -0.19 | -0.01 | 0.85 | 0.85 | 0.05 | |

**(b)**

| **Wet season** | C/N | PH | $NH_4^+$-N | $NO_3^-$-N | MBC | MBN | TN | TP | SWC | SOC | AOA | AOB | *nirK* | *nosZ* | $R_m$ | $R_n$ | $N_2O$ | $R_I$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C/N | | 0.63 | 0.56 | 0.47 | 0.15 | 0.64 | 0.69 | 0.88 | 0.86 | 0.00 | 0.67 | 0.18 | 0.17 | 0.18 | 0.63 | 0.63 | 0.05 | 0.45 |
| PH | 0.10 | | 0.00 | 0.00 | 0.19 | 0.00 | 0.01 | 0.48 | 0.00 | 0.18 | 0.32 | 0.00 | 0.32 | 0.00 | 0.00 | 0.00 | 0.15 | 0.00 |
| $NH_4^+$-N | -0.13 | 0.69 | | 0.00 | 0.04 | 0.00 | 0.06 | 0.81 | 0.00 | 0.07 | 0.25 | 0.01 | 0.79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $NO_3^-$-N | -0.15 | -0.78 | -0.76 | | 0.24 | 0.00 | 0.00 | 0.62 | 0.00 | 0.09 | 0.15 | 0.01 | 0.77 | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 |
| MBC | 0.30 | -0.27 | -0.43 | 0.25 | | 0.00 | 0.05 | 0.48 | 0.00 | 0.01 | 0.68 | 0.77 | 0.41 | 0.09 | 0.01 | 0.01 | 0.01 | 0.01 |
| MBN | 0.10 | -0.65 | -0.71 | 0.77 | 0.61 | | 0.00 | 0.10 | 0.00 | 0.00 | 0.21 | 0.25 | 0.99 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 |
| TN | -0.09 | -0.51 | -0.39 | 0.64 | 0.40 | 0.71 | | 0.12 | 0.00 | 0.00 | 0.38 | 0.41 | 0.95 | 0.13 | 0.01 | 0.01 | 0.07 | 0.03 |
| TP | -0.03 | -0.15 | -0.05 | 0.11 | 0.15 | 0.35 | 0.32 | | 0.26 | 0.30 | 0.28 | 0.54 | 0.99 | 0.29 | 0.34 | 0.34 | 0.92 | 0.24 |
| SWC | 0.04 | -0.65 | -0.69 | 0.74 | 0.59 | 0.91 | 0.77 | 0.24 | | 0.00 | 0.09 | 0.37 | 1.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| SOC | 0.68 | -0.28 | -0.38 | 0.36 | 0.53 | 0.60 | 0.66 | 0.22 | 0.59 | | 0.82 | 0.63 | 0.35 | 0.02 | 0.03 | 0.03 | 0.00 | 0.03 |
| AOA | 0.09 | 0.21 | 0.24 | -0.30 | 0.09 | -0.27 | -0.19 | -0.23 | -0.36 | -0.05 | | 0.24 | 0.75 | 0.77 | 0.26 | 0.26 | 0.85 | 0.30 |
| AOB | 0.28 | 0.59 | 0.50 | -0.49 | -0.06 | -0.24 | -0.17 | -0.13 | -0.19 | 0.10 | 0.25 | | 0.34 | 0.34 | 0.02 | 0.02 | 0.87 | 0.02 |
| *nirK* | -0.29 | -0.21 | -0.06 | 0.06 | 0.18 | 0.00 | 0.01 | 0.00 | 0.00 | -0.20 | -0.07 | -0.20 | | 0.51 | 0.38 | 0.38 | 0.46 | 0.34 |
| *nosZ* | -0.29 | 0.64 | 0.63 | -0.58 | -0.35 | -0.66 | -0.32 | -0.22 | -0.57 | -0.47 | -0.06 | 0.20 | 0.14 | | 0.00 | 0.00 | 0.02 | 0.00 |
| $R_m$ | 0.10 | -0.56 | -0.64 | 0.68 | 0.50 | 0.71 | 0.50 | 0.20 | 0.59 | 0.45 | -0.24 | -0.47 | 0.19 | -0.59 | | 0.00 | 0.04 | 0.00 |
| $R_n$ | 0.10 | -0.56 | -0.64 | 0.68 | 0.50 | 0.71 | 0.50 | 0.20 | 0.59 | 0.45 | -0.24 | -0.47 | 0.19 | -0.59 | 1.00 | | 0.04 | 0.00 |
| $N_2O$ | 0.40 | -0.31 | -0.55 | 0.38 | 0.51 | 0.41 | 0.37 | 0.02 | 0.53 | 0.56 | 0.04 | -0.04 | -0.16 | -0.47 | 0.43 | 0.43 | | 0.03 |
| $R_I$ | 0.16 | -0.56 | -0.64 | 0.63 | 0.49 | 0.67 | 0.45 | 0.25 | 0.57 | 0.45 | -0.22 | -0.49 | 0.20 | -0.58 | 0.98 | 0.98 | 0.44 | |

**Figure Legends**

Fig. 1. Changes in soil $NH_4^+$-N (a) and $NO_3^-$-N (b) contents in the soils at different samplings. Bars represent standard errors of the mean (n=3). Significance levels are indicated by $*P < 0.05$, $**P < 0.01$.

Fig. 2. The variation in the *in situ* mineralization rate ($R_m$) (a) and nitrification rate ($R_n$) (b) in the soils at different samplings. The $R_m$ (c) and $R_n$ (d) in the dry and wet seasons. Bars represent standard errors of the mean (n=3). Significance levels are indicated by $*P < 0.05$, $**P < 0.01$.

Fig. 3. Dynamics of the *in situ* $NH_4^+$-N(a), $NO_3^-$-N(b) and total inorganic N leaching rates ($R_l$) (c) in the soils at different samplings. d. The correlation between the rates of nitrate leaching and inorganic nitrogen leaching ($R_l$). e. The variation in $R_l$ in the dry and wet season. f. The rates of annual $N_2O$ emission with N addition. Bars represent standard errors of the mean (n=3). Significance levels are indicated by $*P < 0.05$, $**P < 0.01$.

Fig. 4. Responses of functional genes (AOA *amoA*(a), AOB *amoA*(b), *nirK*(c), and *nosZ*(d)) to N deposition. Bars represent standard errors of the mean (n=3). Significance levels are indicated by $*P < 0.05$, $**P < 0.01$.

Fig. 5 Redundancy analysis (RDA) among environmental variables ($NH_4^+$-N, $NO_3^-$-N, pH, SOC, TN, C/N and SWC), functional genes (AOA *amoA*, AOB *amoA*, *nirK*, and *nosZ*) and soil N transformation rates ($R_m$, $R_n$, $R_l$ and $N_2O$ emission) in the wet season. Values on the axes indicated the percentages of total variation explained by each axis.

Fig. 6. Directed graph of the partial least squares path model (PLS-PM) of the inorganic N ($NH_4^+$-N and $NO_3^-$-N), soil conditions (pH, SOC, TN, C/N and SWC), microbial biomass (MBC and MBN), and the abundances of functional genes (AOA *amoA*, AOB *amoA*, *nirK*, and *nosZ*) effects on soil N transformation rates ($R_m$, $R_n$, $R_l$ and $N_2O$ emission) in the wet season. Path coefficients and explained variability ($R^2$) reflecting in the width of the arrow were calculated after 1000 bootstraps. The blue and red representing positive and negative effects, respectively. Solid arrows indicated $P < 0.05$; and dashed arrows indicate $P > 0.05$. The model was assessed using the Goodness of Fit (GoF). $* P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Fig. 1

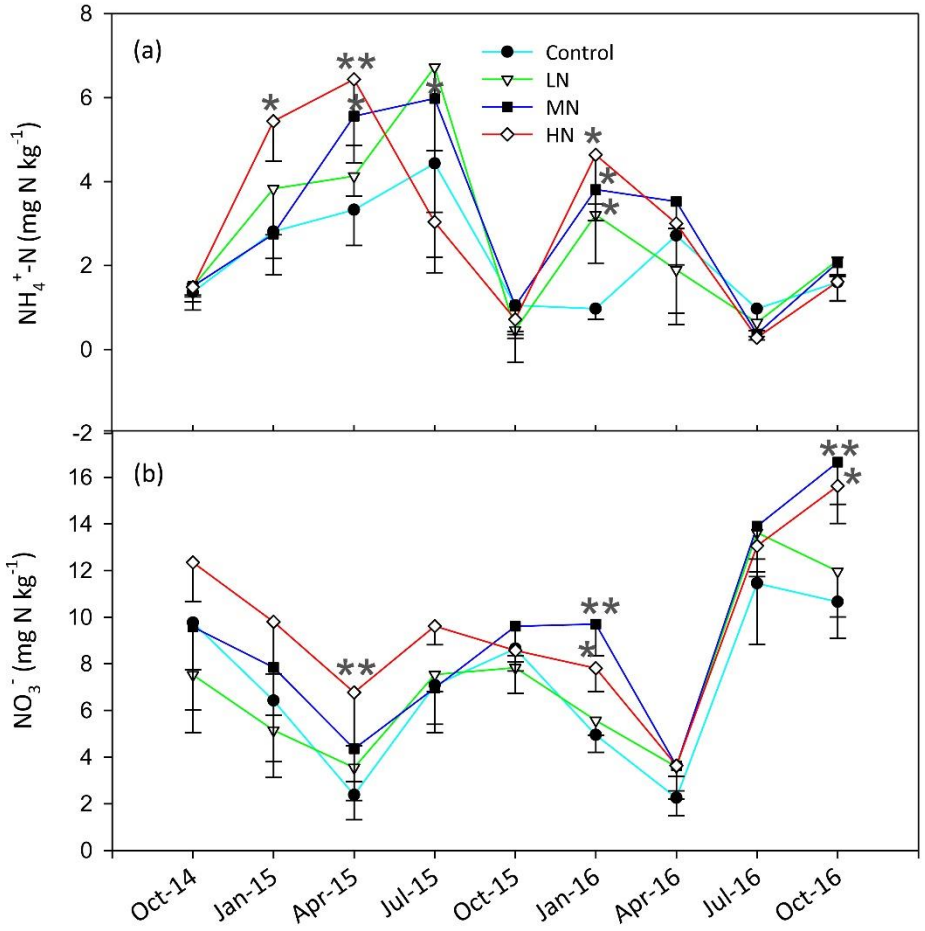

(a)
Control
LN
MN
HN
$NH_4^+$-N (mg N $kg^{-1}$)
(b)
$NO_3^-$ (mg N $kg^{-1}$)
Oct-14
Jan-15
Apr-15
Jul-15
Oct-15
Jan-16
Apr-16
Jul-16
Oct-16

Fig. 2

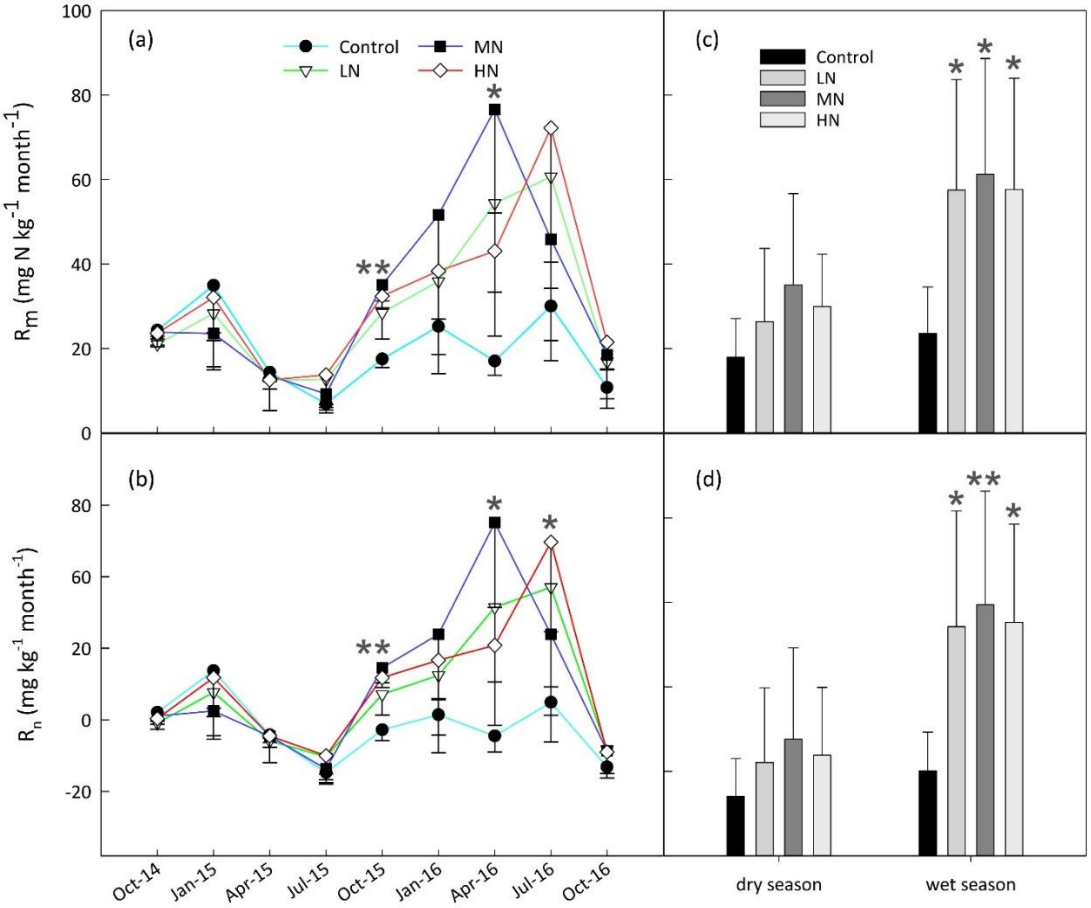

(a)
Control
LN
MN
HN
(b)
(c)
(d)
$R_m$ (mg N kg$^{-1}$ month$^{-1}$)
$R_n$ (mg kg$^{-1}$ month$^{-1}$)
Oct-14
Jan-15
Apr-15
Jul-15
Oct-15
Jan-16
Apr-16
Jul-16
Oct-16
dry season
wet season

Fig. 3

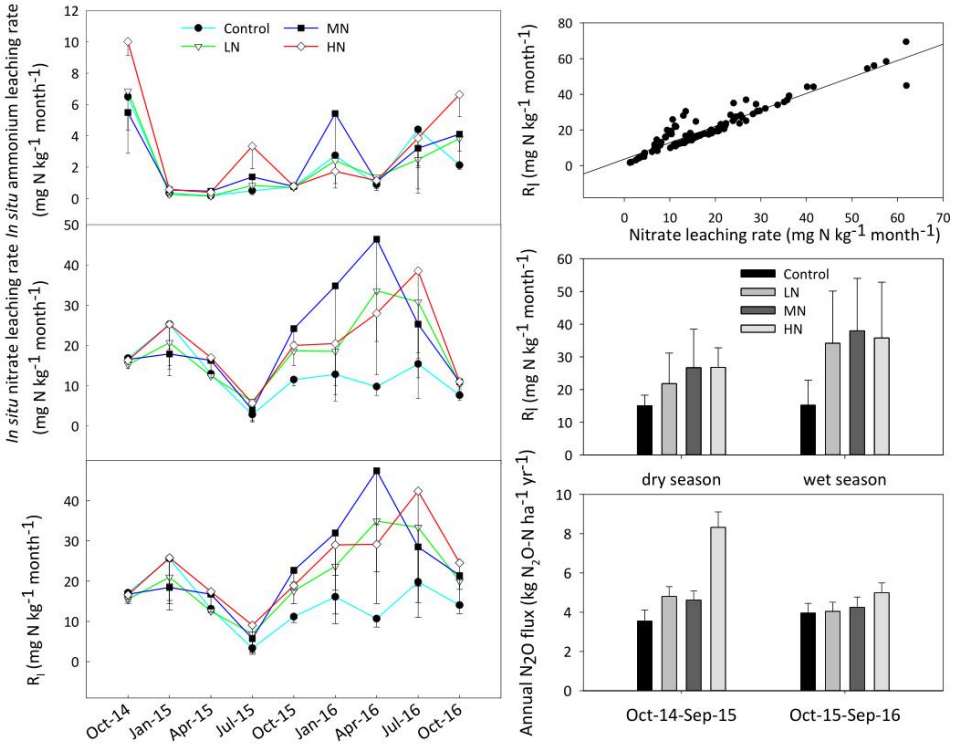

In situ nitrate leaching rate (mg N kg-1 month-1)
In situ ammonium leaching rate (mg N kg-1 month-1)
Control
LN
MN
HN
Ri (mg N kg-1 month-1)
Oct-14
Jan-15
Apr-15
Jul-15
Oct-15
Jan-16
Apr-16
Jul-16
Oct-16
Ri (mg N kg-1 month-1)
Nitrate leaching rate (mg N kg-1 month-1)
Ri (mg N kg-1 month-1)
dry season
wet season
Annual N2O flux (kg N2O-N ha-1 yr-1)
Oct-14-Sep-15
Oct-15-Sep-16

Fig. 4

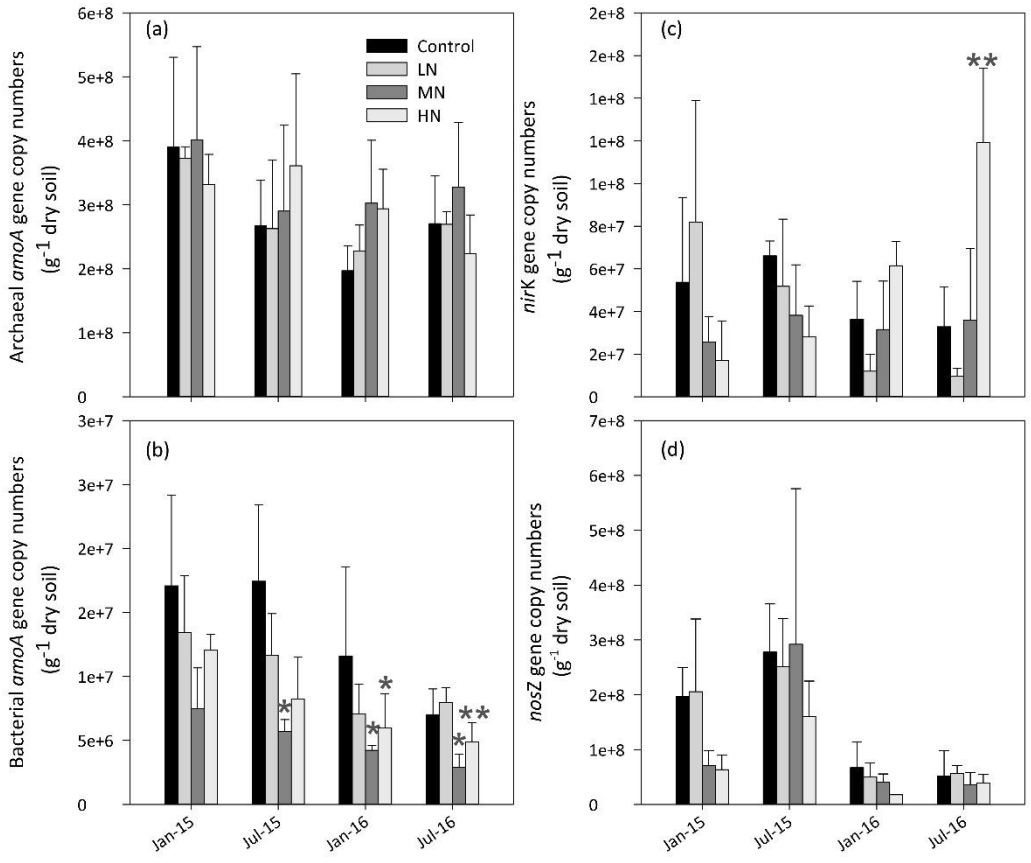

(a)
Control
LN
MN
HN
Archaeal amoA gene copy numbers (g-1 dry soil)
(b)
Bacterial amoA gene copy numbers (g-1 dry soil)
(c)
nirK gene copy numbers (g-1 dry soil)
(d)
nosZ gene copy numbers (g-1 dry soil)
Jan-15
Jul-15
Jan-16
Jul-16

Fig. 5

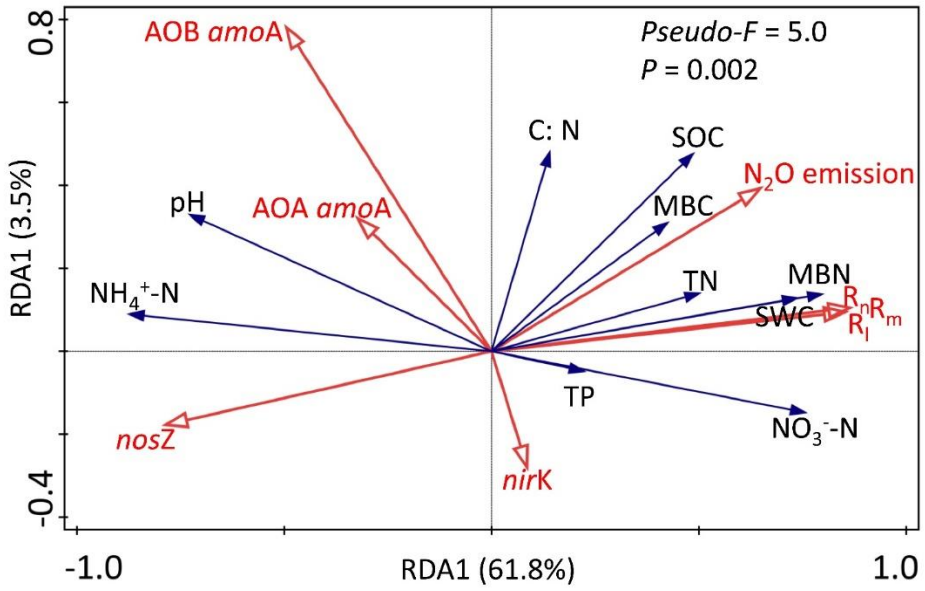
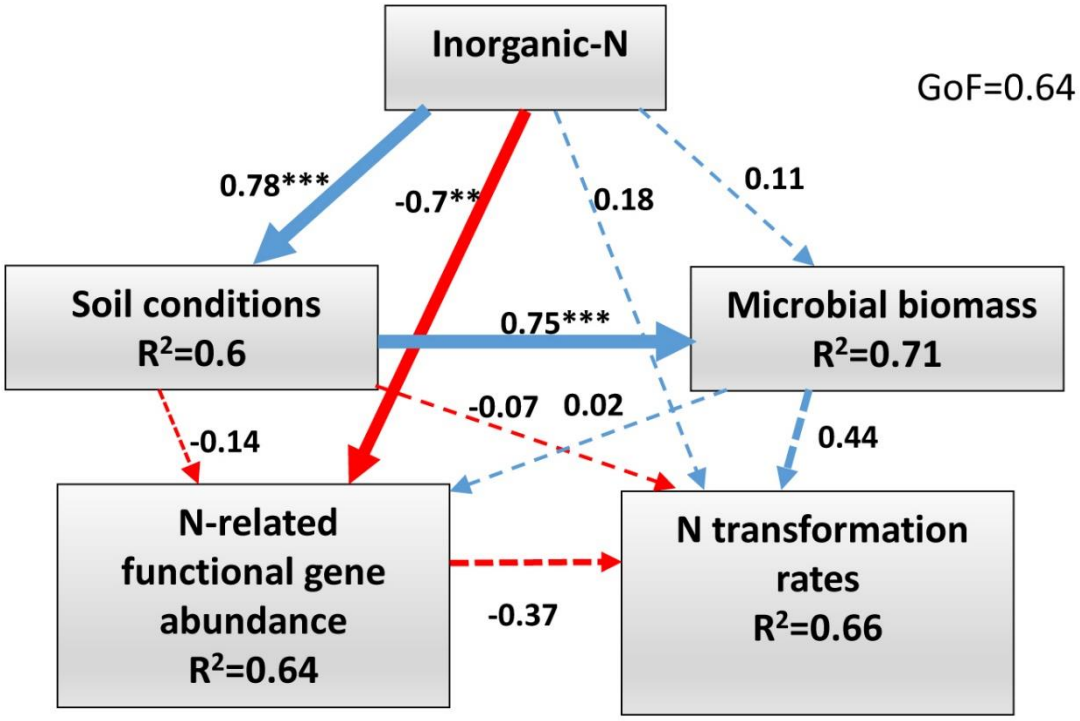

AOB amoA
Pseudo-F = 5.0
P = 0.002
C: N
SOC
N2O emission
pH
AOA amoA
MBC
TN
MBN
NH4+-N
SWC
Rn
Rm
Rl
TP
NO3--N
nosZ
nirK
0.8
-0.4
RDA1 (3.5%)
-1.0
RDA1 (61.8%)
1.0

Fig. 6


Inorganic-N
GoF=0.64
0.78***
-0.7**
0.18
0.11
Soil conditions
R²=0.6
0.75***
Microbial biomass
R²=0.71
-0.07
0.02
-0.14
0.44
N-related functional gene abundance
R²=0.64
-0.37
N transformation rates
R²=0.66

5