Author Response to Interactive Comment on "Interrelationships among soil nitrogen transformation rates, functional gene abundance and soil properties in a tropical forest with exogenous N inputs" *by Yanxia Nie et al.*

Yanxia Nie and co-authors
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Response to Anonymous Referee #1

Reviewer comment: *This paper examined the effect of N addition on soil N transformation processes. The information is valuable to our understanding of how increasing N deposition could change soil microbes and the N process they drive. However, I believe the paper should be significantly revised before publication.*

Response: Thank you for the positive evaluation of our work. The manuscript has been revised based on the suggestions of yours and another reviewer's. We hope that you would find the revision satisfactory.

Reviewer comment: *First, the difference between net N process and gross N process should be carefully discussed.*

Response: In this study, we actually only measured and reported the net N mineralization and nitrification rates estimated using the field incubation method, which are different from the gross N mineralization and nitrification rates often estimated using the ^{15}N dilution method as the reviewer pointed out. We carefully distinguished them in discussions.

Reviewer comment: *Second, the discussion section is still the re-statement of the findings, but the underlying mechanisms of the findings were not analyzed enough. Especially, if there are inconsistent results currently in different studies, it is better to explain why the difference was observed. If this study only presents the difference, it only increases the uncertainty of current findings, but could not contribute to improve our understanding of the current findings.*

Response: Thanks for the constructive suggestion. We have revised the discussion section by eliminating the repetition of results and providing more mechanistic explanations for the different results found between our study and previous studies. For example, in the last paragraph of page 14, we compared our results with those of a previous study and explained why different results were obtained between the two studies.

Specific comments:

P2L9-10: This sentence is not clear. Rm and Rn could be driven by soil microbes, but what do you mean by saying they were driven by higher microbial biomass? You mean positive relationship between Rm and MBC?

Response: Yes, we meant to say there were significantly positive relationships between net N processes (R_m and R_n) and microbial biomass (MBC and MBN), but we used an inaccurate word as the reviewer noticed. We have corrected the sentence as "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" (see P2L9-10).

P2L13: Rm and Rn are only net N transformation rates, I don't think they are equal to soil N availability. Inorganic N content is a better proxy of soil N availability.
Response: Yes, R_m and R_n are the net N mineralization and nitrification rates estimated as the difference of inorganic N contents measured at the beginning and the end of the *in situ* incubation divided by the duration of the incubation period. So these rates do not directly reflect soil N availability; they reflect changes in soil inorganic N content over a period of time (in our study 30 days). We have corrected the sentence as "N additions significantly facilitated R_m, R_n, R_l and N_2O emission" (see P2L13).

P2L18: significantly
Response: Thanks! The correction has been made as suggested (see P2L20).

P2L20: what do you mean by saying a rate is "delayed"?
Response: In this study, we observed that the responses of soil net N transformation rates (i.e., *in situ* R_m and R_n) to N additions were not significant in the first year but became so in the second year. We therefore meant to express that we observed a delayed response. But the description was not clear enough to readers. We have revised the sentence as "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" in P2L6.

P3L15-30: this paragraph listed out some papers with different results. However, it is better to summarize these results and analyze why these results were different. For example, there are more similar studies available, why did the authors choose to mention these single papers? Did they all examined net N transformation rate?

Response: In this paragraph, we aimed to make two points: 1) relatively fewer studies addressing N addition effects on soil N transformations have been conducted in tropical forest ecosystems; 2) existing such studies have received inconsistent results for a variety of reasons. We listed 3 pairs of such N addition experiments that were conducted in the same region but differed in forest age, duration of N addition, and soil properties. We further clarified the descriptions and added a summarizing sentence to point out the main factors affecting soil N transformation responses to N additions in tropical forests. We also specified whether net or gross N transformation rates were measured in these studies (see P3L15-P4L3).

P4L15-24: Yes, gross N transformation rate is controlled by environmental factors and microbial properties. However, net N transformation rate is the results of changes in both input and output. If the gross N production rate is increased, or the N consumption rate is reduces, both could cause the increase of a net N rate. Therefore, it is better to differentiate gross N transformation and net N transformation in the introduction section.
Response: Thank you very much for the suggestions. We clearly differentiated the

gross and net N transformation rates in the Introduction section during this revision (see P4L25-34). The net N transformation rates assessed in our field incubation study are actually different from the gross N transformation rates assessed with the ^{15}N dilution method in lab incubations. Please also see our response to your second comment for further clarifications between the *in situ* net and the lab gross N transformation rates.

P4L25-L30: Again, N availability is about N pool size, while N transformation is about N dynamic. They are not the same thing.

Response: Agreed. We have corrected it as "net N transformation processes (i.e., N mineralization and nitrification) and nitrate leaching and N_2O emission" (see P5L2-4).

P6L15: More information on N2O emission is needed. How many times were N2O emission measured in each month? How was annual rate calculated?

Response: Following your suggestion, more information on N_2O emission measurements and calculations have been added during this revision (P7L23-30). Briefly, soil N_2O emissions were monitored using the closed chamber method. The gas samples were taken twice each month from October 2014 to September 2016, and N_2O concentrations were analyzed with a gas chromatograph. The rate of N_2O emission 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}); ρ 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 (see P7L23-P8L1).

P6L27: was a dividing factor used to calculate MBC and MBN?

Response: Yes, we used 0.45 and 0.54 as the conversion factors for MBC and MBN, respectively. We have added the information and relevant citations (Brookes et al. 1985, Soil Biology and Biochemistry, 17: 837-842; Joergensen et al. 2011, Soil Biology and Biochemistry, 43: 873-876) in this revision (see P8L11-13).

P6: It should be clarified that N inorganic N was added into the PVC tubes when N addition treatment was carried out.

Response: This is a field N addition experiment. N solutions were sprayed evenly on the soil surface in plots of 225 m^2, as well as into the incubation PVC tubes installed in the 0-10 cm soil layer. We have added more specific information on the time of N additions and the field incubation occasions. Briefly, N additions were conducted at the

end of each month (around 24th) starting in September 2014. The field incubations for assessing net N transformation rates were conducted 9 times from September 2014 through October 2016, i.e., September 2014, December 2014, March 2015, June 2015, September 2015, December 2015, March 2016, June 2016, and September 2016. Each incubation was started a couple of days before the N addition date and lasted for 30 days (see P6L26-31).

P7L8: repeated measures ANOVA should be used.

Response: Agreed. Two-way repeated measures ANOVA was performed to examine the effects of N additions on soil NH_4^+-N and NO_3^--N contents, N transformation rates and functional genes abundance over time. The results (Table S4 and S5) were listed in the section of supplementary material. We also added the corresponding description in the sections of Results and Discussion (see P8L30-31, P9L28-30, P10L10-11 and P10L24-25).

P7L14: Is the premise of the PLS-PM method satisfied?

Response: The Partial Least Squares Path Modeling (PLS-PM) method, a particularly useful statistical method for illuminating cause and effect relationships among observed and latent variables (Tenenhaus et al. 2005, Computational statistics and data analysis, 48: 159-205), is often used to explore the effects of environmental variables on soil microbial communities and N transformation process and further evaluate potential causal relationships between the variables (Fan et al. 2019, Soil Biology and Biochemistry, 130: 82-93; Dai et al. 2019, Geoderma, 337: 1116-1125). The premise of using this method is the small sample size ($n < 200$), and we are assuming that all latent variables are relevant. The model is assessed using the Goodness of Fit (GoF) statistic, and the value of goodness of fit in this study which is acceptable (Sanchez. 2013, PLS Path Modeling with R, 1-222).

P10L5: Again, the difference between gross rate and net rate should be discussed. The promotion of net N mineralization was due to the promotion of gross N mineralization? or due to the reduction in immobilization? or other Loss fluxes?

Response: As we responded before, the net N transformation rates measured in this study are different from the gross N transformation rates in terms of both quantity and the methodology used: net rates in our study are assessed from field incubation whereas gross rates are often assessed with the ^{15}N dilution method in lab incubation. Based on a recent ^{15}N dilution lab incubation study using the soil samples collected from the same experimental plots as in this study, Han et al. (2018) found that the gross N mineralization rate was stimulated whereas the gross N immobilization rate was suppressed by the N additions (Science of the Total Environment, 626: 1175-1187). Therefore, the stimulation of the net N mineralization rate observed in the second year of this study might be caused by both the increased gross N mineralization rate and the reduced gross immobilization rate. However, to establish a more rigorous link between the *in situ* net rates and lab incubation gross rates, a lab incubation on net rates as done in Lovett et al. (2004, Biogeochemistry, 67: 289-308) should be helpful. We have added

these descriptions at the end of the first discussion paragraph (see P11L30-P12L7).

P11L10: NH3 should be NH4+?

Response: No! Here, it was ammonia (NH_3) rather than ammonium (NH_4^+) (see P13L15). NH_3 was the direct substrate to ammonia monooxygenase (Suzuki, 1974, journal of bacteriology, 120: 556-558). However, in the acidic soils, NH_3 substrate availability significantly below the demand of AOB, but AOA had higher substrate affinity (Stopnišek et al., 2010, Applied and Environmental Microbiology, 76: 7626).

P11L14: I wonder if the authors could dig more on the reasons of different findings rather than just saying the difference was due to different systems. If this study cannot contribute to our understanding of the reasons of current different findings, this study can only increase the uncertainty of our understand on N cycling.

Response: In this paragraph, we mainly wanted to explain why N additions had a positive (or neutral) impact on AOA abundance but a negative impact on AOB abundance by comparing with the results from a long-term (6-year) N addition experiment in a similar forest. Both decreased pH and NH_4^+ availability were the potential major contributors. We revised the whole paragraph by eliminating some repetitions of our results and the case study that is less comparable to our system. For example, the case study from the temperate steppe ecosystem (Zhang et al., 2018a, Applied and Soil Ecology, 130: 241-250), since it is a semiarid ecosystem with the dominant limiting factor being water availability (see P12L34-P13L19).

P11L23-24: what is the climate of the Masson forest in Li et al. 2019? Because nosZ is mainly affected by soil moisture conditions, it is important to know the climate information.

Response: Thanks for your suggestion! The Masson forest site has a mean annual temperature of 17 °C and mean annual precipitation of 1200–1400 mm. We have provided this information in the revision (see P13L37-P14L1).

P12L1-5: The authors only stated the results again, but did not discuss why the controlling factors were different between the two seasons. I imagine soil C:N would not change seasonally due to the large pool size. Why wasn't it a controlling factor in the wet season?

Response: As the reviewer assumed, soil C:N ratio indeed did not exhibit a seasonal variation (Table. S3), but it was decreased by the N additions, and the decreased C:N ratio was the dominant cause for the increased Rm and Rn in the dry season. According to the RDA analysis, soil C:N ratio was also a controlling factor in the wet season. However, the importance of the C:N ratio in affecting N transformation rates was lower than the other factors such as microbial biomass and soil water content (Fig. 5). We therefore did not consider it as a dominant influential factor in the wet season (see P14L16-24).

P12L20-35: I think some of the discussions here should be mentioned earlier and this section should be re-organized to be more logical. For example, the difference between gross and net rate; the effect of NosZ on N_2O, the effect of moisture (wet season) on

NosZ and N_2O. These information are important factors for understanding the underlying mechanisms of the findings and should be carefully analyzed when the findings were discussed. The whole paragraph need to be reorganized according to this suggestion.

Response: Following your suggestion, we have discussed the difference between gross and net rates, the effect of *nosZ* gene abundance on N_2O emission, and the effect of moisture (wet season) on *nosZ* gene abundance and N_2O emission in the first (see P11L30-P12L7) and fifth paragraph (see P13L24-P14L6) of the Discussion section. We further clarified the discussions on the comparison between our findings and those of Isobe et al. (2012, FEMS Microbiology Ecology, 80: 193-203). Hopefully these revisions would make the section reads more logical (see P15L4-17).

P13L5-10: For net N mineralization, it could be possible that both gross N mineralization and immobilization were suppressed, while net N mineralization did not change much. Then it does not mean the negative effects of N addition on soil microbes did not affect N transformation.

Response: We specified that the N mineralization and nitrification rates we were referring to was the net N transformation rates, which were measured using the field incubation method in this study. We agree with the reviewer that N addition can alter both gross N mineralization rate and gross immobilization rate without altering the net rate, since net rate (e.g., net N mineralization rate) conceptually is the balance between gross mineralization rate and gross immobilization rate. Therefore, unchanged net rates do not mean unchanged gross rates, which were possibly suppressed because of the negative effects of N additions on soil microbes as the reviewer pointed out (see P15L28-29).

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Response to Anonymous Referee #2

Reviewer comment: Due to the complexity of nitrogen cycling in terrestrial ecosystems, it deserves to explore how elevated nitrogen deposition affects soil N transformations in the N-rich soil of tropical forests. Overall, this manuscript was well written and easy to read, but the current version is suffering from some critical defects.

Response: Thanks for the positive evaluation to our work! We carefully revised the manuscript based on your suggestions. Our point-by-point responses to your comments are listed below. Hope you would find these revisions satisfactory.

Reviewer comment: First, this study measured the net mineralization and nitrification, completely different from gross mineralization and nitrification. To this point, the title of this study is not appropriate, because net mineralization and nitrification actually include the balance of various transformation processes such as ammoniation and immobilization, which conceals real nitrogen transformation processes.

Response: Agreed. Net mineralization and nitrification rates essentially measures the net temporal changes in the pool size of inorganic N (NO_3^- and NH_4^+) contents within the incubation period (in our case, 30 days). The limitation of field-assessed net rates can not disentangle the detailed gross transformation rates actually happening simultaneously. We therefore specified the N transformation rates as 'net N transformation rates' in the title and throughout the manuscript during this revision. In another study from our lab, Han et al. (2018) reported the responses of gross rates to N additions (Science of the Total Environment, 626: 1175-1187). We mentioned some of their results in our discussions.

Reviewer comment: Second, the descriptions in Methods are not detailed and thus affect understanding of the results. For example, the descriptions about the specific time for nitrogen addition and sampling soil cores for net mineralization were unclear. Considering net mineralization is the difference of ammonium concentrations between 30 days, the time for nitrogen addition and the sampling of two soil cores is very important. If the sampling of second soil cores was just after nitrogen addition, mineralization could be overestimated because added N contributed to increase in soil ammonium concentrations.

Response: N additions were applied on the 24th of each month from September 2014 through October 2016. The incubations were carried out 9 times in September 2014, December 2014, March 2015, June 2015, September 2015, December 2015, March 2016, June 2016, and September 2016. Each incubation was started a couple of days before the N addition date and lasted for 30 days. We have provided these methodological details in the revised manuscript as the reviewer suggested (see P6L26-31).

Reviewer comment: Third, it is well known that nitrogen addition will lead to soil acidification. However, this study did not separate from inorganic nitrogen input from its acidification (also see Fig. 6). This strongly reduces the importance of this study, e.g. both low pH and higher inorganic nitrogen concentrations can show negative effects on nitrogen transformations.

Response: In the 2-year study period, we monitored the changes in both soil pH and inorganic N (NH_4^+-N and NO_3^--N) contents after N additions and analyzed the relationship between these important factors and net N transformation rates. No significant relationship was found between them in the dry season (Table 1a). However, in the wet season, the net N transformation rates (R_m and R_n) had significantly positive correlations with NO_3^--N content, but had significantly negative relationships with soil pH and NH_4^+-N contents (Table 1b and Fig. 5). Since changes in pH actually was

induced by the nitrogen additions, we were therefore not able to separate the N addition effects from the acidification effects with our experimental design (only N input was manipulated). Further studies manipulating both soil acidification and N addition at the same time might be helpful in teasing out the two kinds of effects.

Reviewer comment: Fourth, it is very good to include the measurements of N-related functional gene abundance, but it is a pity that N-related functional gene abundance was not related with the specific nitrogen transformation processes. As a result, it is difficult to make a microbial mechanism explanation for net mineralization and nitrification. Before the manuscript is accepted to publish, the above issues should be well clarified.

Response: Yes, we only found N_2O emission exhibited a significantly relationship with *nosZ* gene abundance in this study. We did not find a significant relationship between the AOA abundance and net N mineralization rates. The main reason may be that net N mineralization rate actually measures the net temporal changes in inorganic N pool sizes, which are governed by several specific gross input and output rates such as gross mineralization and immobilization. It is possible that the functional genes abundance may have closer relationships with the gross N transformation rates. Some of such relationships have been reported in a recent study by Han et al. (2018) using soil samples taken from the same experimental plots as in this study. We have added these descriptions in the revised discussion to further explore the relationships between functional gene abundance and N transformation rates (see P11L31-P12L7).

Short-term N addition accelerates net N mineralization and nitrification in a tropical forest soil

~~Interrelationships among soil nitrogen transformation rates, functional gene abundance and soil properties in a tropical forest with exogenous N inputs~~

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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 tropical 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 negligibledelayed 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.The R_m and R_n were predominately driven by the lower C:N ratio under N addition in the dry season but by 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 soil N availability (R_m and R_n) and N loss (R_l and N_2O emission), which had a stimulating effect on N transformations. 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 significantlysignificant 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.

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 ~~have~~ reported the alteration of N transformation rates after N additions~~, but these studies~~ have ~~had~~ received inconsistent results for tropical forest ecosystems~~due to the different soil types, soil ages, N status and duration of N additions in tropical forest ecosystems (Lohse and Matson, 2005;Corre et al., 2010;Chen et al., 2016)~~. For example, ~~I~~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). ~~Lohse and Matson (2005) reported that first-time and long-term N additions did not change the rate of net N mineralization (R_m) but significantly increased the net nitrification rate (R_n) and delayed the nitrate leaching rate in a 4.1-million-year-old N-rich and phosphorus (P)-limited forest soil. Corre et al. (2010) documented that the~~ The N addition effects on ~~difference in soil~~ 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, ~~Chen et al. (2016) found that~~ 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_I were observed with a 3-year N addition in ~~the~~ 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, ~~tree species,~~ soil texture and pH (Templer et al., 2005;Chen et al., 2017;~~Ribbons et al., 2018;~~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 (~~Templer et al., 2012;~~Li et al., 2014;Liu et al., 2017~~;Fujii et al., 2018~~). 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. We hereby investigate (1) the effects of short-term N addition on net N transformation processes (i.e., N mineralization and nitrification), nitrate leaching and N_2O emission~~N availability (i.e., N mineralization and nitrification) and N loss (i.e., nitrate leaching and N_2O emission)~~ in N-rich tropical forest soil; (2) the correlations between the variation in soil functional microbial abundances and the corresponding N transformation rates; and (3) the seasonal patterns of N transformations with different soil temperatures and moisture in the dry and wet seasons.

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 ~~old-growth and highly weathered~~ evergreen broad-leaved forest with the age of about 110 ~~history of more than 400~~ 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). 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} $year^{-1}$). 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 ~~October~~ 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:

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

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

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

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}); ρ 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 metre 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 process (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_I 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_I 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 1.7×10^8 to 5.2×10^8 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 genes are inapparent 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 ~~Fig.~~ 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 ~~only for a short term (~~from October 2015 to July 2016~~,~~ (~~-~~Fig. 2a, b and ~~Fig.~~ 3c). ~~Therefore, our study~~ is data~~in the tropical forest~~ provides evidence of the stimulating effects of N inputs on net N transformation processes (i.e., *in situ* R_m~~,~~ and R_n ~~and R_l~~) in tropical forests. In a recent lab 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 lab-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, lab-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 ± 6.5 and 45.1 ± 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 ± 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 ~~implying that increasing N deposition should exist in natural forest ecosystems.~~ In addition, the rates of N_2O emissions (95.0 ± 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 ~~the acidic~~our younger

broadleaf forest as well ~~forest soil (Isobe et al., 2012)~~. The two ammonia oxidizers showed different response patterns to N additions; ~~The~~ the abundance of AOA did not differ statistically significantly among the ~~slightly increased with 10 months of N addition, but no significant decrease was found in all~~four N treatments but showed an increasing trend as N addition level increased (Fig. 4a); contrastingly~~.~~ ~~On the other hand,~~ the abundance of AOB were significantly decreased ~~in~~ by the MN- and HN-treat~~ed plots compared to that in the control plots~~ments (Fig. 4b). These results are similar to what have been found in ~~A~~a previous study ~~partially confirmed that~~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~~se phenomena~~ 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). ~~A previous study documented that a 9-year N addition had no influence on soil N-related functional gene copy numbers in a temperate steppe (Zhang et al., 2018a). These differences may be due to the different soil types and N statuses (N-limited or N-rich) in different ecosystems.~~

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 *nosZ* gene abundance in the wet season (Table 1b). It supports the previous findings that decreased *nosZ* 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). ~~To some extent, the abundances of denitrifiers were related to their corresponding N transformation process.~~ 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* ~~*nirK*~~ 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 ~~N availability (~~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). ~~Among them, t~~The higher soil microbial biomass and SWC in the wet season ~~was higher due to the higher SWC and temperature relative to that in the dry season in the forest soil (Deng et al., 2012), which~~ could ~~drive~~ facilitate N mineralization and nitrification ~~(Templer et al., 2005)~~ and then ~~form~~ cause ~~more~~ 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 *nosZ*-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 *nosZ* 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 ~~2b~~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. ~~There was no positive relationship between AOA abundance and Rn in the study period (Table 1), which disagreed with the results of a previous study showing that the abundance of AOA *amoA* was significantly correlated with gross nitrification rates in this acidic forest soil using the ^{15}N isotope dilution method (Isobe et al., 2012). This difference could be possibly explained by the net nitrification measurements underestimating gross nitrification (Li et al., 2018b). Furthermore, N_2O emissions had significantly negative correlation with *nosZ* gene abundance (R = -0.47, P < 0.05) in the wet season (Table 2b). It was reported that decreasing *nosZ* gene abundance with N addition was the major factor resulting in low N_2O consumption and high N_2O emissions in the acidic forest soil (Zhang et al., 2008a).~~ 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, ~~T~~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.

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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References

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited, Bioscience, 48, 921-934, https://doi.org/10.2307/1313296, 1998.

Aber, J. D., Nadelhoffer, K. J., Steudler, P., and Melillo, J. M.: Nitrogen saturation in northern forest

ecosystems, Bioscience, 39, 378-386, https://doi.org/10.2307/1311067, 1989.
Booth, M. S., Stark, J. M., and Rastetter, E.: Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data, Ecol. Monogr., 75, 139-157, https://doi.org/10.1890/04-0988, 2005.
Braker, G., Zhou, J. Z., Wu, L. Y., Devol, A. H., and Tiedje, J. M.: Nitrite reductase genes (*nirK* and *nirS*) as functional markers to investigate diversity of denitrifying bacteria in Pacific northwest marine sediment communities, Appl. Environ. Microbiol., 66, 2096-2104, https://doi.org/10.1128/AEM.66.5.2096-2104.2000, 2000.
Brookes, P. C., Landman, A., Pruden, G., and Jenkinson, D. S.: Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil, Soil Biol. Biochem., 17, 837-842, https://doi.org/10.1016/0038-0717(85)90144-0, 1985.
Chen, H., Gurmesa, G. A., Zhang, W., Zhu, X. M., Zheng, M. H., Mao, Q. G., Zhang, T., and Mo, J. M.: Nitrogen saturation in humid tropical forests after 6 years of nitrogen and phosphorus addition: hypothesis testing, Funct. Ecol., 30, 305-313, https://doi.org/10.1111/1365-2435.12475, 2016.
Chen, H., Li, D. J., Zhao, J., Xiao, K. C., and Wang, K. L.: Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes : A meta-analysis, Agric. Ecosyst. Environ., 252, 126-131, https://doi.org/10.1016/j.agee.2017.09.032, 2018.
Chen, J., Xiao, G. L., Kuzyakov, Y. K., Jenerette, G. D., Ma, Y., Liu, W., Wang, Z. F., and Shen, W. J.: Soil nitrogen transformation responses to seasonal precipitation changes are regulated by changes in functional microbial abundance in a subtropical forest, Biogeosciences, 14, 2513-2525, https://doi.org/10.5194/bg-14-2513-2017, 2017.
Chen, J., Kuzyakov, Y., Jenerette, G. D., Xiao, G., Liu, W., Wang, Z., and Shen, W.: Intensified Precipitation Seasonality Reduces Soil Inorganic N Content in a Subtropical Forest: Greater Contribution of Leaching Loss Than N_2O Emissions, Journal of Geophysical Research: Biogeosciences, 124, 494-508, https://doi.org/10.1029/2018jg004821, 2019.
Chen, Z., Luo, X. Q., Hu, R. G., Wu, M. N., Wu, J. S., and Wei, W. X.: Impact of long-term fertilization on the composition of denitrifier communities based on nitrite reductase analyses in a paddy soil, Microb. Ecol., 60, 850-861, https://doi.org/10.1007/s00248-010-9700-z, 2010.
Cheng, Y., Wang, J., Chang, S. X., Cai, Z. C., Müller, C., and Zhang, J. B.: Nitrogen deposition affects both net and gross soil nitrogen transformations in forest ecosystems: A review, Environ. pollut. (Barking, Essex : 1987), 244, 608-616, https://doi.org/10.1016/j.envpol.2018.10.054, 2019.
Contosta, A. R., Frey, S. D., and Cooper, A. B.: Seasonal dynamics of soil respiration and N mineralization in chronically warmed and fertilized soils, Ecosphere, 2, 21, https://doi.org/10.1890/es10-00133.1, 2011.
Corre, M. D., Veldkamp, E., Arnold, J., and Wright, S. J.: Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama, Ecology, 91, 1715-1729, https://doi.org/10.1890/09-0274.1, 2010.
Fang, H. J., Yu, G. R., Cheng, S. L., Zhu, T. H., Zheng, J. J., Mo, J. M., Yan, J. H., and Luo, Y. Q.:

Nitrogen-15 signals of leaf-litter-soil continuum as a possible indicator of ecosystem nitrogen saturation by forest succession and N loads, Biogeochemistry, 102, 251-263, https://doi.org/10.1007/s10533-010-9438-1, 2011.

Fang, Y. T., Gundersen, P., Mo, J. M., and Zhu, W. X.: Input and output of dissolved organic and inorganic nitrogen in subtropical forests of South China under high air pollution, Biogeosciences, 5, 339-352, https://doi.org/10.5194/bg-5-339-2008, 2008.

Fang, Y. T., Gundersen, P., Mo, J. M., and Zhu, W. X.: Nitrogen leaching in response to increased nitrogen inputs in subtropical monsoon forests in southern China, For. Ecol. Manage., 257, 332-342, https://doi.org/10.1016/j.foreco.2008.09.004, 2009a.

Fang, Y. T., Yoh, M., Mo, J. M., Gundersen, P., and Zhou, G. Y.: Response of Nitrogen Leaching to Nitrogen Deposition in Disturbed and Mature Forests of Southern China, Pedosphere, 19, 111-120, https://doi.org/10.1016/s1002-0160(08)60090-9, 2009b.

Fu, M. H., Xu, X. C., and Tabatabai, M. A.: Effect of pH on nitrogen mineralization in crop-residue-treated soils, Biol. Fert. Soils, 5, 115-119, 1987.

Fu, X., Liu, X., Li, Y., Shen, J., Wang, Y., Zou, G., Li, H., Song, L., and Wu, J.: Wet-season spatial variability in N_2O emissions from a tea field in subtropical central China, Biogeosciences, 12, 3899-3911, https://doi.org/10.5194/bg-12-3899-2015, 2015.

~~Fujii, K., Yamada, T., Hayakawa, C., Nakanishi, A., and Funakawa, S.: Another bottleneck for nitrogen mineralization in temperate forest soils: Arginine metabolism in microorganisms, Soil Biol. Biochem., 126, 22-30, https://doi.org/10.1016/j.soilbio.2018.08.005, 2018.~~

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z. C., Freney, J. R., Martinelli, L. A., Seitzinger, S. P., and Sutton, M. A.: Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions, Science, 320, 889-892, https://doi.org/10.1126/science.1136674, 2008.

Gao, W. L., Kou, L., Yang, H., Zhang, J. B., Müller, C., and Li, S. G.: Are nitrate production and retention processes in subtropical acidic forest soils responsive to ammonium deposition?, Soil Biol. Biochem., 100, 102-109, https://doi.org/10.1016/j.soilbio.2016.06.002, 2016.

Geisseler, D., Horwath, W. R., Joergensen, R. G., and Ludwig, B.: Pathways of nitrogen utilization by soil microorganisms - A review, Soil Biol. Biochem., 42, 2058-2067, https://doi.org/10.1016/j.soilbio.2010.08.021, 2010.

Gundersen, P., Emmett, B. A., Kjonaas, O. J., Koopmans, C. J., and Tietema, A.: Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data, For. Ecol. Manage., 101, 37-55, https://doi.org/10.1016/s0378-1127(97)00124-2, 1998.

Gurmesa, G. A., Lu, X. K., Gundersen, P., Mao, Q. G., Zhou, K. J., Fang, Y. T., and Mo, J. M.: High retention of ^{15}N-labeled nitrogen deposition in a nitrogen saturated old-growth tropical forest, Glob. Change Biol., 22, 3608-3620, https://doi.org/10.1111/gcb.13327, 2016.

Hall, S. J., and Matson, P. A.: Nitrogen oxide emissions after nitrogen additions in tropical forests, Nature, 400, 152-155, https://doi.org/10.1038/22094, 1999.

Hall, S. J., and Matson, P. A.: Nutrient status of tropical rain forests influences soil N dynamics after N additions, Ecol. Monogr., 73, 107-129, https://doi.org/ 10.2307/3100077, 2003.

Han, X. G., Shen, W. J., Zhang, J. B., and Müller, C.: Microbial adaptation to long-term N supply prevents large responses in N dynamics and N losses of a subtropical forest, Sci. Total Environ., 626, 1175-1187, https://doi.org/10.1016/j.scitotenv.2018.01.132, 2018.

He, J. Z., Shen, J. P., Zhang, L. M., Zhu, Y. G., Zheng, Y. M., Xu, M. G., and Di, H. J.: Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices, Environ. Microbiol., 9, 2364-2374, https://doi.org/10.1111/j.1462-2920.2007.01358.x, 2007.

He, J. Z., Hu, H. W., and Zhang, L. M.: Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils, Soil Biol. Biochem., 55, 146-154, https://doi.org/10.1016/j.soilbio.2012.06.006, 2012.

Hietz, P., Turner, B. L., Wanek, W., Richter, A., Nock, C. A., and Wright, S. J.: Long-term change in the nitrogen cycle of tropical forests, Science, 334, 664-666, https://doi.org/10.1126/science.1211979, 2011.

Hu, H. W., Zhang, L. M., Dai, Y., Di, H. J., and He, J. Z.: pH-dependent distribution of soil ammonia oxidizers across a large geographical scale as revealed by high-throughput pyrosequencing, J. Soils Sediments, 13, 1439-1449, https://doi.org/10.1007/s11368-013-0726-y, 2013.

Isobe, K., Koba, K., Suwa, Y., Ikutani, J., Fang, Y. T., Yoh, M., Mo, J. M., Otsuka, S., and Senoo, K.: High abundance of ammonia-oxidizing archaea in acidified subtropical forest soils in southern China after long-term N deposition, FEMS Microbiol. Ecol., 80, 193-203, https://doi.org/10.1111/j.1574-6941.2011.01294.x, 2012.

Jan, M. T., Roberts, P., Tonheim, S. K., and Jones, D. L.: Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils, Soil Biol. Biochem., 41, 2272-2282, https://doi.org/10.1016/j.soilbio.2009.08.013, 2009.

Joergensen, R. G., Wu, J., and Brookes, P. C.: Measuring soil microbial biomass using an automated procedure, Soil Biology and Biochemistry, 43, 873-876, https://doi.org/10.1016/j.soilbio.2010.09.024, 2011.

Kemmitt, S. J., Wright, D., Goulding, K. W. T., and Jones, D. L.: pH regulation of carbon and nitrogen dynamics in two agricultural soils, Soil Biol. Biochem., 38, 898-911, https://doi.org/10.1016/j.soilbio.2005.08.006, 2006.

Koehler, B., Corre, M. D., Veldkamp, E., Wullaert, H., and Wright, S. J.: Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input, Global Change Biology, 15, 2049-2066, https://doi.org/10.1111/j.1365-2486.2008.01826.x, 2009.

Levy-Booth, D. J., Prescott, C. E., and Grayston, S. J.: Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems, Soil Biology & Biochemistry, 75, 11-25, https://doi.org/10.1016/j.soilbio.2014.03.021, 2014.

Li, M., Zhou, X. H., Zhang, Q. F., and Cheng, X. L.: Consequences of afforestation for soil nitrogen dynamics in central China, Agric. Ecosyst. Environ., 183, 40-46, https://doi.org/10.1016/j.agee.2013.10.018, 2014.

Li, X. J., Yang, H. T., Shi, W. L., Li, Y. F., and Guo, Q.: Afforestation with xerophytic shrubs accelerates soil net nitrogen nitrification and mineralization in the Tengger Desert, Northern China, Catena, 169, 11-20, https://doi.org/10.1016/j.catena.2018.05.026, 2018.

Li, Y., Chen, Z., He, J. Z., Wang, Q., Shen, C. C., and Ge, Y.: Ectomycorrhizal fungi inoculation alleviates simulated acid rain effects on soil ammonia oxidizers and denitrifiers in Masson pine forest, Environ. Microbiol., 21, 299-313, https://doi.org/10.1111/1462-2920.14457, 2019a.

Li, Z. L., Tian, D. S., Wang, B. X., Wang, J. S., Wang, S., Chen, H. Y. H., Xu, X. F., Wang, C. H., He, N. P., and Niu, S. L.: Microbes drive global soil nitrogen mineralization and availability, Glob. Change Biol., 25, 1078-1088, https://doi.org/10.1111/gcb.14557, 2019b.

Liu, B. B., Frostegard, A., and Bakken, L. R.: Impaired reduction of N_2O to N_2 in acid soils is due to a posttranscriptional interference with the expression of *nos*Z, mBio, 5, 10, https://doi.org/10.1128/mBio.01383-14, 2014.

Liu, X. J., Zhang, Y., Han, W. X., Tang, A. H., Shen, J. L., Cui, Z. L., Vitousek, P., Erisman, J. W., Goulding, K., Christie, P., Fangmeier, A., and Zhang, F. S.: Enhanced nitrogen deposition over China, Nature, 494, 459-462, https://doi.org/10.1038/nature11917, 2013.

Liu, Y., Wang, C. H., He, N. P., Wen, X. F., Gao, Y., Li, S. G., Niu, S. L., Butterbach-Bahl, K., Luo, Y. Q., and Yu, G. R.: A global synthesis of the rate and temperature sensitivity of soil nitrogen mineralization: latitudinal patterns and mechanisms, Glob. Change Biol., 23, 455-464, https://doi.org/doi:10.1111/gcb.13372, 2017.

Lohse, K. A., and Matson, P.: Consequences of nitrogen additions for soil losses from wet tropical forests, Ecol. Appl., 15, 1629-1648, https://doi.org/10.1890/03-5421, 2005.

Lovett, G. M., Weathers, K. C., Arthur, M. A., and Schultz, J. C.: Nitrogen cycling in a northern hardwood forest: Do species matter? Biogeochemistry, 67: 289-308, https://doi.org/doi:10.2307/1469754, 2004.

Lu, X. K., Mao, Q. G., Gilliam, F. S., Luo, Y. Q., and Mo, J. M.: Nitrogen deposition contributes to soil acidification in tropical ecosystems, Glob. Change Biol., 20, 3790-3801, https://doi.org/10.1111/gcb.12665, 2014.

Mao, Q. G., Lu, X. K., Zhou, K. J., Chen, H., Zhu, X. M., Mori, T. K., and Mo, J. M.: Effects of long-term nitrogen and phosphorus additions on soil acidification in an N-rich tropical forest, Geoderma, 285, 57-63, https://doi.org/10.1016/j.geoderma.2016.09.017, 2017.

Mo, J. M., Brown, S., Xue, J. H., Fang, Y. T., and Li, Z. A.: Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China, Plant Soil, 282, 135-151, https://doi.org/10.1007/s11104-005-5446-7, 2006.

Mooshammer, M., Wanek, W., Hammerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K. M., Zechmeister-Boltenstern, S., and Richter, A.:

Adjustment of microbial nitrogen use efficiency to carbon: nitrogen imbalances regulates soil nitrogen cycling, Nat. Commun., 5, 7, https://doi.org/10.1038/ncomms4694, 2014.

Nave, L. E., Vance, E. D., Swanston, C. W., and Curtis, P. S.: Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization, Geoderma, 153, 231-240, https://doi.org/10.1016/j.geoderma.2009.08.012, 2009.

Nicol, G. W., Leininger, S., Schleper, C., and Prosser, J. I.: The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria, Environ. Microbiol., 10, 2966-2978, https://doi.org/10.1111/j.1462-2920.2008.01701.x, 2008.

Nie, Y. X., Li, L., Isoda, R., Wang, M. C., Hatano, R., and Hashidoko, Y.: Physiological and genotypic characteristics of nitrous oxide (N_2O)-emitting *Pseudomonas* species isolated from dent corn Andisol farmland in Hokkaido, Japan, Microbes Environ., 31, 93-103, https://doi.org/10.1264/jsme2.ME15155, 2016.

Ning, Q. S., Gu, Q., Shen, J. P., Lv, X. T., Yang, J. J., Zhang, X. M., He, J. Z., Huang, J. H., Wang, H., Xu, Z. H., and Han, X. G.: Effects of nitrogen deposition rates and frequencies on the abundance of soil nitrogen-related functional genes in temperate grassland of northern China, J. Soils Sediments, 15, 694-704, https://doi.org/10.1007/s11368-015-1061-2, 2015.

Ollivier, J., Töwe, S., Bannert, A., Hai, B., Kastl, E.-M., Meyer, A., Su, M. X., Kleineidam, K., and Schloter, M.: Nitrogen turnover in soil and global change, FEMS Microbiol. Ecol., 78, 3-16, https://doi.org/10.1111/j.1574-6941.2011.01165.x, 2011.

Patel, K. F., and Fernandez, I. J.: Nitrogen mineralization in O horizon soils during 27 years of nitrogen enrichment at the Bear Brook Watershed in Maine, USA, Environ. Monit. Assess., 190, 14, https://doi.org/10.1007/s10661-018-6945-3, 2018.

Petersen, D. G., Blazewicz, S. J., Firestone, M., Herman, D. J., Turetsky, M., and Waldrop, M.: Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska, Environ. Microbiol., 14, 993-1008, https://doi.org/10.1111/j.1462-2920.2011.02679.x, 2012.

Reichmann, L. G., Sala, O. E., and Peters, D. P. C.: Water controls on nitrogen transformations and stocks in an arid ecosystem, Ecosphere, 4, 17, https://doi.org/10.1890/es12-00263.1, 2013.

~~Ribbons, R. R., Kepfer-Rojas, S., Kosawang, C., Hansen, O. K., Ambus, P., McDonald, M., Grayston, S. J., Prescott, C. E., and Vesterdal, L.: Context-dependent tree species effects on soil nitrogen transformations and related microbial functional genes, Biogeochemistry, 140, 145-160, https://doi.org/10.1007/s10533-018-0480-8, 2018.~~

Shen, J. P., Zhang, L. M., Zhu, Y. G., Zhang, J. B., and He, J. Z.: Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam, Environ. Microbiol., https://doi.org/10, 1601-1611, 10.1111/j.1462-2920.2008.01578.x, 2008.

Shi, X. Z., Hu, H. W., Wang, J. Q., He, J. Z., Zheng, C. Y., Wan, X. H., and Huang, Z. Q.: Niche separation of comammox Nitrospira and canonical ammonia oxidizers in an acidic subtropical forest soil under

long-term nitrogen deposition, Soil Biol. Biochem., 126, 114-122, https://doi.org/10.1016/j.soilbio.2018.09.004, 2018.

Song, Y. Y., Song, C. C., Hou, A. X., Ren, J. S., Wang, X. W., Cui, Q., and Wang, M. Q.: Effects of temperature and root additions on soil carbon and nitrogen mineralization in a predominantly permafrost peatland, Catena, 165, 381-389, https://doi.org/10.1016/j.catena.2018.02.026, 2018.

Tang, Y. C., Zhang, X. Y., Li, D. D., Wang, H. M., Chen, F. S., Fu, X. L., Fang, X. M., Sun, X. M., and Yu, G. R.: Impacts of nitrogen and phosphorus additions on the abundance and community structure of ammonia oxidizers and denitrifying bacteria in Chinese fir plantations, Soil Biol. Biochem., 103, 284-293, https://doi.org/10.1016/j.soilbio.2016.09.001, 2016.

Tang, Y. G., Yu, G. R., Zhang, X. Y., Wang, Q. F., Tian, D. S., Tian, J., Niu, S. L., and Ge, J. P.: Environmental variables better explain changes in potential nitrification and denitrification activities than microbial properties in fertilized forest soils, Sci. Total Environ., 647, 653-662, https://doi.org/10.1016/j.scitotenv.2018.07.437, 2019.

Templer, P. H., Groffman, P. M., Flecker, A. S., and Power, A. G.: Land use change and soil nutrient transformations in the Los Haitises region of the Dominican Republic, Soil Biol. Biochem., 37, 215-225, https://doi.org/10.1016/j.soilbio.2004.07.031, 2005.

~~Templer, P. H., Mack, M. C., Chapin, F. S., Christenson, L. M., Compton, J. E., Crook, H. D., Currie, W. S., Curtis, C. J., Dail, D. B., D'Antonio, C. M., Emmett, B. A., Epstein, H. E., Goodale, C. L., Gundersen, P., Hobbie, S. E., Holland, K., Hooper, D. U., Hungate, B. A., Lamontagne, S., Nadelhoffer, K. J., Osenberg, C. W., Perakis, S. S., Schleppi, P., Schimel, J., Schmidt, I. K., Sommerkorn, M., Spoelstra, J., Tietema, A., Wessel, W. W., and Zak, D. R.: Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of N-15 tracer field studies, Ecology, 93, 1816-1829, https://doi.org/10.1890/11-1146.1, 2012.~~

Tian, P., Zhang, J. B., Muller, C., Cai, Z. C., and Jin, G. Z.: Effects of six years of simulated N deposition on gross soil N transformation rates in an old-growth temperate forest, J. For. Res., 29, 647-656, https://doi.org/10.1007/s11676-017-0484-6, 2018.

Vance, E. D., Brookes, P. C., and Jenkinson, D. S.: An extraction method measuring soil microbial biomass-C, Soil Biol. Biochem., 19, 703-707, https://doi.org/10.1016/0038-0717(87)90052-6, 1987.

Wang, C., Liu, D. W., and Bai, E.: Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition, Soil Biol. Biochem., 120, 126-133, https://doi.org/10.1016/j.soilbio.2018.02.003, 2018a.

Wang, C., Lu, X. K., Mori, T., Mao, Q. G., Zhou, K. J., Zhou, G. Y., Nie, Y. X., and Mo, J. M.: Responses of soil microbial community to continuous experimental nitrogen additions for 13 years in a nitrogen-rich tropical forest, Soil Biol. Biochem., 121, 103-112, https://doi.org/10.1016/j.soilbio.2018.03.009, 2018b.

Wang, Z. H., Meng, Y., Zhu-Barker, X., He, X. H., Horwath, W. R., Luo, H. Y., Zhao, Y. P., and Jiang, X. J.: Responses of nitrification and ammonia oxidizers to a range of background and adjusted pH in

purple soils, Geoderma, 334, 9-14, https://doi.org/10.1016/j.geoderma.2018.07.038, 2019.

Yao, H. Y., Gao, Y. M., Nicol, G. W., Campbell, C. D., Prosser, J. I., Zhang, L. M., Han, W. Y., and Singh, B. K.: Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils, Appl. Environ. Microbiol., 77, 4618-4625, https://doi.org/10.1128/aem.00136-11, 2011.

Yu, Q., Duan, L., Yu, L. F., Chen, X., Si, G. Y., Ke, P. P., Ye, Z. X., and Mulder, J.: Threshold and multiple indicators for nitrogen saturation in subtropical forests, Environ. Pollut., 241, 664-673, https://doi.org/10.1016/j.envpol.2018.06.001, 2018.

Zhang, C. J., Yang, Z. L., Shen, J. P., Sun, Y. F., Wang, J. T., Han, H. Y., Wan, S. Q., Zhang, L. M., and He, J. Z.: Impacts of long-term nitrogen addition, watering and mowing on ammonia oxidizers, denitrifiers and plant communities in a temperate steppe, Appl. Soil Ecol., 130, 241-250, https://doi.org/10.1016/j.apsoil.2018.06.017, 2018a.

Zhang, L. M., Hu, H. W., Shen, J. P., and He, J. Z.: Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils, Isme J., 6, 1032-1045, https://doi.org/10.1038/ismej.2011.168, 2012.

Zhang, M. Y., Wang, W. J., Wang, D. J., Heenan, M., and Xu, Z. H.: Short-term responses of soil nitrogen mineralization, nitrification and denitrification to prescribed burning in a suburban forest ecosystem of subtropical Australia, Sci. Total Environ., 642, 879-886, https://doi.org/10.1016/j.scitotenv.2018.06.144, 2018b.

Zhang, T. A., Chen, H. Y. H., and Ruan, H. H.: Global negative effects of nitrogen deposition on soil microbes, Isme J., 12, 1817-1825, https://doi.org/10.1038/s41396-018-0096-y, 2018c.

Zhang, W., Mo, J. M., Yu, G. R., Fang, Y. T., Li, D. J., Lu, X. K., and Wang, H.: Emissions of nitrous oxide from three tropical forests in Southern China in response to simulated nitrogen deposition, Plant Soil, 306, 221-236, https://doi.org/10.1007/s11104-008-9575-7, 2008a.

Zhang, X. L., Wang, Q. B., Li, L. H., and Han, X. G.: Seasonal variations in nitrogen mineralization under three land use types in a grassland landscape, Acta Oecol.-Int. J. Ecol., 34, 322-330, https://doi.org/10.1016/j.actao.2008.06.004, 2008b.

Zhao, H. B., Peng, S. L., Chen, Z. Q, Wu, Z. M., Zhou, G. Y., Wang, X., and Qiu, Z. J.: Abscisic acid in soil facilitates community succession in three forests in China, J. Chem. Ecol., 37, 785-793, https://doi.org/10.1007/s10886-011-9970-z, 2011.

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

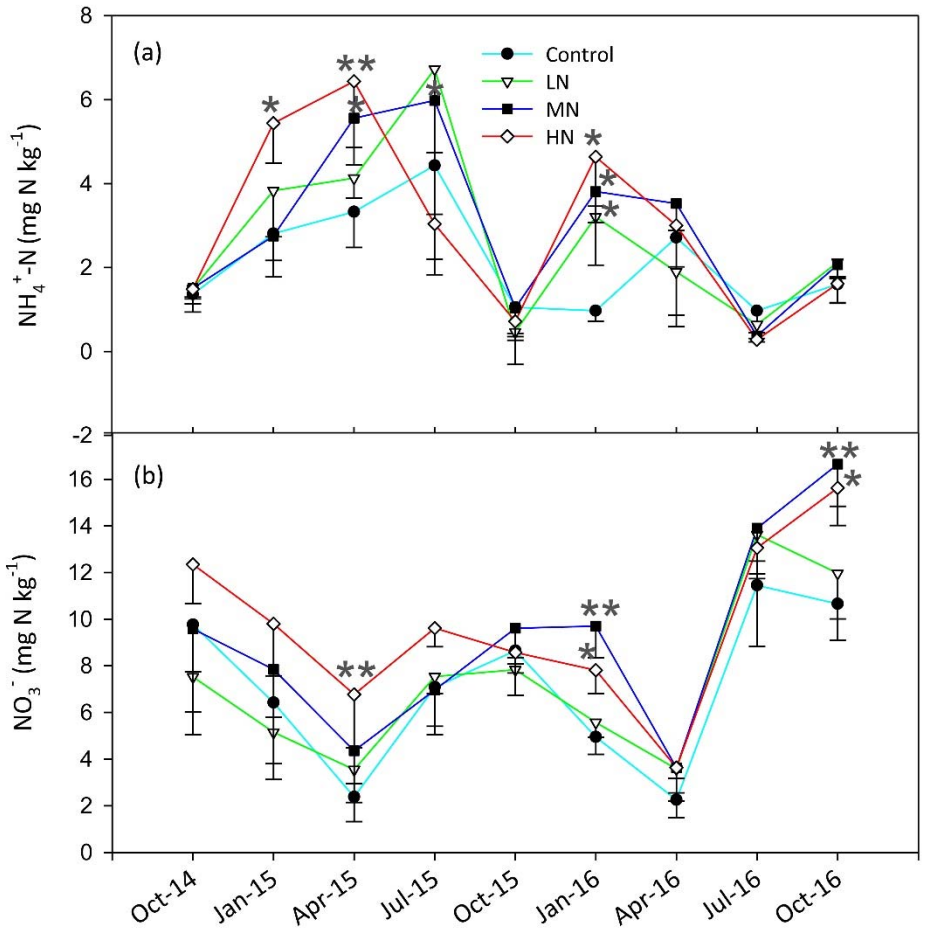

(a)
(b)
Control
LN
MN
HN
NH_4^+-N (mg N kg^{-1})
NO_3^- (mg N kg^{-1})
Oct-14
Jan-15
Apr-15
Jul-15
Oct-15
Jan-16
Apr-16
Jul-16
Oct-16

Fig. 2

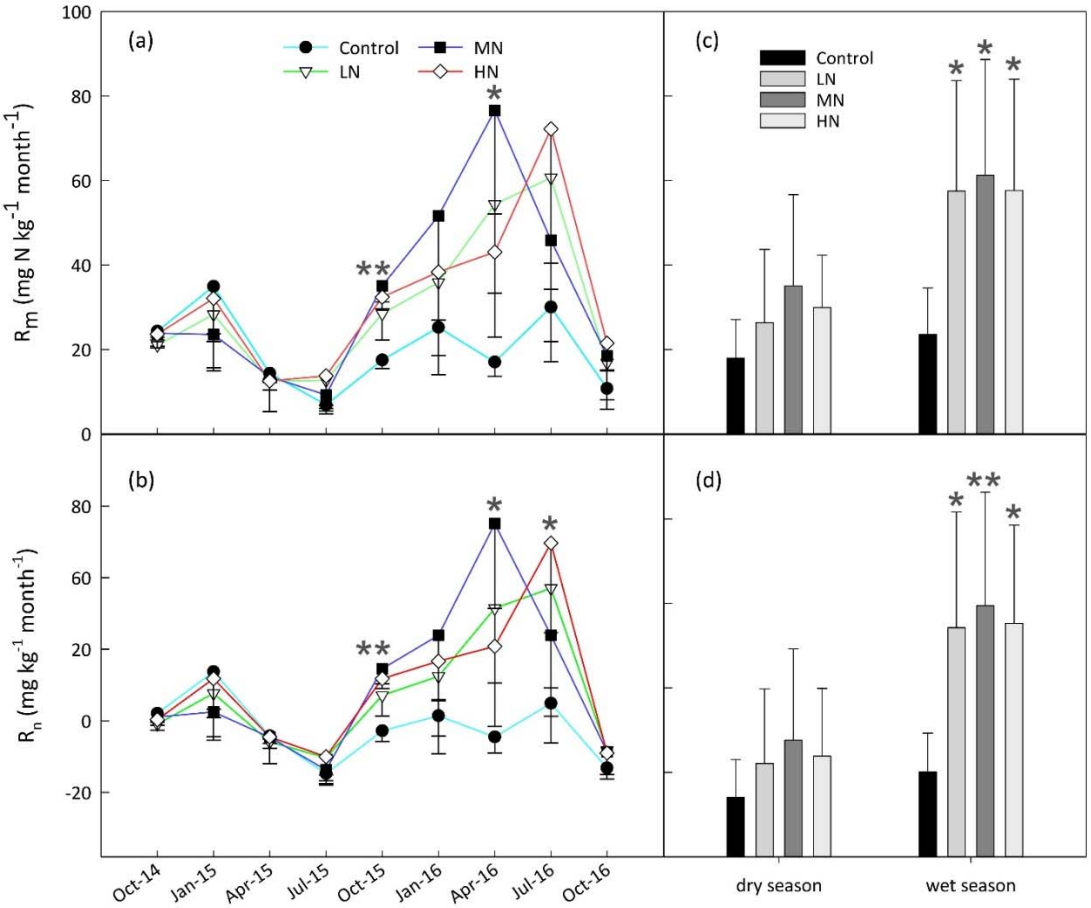

(a)
Control
LN
MN
HN
(b)
(c)
(d)
R_m (mg N kg^{-1} month^{-1})
R_n (mg kg^{-1} month^{-1})
Oct-14
Jan-15
Apr-15
Jul-15
Oct-15
Jan-16
Apr-16
Jul-16
Oct-16
dry season
wet season

Fig. 3

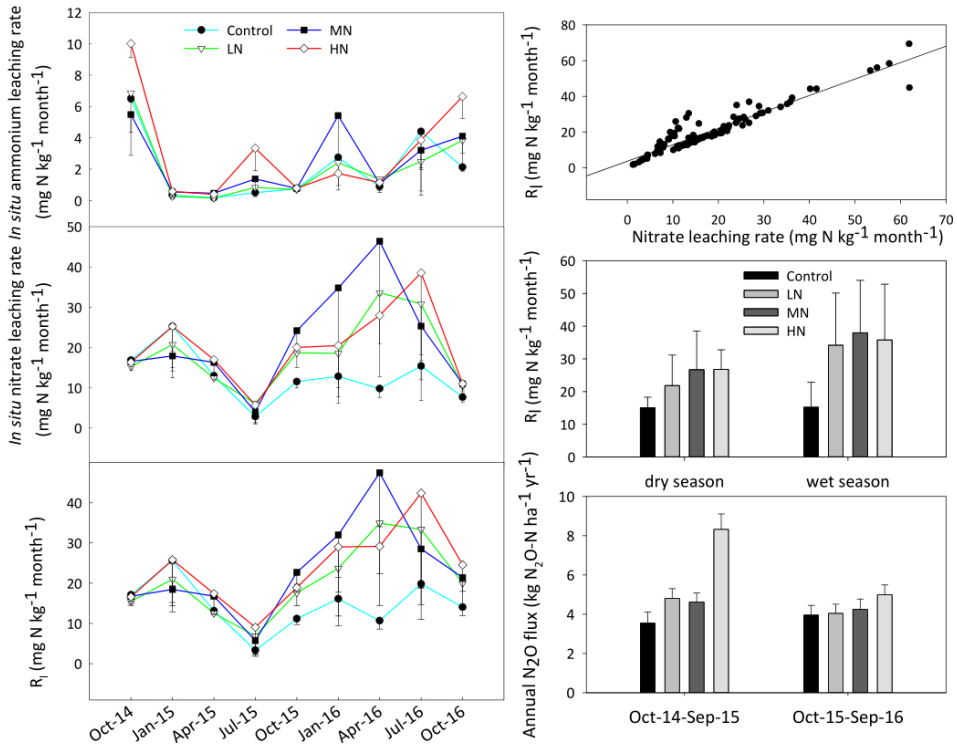

Control
LN
MN
HN
In situ nitrate leaching rate In situ ammonium leaching rate (mg N kg-1 month-1)
Ri (mg N kg-1 month-1)
Oct-14
Jan-15
Apr-15
Jul-15
Oct-15
Jan-16
Apr-16
Jul-16
Oct-16
Ri (mg N kg-1 month-1)
Nitrate leaching rate (mg N kg-1 month-1)
Ri (mg N kg-1 month-1)
dry season
wet season
Annual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

Fig. 4

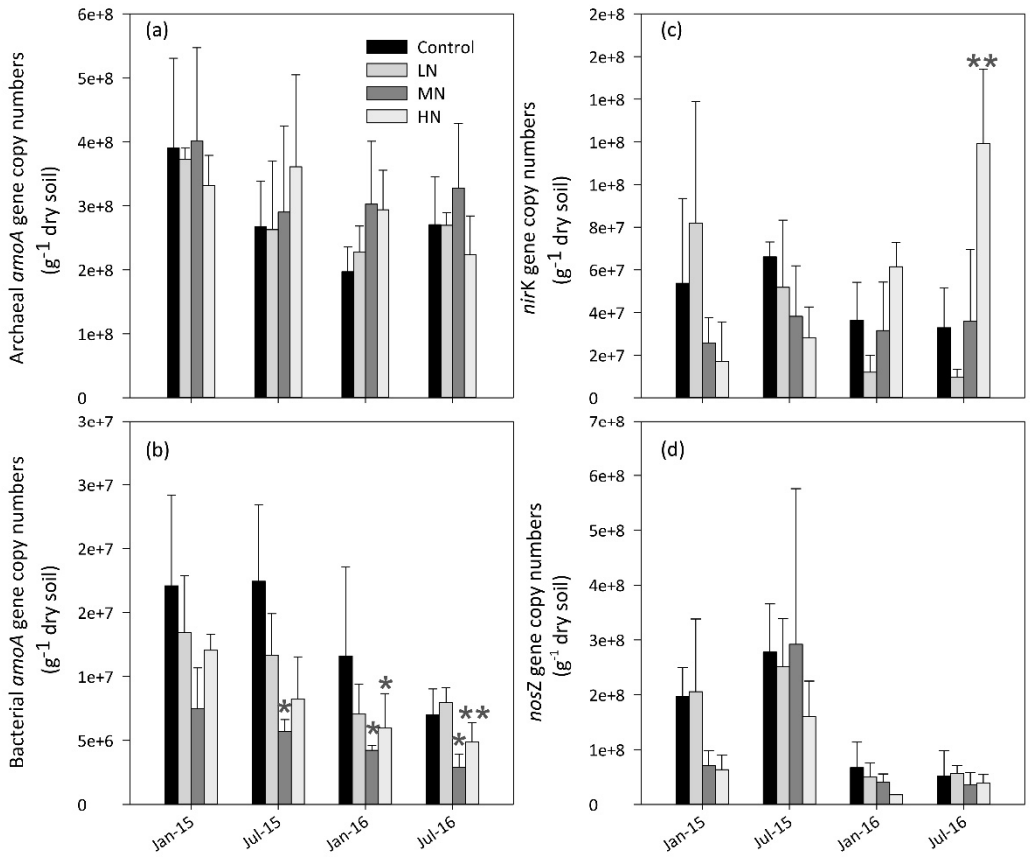

(a)
Control
LN
MN
HN
Archaeal amoA gene copy numbers (g-1 dry soil)
(b)
Bacterial amoA gene copy numbers (g-1 dry soil)
(c)
nirK gene copy numbers (g-1 dry soil)
(d)
nosZ gene copy numbers (g-1 dry soil)
Jan-15
Jul-15
Jan-16
Jul-16

Fig. 5

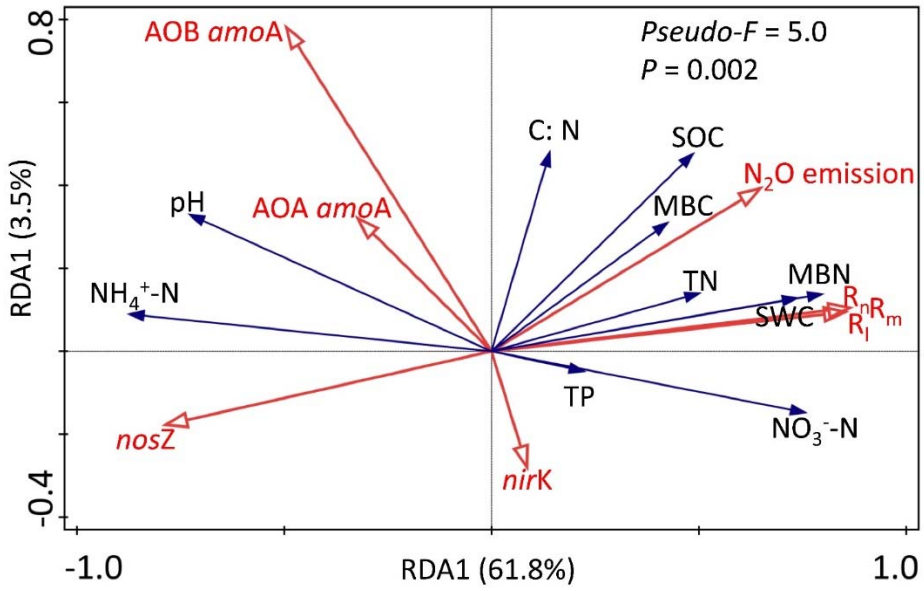
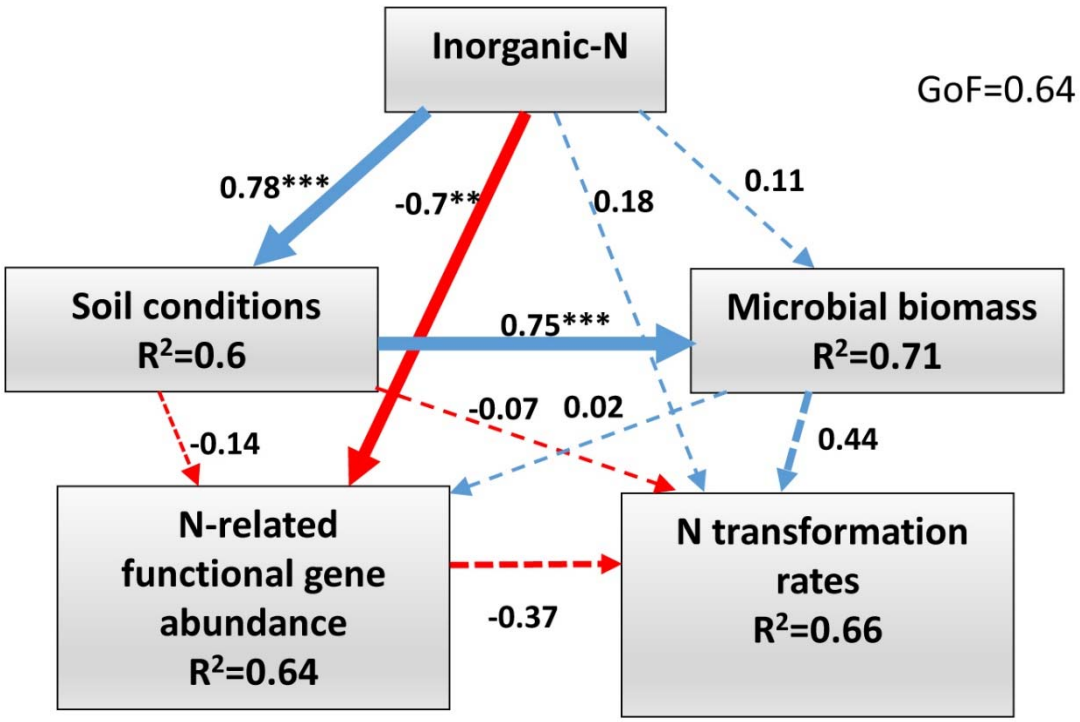

AOB amoA
Pseudo-F = 5.0
P = 0.002
C: N
SOC
N2O emission
pH
AOA amoA
MBC
TN
MBN
NH4+-N
SWC
Rn
Rm
Rl
TP
NO3--N
nosZ
nirK
0.8
-0.4
RDA1 (3.5%)
-1.0
RDA1 (61.8%)
1.0

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