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Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment

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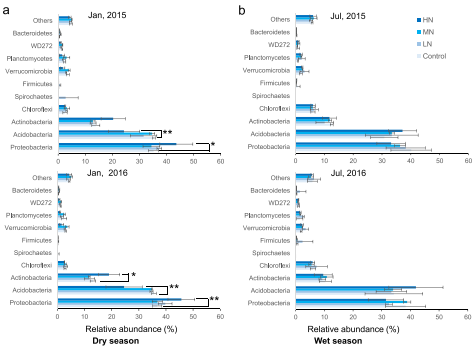
HIGHLIGHTS

- High nitrogen (N) addition reduced bacterial diversity in a tropical forest soil.
- High N addition also altered the soil bacterial community composition (BCC).
- The relative abundance of copiotrophic bacteria was enhanced by the addition of N.
- NH_4^+-N content was a dominant predictor for BCC in the severely acidic forest soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil pH is a dominant factor affecting bacterial community composition in acidic, neutral, and alkaline soils but not in severely acidic soils (pH < 4.5). We conducted a nitrogen (N) addition experiment in the field in severely acidic forest soil to determine the response of the soil bacterial community and identified the dominant factor in determining community composition. Using a high-throughput Illumina HiSeq sequencing platform, we found that high levels of N addition significantly decreased soil bacterial diversity and altered the composition of the soil bacterial community. The addition of nitrogen increased the relative abundance of copiotrophic taxa (*Proteobacteria* and *Actinobacteria* phyla) but decreased the relative abundance of oligotrophic taxa (*Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*, and *WD272*). In particular, the relative abundance of N-cycling-related microbes (e.g., *Burkholderia* and *Rhizomicrobium* genera) also increased upon addition of N. Our correlation analysis showed that soil ammonium nitrogen concentration, rather than pH or nitrate nitrogen concentration, was a key environmental parameter determining the composition of the soil bacterial community. However, these bacterial response behaviors were observed only in the dry season and not in the wet season, indicating that high temperature and precipitation in the wet season may alleviate the impact of the addition of N

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on soil bacterial diversity and community composition. These results suggest that the soil bacterial community shifted to copiotrophic taxa with higher N demands under increased N addition in severely acidic forest soil.

1. Introduction

Globally, elevated atmospheric nitrogen (N) deposition resulting from anthropogenic activities has ranged from 15 to 187 Tg N yr^{-1} over the past 145 years, and it is predicted to reach 200 Tg N yr^{-1} over the next 30 years (Galloway et al., 2008). Excessive N input has many adverse effects on the structure and functioning of terrestrial ecosystems, such as soil acidification (Van breemen et al., 1984; Bouwman et al., 2002; Lu et al., 2014), plant growth restriction (Berendse et al., 2001), plant diversity reduction (Stevens et al., 2004; Lu et al., 2010), and alterations in the composition of the soil microbial community (Fierer et al., 2012; Ramirez et al., 2012). Soil microorganisms play a critical role in the maintenance of soil fertility and ecosystem function (Falkowski et al., 2008), and thus monitoring the responses of the soil microorganisms to N deposition is conducive to evaluation of variations in ecosystem processes driven by soil microbiota (Li et al., 2017).

Various responses to exogenous N enrichment have been observed in soil microbial communities. Some studies have shown that addition of N altered the composition of the soil microbial community (Nemergut et al., 2008; Fierer et al., 2012; Ramirez et al., 2012; Yao et al., 2014; Li et al., 2016). Some studies found N enrichment to have no impact on the soil microbial community (Freedman et al., 2015; McHugh et al., 2017). For instance, Freedman et al. (2015) reported that 20 years of experimental N addition (30 kg N ha^{-1} yr^{-1}) did not change the relative abundance of bacteria phyla in northern hardwood forest stands. Fierer et al. (2012) documented that N enrichment significantly decreased the soil bacterial diversity in farmland but not in grassland. Relatively few studies on the responses of soil microbial community composition to elevated N deposition have been done in tropical and subtropical forests (Liu et al., 2013; Li et al., 2015; Fang et al., 2011). In one such study, Li et al. (2015) reported that short-term N addition did not change the fungi/bacteria ratio (F/B) in the soil (2015), whereas Liu et al. (2013) found that addition of N significantly increased the F/B ratio in the tropical forest. In contrast, Tian et al. (2017) showed that addition of N decreased the F/B ratio in the subtropical forest. Results have been contradictory with respect to the pronounced variation in microbial responses across different soil and vegetation types.

Within a soil microbial community, different taxonomic or functional microbial groups may also exhibit differentiated responses. It has been hypothesized that elevated N addition should reduce the abundance of oligotrophic taxa but increase the abundance of copiotrophic taxa (Fierer et al., 2007; Ramirez et al., 2010; Fierer et al., 2012; Ramirez et al., 2012; Li et al., 2016; Ling et al., 2017). Generally, decreased relative abundance of oligotrophic taxa is represented by taxonomic groups of *Acidobacteria*, *Verrucomicrobia*, *Nitrospira*, and *Chloroflexi* phyla, and increased relative abundance of copiotrophic taxa are represented by *Proteobacteria*, *Actinobacteria*, and *Firmicutes* with added N (Ramirez et al., 2012; Ling et al., 2017). However, Li et al. (2016) found that added N to have no effect on the relative abundance of *Actinobacteria* and *Verrucomicrobia* in a natural steppe system. The mechanistic links are still unclear with respect to the responses of soil bacteria to N enrichment at phylogenetic, taxonomic, and community levels.

Many environmental factors have been found to be correlated with variations in soil microbial community composition, such as soil moisture, pH, total organic carbon, total nitrogen, inorganic nitrogen content, climate change, and aboveground vegetation (Högberg et al., 2007; Yang et al., 2013; Yao et al., 2014; Lladó et al., 2017). Of these, pH was considered the most important factor in predicting the alteration of soil bacterial community composition (Lauber et al., 2009). A previous study found that N addition resulted in a lowered soil pH in a tropical forest after long-term N addition (Lu et al., 2014). It is widely accepted that soil bacterial community composition is affected by soil pH across biomes and regions, with soil pH values ranging from 4.5 to 8.5 (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010; Fernandez-Calvino et al., 2012; Yun et al., 2016; Wu et al., 2017). However, no relationship between soil bacterial community composition and soil pH has been found when the pH is below 4.5 (Rousk et al., 2010).

In this study, we conducted a field N addition experiment in severely acidic tropical forest soil (pH < 4.0). Throughout the first two years of the experiment, bacterial diversity and community composition were examined using 16S rRNA gene-based barcoded pyrosequencing analysis, and soil physicochemical properties were assessed periodically. We aimed to address the following questions: 1) Would copiotrophic bacteria be stimulated and oligotrophic bacteria be suppressed by N addition in the tropical forest soil? 2) How would different taxonomic and phylogenetic bacterial groups respond to the addition of N? 3) Which factors determine the composition of the bacterial community in severely acidic forest soil?

2. Materials and methods

2.1. Site description

Dinghushan Biosphere Reserve (DBR) is located in the city of Zhaoqing, Guangdong Province, in southern China (112°10′ E, 23°10′ N), where we conducted an N addition experiment to simulate N deposition in a tropical forest soil. Evergreen broad-leaved forest >400 years old is the typical zonality vegetation of DBR, with an annual average temperature of 20.9 °C and mean annual precipitation of 1956 mm (Yan et al., 2006). The study site is arranged along an altitudinal gradient from 300 to 355 m above sea level, consists of *Castanopsis chinensis* as the obviously dominant tree species and is influenced by a typical south tropical monsoon climate. At this site, the wet season is concentrated between April and September (approximately 80% rainfall). In contrast, the dry season lasts from October to March (approximately 20% rainfall). In addition, the soil of this region has a pH below 4.0 and is a severely acidic lateritic red loam (Zhang et al., 2008).

2.2. Experimental design and soil sampling

Gradient concentrations of NH_4NO_3 were applied at four levels: Control (ambient: 33.5 kg N ha^{-1} yr^{-1}); LN (low nitrogen: ambient + 35 kg N ha^{-1} yr^{-1}), MN (medium nitrogen: ambient +70 kg N ha^{-1} yr^{-1}), and HN (high nitrogen: ambient + 105 kg N ha^{-1} yr^{-1}). NH_4NO_3 was applied to simulate N deposition because the nitrogen deposited from the atmosphere onto the earth surface is mainly composed of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^- N) all over the world (Fierer et al., 2012; Ramirez et al., 2012). The four N addition levels was applied to simulate the doubled (LN), tripled (MN) and quadrupled ambient N deposition rate (33.5 kg N ha^{-1} yr^{-1}) in the region (Fang et al., 2015). A total of 12 (3 replicates per treatment × 4 treatments) randomly scattered plots (15 m × 15 m per plot) were established beginning in October 2013. Different doses of N (NH_4NO_3) solution were sprayed over the plots at the end of every month beginning in September 2014. From each plot, two layers of soil samples (0–10 cm and 10–20 cm in depth) were collected using 5-core soil

sampling (each soil sample was collected from five separate cores from each plot and mixed for further analysis) in both January (dry season) and July (wet season) of each year from 2015 to 2016. Soil samples were ground to pass through a 2-mm sieve after removing the roots, litter and stones and divided into two portions. One portion (approximately 200 g) was used to analyze soil properties, and the other portion (approximately 20 g) was stored at −80 °C for further molecular biological analysis.

2.3. Measurement of soil properties and soil microbial biomass

Soil pH was determined using a pH meter with a glass electrode (Horiba F—71S, Japan) (ratio of soil to water, 1:2.5 dry wt/v). Soil organic matter (SOM) was analyzed using an external heating method with potassium dichromate ($K_2Cr_2O_7$) (Houba et al., 1989). To examine the total N and phosphorus, semi-micro Kjeldahl digestion and molybdenum antimony colorimetric approaches were carried out, respectively (Bremner, 1996). Soil NH_4^+-N and NO_3^--N were extracted with 1 M KCl and the indophenol-blue colorimetric and double wavelength (220 nm and 275 nm) methods, respectively, and their concentrations were measured using a spectrophotometer (UV-6000, China) (Zhao et al., 2017). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the chloroform fumigation-incubation method (Vance et al., 1987). A 10-g fresh soil sample was transformed to an 80-ml centrifugal tube after 24 h of chloroform fumigation in a glass vacuum dryer at room temperature in the dark, and then 40 ml of 0.5 M K_2SO_4 was added. The leaching liquor was filtered after shaking for 1 h at 200 rpm in an oscillator, diluted 10-fold with distilled water, and measured on a total organic (TOC) analyzer (Shimadzu TOC-VCSH Analyzer). Additionally, the corresponding soil samples without fumigation as a control were extracted by 0.5 M K_2SO_4 and analyzed as described above.

2.4. Analysis of soil bacterial communities

Soil DNA extraction was performed using the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). The DNA concentration was measured using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). To identify the members of the soil bacterial communities, the paired primers of 515F: GTG CCA GCM GCC GCG GTA A and 806R: GGA CTA CHV GGG TWT CTA AT with a barcode were used to amplify the conserved region V4 of the 16S rRNA gene. Paired-end sequencing of PCR amplicons was performed on an Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA, USA). Paired-end reads were assembled using FLASH (http://ccb.jhu.edu/software/FLASH/) (Magoč and Salzberg, 2011) to obtain raw tags. The data were filtered using QIIME (V1.7.0, http://qiime.org/index.html) (Caporaso et al., 2010). Chimera were removed using the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html) (Edgar et al., 2011) to obtain the effective tags. Sequences with ≥97% identity were clustered to the same operational taxonomic units (OTUs). The taxonomic information of each OTU was annotated using the Ribosomal Database Project (RDP) (Wang et al., 2007). Phylogenetic relationships between bacterial communities were constructed using MUSCLE software (Version 3.8.31, http://www.drive5.com/muscle/). The relative abundance (%) of individual taxa for each community was normalized using a standard sequence. Alpha biodiversity was evaluated according to the abundance-based indices of observed species, phylogenetic distance (PD) whole tree, Chao1, Shannon and Simpson to analyze the complexity of bacterial species diversity for each sample using QIIME (Version 1.7.0). Beta diversity (unweighted and weighted UniFrac distances) was calculated based on a subset of randomly selected sequences per community to estimate differences in species complexity between samples with QIIME software (Version 1.7.0).

2.5. Statistical analysis

Statistical analyses were performed using ANOVA and Student's *t*-test. All significant variance between treatments was calculated using SPSS software (version 18.0). Principal coordinate analysis (PCoA) was carried out using the R "ade4," "ggplot2" and "grid" packages (version 3.3.3). Redundancy discriminate analysis (RDA) was performed using CANOCO 5.0 software (Wageningen UR, Netherlands). The correlation coefficients of soil bacterial communities and environmental parameters were calculated using Past software (version 2.16).

2.6. Data accession numbers

All the raw Hiseq sequencing data (.fastq files) were submitted to the Sequence Read Archive (SRA) at National Center for Biotechnology Information (NCBI) under accession number PRJNA413736 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA413736) using Aspera connect tool (version 3.7.4.147728).

3. Results

3.1. Change in soil physicochemical properties and microbial biomass under N addition

N addition increased soil NO_3^--N concentration across the two-year period but significantly increased soil NH_4^+-N concentration in the dry season ($P < 0.05$), indicating that ammonia volatilization and the transformation of NH_4^+-N to NO_3^--N via nitrification occurred more rapidly in the wet season than in the dry season. In addition, high and medium N addition slightly decreased soil pH, soil organic carbon (SOC) and C/N ratio compared to those of the control plots, but no significant difference was observed ($P > 0.05$). In addition, the values of MBN and MBC were slightly higher in the wet season than in the dry season (Table 1).

3.2. Significant alteration of soil bacterial community composition by nitrogen addition

In the dry season, N addition was considered the main factor in the first principle coordinate axis (PCo1) based on the weighted UniFrac distance matrix, contributing 58.65% of the total variation. The bacterial community composition in control, low-N-addition and medium-N-addition plots was slightly different, but a large difference was found between these plots and high-N-addition plots (Fig. 1a). In contrast, no obvious difference between the control and N addition plots was observed in the wet season ($P > 0.05$) (Fig. 1b).

Totally, 9420 OTUs were obtained from a total of 6,430,548 high quality and chimera-free reads by Hiseq sequencing of 16S rRNA gene amplicons, with an average of 59,513 to 122,964 reads per sample. High N addition significantly decreased soil bacterial alpha-diversity according to the observed species and Shannon's diversity index in the dry season ($P < 0.05$). However, the control and high-N-addition plots did not show a significant difference in the bacterial alpha-diversity ($P > 0.05$) in the wet season (Table 2).

At the phylum level, high N addition significantly changed the soil bacterial composition. The relative abundance (% of sequence) of *Acidobacteria* significantly decreased with high N addition ($P < 0.01$) in the dry season. Similarly, high N addition also decreased the relative abundance of *Verrucomicrobia*, *Planctomycetes* and *WD272* phyla in the dry season. In contrast, high N addition significantly increased the relative abundance of *Proteobacteria* and *Actinobacteria* ($P < 0.05$) in the dry season (4 and 16 months after N addition) (Fig. 2a). Nevertheless, there was no significant difference across the N gradients in the wet season (Fig. 2b).

At the genus level, the relative abundance of Unidentified_*Acidobacteria* and *Acidobacteriaceae* subgroup 1 belonging to *Acidobacteria* phyla significantly decreased under high N addition in

Table 1
Responses of soil properties and microbial biomass to nitrogen addition. Shown is the mean value ± standard error ($n = 3$). Different letters represent significant difference (one-way ANOVA, $P < 0.05$, LSD post hoc analysis) among different levels of N addition. Note: All the results were collected from surface soil samples (0–10 cm) except for special instructions.

	Dry season							
	Jan, 2015				Jan, 2016			
	Control	LN	MN	HN	Control	LN	MN	HN
pH	3.73a (0.04)	3.77a (0.02)	3.72a (0.04)	3.73a (0.02)	3.78a (0.07)	3.81a (0.05)	3.68a (0.07)	3.76a (0.03)
TN (mg g^{-1})	2.9a (0.41)	2.43a (0.17)	2.73a (0.36)	2.63a (0.17)	2.31a (0.44)	1.88a (0.22)	2.22a (0.15)	2.08a (0.38)
SOC	52.3a (7.5)	43.8a (2.4)	50.5a (7.02)	42.4a (3.5)	38.0a (3.7)	24.8a (5.7)	25.2a (3.5)	32.1a (9.8)
C/N	18.0a (0.5)	18.0a (0.3)	18.5a (0.2)	16.1a (1.1)	16.7a (2.3)	13.2a (2.5)	11.3a (0.9)	15.2a (2.4)
TP (mg g^{-1})	0.26a (0.01)	0.24a (0.03)	0.24a (0.00)	0.24a (0.02)	0.28a (0.21)	0.15a (0.03)	0.15a (0.03)	0.14a (0.02)
SWC (%)	38.7a (1.2)	34.4a (1.6)	36.9a (5.6)	35.5a (1.5)	43.19a (1.4)	39.19a (3.8)	43.48a (5.9)	39.06a (1.1)
NH_4^+-N (mg kg^{-1})	2.81b (1.03)	3.83ab (1.10)	2.75b (0.58)	5.44a (0.95)	0.97b (0.25)	3.21a (1.15)	3.81a (0.74)	4.64a (1.18)
NO_3^--N (mg kg^{-1})	6.42a (0.63)	5.15a (2.02)	7.85a (4.05)	9.8a (2.23)	4.95c (0.75)	5.57c (0.63)	9.7a (1.35)	7.81b (1.00)
MBC (mg kg^{-1})	508.8a (161.9)	447.5a (88.4)	466.6a (162.8)	382.1a (39.3)	435.3b (60.6)	421.2b (24.3)	525.5a (33.1)	419.2b (5.4)
MBN (mg kg^{-1})	72.7a (20.4)	70.4a (15.7)	58.0a (13.3)	60.6a (11.1)	74.0a (6.3)	67.3a (18.2)	88.2a (8.2)	64.9a (1.3)

the dry season ($P < 0.05$, and $P < 0.01$ respectively). However, high N addition dramatically increased the relative abundance of *Acidothermus* and *Mycobacterium* genera affiliated with *Actinobacteria* under high N addition, especially in the dry season ($P < 0.05$). Moreover, N addition increased the relative abundance of the *Rhizomicrobium* genus belonging to *Alphaproteobacteria* in the same season ($P < 0.05$), suggesting that the *Rhizomicrobium* genus requires greater N availability. The relative abundance of the *Burkholderia* genus belonging to *Betaproteobacteria* was positively associated with N addition. In addition, the relative abundance of the *Acidibacter* genus belonging to *Gammaproteobacteria* increased with high N addition ($P < 0.05$) (Fig. 3a). However, the relative abundance of the *Acidobacterium* genus belonging to *Acidobacteria* significantly increased in the high-N-addition plots compared with the control plots ($P < 0.01$) in the wet season (Fig. 3b).

3.3. The correlations between soil bacterial communities and environmental parameters

RDA indicated that soil inorganic N availability especially that of NH_4^+-N, was the most important factor influencing the acidic soil bacterial community composition in the dry season. In contrast, this pattern was not detected in the wet season, which was possibly attributed to the relatively lower variation in inorganic availability between the control and N-addition plots (Fig. 4). In the dry season, correlation analysis showed that the dominant bacterial phyla of *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes* and *WD272* were negatively correlated with the soil NH_4^+-N content ($r = -0.623$, $P = 0.001$; $r = -0.480$, $P = 0.018$; $r = -0.378$, $P = 0.069$; and $r = -0.392$, $P = 0.058$, respectively). However, the *Proteobacteria* phyla were positively correlated with the soil NH_4^+-N content ($r = 0.477$, $P = 0.018$). The change in the relative abundance of *Actinobacteria* phyla was affected by soil NH_4^+-N and NO_3^--N availability. However, for all the phyla, the variation in community composition caused by soil pH was negligible (Fig. 5).

4. Discussion

4.1. Nitrogen enrichment induces a more copiotrophic bacteria-dominated community

Pyrosequencing analyses revealed that *Acidobacteria*, *Proteobacteria* and *Actinobacteria* were dominant taxa in the acidic tropical forest soil

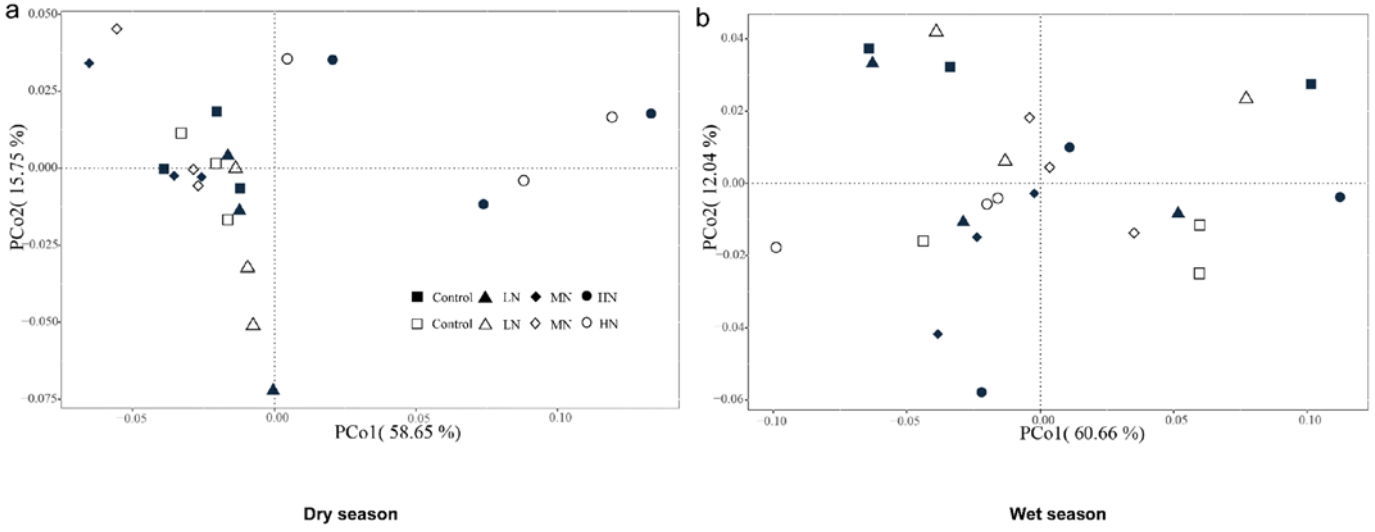

Fig. 1. Principal coordinates analysis (PCoA) based on the weighted Fast UniFrac metric performed in the dry and wet seasons. Solid and hollow shapes represent the sampling times of 2015 and 2016, respectively.

Wet season							
Jul, 2015				Jul, 2016			
Control	LN	MN	HN	Control	LN	MN	HN
3.9a (0.03)	3.9a (0.01)	3.84b (0.02)	3.8b (0.02)	3.78a (0.05)	3.76a (0.06)	3.71a (0.09)	3.68a (0.03)
1.86a (0.13)	1.7a (0.38)	1.99a (0.52)	1.89a (0.32)	2.38a (0.28)	2.41a (0.18)	2.32a (0.03)	2.38a (0.25)
25.5a (3.3)	25.8a (4.7)	25.7a (1.7)	27.6a (2.9)	42.3a (1.4)	35.0a (9.2)	31.0a (9.0)	33.7a (9.5)
13.6a (0.8)	15.3a (1.0)	13.3a (2.4)	14.7a (1.0)	17.9a (2.5)	14.5a (3.3)	13.4a (3.9)	14.1a (3.2)
0.23a (0.02)	0.24a (0.04)	0.24a (0.01)	0.23a (0.04)	0.24a (0.05)	0.3a (0.16)	0.24a (0.11)	0.25a (0.04)
35.9a (1.9)	33.8a (1.8)	34.7a (3.4)	33.7a (1.0)	47.6a (1.0)	45.5a (0.6)	45.8a (6.0)	45.8a (2.6)
4.43a (2.23)	6.73a (3.46)	5.98a (1.24)	3.04a (1.21)	0.97a (0.66)	0.64a (0.19)	0.37a (0.06)	0.28a (0.05)
7.08a (1.67)	7.54a (0.73)	6.96a (1.91)	9.62a (0.8)	11.45a (2.62)	13.63a (1.89)	13.9a (1.96)	13.06a (0.57)
504.5a (25.1)	521.2a (57.4)	464.8a (56.0)	498.2a (33.6)	671.0a (52.8)	540.0a (187.7)	690.3a (326.7)	800.7a (216.1)
49.4a (0.9)	51a (6.1)	47.2a (4.4)	49.7a (3.5)	130.8a (45.3)	122.7a (24.8)	123.2a (21.2)	119.1a (8.5)

(Fig. 2). Among these taxa, the relative abundance of *Acidobacteria*, the representative phylum of oligotrophic taxa, significantly decreased in the plots with the highest level of N addition in the dry season. In contrast, the relative abundance of copiotrophic taxa, including *Proteobacteria* and *Actinobacteria* phyla, significantly increased in the high-N-addition plots. These findings agreed with those of previous studies across different soil types (Ramirez et al., 2010; Fierer et al., 2012; Ramirez et al., 2012; Yao et al., 2014). Hence, our findings for the surface soil layer (0–10 cm) also supported the copiotrophic hypothesis proposed by Fierer et al. (2007). However, our findings for the subsurface soil layer (10–20 cm) did not support the hypothesis - no statistically significant changes in copiotrophic bacterial abundance were detected between the control and N-addition plots in the dry season ($P > 0.05$) (Fig. S1), possibly due to the small change in soil NH_4^+-N concentration after N addition (Fig. S2) and little disturbance of other environmental factors.

Changes in soil bacterial community composition are often associated with changes in the function of ecosystems. For instance, elevated N addition produced a significant decrease in methane oxidation (Neff et al., 1994). In addition, Bodelier and Laanbroek (2004) noted that N application to acid forest soil strongly decreased the uptake of atmospheric methane. Members of the *Verrucomicrobia* phylum can oxidize methane in acidic soils (Dunfield et al., 2007). One genera of *Candidatus Methylacidiphilum,* which was affiliated with the *Verrucomicrobia* phylum in this study, exhibited a decreased trend of the relative abundance in the high-N-addition plots in the dry season (Jan, 2016) (Fig. S3). The *Methylobacterium* genus belonging to the *Proteobacteria* phylum was not widespread in the acidic tropical forest soil, especially in the dry season (data not shown). Consequently, changes in the relative abundance of *Candidatus Methylacidiphilum* genera are closely related to the process of methane oxidation in acidic forest soil. In addition, our findings also showed that N addition caused an increase in the relative abundance of *Burkholderia* and *Rhizomicrobium* genera, which are possible N-cycling-related bacterial taxa of denitrifiers (Fig. 3) (Nishizawa et al., 2014; Nie et al., 2015) and the N_2 fixation group (Kodama and Watanabe, 2011).

4.2. Ammonium nitrogen concentration as a determinant factor for soil bacterial community composition

It is well established that soil pH is a strong predictor of soil bacterial community composition but not apply for the severely acidic soils (pH < 4.5) (Fierer and Jackson, 2006; Jones et al., 2009; Lauber et al., 2009; Rousk et al., 2010). In this study, we confirmed that N addition directly affected soil bacterial community composition through soil inorganic N availability rather than through soil pH under the acidic tropical forest soil, which agreed with a recent study in a temperate steppe ecosystem (Zeng et al., 2016). In particular, our study indicated that short-term N addition led to a quite narrow pH decrease in soil with a lower pH level. Hence, compared with soil NH_4^+-N concentration, the effects of soil pH on soil bacterial community composition could be considered minimal. Moreover, from a global perspective, Leff et al. (2015) also reported that the small pH change was not related to the variation in the relative abundance of dominant bacterial taxa in grasslands.

Our results suggest a significant relationship between the relative abundance of the soil bacterial community and the soil NH_4^+-N concentration rather than the soil pH in the severely acidic tropical forest soil (Fig. 5). To the best of our knowledge, NH_4^+-N is the preferred nutrient element for most bacteria (Merrick and Edwards, 1995). Although a high NH_4^+-N concentration is toxic to some bacterial groups, most of bacterial communities especially of those copiotrophic taxa could have a high abundance under such conditions (Müller et al., 2006). The relationship between soil NH_4^+-N content and bacterial community composition and diversity was previously investigated. In a *Leymus chinensis* steppe, Yao et al. (2014) found that soil NH_4^+-N concentration, along with soil pH, TOC and total N (TN), are important factors for predicting soil microbial biomass and dominant bacterial community composition using RDA analysis. In addition, Zeng et al. (2016) also discovered that NH_4^+-N availability and soil pH could change the soil bacterial community composition and diversity using structural equation modeling. In addition, it was found that soil NH_4^+-N concentration was significantly related to soil bacterial community composition in a grassland (Zhang and Han, 2012). Furthermore, previous study confirmed that the relative abundance of the *Acidobacteria* and *Proteobacteria* phyla was sensitive to soil NH_4^+-N concentration (Liu et al., 2016).

4.3. Seasonal variations in bacterial community responses to N addition

Different from the dry season, soil NH_4^+-N concentration was not significantly increased between the control and N-addition plots ($P > 0.05$) in the wet season (Table 1). Accordingly, the bacterial community composition did not exhibit a significant change between treatments in this season. Overall, the differential responses of soil bacterial communities to N addition were typical between the dry and wet seasons in the acidic forest soil. A rare effect of N addition on soil bacterial community composition and diversity was observed in the wet season mainly due to the high rate of inorganic N leaching with high rainfall and ammonia volatilization with high temperature (Mo et al., 2008). In addition, increased precipitation previously alleviated the influence of N addition on soil bacterial communities (Li et al., 2016). Based on the above information, precipitation, high rate of ammonia volatilization and high temperature in the wet season to some extent indirectly hindered the shift of soil bacterial communities to copiotrophic taxa. Taken together, soil bacterial diversity and community composition were more or less affected by

Table 2
Effects of N addition on bacterial alpha-diversities. Shown is the mean value ± standard error (n = 3). Different letters represent significant differences (one-way ANOVA, $P < 0.05$, LSD post hoc analysis) across N gradients. PD_Whole_tree represents the abbreviation of phylogenetic distance whole tree.

	Dry season								Wet season							
	Jan, 2015				Jan, 2016				Jul, 2015				Jul, 2016			
	Control	LN	MN	HN	Control	LN	MN	HN	Control	LN	MN	HN	Control	LN	MN	HN
Observed species	2156a (203)	2371ab (97)	2186ab (80)	1999b (58)	2067ab (38)	2164a (127)	2117a (91)	1896b (40)	2148a (155)	2045a (38)	2044a (70)	2095a (80)	2291a (231)	2181a (75)	2216a (35)	2133a (90)
Shannon	8.8ab (0.16)	8.93a (0.04)	8.89a (0.05)	8.6b (0.05)	8.78a (0.08)	8.73a (0.04)	8.84a (0.03)	8.53b (0.03)	8.46a (0.19)	8.57a (0.13)	8.54a (0.17)	8.59a (0.19)	8.95a (0.09)	8.88a (0.04)	8.92a (0.03)	8.88a (0.05)
Chao1	2728a (423)	3046a (324)	2795a (370)	2451a (207)	2434a (110)	2737a (373)	2570a (275)	2222a (29)	2860a (320)	2593a (169)	2695a (82)	2694a (207)	2896a (508)	2646a (325)	2905a (58)	2676a (261)
PD_whole_tree	161.1b (10.4)	181.8a (8.5)	166.9ab (6.3)	153.3b (4.8)	154.4a (1.1)	169.1a (9.3)	158.3ab (4.8)	145.4b (1.7)	166.5a (14.6)	154.0a (4.1)	156.6a (5.7)	158.7a (5.0)	175.5a (22.0)	161.0a (4.9)	163.5a (3.2)	160.8a (7.0)

the interaction of environmental characteristics, including precipitation and temperature. Therefore, seasonal variation needs to be investigated to confirm the response of soil bacterial communities to N addition to offer sufficient evidence.

5. Conclusions

Addition of large amounts of N reduced the diversity of soil bacterial communities and resulted in an increased abundance of copiotrophic bacterial communities in the dry season in severely acidic tropical forest soil. Interestingly, the N enrichment effects were positive on some N-cycling-related bacteria taxa (e.g., *Burkholderia* and *Rhizomicrobium*) but negative on some C-cycling-related bacteria taxa (e.g., *Candidatus Methylacidiphilum*). Among environmental factors, soil NH_4^+-N concentration was positively associated with a shift in the soil bacterial community composition from oligotrophic taxa to copiotrophic taxa, indicating that soil NH_4^+-N content is a dominant environmental factor for predicting the composition of the bacterial community in severely acidic forest soils. Additionally, high precipitation and temperature in the wet season slowed this transformation of the bacterial community.

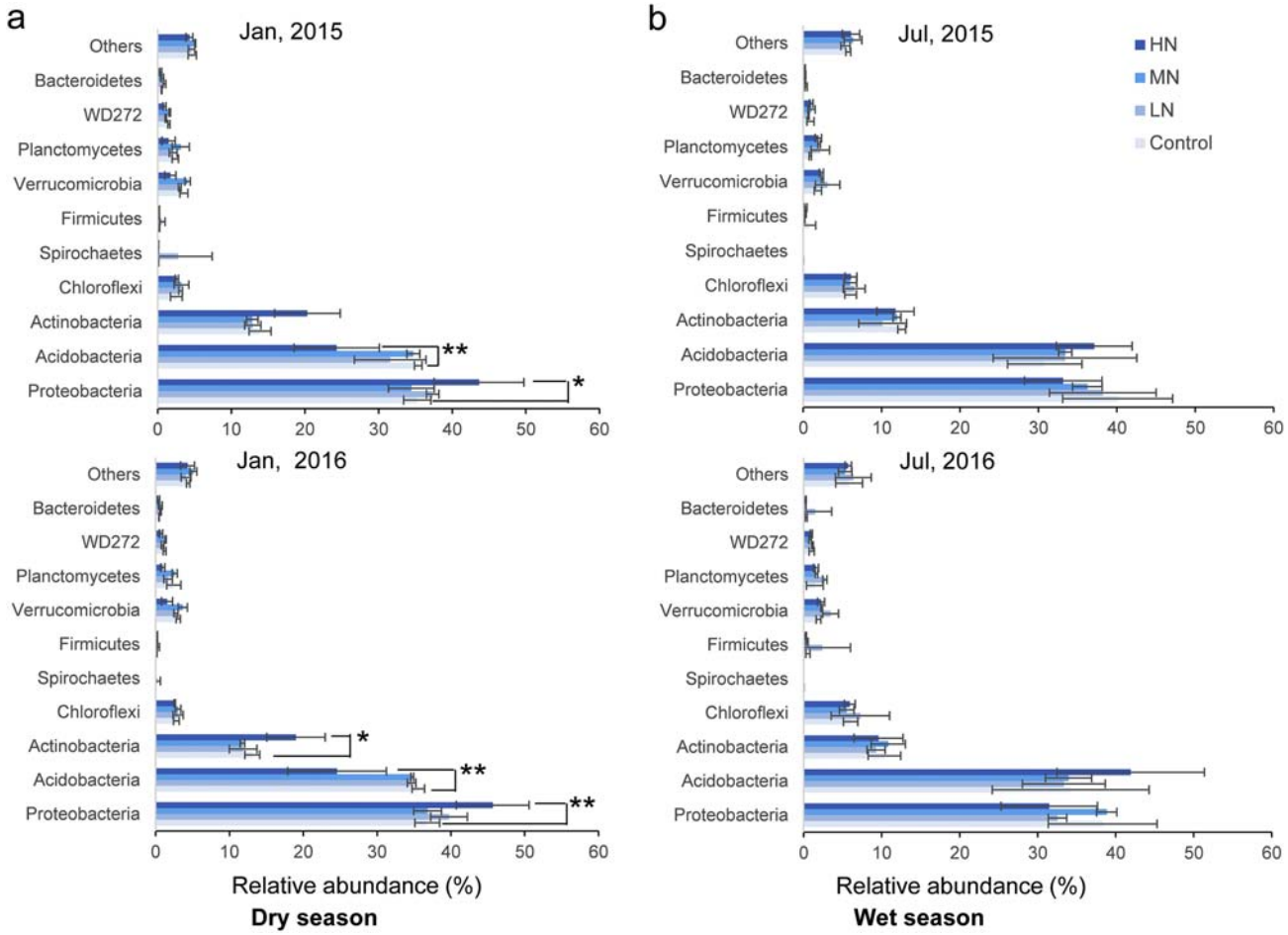

Fig. 2. Relative abundance of the dominant bacterial group under different levels of N addition at the phylum level. Significance is indicated by $^*P < 0.05$ and $^{**}P < 0.01$.

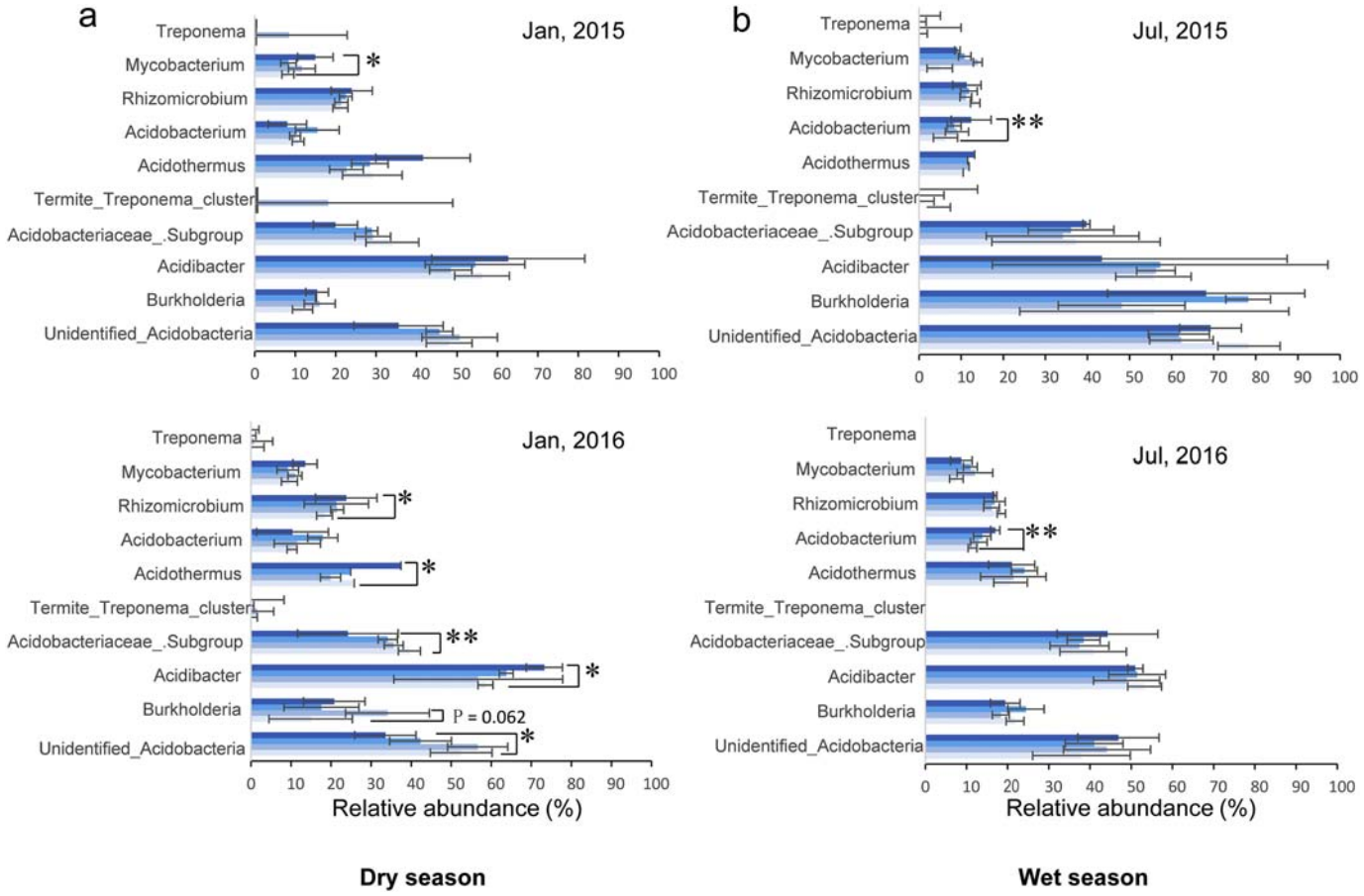

Fig. 3. Relative abundance of the dominant bacterial group under different levels of N addition at the genus level. Significance is indicated by $^{*}P < 0.05$ and $^{**}P < 0.01$. Note: Fig. 2 and Fig. 3 share a common legend.

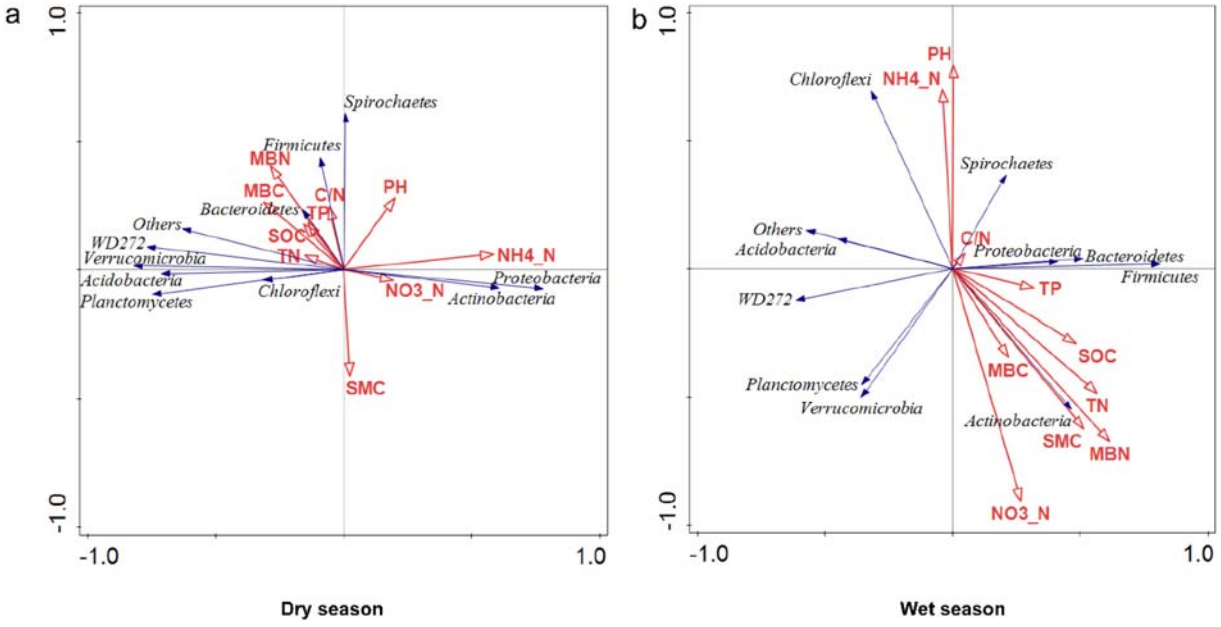

Fig. 4. Redundancy discriminate analysis (RDA) plots illustrating the relationships between the dominant bacteria phyla and soil physicochemical properties between the dry and wet seasons.

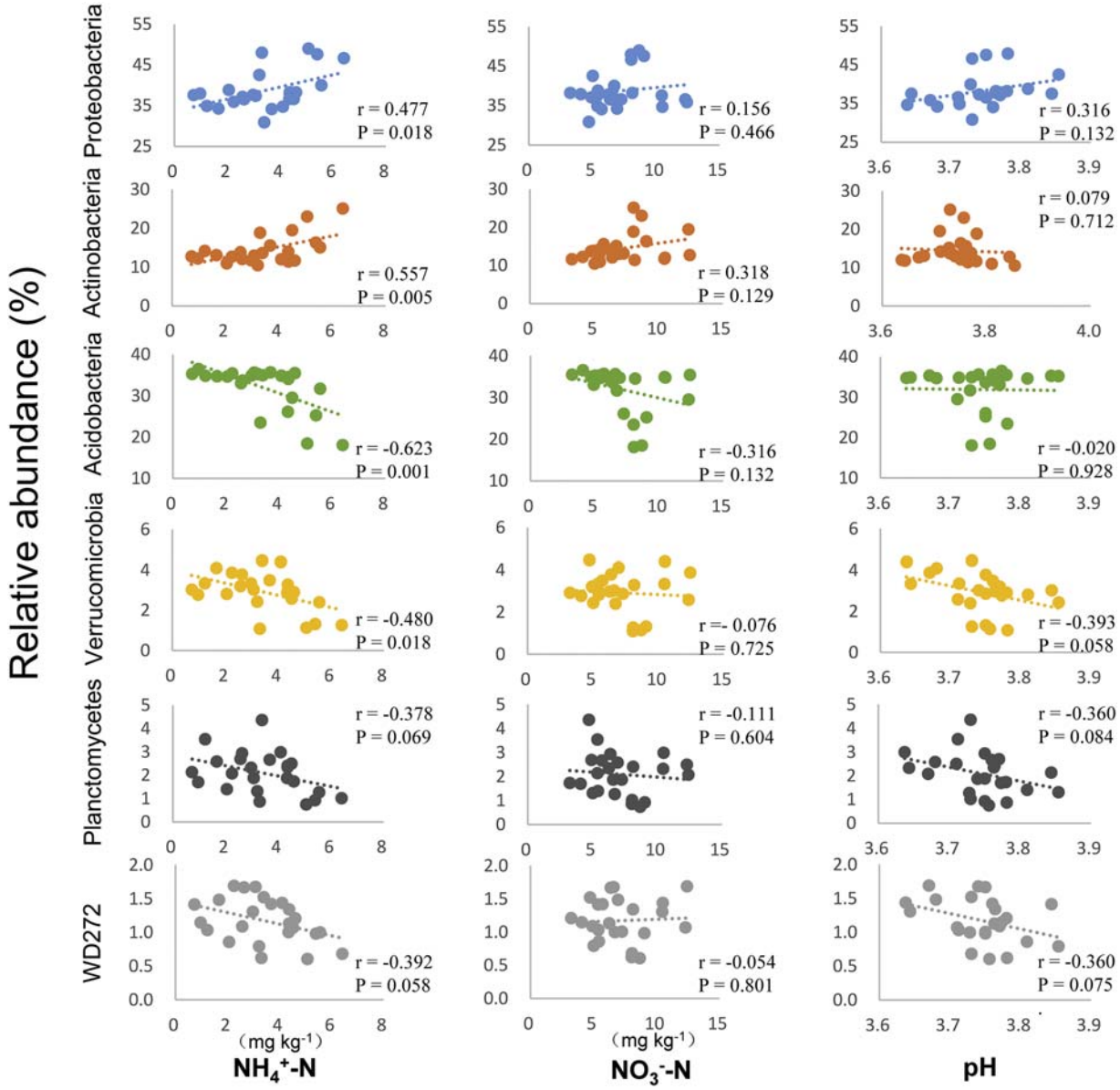

Fig. 5. Correlation of soil properties (NH_4^+-N, NO_3^--N and pH) and the relative abundance of the dominant bacterial community in the dry season.

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.12.142.

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