Fungal communities are more sensitive to the simulated environmental changes than bacterial communities in a subtropical forest: the single and interactive effects of nitrogen addition and precipitation seasonality change

Dan He
Zhiming Guo
Weijun Shen
Lijuan Ren
Dan Sun
Qing Yao
Honghui Zhu (zhuhh_gdim@163.com)
Institute of Microbiology Guangdong Academy of Sciences

Research Article

Keywords: Nitrogen addition, Precipitation seasonality change, Soil microbial communities, Subtropical forests, Soil enzymes activities

Posted Date: May 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1633278/v1

Abstract

Increased nitrogen (N) deposition and changes in precipitation seasonality can greatly impact soil microbial communities in tropical/subtropical forests. Although knowledge about the effects of a single factor on soil microbial communities is growing rapidly, little is understood about the interactive effects of these two environmental change factors. In this study, we investigated the responses of soil bacterial and fungal communities to the simulated environmental changes (nitrogen addition, precipitation seasonality change, and their combination) in a subtropical forest in South China. The interaction between N and water treatments was significant for affecting some soil physicochemical properties (such as pH, soil water and NO_3^- contents). Fungi were more susceptible to treatment than bacteria in a variety of community traits (alpha, beta diversity, and network topological features). The N and water treatments act antagonistically to affect fungal alpha diversity, and the interaction effect was detected significant for the dry season. The topological features of the meta-community (containing both bacteria and fungi) network overrode the alpha and beta diversity of bacterial or fungal communities in explaining the variation of soil enzyme activities. The associations between Ascomycota fungi and Gammaproteobacteria or Alphaproteobacteria might be important in mediating the inter-kingdom interactions. In summary, our results suggested that fungal communities were more sensitive to N addition and precipitation seasonality change (and their interaction) than bacterial communities, and the treatments' effects were more prominent in the dry season, which may have great consequences in soil processes and ecosystem functions in subtropical forests.

Introduction

Water and nitrogen (N) are the basic elements that affect soil microorganisms and biogeochemical processes. Since the last century, intensified anthropogenic activities and industrial production have caused global changes in the distribution and cycling of both water and N [1, 2]. Global ecosystems are thus subjected to more variable rainfall patterns and enhanced atmospheric N depositions [3]. Great changes in water potential and/or reactive N levels in soil may be challenging for the growth and functions of microorganisms. As the two environmental factors that often change simultaneously, N deposition (addition) and precipitation seasonality can solely and interactively affect soil microbial communities and ecosystem functions, while the knowledge remains poorly understood.

Bacteria and fungi, the two main microbial groups in soil, have disparate characteristics in cell structure and physiology, likely posing differential response patterns to environmental changes. Bacterial or fungal communities could be sensitive [4–6] or unaffected [7–9] to the changes in precipitation patterns. Of the studies that tracked both bacteria and fungi, some suggested that the diversity or composition of bacteria were more influenced by soil water content [10, 11], while others supported that fungal communities were more responsive to soil moisture changes [12, 13]. This inconsistency may be attributed to ecosystem types, methods to determine diversity, and experimental designs, such as precipitation change settings [11, 14]. N deposition/enrichment usually has negative effects on bacteria and fungi in terms of diversity and biomass [15, 16], or, in a few cases, has no significant effects [17]. The changes in soil

physicochemical properties (e.g., pH and available phosphorus), and aboveground plant communities caused by N deposition are often related to changes in microbial communities [16]. In some cases N enrichment had similar effects on bacterial and fungal diversity [18]; in other cases, N enrichment caused a higher magnitude of changes for either bacterial [19] or fungal [20] communities. Ecosystem type, soil original fertility levels, and sampling season often interact with the N treatment and may contribute to the inconsistencies in different studies [21–23].

N addition could interact with the precipitation-level to affect the decomposition process in soil in a tropical dry forests [24]. In the arid or semiarid ecosystems, the interactions between N and water in affecting microbial communities such as the total phospholipid fatty acid (PLFA) concentrations, fungi-to-bacteria ratio, diversity, and biomass, were frequently reported [21, 25–26]. In the arid/semiarid environment where both water and N are frequently limited, the changes in precipitation and N might interact strongly to influence microbial diversity [27]. In addition to microbial diversity and biomass, the co-occurrence network between different members of the microbial community can also be shaped by environmental changes. Though the co-occurrence network could not directly represent real inter-species interaction [28], network structure has become a new dimension in exploring microbial communities and inferring the relationships between different community members [29–31]. Both bacterial and fungal community networks could be affected by the water and N treatments. In temperate ecosystems, soil bacterial network structures were more responsive to drought than did fungal networks [32–33], which contrasts with the study conducted in a subtropical forest where fungal networks changed more to an altered precipitation seasonality [13]. In another study conducted in a desert steppe, water reduction and addition resulted in contrasting effects on bacterial diversity and network structure; and N addition weakened the effect of water addition on bacterial communities [34]. However, little was known about the microbial communities' responses to the combination of water and N changes in tropical/subtropical forests.

Tropical and subtropical forests are central spots of global biodiversity and carbon storage [35], and also are hotpots sensitive to changes in precipitation patterns and N deposition [36]. Compared to temperate and arid ecosystems, studies on the wet tropical/subtropical forests in response to global changes are relatively fewer. In South China, subtropical forest ecosystems are facing the challenges of both enhanced N deposition and precipitation pattern change. The prominent divergence of dry and wet seasons in the subtropical forests may complicate the responses of soil physicochemical properties and microbial communities to N and water treatments, while the detailed mechanisms were scarcely understood. Previous studies have indicated that in the future, the subtropical forests in South China would be subjected to growing N deposition and changes in precipitation seasonality (drier dry season and wetter wet season with the total precipitation unchanged) [37]. How soil bacterial and fungal communities respond to the sole and interactive effects of N and water treatments was largely unknown, which hinders our understanding of soil functions and ecological processes in subtropical forests. Here, we conducted a field experiment simulating enhanced N deposition and precipitation seasonality change as future climate scenarios in the subtropical forests in South China. We aimed to determine (1) how soil bacterial and fungal communities respond to water and/or N treatments in terms of community diversity,

composition, and network topological features; and (2) whether there are interactive effects of water and N treatments on soil physicochemical properties and different microbial community traits. Our study indicated that season overwhelmed treatment in affecting soil physicochemical properties and microbial communities. In the dry season, the effects of N and water treatments and their interaction were more prominent. Fungal communities were more sensitive to the single and interactive effects of nitrogen addition and precipitation seasonality change than bacterial communities in the subtropical forest.

Methods

Site information and experimental design

The experimental site was located in a subtropical forest at the Heshan National Field Research Station of Forest Ecosystem (112° 50' E, 22° 34' N). The forest is an evergreen monsoon subtropical forest with two dominant tree species, *Schima superba* and *Michelia macclurei* [38]. The climate in the region where the station is situated is characterized by distinct dry and wet seasons, usually expanding from October to March and April to September, respectively. The mean annual air temperature is 21.7 ℃, and the mean annual precipitation is 1700 mm [39–40]. The soil type is Ultisol as per the USDA soil taxonomy classification [41].

A total of 16 plots were established in 2018. The total area was nearly 1 ha, and the mean slope of these plots was 15°. The two factors, enhanced N deposition (N) and precipitation seasonality change (PC), and their combination (NPC) and control (C) were randomly assigned to four plots in each of the four blocks (Fig. S1). At the time of establishment, the above-ground plant community compositions and below-ground soil physicochemical properties did not show significant variations in plots from different treatments (data unpublished). The plot size is 12×12 m^2, with a distance of at least 2 m from each other. For the N and NPC treatments, solutions of NH_4NO_3 were sprayed into surface soil at the beginning of each month (on one of the first several days with no rain), with an annual dose of 100 kg N hm^{-2}. For the PC and NPC treatments, a set of steel frames with a height of 1.5 m above ground was set up to support throughfall reduction shelters and water-adding sprinklers (Fig. S1). To minimize the lateral water flow and interference from other plots, the four sides of each plot were trenched using 1-m height polyvinyl chloride boards to a depth of 60–80 cm. We adopted a throughfall exclusion rate of 67% as per the other studies that were used in the tropical forests [42–43]. Ten to twelve polyethylene sheets, covering a total shade area of 67% of the plot area, were spread to reduce the throughfall during the dry season (from September 15 to April 14) (Fig. S1). These sheets are of 95% light transparency to minimize the shading effect. The excluded rain flowed along with the sheets into the polyvinyl chloride troughs at the lower slope and was drained out of the plots (Fig. S1). Automatic rain gauges (Davis Instrument, MD, USA) were installed at the plot (area without shelters) to record the amount of throughfall. During the wet season, water with 67% of the recorded amount of throughfall in the dry season was added to the PC and NPC treatment plots. In each plot, water was added through 25 automated sprinklers at the center of the steel frames, with a spraying diameter of 2 m and showering ca. 50 L of water per hour (Fig. S1). The

throughfall was 454.08 mm in the dry season in the first hydraulic year. For each plot from the PC and NPC treatment, 10.90 m^3 of water was added at the end of each month from May to August 2019. The added water was pumped from a nearby pond, which in general had lower organic C and N contents than and similar pH values to the throughfall. Such differences in water chemistry were considered to have a neglectable impact on the treatment effects [38, 40].

Sampling and determination of soil physicochemical properties and enzyme activities

To understand the quick responses of soil microbes to short-term environmental changes, samples were taken during both dry and wet seasons in the first hydraulic year (September 15, 2018-September 14, 2019). The upper 0–15 cm soil layers were sampled from each plot on December 13, 2018, and July 12, 2019, by soil augers with a diameter of 5 cm. Soil samples were put in clean plastic bags and transferred to the laboratory within 8 hours in a heat-insulated box with ice. The samplings were performed at least 10 days after water or N addition. In the laboratory, the soils were sieved through a 2-mm sieve, and the five soil cores randomly taken from each plot were homogenized into a composite soil sample. A subset of the soil sample was stored at 4 ℃ for chemical and enzymatic analyses. All analyses were done in less than 2 weeks. A subset of the soil sample was stored at -80 ℃ for DNA extraction and sequencing.

Soil water content (SWC) was determined by the weight method after drying the fresh soil at 105°C for 24 h. Soil pH was measured in an air-dried soil/water suspension (1:2.5, w/w) using a pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland). Total organic carbon (TOC) was determined using the $K_2Cr_2O_7$ titration method. Dissolved organic carbon (DOC) was measured with a TOC analyzer (Shimadzu, Kyoto, Japan) after filtering the extracts of 10 g of fresh soil in a 0.5 M K_2SO_4 solution. Total N (TN) was measured by the indophenol blue colorimetric method. Total phosphorous (TP) was measured by the molybdenum antimony blue colorimetric method [44]. Available phosphorus (AvaiP) was measured by Olsen's method [45]. Soil microbial biomass was determined using the fumigation extraction method [46]. The conversion coefficient used to calculate the microbial biomass C (MBC) was 0.45 [47].

The potential activities of four basic soil enzymes (β-1,4-glucosidase (BG), β-1,4-N-acetyl-glucosaminidase (NAG), alkaline phosphatase (ALP), and acidic phosphatase (ACP) were determined using fluorimetric assays in 96-well black microplates [48]. One gram of fresh soil was added to 100 ml acetate buffer (pH 4.0, which was chosen to approximate the original soil pH). Fluorescence was measured using a spectrofluorometer (TECAN, Salzburg, Austria). The wavelengths were set at 360 nm and 450 nm for excitation and emission, respectively. The enzyme activity (nmol g^{-1} h^{-1}) was calculated after correction for the negative control and quenching [49].

Soil DNA extraction and rRNA gene sequencing

Microbial DNA was extracted from nearly 0.5 gram soil with the MP soil extraction kit (MP medicals, USA) as per the manufacturer's protocol. The quantity and purity of extracted DNA were checked in a Nanodrop One spectrophotometer. The primers 341F/806R (5'-CCTACGGGAGGCAGCAG-3'/5'-

GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3-V4 region of the bacterial 16S rRNA gene. The primers ITS3NGS/ITS4NGS (5'-CATCGATGAAGAACGCAG-3'/5'TCCTSSGCTTAN

TDATATGC-3') were used to amplify the ITS2 region of the fungal ITS rRNA gene. All amplicon library preparation and sequencing were performed in the MagiGene Company (Guangzhou, China).

Bioinformatic analysis of sequences

The paired-end reads from the sequencing company were mainly processed with the software Mothur v1.44 [50]. Briefly, the forward and reverse reads were merged with the command 'make.contigs' with the default parameter. For bacteria, the sequences with an average quality score lower than 25 and a length shorter than 400 were discarded with the command 'trim.seqs'. Then, the sequences were further quality-controlled with the 'pre.cluster' command. The 'unoise' method was chosen, and sequences were refined with the threshold of 4 bases' difference [51]. Then, all the sequences were compared with Silva v132 to obtain taxonomic information [52]. Those sequences not affiliated with the bacterial domain were discarded. OTUs were then calculated with the command 'cluster' and the method 'agc' and a cut-off of 0.03. For fungi, the sequences with an average quality score lower than 25 and a length shorter than 300 were discarded. The sequences containing the ITS2 region of rRNA were then extracted with the software 'ITSx' [53]. Then, the sequences were processed with the command 'pre.cluster' in Mothur. All the sequences were compared to the UNITE database to obtain taxonomy information [54]. Those sequences not affiliated with fungi were discarded. OTUs were then calculated with the command 'cluster' and the method 'agc' and a cut-off of 0.02 in Mothur [55]. The original OTU table for the bacterial or fungal community was made with the command 'make.shared' in Mothur.

Meta-networks of soil microbial communities

To construct the co-occurrence networks, the top 1000 abundant bacterial and fungal OTUs were pooled together to represent a 'meta-community'. The Spearman correlation coefficients among OTUs and the significance values adjusted with the "Benjamini and Hochberg" [56] method were calculated with the 'WGCNA' [57] and 'multtest' [58] packages in R software 4.1.1 [59]. The meta-community network was built based on the correlation coefficients and adjusted *P* values. The cutoff of correlation coefficients (0.68) was detected with the "RMThreshold" package [60] based on the random matrix theory [61]. The cut-off of the adjusted *P* value was set as 0.001. The deconvolution method was used to detect the edges arising from the direct interactions in the network [62]. The edge tables connecting those significantly correlated OTUs were exported and then loaded into R. The downstream analyses of network features calculation and network graph visualization were performed with the "igraph" package [63]. Sub-networks were extracted from the meta-network by attaining the OTUs that occurred in the specified groups.

Statistical analyses

The grouping effects of treatment and season on alpha diversity, soil physicochemical properties, soil enzyme activities, and network features were checked for significance with the permutation-based two-way analysis of variance (ANOVA). The significance of treatment and season in affecting community

compositional change (beta diversity) was checked with permutation-based multivariate analysis of variance (*adonis* in R). For pairwise comparisons of alpha diversity, soil physicochemical properties, soil enzymatic properties, and network topological features, permutation-based pairwise *t* tests were performed with the 'FDR' correction methods for multiple comparisons. The Bray–Curtis dissimilarities were calculated on the square-rooted abundance values across all samples and then visualized with the nonmetric multidimensional scaling (NMDS) plot. The scaled values of soil physicochemical properties (except the pH which kept no change) and enzyme activities were put into the correlation analyses with the "Spearman" method. The Euclidean distances of the scaled values of alpha diversity and network features, and the Bray-Curtis distances of community compositions were correlated with each of the soil physicochemical properties and enzyme activities with the "mantel.test" function in R. The results of the above correlation analyses and Mantel tests were visualized with the "quickcor" function in R. We also checked the relative contributions of soil physicochemical properties and different community traits (the alpha and beta diversity of the bacterial and fungal communities respectively, and the network-level topological features of the meta-community networks, between bacteria and fungi networks, within-bacteria networks, and within-fungi networks) to the variations of soil basic functions (represented by the 4 determined soil enzyme activities), which were done with the analyses of multiple regression on distance matrices (MRM) [64].

To check the interactive effects of N and water treatments in affecting soil physicochemical properties, enzyme activities, microbial community alpha and beta diversity, and network features, the two-way analyses of variance were done, for which the samples were given two dummy variables set as "N" and "W". In specific, C samples were set as 0, 0; N were samples set as 1, 0; PC samples were set as 0, 1; NPC samples were set as 1, 1 for the "N" and "W" variables, respectively. All the aforementioned statistical analyses were performed with the "vegan" [65], "lmPerm" [66], "rcompanion" [67], "ggcor" [68], and "ecodist" [69] packages in R.

Results

soil physicochemical properties and enzyme activities

We examined 10 soil physicochemical properties and 4 soil enzyme activities (Fig. 1) in the surface soil from the experimental plots. Most of them showed obvious seasonal changes, while the treatment affected these variables less than the season. The contents of SWC, SOC, NH_4^+-N, AvaiP and all the 4 enzyme activities (BG, NAG, ALP, and ACP) had significantly higher values in the wet season, whereas soil pH, the contents of MBC, TN, and TP showed significantly higher values in the dry season (permutation-based Two-way ANOVA, all cases, $P < 0.05$).

In the dry season, the PC and NPC treatments substantially decreased (with a magnitude of over 25%) the SWC compared with the control. However, in the wet season, there were no significant differences in SWC between different treatments. In the dry season, the N treatment caused significantly lower pH than the other 3 treatments (permutation-based pairwise *t* test, $P < 0.05$), while in the wet season, the N treatment

affected soil pH less than the PC and NPC treatments. No significant changes in the 4 measured enzyme activities were detected between different treatments in either the dry or wet season.

Statistically, we found that in the dry season, both soil SWC and pH were significantly affected by the N treatment, the water treatment and their interaction; The N treatment also significantly influenced soil AvaiP content; The water treatment also significantly influenced the contents of soil DOC, NH_4^+ and soil BG activity (permutation-based Two-way ANOVA, all cases, $P < 0.05$). In the wet season, the contents of soil DOC and NH_4^+ were significantly influenced by the N treatment. Soil pH was significantly affected by the water treatment. The contents of soil MBC and NO_3^- were significantly affected by the interaction of N and water treatments (permutation-based Two-way ANOVA, all cases, $P < 0.05$) (Table S1).

Alpha diversity of soil bacterial and fungal communities

After denoising steps, we obtained a total of 2,487,788 bacterial and 1,049,880 fungal high-quality sequences. To reduce the biases caused by uneven sequencing effort, all bacterial and fungal samples were rarefied to 26136 and 13143 sequences, respectively. There were 6455 (ranging from 961 to 1424) bacterial and 1514 (ranging from 265 to 412) fungal OTUs for all the samples. For bacterial communities, the alpha diversity (both Shannon diversity and Pielou's evenness) showed significant seasonal changes with lower values in the wet season (permutation-based two-way ANOVA, $P < 0.05$), while treatment showed no significant effects. For fungal communities, season and the interaction of season and treatment significantly affected the Shannon diversity and OTU richness (permutation-based two-way ANOVA, $P < 0.05$), while treatment showed no significant effects. In the dry season, the PC and N treatments significantly decreased fungal diversity compared with the control group (permutation-based *t* test, $P < 0.05$), while in the wet season, no treatments caused significant changes of fungal diversity compared with the control (Fig. 2). Statistically, we found that only in the dry season, the interaction of the N and water treatments significantly affected the alpha diversity (both Shannon diversity and Pielou's evenness) of fungal communities (Table 1).

Table 1
The effects of N addition (N treatment), precipitation seasonality change (water (W) treatment), and their interaction (N×W) in affecting microbial community alpha and beta diversity. All the samples were given two dummy variables set as “N” and “W”. In specific, C samples were set as 0, 0; N were samples set as 1, 0; PC samples were set as 0, 1; NPC samples were set as 1, 1 for the “N”and “W” variables, respectively. The significance value for alpha diversity was calculated from the permutation-based Two-way analysis of variance. The significance value for beta diversity was calculated with the “adnois” function in the “vegan” package in R. Significant values were indicated by the bold *P* values ($P < 0.05$)

		Bacteria				**Fungi**			
		alpha		*beta*		*alpha*		*beta*	
		F	*P*	R^2	*P*	F	*P*	R^2	*P*
Dry season									
	N	0.663	0.431	0.073	0.178	0.439	0.520	0.068	0.385
	W	0.125	0.729	0.063	0.612	0.895	0.363	0.072	0.242
	N×W	0.442	0.519	0.058	0.909	11.505	**0.005**	0.081	0.125
Wet season									
	N	0.042	0.841	0.067	0.400	0.110	0.746	0.077	0.179
	W	0.507	0.490	0.060	0.714	2.523	0.138	0.058	0.745
	N×W	0.322	0.581	0.073	0.211	0.825	0.382	0.064	0.537

Taxonomy distributions and compositions of soil bacterial and fungal communities

A total of 38 bacterial subphyla and 39 fungal classes were detected in our study. Among them, the top 7 bacterial and fungal groups comprised 91.3% and 89.2% of all the bacterial and fungal sequences, respectively. In general, season affected the relative abundances of these top microbial groups more than treatment did (Fig. 3(b, d)). The relative abundances of Acidobacteria and Alphaproteobacteria increased significantly from the dry season to the wet season; while the relative abundances and OTU richness of Actinobacteriota, Verrucomicrobiota, and Planctomycetota decreased significantly from the dry season to the wet season (permutation-based Two-way ANOVA, $P < 0.05$). The N treatment significantly enhanced the relative abundances and OTU richness of Gammaproteobacteria in the wet season. The NPC treatment significantly increased the relative abundances and OTU richness of Planctomycetota in the dry season compared with the control (permutation-based *t* test, $P < 0.05$) (Fig. S2(a, c)). For the fungal class, the relative abundances of Dothideomycetes, unclassified Ascomycota, and Sordariomycetes increased significantly from the dry season to the wet season. The relative abundance and OTU richness of Eurotiomycetes decreased significantly from the dry season to the wet season (permutation-based two-way ANOVA, $P < 0.05$). The PC treatment in the dry season significantly enhanced the relative abundance of Eurotiomycetes, and in the wet season significantly enhanced the OTU richness

(permutation-based *t* test, $P < 0.05$). In the dry season, The N treatment significantly reduced the OTU richness of Agaricomycetes, unclassified Ascomycota, Eurotiomycetes, and Sordariomycetes; the PC treatment significantly decreased the OTU richness of Dothideomycetes, unclassified Ascomycota and Sordariomycetes compared with the control (permutation-based *t* test, all cases, $P < 0.05$) (Fig. S2(b, d)). Statistically, both the relative abundance and OTU richness of the Plantomycetota were significantly affected by the N treatment in the dry season. There were more taxa significantly affected by the N and water treatments (and their interaction) in the dry season than in the wet season (Table S2).

The OTU compositions of the soil bacterial and fungal communities all showed significant seasonal changes (two-way Adonis, $P < 0.001$) (Fig. 3(a, c)). In general, the treatment caused marginal significant effects on the changes of both bacteria and fungal communities, and the effect was slightly greater for fungi ($P = 0.04$) (Two-way Adonis, $P < 0.1$, Fig. 3(a, c)). The volcano plots showed that fungal communities changed more than bacterial communities to different treatments in comparisons with the control, as indicated by the higher ratios of OTUs that were significantly enriched or depleted (Fig. S3). Statistically, no significant effects of the N or water treatment (or their interaction) were detected on the divergence of community compositions (beta diversity) (Table 1).

Microbial community co-occurrence networks

We constructed a meta-community network based on the combined community comprised of the top 1000 bacterial and top 1000 fungal OTUs from all samples (Fig. 4). The meta-network was finally comprised of 391 bacterial OTUs and 556 fungal OTUs, developing 1162 within-bacteria links (60.9% positive relationship), 732 within-fungi links (95.6% positive relationship), and 593 between bacteria and fungi links (57.7% positive relationship).

By preserving the OTUs occurring in a specific group, the sub-networks can be generated from the meta-community network. Generally, the basic node-level network features (the descriptions of the node-level and network-level features were shown in Table S3) did not change greatly between different treatments or different seasons. The exception is that for the within-fungi network, season significantly affected all the 4 node-level features, and treatment significantly affected the 4 features except the proportion of positive edges (PPE) (permutation-based Two-Way ANOVA, $P < 0.05$). The N treatment caused the lowest closeness in both seasons and the highest betweenness in the within-fungi network in the wet season. The NPC treatment caused the highest closeness in both seasons in the within-bacteria network (Fig. 5). For the network-level topological features, seasonal differences were more obvious than among-treatment differences. The N treatment tended to cause higher divergence in these features than the other treatments (Fig. S4). For example, the edge number in the dry season was significantly higher in the N treatment compared with the control in the within-bacteria network. The diameter and cluster number in the dry season were lowest in the N treatment in the bacteria-fungi (between bacteria and fungi) network. The centralized degree in the dry season was the highest in the N treatment in the within-fungi network. For the effects of the N, water treatments, and their interaction, we found that these factors had greater effects on the network-level features in the dry season than in the wet season. The interaction of the N and water treatments prominently affected the network features in the within-fungi and within-bacteria

networks in the dry season, and the within-bacteria networks in the wet season (Table S4). The water treatment had no significant effect on any of the network features in the wet season.

Links between microbial community traits, soil physicochemical properties, and enzyme activities

For the soil physicochemical properties, mainly the SWC, NH_4^+, and AvaiP correlated positively with each other; mainly the TN, TP, and MBC correlated positively with each other. The Mantel test showed that the alpha diversity of bacterial community correlated significantly with the contents of MBC, NH_4^+, TP, and AvaiP, and the activities of BG and ALP; The alpha diversity of fungal community mainly correlated with the SWC, and ALP activity; The beta diversity (compositional change) of bacteria correlated significantly with the contents of SWC, MBC, TN, TP, and AvaiP, and the activities of BG and ALP; The beta diversity of fungi correlated significantly with the contents of SWC, MBC, TN and TP, and ALP activity. Both the topological features of the meta-community network and the bacteria-fungi network correlated significantly with the contents of SWC, MBC, NH_4^+ and AvaiP, and the activities of all 4 enzymes; The topological features of the within-bacteria network correlated mainly with the contents of SWC and AvaiP, and the activities of all 4 enzymes; There were no soil physicochemical properties or enzyme activities that were significantly correlated with the topological features of the within-fungi network (Fig. 6).

To test the relative importance of soil physicochemical properties, and different aspects of microbial community traits (alpha, beta diversity, and network topological features) in affecting soil enzyme activities, the MRM analyses were conducted. The results showed that, by order, the soil physicochemical properties, the meta-community network features, the bacterial alpha diversity, the bacteria-fungi network features, the within-bacteria network features, and the bacterial beta diversity mainly explained the variations of soil enzyme activities. The fungal alpha diversity, the within-fungi network features, and the fungal beta diversity explained relatively minor parts (Table 2).

Table 2

The results of the MRM analyses that linked the soil physicochemical properties, microbial community traits (alpha, beta diversity, and network features) to the variations of soil enzyme activities. Before the analyses were done, the soil enzyme activities, soil physicochemical properties (except the pH which kept no change), and the community traits (except the beta diversity) were both scaled to zero mean and unit variance. The Euclidean distances of soil enzyme activities and community traits (except the beta diversity) were then used in the formula in the MRM function. For the beta diversity of bacteria or fungi, the Bray-Curtis distance was used in the formula. Significant values were indicated by the bold *P* values ($P < 0.05$)

Distance of	R^2 (individually, ordered by value)	*P*	R^2 (Cumulatively, added sequentially)	*P*
Soil physicochemical properties	0.350	**0.001**	0.350	**0.001**
Meta-community network features	0.147	**0.001**	0.375	**0.001**
Bacterial alpha diversity	0.112	**0.001**	0.383	**0.001**
Bacteria-fungi network features	0.107	**0.001**	0.458	**0.001**
Bacterial beta diversity	0.085	**0.001**	0.461	**0.001**
Within-bacteria network features	0.079	**0.001**	0.466	**0.001**
Fungal alpha diversity	0.011	0.060	0.488	**0.001**
Within-fungi network features	0.006	0.262	0.488	**0.001**
Fungal beta diversity	0.001	0.752	0.497	**0.001**

Discussion

Different from previous studies containing only a single N or water treatment in tropical/subtropical forests, our study involved both the nitrogen addition and precipitation seasonality change (Fig. S1), so the single and interactive effects of the two factors could be checked. The one-year manipulation of the nitrogen and water treatments did not significantly change the above-ground tree communities and biomass in comparison with the control (data unpublished). Yet, as the sensitive ecological groups, soil physicochemical properties and microbial communities showed significant responses to the N and water treatments, which showed great fluctuations between the dry and wet seasons.

The effects of season and treatment in affecting soil physicochemical properties, enzyme activities, and microbial communities

We observed that the seasonal changes were prominent for soil physicochemical properties, soil enzyme activities, and all the aspects of bacterial and fungal community traits. The effects of treatments were

generally less than the season (Figs. 1–3, Fig. 5, and Fig. S2). Other studies have also found such strong seasonal effects on soil physicochemical properties or soil microbial communities [70–72]. In the subtropical forests of southern China, the climate is characterized by the divergence of the dry season in autumn-winter and the wet season in spring-summer [37]. The disparate water and temperature situations between the dry and wet seasons could have acclimated soils to exhibit different adaptive traits in different seasons. Remarkably, the SWC differed significantly between the dry and wet seasons, which correlated significantly with almost all the traits of bacterial and fungal communities (Fig. 6). Another explanation for the season's dominance in affecting soil microbial communities was that the treatments were only conducted for 1 year in our study. The long-term effects of nitrogen addition and precipitation change could cause a great magnitude of changes in soil respiration and microbial communities [73].

By introducing the dummy variables for the N and water treatments, we did the statistical tests of the two factors and their interaction in affecting soil physicochemical properties and soil microbial communities. For fungal alpha diversity, their interaction played a significant role in the dry season (Table 1). Interestingly, both the N and water treatments tended to reduce the alpha diversity of fungi, while their interaction acted antagonistically to alleviate the decrease of the fungal diversity (Fig. 2). The opposite effect of the interaction of N and water treatments to any of the sole treatments was also observed in one desert ecosystem [74]. In a drought environment, the water and nitrogen could be both limited (as the diffusive capacity of N in soil is hindered), while the addition of exogenous N possibly alleviate the N limitation (Fig. 1) [75], thus an antagonistic interaction could be observed for N addition and precipitation reduce in the dry season [76].

Like the situation for the fungal diversity, the season dependence of the N and water treatments' (and their interaction's) effects were also observed for the soil physicochemical properties and network topological features (Tables S1 and S4). In the dry season, the water and N treatments and their interaction had more significant effects on soil properties (mainly the SWC and pH) and microbial co-occurrence networks (mainly the within-fungi network and the bacteria-fungi network) than in the wet season. This season-dependence of water and N treatments' effects were not only in the subtropical forest, but also observed in a temperate desert [21]. In the dry season, the PC treatment (rainfall reduction) caused drastic changes in the SWC (Fig. 1), which served as one primary property that affected other soil physicochemical properties and microbial community traits (Fig. 6). On the contrary, the PC treatment in the wet season (rainfall increase) did not cause significant changes in the SWC; and the TN and NO_3^- did not change significantly when both the N and water additions were applied (the NPC treatment) (Fig. 1), which implicated high NO_3^- leaching loss due to the enhanced surface runoff and interflow in the NPC plots (data unpublished). The enhanced hydrologic leaching may downsize the interaction strength of N and water treatments in the wet season [26].

Fungal communities were more sensitive to short-term nitrogen and water treatments

Though statistically no significant effects of the sole N or water treatment were detected for the alpha and beta diversity of either fungal or bacterial communities (Table 1), we found that in comparison with the control, the N and PC treatments caused significantly lower alpha diversity in fungal but not bacterial communities in the dry season (Fig. 2); and fungal community compositions changed more than bacterial community compositions (Fig. 3 and Fig. S3). There were also more significant changes between different treatments in both the node-level and network-level topological features in the within-fungal networks than in the within-bacterial networks (Fig. 5 and Fig. S4). So it was rational to address that fungi were more sensitive than bacteria to the short-term N and water treatments. Similar results showing that fungal communities were more sensitive to the N or water treatment had been observed in forest ecosystems in other studies [13, 20, 18, 77]. There were also some studies conducted in grassland or desert ecosystems, in which bacteria rather than fungi were more sensitive to the N or water treatment [78–79]. This inconsistency might be associated with the difference in ecosystem types, while the underlying mechanisms are still to be inspected.

The PC treatment in the dry season (water reduction) caused a low soil water content (with a mean of 18.5%), corresponding to a value of less than − 0.4 Mpa of soil water potential and nearly 30% water holding capacity in the subtropical forest soil [80–81], which might represent a water condition causing mild drought stress for soil microbes [82]. Fungi are more complex, larger organisms, which mainly live in large soil pores. The moderate drought stress (less water in large soil pore) might more readily affect the fungal community than bacteria which lives in a finer scale and often develop biofilms in soil [83]. In the dry season, the N treatment caused lower pH, SWC and AvaiP compared with the control. The contents of soil pH, SWC, and AvaiP were positively correlated (marginally significantly, $P < 0.1$) with fungal Shannon diversity, while having no significant relationships with bacterial Shannon diversity (Figs. S5-6). That fungal communities were more sensitive than bacteria to nitrogen deposition had been indicated by a meta-analysis study, which was generally consistent across global terrestrial ecosystems [16]. Our results also indicated that the interaction of N and water treatments in the dry season could cause significant effects on the OTU richness of the taxa Agaricomycetes, unclassified Ascomycota, Eurotiomycetes, and Sordariomycetes, while had no significant effects on bacterial main taxa (Table S2). Though both the N and PC treatments tended to decrease the OTU richness of these fungal taxa, their interaction (in the NPC treatment) act antagonistically to alleviate the decreasing effects (Fig. S2).

In addition to the alpha diversity and community compositions, the network topological features of fungi were also more sensitive to the N and water treatments than those of bacteria (Fig. 5, Fig. S4 and Table S4). In the dry season, the fungal members might have sparser relationships (lower closeness) and less interaction influence (lower betweenness) in the N treatment; while in the wet season, fungal members might develop sparser relationships and have higher interaction influence in the N treatment in comparisons with the control (Fig. 5) [84]. N addition might down-regulate the potential cooperation between different fungal species in acquiring N, while in the wet season, the significantly higher N content might favor the growth efficiency and biomass of some fungal species [85], which might exert a higher influence capacity on other members in the community.

The contributions of different soil factors to the variations of enzyme activities

Soil functions, which are often represented by the enzyme activities in soils, are closely linked with microbial activities [86]. In this study, four enzymes related to carbon, nitrogen, and phosphorous cycling were included to represent the basic yet sensitive soil functions to environmental change. Similar to soil physicochemical properties, the seasonal dynamics of soil enzyme activities were more apparent than the differences between different treatments (Fig. 1). In the wet season, all 4 enzyme activities were higher than those in the dry season. The 4 enzymes were highly correlated with each other in their activities and were all significantly correlated with soil water content (Fig. 6). Such positive relationships between soil moisture with the activities of BG and NAG were also observed in other studies [87–88]. For a certain gradient, higher water availability may result in more active microbial populations and higher enzymatic activities [89–90].

For the short-term simulated environmental changes, soil physicochemical properties explained a greater part of the variations of enzyme activities than the community traits (Table 2 and Fig. 6). Soil physicochemical properties, such as soil water content, pH, NH_4^+, and AvaiP were significantly correlated with enzyme activities (Fig. 6), and may readily affect enzyme activities through the regulation of reaction conditions or substrate concentrations. Soil physicochemical properties had been suggested as the primary regulators of soil enzyme activities which overwhelmed plant diversity or agronomic management [91–92]. Due to the widespread functional redundancy among different microbial taxa, the changes in microbial community traits (e.g., compositional change) may have less influential capacity on soil enzyme activities [93] (Table 2). What's more, different community traits of bacteria and fungi may have differential influential effects on soil functions (Table 2 and Fig. 6). The network structure of the meta-community explained a higher proportion of variations of enzyme activities than the alpha or beta diversity of bacteria and fungal communities. This implicated the importance of microbial connections or interactions in affecting soil enzyme activities and ecosystem functions [94].

Our results also suggested that the inter-kingdom microbial associations possibly had great effects in affecting soil enzyme activities (even larger than the effects of within-kingdom associations) (Table 2). Bacteria and fungi may interact far more often than previously thought [95]. They can establish close physical associations ranging from seemingly disordered polymicrobial communities to highly specific symbiotic relationships, such as fungal hyphae and bacterial cells; Their interactions were suggested to be important in gut health, rumen ecosystem functions, and also in biogeochemical processes [96]. The cooperations between bacteria and fungi in degrading litters were also well known [97]. Our results revealed that the links between Ascomycota with a variety of bacteria (such as those from Gammaproteobacteria, Alphaproteobacteria and Verrucomicrobiota) might be important in mediating the interactions between fungi and bacteria (Table S5). Ascomycota fungi could interact with bacteria through the hyphae or inter-kingdom gene transfer, which promoted nutrient transportation and enzyme activities [98–100]. Take the most important edge in the between bacteria and fungi network (Table S5)

for example; The Archaeorhizomyces are global distributed fungi, which live in soil or around hardwoods roots. It may play great roles in nutrient turnover and can establish links with other fungi or bacteria [101–102]. The uncultured KF-JG30-C25 was also found to have many links with other fungi (such as the Ascomycota), and their potential interactions may contribute to the assimilation of acidobacterial extracellular polymeric substances [103]. Individually, bacterial diversity (alpha and beta) and network features were more important than those of fungi in explaining the variations of enzyme activities. This may be due in part to the fact that bacteria may be more effective (higher biomass-specific activities) than fungi in regulating enzyme activities [97]. For a specific season, the relative abundances of 4 bacterial taxa (Acidobacteriota, Gammaproteobacteria, Planctomycetota, Verrucomicrobiota), but only 1 fungal taxa (Eurotiomycetes) were significantly correlated with enzyme activities (Figs. S7-11). Besides, the 4 determined enzymes were mainly corresponding to the degradation of labile organics, which were preferentially linked with bacteria's functions [104]. It is possible that when including the enzymes specific for the recalcitrant carbon such as lignin, the importance of fungal community traits might arise in explaining the variation of enzyme activities.

Conclusions

The interactions between N and water treatments are complex in affecting soil abiotic and biotic properties, which can be affected by the ecosystem type and the environmental settings. In our study, the precipitation setting was to simulate the predicted future precipitation patterns in South China (drier dry season and wetter wet season with the total precipitation amount unchanged). In the dry season, the rainfall reduction and nitrogen addition treatments interact in an antagonistic way to cause minor changes of soil biotic and abiotic properties than the sole treatments. This could be partly attributed to that the water reduction in the dry season enhanced N limitation, which was alleviated directly by the N addition. In the wet season, the interaction between N and water treatments were weaker than in the dry season. This may be due to the high hydrologic leaching caused by the water addition in the wet season. Fungi, rather than bacteria, showed more sensitive to the N and water treatments (and their interaction) at the community level. Fungal communities might be more readily affected by the intermediate water stress, and show stronger responses to physicochemical changes caused by the N addition. We also found that the topological features of the meta-community network might be important in explaining the variation of enzyme activities across the samples. Though there lies gaps between co-occurrence network with true interaction, our results implicated that the inter-kingdom associations (cooperations) between fungi and bacteria might be important in affecting soil enzyme activities, which should be considered along with the traditional diversity index when linking microbial community traits with soil processes and ecosystem functionality.

Declarations

Acknowledgments

We would like to thank Zhipeng Chen, Shaoyun Lv, Shengxing Fu, Xiangping Tan, Yanxia Nie, Xiaoge Han, Yaya Wang and Qingshui Yu for their kind help in the laboratory and fieldwork.

Funding

This study was granted by the National Natural Science Foundation of China (31800439, 31425005, 32171517), the GDAS' Special Project of Science and Technology Development (2021GDASYL-20210103015, 2020GDASYL-20200301003). The authors declare that there was no conflict of interest.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dan He, Zhiming Guo, Dan Sun and Lijuan Ren. The first draft of the manuscript was written by Dan He and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The original bacterial sequences files were deposited in the sequence read archive (https://submit.ncbi.nlm.nih.gov/subs/sra/) under the Biosample numbers SAMN19238034 to SAMN19238065. The original fungal sequences files were deposited in the sequence read archive under the Biosample numbers SAMN19238633 to SAMN19238664. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Arnell NW (1999) Climate change and global water resources. Glob Chang Biol 9:S31–S49. https://doi.org/10.1016/S0959-3780(99)00017-5
2. Fowler D, Coyle M, Skiba U, Sutton MA, Cape JN, Reis S, Sheppard LJ, Jenkins A, Grizzetti B, Galloway JN, Vitousek P, Leach A, Bouwman AF, Butterbach-Bahl K, Dentener F, Stevenson D, Amann M, Voss M (2013) The global nitrogen cycle in the twenty-first century. Philos Trans R Soc Lond B Biol Sci 368:20130164
3. Schuster M (2015) Increased rainfall variability and N addition accelerate litter decomposition in a restored prairie. Oecologia 180:645–655
4. Hawkes CV, Kivlin SN, Rocca JD, Huguet V, Thomsen MA, Suttle KB (2011) Fungal community responses to precipitation. Glob Chang Biol 17:1637–1645
5. Classen AT, Cregger MA (2012) Soil microbial community response to precipitation change.American Geophysical Union Fall Meeting2012 abstract id:B12C-08

6. Zhang H, Liu W, Kang X, Cui X, Wang Y, Zhao H, Hao Y (2019) Changes in soil microbial community response to precipitation events in a semi-arid steppe of the Xilin river basin China. J Arid Land 11:97–110
7. McHugh TA, Koch GW, Schwartz E (2014) Minor changes in soil bacterial and fungal community composition occur in response to monsoon precipitation in a semiarid grassland. Microb Ecol 68:370–378
8. Paradis C, Mahmoudi N, Driver D, O'Dell K, Fortney J, Jagadamma S, Hazen TC (2015) Soil microbial respiration and community structure in response to severe drought and precipitation events. The Second Southeastern Biogeochemistry Symposium Atlanta GA
9. Waring B, Hawkes CV (2018) Ecological mechanisms underlying soil bacterial responses to rainfall along a steep natural precipitation gradient. FEMS Microbiol Ecol 94:fiy001. https://doi.org/10.1093/femsec/fiy001
10. Zeglin LH, Bottomley PJ, Jumpponen A, Rice CW, Arango M, Lindsley A, Myrold DD (2013) Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. Ecology 94:2334–2345
11. Jansson JK, Hofmockel KS (2020) Soil microbiomes and climate change. Nat Rev Microbiol 18:35–46
12. Kaisermann A, Maron P, Beaumelle A, Lata L J, C (2015) Fungal communities are more sensitive indicators to non-extreme soil moisture variations than bacterial communities. Appl Soil Ecol 86:158–164
13. He D, Shen W, Eberwein J, Zhao Q, Ren L, Wu QL (2017) Diversity and co-occurrence network of soil fungi are more responsive than those of bacteria to shifts in precipitation seasonality in a subtropical forest. Soil Biol Biochem 115:499–510
14. Beier C, Beierkuhnlein C, Wohlgemuth T, Penuelas J, Emmett B, Körner C, Janssens IA (2012) Precipitation manipulation experiments–challenges and recommendations for the future. Ecol Lett 15:899–911
15. Liu C, Dong Y, Sun Q, Jiao R (2016) Soil bacterial community response to short-term manipulation of the nitrogen deposition form and dose in a chinese fir plantation in southern china. Water Air Soil Poll 227:447
16. Zhang T, Chen HYH, Ruan H (2018) Global negative effects of nitrogen deposition on soil microbes. ISME J 12:1817–1825
17. Purahong W, Wubet T, Kahl T, Arnstadt T, Hoppe B, Lentendu G, Buscot F (2018) Increasing N deposition impacts neither diversity nor functions of deadwood-inhabiting fungal communities but adaptation and functional redundancy ensure ecosystem function. Environ Microbiol 20:1693–1710
18. Yan G, Xing Y, Xu L, Wang J, Dong X, Shan W, Wang Q (2017) Effects of different nitrogen additions on soil microbial communities in different seasons in a boreal forest. Ecosphere 8:e01879
19. Mueller R, Belnap J, Kuske C (2015) Soil bacterial and fungal community responses to nitrogen addition across soil depth and microhabitat in an arid shrubland. Front Microbiol 6:891

20. Wang J, Shi X, Zheng C, Suter H, Huang Z (2021) Different responses of soil bacterial and fungal communities to nitrogen deposition in a subtropical forest. Sci Total Environ 755:142449
21. Huang G, Li L, Su Y, Li Y (2018) Differential seasonal effects of water addition and nitrogen fertilization on microbial biomass and diversity in a temperate desert. CATENA 161:27–36
22. Zhao A, Liu L, Xu T, Shi L, Xie W, Zhang W, Chen B (2018) Influences of canopy nitrogen and water addition on am fungal biodiversity and community composition in a mixed deciduous forest of China. Front Plant Sci 9:01842. https://doi.org/10.3389/fpls.2018.01842
23. Ma S, Chen G, Tang W, Xing A, Chen X, Xiao W, Fang J (2021) Inconsistent responses of soil microbial community structure and enzyme activity to nitrogen and phosphorus additions in two tropical forests. Plant Soil 460:453–468. https://doi.org/10.1007/s11104-020-04805-9
24. Bejarano M, Crosby MM, Parra V, Etchevers JD, Campo J (2014) Precipitation regime and nitrogen addition effects on leaf litter decomposition in tropical dry forests. Biotropica 46:415–424
25. Bi J, Zhang N, Liang Y, Yang H, Ma K (2012) Interactive effects of water and nitrogen addition on soil microbial communities in a semiarid steppe. J Plant Ecol 5:320–329
26. Sun L, Qi Y, Dong Y, He Y, Peng Q, Liu X, Cao C (2015) Interactions of water and nitrogen addition on soil microbial community composition and functional diversity depending on the inter-annual precipitation in a Chinese steppe. J Integr Agr 14:788–799
27. Shi L, Zhang H, Liu T, Mao P, Zhang W, Shao Y, Fu S (2018) An increase in precipitation exacerbates negative effects of nitrogen deposition on soil cations and soil microbial communities in a temperate forest. Environ Pollut 235:293–301
28. Goberna M, Verdú M (2022) Cautionary notes on the use of co-occurrence networks in soil ecology. Soil Biol Biochem 166:108534
29. Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10:538–550
30. Layeghifard M, Hwang DM, Guttman DS (2017) Disentangling interactions in the microbiome: a network perspective. Trends Microbiol 25:217–228
31. Matchado MS, Lauber M, Reitmeier S, Kacprowski T, Baumbach J, Haller D, List M (2021) Network analysis methods for studying microbial communities: A mini review. Comput Struct Biotec 19:2687–2698
32. de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, Bardgett RD (2018) Soil bacterial networks are less stable under drought than fungal networks. Nat Commun 9:3033
33. Zhang J, Liu S, Liu C, Wang H, Luan J, Liu X, Niu B (2021) Soil bacterial and fungal richness and network exhibit different responses to long-term throughfall reduction in a warm-temperate oak forest. Forests 12:165
34. Jia M, Gao Z, Gu H, Zhao C, Liu M, Liu F, Xie L, Wang L, Zhang G, Liu YHan G (2021) Effects of precipitation change and nitrogen addition on the composition diversity and molecular ecological network of soil bacterial communities in a desert steppe. PLoS ONE 16:e0248194

35. Brown S (1996) Tropical forests and the global carbon cycle: estimating state and change in biomass density. In: Apps MJ, Price DT (eds) Forest ecosystems forest management and the global carbon cycle. NATOASI series (series I: global environmental change). Springer Berlin, Heidelberg, pp 135–144
36. Zuidema P, Baker A, Groenendijk PJ, Schippers P, van der Sleen P, Vlam P, Sterck M F (2013) Tropical forests and global change: filling knowledge gaps. Trends Plant Sci 18:413–419
37. Zhou G, Wei X, Wu Y, Liu S, Huang Y, Yan J, Meng Z (2011) Quantifying the hydrological responses to climate change in an intact forested small watershed in Southern China. Global Change Biol 17:3736–3746
38. Chen J, Xiao G, Kuzyakov Y, Jenerette GD, Ma Y, Liu W, Wang Z, Shen W (2017) Soil nitrogen transformation responses to seasonal precipitation changes are regulated by changes in functional microbial abundance in a subtropical forest. Biogeosciences 14:2513–2525
39. Wang F, Li Z, Xia H, Zou B, Li N, Liu J, Zhu W (2010) Effects of nitrogen-fixing and non-nitrogen-fixing tree species on soil physicochemical properties and nitrogen transformation during forest restoration in southern China. Soil Sci Plant Nutr 56:297–306
40. Zhao Q, Jian S, Nunan N, Maestre FT, Tedersoo L, He J, Wei H, Tan X, Shen W (2017) Altered precipitation seasonality impacts the dominant fungal but rare bacterial taxa in subtropical forest soils. Biol Fert Soils 53:231–245
41. Soil Survey Staff (2010) Keys to soil taxonomy. Unite States Department of Agriculture-Natural Resources Conservation Service, Washington DC
42. Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P (2008) Drought effects on litter fall wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. Philos T Roy Soc B 363:1839–1848
43. da Costa A, Galbraith D, Almeida S, Portela BT, Da CM, Silva JJA, Braga AP, de Gonçalves PH, de Oliveira AA, Fisher R (2010) Effect of 7 year of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. New Phytol 187:579
44. Fang YT, Gundersen P, Mo JM, Zhu WX (2008) Input and output of dissolved organic and inorganic nitrogen in subtropical forests of South China under high air pollution. Biogeosciences 5:339–352
45. Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Buxton RH, Miller Keeney DR (ed) Methods of soil analysis Part 2, 2nd edition. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin USA, pp 403–430
46. Vance ED, Brooks PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass. Soil Biol Biochem 19:703–707
47. Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) 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
48. German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397

49. Saiya-cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biol Biochem 34:1309–1315
50. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Weber CF (2009) Introducing mothur: open-source platform-independent community-supported software for describing and comparing microbial communities. Appl Environ Microb 75:7537–7541
51. Edgar R (2016) UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. BioRxiv:081257. https://doi.org/10.1101/081257
52. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Glöckner FO (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596
53. Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, Nilsson RH (2013) Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol Evol 4:914–919
54. Abarenkov K, Henrik Nilsson R, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Kõljalg U (2010) The UNITE database for molecular identification of fungi–recent updates and future perspectives. New Phytol 186:281–285
55. Tedersoo L, Bahram M, Cajthaml T, Põlme S, Hiiesalu I, Anslan S, Koricheva J (2016) Tree diversity and species identity effects on soil fungi protists and animals are context dependent. ISME J 10:346–362
56. Benjamini Y, Krieger AM, Yekutieli D (2006) Adaptive linear step-up procedures that control the false discovery rate. Biometrika 93:491–507
57. Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9:559. https://doi.org/10.1186/1471-2105-9-559
58. Pollard KS, Dudoit S, van der Laan MJ (2005) Multiple testing procedures: R multtest package and applications to genomics. In: Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S (eds) Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Statistics for Biology and Health Series. Springer, pp 251–272
59. R Core Team (2021) R: ALanguage and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org (accessible on May 7th 2022)
60. Menzel U (2016) RMThreshold: Signal-noise separation in random matrices by using eigenvalue spectrum analysis. R package version 1.1. https://CRAN.R-project.org/package=RMThreshold (accessible on May 7th 2022)
61. Luo F, Zhong J, Yang Y, Scheuermann RH, Zhou J (2006) Application of random matrix theory to biological networks. Phys Lett A 357:420–423
62. Feizi S, Marbach D, Médard M, Kellis M (2013) Network deconvolution as a general method to distinguish direct dependencies in networks. Nat Biotechnol 31:726–733
63. Csardi G, Nepusz T (2006) The igraph software package for complex network research. InterJournal 1695:1–9. https://igraph.org

64. Lichstein J (2007) Multiple regression on distance matrices: A multivariate spatial analysis tool. Plant Ecol 188:117–131
65. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan (accessible on May 7th 2022)
66. Wheeler B, Torchiano M (2016) lmPerm: permutation tests for linear models. R package version 2.1.0. https://CRAN.R-project.org/package=lmPerm (accessible on May 7th 2022)
67. Mangiafico S (2021) rcompanion: functions to support extension education program evaluation. R package version 2.4.1. https://CRAN.R-project.org/package=rcompanion (accessible on May 7th 2022)
68. Huang H, Zhou L, Chenk J, Wei T (2020) ggcor: extended tools for correlation analysis and visualization.R package version0.9.8. https://github.com/houyunhuang/ggcor (accessible on May 7th 2022)
69. Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. J Stat Softw 22:1–19
70. Bardgett RD, Lovell RD, Hobbs PJ, Jarvis SC (1999) Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biol Biochem 31:1021–1030
71. Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JAM, Cregger MA, Moorhead LC, Patterson CM (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6:1–21
72. Schutter M, Sandeno J, Dick R (2001) Seasonal soil type and alternative management influences on microbial communities of vegetable cropping systems. Biol Fert Soils 34:397–410
73. Li J, Xie J, Zhang Y, Dong L, Shangguan Z, Deng L (2022) Interactive effects of nitrogen and water addition on soil microbial resource limitation in a temperate desert shrubland. Plant Soil. https://doi.org/10.1007/s11104-022-05371-y
74. van de Weg MJ, Shaver GR, Salmon VG (2013) Contrasting effects of long term versus short-term nitrogen addition on photosynthesis and respiration in the Arctic. Plant Ecol 214:1273–1286
75. Plett DC, Kosala R, Melino VJ, Noriyuki K, Yusaku U, Kronzucker HJ (2020) The intersection of nitrogen nutrition and water use in plants: new paths toward improved crop productivity. J Exp Bot 71:4452–4468
76. Yan G, Xing Y, Lü XT, Xu L, Zhang J, Dai G, Luo W, Liu G, Dong X, Wang Q (2019) Effects of artificial nitrogen addition and reduction in precipitation on soil CO2 and CH4 effluxes and composition of the microbial biomass in a temperate forest. Eur J Soil Sci 70:1197–1211
77. Zhou Z, Wang C, Luo Y (2020) Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nat Commun 11:3072
78. Zhang K, Shi Y, Jing X, He J-S, Sun R, Yang Y, Shade A, Chu H (2016) Effects of short-term warming and altered precipitation on soil microbial communities in alpine grassland of the Tibetan plateau. Front Microbiol 7:1032

79. She W, Bai Y, Zhang Y, Qin S, Feng W, Sun Y, Zheng J, Wu B (2018) Resource availability drives responses of soil microbial communities to short-term precipitation and nitrogen addition in a desert shrubland. Front Microbiol 9:186. https://doi.org/10.3389/fmicb.2018.00186
80. Ni GY, Zhao P, Zhu LW, Niu JF, Zhao XH, Zeng XP (2015) Hydraulic responses of whole tree transpiration of Schima superba to soil moisture in dry and wet seasons. Sheng Tai Xue Bao 35:652–662
81. Zhou WP, Shen WJ, Li YE, Hui DF (2017) Interactive effects of temperature and moisture on composition of the soil microbial community. Eur J Soil Sci 68:909–918
82. Harris R (1981) Effect of water potential on microbial growth and activity. In: Parr J, Gardner W, Elliott L (eds) Water potential relations in soil microbiology. Soil Science Society of America Special Publications, Madison, Wisconsin USA, pp 23–96. https://doi.org/10.2136/sssaspecpub9.c2
83. Flemming HC, Wuertz S (2019) Bacteria and archaea on Earth and their abundance in biofilms. Nat Rev Microb 17:247–260
84. Ma B, Wang H, Dsouza M, Lou J, He Y, Dai Z, Gilbert JA (2016) Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. ISME J 10:1891–1901
85. Di Lonardo DP, van der Wal A, Harkes P, De Boer W (2020) Effect of nitrogen on fungal growth efficiency. Plant Biosyst 154:433–437
86. Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Marinari SARA (2012) Soil enzymology: classical and molecular approaches. Biol Fert Soils 48:743–762
87. Wang R, Dorodnikov M, Shan Y, Zhang Y, Yong J (2015) Responses of enzyme activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland. Soil Biol Biochem 81:159–167
88. Zuccarini P, Asensio D, Ogaya R, Sardans J, Peuelas J (2020) Effects of seasonal and decadal warming on soil enzymatic activity in a P-deficient mediterranean shrubland. Global Change Biol 26:3698–3714
89. Sheik C, Beasley W, Elshahed M et al (2011) Effect of warming and drought on grassland microbial communities. ISME J 5:1692–1700. https://doi.org/10.1038/ismej.2011.32
90. Zhang N, Wan S, Guo J, Han G, Gutknecht J, Schmid B, Yu L, Liu W, Bi J, Wang Z, Ma K (2015) Precipitation modifies the effects of warming and nitrogen addition on soil microbial communities in northern Chinese grasslands. Soil Biol Biochem 89:12–23
91. Durrer A, Gumiere T, Taketani RG, Costa DP, Pereira e Silva MD C, Andreote FD (2017) The drivers underlying biogeographical patterns of bacterial communities in soils under sugarcane cultivation. Appl Soil Ecol 110:12–20
92. Kompała-Bąba A, Bierza W, Sierka E, Błońska A, Besenyei L, Woźniak G (2021) The role of plants and soil physicochemical properties in the enzyme activities of substrates on hard coal mine spoil heaps. SCI REP-UK 11:5155

93. Louca S, Polz M, Mazel F, Albright F, Huber MB, O'Connor JA, Parfrey MI L, W (2018) Function and functional redundancy in microbial systems. Nat Ecol Evol 2:936–943
94. Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA (2019) Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nat Commun 10:4841
95. Robinson AJ, House GL, Morales DP, Kelliher JM, Gallegos-Graves L, LeBrun ES, Davenport KW, Palmieri F, Lohberger A, Bregnard D, Estoppey A, Buffi M, Paul C, Junier T, Hervé V, Cailleau G, Lupini S, Nguyen HN, Zheng AO, Gimenes LJ, Bindschedller S, Rodrigues DF, Werner JH, Young JD, Junier P, Chain PSG (2021) Widespread bacterial diversity within the bacteriome of fungi. Comms Biol 4:1168
96. Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011) Bacterial-fungal interactions: hyphens between agricultural clinical environmental and food microbiologists. Microbiol Mol Biol R 75:583–609
97. Romaní AM, Fischer H, Mille-Lindblom C, Tranvik LJ (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87:2559–2569
98. Lawrence DP, Kroken S, Pryor BM, Arnold AE (2011) Interkingdom gene transfer of a hybrid NPS/PKS from bacteria to filamentous ascomycota. PLoS ONE 6:e28231
99. Shaffer JP, Carter ME, Spraker JE, Clark M, Smith BA, Hockett KL, Baltrus DA, Arnold AE (2022) Transcriptional profiles of a foliar fungal endophyte (Pestalotiopsis Ascomycota) and its bacterial symbiont (Luteibacter Gammaproteobacteria) reveal sulfur exchange and growth regulation during early phases of symbiotic interaction. Msystems:e00091-22
100. Wei Y, Wu D, Wei D, Zhao Y, Wu J, Xie X, Zhang R, Wei Z (2019) Improved lignocellulose-degrading performance during straw composting from diverse sources with actinomycetes inoculation by regulating the key enzyme activities. Bioresource Technol 271:66–74
101. Pinto-Figueroa EA, Seddon E, Yashiro E, Buri A, Niculita-Hirzel H, Van der Meer JR, Guisan A (2019) Archaeorhizomycetes spatial distribution in soils along wide elevational and environmental gradients reveal co-abundance patterns with other fungal saprobes and potential weathering capacities. Front Microbiol 10:656
102. Mafa-Attoye TG, Borden KA, Alvarez DO, Thevathasan N, Isaac ME, Dunfield KE (2022) Roots alter soil microbial diversity and interkingdom interactions in diversified agricultural landscapes. Oikos. https://doi.org/10.1111/oik.08717
103. Costa OY, Pijl A, Kuramae EE (2020) Dynamics of active potential bacterial and fungal interactions in the assimilation of acidobacterial EPS in soil. Soil Biol Biochem 148:107916
104. Xu Z, Yu G, Zhang X, Ge J, He N, Wang Q, Wang D (2015) The variations in soil microbial communities enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai Mountain. Appl Soil Ecol 86:19–29

Figures

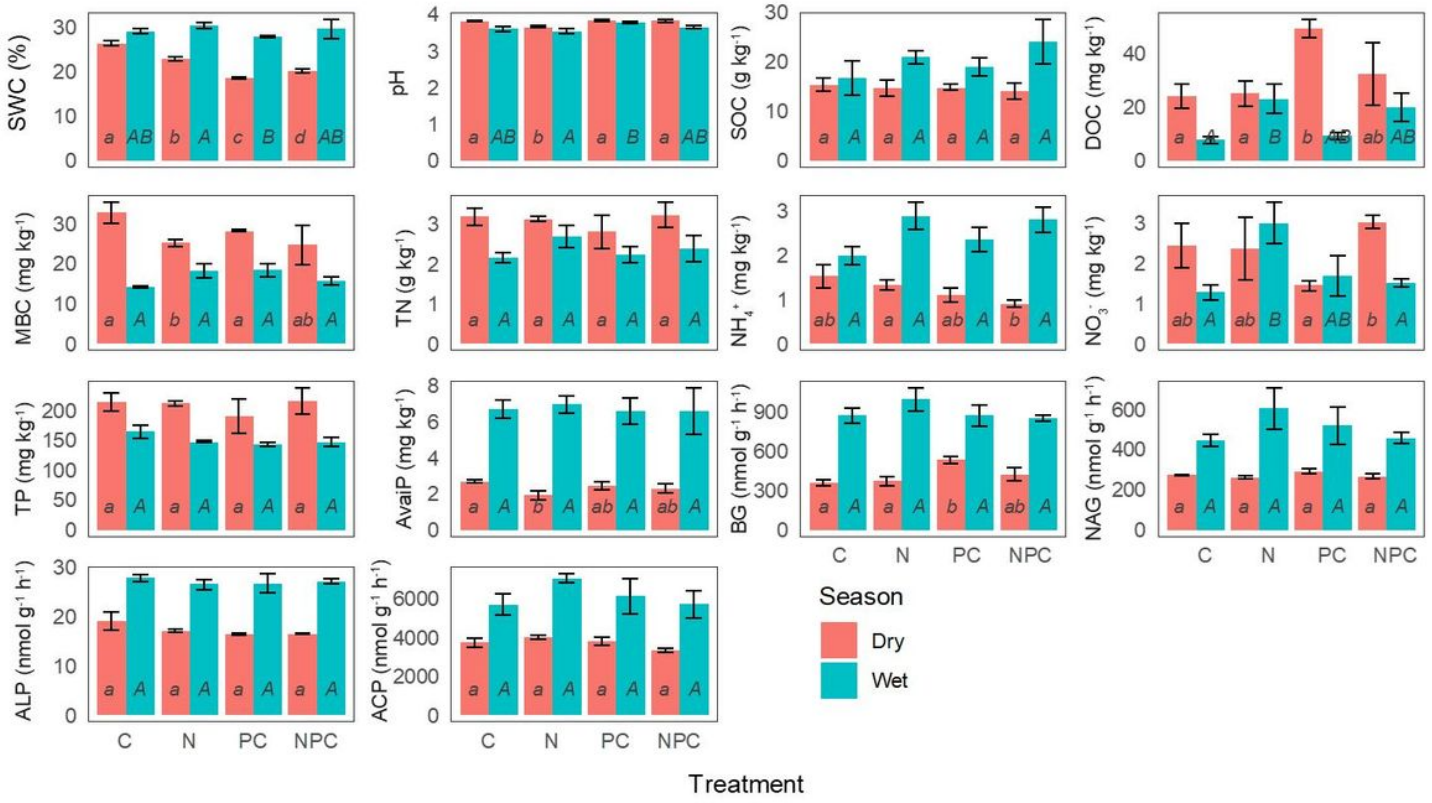

Figure 1

The soil physicochemical properties and enzymatic activities in different sampling seasons and different treatments. Within each season, sharing no letters on the bars denotes significant differences between different treatments. SWC, soil water content; SOC, soil organic carbon; DOC, dissolved organic carbon; MBC, microbial biomass carbon; TN, total nitrogen; TP, total phosphorous; AvaiP, available phosphorous; BG, β-1,4-glucosidase; NAG, β-1,4-N-acetyl-glucosaminidase; ALP, alkaline phosphatase; ACP, acidic phosphatase. For treatments, C, control; N, nitrogen addition; PC, precipitation seasonality change; NPC, the combination of precipitation seasonality change and nitrogen addition

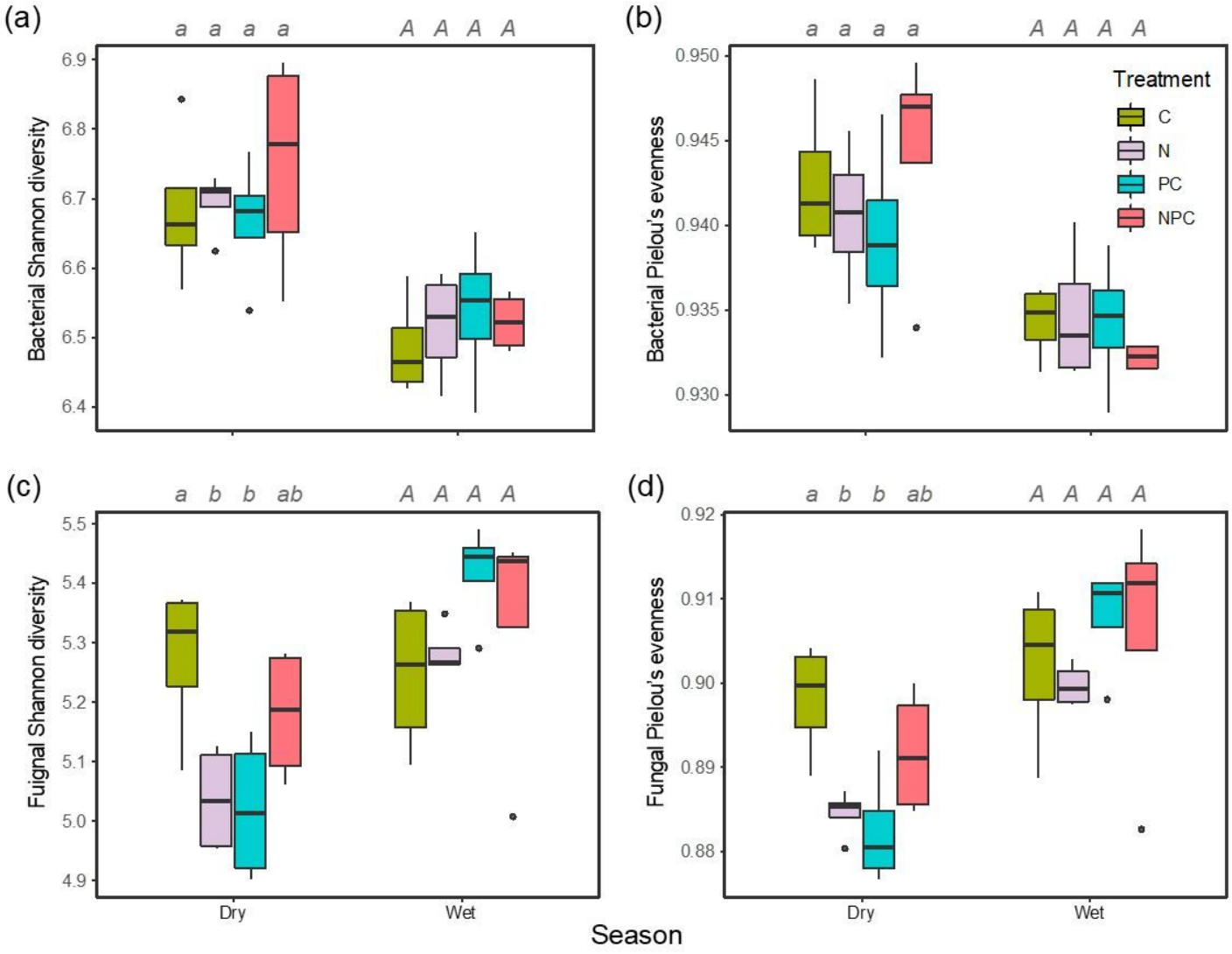

Figure 2

The alpha diversity of bacterial (a, b) and fungal communities (c, d). The box is drawn to represent values from the 1/4 quantile to the 3/4 quantile. The black horizontal bars denote the medians of the diversity values. Whiskers and black solid circles represent the 95% CI values and outliers, respectively. No same letters above the boxes denote significant differences between different treatments. The tests were done respectively, for the dry and wet seasons

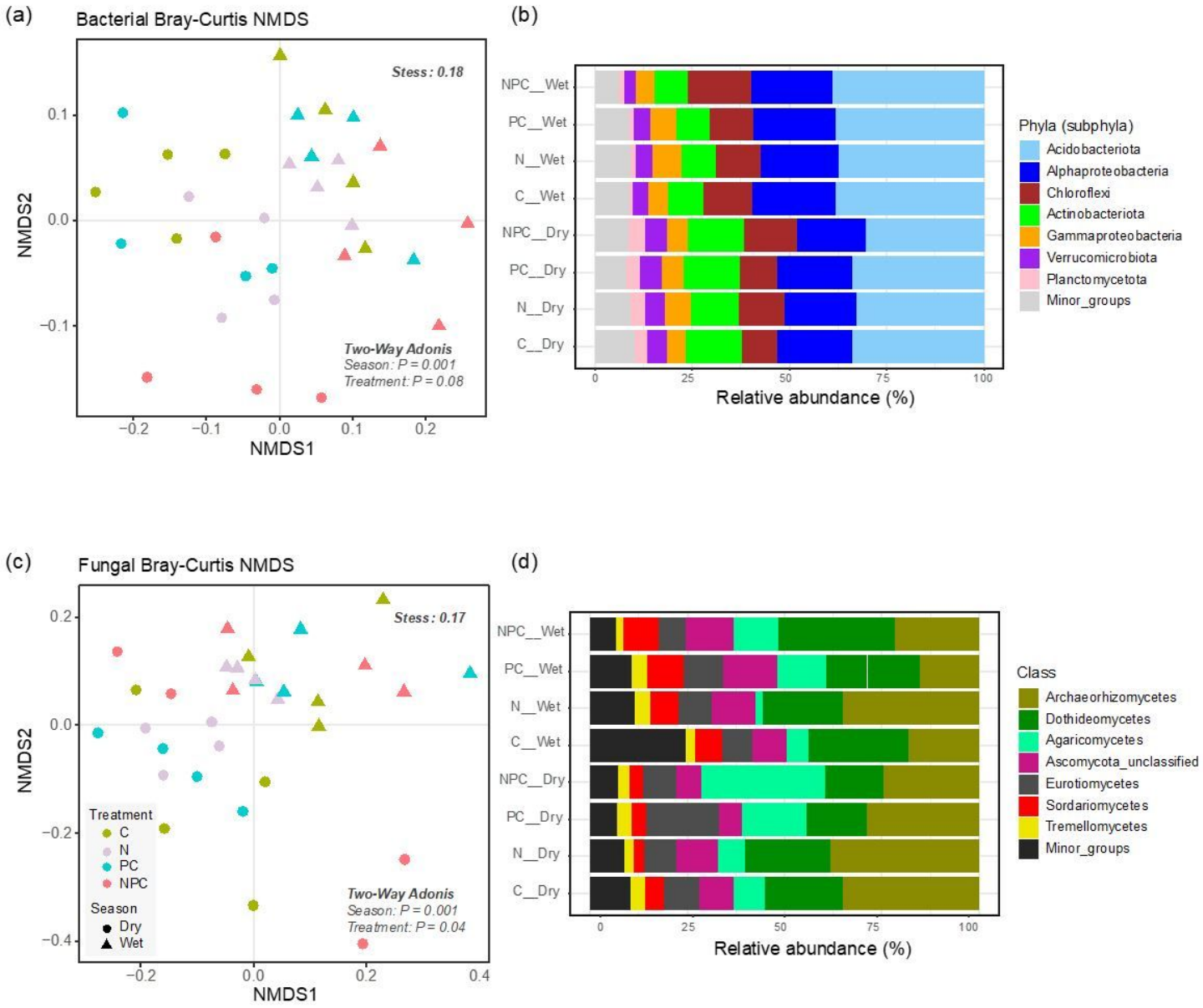

Figure 3

The differentiation of community compositions for bacteria (a, b) and fungi (c, d) in different treatments. The OTU compositional differences between different treatments were displayed in the NMDS plots for bacteria (a) and fungi (c). The relative abundances for the top seven bacterial (b) and fungal (d) taxa were shown

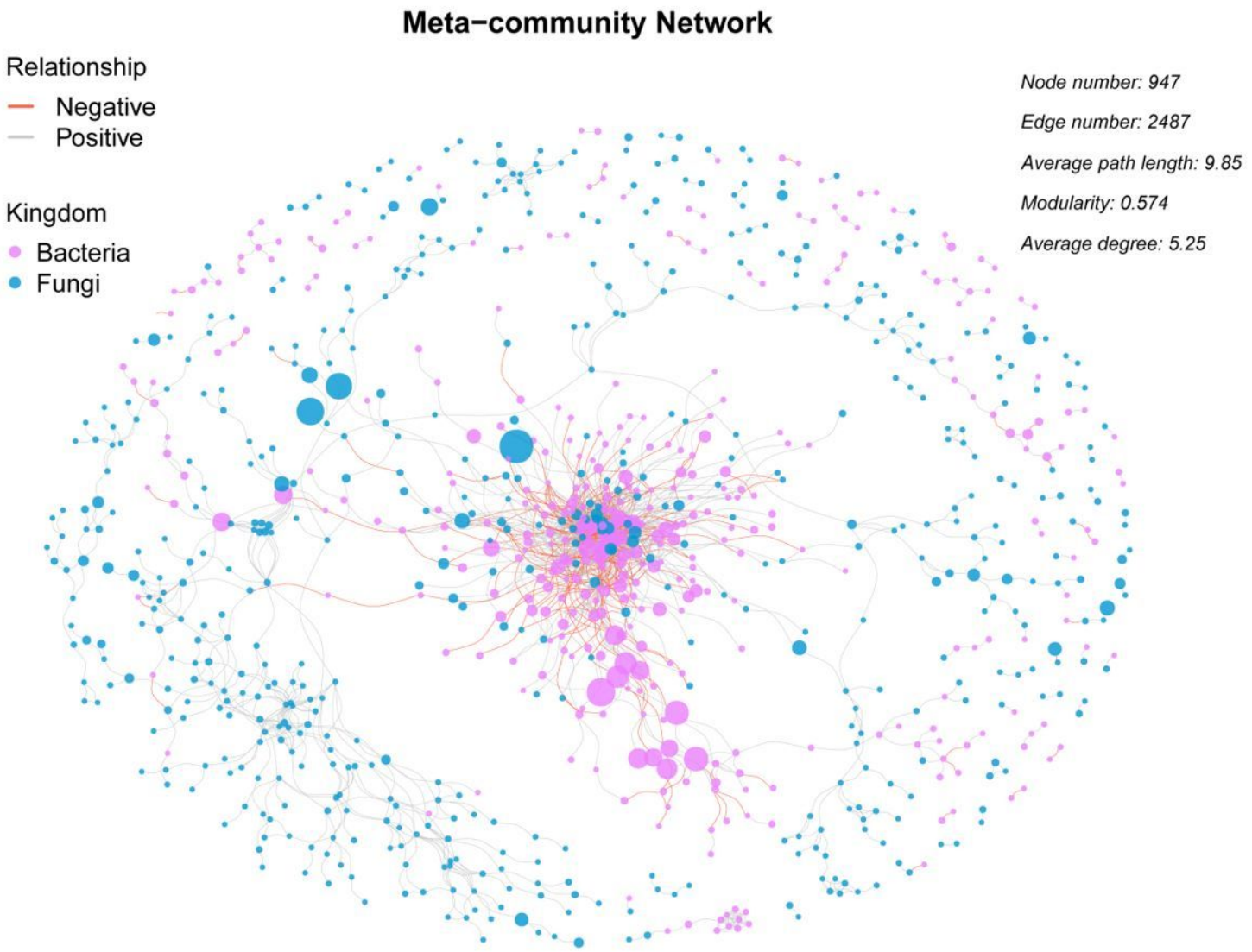

Figure 4

Network graphs for the meta-community in different treatments. The graphs were drawn with the ‘igraph’ in R with the layout of Fruchterman-Reingold. The size of node is proportional to the square-rooted abundance of the OTU

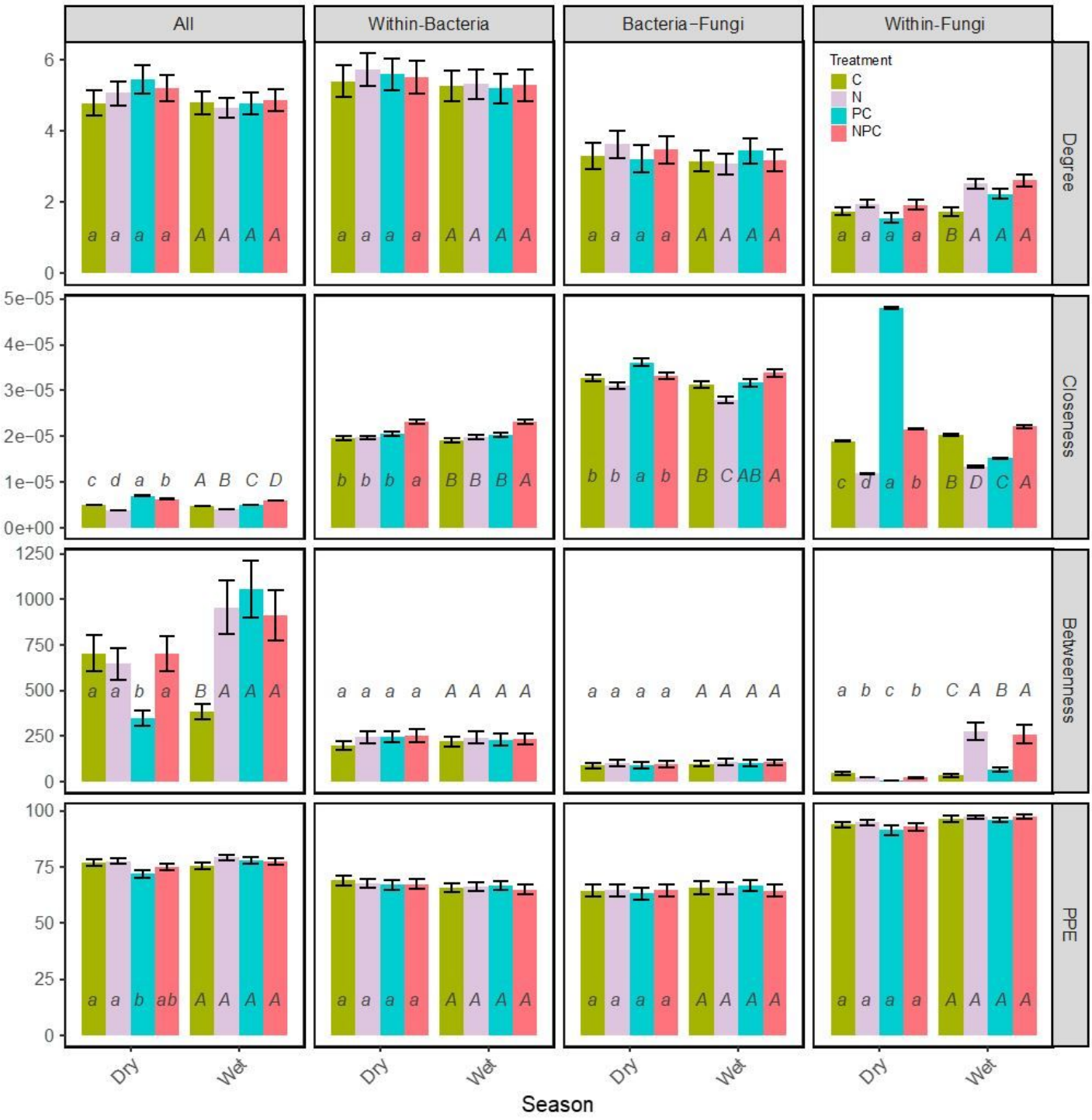

Figure 5

The basic node-level features of different networks. Degree is the number of neighbors for a specific OTU. Closeness is defined by the inverse of the average length of the shortest paths to/from all the other vertices in the graph. Betweenness is the number of shortest paths between any two nodes in the graph passing through that node. PPE is the proportion of positive edges in all of the edges (links) for a specific OTU in the network. Within each season, sharing no letters on the bars denotes significant differences between different treatments

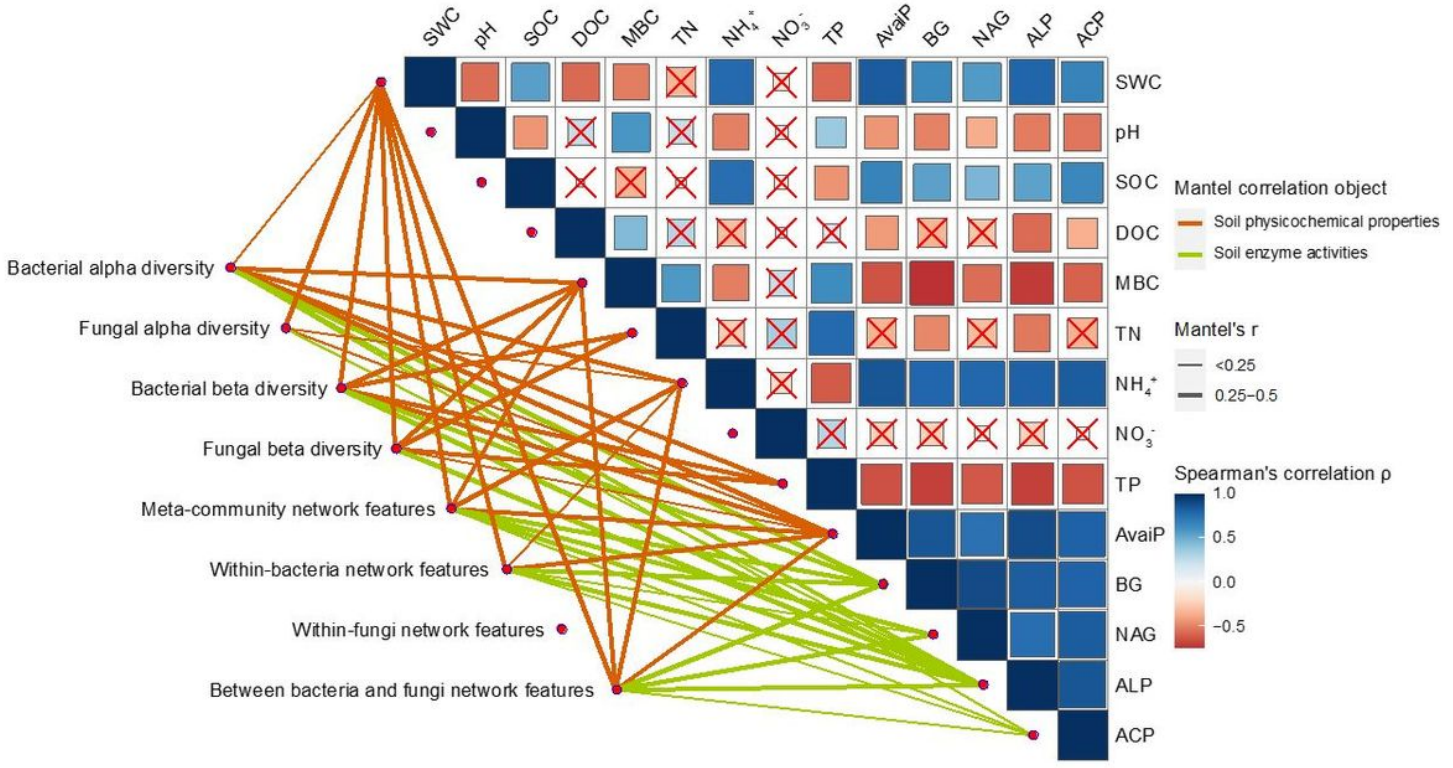

Figure 6

The correlations between soil physicochemical properties, enzyme activities, and different aspects of community traits. The Spearman correlation coefficients between different soil physicochemical properties and enzyme activities were shown in the right triangular plot. The mantel correlation result was shown in the left line plot. To do the mantel analyses, the scaled values of soil physicochemical properties (except the pH which kept no change) and enzyme activities were used; The alpha diversity and network features were scaled before calculating the Euclidean distances. For beta diversity, the Bray-Curtis distance based on the square-rooted community abundance was used. The meanings of the abbreviations for soil physicochemical properties and enzyme activities could be found in the main text and the legend of Fig. 1

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HeetalSupplementaryMaterialsME.pdf