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 He1 · Zhiming Guo2 · Weijun Shen3 · Lijuan Ren4 · Dan Sun2 · Qing Yao5 · Honghui Zhu1

Received: 7 May 2022 / Accepted: 28 July 2022 / Published online: 4 August 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
Increased nitrogen deposition (N factor) and changes in precipitation patterns (W factor) 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 short-term simulated environmental changes (nitrogen addition, precipitation seasonality change, and their combination) in a subtropical forest in South China. The interaction between N and W factors was detected significant for affecting some soil physicochemical properties (such as pH, soil water, and NO3- 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 W factors 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 and W factors (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.

Keywords Nitrogen addition · Precipitation seasonality change · Soil microbial communities · Subtropical forests · Soil enzyme activities

One sentence summary: Fungal communities are more sensitive to the single and interactive effects of nitrogen addition and precipitation seasonality change.
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 precipitation 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. 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 and fungal communities could be sensitive [4–6] or unaffected [7–9] to the changes in precipitation patterns and nitrogen levels [10–12]. Fungal communities might be more sensitive than bacterial communities to non-extreme soil moisture variations [13], while it showed more resistance to extreme desiccation and re-wetting [14]. In arid shrubland or wetlands, bacterial communities were more sensitive, while in subtropical forests fungal communities were more sensitive to nitrogen depositions [15–17]. It was still hard to generalize a common law to decide whether fungi or bacteria are more sensitive to N deposition (N factor) and/or precipitation change (W factor). The inconsistency may be attributed to ecosystem types, soil original fertility levels, and experimental designs [18, 19].

Growing evidences have shown that multiple drivers of global change interact in complex ways that are not predicted by the additive effects of individual drivers. The interaction types can be divided to synergistic, antagonistic, and simply additive [20]. As the two environmental factors that often change simultaneously [21], N deposition (addition) and altered precipitation can solely and interactively affect soil microbial communities and ecosystem functions. In the arid/semiarid ecosystems where both water and N are frequently limited, the changes in precipitation and N level might interact strongly (synergistically or antagonistically) to influence microbial diversity, biomass, and fungi-to-bacteria ratio [22, 23]. Bacterial communities showed different sensibility to reduced and increased precipitation in a desert steppe, and the effects of water addition were weakened by nitrogen deposition [23]. In tropical/subtropical ecosystems, whether the interaction between the two factors is significant for microbial communities and what the interaction types are, were seldom understood. In addition to the diversity, the co-occurrence network between different members in a community has become a new dimension in exploring microbial community changes. Though the co-occurrence network does not represent real inter-species interaction [24], it has its own merit for inferring the relationships between different community members [25–27]. Both bacterial and fungal community networks could be affected by the N and W factors. In temperate ecosystems, soil bacterial network structures were more susceptible to drought than did fungal networks [28, 29], which contrasts with one study conducted in a subtropical forest where fungal networks changed more to precipitation seasonality change [30]. The responses of community diversity and network may be different to environmental changes, for example, soil bacterial diversity showed no significant change but the network structure changed significantly in different seasons in a maize cropland [31]. However, the responses of community network structure to the interaction of different environmental factors (e.g., N and W factors) were not understood for either bacterial or fungal communities.

As a good proxy for microbial functions in soil, soil enzymatic activities were often determined along with microbial community studies. The relationships between diversity with soil functions have been frequently reported. Generally, greater diversity ensures high enzymatic potential, though the functional redundancy among different members may obscure their relationships. The compositional changes of community may or may not explain more than the diversity for the enzymatic variations [32]. Network structure also has good correlations with soil enzyme activities. For example, some module with special keystone species correlated significantly with the activities of laccase in soil [33]. Despite a good collection of literature reporting microbial community traits with soil enzyme activities, there lacks studies to compare different community traits of both bacteria and fungi in correlation with soil enzymatic activities. An inspection of different community traits (diversity, composition, and network structure) for both bacteria and fungi, and their relationships with soil enzymatic activities would have the potential to enhance the predictive capacity for soil functions and ecosystem processes in context of global changes.

Tropical and subtropical forests are central spots of global biodiversity and carbon storage, and also hotspots sensitive to changes in precipitation patterns and N deposition [33]. In South China, subtropical forest ecosystems are facing the challenges of both enhanced N deposition and precipitation pattern change. 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) [34]. How soil bacterial and fungal communities respond to the sole and interactive effects of N and W factors 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 N and/or W factors in terms of community diversity, composition, and network topological features; and (2) whether there are interactive effects of N and W factors on soil physicochemical properties and different microbial community traits. We hypothesized, (1) the interaction of N and W factors could be significant in driving some traits of bacterial and fungal communities. (2) Fungal communities may be more sensitive than bacterial communities to the changes in N level and precipitation in the subtropical forest. Our study would track both fungal and bacterial communities in a variety of traits. The results would deepen our understanding of soil biodiversity, functions, and ecosystem processes in the context of global changes.

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* [35]. The climate in the region 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 °C, and the mean annual precipitation is 1700 mm [36, 37]. The soil type is Ultisol as per the USDA soil taxonomy classification [38].

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 m², with a distance of at least 2 m from each other. For the N and NPC treatments, solutions of NH₄NO₃ were sprayed into soil at a height of nearly close to the forest floor at the beginning of each month (on one of the first several days with no rain), with an annual dose of 100 kg N ha⁻¹. 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 exclusion 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 [39, 40]. 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 m³ 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 [35–37].

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). Five upper 0–15-cm soil samples were randomly taken from each plot on December 13, 2018, and July 12, 2019, by soil augers with a diameter of 5 cm. The soil samples were pooled together in one clean plastic bag for one composite sample and transferred to the laboratory within 8 h 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. A subset of the soil sample was stored at 4 °C for chemical and enzymatic analyses. All analyses were done in less than 2 weeks. A subset of the soil sample was stored at −80 °C 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 K₂Cr₂O₇ 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 K₂SO₄ solution. Total N (TN) was measured by the indophenol blue colorimetric method. Total phosphorous (TP) was measured by the molybdenum antimony blue colorimetric method [41]. Available phosphorus (AvaiP) was measured by Olsen’s method [42]. Soil microbial biomass was determined using the fumigation extraction method [43]. The conversion coefficient used to calculate the microbial biomass C (MBC) was 0.45 [44].

Our study also analyzed 4 soil enzyme activities involved in carbon-, nitrogen-, and phosphorous-rich compound degradations, which often exhibit stoichiometric relationships. The activities of β-1,4-glucosidase (BG; EC: 3.2.1.21; C-cycle), β-1,4-N-acetyl-glucosaminidase (NAG; EC: 3.2.1.30; N-cycle), and acid and alkaline phosphatases (ACP; EC 3.1.3.2 and ALP; EC 3.1.3.1, respectively; P-cycle) were determined using fluorimetric assays in 96-well black microplates [45]. 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 [46].

**Soil DNA Extraction and rRNA Gene Sequencing**

Total genomic DNA was extracted from nearly 0.5-g 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′-CCTACGCGGA GGCAGCAG-3′/5′-GGACTACHVGGGTWTCTAAAT-3′) were used to amplify the V3-V4 region of the bacterial 16S rRNA gene. The primers ITS3NGS/ITS4NGS (5′-CCTACGGGATTGCGTAATCATC-3′/5′-GGACTACHVGGGTWTCTAAAT-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 [47]. 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. Then, all the sequences were compared with Silva v132 to obtain taxonomic information [48]. Those sequences not affiliated with the bacterial domain were discarded. OTUs were then calculated with the command “cluster” and the method “age” 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” [49]. 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 [50]. Those sequences not affiliated with fungi were discarded. OTUs were then calculated with the command “cluster” and the method “age” and a cut-off of 0.02 in Mothur. 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” method were calculated with the “WGCNA” and “multtest” packages in R software 4.1.1 [51]. 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 based on the random matrix theory [52]. 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 [53]. 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. 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 indicator
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) [54].

To check the interactive effects of N and W factors 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,” “lmPerm,” “rcompanion,” “ggcor,” and “ecodist” packages in R. These and aforementioned R packages can be found in the R package repository (https://cran.r-project.org/web/packages/).

**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, 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.
and ACP) had significantly higher values in the wet season, whereas soil pH and the contents of MBC, TN, and TP showed significantly higher values in the dry season (permutation-based two-way ANOVA, all cases, \( P < 0.05 \)). Only in the dry season, the PC and NPC treatments substantially decreased (with a magnitude of over 25%) the SWC compared with the control; and the N treatment significantly reduced soil pH (permutation-based pairwise \( t \) test, \( P < 0.05 \)). No significant changes in the 4 measured enzyme activities were detected during 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 factor, the W factor, and their interaction. The N factor also significantly influenced soil DOC, \( \text{NH}_4^+ \), and soil BG activity (permutation-based two-way ANOVA, all cases, \( P < 0.05 \)). In the wet season, the contents of soil DOC and \( \text{NH}_4^+ \) were significantly influenced by the N factor. Soil pH was significantly affected by the W factor. The contents of soil MBC and \( \text{NO}_3^- \) were significantly affected by the interaction of N and W factors (permutation-based two-way ANOVA, all cases, \( P < 0.05 \)) (Table S1).

### Alpha Diversity of Soil Bacterial and Fungal Communities

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 significantly lower values in the wet season (permutation-based two-way ANOVA, \( P < 0.05 \)), but no significant differences between different treatments. For fungal communities, the PC and N treatments significantly decreased fungal diversity compared with the control (permutation-based \( t \) test, \( P < 0.05 \)) in the dry season (Fig. 2). Statistically, we found that only in the dry season, the interaction of the N and W factors significantly affected the alpha diversity of fungal communities (Table 1).

### 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 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, \( P < 0.05 \)). In the dry season, the PC treatment significantly enhanced the relative abundance of Eurotiomycetes, and the N treatment significantly reduced the OTU richness of Agaricomycetes, unclassified Ascomycota, Eurotiomycetes, and Sordariomycetes (permutation-based \( t \) test, all cases, \( P < 0.05 \)) (Fig. S2(b, d)). Statistically, both the relative abundance and OTU richness of Planctomycetota were significantly affected by the N factor in the dry season. There were more taxa significantly affected by the N and W factors (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 bacterial 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).

### 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. S4). 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 within-fungi network showed more changes than the within-bacteria network in different seasons or treatment. Season significantly affected all the 4 node-level features, and treatment significantly affected 3 out of the 4 features...
Fungal Communities Are MoreSensitive to the Simulated EnvironmentalChanges than Bacterial Communities

Fig. 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.

Table 1 The effects of N addition (N factor), precipitation seasonality change (water (W) factor), and their interaction (N×W) in affecting microbial community alpha and beta diversity. 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 “adonis” function in the “vegan” package in R. Significant values were indicated by the bold P values (P < 0.05).

|          | Bacteria |          | Fungi |
|----------|----------|----------|-------|
|          |         | alpha    |         | alpha    |         |
|          |         | beta     |         | beta     |         |
|          |         | F        | P      | R²       | P      | F        | P      | R²       | 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    |
of the within-fungi network (permutation-based two-way ANOVA, $P < 0.05$) (Fig. 4). The N treatment significantly reduced the betweenness and closeness for the within-fungi network in the dry season. For the network-level topological features, seasonal differences were more obvious than among-treatment differences (Fig. S5). For the N, W factors, 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 W factors 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).

**Fig. 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.

**Links Between Microbial Community Traits, Soil Physicochemical Properties, and Enzyme Activities**

For the soil physicochemical properties, mainly the SWC, $\text{NH}_4^+$, and AvaiP correlated positively with each other (Pearson correlation, $r > 0.78$, $P < 0.001$). The TN, TP, and MBC correlated positively with each other (Pearson correlation, $r > 0.58$, $P < 0.001$). The Mantel test showed that bacterial alpha diversity correlated significantly with the contents of MBC, $\text{NH}_4^+$, TP, and AvaiP, and the activities of BG and ALP. Fungal alpha diversity community correlated significantly with the SWC and ALP activity. Bacterial beta diversity correlated significantly with the contents of SWC, MBC, TN, TP, and AvaiP, and the
Fungal Communities Are More Sensitive to the Simulated Environmental Changes than Bacterial…

activities of BG and ALP. Fungal beta diversity 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, \( \text{NH}_4^+ \) 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. 5). The MRM analysis results

Fig. 4 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
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 and 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** |

Fig. 5 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 can be found in the main text and the legend of Fig. 1.
Discussion

Different from previous studies containing only a single N or W factor 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 1-year manipulation of the nitrogen deposition and precipitation seasonality 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 microbial communities showed immediate responses to the N and W factors, which showed great fluctuations between the dry and wet seasons. The strength of W factor were obviously stronger than that of N factor in affecting soil physicochemical traits in the dry season (Table S1), reflecting the overwhelming effects of precipitation reduce on soil SWC and other associated traits. However, no great differences were detected between the N and W factors for their effects on microbial community traits (Table 1). 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. 5). 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 (in 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 W factors in the wet season [55].

Fungal Community Diversity Were More Sensitive to N and W Factors

In the subtropical forests of southern China, the climate is characterized by the divergence of the dry and wet seasons [34], which caused great seasonal changes of soil abiotic properties and microbial communities. By introducing the dummy variables for the N and W factors, we did the statistical tests of the two factors and their interaction in affecting soil physicochemical properties and soil microbial communities. The results showed that fungal communities showed more sensitive to the N and W factors and their interaction than bacterial communities, which could be reflected in both alpha and beta diversity. Similar results showing that fungal communities were more sensitive to the N or W factor had been observed in forest ecosystems in other studies [16, 30, 56, 57]. The reasons might be, first, 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 [58, 59], which might represent a water condition causing mild drought stress for soil microbes [60]. The moderate drought stress (less water in large soil pore) may more readily affect fungal community than bacteria which lives in a finer scale and often develop biofilms in soil [61]. Second, 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 the N factor had been indicated by a meta-analysis study, which was generally consistent across global terrestrial ecosystems [11]. The N or PC treatment in the dry season could reduce the OTU richness of Agaricomycetes, Eurotiomycetes, and Sordariomycetes, which revealed that some species within these classes might have low N nutrition demand or less drought tolerance (e.g., [62]), while the interaction of N and W factor acts antagonistically to alleviate the decreasing effects (Fig. S2). The mechanistic explanation may lie in that, 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 alleviates the N limitation (Fig. 1) [63]; thus, an antagonistic interaction could be observed for N addition and precipitation reduce in the dry season [64]. This antagonistic interaction between the N and W factors was also observed in one desert ecosystem [65].

Fungal Community Networks Were More Sensitive to N and W Factors

More significant changes between different treatments for both the node-level and network-level topological features in the within-fungi networks were found than in the within-bacteria networks (Fig. 4, Fig. S5 and Table S4), which revealed that fungal community networks were more sensitive to the N or W factors. This result partly disagreed with one study conducted in a grassland, in which soil bacterial network showed less stable than fungal network when subjected to drought treatment [66]. The accordant point between this study with de Vries’ study is that the community networks with more negative edges (fungal networks in de Vries’ study but bacterial networks in this study) showed more stable to environmental changes. Ecological networks consisted with strong interactions might be less stable than those consisted with weak interactions [67]. Members connected...
with positive links in one community may respond in tandem to environmental fluctuations, resulting in positive feedback and co-oscillation [68]. 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. 4) [69]. N addition might downregulate the potential cooperation between different fungal species in acquiring N, while in the wet season, the higher N content (Fig. 1) might favor the growth efficiency and biomass of some fungal species [70], which might exert a higher influence capacity on other members in the community.

The Inter-kingdom Network Explained Great Part of Soil Enzyme Variations

Soil functions, which are often represented by the enzyme activities in soils, are closely linked with microbial activities [71]. 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). 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. 5). Soil physicochemical properties, such as soil water content, pH, NH$_4^+$, and AvaiP, were significantly correlated with enzyme activities (Fig. 5) and may readily affect enzyme activities through the regulation of reaction conditions or substrate concentrations. 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 [72] (Table 2). The network structure of the meta-community explained a higher proportion of variations of enzyme activities than the alpha or beta diversity of bacterial and fungal communities (Table 2 and Fig. 5). This implicated the importance of microbial connections or interactions in affecting soil enzyme activities and ecosystem functions [73].

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 [74]. 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 [75]. The cooperations between bacteria and fungi in degrading litters were also well known [76]. 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 [77–79]. Take the most important edge in the bacteria-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 [80, 81]. 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 [82]. Individually, bacterial diversity (alpha and beta) and network features were more important than those of fungi in explaining the variations of enzyme activities (Table 2, Fig. S6-S12). 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 [76]. For a specific season, the relative abundances of 4 bacterial taxa (Acidobacteriota, Gammaproteobacteria, Planctomycetota, Verrucomicrobiota), but only 1 fungal taxa (Eurotiales) were significantly correlated with enzyme activities (Fig. S8-S12). Besides, the 4 determined enzymes were mainly corresponding to the degradation of labile organics, which were preferentially linked with bacteria’s functions [83]. 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 W factors 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 (such as pH, SWC, and DOC) and abiotic properties (fungal alpha diversity) 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 W factors was 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 W factors (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 were important in explaining the variation of enzyme activities across the samples. Though there lie gaps between co-occurrence network with true interaction, our results implicated that the inter-kingdom associations (cooperations) between fungi and bacteria might contribute greatly to soil enzyme activities, which should be considered along with the traditional diversity index when linking microbial community traits with soil processes and ecosystem functionality.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00248-022-02092-8.

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

**Author Contribution** 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.

**Funding** This study was granted by the National Natural Science Foundation of China (31800439, 31425005, 32171517) and the GDAS’ Special Project of Science and Technology Development (2021GDA-SYL-20210103015, 2020GDASYL-20200301003).

**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 analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of Interest** The authors declare no competing interests.

**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 Meeting 2012, 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

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/femsme/fiy001

10. 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

11. Zhang T, Chen HYH, Ruan H (2018) Global negative effects of nitrogen deposition on soil microbes. ISME J 12:1817–1825

12. Purahong W, Wubet T, Kahl T, Arnstadt T, Hoppe B, Lentendu G, Buscott 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

13. Kaisermann A, Maron PA, Beaumelle L, Lata JC (2015) Fungal communities are more sensitive indicators to non-extreme soil moisture variations than bacterial communities. Appl Soil Ecol 86:158–164

14. Barnard R, Osborne C, Firestone M (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J 7:2229–2241

15. 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

16. 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

17. Hu Y, Chen M, Yang Z, Cong M, Zhu X, Jia H (2022) Soil microbial community response to nitrogen application on a swamp meadow in the arid region of central Asia. Front Microbiol 12:797306

18. 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

19. Jansson JK, Hofmockel KS (2020) Soil microbiomes and climate change. Nat Rev Microbiol 18:35–46
20. Yue K, Yang W, Peng Y, Peng C, Tan B, Xu Z, Zhang L, Ni X, Zhou W, Wu F (2018) Individual and combined effects of multiple global change drivers on terrestrial phosphorus pools: a meta-analysis. Sci Total Environ 630:181–188
21. Jamieson MA, Quintero C, Blumendhal DM (2013) Interactive effects of simulated nitrogen deposition and altered precipitation patterns on plant allelochemical concentrations. J Chem Ecol 39:1204–1208. https://doi.org/10.1007/s10886-013-0340-x
22. 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
23. Jia M, Gao Z, Gu H, Zhao C, Liu M, Liu F, Xie L, Wang L, Zhang G, Liu Y, Han 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
24. Goberna M, Verdu M (2022) Cautionary notes on the use of co-occurrence networks in soil ecology. Soil Bio Biochem 166:108534
25. Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10:538–550
26. Layeghifard M, Hwang DM, Guttman DS (2017) Disentangling interactions in the microbiome: a network perspective. Trends Microbiol 25:217–228
27. Matchado MS, Lauber M, Reitmeier S, Kacprowski T, Baumgart J, Haller D, List M (2021) Network analysis methods for studying microbial communities: a mini review. Comput Struct Biotechnol 19:2687–2698
28. 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
29. 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
30. He D, Shen W, Eberwein J, Zhao Q, Ren L, Wu QL (2017) Divergence of microbial communities in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs. Biogeochemistry 117:5–21
31. Zuidema PA, Baker PJ, Groenendijk P, Schippers P, van der Steen P, Vlam M, Sterck F (2013) Tropical forests and global change: filling knowledge gaps. Trends Plant Sci 18:413–419
32. Zai J, Xiao G, Kuzjakov 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. Biogesosciences 14:2513–2525
33. 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
34. 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
35. Chen J, Xiao G, Kuzjakov 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. Biogesosciences 14:2513–2525
36. 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
37. 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
38. Soil Survey Staff (2010) Keys to soil taxonomy. Unite States Department of Agriculture-Natural Resources Conservation Service, Washington DC
39. 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
40. da Costa ACL, Galbraith D, Almeida S, Portela BTT, da Costa M, Silva JJA, Braga AP, de Gonçalves PHL, de Oliveira AA, Fisher R, Phillips OL, Metcalfe DB, Levy P, Meir P (2010) Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. New Phytolet 187:579
41. 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. Biogesosciences 5:339–352
42. 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
43. Vance ED, Brooks PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass. Soil Bio Biochem 19:703–707
44. 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 Bio Biochem 17:837–842
45. German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Bio Biochem 43:1387–1397
46. 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 Bio Biochem 34:1309–1315
47. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Holister EB, Weber CF (2009) Introducing mothur: open-source platform-independent community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
48. 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
49. 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
50. Abarenkov K, Henrik Nilsson R, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Kõljalg U (2010) The UNITE database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596
51. R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
52. 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
53. 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 Phytolet 186:281–285
54. Lichstein J (2007) Multiple regression on distance matrices: A multivariate spatial analysis tool. Plant Ecol 188:117–131
55. 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
56. 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
57. 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
58. 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. Acta Ecológica Sinica (Sheng Tai Xue Bao) 35:652–662. https://doi.org/10.5846/stxb201305070962
59. 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
60. Harris R (1981) Effect of water potential on microbial growth. Soil Sci 70:1197–1211. https://doi.org/10.1111/jeos.12812
61. Lichstein J (2007) Multiple regression on distance matrices: A multivariate spatial analysis tool. Plant Ecol 188:117–131
62. Yusef HM, Allam M (1967) The carbon and nitrogen nutrition of certain fungi. Can J Microbiol 13:1097–1106
63. Plett DC, Kosala R, Melino VJ, Niriuyuki K, Yusuak U, Kroczukier HJ (2020) The interaction of nitrogen and water use in plants: new paths toward improved crop productivity. J Exp Bot 71:4452–4468
64. Yan G, Xing Y, Li 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. https://doi.org/10.1111/jeos.12812
65. 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 475:361–378. https://doi.org/10.1007/s11104-022-05371-y
66. de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, Hallin S, Kaisermann A, Keith AM, Kretzschmar M, Lemanceau P, Lumini E, Mason KE, Oliver A, Ostle N, Prosser JI, Sarniguet A, Schima KL, Baltrus DA, Arnold AE (2022) Transcriptional profiles of a fossil fungal endophyte (Pestalotiopsis Ascomycota) and its bacterial symbiont (Luteibacter Gammaproteobacteria) reveal sulfur exchange and growth regulation during early phases of symbiotic interaction. Mysystems:00091-22
67. 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
68. Pinto-Figueroa EA, Seddon E, Yashiro E, Buri A, Nicolita-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. Frontiers in Microbiology 10:626
69. 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:08717. https://doi.org/10.1111/oik.08717
70. Costa OY, Pijl A, Kuramae EE (2020) Dynamics of active potential bacterial and fungal interactions in the assimilation of acido-bacterial EPS in soil. Soil Biol Biochem 148:107916
71. 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

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.