Richness contributes higher on soil microbial community resistance while nutrient contributes higher on resilience to a thermal stress

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Abstract

Soil microbial communities play irreplaceable roles in regulating environment. However, global climate change induced extreme weather events were reported capable reshape microbial community composition, which exert possible influences on the environment regulation function. Unfortunately, the compositional response of microbial community to extreme weather events and the potential factors regulating this response were still scarcely explored up to date. To fill the gap, here we sampled soils from 5 different type ecosystems and conducted a simulated extreme high-temperature stress experiment. The results showed that the complex ecosystems (like forests) had higher richness reduction than simple ecosystems (like bare land) during stress stage, most bacterial community richness kept decreasing while most fungal community richness were relatively unchanged during recovery stage. Despite the fungal communities in different types of ecosystems showed different and specific changes in the face of stress during the stress and recovery stage, the relative abundances of α-*Proteobacteria* were enhanced by stress in simple ecosystems but decreased in complex ecosystems, and the recovery stage of simple ecosystems were characterized by the trade-off between α-, β- and γ-*Proteobacteria* while complex ecosystems were by *Firmicutes*. Further analysis showed that the simple ecosystems tend to possess higher resistance and lower resilience while the complex ecosystems displayed the opposite trend. Resistance and resilience were significantly negatively correlated. Structural equation modeling showed that richness provided a higher contribution to bacterial and fungal resistance than to resilience while nutrients provided a higher contribution to resilience than to resistance. This research explored the influence of extreme high temperature stress on soil microbial community and the resistance and resilience of microbial community different types ecosystems to the stress, highlighted the respectively greater relative contribution of richness and nutrients offered to resistance and resilience, which provided valuable information for future prediction on microbial community response to climatic stresses.

1 Introduction

Soil microbial community is a core component of terrestrial ecosystem, which performs several ecological functions such as greenhouse gases emission, the regulation of nutrients in agricultural soils and the transformation of pollutants released by humans, and plays an important role in regulating the earth's environment (Jansson and Hofmockel, 2020). Microbial communities possess extremely high richness and the microbe are capable to reproduce in relative short time range, which lead microbial communities sensitive to environmental variation (Jansson and Hofmockel, 2020; de Vries and Ashley, 2013). The increasing frequency and extent of stresses caused by climate change are threatening microbial community biodiversity and potential function around the world (Fei et al., 2018; Newbold et al., 2020; Marta et al., 2021). However, the impact of extreme weather events on soil microbial communities has not been fully investigated, nor have the factors affecting the specific response of microbial communities been fully described, which is unfavourable for the comprehensive understanding and correct assessment to the persistance of microbial community function and the feedback to environment change.

The extent of change in community composition and function caused by stresses is highly related to community stability, a property reported governed by several biotic and abiotic factors (de Vries and Ashley, 2013). Species richness composing the community and the nutrients surround the community are reported influential to the stability of a certain community (Sankaran and McNaughton, 1999; de Vries and Ashley, 2013; Bastida et al., 2017; O'Brien et al., 2017). However, unfortunately, there have been contradictory conclusions and opinions as to whether richness and environmental factors benefit community stability, or reduce it, when facing climatic stresses. A tropical forest study showed that plant community resistance to heat stress was enhanced by higher richness as the higher richness of plant neighbours in the surrounding area significantly enhanced the number of seedlings that survived under drought conditions (O'Brien et al., 2017). While a field control experiment of savanna grassland in India reported that the composition of a plant community with higher richness was easier to change when exposed to drought stress and showed lower resistance (Sankaran and McNaughton, 1999). Sufficient nutrients and favorable moisture could increase individual resistance of microorganisms to drought stress and thus might enhance community compositional stability (Bastida et al., 2017). While favorable environmental conditions were also thought to be an accelerator for relative abundance trade-offs among different populations that compose the community, and thus decrease compositional stability to climate stresses (de Vries and Ashley, 2013).

Differences in the components that comprise community stability is a potential reason for the above contradictory research conclusions. Community stability is a multidimensional quantity that is composed of many different components, such as the widely studied components of resistance and resilience (Gonze et al., 2017). Different communities may rely on different stability components to maintain an unchanged composition when facing stressors, with no single component capable of completely influencing the true compositional stability of a specific community (Hillebrand et al., 2018). For instance, either high resistance algal communities or high resilience algal communities are capable of nearly maintaining their original state after stress (Hillebrand et al., 2018), showing high compositional stability. Thus, if richness and environmental factors show different effects on resistance and resilience, while at the same time the communities studied rely mainly on either resistance or resilience, contradictory results may be observed. However, current research on compositional stability to climate stresses is mainly focused on artificial communities composed of specific species (Pennekamp et al., 2018), or focused on communities from a single ecosystems (Sankaran and McNaughton, 1999; Bastida et al., 2017; O'Brien et al., 2017). And it's quite necessary to explore the differences of community stability components from different ecosystems and resolve their relationship to richness and nutrient conditions, to get deeper understanding on community response and stability to climate stresses.

To fill the gap mentioned above, we selected soils samples from 5 different successional stage ecosystems and tested the stability of the microbial community through a simulated high temperature stress incubation. The 5 ecosystems were all located in a region of less than 3km×0.5km with less than 130 m in elevation change over the study tract (Li and Xiong, 1995), that indicated they possess same climate background conditions so as to eliminate errors caused by community acclimation to different background stresses when stability components were tested (Canarini et al., 2021). The ecosystems

selected had significant differences in soil microbial community richness and soil physical-chemical properties, which offered good natural background for the study (Li and Xiong, 1995). In this study, we tested the hypothesis that: Soil microbial communities in complex ecosystems possess higher resistance but lower resilience, which is similar to macro-communities. Richness enhance resistance but lower resilience. Nutrient lower resistance but enhance resilience.

2 Materials And Methods

2.1 Sampling and experiment

The soils were sampled from Gongga Snow Mountain, on the eastern edge of the Tibet plateau, Sichuan province, China. We selected different ecosystems (Fig.S1), bare soil (29°34’37”N, 101°59’25”E, 2961m), grass (29°34’48”N, 101°59’33”E, 2955m), shrub (29°34’60”N, 101°59’42”E, 2934m), deciduous forest (29°34’16”N, 101°59’55”E, 2924m), and coniferous (pine) forest (29°34’23”N, 102°00’06”E, 2893m) in June 2018 (Li and Xiong, 1995; Jiang et al., 2018). The main vegetation species and types of the sampling sites were described in detail in previous studies (Li and Xiong, 1995; Jiang et al., 2018). The dominant species of grassland stage are *Oxytropis caerulea* and *Spenceria ramalana*. The dominant species of shrub stage are *Hippophae rhamnoides Linn* and *Salix cupularis*. The dominant species of deciduous forest stage are *Alnus cremastogyne* and *Salix cupularis*. The dominant species of coniferous forest stage are *Picea asperata Mast* and *Abies fabri*(*Mast.*)*Craib*. The mean annual precipitation (MAP) is about 2000mm, the mean annual temperature (MAT) is about 4.2°C, the highest temperature in summer is around 25°C, the mean annual air humidity is over 90% (Lv and Wang, 2008). And the MAT, MAP and soil temperature showed an increasing trend while evaporation showed a decreasing trend since 1988 (Lv and Wang, 2008). 3 samples were taken in each ecosystem (total of 15 for the 5 ecosystems), each was taken from a 10m×10m square and were composed of 5 subsamples (about 1kg each subsample) from the 4 corners and the center of the square. All samples were passed through 2mm sieves with large plant litters and small animal bodies (e.g. insects and earthworms) picked out by hand, and lastly around 5kg mixed samples were stored for each sampling square. Samples were transported on ice to Beijing as soon as possible.

Fifteen incubations were set up for each ecosystem (5 per soil sample). For each incubation, 5g (dry weight) of soil were incubated in 50ml glass bottles under 60% water holding capacity (WHC) and 15°C, this amount of soil was chosen so as to create a soil layer in thin enough in the bottom of the bottle that the community could evenly receive stress and other environment factors, while there was still enough for molecular analysis. Every 12h the lids of the bottles were opened to allow complete exchange of the air by soft hairdryer and to adjust water content by adding sterilized deionized water with fine-long needle-injectors, and reweighing to the original weight. Incubations were reactivated under 60% WHC and 15°C for 3 days before beginning the experiment. Before the beginning of the stress incubation, we sampled 1 original sample (O), the other incubations were divided into high temperature stress treatment and control groups. The stress treatment groups were transferred to 25°C for 3 days while the control groups were incubating under the original temperature condition. We then sampled 1 incubation for treatment (T1)

and control (U1) groups. The remaining temperature stress treatment incubations were returned to the original temperature condition (15°C) and the incubations for both treatment and control groups were incubated an additional 7 days for recovery. Then we sampled both treatment (T2) and control (U2) groups at the end of the 10th day. In total 75 incubations were conducted (Fig. S2A).

Total carbon (TC) and total nitrogen (TN) contents were measured using an elemental analyzer (VarioMAX, Elementar, Germany) (Guo et al., 2017; Zhou et al., 2022). Soil pH was measured using a pH meter (FE20-FiveEasyTM pH, MettlerToledo, Germany) after shaking a soil:water (1:2.5 w/v) suspension for 30 min (Guo et al., 2017). Ammonium (NH_4^+-N) and nitrate (NO_3^{--}N) were extracted at a ratio of 10g fresh soil to 50mL 2M KCl. After shaking for 1h, NH_4^+-N, NO_3^--N contents in the filtered extracts were analyzed using a continuous flow analytical system (San + + System, Skalar, Holland) (Zhou et al., 2022). Total phosphorus content (TP) was measured using the alkali fusion–Mo-Sb Anti spectrophotometric method (Guo et al., 2017; Zhou et al., 2022).

2.2 DNA extraction and amplicon sequencing

Soil DNA was extracted using the FastDNA™ SPIN kit (MP Biomedicals) according to the manufacturer's protocol, after which the concentration and quality were checked using a Nano-100, a NanoDrop Spectrophotometer. Primers targeting the V3-V4 region of the16S rRNA gene of bacteria (Mori et al., 2014), 338F (5´-ACTCCTACGGGAGGCAGCA-3´) and 806R (5´-GGACTACHVGGGTWTCTAAT-3´). The PCR reaction consisted of 25 μl 2×Primer Taq (Takara, RR902A), 1 μl F-Primer (10 mM) and 1 μl R-Primer (10 mM), 3 μl DNA and 20 μl dd-H_2O. The PCR program was 94°C 5 min, 31 cycles of (94°C 30 s, 52°C 30 s, 72°C 45 s), 72°C 10 min. The ITS2 region of fungi (Toju et al., 2012), 3F (5´-GCATCGATGAAGAACGCAGC-3´) and 4R (5´-TCCTCCGCTTATTGATATGC-3´), were chosen for amplification. The PCR reaction consisted of 6 μl 10×E×Taq Buffer, 6 μl dNTP, 0.6 μl BSA, 0.3 μl E×Taq, 1 μl DNA, 1.2 μl F-Primer, 1.2 μl R-Primer, and 43.7 μl dd-H_2O. The PCR program was 94°C 5 min, 25 cycles of (94°C 30 s, 55°C 30 s, 72°C 30 s), 72°C 7 min. PCR was run on a Biorad 1000, and the ending temperature was 16°C. PCR products were mixed in equimolar ratios according to the GeneTools Analysis Software (Version 4.03.05.0, SynGene). Next, the mix of PCR products was purified using an E.Z.N.A. Gel Extraction Kit (Omega, USA). Sequencing libraries were generated using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, MA, USA). The library was sequenced on an Illumina Nova6000 platform and 250 bp paired-end reads were generated (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China). Magichand Cloud Platform (http://www.magichand.online) was used to conduct all of the sequence bioinformatics scripts. Sequences were trimmed of barcodes and merged with a minimum overlap length of 20 bp into full-length sequences by FLASH after removing barcodes and primers, to create pair-ended sequences (Magoč and Salzberg, 2011; Kai et al., 2017). UPARSE was used to remove chimeras and cluster sequences into operational taxonomic units (OTUs) defined at the 97% similarity level (Edgar, 2013). The bacterial OTUs were annotated according to Sliva-138 database while the fungal OTUs were annotated according to UNITE-8.0 database. The total number of bacterial (75526 reads) and fungal (39697 reads) sequences were randomly selected for all samples

to form evenly-resampled OUT table for further analysis according to the least read numbers. The raw sequences were uploaded to NCBI and the projects number are listed on Table S1.

2.3 Statistics

Calculations of richness, Shannon index, and principal coordinates analysis (PCoA) based on Bray-Curtis distances at OTU-level were all conducted with the online platform (http://www.magichand.online). Resistance was defined as the log10 transformed ratio of Bray-Curtis distance between O-U1 to Bray-Curtis distance between T1-U1, the resilience was defined as log10 transformed ratio of Bray-Curtis distance between T1-U1 to Bray-Curtis distance between T2-U2, and detailed calculation, principles and models are attached in supplementary accordingly (Fig. S2B and C). The significant difference of basic soil properties and diversity indices among ecosystems were checked using one-way ANOVA test. Structural equation modeling (SEM) was conducted by amos software with SPSS 24.0. In SEM, the coordinates of the main axes (PC1) from PCoA analysis results for bacterial and fungal community were selected as community structure indicators, Shannon index was selected as diversity indicators, environmental factors were CCA (canonical correspondence analysis) dimensional reduced according to Pearson correlation matrix (Ramette, 2007; Kline et al., 2011; Xie et al., 2020).

3 Results

Our results found that there were significant differences in the basic abiotic and biotic properties of the different ecosystems. The basic soil environmental conditions are shown in Fig. S3, basic bacterial and fungal community α-diversity in Fig. S4.

3.1 The richness variation on bacterial and fungal community

During stress stage, bacterial richness T1-U1 were all negative in five ecosystems (Fig. 1). High temperature stress caused most richness decrease in deciduous forest (-1350), which was magnitude significantly higher than other ecosystems ($p < 0.05$). From bare land, grassland, shrub and pine forest, the reduction on richness increased weakly ($p > 0.05$), the minimum reduction was − 75 and the maximum reduction was − 575. During recovery stage, bacterial richness T2-U2 showed negative value in most ecosystems but in bare land. The grassland and deciduous forest reduced about 550, pine forest and shrub reduced about 810 and 970, respectively. However, fungal richness showed different variation pattern to bacterial richness. During stress stage, stress increased grassland and shrub fungal richness 48 and 57, respectively. but decreased bare land, deciduous and pine forest 48, 116 and 105, respectively. The former two were significantly higher than latter three ($p < 0.05$). During recovery stage, fungal richness in shrub showed significant reduction (-162) than other ecosystems ($p < 0.05$). While the other 4 ecosystems showed no significant differences in fungal richness variation (T2-U2).

3.2 The composition variation on bacterial and fungal community

Extreme high temperature caused significant variation in the soil bacterial community composition during stress stage (U1 and T1) and recovery stage (U2 and T2) (Fig. 2). For bare land, the relative abundances (RA) of α-*Proteobacteria* was 37.1 ± 0.8% in T1, which is about 0.35 times higher than U1 (27.4 ± 3%), but RAs of *Bacteroidetes* and *Actinobacteria* were significantly lower in T1 rather than U1. During recovery stage, the RA of α-*Proteobacteria* in T2 (12.3 ± 2.1%) decreased to only about 1/3 of U2, on the contrary, the RA of γ-*Proteobacteria* was 57.7 ± 5.9% in T2, which was nearly 1.5 times of U2 (36.3 ± 2.8%). For grassland, α-*Proteobacteria* also increased RA in T1 (37.8 ± 4.2%) to about 0.26 times higher than U1 (30.0 ± 3.9%). While the RA of *Actinobacteria* in T1 was 11.8 ± 2.4%, which was about 0.4 times of U1 (27.6 ± 5.0%). After recovery, rare phylum δ-*Proteobacteria* possessed 24.6 ± 6.4% RA in T2, which is as high as 5 times of U2. The RA of α-*Proteobacteria* in T2 is less than half of U2 (34.3 ± 4.0%), but the RA of γ-*Proteobacteria* in T2 (31.3 ± 8.1%) was about 2 times of U2. For shrubs, high temperature stress also caused about 0.25 times increase in RA of α-*Proteobacteria* in T1, *Bacteroidetes* also 1 time higher in T1 (19.2 ± 2.3%) than U1 (9.2 ± 4.1%). But RA of *Actinobacteria* in T1 was only about 0.51 times of U1 (36.3 ± 12.7%). After recovery, RA of α-*Proteobacteria* in T2 was only about 1/3 of U2 (35.5 ± 0.9%), and RA of *Firmicutes* in T2 expanded into 58.8 ± 2.7%, much higher than U2 (2.7 ± 0.5%). For deciduous tree forest, the RA of *Firmicutes* expanded during stress stage and reached to as high as 54.7 ± 4.3% in T1 (U1 was only 2.5 ± 0.5%), and after recovery, T2 and U2 increased the RA of *Firmicutes* to 86.5 ± 1.4% and 74.3 ± 2.3%, respectively. And different to former 3 ecosystems, the RA of α-*Proteobacteria* in T1 was only half of U1 (18.2 ± 4.1%). For pine forest, high temperature treatment offered decreasing effects for α-*Proteobacteria* RA and increasing effects for *Firmicutes* RA in both stress stage and recovery stage, RA of γ-*Proteobacteria* in T1 increased around 0.6 times higher than U1 (16.2 ± 3.1%), RA of *Acidobacteria* in T1 was only 0.7 times of U1 (24.7 ± 0.9%). After recovery, *Firmicutes* RA reached to 68.6 ± 5.7% in U2 (0.34 times higher than T2). The response of bacterial community to high temperature stress varies in ecosystems, but we still found that for relatively simple ecosystems like bare land, grassland and shrub, RAs of α-Proteobacteria were higher in treatment group than control. But in relatively complex ecosystems like tree and pine forest, lower in treatment group than control. In recovery stage, simple ecosystems were characteristic as the increasing RA of γ-*Proteobacteria* and δ-*Proteobacteria*, while complex ecosystems were characteristic as the expanding of *Firmicutes* RA (though RA in simple ecosystems also enhanced, the abundances is not higher enough to be a main phylum). PCoA analysis (OTU level) found that high temperature stress lead significant community composition variation between treatment group and control group (ANOSIM test $R = 0.199$, $p < 0.001$).

The PCoA analysis showed that fungal community composition (at OTU level) was significantly altered by high temperature stress (Fig. 3), and different ecosystems behaved differently (ANOSIM test $R = 0.932$, $p < 0.001$). For bare land, the main order was Unclassified (64 ± 3.4% in O), and high temperature stress did not cause significant variation on T1 (67.6 ± 7.4%) and U1 (69.9 ± 1.4%). After recovery, however, T2 had Unclassified RA of 84 ± 4.0%, which were 0.33 times higher than U2. For grassland, Unclassified RA in T1 was only about 0.75 times of U1 (51.3 ± 7.7%). After recovery, RAs of rare orders like Others, *Archaeorhizomycetales*, *Helotiales* were lower in T2 than U2, while RA of Unclassified in T2 (50.8 ± 1.9%) exceeded 14.7% over U2. The main fungal order for Shrub was originally *Archaeorhizomycetales* (63.1 ±

1.6% in O), and during stress stage and recovery stage, the RA of *Archaeorhizomycetales* were replaced by Unclassified, the RA of *Archaeorhizomycetales* in T1 was 0.1 times lower than U1 (65.5 ± 1.2%), and RA in T2 was 16.7% lower than U2 (45.0 ± 0.8%). On the contrary, Unclassified RA in T1 was 1.2 times of U1 and Unclassified RA in T2 was 1.5 times of U2. At the same time, RA of Others in T2 also increased to 16.2 ± 0.9% during recovery, which was about 0.35 times higher than U2. For deciduous forest, high temperature stress caused significant increase of Unclassified RA (34.1 ± 3.6% and was 0.25 times higher than U1) and decrease of *Archaeorhizomycetales* RA (24.1 ± 2.9% and was 0.18 times lower than U1). After recovery, the RA of Unclassified kept increasing to about 40% for both T1 and U1, while RA of *Archaeorhizomycetales* in T2 exceeded U2 nearly 0.2 times. The most varied fungal order in pine forest was *Filobasidiales*. high temperature sharply in enhanced RA of to *Filobasidiales* 22.9 ± 11.2% in T1, which was 3 times of U1; while after recovery, it was 2% in T2 (only 0.25 times of U2).

1.3 Differences of stability components among terrestrial ecosystems

As depicted in Fig. 4, the ecosystem with lowest bacterial resilience was shrub (-0.86), which was not significantly different from grass and bare soil ($P > 0.05$), but was significantly lower than deciduous forest (0.06) and coniferous forest (-0.15, $P < 0.05$). Similarly, fungal resilience was lowest in shrub (-0.68), then bare soil (-0.58), and both were significantly lower than coniferous forest (0.46, $P < 0.05$), but not significantly lower than grass and deciduous forest ($P > 0.05$). Bacterial resistance was highest in shrub (0.46) and lowest in coniferous forest (-0.70), and these two ecosystems were significantly different ($P < 0.05$) from each other while the other 3 showed no significant differences between them ($P > 0.05$). The highest fungal resistance was observed in bare soil (0.28), and was significantly higher than in the coniferous forest (-0.72, $P < 0.05$). Microbial resistance showed a decreasing trend with vegetation types from bare soil, grass, shrub, deciduous forest, to coniferous forest while the microbial resilience showed an increasing trend. Linear regression analysis showed a significantly negative relationship between resistance and resilience in both the bacterial (Fig. 1B, $P < 0.05$, $R^2 = 0.42$, slope=-0.51) and fungal (Fig. 4C, $P < 0.05$, $R^2 = 0.62$, slope=-0.91) communities, which indicated a trade-off between microbial community resistance and resilience.

3.2 SEM analysis

In order to further analyze the relative contribution of richness and nutrients to microbial community stability components, we use SEM to resolve the effect of nutrients, richness, Shannon index diversity, community composition to stability. The four SEMs all had qualified parameters, such that the $\chi^2/df < 2$, NFI (normed fit index) > 0.95, CFI (comparative fitness index) > 0.95, GFI (goodness fit index) > 0.95, RMSEA (root square mean error of approximation) < 0.05, $P < 0.05$ which indicate parsimonious and plausible modelling. The results showed that richness offered − 0.207 and − 0.409 standard total effect on bacterial and fungal community resistance, respectively (Fig. 5A and B). However, richness offered only 0.141 and − 0.023 standard total effect on bacterial and fungal community resilience, respectively (Fig. 5C and D). Nutrients offered − 0.125 and − 0.019 total effect on resistance, respectively (Fig. 5A and

B). While nutrients offered as high as 0.535 and 0.279 on resilience, respectively (Fig. 5C and D). The richness had a higher standard total effect on resistance rather than on resilience. But nutrients had higher standard total effect on resilience rather than on resistance. The effect of nutrients offered to resistance were negative while the effects to resilience were positive (Fig. 5), which indicated sufficient material and energy is unfavorable for maintaining an unchanged community structure under stresses but capable to accelerate recovery after removal of stressors. Similarly, richness by having negative standard total effect on resistance also indicated that high richness is detrimental for maintaining stable community composition (Fig. 5A and B). Besides richness and nutrients, composition showed significant standard total effects on bacterial resistance (-0.603), fungal resistance (-0.433), bacterial resilience (0.482) and fungal resilience (0.713).

4 Discussion

4.1 The response of microbial community richness and composition to high temperature stress

This study investigated the effects of extreme high temperature stress on soil microbial communities from different terrestrial ecosystems and the factors affecting the stability of microbial communities. We found that high temperature stress mainly caused a reduction in the richness of bacterial and fungal communities. During stress stage, the abundance of complex ecosystems is reduced to a greater extent than that of simple systems (Fig. 1). Complex systems inherently have higher richness (Fig. S4), resulting in more species crowding into the same ecological niche amplitude, and thus, stress is more likely to erase greater numbers of species (Pianka, 1981; Pinsky, 2019). Most bacterial communities show a further reduction in richness during recovery stage, which is also potentially related to the secondary extinctions caused by the disappearance of cooperative members in complex inter-collaborative networks (May and MacDonald, 1978; Damore and Gore, 2012). In contrast, most fungal communities didn’t show such secondary extinctions during the recovery stage, which was potentially related to the fact that fungi are more adapted to terrestrial habitats (de Vries and Ashley, 2013). And the fungal networks were also reported more robust than bacterial networks under climatic stress (de Vries et al., 2018), which reduced the possibility of further extinction induced by change of interaction (May and MacDonald, 1978; Damore and Gore, 2012).

The composition of bacterial and fungal communities in different ecosystems show distinctive trajectories of change in response to extreme high temperature stress (Figs. 2 and 3). The α-*Proteobacteria* in simple ecosystems increased RA during stress stage while decreased RA during the recovery stage (Fig. 2). This was possibly due to simple ecosystems were relative scarcity of carbon and nitrogen nutrients (Fig. S3) and were unfavorable for r-strategic microorganisms such as α-*Proteobacteria* (de Vries and Ashley, 2013). While high temperatures promote substrate use by microorganisms and increase the dominance of r-strategic microorganisms (de Vries and Ashley, 2013). In contrast, α-*Proteobacteria* RAs in complex ecosystem were reduced during both the stress and recovery stages and

were largely replaced by phylum such as *Firmicute* (Fig. 2). Complex ecosystems had relatively higher carbon and nitrogen resources (Fig.S3), and higher lignin content (Li and Xiong, 1995; Jiang et al., 2018), which were may create more relatively anaerobic microsites and enrich lignin-intimate microbes at high temperatures (Wu and He, 2013; Kato et al., 2015). The changes in fungal communities under stress are more specific than those in bacterial communities (Figs. 2 and 3). Fungal communities are more dependent on vegetation type and fungi are dispersal limited than bacteria, leading to greater differences in the original species composition of fungal communities in different ecosystems and, in turn, to differential cultural succession trajectories (de Vries and Ashley, 2013; de Vries et al., 2018).

This research showed that extreme high temperature stress significantly influenced the richness and community structure of bacterial and fungal communities, but it was difficult to derive a consistent pattern for compositional change. To further elucidate the rules for soil microbial response to climate extremes, we analyzed the microbial community stability and the factors influence the stability. We found a rough trend that forests had higher resilience, but lower resistance to simulated extreme high-temperature stress, than shrub, grassland, and bare soil (Fig. 4A), which was opposite to the trend displayed by plant communities where complex systems were likely to have higher resistance and lower resilience (Isbell et al., 2015). We suggest that such differences arise from the reproductive rates, physiological resistance, and differences in richness between plants and microbes (Curtis, 2006; Konopka, 2006). Compared to microbes, plants have much lower reproductive rates and richness (Curtis, 2006; Konopka, 2006). When faced with short term high temperature stress, on the order of only several days, the dominant forest plant keystone population was likely to remain unchanged unless large scale regional death occurred, and thus appearing to have high resistance (Curtis, 2006; Konopka, 2006). However, microbes have extremely high richness as compared to plants in terrestrial ecosystems, and are able to reproduce in a timespan as short as hours or even minutes (Curtis, 2006; Konopka, 2006). These indicate that competitive functional groups with different temperature range tolerances could potentially replace the original dominant groups and thus led to lower resistance of the microbial community (Kai et al., 2017; Pinsky, 2019). However, with keystone species dominated by organisms with high stress tolerance, such as the communities from bare soil (Fig. 4A) that are exposed to high day-night temperature variation, dry-wet alteration, and high ultra-violate, could exhibit high resistance because those keystone species were not likely to change (Remias et al., 2012; Harrison and LaForgia, 2019).

4.2 The resistance and resilience of microbial community to high temperature stress

We found a strong negative linear relationship between microbial community resistance and resilience, which indicated a trade-off between resistance and resilience of microbial communities in the ecosystems studied here (Fig. 4B and Fig. 4C). This is plausible and inevitable from both basic logic and an evolutionary perspective. The essence of resistance and resilience is the ability of altering the relative abundance of species as conditions change (de Vries and Ashley, 2013). Thus, a microbial community that is more readily prone to change simultaneously has less resistance and higher resilience, and vice versa (Miller and Chesson, 2009; Griffiths and Philippot, 2013). From an evolutionary perspective,

communities need to coordinate the functions of different components to ensure the continuation of key ecological processes for survival of the community under variable environmental conditions. This can be realized by assigning key functions to a few stress tolerant functional species (such as the high resistance community in bare soil) (Craine et al., 2013), or to alternative functional groups composed of different stress tolerant members that are capable to quickly reproduce under certain environmental conditions (like the low resistance community in forests) (Whitham et al., 2006; Walworth et al., 2020), that can be treated as the trade-off between K-strategy members and r-strategy members of community evolution or succession. However, to simultaneously possess K- and r-strategy wastes energy and is an evolutionary dead-end (Whitham et al., 2006; Li et al., 2020; Walworth et al., 2020). Thus, a microbial community prone to have a higher resistance or a higher resilience rather than both.

4.3 The factors influencing microbial community resistance and resilience

Our results found that richness had higher total effects on resistance rather than on resilience while environmental factors had higher total effects on resilience rather than on resistance, which indicated that richness and environmental factors had biases for influencing different stability components (Fig. 5). To better understand these biases, we need to elucidate the relationship between richness and resistance, and the relationship between nutrients and resilience.

The results showed that high richness was unfavorable for high resistance as it exerted negative total effects on resistance, and this was supported by several field and theoretical studies (Fig. 5). Certain stressors may cause the simultaneous extinction of more species in higher richness community if they belonged to the same niche (Kalmykov and Kalmykov, 2012). A recent study reported that stress decreased not only the dominance of *Tricholoma matsutake* but also the dominance of its competitors (Zhou et al., 2021). Also, higher richness offered higher possibility for the trade-off of relative abundances among functionally similar groups when facing stressors (Louca et al., 2018; Pinsky, 2019). Both situations are unfavorable for maintaining an unchanged community composition under stress (Kalmykov and Kalmykov, 2012; Louca et al., 2018; Pinsky, 2019). In our study, richness had higher total effects on inhibiting resistance than on accelerating resilience (Fig. 5), however we still lack reasonable ecological theory offering plausible explain. We believe this might be linked to the relatively isolated incubation strategy utilized here. A complete recovery requires the original species occupying their original functions. However, if several species became extinct due to stress and were unable to be reintroduced into the community during the recovery state, the community would need to use other species to fill those functional roles (Louca et al., 2018). Though higher richness would indicate the availability of more potential replacement species to accelerate recovery (a kind of positive effect) (Louca et al., 2018), it would also indicated a more or less incomplete recovery (a kind of negative effect) (Hillebrand et al., 2018). Thus, the total effect from richness on resilience was the balance of those opposing effects (we should note these two opposing effects might not be the opposite direct and indirect effect in SEM), which potentially explains why richness had lower total effects on accelerating resilience than on inhibiting resistance.

Our results found that nutrient sufficient condition was unfavorable for resistance but favorable for resilience (Fig. 5). As discussed above, in a microbial community which has highly richness, functional redundancy and fast individual reproduction rate, stress (like temperature change in this study) may cause significant change to population reproduction rate and vary the relative abundances of several species within few days (Curtis, 2006; Prosser et al., 2007). Also, a nutrient sufficient condition would accelerate the compositional change under stress (lower resistance) as well as the compositional change after removing the stress (higher resilience). Interestingly, nutrients offered less inhibiting effect on resistance but higher accelerating effect on resilience (Fig. 5). We suggested this was caused by ecological memory shaped in the 17°C field site (sampling sites) and the reactivation stage temperature (17°C), such that the microbial community had acclimated, compositionally and functionally, to environmental conditions under 17°C (Veen et al., 2015; Canarini et al., 2021). Though stresses forced the community to change to a state which operates more efficiently under 25°C, several environmental factors were unlikely to be changed by only a few days of stress exposure, such as the chemical composition of litters (Veen et al., 2015), leading the community to still more suitable work under the original state. Thus, nutrients had higher accelerating effect on returning from a stressed state to original state rather than accelerating from the original state to a stressed state. Above all, due to the high richness and functional redundancy of the microbial community and the ecological memory under the original state, richness had a greater contribution to resistance than resilience, and environmental factors had a greater contribution to resilience than resistance.

Since different microbial communities rely on high resistance or resilience to maintain their stability, and resistance and resilience are respectively influenced by richness and nutrients mainly, we are then capable to estimate the stability of communities in the face of climate extremes according to richness and nutrients information (Fig. 6). The relatively high richness communities in eutrophic ecosystem (such as forest) possess considerable stability to extreme climate stresses due to high resilience. And the relatively low richness communities in oligotrophic ecosystem (such as bare land) also possess considerable stability due to high resistance. The relatively low richness communities in eutrophic ecosystem were seldom reported in natural ecosystems and theoretically hard to exist. The relatively high richness communities in oligotrophic ecosystem were potentially most sensitive to extreme climate change and the region may be the hotspots for ecosystem degradation, such as grassland and shrub (Fig. 4), which was supported by alpine grassland related researches (Borer et al., 2017).

5 Conclusion

In this study, the soil bacterial communities and fungal communities from different ecosystems were selected and their resistance and resilience to high temperature stress were tested by a simulated experiment. The results support that the microbial communities in more complex ecosystems (forests) are prone to possess lower resistance and higher resilience than in less complex ecosystems (bare ground, grassland, shrub), and no ecosystems would have microbial communities that possess high resistance and resilience simultaneously. Resistance was mainly influenced by richness (an inner property of community itself) and resilience was mainly by nutrients (the ambient material and energy

supply), respectively. This study confirmed that microbial communities adopted different strategy when facing extreme climate stresses, which offers new insight and valuable guides for stability assessment, climate change sensitive hotspots evaluation and conservation manipulation.

Declarations

Data availability

The sequencing data had been uploaded on NCBI and the corresponding project numbers were list on Table S1.

Competing interests

The authors declare no competing interests.

Author contributions

All authors contributed intellectual input and assistance to this study and manuscript preparation. Design, H.Z., A.Z. and G.Z.; methodology, H.Z.; software, G.Z. and H.Z.; validation, H.Z., R.C. and A.Z.; formal analysis, H.Z. and A.Z.; investigation and resources, H.Z., A.Z. and G.Z.; H.Z. wrote the manuscript with help from A.Z., G.L., and G.Z. All authors contributed intellectual input and assistance to this study and manuscript preparation.

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Figures

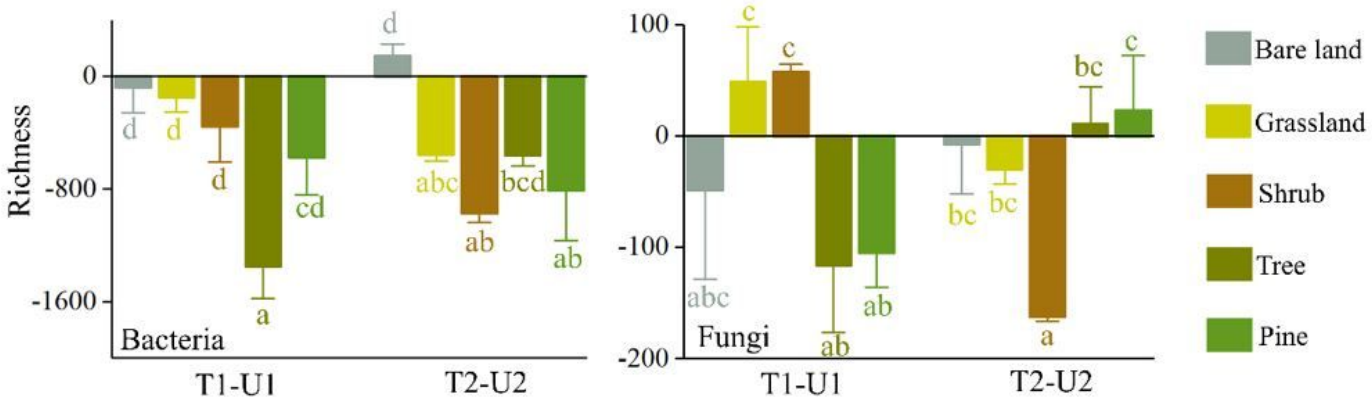

Figure 1

The variation on richness at stress stage and recovery stage. Different letters indicate significant statistic differences (one-way ANOVA and Tukey's test, $p < 0.05$).

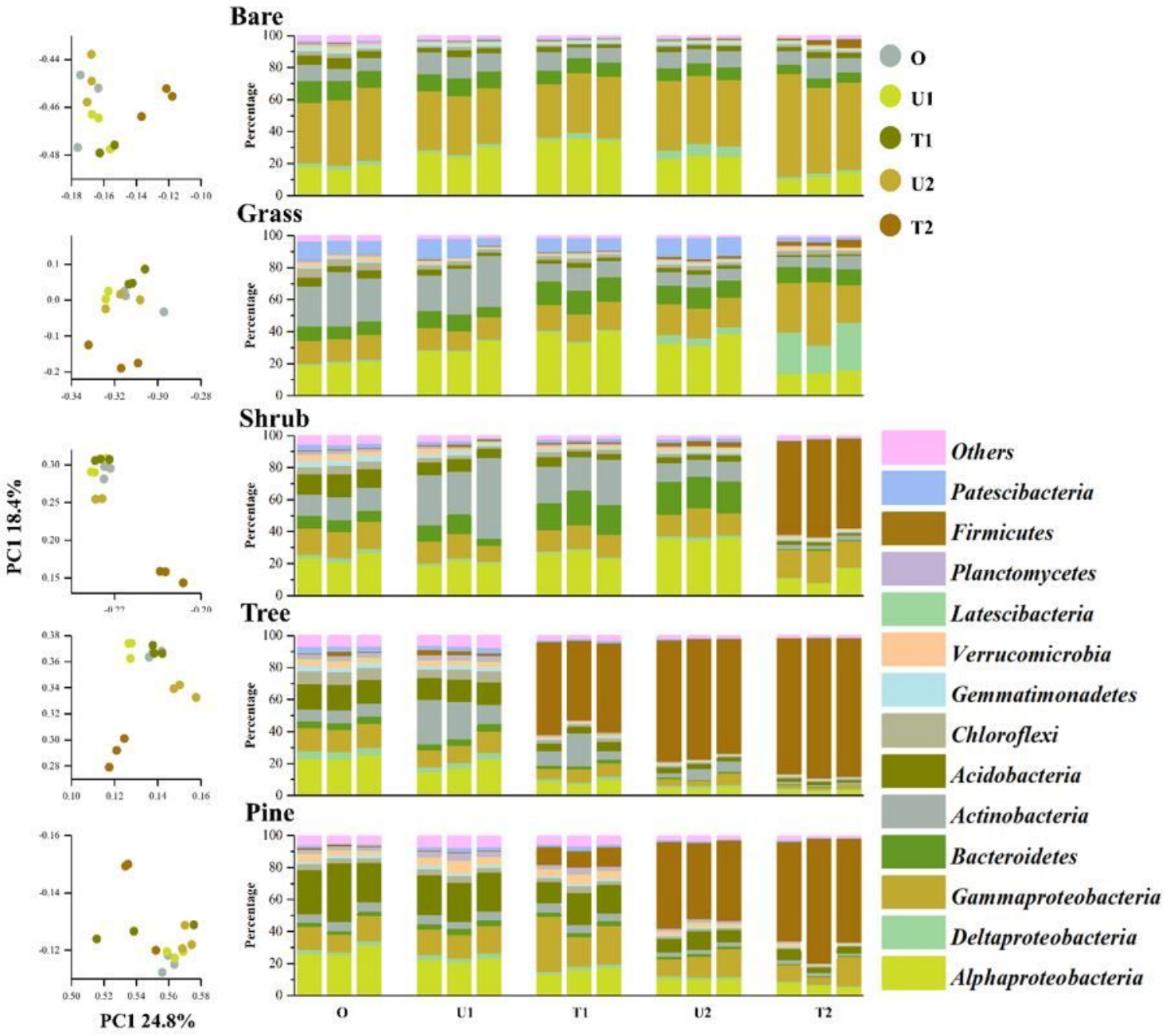

Figure 2

The bacterial community composition at phylum level of different ecosystems (right side) and OTU level-PCoA analysis for composition variation of different ecosystems (left side)

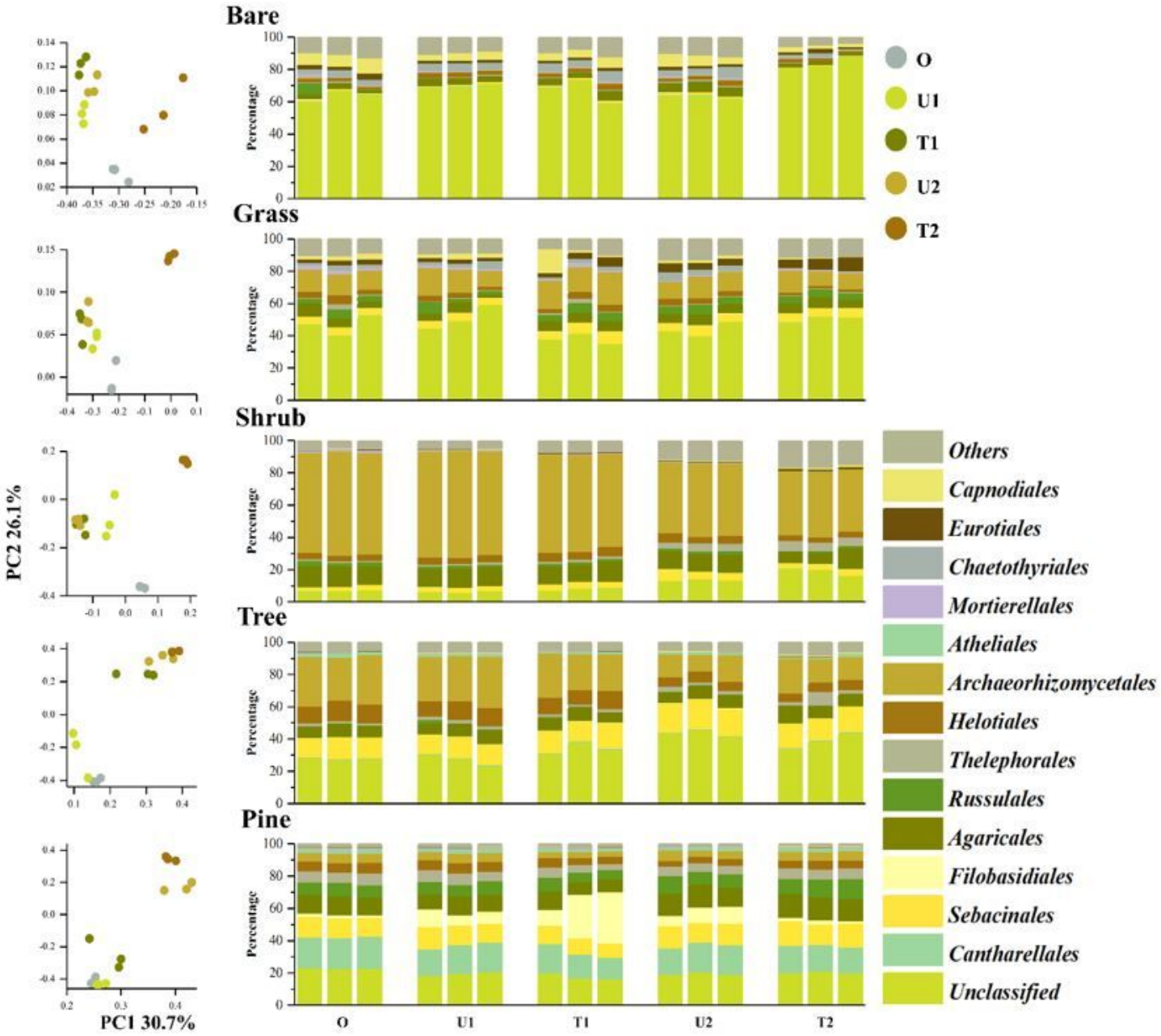

Figure 3

The fungal community composition at order level of different ecosystems (right side) and OTU level-PCoA analysis for composition variation of different ecosystems (left side)

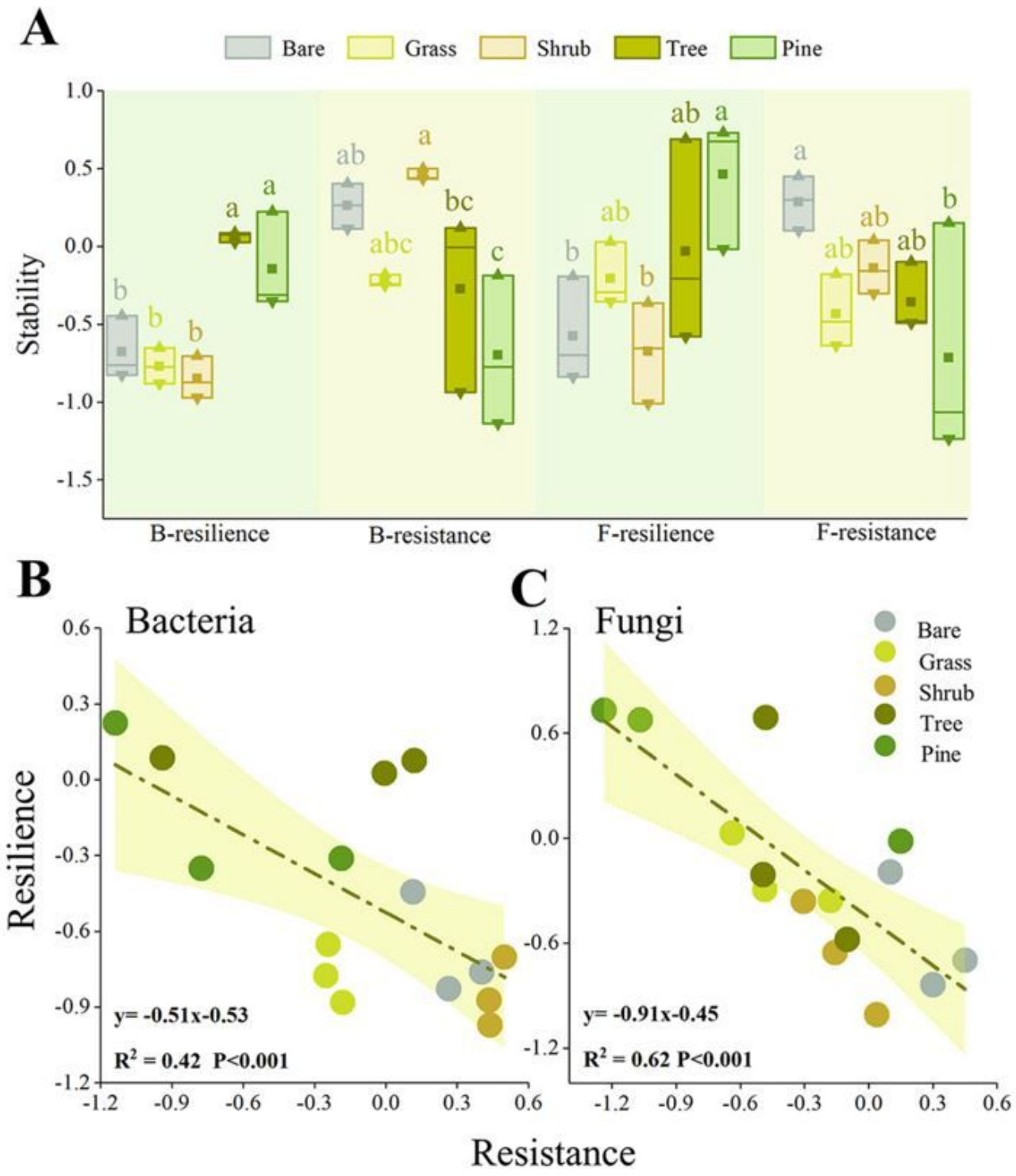

Figure 4

The resistance and resilience of microbial communities from different terrestrial ecosystems. Fig. 4A depicts the magnitude of resistance and resilience of the bacterial and fungal communities among bare soil, grass, shrub, deciduous forest, and coniferous forest. Different letters indicate significance ($P < 0.05$; one-way ANOVA Tukey's test). Fig. 4B and 4C depict the negative relationship between resistance and resilience of the bacterial and fungal communities, respectively.

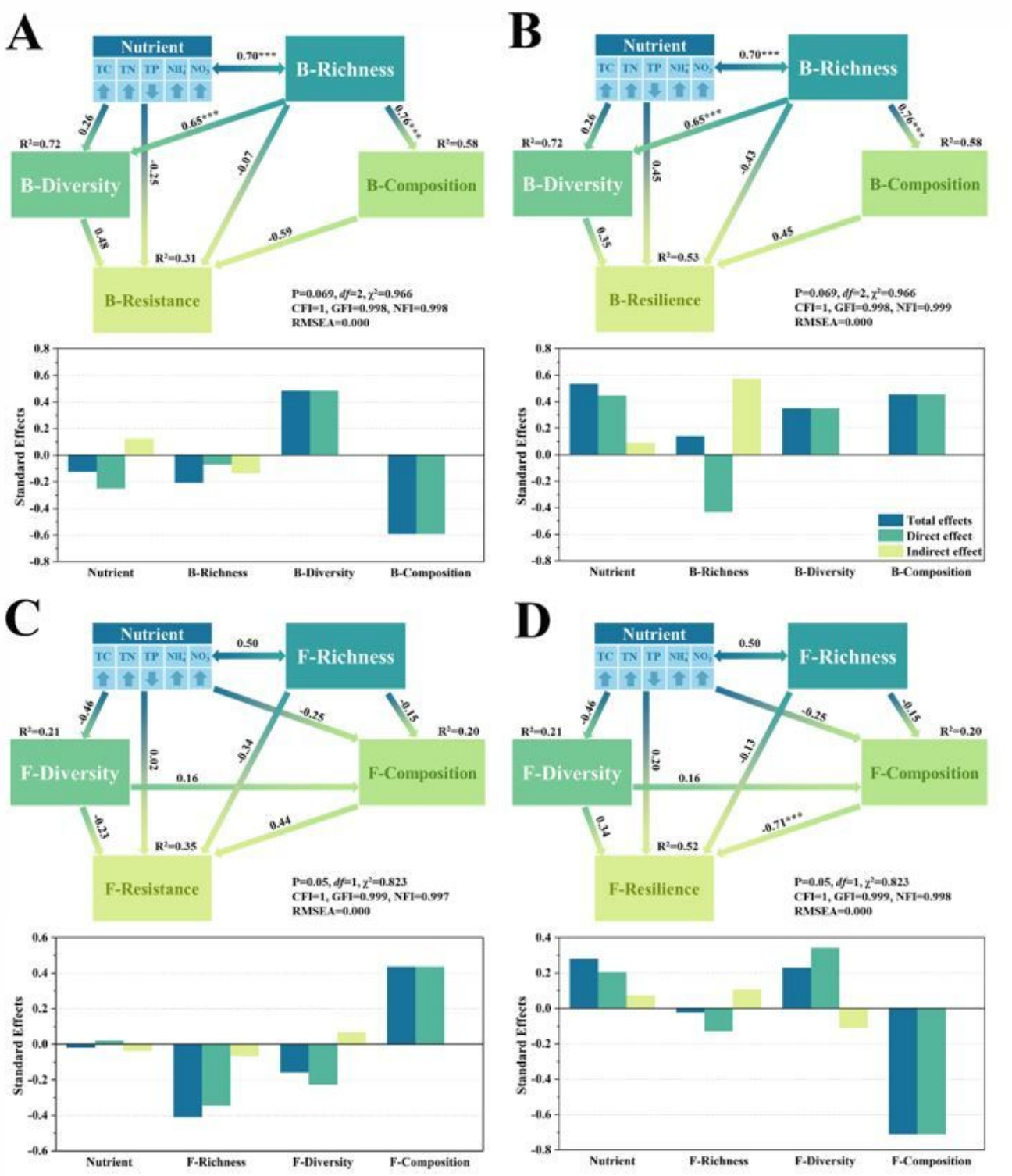

Figure 5

The SEM resolving factor contribution on microbial stability components. Figure 5A, 5B, 5C and 5D depict bacterial resistance, bacterial resilience, fungal resistance and fungal resilience. The solid line and dashed line indicate positive and negative correlation, respectively. *** indicates significant correlation under $P < 0.001$. The up and down arrows in “Nutrients” indicate positive and negative extraction, respectively.

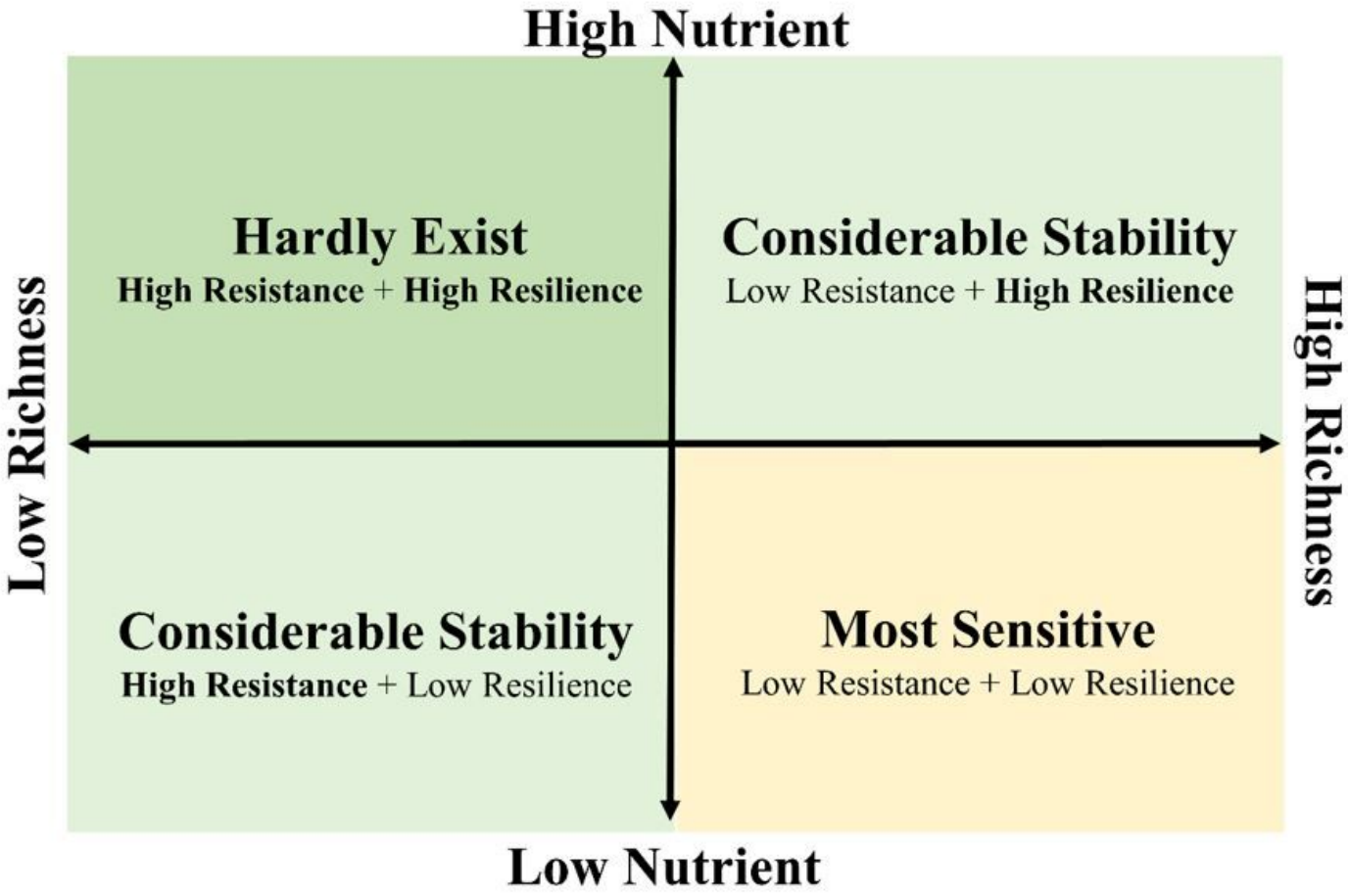

Figure 6

The soil microbial community stability of different richness and nutrient

Supplementary Files

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