



Structural variability in the bulk soil, rhizosphere, and root endophyte fungal communities of *Themeda japonica* plants under different grades of karst rocky desertification

Ming Tang · Jie Liu · Wenpeng Hou · Reed M. Stubbendieck · Han Xiong · Jie Jin · Jiye Gong · Chen Cheng · Xiaoxin Tang · Yinglong Liu · Zhaofeng Li · Jianfeng Wang · Yin Yi

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Abstract

Background and aims Karst rocky desertification (KRD) influences soil properties and plant species. Soil microbes are important factors in maintaining ecosystem stability. However, little is known about the role of fungi in adaptation of plants to KRD.

Methods Fungi colonized in bulk soil, rhizosphere, and roots of *Themeda japonica* at strong and slight KRD were analyzed by ITS2 amplicon sequencing. The

relationship between soil nutrients and fungal diversity was estimated by redundancy analysis (RDA) and Spearman analysis.

Results AN, NN, SOC, TN, TP content and pH in strong KRD soil were higher than those with slight KRD. Rhizosphere with slight KRD had higher fungal richness and diversity than it with strong KRD, but there was no difference in root endophyte between KRD grades. The bulk soil with slight KRD had higher fungal

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M. Tang · J. Liu · H. Xiong · J. Gong · X. Tang · Z. Li · Y. Yi (✉)

Key Laboratory of National Forestry and Grassland Administration on Biodiversity Conservation in Karst Mountainous Areas of Southwestern China, Guizhou Normal University, Guiyang, Guizhou 550025, People's Republic of China
e-mail: yiyin@gznu.edu.cn

M. Tang
e-mail: 8043484@qq.com
J. Liu
e-mail: liujie791204@126.com
H. Xiong
e-mail: 530999850@qq.com
J. Gong
e-mail: 583823121@qq.com
X. Tang
e-mail: txxfull@163.com
Z. Li
e-mail: 850926056@qq.com

Industry Innovation; Ministry of Agriculture and Rural Affairs; Engineering Research Center of Grassland Industry; Ministry of Education; College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730000, China
e-mail: wangjf12@lzu.edu.cn

W. Hou
e-mail: houwp19@lzu.edu.cn
C. Cheng
e-mail: chengch20@lzu.edu.cn
Y. Liu
e-mail: 719520183@qq.com

R. M. Stubbendieck
Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA
e-mail: stubbendieck@wisc.edu

J. Jin
School of Life Sciences, Lanzhou University, Lanzhou, Gansu 730000, People's Republic of China
e-mail: jinj2015@lzu.edu.cn

W. Hou · C. Cheng · Y. Liu · J. Wang (✉)
State Key Laboratory of Grassland Agro-ecosystems; Center for Grassland Microbiome; Key Laboratory of Grassland Livestock

richness compare to strong KRD. The fungal communities in bulk soil, rhizosphere, and root between plants at different KRD grades were significantly different. In addition, the fungal communities of rhizosphere were sensitive to the change of KRD environment. Ascomycota and Basidiomycota were the predominant phyla in bulk soil, rhizosphere and root endophyte at strong and slight KRD. SOC, AN and pH influenced the composition of fungal communities at strong KRD. In contrast, TN and AN had a negative impact on richness.

Conclusion Our results suggest that fungal communities of rhizosphere may play a role in adaptation of *T. japonica* to KRD and may contribute to promote plant growth and ecological performance in karst areas.

Keywords Karst rocky desertification · Fungal community structure and diversity · Soil nutrients · Rhizosphere fungi · Root endophyte · Karst areas

Introduction

Ecosystems with rocky desertification possess poor soil quality due to low levels of available phosphorus and moisture, which limit plant growth (Wang et al. 2004; Jiang et al. 2014). According to State Forestry Administration's second monitoring report, on rocky desertification, in 2012 there was approximately 120,000 km² of rocky desertification in China (Jiang et al. 2014). Since the 1950s, anthropogenic activity has led to the rise of rocky desertification in karst areas of China, especially in the Guizhou province. This karst rocky desertification (KRD) is a serious ecological threat, leading to vegetation loss and soil erosion (Wang et al. 2004; Jiang et al. 2014). In recent decades, the local government has encouraged farmers to restore the ecological degradation that has occurred in karst regions by growing plants that mediate soil restoration (Qi et al. 2013). Previous studies on soil in areas with typical KRD had been confined to describing their basic chemical and physical properties (Peng et al. 2013; Sun et al. 2019; Tang et al. 2013). For example, soils isolated from regions with more severe KRD possess higher absolute levels of carbon, nitrogen, and phosphorus (Wang et al. 2018) and low molecular weight organic acids (Li et al. 2012). Given that organic acids can dissolve limestone and accelerate KRD and are often produced by soil microbes (Qi et al. 2018), there is likely a connection between microbial communities and KRD. Indeed, there has been recent interest on the community structure,

diversity and function of microbes in karst regions. Across terrestrial environments, microbes contribute to essential ecosystem functions. In karst regions, microbes are critical for nutrient cycling (Hu et al. 2016; Zhang et al. 2006), altering soil nutrients and quality (Chen et al. 2018; Peng et al. 2020; Sheng et al. 2018; Wang et al. 2018), and regulating soil enzymes (Jin et al. 2016; Li et al. 2014b; Liu et al. 2010; Pan et al. 2018; Zhou et al. 2007).

In addition, soil microbes play important roles in restoration of vegetation (Xue et al. 2017; Yuan et al. 2011), restoration of the different grades KRD (Wang et al. 2016), and the changes of land use types (Xiao et al. 2017) in karst regions. The previous study showed that Shannon-Wiener and richness index of soil fungi were higher in the severe and extremely severe KRD soils than that in potential, slight and moderate KRD. However, the Shannon and richness index of soil fungi in moderate KRD soil were the lowest (Wang et al. 2016). The structure of soil microbial communities in karst regions has been shown to vary depending on local vegetation type, suggesting that plants may influence microbial community structure by modifying environmental and soil properties. For instance, communities of bacteria and fungi in soil near broad leaved trees differed from communities in soil near shrubs and shrubs-grasses (Li et al. 2014a; Xue et al. 2017). The structure and diversity of fungal communities varies between soils isolated from regions slight KRD and strong KRD (Wang et al. 2016), but fungal communities in karst regions are not as well-studied as bacterial communities, especially in rhizosphere and plant roots. Furthermore, it is not currently known what effect interactions between fungi and plants in karst regions contributes to KRD or its remediation.

The amounts of soil microbial biomass C, N and P were higher in potential KRD than those of light KRD, moderate KRD and intensive KRD, but the changes of microbial communities was not consistent with the change of KRD degrees (Xie et al. 2015a). Another study demonstrated that the contents of C, N and P in nil KRD soil exceeded those in slight, moderate and severe KRD, but the soil K content for severe KRD was higher than those for the others. Further, the ratios of stoichiometry vary with the degrees of the KRD (Wang et al. 2018). The soil properties in different KRD degrees were markedly different, and along with the increase of KRD, soil properties had a change process from degradation to improvement (Sun et al. 2019). In addition, the contents of organic acids with low-molecular-weight (LMW) were different in the different

grade of KRD, and their contents reduced with the KRD aggravation. Importantly, it was shown that the contents of soil organic acids with LMW probable were one of the key role in the formation of KRD (Li et al. 2012).

Restoration of vegetation in karst regions adheres to the typical pattern of secondary succession: reestablishment of grasses, which is then followed by shrubs, short-lived pioneer trees, and ultimately climax forests (Liang et al. 2015). Therefore, the reestablishment of grasses is essential for the restoration of these ecosystems. However, a critical, and often overlooked component in this ecological succession are soil microbes. Given the roles that microbes play in terrestrial ecosystems, it is probable that interactions between grasses and soil microbes will influence the outcome of efforts to restore vegetation (Xue et al. 2017). However, it is not currently well understood how interactions between soil microbes, in particular fungi, and grasses contribute to the restoration of karst regions. To better comprehend the role fungi play in adaptation of grasses to KRD, we focused on characterizing the fungi present in bulk soil, within rhizosphere, and in root endophyte communities of the perennial grass *Themeda japonica*. This grass is widely distributed in karst regions in southwest China, including areas with varying degrees of KRD. By defining the differences in soil chemistry and the fungal communities of *T. japonica* from regions with different levels KRD, we will inform our understanding of how grasses grow during the recovery from strong to slight KRD and identify fungal taxa that may influence this recovery, which helps to deeply understand the roles of *T. japonica* during the process of rocky desertification restoration, and it is helpful to understand the microbial function of grasses establishment in karst rocky desertification environment. Therefore, the purpose of our study was to understand (1) the effect of various degrees of KRD on soil nutrients contents, (2) the influence of the different grades of KRD on the diversity and structure of bulk soil, rhizosphere, and endophytic fungal communities in *T. japonica*, and (3) define the relationship between nutrients and the diversity/structure of bulk soil, rhizosphere and endophytic fungi communities near and within *T. japonica*.

Materials and methods

Description of trial site and soil sampling method

Our study was carried out in Guanling Buyi and Miao Autonomous County, Anshun City, Guizhou Province,

Southwest China (Altitude 1439 m, 105° 34' E, 25° 43' N), which is a typical karst area. The mean annual rainfall is about 1200 mm yr⁻¹, the mean annual temperature is about 17 °C, and the frost-free period is about 288 days. According to the classification method of Xiong et al. (2002), we selected two sites to represent slight and strong KRD grades. The dominant plant species of sites with the slight KRD are *T. japonica*, *Capillipedium parviflorum* and *Bidens pilosa* L. Whereas *T. japonica* was the sole dominant species of sites with strong KRD. We chose to compare *T. japonica* under the two KRD grades in a completely randomized design with five replicates to generate ten 4 m × 5 m plots, with ~20 m spacing between each plot at different KRD grades. We collected three individual bulk soil, rhizosphere, and root samples from around *T. japonica* plants in each plot. The depth of 0–15 cm bulk soil were collected at a location of 15 cm away from the plants. For every plot, we blended three individual bulk soil, rhizosphere and root samples into a composites of each type, respectively. We removed visible roots and stones from bulk soil samples using a 2 mm sieve before sample preparation. Subsequently, we placed a part of each soil samples into a sterile 50 mL aseptic centrifuge tube, which was frozen in liquid nitrogen tank and stored at -80 °C until we extracted DNA. We stored another part of the bulk soil sample in plastic bags on ice and returned to the laboratory. We air dried these soil samples to determine nutrients content and pH. Similarly, we transported rhizosphere and root samples to the laboratory in a sterile plastic bag on ice bag.

We used a slight modification of the procedure in McPherson et al. (2018) to obtain rhizosphere samples and to disinfect root surfaces for isolating DNA from endophyte communities. In brief, we sterilized scissors in 75% ethanol, and then cut various roots to 7–8 cm in length. We then placed the excised roots into marked 50 ml centrifuge tubes, which contain 35 ml of autoclaved phosphate buffer (PBS; 130 mM NaCl, 7 mM Na₂HPO₄, 3 mM NaH₂PO₄, pH 7.4). After shaking the centrifuge tubes for 2 min to release rhizosphere soil from the root surface, we removed the roots, after drying it on a paper towel, placed it into a new 50 ml centrifuge tubes on ice. We resuspended the whole rhizosphere soil samples by shaking then centrifuged the samples at 3000 g for 5 min at 25 °C. We discarded the supernatant, and stored the pellet in 50 ml centrifuge tubes on ice. We added 1.5 ml sterile phosphate buffer to the rhizosphere and resuspended the

pellet with an oscillator vortex. Immediately, we transferred the resuspended pellet into 2 ml centrifuge tubes, and centrifuged the samples at 3000 g for 2 min at 25 °C. We removed the supernatant, dried the pellet, and stored samples at –80 °C until we extracted DNA. To samples root endophytic fungi, we surface sterilized root and then placed them into 50 ml centrifuge tubes with 35 ml 50% bleaching agent (Tianjin Beichen Fangzheng Reagent Factory, available chlorine concentration: 10%) + 0.01% Tween-20, shook them for 1 min. We then discarded the bleach wash and added 35 ml 75% ethanol and shook the roots as above. Finally, we removed the 75% ethanol wash, added 35 mL sterile water, and shook the roots as above. We repeated the water rinse five times. We store sterilized root samples at –80 °C until we extracted DNA. To ensure that the samples root surface were fully disinfected, the last water rinse was plated, and we observed no bacterial growth. In addition, we analyzed the sterile water used in the last rinse by two-step PCR (ITS4 and ITS1F were primer for first PCR; ITS4 and fITS7 were primer for second), and the surface sterilization effect was taken as the negative control. We did not detect PCR products, indicating the availability of surface sterilization.

Soil nutrients analyses

We used the assay described by Nelson and Sommers (1982) to determine AP (available phosphorous) and SOC (soil organic carbon) in bulk soil samples. We used the assay described by Zhao et al. (2014) to determine soil total nitrogen (TN), NN (nitrate nitrogen) ammonium nitrogen (AN) and total phosphorus (TP). We determined soil pH by suspending soil in water (1:2.5 w/v ratio) and using a laboratory pH meter.

DNA extraction and amplicon sequencing of fungal ITS2

We extracted DNA from bulk soil, rhizosphere, and root samples using the Power Soil DNA Separation Kit (MoBio, Carlsbad, USA) following the manufacturer's instructions. We used ~300 mg of bulk soil and rhizosphere and ~500 mg of root samples for DNA extraction. We amplified the fungal internal transcribed spacer 2 (ITS2) region of rRNA gene using two-step PCR for amplicon sequencing. In the first step, the ITS region was amplified by PCR with primers ITS4 according to White et al. (1990) and on the basis of Gardes and Bruns

(1993), ITS1F was performed in a 10 µL reaction solution, including 0.2 µL each primer (10 µM), 0.2 µL Toptaq DNA polymerase, 0.8 µL each dNTP (25 mM), 1 µL 10 × Toptaq buffer, 2 µL template DNA and ddH₂O up to 10 µL. We used the following PCR cycling conditions: 94 °C for 2 min, then 28 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. We used the PCR product from the first reaction as a template for a second PCR. In this reaction, we used primers ITS4 and fITS7 (Ihrmark et al. 2012), and the same cycling conditions as above. We performed three technical replicates to minimize the bias of amplicon generated in the PCR products. The PCR products were sequenced using an Illumina MiSeq PE 250 platform at Genesky Biotechnologies Inc., Shanghai, 201,315 (China).

Analysis of bioinformatics

To eliminate low-quality sequences, we filtered original sequences using QIIME version 1.7.0 (Caporaso et al. 2010). The average mass fraction was less than 20, there was no effective primer sequence or bar code sequence, the base was not clear, and the length was less than 250 bp. For the remaining sequences, we extracted the ITS2 region using the fungal ITSx software (Bengtsson-Palme et al. 2013), and then used the command of chimera. Uchime in MOTHUR version 1.31.2 to check for potential chimeras (Schloss et al. 2009). We clustered non-chimeric sequences at 97% sequence similarity level into operational taxonomic units (OTUs) using the UPARSE pipeline in USEARCH version 8.0 (Edgar 2013) after canceling replication and discarding all singletons.

Statistical analysis

We calculated richness and Shannon indices with R (version 3.2.2), and richness represented the number of unique OTUs in each sample. We performed principle coordinate analysis (PCoA), heatmap generation, Spearman's rank correlation analysis and redundancy analysis (RDA) by using R. In addition, permutational multivariate two-way analysis of variance (PERMANOVA) was carried out to estimate the significant differences of fungal communities based on Bray-Curtis distances. We used two-way ANOVA to determine the impacts of the different grades KRD (DGK) and the different sites (DS) on richness and Shannon

diversity using SPSS (version 17.0). To test for differences in richness and Shannon indices, TN, TP, SOC, NN, AP, AN and pH, we used independent Student's *t*-tests at $P < 0.05$ in SPSS.

Results

Soil nutrients are decreased in slight KRD sites relative to strong KRD sites

We found that the SOC content ($P < 0.001$), TN content ($P < 0.001$), TP content ($P = 0.010$), NN content ($P = 0.002$), AN content ($P < 0.001$), and pH ($P = 0.004$) in site with strong KRD were significantly higher than in sites with slight KRD. There was no significant difference in the AP content between slight KRD and strong KRD ($P = 0.081$) (Fig. 1). The SOC and NN content were most affected by the KRD grade and were 60% and 28,200% higher, respectively, in sites with strong KRD. The AN, TN, and TP content were 177%, 165%, and 63% higher, respectively, in sites with strong KRD. Further, pH was 7.2% higher in sites with strong KRD.

KRD grade and sampling site affect fungal community diversity

After removing ambiguous, short and low-quality reads and singleton OTUs, 3973 unique fungal OTUs were obtained from 30 samples available for community analysis. We found significant differences in the richness and Shannon indices of fungal community between different grades of KRD (DGK) ($P = 0.003$) and between different sites (DS) ($P < 0.001$) (Table 1). Further, we found the interaction DGK \times DS significantly impacted richness ($P = 0.009$) (Table 1). There was significantly higher fungal richness in the bulk soil and rhizosphere samples of sites with slight KRD compared to sites with strong KRD (Fig. 2a). In contrast, there was no difference in richness and Shannon between root endophyte communities when comparing different KRD grades (Fig. 2a, b). The Shannon diversity of rhizosphere fungal communities was modestly higher in sites with slight KRD compared to sites with strong KRD, but there was no effect of KRD grade on the diversity of bulk soil and root endophyte communities (Fig. 2b). In addition, fungal richness and Shannon had significantly difference among the different site (bulk

soil, rhizosphere, root samples) under slight and strong KRD, respectively (Fig. 2a, b).

KRD grade and sampling site affect fungal community composition at the phylum and genus levels

In all communities that we assessed, regardless of sampling location or KRD grade, members of the fungal phyla Ascomycota and Basidiomycota dominated, though the latter phylum was less predominant in sites with strong KRD (Fig. 3). In addition, we identified that members of the fungal phyla Glomeromycota and Mortierellomycota in bulk soil and rhizosphere communities from both KRD grades, but not among root endophyte communities. Further, we found that members of the phylum Chytridiomycota was present in the bulk soil and rhizosphere communities of sites with strong KRD, but were absent from all other communities that we analyzed (Fig. 3). Ascomycota (67.3%), Basidiomycota (26.3%), Glomeromycota (2.6%) and Mortierellomycota (2.0%) were the main fungal phyla with relative abundances more than 1% at bulk soil under slight KRD; similarly, the relative abundances greater than 1% of dominant fungal phyla were Ascomycota (80.0%), Basidiomycota (12.9%), Glomeromycota (1.8%) and Mortierellomycota (2.4%) in rhizosphere under slight KRD; and the relative abundances greater than 1% of dominant fungal phyla were Ascomycota (65.7%) and Basidiomycota (33.5%) at root endophyte fungi under slight KRD. Importantly, the Mortierellomycota was not found in root endophyte fungi under slight KRD (Fig. 3a). Markedly, Ascomycota and Basidiomycota were more abundant in bulk soil, rhizosphere soil and endophyte fungi in root. Additionally, the relative abundances greater than 1% of principal fungal phyla were Ascomycota (76.7%), Basidiomycota (9.8%), Glomeromycota (4.2%), Mortierellomycota (3.5%) and Chytridiomycota (1.3%) at bulk soil under strong KRD; similarly, the relative abundances greater than 1% of dominant fungal phyla were Ascomycota (78.2%), Basidiomycota (6.6%), Glomeromycota (2.2%), Mortierellomycota (1.9%) and Chytridiomycota (1.7%) at rhizosphere soil under strong KRD; and the relative abundances greater than 1% of dominant fungal phyla were Ascomycota (94.5%), Basidiomycota (2.1%) and Glomeromycota (1.2%) at root endophyte fungi under strong KRD. Importantly, the Mortierellomycota was not found in root endophyte fungi under strong KRD (Fig. 3a). The

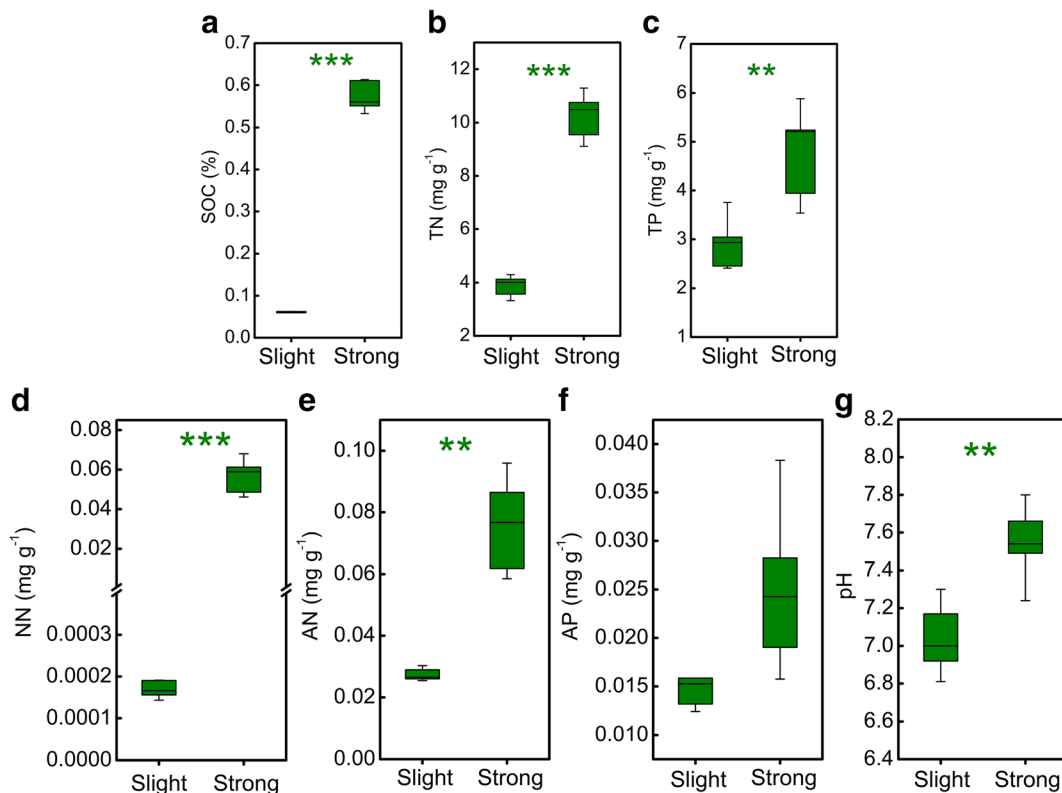


Fig. 1 Effect of the different grade KRD on soil nutrients contents. (a) SOC, (b) TN, (c) TP, (d) NN, (e) AN, (f) AP, (g) pH. ** and *** refer to significant difference at $P < 0.01$ and $P < 0.001$ between strong KRD and slight KRD (independent t -test), respectively

different grades KRD (DGK) had a significant effect on Ascomycota ($P = 0.002$), Basidiomycota ($P < 0.001$) and Chytridiomycota ($P < 0.001$), and the different sites (DS) had an effect on Ascomycota ($P < 0.001$), Chytridiomycota ($P < 0.001$), Glomeromycota ($P = 0.003$) and Mortierellomycota ($P < 0.001$), but the interaction of DGK \times DS had clearly effect on Ascomycota ($P = 0.005$), Basidiomycota ($P = 0.016$) and Mortierellomycota ($P = 0.034$) (Table 2).

Table 1 Results of two-way ANOVA for the effects of the Different Grade KRD (DGK) and Different Sites (DS) on Richness and Shannon

Treatments	df	Richness		Shannon	
		F-value	P	F-value	P
DGK	1	10.6	.003	1.4	.241
DS	2	272.6	<0.001	106.9	<0.001
DGK \times DS	2	5.7	.009	0.6	.537

When we investigated the fungal communities of *T. japonica* at the genus-level, we observed more variation in the patterns of classified genera between different sites and KRD grades (Fig. 3b). As examples, the genus *Fusarium* (Phylum Ascomycota) was observed in all communities, except root endophytes at slight KRD. The genus *Cyphellophora* (Phylum Ascomycota) was found in all rhizosphere and root communities, but not in bulk soil. Finally, *Mortierella* (Phylum Zygomycota) was only found within the soil and rhizosphere, except in bulk soil at slight KRD. Our results showed that the principal fungal genera with relative abundances more than 1% were *Hygrocybe* (10.2%), *Geoglossum* (7.4%), *Fusarium* (2.0%) and *Clavaria* (1.9%) at bulk soil under the slight KRD; the relative abundances were greater than 1% of dominant fungal genera were *Geoglossum* (9.0%), *Fusarium* (4.4%), *Hygrocybe* (2.5%), *Mortierella* (2.3%), *Ochroconis* (1.6%), *Cyphellophora* (1.2%) and *Cladosporium* (1.1%) at rhizosphere soil under the slight KRD; the relative abundances greater than 1% of dominant fungal genera were *Periconia*

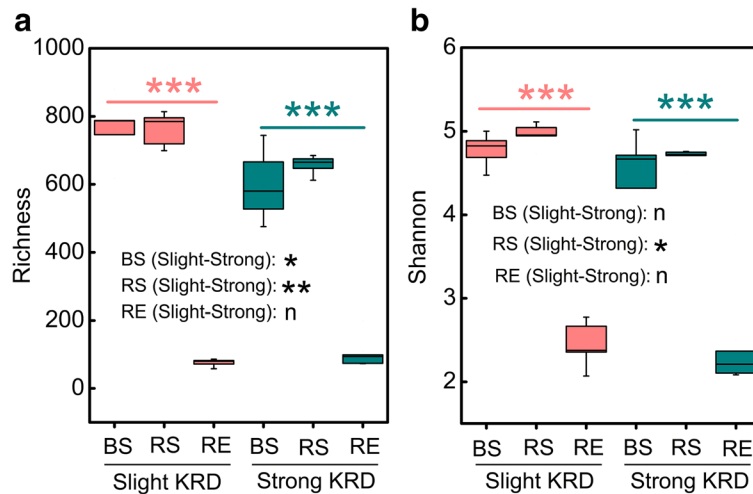


Fig. 2 Effect of the different grade KRD on the richness and Shannon of fungal communities in bulk soil, rhizosphere and root endophyte, (a) Richness, (b) Shannon. *** above pink line and green line show significant difference at $P < 0.001$ among the bulk soil, rhizosphere and root endophyte in slight KRD and strong KRD, respectively (ANOVA); * and ** after BS/RS/RE (Strong-Slight) indicate obvious difference at $P < 0.05$ and $P < 0.01$ at bulk

(27.8%), *Mycena* (15.1%), *Coprinopsis* (8.2%), *Cyphellophora*, (1.9%) and *Cladosporium* (1.3%) at the root endophyte fungi under the slight KRD (Fig. 3b). In addition, the relative abundances greater than 1% of dominant fungal genera were *Mortierella* (3.4%), *Periconia* (3.3%) and *Fusarium* (2.9%) at the bulk soil under the strong KRD; the relative abundances greater than 1% of dominant fungal genera were *Fusarium* (5.8%), *Periconia* (5.4%), *Mortierella* (1.9%), *Ochroconis* (1.3%) and *Cyphellophora* (1.1%) at rhizosphere soil under the strong KRD. Further, we found that the relative abundances greater than 1% of dominant fungal genera were *Periconia* (33.4%), *Cyphellophora* (6.5%), *Myrmecridium* (6.5%), *Sigarispora* (3.5%), *Magnaportheopsis* (2.7%), *Edenia* (2.2%), *Fusarium* (2.2%) and *Exophiala* (1.6%) at the root endophyte fungi under the strong KRD (Fig. 3b).

To explore how fungal communities varied with sampling site and KRD grade, we performed a PCoA on the calculated Bray-Curtis distances between each site. Together, the first two principal coordinates accounted for 41.58% of the total variation. The first principal coordinate (27.01%) separated communities from the bulk soil and rhizosphere away from the root endophyte communities. Further, the second principal coordinate (14.57%) separated communities on the basis of KRD grade. However, the differences between

soil, rhizosphere and root endophyte between strong KRD and slight KRD, respectively (independent t -test), n indicates no statistical significance of difference between strong KRD and slight KRD at bulk soil, rhizosphere and root endophyte, respectively (independent t -test). BS: bulk soil, RS: rhizosphere soil, RE: root endophyte

root endophyte communities at each KRD grade were less pronounced than those between the soil and rhizosphere (Fig. 4). Obviously, slight and strong KRD were able to significantly ($R^2 = 0.122$, $P = 0.001$) separate in the soil of bulk and rhizosphere and root endophyte, respectively (Table 3). In addition, the different site were able to significantly ($R^2 = 0.187$, $P = 0.001$) separate in the bulk soil, rhizosphere and root endophyte, respectively (Table 3), further, the interaction of $DGK \times DS$ had significant ($R^2 = 0.048$, $P = 0.021$) effect on fungal communities of bulk soil, rhizosphere and root endophyte (Table 3). The heatmap at the genus level also suggested patterns of fungal communities in bulk soil, rhizosphere and root endophyte similar to the grouping pattern observed in PCoA (Fig. 5). Most of the top 100 genera in bulk soil and rhizosphere soil had a higher relative abundance, compared to root endophytes (Fig. 5).

Differences in the levels of soil nutrients are correlated with changes in fungal community composition at the phylum-level

To better understand correlations between soil nutrient content and fungal community composition, we performed a redundancy analyses (RDA). Together, the first two axes explained 88.61% of the total variance between bulk soil

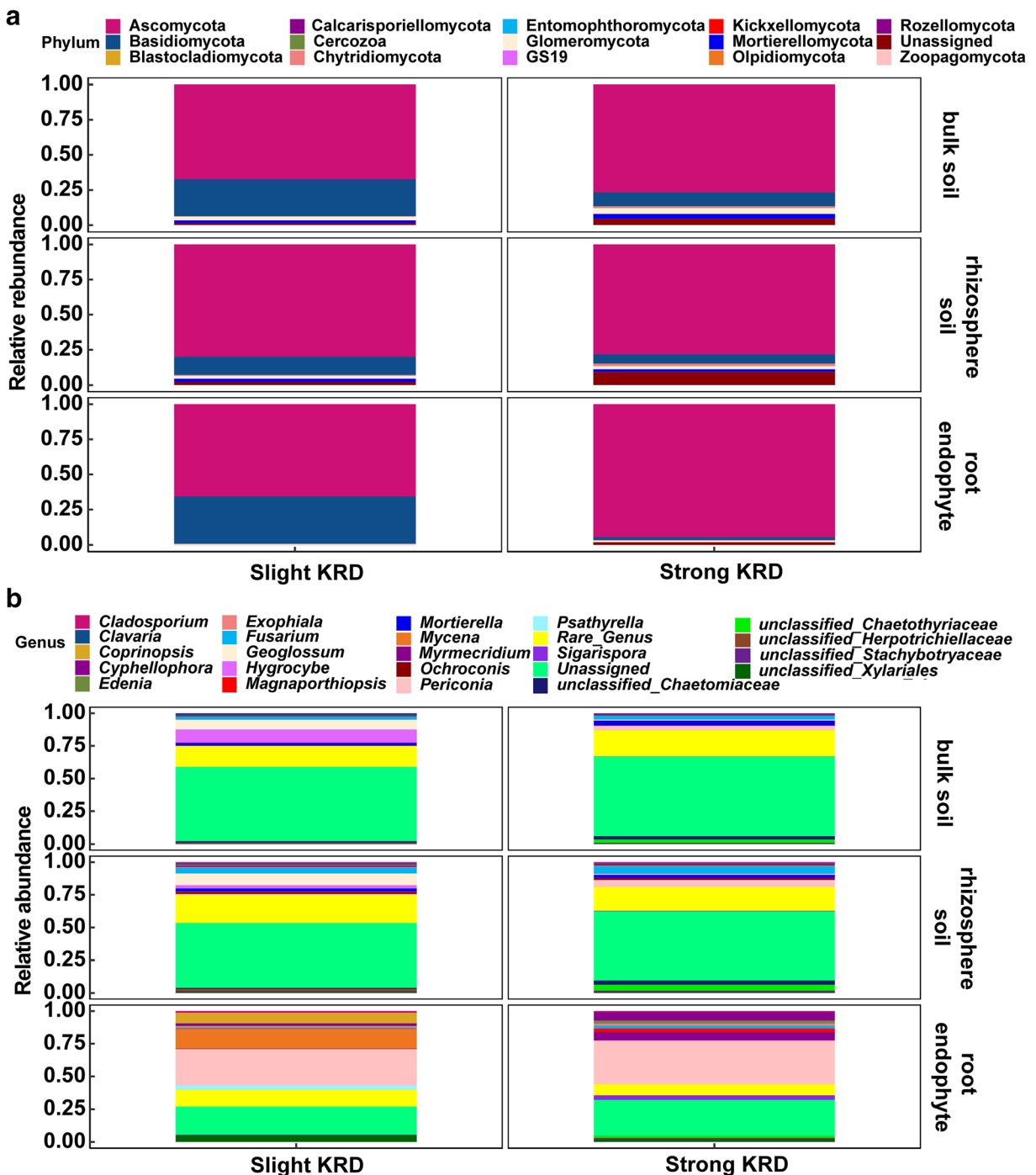


Fig. 3 Relative abundances of the fungal communities of bulk soil, rhizosphere and root endophyte. (a) phylum, (b) genus

fungal communities at the different KRD grades (Fig. 6a). The RDA demonstrated that SOC, TN, TP, NN, AN and AP content, and pH were positively correlated with the phyla Ascomycota, Chytridiomycota, Glomeromycota and Mortierellomycota, and inversely correlated with the

phylum Basidiomycota (Fig. 6a). In particular, AN content ($P = 0.041$) and pH ($P = 0.047$) were the top two factors correlated with the differences in the composition of the bulk soil fungal communities, explaining 21.27% and 19.25% of variance, respectively, of the observed variation

Table 2 Results of two-way ANOVA for the effects of the Different Grade KRD (DGK) and the Different Sites (DS) on Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Mortierellomycota

Treatments	df	Ascomycota		Basidiomycota		Chytridiomycota		Glomeromycota		Mortierellomycota	
		F-value	<i>P</i>	F-value	<i>P</i>	F-value	<i>P</i>	F-value	<i>P</i>	F-value	<i>P</i>
DGK	1	12.2	.002	30.4	<0.001	14.5	<0.001	2.7	.115	1.5	.240
DS	2	2.2	<0.001	2.8	.081	10.7	<0.001	7.6	.003	28.9	<0.001
DGK × DS	2	6.6	.005	5.0	.016	3.1	.063	0.5	.636	3.9	.034

between KRD grades. Similarly to bulk soil, the first two RDA axes explained 82.26% of the total variance between rhizosphere fungal communities at the different KRD grades (Fig. 6b). The AN, AP, NN, SOC, TN, and TP content and pH were positively correlated with the phyla Chytridiomycota and Glomeromycota. However, unlike bulk soil, these factors were inversely correlated with the phyla Ascomycota and Mortierellomycota, in addition to Basidiomycota (Fig. 6b). In particular, AN ($P = 0.058$) and SOC ($P = 0.075$) content were the top two factors correlated with the differences in the composition of the fungal rhizosphere communities, explaining 21.58% and 19.88% of variance, respectively, of the observed variation between KRD grades. For root endophyte communities at the different KRD grades, the first two RDA axes explained 76.1% of the total variance. The AN, AP, NN, SOC, TN, and TP content and pH were positively correlated with the phyla Ascomycota, Chytridiomycota, and Glomeromycota. In contrast, these factors were negatively correlated with the phyla Basidiomycota and Mortierellomycota (Fig. 6c). In particular, the SOC ($P =$

0.009) and NN ($P = 0.012$) content were the top two factors correlated with the differences in the composition of the fungal root endophyte communities, explaining 17.84% and 17.03% of variance, respectively, of the observed variation between KRD grades.

Correlations of fungal community richness and Shannon indices with soil nutrients

The results of Spearman coefficients found that clear correlations between the bulk soil fungal diversity and soil nutrients (Fig. 7). The changes of richness of fungal communities in bulk soil were correlated to the TN ($P = 0.019$) and AN ($P = 0.026$), but the left nutrients were not strong factors correlate to richness (Fig. 7a); further, we found that Shannon indices changes were not a strong factor correlate to the soil nutrients at bulk soil (Fig. 7a); interestingly, Beta diversity (PCoA1) was correlated to the TN ($P = 0.029$), TP ($P = 0.049$), SOC ($P = 0.021$), NN ($P = 0.025$), AN ($P = 0.04$) and AP ($P = 0.028$), but pH was not a strong factor correlate to

Fig. 4 Principal coordinate analysis (PCoA) of fungal communities (Bray-Curtis) for bulk soil, rhizosphere and root endophyte

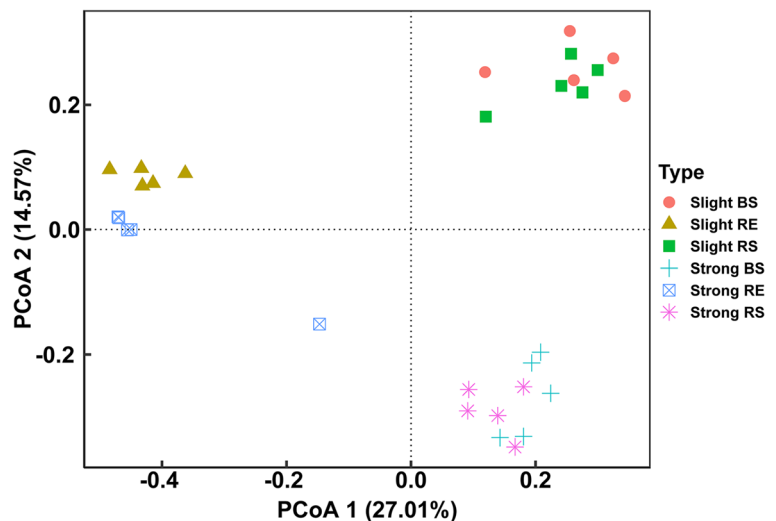


Table 3 The statistical test of permutational multivariate two-way analysis of variance (PERMANOVA) to analyze the differences of fungal communities between different grades KRD (DGK) and different site (DS)

Treatments	df	PERMANOVA	
		Bray-Curtis	
		R ²	P
DGK	2	0.122	.001
DS	1	0.187	.001
DGK×DS	2	0.048	.021

microbial richness, Shannon and PCoA1 (Fig. 7a) at bulk soil. Our results showed that fungal community richness was correlated to TN ($P = 0.002$), TP ($P = 0.008$), SOC ($P = 0.003$), NN ($P = 0.002$), AN ($P = 0.034$) and pH ($P = 0.045$), but AP was not a strong factor correlate to richness and Shannon in rhizosphere (Fig. 7b); the Shannon was correlated to TN ($P = 0.017$), SOC ($P = 0.013$), NN ($P = 0.024$) and AN ($P = 0.008$), but TP, AP and pH were not strong factor correlate to Shannon in rhizosphere (Fig. 7b); Beta diversity (PCoA) was correlated to the TP ($P = 0.028$) and AP ($P = 0.009$), but TN, SOC, NN, AN pH were not strong factor correlate to PCoA1 (Fig. 7b) in rhizosphere. Nevertheless, we found that the Shannon and Beta (PCoA) were not strong factor correlate to all the nutrients at root endophyte (Fig. 7c).

Correlations of fungal phyla with soil nutrients

Our results showed that Basidiomycota only was negatively correlated to NN ($P = 0.037$); Chytridiomycota was positively correlated to SOC ($P = 0.026$), NN ($P = 0.04$) and AN ($P = 0.03$); Mortierellomycota was positively correlated to AN ($P = 0.012$) and pH ($P = 0.004$) at bulk soil (Fig. 8a). In addition, for the phyla of rhizosphere, Basidiomycota was negatively correlated to TN ($P = 0.050$), SOC ($P = 0.018$), NN ($P = 0.027$) and AN ($P = 0.020$); Chytridiomycota was positively correlated to TN ($P = 0.016$), SOC ($P = 0.011$), NN ($P = 0.016$) and AN ($P = 0.010$); however, Ascomycota, Glomeromycota and Mortierellomycota were not strong factor correlate to all the nutrients at rhizosphere soil (Fig. 8b). For the phyla of root endophyte, Ascomycota was positively correlated to TN ($P = 0.003$), TP ($P = 0.032$), SOC ($P = 0.003$), NN ($P = 0.002$) and AN ($P =$

0.021); Basidiomycota was negatively correlated with TN ($P = 0.001$), TP ($P = 0.015$), SOC ($P = 0.001$), NN ($P = 0.001$) and AN ($P = 0.005$); but the Mortierellomycota, Chytridiomycota and Glomeromycota were not strong factor correlate to all the nutrients (Fig. 8c).

Discussion

Karst ecosystems are unstable and may easily degenerate, loss of nutrients and decrease of water holding capacity, ultimately resulting in soil loss and deforestation. It is difficult for karst ecosystems to recover from this desertification, due to anthropogenic activity. Therefore, KRD is becoming more prevalent in southwest China (Huang et al. 2009; Peng et al. 2013; Tang et al. 2013). One of the first steps in the recovery of KRD is the reestablishment of grasses. The effect of KRD on plant communities has been well studied. However, the role that fungal communities play during KRD recovery is not well-understood. Therefore, in the present study, we evaluated the structure and diversity of bulk soil, rhizosphere, and root endophyte fungal communities, investigated the relationship between soil nutrients and these communities, and discussed the possible roles of dominant fungi in *T. japonica* plants under slight and strong KRD. Together, our results contribute to understanding of the role of fungal communities in plant adaptation to different grades of KRD.

During the development and progression of KRD, these ecosystems undergo overt changes in the amount of bedrock exposure and in the type of vegetation that grow (Xie and Wang 2006). Consequently, these processes may lead to changes in the soil nutrient content, which may influence fungal communities. For instance, it has been previously demonstrated that increased AN, NN, SOC, TN, and TP content are negatively correlated with soil fungal diversity (Gong et al. 2019) and that SOC content drives the diversity of fungal communities (Yang et al. 2017b). In our study, we found that TN, TP, SOC, NN and AN content, and pH were significantly higher in soil from regions with strong KRD compared to soil from regions with slight KRD. These results were consistent with previous work that measured higher organic material, TN, and pH (Qi et al. 2018) in soil from regions with strong KRD. These observations can be explained due to the dissolution of limestone in karst regions, which raises soil pH through calcium carbonate

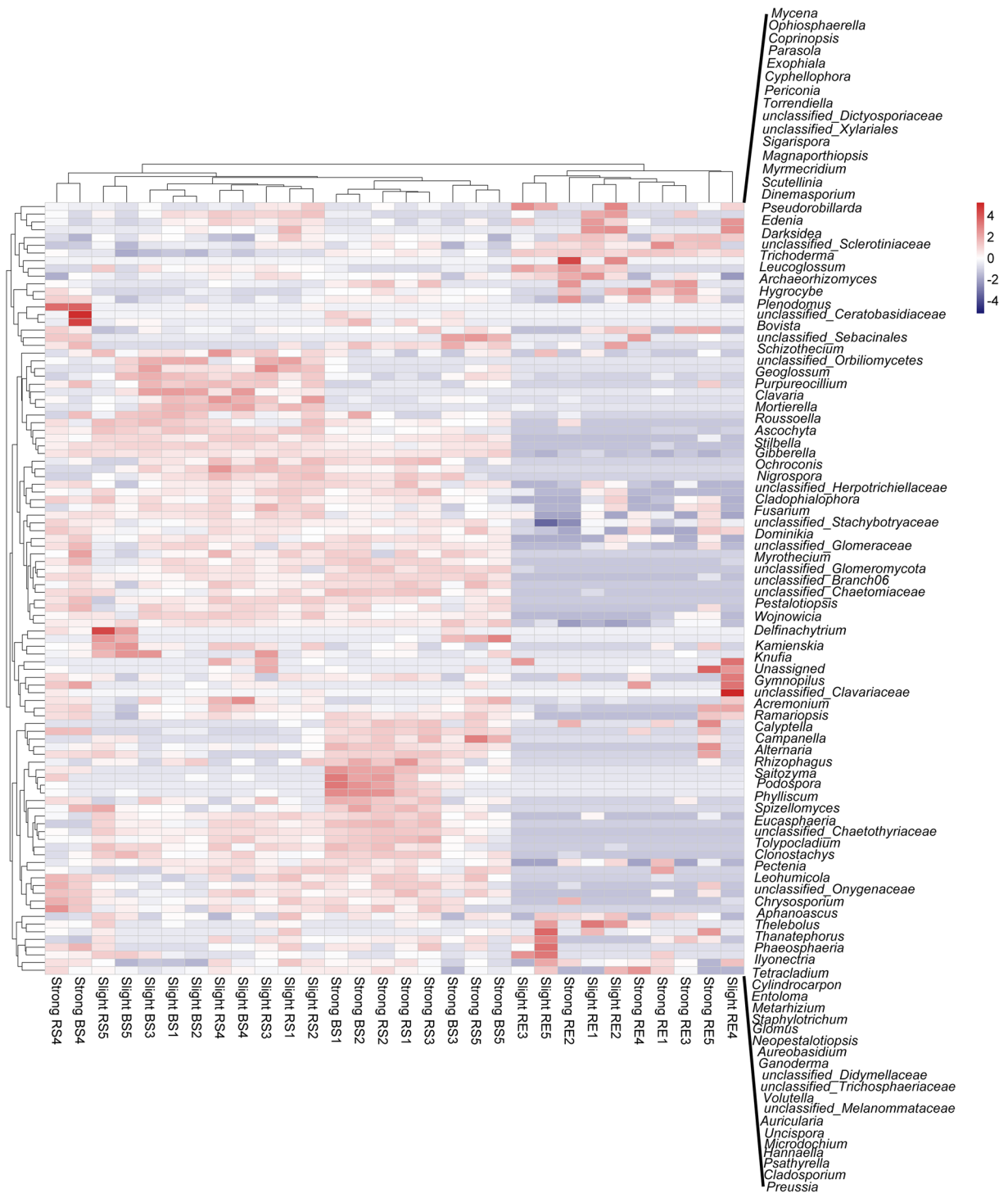


Fig. 5 The heatmap of the distributions of the top 100 fungal genera in richness in bulk soil, rhizosphere and root endophyte at strong KRD and slight KRD

release and increases levels of soil organic material (Zhong et al. 2006). Indeed, increased soil pH is the

most common indicator of desertification in karst soil (Xie et al. 2015b) and is known to influence soil fungal

Fig. 6 Redundancy analysis (RDA) to estimate the relationship between soil nutrients (red arrows) and fungal phyla in the bulk soil (a), rhizosphere (b), and root endophyte (c), respectively

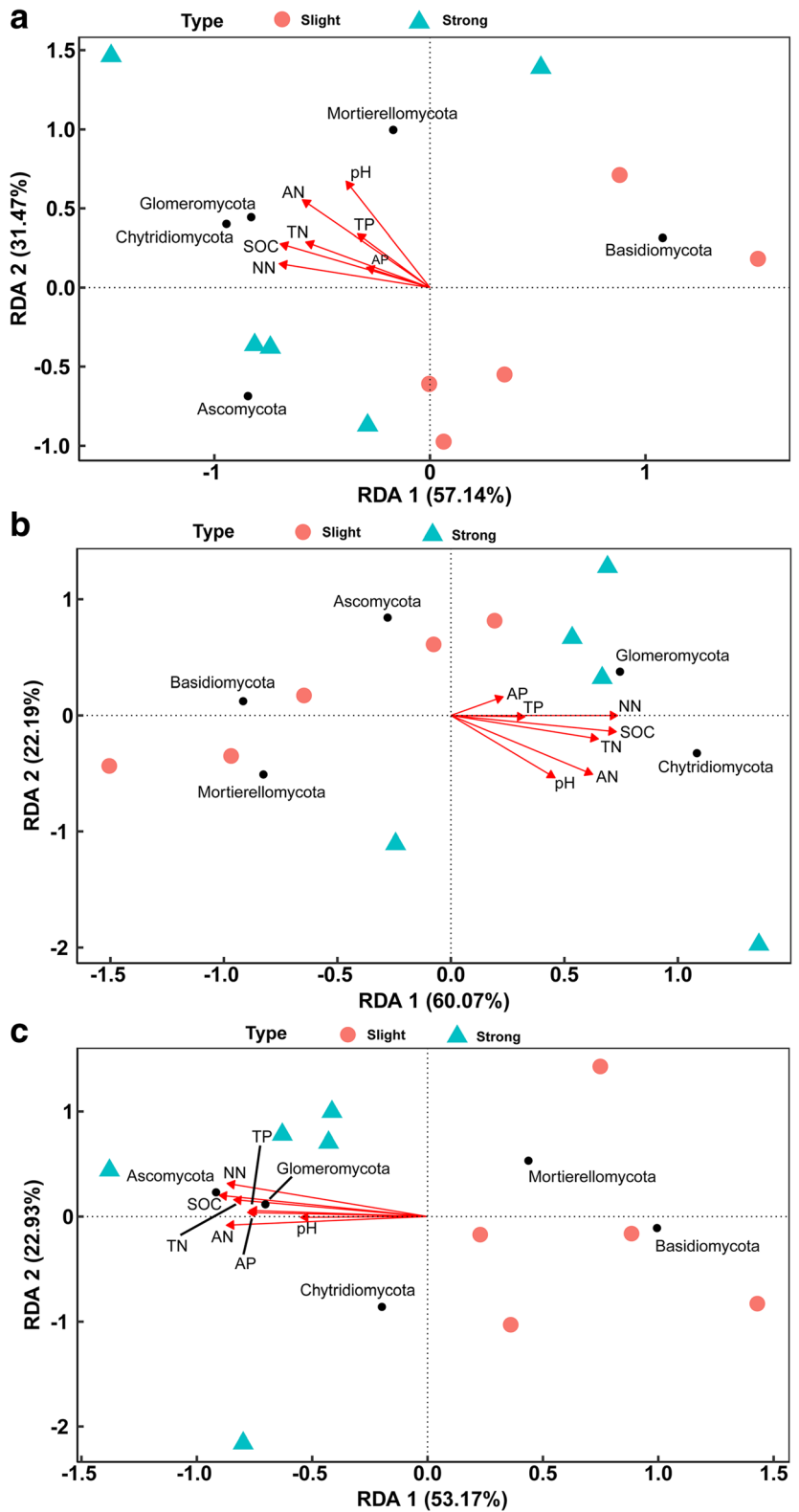
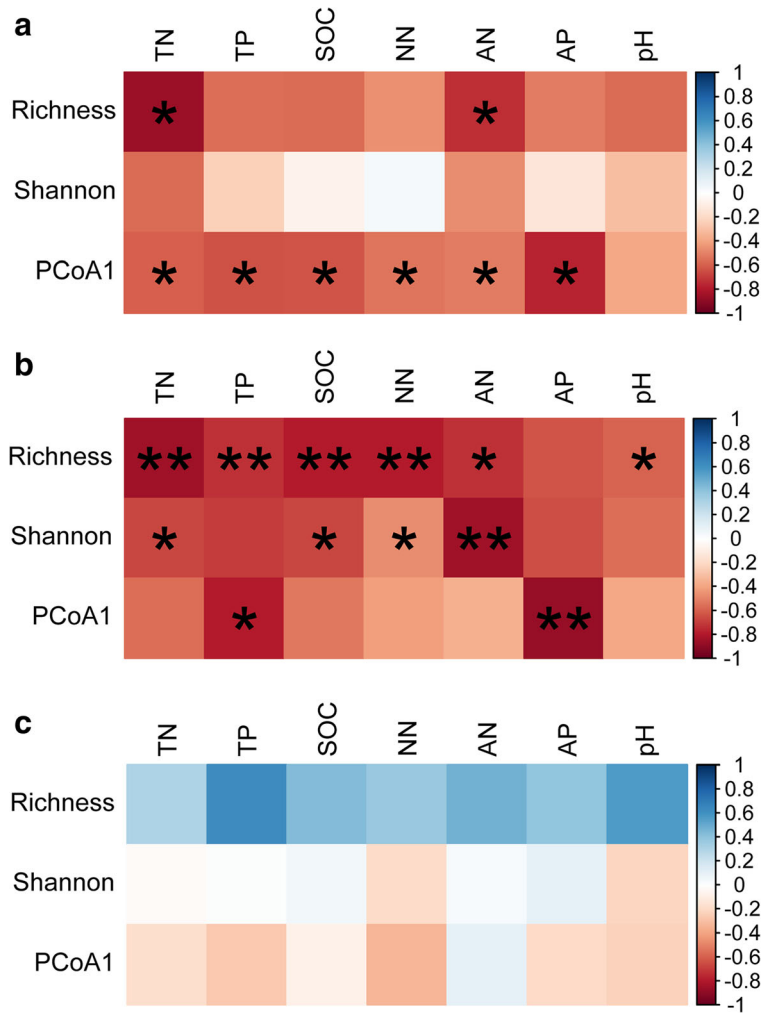


Fig. 7 Spearman’s rank correlation coefficients between soil nutrients and fungal diversity (Richness, Shannon and PCoA) (black dots) in the bulk soil (a), rhizosphere (b), and root endophyte (c), respectively

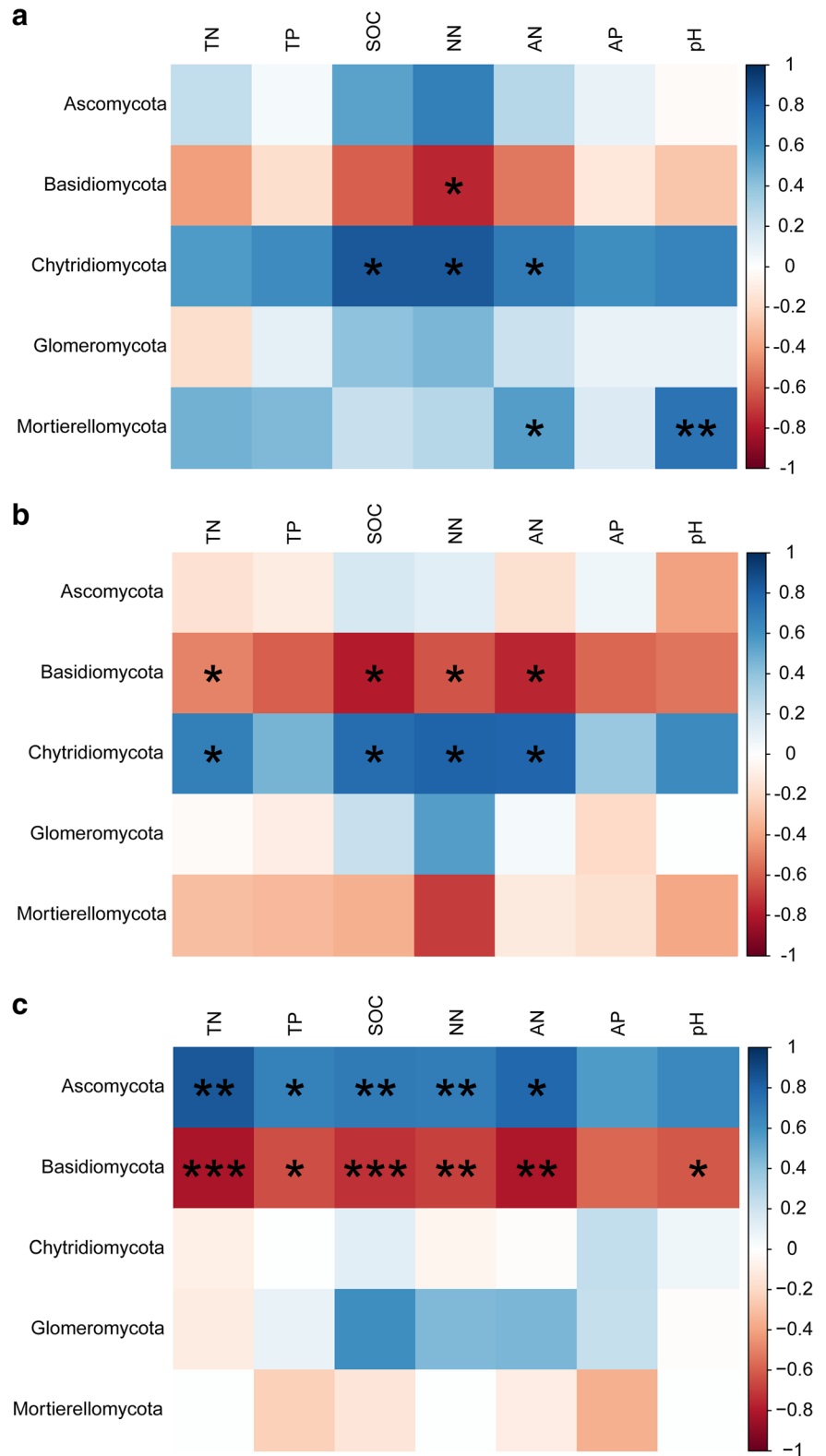


communities (Essel et al. 2019). However, in contrast to previous reports, we observed no differences in AP from soil collected from sites at the two different KRD grades and found higher SOC, TN, and TP content in soil from regions with strong KRD (Xie et al. 2015a; Qi et al. 2018). These inconsistencies may be due to differences in local soil microbial communities (e.g., nitrogen-fixing bacteria) or due to our sampling in the proximity of *T. japonica* plants instead of shrub-grassland (Qi et al. 2018).

In karst regions with severe desertification, plant diversity and coverage is lower than in regions with less severe KRD (Zuo et al. 2015; Yang et al. 2017a). Similarly, our study found the diversity of fungal communities in bulk soil and rhizosphere tended to be lower in regions with strong KRD. In general, across terrestrial ecosystems, plant richness is positively correlated to

fungal richness (Yang et al. 2017a), the diversity and coverage of plant species were higher in slight KRD than in strong KRD, therefore, the richness of soil fungi in the slight KRD was higher than the counterpart in the strong KRD in the present study. One possible explanation for the interconnected relationship between fungi and plants is that the levels of soil nutrients or pH regulate fungal communities, which in turn influence plant communities. By controlling the levels and types of available nutrients and enzymes, microbes play a central role in regulating soil fertility and influencing plant growth (Xie et al. 2019; Yang et al. 2020). For instance, the levels of bulk nutrients and low molecular weight organic acids in the soil have been previously demonstrated to forecast the response of karst ecosystems to future degradation (Wang et al. 2014). Therefore, understanding how plant-associated fungal

Fig. 8 Spearman’s Rank correlation coefficients between soil nutrients and the relative abundance of fungal phyla in the bulk soil (black dots) (a), rhizosphere (b) and root endophyte (c), respectively



communities change in response to KRD may enable us to identify specific fungal taxa to prevent future degradation or promote the growth of vegetation and contribute to the remediation of regions with strong KRD. The severity of desertification of soils in karst regions is known to influence fungal communities. However, as far as we knew, this is the first study to characterize fungal communities of the same plant species (*T. japonica*) found at different KRD grades. The fungal phyla Ascomycota, Basidiomycota, and to a lesser extent, Mortierellomycota dominated all communities that we analyzed, regardless of sampling location or KRD grade. This pattern was similar to what has been previously observed in the fungal communities of arid farmlands, which are populated by the same phyla (Li and Wu 2018). Members of the phyla Ascomycota and Mortierellomycota can decompose organic matter rapidly in rhizosphere and their richness is known to be regulated by soil nutrients (Bastida et al. 2013). We found that the relative abundance of Ascomycota and Mortierellomycota was higher in bulk soil isolated from regions with strong KRD, but lower in rhizosphere under these same conditions. One potential function of Ascomycota in soils probably promote soil nitrogen accumulation through secreting more enzymes of degrade plants (Challacombe et al. 2019).

In soil from regions with strong KRD, the predominant fungal genera were previously reported to be *Gibberella* and *Fusarium*, whereas the genera *Aspergillus*, *Geomyces*, and *Fusarium* dominated soil communities in regions with slight KRD (Wang et al. 2016). In our study, we found significant differences in fungal community structures in bulk soil, rhizosphere and root between samples taken from regions with different KRD grades. Specifically, in samples from regions with slight KRD, we found that the genera *Geoglossum* and *Hygrocybe* dominated in bulk soil, *Geoglossum* and *Fusarium* dominated in rhizosphere, and *Mycena* and *Periconia* dominated in roots. In contrast, in samples from regions with strong KRD, we found the genera *Fusarium* dominated with these sites in strong KRD. We noted here that these specific genera were not consistent with a previous study. The inconsistencies may be due to the difference of plant diversity and soil properties between sampling locations (Rad et al. 2009). The differences between fungal communities isolated at different KRD grades was more pronounced at the phylum level, in part due to the limited genus-level resolution of the ITS2 amplicon primers used in this study.

The diversity of plants varies with different grades of KRD (Xiong et al. 2002), one study showed that plant richness increased the diversity of soil fungal communities (Shen et al. 2021), the plant richness of slight KRD is higher than counterpart of strong KRD, therefore, the richness of fungal communities in slight KRD was higher than counterpart of strong KRD at bulk soil and rhizosphere. However, the richness of root endophyte fungal communities has no significant difference between two grades KRD, in the long-term evolution process of plants, even in different growth environments, the changes of endophyte fungal communities may not be great. Because the environment has a great influence on rhizosphere microorganisms (Zhang et al. 2017). The water holding capacity of soil in karst area is weak, and plants have developed root structure in order to grow, therefore, rhizosphere and bulk soil are closely connected, and maybe the richness of fungal communities between bulk soil and rhizosphere has no significant difference. The structure of bulk soil, rhizosphere, and root endophyte fungal communities are normally and closely correlated with environmental factors such as soil nutrient levels, soil enzymes and climate (Blaalid et al. 2014; Yu et al. 2013; Gong et al. 2019). In particular, resource availability can drive changes in communities of root-associated fungi (Liu et al. 2015). For example, the structure of root-associated fungal communities in *Salix arctica* are strongly correlated with soil AN, AP, NN content, and pH at different sites (Fujimura et al. 2008). Consistent with this idea, we found both that the absolute nutrient content and the relationship between soil nutrients and fungal community structure varied at sites with different KRD grades. In particular, we found that AN and SOC content, and pH were the most important factors that influenced the major phyla differences between bulk soil, rhizosphere, and root endophyte fungal communities. We speculate that the increased levels of nutrients available in the strong KRD environments or altered root exudates by *T. japonica* plants growing in different environments could directly affect the structure and diversity of the fungal communities. In addition, one study showed that diverse soil properties and potential interactions among taxa in the processes of vegetation restoration may influence the structure and diversity of soil bacterial communities in KRD areas (Xue et al. 2017). The restoration of KRD is accompanied by vegetation restoration, therefore, bacterial communities probably also played an important role during the processes of KRD restoration.

The diversity of soil bacterial communities was negatively related with soil nutrients (SOC and TN) (Xue et al. 2017), probably, the composition and diversity of bulk soil, rhizosphere and root in *T. japonica* between the different grades KRD were different, in the future, we should also focus on the function of bacteria during the *T. japonica* plants adapt the different grades KRD. In addition, the bacterial-fungal interactions are also important and cannot be overlooked, therefore, we should also focus on investigating both bacterial-fungal interactions and their functions in the future.

In summary, the key findings of our study are that the soil nutrients (SOC, TN, TP, AN and NN) content and pH of the strong KRD areas were higher than those of the slight KRD. In addition, the richness of bulk soil and rhizosphere fungal communities in the strong KRD were lower than in the slight, and the Shannon index of rhizosphere fungal communities in the strong KRD was lower than in the slight KRD. Ascomycota and Basidiomycota were dominant fungal phyla at bulk soil, rhizosphere and root under the slight KRD and the strong KRD. Importantly, the Mortierellomycota was not found in root endophyte fungi under the slight KRD; Olpidiomycota only existed in bulk soil and rhizosphere under the strong KRD. The results of PCoA showed that the fungal communities separate in bulk soil, rhizosphere and root endophyte under slight KRD and the strong KRD. SOC and AN content, and pH were central factors to drive the change of dominant fungal phyla in bulk soil, rhizosphere and root endophyte. In addition, TN and AN were negatively correlated with the richness index of fungal communities in bulk soil and rhizosphere; TP and AP were negatively correlated with the beta diversity of fungal communities in bulk soil and rhizosphere; however, all soil nutrient and soil pH were no correlation with richness, Shannon and PCoA1 of fungal communities in root endophyte. Our findings highlighted the different responses of fungal communities to the grade of KRD, and the compartment (bulk soil, rhizosphere, root), and further shed light on the structure and diversity of fungal communities and their relationship with soil nutrients during the process of rocky desertification restoration, which provides a theoretical basis for rocky desertification control.

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Declarations

Competing interests The authors declare that they have no competing interests.

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