Particular microbial taxa rather than total microbial diversity best predict the vertical profile variation in soil multifunctionality in desert ecosystems

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June 10, 2020

Abstract

In desert ecosystems, the desertification process is characterized by increasing attenuation of plant productivity and deterioration of soil habitats, leading to enhanced environmental stress gradients for soil microbiomes. Despite the significance of microbial communities for multifunctionality in terrestrial ecosystems, the feedback dynamics of microbiomes and their contributions to maintaining subsurface soil multifunctionality as desertification progresses have yet to be evaluated. Here, we used three sites with different desertification stages and investigated the variation trends of microbiomes in soil profiles (0-100 cm) and their contributions to regulating multifunctionality. We first confirmed that multifunctionality did not exhibit a significant difference between superficial soils (0-20 cm) and deep soils (20-100 cm) and slightly decreased as soil depth increased throughout the entire profile. Desertification progression drove distinct variation trends of microbiomes in vertical soil profiles. Soil bacterial communities received on average more positive and progressive feedback from desertification development than fungal and archaeal communities, characterized by significant variation in bacterial alpha- and beta-diversity and slight variation in fungal and archaeal alpha- and beta-diversity. The most abundant phyla in the microbiomes did not vary between the superficial and deep soils at any desertification stage. Significant declines in microbial clades within Acidobacteria are an important feature as desertification proceeds. Particular microbial taxa rather than total microbial diversity best predict and explain the vertical profile variation in soil multifunctionality in desert ecosystems. Our results highlight the significance of microbial community composition in subsurface soils for regulating multifunctionality in desert ecosystems.

1. INTRODUCTION

Desert ecosystems, the arid areas (approximately 45.6 million km^2, covering at least 35% of Earth's land surface) most environmental sensitive to changes in climate and human disturbances, are suffering from increasing land degradation and biological diversity loss (Bastin et al., 2017; Schimel, 2010; Wang et al., 2019a). In arid and semiarid regions, fragile steppe grassland ecosystems are especially prone to desertification, which results in the destruction of plant communities, soil physical texture, and nutrient losses. Restoring and rehabilitating desertification grassland has been a serious issues in ecology (D'Odorico et al., 2019). Soil microorganisms have a predominant potential to reveal and regulate the changes in soil ecosystem functions and services, so characterizing the microbial successional patterns and their interactions with plants and soils is essential for increasing our understanding of the mechanisms of ecological restoring and rehabilitating, improving our capacity to predict the responses of ecosystems to human disturbance, and optimizing the design of large-scale restoration projects.

Soil multifunctionality has been widely used to quantify and compare the ability of various ecosystems to support overall functionality (Manning et al., 2018). Numerous studies have widely emphasized the signifi-

cance of multifunctionality in superficial soils (top ˜20 cm) due to their predominant ecological importance (Delgado-Baquerizo et al., 2017; Delgado-Baquerizo et al., 2016; Wagg et al., 2014; Wagg et al., 2019; Zheng et al., 2019), where the density, activity, and diversity of microorganisms are higher than those in deep soils (i.e., below 20 cm) (Fierer et al., 2003). However, due to the large bulk of soil profiles, deep soils may accumulate abundant soil nutrients, leading to an enhanced ability to provide multifunctionality during ecosystem development. For example, compared to superficial soils, deep soils in typical forest and grassland ecosystems can store over two-thirds the organic carbon and nearly equal amounts of phosphorus due to the long-term input from plant roots and soil leaching (Balesdent et al., 2018; Upton et al., 2020; Zhou et al., 2018). Indeed, increasing evidence has confirmed that a large percentage of soil multifunctionality is hidden in deep soils (Jiao et al., 2018; Upton et al., 2020). Intriguingly, in desert ecosystems, the desertification process can result in a decrease in the water-holding capacity of superficial soils and a steady increase in the soil leaching capacity (D'Odorico et al., 2013; D'Odorico et al., 2019; Neilson et al., 2017), leading to the enhanced downward elemental (i.e., carbon (C), nitrogen (N), and phosphorus (P)) transfer responsible for the persistent accumulation of nutrients in deep soils. Thus, it is expected that deep soil multifunctionality in desert ecosystems plays increasingly important roles in regulating and buffering overall ecological service functions.

Soil microbial diversity and community composition drive and determine soil multifunctionality in various ecosystems (Delgado-Baquerizo et al., 2016; Wagg et al., 2014; Zheng et al., 2019). Different influences of biodiversity and community composition on soil multifunctionality have been investigated (Delgado-Baquerizo et al., 2017; Wagg et al., 2014). For example, the bacterial and fungal diversity in superficial soils (top ˜20 cm) positively influenced multifunctionality (Wagg et al., 2019), especially in arid ecosystems (Delgado-Baquerizo et al., 2016), while particular taxa of bacteria and fungi but not their total richness and abundance determined the resistance of superficial soil multifunctionality in arid ecosystems (Delgado-Baquerizo et al., 2017). Furthermore, although such vast soil microbes in superficial soils may play a central role in driving nutrient turnover with supportive effects on soil multifunctionality, microbial diversity and particular microbial attributes in deep soils share indispensable ecological drivers (Delgado-Baquerizo et al., 2019; Jiao et al., 2018). Indeed, deep soil contains a significant portion (35% ˜ 50%) of total microbial diversity and biomass, suggesting its non-negligible ecological functions (Eilers et al., 2012). Increasing evidence, based mostly on results from ecosystems with typical development (reforested ecosystems) and grassland ecosystems with high rainfall, suggests that particular microbial attributes (i.e., individual species, particular communities and their cooperation) rather than alpha-diversity levels (i.e., total abundance, species richness, and the Shannon index) may play more important roles in driving deep soil nutrient (i.e., C, N, and P) cycling with direct feedback effects on soil multifunctionality (Jiao et al., 2018; Upton et al., 2020), as particular microbial attributes compose significant regulators of microbial growth and interactions and predominant functional attributes (e.g., soil C cycling or N mineralization) (Delgado-Baquerizo et al., 2017). In contrast to the above ecosystems with typical development and less drought stress, ecosystems undergoing desertification display enhanced environmental stress gradients (i.e., decreasing plant productivity and soil nutrients and increasing soil leaching), while the dynamics of soil microbiomes and their contributions to regulating deep soil multifunctionality remain largely unexplored, particularly the contributions of particular microbial attributes.

Herein, we assess the importance of vertical soil microbiomes and their contributions to multifunctionality during the desertification process in desert ecosystems. We used three sites in different typical desertification stages (potential, moderate, and severe desertification) that represent the process of desertification. The multifunctionality and diversity of soil bacteria, fungi, and archaea were investigated at soil depths of 0-100 cm. We tested the hypotheses that (1) multifunctionality in deep soils (20-100 cm) would, at least partially, be equally as important as that of superficial soils (0-20 cm) in the desert ecosystem and (2) the combined effects of the particular species richness of soil microbiomes would better predict multifunctionality in desert ecosystems, especially in deeper soil profiles.

2. METHODS

2.1 Study site

This study was conducted in the Mu Us Desert, south of the Ordos Plateau of China, with an area of approximately $4 \times 10^4 km^2$ (N38°55', E 109°53'). The average altitude is between 1100 and 1300 m. The climate is semi-arid (rainfall: 440 mm/year), and the mean annual temperature is 7.7°C. The soil is mainly composed of sand and loessal soil. The vegetation is mainly composed of xerophytes such as *Artemisia desertorum* , *Hedysarum laeve* ,*Psammochloa villosa* , and *Hedysarum scoparium* .

Based on standardized vegetation coverage criteria (Guo-dong, 2004), sites at three different desertification stages were selected as sampling sites using a Global Positioning System (GPS) unit, including potential desertification (PD), moderate desertification (MD), and severe desertification (SD) (Table S1). In July 2018, we built ten replicate plots (20 m × 20 m) for PD and MD due to the high variability of vegetation cover and edatope, respectively. We built five replicate plots (20 m × 20 m) for the SD stage due to the low and indistinctive vegetation cover and edatope. These 25 plots were randomly selected at each site, with an interval of at least 200 m. Nine subsamples were obtained by S -type sampling and mixed into one sample from each soil layer (0 - 10, 10 - 20, 20 - 30, 30 - 60, and 60 - 100 cm) using a soil auger with a diameter of 5 cm. Each soil sample (125 samples in total) was fully mixed and divided into two parts. One part was stored at -80°C until DNA extraction, and the other part was air dried for physicochemical analysis.

2.2 Edaphic variables and functional genes

Soil physicochemical analysis of organic carbon (OC), total nitrogen (TN), nitrate (NO_3–N), ammonium (NH_4^+-N), total phosphorus (TP), available phosphorus (AP), pH, electrical conductivity (EC), bulk density (BD) and soil moisture was conducted by using standard methods described elsewhere (Bao, 2000; Lozano et al., 2014; Wang et al., 2017). Microbial DNA was extracted from 0.6˜1.0 g of each desert soil (125 samples) by using the MP Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. Total Ca^{2+} was measured by flame atomic absorption spectrophotometry (AAS), and Zn^{2+} was measured sequentially by graphite furnace atomic AAS on a Hitachi Z-2000 (Hitachi, Tokyo, Japan).

The abundance of functional genes associated with key microbial processes was quantified to assess multifunctionality. The abundances of functional genes associated with the C fixation process (*CBBL*gene), N fixation process (*nifH* gene), P dissolution process (*phoD* gene), N mineralization process (*apr* and *chiA*genes), NH_4^+-N transformation process (*AOA* and *AOB* genes), NH_4^+-N loss process (*anammox*gene), and NO_3^--N loss process (denitrification, including *nirK* , *nirS* , *qnorB* , and*nosZ* genes) were quantified by a real-time PCR system (QuantStudio 6 Flex, Thermo Fisher, Singapore City, Singapore). These genes were described elsewhere (Levy-Booth et al., 2014; Li et al., 2018; Wang et al., 2017). Detailed information on the gene primers is given in Table S2. Reaction mixtures (20 μL) contained 10 μL of SYBR® Premix ExTaq (Dining), 0.5 μL of DNA, 0.5 μL of primers (forward and reverse) (20 μM), and 8.5 μL of sterile distilled water. At the same time, we tested the amplification specificity by constructing a dissolution curve.

Amplicon libraries were constructed according to the dual indexing strategy. The V4-V5 hypervariable regions were targeted using primer pair 515F (GTGCCAGCMGCCGCGGTAA)/907R (CCGTCAATTCCTTTGAGTTT) for the bacterial 16S rRNA gene and primer pair Arch519F (CAGCCGCCGCGGTAA)/Arch915R (GTGCTCCCCCGCCAATTCCT) for the archaeal 6S rRNA gene. The fungal ITS1 gene was amplified by the primer pair ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG)/ITS2-2043R (GCTGCGTTCTTCATCGATGC). All the samples were sequenced on the Illumina MiSeq (300-bp paired-end reads) platform (Illumina Inc., San Diego, USA) at Personal Biotechnology Co., Ltd. (Shanghai, China). As a standard data analysis

method, the acquired sequences were filtered for quality, and any chimeric sequences were removed with

the Quantitative Insights into Microbial Ecology (QIIME, v 1.8.0, http://qiime.org/) tool. These original sequences were filtered for quality and assigned to operational taxonomic units (OTUs) (DeSantis et al., 2006). The OTUs with fewer than two sequences were first removed, and then the OTUs were classified within the SILVA database (release 132, http://www.arb-silva.de) for bacteria and archaea and the UNITE database (release 7.2, http://unite.ut.ee/index.php) for fungi. The soil microbiome dataset has been deposited in the NCBI Sequence Read Archive under accession number PRJNA541211.

2.3 Assessing multifunctionality

Multifunctionality has been broadly defined as the simultaneous provisioning of multiple functions and services (Manning et al., 2018). These processes involve, among others, soil nutrient turnover (i.e., OC and TN contents, N and P availability, and N mineralization and loss) and primary production (i.e., net primary productivity) (Manning et al., 2018; Wagg et al., 2019). Compared to superficial soils, deep soils are mainly anaerobic and low-temperature environments. Although maximal nutrient turnover rates can be measured using indoor culture experiments, it may be largely inappropriate to adopt indoor culture experiments to measure nutrient turnover rates in deep soils. The abundance of functional genes has been identified as the predominant variable to predict potential nutrient turnover rates (Attard et al., 2011; Graham et al., 2014; Petersen et al., 2012; Wang et al., 2017). Thus, to summarize the changes in the overall functioning of deep soils, the abundance of functional genes affiliated with the nutrient turnover process was used to indirectly characterize their potential functioning capacity (de Vries et al., 2018; Graham et al., 2014; Li et al., 2018; Zhi et al., 2015). In desert ecosystems, the desertification process is characterized by the long-term deterioration of soil multifunctionality and appearance of environmental stress gradients for soil microorganisms (Fierer, 2017; Ravi et al., 2010). Thus, functional gene abundance is likely to be more accurate than other metrics as a long-term proxy and predictor of the in-site functioning capacity in deep soils (Li et al., 2018; Wang et al., 2019b). Based on well-known methods, the abundance of functional genes associated with functional processes (i.e., C, N and P transformation) was organized into a multifunctionality index to obtain and summarize a multidimensional multifunctionality index in deep soils. In total, we quantified multifunctionality using related variables as follows: C functions (*CBBL*), N functions (TN, NH_4^+-N, NO_3^--N, *nifH* , *apr* ,*chiA, AOA* , *AOB, anammox* , *nirK* ,*nirS* , *qnorB* , and *nosZ*), P functions (TP, AP, and*phoD*), Ca^{2+} and Zn^{2+}.

There are several approaches to quantify multifunctionality (i.e., single functions, averaging, and the threshold approach) (Manning et al., 2018). Because each approach possesses different strengths and weaknesses, we adopted two distinct approaches (single functions and the averaging approach) to quantify and assess multifunctionality. We normalized and standardized each nutrient cycling variable and functional gene abundance using Z-score transformation (Manning et al., 2018). These standardized ecosystem functions were then averaged to obtain an overall multifunctionality index and single-function index (i.e., C process, N process, and P process). This index is broadly adopted in multifunctionality research and provides a straightforward measure of the ability of different communities to sustain multifunctionality (Manning et al., 2018; Wagg et al., 2019). In addition, functional gene abundances for NH_4^+-N and NO_3^--N loss were inverted, as higher abundances of these genes are undesired functions (i.e., a lower abundance of N loss indicates high N retention and better ecosystem functioning, while a higher abundance indicates dysfunction and higher N loss).

2.4 Data analysis

Z-score values of ecosystem functions and related variables were calculated using SPSS (IBM SPSS Statistics 25.0). The most abundant phyla and alpha-diversity indexes (sobs, ACE, Shannon, and phylogenetic diversity) of soil bacteria, archaea, and fungi were calculated in the R environment (v3.5.3; http://www.r-project.org/) using MOTHUR R package (https://mothur.org/wiki/calculators/), and their variations with increasing soil depth were determined using linear least-squares regression models (OriginLab Corporation, One Roundhouse Plaza, Suite 303, Northampton, MA 01060, United States). The beta-diversity of the

bacteria, archaea, and fungi was quantified by using two axes from a nonmetric multidimensional scaling (NMDS) analysis of Bray-Curtis dissimilarities within the OTU community matrix (Daum & Schenk, 1997). Similarity values among the sites were examined via the ANOSIM and ADONIS test ($P < 0.001$). The vertical spatial decay relationships were calculated as the linear least-squares regression relationships between soil depth and microbial community similarity (Jiao et al., 2018).

Classification random forest (RF) analysis was adopted to identify the importance of each predictor of soil multifunctionality (Wagg et al., 2014). In these RF models, soil variables (i.e., depth, pH, EC, and BD) and the most abundant phyla and alpha-diversity indexes of bacteria, archaea, and fungi served as predictors of multifunctionality. These analyses were conducted using the randomForest package in the R environment (Delgado-Baquerizo et al., 2016). The significance of the importance of each predictor of multifunctionality was identified by using the rfPermute package for R (https://cran.r-project.org/web/packages/rfPermute/index.html). Structural equation modelling (SEM) was performed to determine the direct and indirect effects of soil variables (i.e., depth, pH, EC, and BD) and the most abundant phyla and alpha-diversity of bacteria, archaea, and fungi on multifunctionality. We used the chi-square (χ^2) test and root MSE of approximation (RMSEA) to assess SEM fit. The model has a good fit when χ^2/df (degrees of freedom) is low ([?] 2) and$P$ is high (> 0.05) and when RMSEA is low ([?] 0.05) and P is high (> 0.05) (Schermelleh-Engel et al., 2003). Furthermore, we evaluated the fit of the models using the Bollen-Stine bootstrap test. Each SEM was fit using AMOS 20.0 (International Business Machines Corporation, Armonk, NY, United States).

3. RESULTS

3.1 Variation in multifunctionality

We first explored the vertical variation in multifunctionality in desert ecosystems. Overall, the multifunctionality index did not exhibit a significant difference between whole superficial soils (0-20 cm) and deep soils (20-100 cm) ($p > 0.05$) and slightly decreased as soil depth increased through the soil profiles (Fig. 1 a, b). We also found that the multifunctionality index significantly decreased along the desertification gradient (Fig. 1 c) but did not show different changes with depth between the superficial and deep soils (Fig. 1 d).

Three individual functional indexes (C, N, and P processes) were evaluated (Figure S1). Overall, the C and N processes were higher at the potential desertification (PD) sites but did not show different changes between the moderate desertification (MD) and severe desertification (SD) sites. However, the P process did not differ between the whole superficial and deep layers along the desertification gradient. The C process did not exhibit a significant difference between superficial soil and deep soil at the MD and SD sites. The N process differed between superficial soil and deep soil at only the SD sites. The C and N processes typically decreased as the soil depth increased throughout the entire profile.

3.2 Variation in soil microbiomes

We acquired a total of 5490761, 7562145, and 5357782 high-quality bacterial, fungal, and archaeal sequences (from the 125 samples), which were classified into 4890, 7723, and 3246

operational taxonomic units (OTUs), respectively. Bacteria were mainly composed of the phyla Actinobacteria (40.06%), Proteobacteria (27.23%), Chloroflexi (8.26%), and Acidobacteria (7.89%). Fungi were mainly composed of the phyla Ascomycota (69.70%), Basidiomycota (9.85%), and Zygomycota (5.39%). Archaea were mainly composed of the phyla Thaumarchaeota (81.34%) and Euryarchaeota (15.27%). Overall, Acidobacteria, Chloroflexi, and Thaumarchaeota typically increased, while Actinobacteria, Proteobacteria, and Euryarchaeota significantly decreased during the process of desertification (Figure S2).

Regarding the soil profiles, distinct variation trends of the microbiomes were observed along the desertification

gradient. First, Ascomycota, Basidiomycota, Euryarchaeota and Thaumarchaeota did not differ between the superficial and deep layers at any desertification stage. In contrast to Proteobacteria, Acidobacteria, Actinobacteria and Chloroflexi in the superficial soils were much richer than those in the deep soils (Figure S3). Second, Acidobacteria, Actinobacteria, Chloroflexi, and Thaumarchaeota significantly decreased and Proteobacteria significantly increased as soil depth increased (Figure S4). The most abundant phyla of bacteria, fungi, and archaea showed distinct variation trends as soil depth increased along the desertification gradient (Figure S5). The significance of the primary phyla composing the microbiomes was confirmed by a Kruskal-Wallis H test and least-squares linear regression analysis.

The alpha-diversity levels of the soil microbiomes were all higher at the PD and MD sites than at the SD sites, except for the Shannon index for archaea (Fig. 2). As soil depth increased, bacterial, fungal and archaeal diversity exhibited different fluctuating trends in the desert ecosystems. The Shannon and phylogenetic diversity indexes of bacterial diversity significantly decreased as soil depth increased, and the phylogenetic diversity index of fungi significantly decreased, but the Shannon diversity index of archaea significantly increased as soil depth increased. Vertical variation tendencies were determined by least-squares linear regression models. In addition, distinct variation tendencies for alpha diversity were detected during the desertification process (Figure S6). The Shannon index of bacteria at the SD sites and the phylogenetic diversity index of bacteria did not exhibit different changes between superficial and deep soils. The alpha diversity of fungi in superficial and deep soils did not exhibit different changes, except at the PD sites. The alpha diversity of archaea in superficial and deep soils did not exhibit different changes, except for the Shannon index at the MD sites.

NMDS analysis suggested that soil microbiomes at different desertification sites formed distinct clusters in ordination space (Fig. 3). The significant spatial variations in species composition were further confirmed by ANOSIM and ADONIS tests (Table S3). These variations were the highest for bacteria, followed by fungi and archaea, suggesting that the bacterial communities were more likely to be reshaped by desertification. We observed different changes in microbiomes between superficial and deep soils (Fig. 3 and Table S4). These changes were larger for bacteria and archaea than for fungi, suggesting that the bacterial and archaeal communities had higher feedbacks with soil depths. The significant variations in beta diversity among different microbiomes were evaluated and identified (Fig. 3). Fungi exhibited the highest beta diversity, suggesting a higher community dispersion level. The vertical spatial variation (VDR) of different microbiomes was compared among the desertification sites. We further found that the VDR slopes of the soil microbiomes at the PD sites were steeper than those at the MD and SD sites. The bacterial and archaeal VDR slopes were significantly steeper than the fungal VDR slopes, suggesting that desertification markedly enhanced the vertical variation in bacterial and archaeal communities but had less effect on the variation in fungal communities.

3.3 Links between the microbial community and multifunctionality

The relationships between the microbial community and multifunctionality in desert ecosystems were explored (Figs. 4 and 5). The dominant bacterial and archaeal phyla were significantly ($P < 0.01$) related to multifunctionality, but the dominant fungal phyla did not exhibit different relationships ($P > 0.05$) (Fig. 4). The soil microbial diversity of bacteria and fungi was positively related to multifunctionality, while archaeal diversity showed the reverse pattern (Fig. 5). Further analyses provided evidence that dominant species (Acidobacteria and Chloroflexi) and the fungal phylogenetic diversity index had significantly stronger and more positive correlations with multifunctionality than did microbial diversity (Shannon and phylogenetic diversity indexes). Furthermore, we found significantly stronger and more positive correlations between microbial diversity and individual functions (i.e., C, N, and P processes), as well as between this diversity and combinations of functions (i.e., C and N, N and P, C and P, and C, N, and P processes) (Table S5). In particular, Acidobacteria, Chloroflexi and fungal phylogenetic diversity were positively and strongly related to the individual functions and combinations of functions.

3.4 Accounting for soil multifunctionality drivers in desert ecosystems

Random forest modelling was adopted to determine and compare the most important predictors of multifunctionality in desert ecosystems. We found that Acidobacteria and fungal phylogenetic diversity were more important than the other multifunctionality predictors (Fig. 6 a). The predominant predictors of multifunctionality differed between superficial and deep soils (Fig. 6 b, c). While the fungal phylogenetic diversity index best predicted multifunctionality in superficial soils, Acidobacteria and Thaumarchaeota were instead pivotal in deep soils. Further, the microbial species index was the predominant predictor of multifunctionality (Fig. 6 d).

Our structural equation modelling (SEM) explained 65.0% of the variance in multifunctionality at the desertification sites (Fig. 7). The microbial species index appeared to have a distinctly stronger total positive effect than the biodiversity index on multifunctionality (Fig. 7 and Figure S7). Depth and soil electrical conductivity (EC) had the strongest total positive effects on multifunctionality, as indicated by the standardized total effects from SEM.

4. DISCUSSION

In this study, we discovered that distinct variation trends of soil microbiomes during desertification regulated the changes in multifunctionality in vertical soil profiles. Notably, particular microbial taxa rather than microbial diversity better predicted the vertical profile variation in multifunctionality in desert ecosystems. Our results highlight the significance of deep soil microbiomes for buffering and regulating the multifunctionality of desert ecosystems.

4.1 Variation in multifunctionality in desert ecosystems

Deep soil multifunctionality was as important as superficial soil multifunctionality in the desert ecosystems (Fig. 1). The results of this study confirmed our first hypothesis. Desertification can lead to the loss of surface soil C, N and P and fine soil particles and decreases in the water-holding capacity and vegetation cover (D'Odorico et al., 2013; Ravi et al., 2010; Ward et al., 2018). Soil pores and water infiltration in superficial soil exhibited continuous increases due to the gradual loss of fine soil particles during desertification (Allington & Valone, 2010; D'Odorico et al., 2007), leading to enhanced nutrient accumulation in deep soils from plant litter and superficial soil nutrients. Furthermore, microbiomes play a vital role in regulating soil multifunctionality by supporting functional processes (i.e., soil nutrient cycling, litter decomposition, and N mineralization), which allow the transfer of materials and carbon energy between above- and belowground communities (Falkowski et al., 2008; Tedersoo et al., 2014). Thus, the simultaneous changes in and coupling of soil physical properties and vegetation and microbiomes jointly contributed to the equivalent multifunctionalities of superficial and deep soils (D'Odorico et al., 2019; Jiao et al., 2018; Ravi et al., 2010). In addition, soil nutrients are mainly derived from the decomposition of plant litter and root in both undisturbed and restored ecosystems (Barber et al., 2017; Lozano et al., 2014). In this study, the plant litter and root biomass gradually decreased during desertification (Figure S8), leading to reduced soil nutrient accumulation from litter and root decomposition, which was largely responsible for the significant decrease in multifunctionality in the vertical soil profiles along the desertification gradient (Fig. 1c).

4.2 Vertical variation in soil microbiomes in desert ecosystems

Our results showed that the desertification process drove distinct variation trends of the microbiomes in the vertical soil profiles. Microbial survival and growth may be severely limited by continuous abiotic stressors (i.e., limited bioavailability of water and C substrates), frequent disturbances (i.e., drying-rewetting events), and heterogeneous distributions of substrates across soil profiles (Fierer, 2017). In desert ecosystems, all these abiotic stressors often synchronously appear during desertification, which is characterized by decreasing

plant biomass and fluctuating soil nutrients and soil structure, leading to enhanced environmental stress gradients that account for the distinct variation in microbiomes in vertical soil profiles (D'Odorico et al., 2013; D'Odorico et al., 2019; Neilson et al., 2017). Furthermore, the phyla Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Ascomycota, Basidiomycota, Thaumarchaeota and Euryarchaeota were the most abundant microbial taxa with distinct responses in the desert ecosystems. These phylum-level profiles were similar to those in other soils and environments (Jiao et al., 2018; Li et al., 2014; Tedersoo et al., 2014; Upton et al., 2020). We further found that bacteria are on average more resilient in the face of disturbances and perturbations because of their relatively fast intrinsic growth rates (Wardle, 2013), suggesting that they are more sensitive to the environmental filtering driven by desertification. As soil depth increased, the bacterial phyla Acidobacteria, Actinobacteria, and Chloroflexi typically declined, while Proteobacteria significantly increased (Figs. S4 and S5). The majority of Acidobacteria, Actinobacteria, Proteobacteria, and Chloroflexi have been suggested to be closely associated with organic substrates (Goldfarb et al., 2011). Except for the response of Proteobacteria, these observed changes were further confirmed by the findings of other studies (Jiao et al., 2018; Li et al., 2014), suggesting that soil pH and nutrient bioavailability are more likely to be the reasons for the decreased relative abundance in soil profiles. In this study, Alpha-, Delta-, and Gammaproteobacteria belonging to the class Proteobacteria were examined (Figure S2). Alpha- and Deltaproteobacteria have been suggested to be negatively associated with increased organic substrates, while Gammaproteobacteria are positively associated with increased organic substrates (Goldfarb et al., 2011). This contrasting pattern could reflect divergent ecological niches and microbial synergism, which are more likely to be the reasons for the enhanced abundance of Proteobacteria in the vertical soil profiles, as reported by Li et al. (2014) in farmland ecosystems. In addition, the most abundant fungal phyla (Ascomycota and Basidiomycota) were less mobile in vertical soil profiles than bacterial and archaeal phyla in the desert ecosystems, similar to previous findings (Tedersoo et al., 2014), suggesting that the richness of fungi and functional groups is not associated with plant productivity and that the plant-soil feedback loop does not typically reshape fungal diversity in different ecosystems. The archaeal phyla Thaumarchaeota and Euryarchaeota mainly drive soil N cycling (Haroon et al., 2013; Leininger et al., 2006). Thus, the gradual decrease in N substrate in vertical soil profiles is more likely to be the reason for the decreased relative abundance of Thaumarchaeota and Euryarchaeota.

Alpha-diversity can characterize the number of microbial taxa within sample sites, while beta diversity can describe the variation trend of microbial composition across sample sites (de Carvalho et al., 2016; Legendre & De Cáceres, 2013). Previous work has suggested that the alpha-diversity (i.e., Shannon and OTU richness indexes) of bacteria and fungi decreases while archaeal diversity typically increases with increasing soil depth in different systems (i.e., grassland, forest and farmland) (Eilers et al., 2012; Jiao et al., 2018). Our results further indicated that bacterial diversity decreased, archaeal diversity increased, and fungal diversity fluctuated with increasing soil depth along a desertification gradient (Fig. 2). These discordant patterns of soil microbiomes were due to their distinct ecological niches and differences in oxygen tolerance, showing that bacteria and fungi are mainly aerobic, while archaea are mainly anaerobic (Haroon et al., 2013; Upton et al., 2020). The oxygen content gradually decreased with soil depth in the desert ecosystems, given the increasing water content with increasing depth resulting from high soil water infiltration (D'Odorico et al., 2007). In addition, ecological restoration characterized by increased availability of soil C and N significantly enhanced the alpha- and beta-diversity of bacteria and archaea but not fungi (Barber et al., 2017; Jiao et al., 2018; Lozano et al., 2014). In contrast to ecological restoration, desertification is characterized by decreasing soil C and N, which may be the reason for the decreased alpha- and beta-diversity of bacteria and archaea in the vertical soil profiles as desertification proceeded. In this study, the indistinctive alpha- and beta-diversity of fungi suggested the stable performance of fungal communities in desert ecosystems (Figs. 2 and 3). Fungi are heterotrophic microorganisms that play fundamental ecological roles as decomposers, such as the decomposition of litter and senescence or death of roots (Tedersoo et al., 2014). In this study, the litter and root supplies decreased as desertification progressed, and this unfavourable and variable habitat was ineffective in completely restraining the growth and enrichment of fungi. The fungal kingdom contains a large proportion of various niche strategies ranging from saprotrophy through mutualism to parasitism across trophic levels (Nilsson et al., 2019). In addition, fungal communities with filamentous growth may

show different interactions because of dispersal limitation and greater tolerance of desiccation (Austin et al., 2004; Fukami et al., 2010; Powell et al., 2015), leading to their co-enrichment and distinct vertical distributions.

4.3 Main predictors of ecosystem multifunctionality in desert ecosystems

Our results indicated that particular microbial phyla rather than total microbial diversity better predicted and explained the vertical profile variation in soil multifunctionality in desert ecosystems. The results of this study confirmed our second hypothesis. Experiments at the microcosm and global scales showed that microbial diversity variables (i.e., Shannon and phylogenetic diversity indexes and OTU richness) are important predictors of multifunctionality and are positively linked to superficial soil multifunctionality (Delgado-Baquerizo et al., 2016; Li et al., 2019; Wagg et al., 2014; Zheng et al., 2019), suggesting that microbial communities with higher richness perform better under varying conditions and better protect against the loss of taxa. However, our knowledge is largely based on microbial diversity and dominance in superficial soil, and less attention has been paid to deep soils of desert ecosystems. Our results suggested that individual bacterial and archaeal species are more important predictors of multifunctionality in desert soils, especially in deep soils (20-100 cm). These individual bacterial and archaeal species in deep soils may play a leading role in driving soil multifunctionality, which to some extent could explain why the process of desertification significantly decreased soil multifunctionality (Fig. 1) and dominant bacterial and fungal phyla maintained synchronous positive feedbacks (Figures. S3, S4, and S5). In contrast to those of bacterial and archaeal taxa, the links between individual species of fungi and ecosystem function are dependent on the presence of other species and a result of multiple interactions (i.e., positive and negative as well as direct and indirect) between the various species that as a whole regulate potential ecosystem functions (Tedersoo et al., 2014; Wagg et al., 2019).

Intriguingly, our results showed a disproportionate role of individual species (i.e., Acidobacteria or their strategic alliances) in multifunctionality, which seems counter-intuitive given their higher than expected ecological importance in soil microbial communities (Figs. 4 and 5). Acidobacteria in soil habitats are considered ubiquitous and physiologically active but are rarely cultured and consequently remain a poorly studied phylum (Goldfarb et al., 2011; Naether et al., 2012). The phylogenetic diversity and relative abundance of Acidobacteria in diverse habitats have suggested their vital role in driving biogeochemical processes and diverse metabolic functions (Naether et al., 2012). High C bioavailability is negatively associated with acidobacterial abundance in various soils (Fierer et al., 2007; Goldfarb et al., 2011), suggesting that Acidobacteria are adapted to habitats with poor substrates and often slow-growing oligotrophs. Indeed, the acidobacterial community can be energetically adapted to C-limited soils and may be predominant in oligotrophic habitats, where decreasing plant biomass results in a decrease in the availability of plant-derived C sources (Castro et al., 2010).

Microbial diversity can maintain and regulate multifunctionality in a variety of ways, suggesting that microbial communities with higher total richness perform better in progressively developed and less-stressed soils (i.e., forest, cropland, and wetland soils) (Delgado-Baquerizo et al., 2016; Jiao et al., 2018; Li et al., 2019; Wagg et al., 2014). Conversely, multifunctionality may be controlled by particular microbial taxa (relative abundance of phylotypes) but not the total richness and abundance of microbial communities in dryland soils (Delgado-Baquerizo et al., 2017). Interestingly, our results further support the notion that particular microbial phyla (microbial species index in Figs. 4 and 5) rather than total microbial diversity best predict and explain the vertical profile variation in soil multifunctionality in desert ecosystems.

5. CONCLUSIONS

Altogether, our findings provide strong empirical evidence that deep soil multifunctionality, which has largely been ignored in desert ecosystems, differs from the multifunctionality of whole superficial soils and slightly

decreases as soil depth increases throughout the entire profile, suggesting fundamental roles in regulating and buffering overall ecological service functions. Particular microbial phyla rather than total microbial diversity can best predict and explain the vertical profile variation in multifunctionality in desert ecosystems. By identifying and characterizing the quantitative relationships between soil microbiomes and multifunctionality in vertical soil profiles, our results advance knowledge of pivotal ecological factors such as microbial community-multifunctionality relationships and will assist microbial ecologists in predicting and explaining the slight variation in ecosystem service functions in desert ecosystems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA23070201) and the National Science Foundation of China (NO. 41830755 and 41807042).

Figure captions

Fig. 1 Variation in multifunctionality in the desert ecosystems. (a): Whole superficial soils (0-20 cm) and deep soils (20-100 cm); (b): vertical soil multifunctionality during the process of desertification; (c): multifunctionality in different desertification stages; (d) superficial and deep soil multifunctionality in different desertification stages. Significant differences between desertification sites were based on a one-way ANOVA followed by an LSD test. Linear least-squares regression relationships between multifunctionality and soil depth were estimated. The bold lines denote the least-squares linear regressions across soil depth, with their 95% confidence intervals (grey-shaded areas). S: slope; PD: potential desertification; MD: moderate desertification; SD: severe desertification.

Fig. 2 Variation in microbial alpha-diversity during desertification progression. Significant differences between desertification sites were based on a one-way ANOVA followed by an LSD test. Vertical variation in the Shannon and phylogenetic diversity indexes for bacterial, archaeal, and fungal communities was estimated via linear least-squares regression. The bold lines denote the least-squares linear regressions across soil depth, with their 95% confidence intervals (grey-shaded areas). S: slope; PD: potential desertification; MD: moderate desertification; SD: severe desertification.

Fig. 3 General patterns of microbial beta-diversity in superficial and deep soils during the process of desertification. NMDS showed the variation in the microbial community for soil bacteria (A), fungi (B), and archaea (C). 95% confidence ellipses are shown around the sites. Differences in beta-diversity among the bacteria, fungi, and archaea were estimated based on a Bray-Curtis distance matrix of all soil samples. Community similarity was calculated based on 1–[dissimilarity of the Bray-Curtis distance metric]. The lines denote the least-squares linear regressions across soil depth, with their 95% confidence intervals (grey-shaded areas). ***: $P< 0.001$. Significant differences of beta-diversity between desertification sites were based on a one-way ANOVA followed by an LSD test.

Fig. 4 Relationships between the dominant phyla of soil microbiomes and multifunctionality.

The red lines denote the least-squares linear regressions with their 95% confidence intervals (grey-shaded areas). S: slope.

Fig. 5 Relationships between microbial diversity and multifunctionality. The red lines denote the least-squares linear regressions with their 95% confidence intervals (grey-shaded areas). S: slope.

Fig. 6 Main predictors of soil multifunctionality in the desert ecosystems. The figure shows the random forest mean predictor importance (%increase in MSE) of soil variables and microbial community and diversity (Shannon and phylogenetic diversity indexes) for multifunctionality for all data sets. The abundances of standardized dominant phyla of soil bacteria (Chloroflexi and Acidobacteria), fungi (Ascomycota and Basidiomycota), and archaea (Thaumarchaeota and Euryarchaeota) were then averaged to obtain an overall microbial species index. These standardized diversity indexes (Shannon and phylogenetic diversity) of soil bacteria, fungi, and archaea were then averaged to obtain an overall biodiversity index. The significance levels of each predictor are as follows: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. EC: electrical conductivity.

Fig. 7 Direct and indirect effects of soil depth, pH, electrical conductivity (EC), bulk density (BD), microbial species index, and biodiversity index on multifunctionality. Structural equation models are shown for all data sets. Numbers adjacent to arrows are indicative of the effect size (bootstrap P value) of the relationship. R^2 denotes the proportion of variance explained. Standardized total effects (direct plus indirect effects) derived from the structural equation models depicted above. The abundances of standardized dominant phyla of soil bacteria (Chloroflexi and Acidobacteria), fungi (Ascomycota and Basidiomycota), and archaea (Thaumarchaeota and Euryarchaeota) were then averaged to obtain an overall microbial species index. These standardized diversity indexes (Shannon and phylogenetic diversity) of soil bacteria, fungi, and archaea were then averaged to obtain an overall biodiversity index.

Additional Files captions

Table S1 Coverage of plant species at the different desertification stages.

Table S2 Primers and thermal profiles used for real-time PCR quantification of the different phylogenetic and functional genes.

Table S3 ANOSIM and *ADONIS* analyses of microbial beta-diversities between desertification stages.

Table S4 ANOSIM and *ADONIS* analyses of microbial beta-diversities between superficial and deep layers.

Table S5 Results of least-squares linear regressions analysing ecosystem functions and community metrics. The multifunctionality index calculated with all possible combinations of one, two and three functions.

Figure S1 Vertical variation in C, N, and P functions during desertification. Significant differences between desertification sites were based on a one-way ANOVA followed by an LSD test. Linear least-squares regression relationships between multifunctionality and soil depth were estimated. The bold lines denote the least-squares linear regressions across soil depth, with their 95% confidence intervals (grey-shaded areas). PD: potential desertification; MD: moderate desertification; SD: severe desertification.

Figure S2 Variation in dominant phyla of soil microbiomes during desertification development.

(A): Bacteria; (b): fungi; (C): archaea. Significant differences between desertification stages were tested by the Kruskal-Wallis H test. PD: potential desertification; MD: moderate desertification; SD: severe desertification.

Figure S3 Variation in dominant phyla of soil microbiomes in superficial and deep soils during desertification. Significant differences between desertification sites were based on a one-way ANOVA followed by an LSD test. PD: potential desertification; MD: moderate desertification; SD: severe desertification.

Figure S4 Relationships between the dominant phyla of soil microbiomes and multifunctionality at all sites. The red lines denote the least-squares linear regressions with their 95% confidence intervals (grey-shaded areas).

Figure S5 Relationships between the dominant phyla of soil microbiomes and multifunctionality at different desertification stages. The red lines denote the least-squares linear regressions with their 95% confidence intervals (grey-shaded areas). S : slope. PD: potential desertification; MD: moderate desertification; SD: severe desertification.

Figure S6 Variation in microbial alpha-diversity in superficial and deep soils during desertification. Significant differences between desertification sites were based on a one-way ANOVA followed by an LSD test. PD: potential desertification; MD: moderate desertification; SD: severe desertification.

Figure S7 Direct and indirect effects of soil depth, pH, electrical conductivity (EC), bulk density (BD), microbial species index or biodiversity index on multifunctionality. Structural equation models are shown for the all data sets. Numbers adjacent to arrows are indicative of the effect-size (bootstrap P value) of the relationship. R^2 denotes the proportion of variance explained. Standardized total effects (direct plus indirect effects) derived from the structural equation models depicted above. The abundances of standardized dominant phyla of soil bacteria (Chloroflexi and Acidobacteria), fungi (Ascomycota and Basidiomycota), and archaea (Thaumarchaeota and Euryarchaeota) were then averaged to obtain an overall microbial species index. These standardized diversity indexes (Shannon and phylogenetic diversity) of soil bacteria, fungi, and archaea were then averaged to obtain an overall biodiversity index.

Figure S8 Changes of plant litter and belowground biomass during desertification development. Significant differences between desertification sites were based on a one-way ANOVA followed by an LSD test. The plant was dug up and the aboveground litter were clipped and dried to obtain the litter biomass, and the roots were washed with tap water and dried at 70 °C for 48 h to obtain belowground biomass.

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