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Fungal community assemblages in a high elevation desert environment: absence of dispersal limitation and edaphic effects in surface soil

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Abstract

Recent studies have shown the significant effects of environmental selection and possible dispersal limitation on soil fungal communities. However, less is known about the role of soil depth in fungal community assemblages, especially under soil environments that are intensely cold, infertile and water-deficient. In Ngari drylands of the Asiatic Plateau, we studied fungal assemblages at two soil depths, using Illumina sequencing of the ITS2 region for fungal identification (0–15 cm as the surface soil and 15–30 cm as the subsurface soil). Fungal diversity in the surface soil was much higher than that in the subsurface soil (P<0.001), and communities differed significantly between the two layers (P=0.001). Neither soil properties nor dispersal limitation could explain variation in the surface-soil fungal community. For the subsurface, by contrast, soil, climate and space explained 27% of variation in fungal community. Collectively, these results point to high dispersal rates and absence of edaphic effects in the surface-soil fungal community assemblage in Ngari drylands. It also suggests that for soil fungi with highly effective dispersal, regional distributions may fit with Bass-Becking's paradigm that 'Everything is everywhere'.

Keywords: fungal community assemblages, soil depth, Ngari drylands, high dispersal, environmental selection, null models.

Highlights

1. Fungal communities differed dramatically between surface and subsurface soils.
2. Surface soil had much higher fungal diversity than subsurface soil.
3. High dispersal contributed to the stochastic distribution of fungi in surface soil.
4. Space and environment structured fungal communities in subsurface soil.

1. Introduction

While extreme cold desert environments may be characterized by paucity of soil nutrients, and challenging climates, they still produce abundant microbial populations (Treonis et al., 2002; Ziolkowski et al., 2013; Yadav et al., 2015), even though they may appear hostile to the most larger living organisms. Perhaps because of their faster evolutionary rates and higher genetic diversity (Whitman et al., 1998; Blackwell 2011; Li et al., 2014), microorganisms are more capable of adapting to harsh environments than macroorganisms, playing pivotal roles in biogeochemical cycling and ecosystem functioning (Yergeau et al., 2007; Chan et al., 2013). Recently, the diversity patterns and community assemblage processes of uncultured microorganisms in extreme environments have gained increasing attention. For example, Frossard et al. (2015) found that the intensity of wetting events and history of soil water regime had an interactive effect on soil microbial community composition in hot Namib Desert, while Shi et al. (2015) reported that soil microbial community composition was shaped by vegetation type on Arctic tundra. Recently, Cox et al. (2016) proposed that Antarctic soil fungal communities shared significantly more species with those in the distant Arctic, suggesting that a few fungal species with great dispersal ability are also able to colonize and dominate in cold and arid environments.

The Ngari region, which we focus on in this study, is located in the western Tibetan Plateau. It is characterized by severe cold, hyperaridity, strong wind and high ultraviolet radiation, and has been called the "arid core" of the Asiatic Plateau (Troll, 1972). Because of its remote location and harsh environments, few people enter this region, and correspondingly studies on its regional biodiversity are limited. The 1976 Interdisciplinary Scientific Expedition of the Chinese Academy of Sciences recorded 241 vascular plant species, and revealed the significant effects of climate, soil and space on plant community composition within the Ngari (Chang and Gauch, 1986). Recently, we reported the soil bacterial distribution in the Ngari, and also found that both environmental selection and dispersal limitation significantly influenced bacterial assemblages in the surface and subsurface soil layers (Chu et al., 2016).

Similarly to soil bacteria, fungi are also essential components of belowground biodiversity (Uroz et al., 2016; Peay et al., 2016), and their various functional guilds maintain a wide range of

ecosystem processes, such as belowground carbon transportation (Klein et al., 2016), plant litter decomposition (Voriskova and Baldrian, 2013), controls on the coexistence and relative abundance of plant species (Lewis, 2010; Rudgers et al., 2010), and effects on plant growth (Parniske, 2008). Nevertheless, niche types, diversity patterns and community assemblage processes tend to differ between soil fungi and bacteria (Rousk et al., 2010; Geremia et al., 2016; Peay et al., 2016). Compared with bacteria, fungi are more capable of decomposing recalcitrant organic materials (Clipson et al., 2006), adapting to soil conditions of low nitrogen and high C:N ratio (Strickland and Rousk, 2010), and tolerating acidic soils (Rousk et al., 2009). As a follow-on from our recent study of soil bacterial distribution in the Ngari (Chu et al., 2016), a broad overview of the range of fungal communities is now appropriate.

High-throughput sequencing (HTS) provides fungal ecologists an efficient approach to studying soil fungal communities and their relationship to the surrounding environment (Lindahl et al., 2013; Balint et al., 2016). In recent and analogous broad scale studies, Shi et al. (2014) found that space, temperature and vegetation significantly affected soil fungal communities of forests in western China, while Pellissier et al. (2014) proposed that soil fungal communities of grasslands were mainly structured by soil properties, temperature and plant communities in the Western Swiss Alps. In an agricultural ecosystem in the black soil zone of northeast China, it was also reported that geographic distance and soil properties structured soil fungal communities (Liu et al., 2015). However, to our knowledge, regional scale studies on fungal community assemblages have rarely been carried out in both cold and hyperarid environments, such as Ngari (Pointing and Belnap, 2012). In this kind of special environment, soil depth may produce a stronger contrast of soil physicochemical and niche properties between the different layers: the surface soil may have higher nutrient availability and arrival of immigrant microbes, whereas the deeper soil may provide a more amenable environment for microbial activity, blocking the harmful UV radiation as well as extreme fluctuations in temperature and water potential. Therefore, based on the above suppositions, it is worth exploring how the fungal diversity, community composition and assembly process vary with soil depth. Moreover, taking into account soil depth can also help us to develop a more comprehensive understanding of soil fungal ecology in such an extreme environment, even

though more than 50% of microbial biomass and biological activity occur in the surface soil for the most soil types (Tedersoo et al. 2014).

In this study, we used Illumina MiSeq platform to sequence fungal communities from samples taken at two soil depths (0–15 cm as the surface soil and 15–30 cm as the subsurface soil) at 13 sites in Ngari, Tibet. The horizontal distance between sites varied from 14 km to 925 km. We addressed the difference in fungal diversity and community composition between different soil layers, as well as the relative roles of stochastic and deterministic processes in community assemblages between layers. Different community assemblage processes should prevail in the surface and subsurface soil. We hypothesize that stochastic processes should dominate fungal assemblages in the surface soil due to high dispersal in harsh environments (Favet et al., 2013; Itani and Smith, 2016), whereas deterministic processes may be expected to dominate fungal assemblages in the subsurface soil, considering its closed microhabitat and stable environment.

In addition, we proposed two ancillary hypotheses on diversity and community composition, respectively. One hypothesis was that fungal diversity is much lower in the subsurface soil relative to the surface soil, considering the reduced immigration and low nutrient availability in deeper soils. The other was that fungal community dissimilarity across the depth profile (0-30 cm) should be comparable to that found over large spatial distances (14-925 km), because niche differentiation caused by soil depth is expected to be at least as strong as any spatial effects of regional geographic distance occurring within the same layer of the desert soil.

2. Materials and Methods

2.1. Soil sampling

Soil samples were collected at 13 sites over a broad area (~ 300 000 km^2) in western Tibet, China during July to August 2011 (Fig. 1). At each site, the surface soil (0–15 cm of depth) was collected from five random locations within a given square plot (10m×10m) and homogenized as a single soil sample, while the corresponding subsurface soil (15–30 cm of depth) was collected simultaneously and then mixed as another soil sample within each site. All the samples collected in the field were packed in sterilized polyethylene bags, and transported to the lab in portable car

refrigerators as quickly as possible. The twenty-six soil samples were then divided into two subsamples: One was stored at 4 °C to determine the soil properties, and the other was stored at -80 °C prior to DNA extraction.

2.2. The collection of environmental data

The environmental data included three geographic variables (latitude, longitude and elevation), four climatic variables (mean annual temperature—MAT, mean annual precipitation—MAP, annual potential evapo-transpiration—PET and annual Aridity), and eight soil properties (pH, soil conductivity, soil moisture—SM, total soil carbon—TC, total soil nitrogen—TN, C:N ratio, dissolved organic carbon—DOC and dissolved total nitrogen—DTN). DTN is the sum of ammonium, nitrate and dissolved organic nitrogen. The measurement of soil properties was described in our recent study on soil bacterial communities (Chu et al., 2016), and soil conductivity was newly determined with a soil to water ratio of 1:5 by conductivity meter (Mettler Toledo FE30, Shanghai, China). MAT and MAP were compiled from the WorldClim database (www.worldclim.org) at 30 arc second resolution. PET and Aridity were obtained from CGIAR-CSI Global-Aridity and Global-PET database (http://www.cigar-csi.org). In addition, net primary productivity—NPP was compiled from the Atlas of the Biosphere (www.sage.wisc.edu/atlas/maps.php).

2.3. DNA extraction and MiSeq sequencing

Total DNA from each sample were extracted under sterile conditions from 0.5 g of soil by using a FastDNA® Spin kit (Bio 101, Carlsbad, CA, USA) according to the manufacturer's instruction, and stored at -40℃. Extracted DNA was diluted to approximately 25 ng/μl with sterilized distilled water and stored at -20℃ until use. Then the diluted DNA were frozen-transported to the Novogene Bioinformatics Technology Co., Ltd., Beijing, China, where they were defrosted, and analyzed using the Illumina MiSeq platform PE250 and the primers ITS3 (5'-GCATCGATGAAGAACGCAGC) / ITS4 (5'-TCCTCCGCTTATTGATATGC) (White et al., 1990). Specifically, soil DNA were amplified by a T100 Thermal Cycler (Bio-rad, USA) in quadruplicate to alleviate environmental PCR bias. PCR system was 30 μl, including 15 μl Phusion Master Mix (New England Biolabs, USA), 1.5 μl each of 2 μM forward and reverse primers, 10 μl DNA template (10 ng μl^{-1}) and 2 μl H_2O. PCR condition was 1

min at 98 °C; 30 cycles of (10 s at 98 °C; 30 s at 52 °C; 30 s at 72 °C); 5 min at 72 °C. Length of the PCR amplicons was ca. 350 bp. Then we mixed equimolar PCR products to produce equivalent sequencing depth, and purified them by using GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific Inc., USA). The amplicons' concentration was determined with a Qubit Fluorometer (Thermo Fisher Scientific Inc., USA). The library was constructed by using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, USA). The sequence data associated with this study were submitted to the European Nucleotide Archive under the accession number PRJEB18012.

2.4. Bioinformatics

Paired-end reads from the original DNA fragments were merged using FLASH V1.2.7 (Magoc and Salzberg, 2011). The original quantity of reads was 1 605 898. QIIME v. 1.9.0 (Caporaso *et al.*, 2010) and cutadapt 1.9.1 (http://dx.doi.org/10.14806/ej.17.1.200) were used to quality filter, trim length, chimera check, cluster and assign sequences. It resulted in 1 403 192 high-quality reads after quality filter (parameters: minlength=280; maxambigs=0, and phred quality threshold=30). Then ITSx 1.0.11 (http://microbiology.se/software/itsx/) was used to remove the flanking large ribosomal subunit (LSU) and 5.8S genes according to the Users' guide (Bengtsson-Palme et al., 2013). We removed the putative chimeric sequences by using a combination of de novo and reference-based Chimera checking, with the flags --non_chimeras_rentention=union (Edgar et al., 2011). After that, the remaining sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using the USEARCH algorithm (Edgar, 2010). All the singletons (clusters of size 1) were removed during the USEARCH clustering process, with the flags –g 2, because some singletons represented artifacts or contaminants, and inflated alpha diversity erroneously (Kunin et al., 2010; Tedersoo et al., 2010). Taxonomy was assigned to fungal OTUs by rdp option in the parallel_assign_taxonmy_rdp.py with mini-confidence of 0.8 (Wang et al., 2007). The reference OTU database were the latest QIIME releases version from UNITE database (Version 7; http://unite.ut.ee). The "dynamic" representative/reference sequence file was used according to the recommendation of the manual (Koljalg et al., 2013). 104 OTUs (779 sequences) unassigned to fungi were removed prior to subsequent analysis. The final result was 1 356 770 fungal sequences

(min: 22 466, max: 92 943, mean: 52 183) covering 5 438 OTUs in 26 soil samples. In order to analyze the alpha and beta diversity of soil fungi at the same sequencing depth, the number of reads was rarefied to 22 466 per sample.

2.5. Statistics

Based on the unrarefied OTU table, fungal community structure was summarized at the phylum level with *summarize_taxa_through_plots.py* script in QIIME. Significant differences in the relative abundance of Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota between the surface and subsurface soil were tested with the Independent t-test using R stats package (R core team, 2017). Before the t-test, the original relative abundance data were arcsine-sqrt transformed to satisfy a normal distribution. We also manually blasted the ten most dominant OTUs in the surface and subsurface soil, respectively, in the GenBank Nucleotide database. Their putative taxonomic names and ecological niches were determined by the pre-stored information of specific GenBank Nos..

The observed OTUs and predicted Chao1 (Chao, 1984) were used to compare fungal alpha diversity between different soil layers. Significant differences in alpha diversity between the two layers were tested with the Independent t-test. Pearson correlation analyses were used to identify the environmental predictors of fungal richness in the two soil layers and for all soil samples using R psych package (Revelle, 2016). The original geographic variables (latitude, longitude and elevation) were not included in the tested environmental variables, because they have no direct effects on soil fungi, and indirectly affect fungal diversity by climate and soil properties (Pellissier et al., 2014; Delgado-Baquerizo et al., 2016). All the environmental variables and diversity indexes meet the criteria for normality and homogeneity of variances by Kolmogorov-Smirnov test and Levene's test, respectively, as implemented in SPSS Statistics 20.0 for windows (IBM-SPSS, Chicago, Illinois, USA).

In terms of fungal beta diversity, the rarefied OTU table was firstly Hellinger-transformed as in other studies (Shi et al., 2014; Balint et al., 2015), and Bray-Curtis dissimilarity index (BC) was calculated by function *vegdist* in R vegan package (Oksanen et al., 2017). Significant differences in Bray-Curtis dissimilarity among three groups (the dissimilarity between two soil layers within each

site, the dissimilarity between different sites within the surface soil, and the dissimilarity between different sites within the subsurface soil) were tested with Games-Howell tests in SPSS Statistics 20.0. Non-metric multidimensional scaling analyses (NMDS) were performed with the *metaMDS* function in vegan package, and the *ordiellipse* function was employed to fit the ellipse lines at the confidence areas (0.95) by two soil layers onto the NMDS ordination. Significant differences in community composition between two soil layers were tested by permutational multivariate analysis of variance (Adonis) and analysis of similarity (ANOSIM) in vegan package.

The environmental dissimilarity was calculated based on Euclidean distance, and geographic distance was calculated based on latitude and longitude in PASSaGE2 (www.passagesoftware.net). We used Mantel tests to identify the environmental drivers of fungal community composition in the two soil layers and for all soil samples. The effects of geographic distance on the surface and subsurface soil fungal communities were tested by the distance decay model, that reflects the decrease in community similarity with increasing geographic distance (Nekola and White, 1999; Hanson et al., 2012). The similarity index was represented by 1-BC, where BC depicted Bray-Curtis dissimilarity index. Mantel tests were also used to examine the significant correlation between fungal communities and geographic distance in the surface and subsurface soil, respectively. In addition, we performed a multivariate analysis—variation partitioning analysis (VPA) to disentangle the effects of soil, climate and space on community composition in the surface and subsurface soil, respectively, as implemented with the function *varpart* in vegan package. Here, PCNM (principal coordinates of neighbour matrices) vectors with significant positive spatial autocorrelation were selected as the proxies of spatial variables (Borcard et al., 2011), and the variables from each part (i.e. soil, climate and space) were forward selected before VPA (McArdle and Anderson, 2001).

The modified Raup-Crick probability metric was used to infer the relative dominance of different community assemblage processes in the surface and subsurface soil, respectively (Chase et al., 2011). This measure compared the deviations in site-to-site dissimilarity from the stochastic null model expectations (3999 randomized matrices). The values ranging from -0.95 to -1 indicated high homogenizing dispersal. The values ranging from -0.95 to 0.95 indicated ecological drift, and

the values ranging from 0.95 to 1 indicated environmental selection. Network-based visualization was performed to discern the relative proportions of the fungal cosmopolitan group (the presence in all sites), endemic group (the presence in one unique site) and shared group (the presence in 2–12 sites) in the surface and subsurface soil layers, respectively. The diagram was generated by Cytoscape 3.4.0 (Cline et al., 2007), and the layouts were based on the same algorithm (Kamada and Kawai, 1989). The OTUs' number and sequences' proportion of endemic group were compared between the surface and subsurface layers with the Independent t-test. For the shared group, the distribution range of every OTU was calculated based on the maximum distance between individuals of that OTU, as implemented with a Perl script (https://github.com/sunhuaibo/yangt_sbb/blob/master/shared_otu_range.pl). The distribution ranges of shared OTUs in the surface and subsurface soil were then displayed with a smooth density estimate and tested by the Independent t-test.

3. Results

3.1. Data characteristics

A total of 5 438 fungal operational taxonomic units (OTUs, all are non-singletons) were retrieved from 1 356 770 high-quality ITS2 sequences in 26 soil samples. There were 856 802 and 499 968 reads in the surface and subsurface soil, respectively. The surface and subsurface soil each had 2 925 and 796 exclusive OTUs, respectively. The number of shared OTUs between the two soil layers was 1 717 (Fig. 1). The most abundant phylum was Ascomycota, accounting for more than 90% of total sequences, followed by Chytridiomycota (~1.9%), Basidiomycota (~1.3%) and Zygomycota (~1.2%). The relative abundance of Ascomycota in the subsurface soil was significantly higher than in the surface soil, while the relative abundances of Basidiomycota and Zygomycota in the surface soil were significantly higher than those in the subsurface soil (Table S1). Blast searches in GenBank for the most 17 abundant OTUs in the surface and subsurface soil showed that they were widely distributed in various habitats and across different continents (Table S2).

3.2. Variation in diversity and community composition between soil layers

Fungal richness in the surface soil was significantly higher than that in the subsurface soil

(*P*<0.001), and this soil depth-based trend was also statistically significant for predicted Chao1 (Fig. 2). Among the measured soil properties, DTN (dissolved total nitrogen) was significant different between the surface and subsurface layers (*P*=0.001, Fig. S1 and Table S3), and fungal richness was significantly positive related to DTN for all samples (Pearson r=0.69, *P*=0.001). In the surface soil, C:N ratio and PET (potential evapo-transpiration) showed the negative correlations with fungal richness, whereas no environmental variables were found to be related to fungal richness in the subsurface soil (Table S4).

An NMDS plot showed a clear differentiation in fungal communities between two soil layers (Fig. 3A), and a closer clustering of the points from within each soil layer, despite the large distances from 14 to 925 kilometers between each site within the same soil layer. Adonis analysis also corroborated that fungal community in the surface soil was significantly different from that in the subsurface soil (R^2=0.23, *P*=0.001). In terms of pairwise Bray-Curtis dissimilarity, community dissimilarity between the two soil layers within each site (0–15 cm vs 15–30 cm, separated by a few centimeters), significantly exceeded that between different sites (spatially separated by many kilometers) in the surface layer (Fig. 3B). However, community dissimilarity between two soil layers within each site equaled that between different sites in the subsurface layer (Fig. 3B).

Mantel tests showed that C:N ratio and Aridity Index significantly affected the community composition for all 26 samples (Table S5). For surface-soil fungal communities, PET was an environmental driver, while soil conductivity, SM (soil moisture), TC (total carbon), C:N ratio, PET and Aridity drove the variation in fungal community in the subsurface soil (Table S5). With increasing geographic distance, community similarity did not significantly decrease in the surface soil, while community similarity significantly decreased with increasing geographic distance in the subsurface soil (Fig. 4). Variation partitioning analysis further confirmed that soil, climate and space cumulatively explained 27% of variation in subsurface-soil fungal communities, which was much greater than the explained variation in surface-soil fungal communities (Fig. S2).

3.3. Different assemblage processes between soil layers

Fungal community turnover (pairwise Bray-Curtis distance between each site) in the surface soil was significantly lower than that in the subsurface soil (Fig. 3B), and community dissimilarity

between each site was significantly lower than expected by the null model (3999 randomized matrices) in the surface soil (Table 1A). Most of the Raup-Crick indexes were -1, which reflected a strong homogenizing-mixing effect on surface-soil fungal assemblages due to high dispersal (Table 1A). In the subsurface layer, 29.5% of Raup-Crick indexes were between 0.95 and 1, and 42.3% of indexes were between -0.95 and 0.95, which indicated that environmental selection coupled with ecological drift was the main determinant for subsurface-soil fungal assemblages (Table 1B).

The network Venn diagram showed that the surface-soil samples (black points) converged at the center, with many shared OTUs (green points) and cosmopolitans (red points) surrounding them, whereas the subsurface-soil samples (black points) were dispersed with respect to sites, and each of them was individually surrounded by their endemic OTUs (yellow points) (Fig. 5A). Statistically, 132 OTUs (i.e. cosmopolitans) were distributed across all the sites in the surface layer, accounting for 60% of sequences. Oppositely, the number of cosmopolitans in the subsurface layer was only 4, accounting for 9.6% of sequences (Fig. 5B). The proportion of endemic OTUs in subsurface was significantly larger than that in surface (Fig. 5C). In addition, the distribution range of OTUs shared among 2–12 sites was significantly larger in the surface soil relative to that in the subsurface soil (P<0.001, Fig. S3).

4. Discussion

We observed a sharp decline in fungal diversity from surface to subsurface soil layers, all across the region (Fig. 2). This was consistent with our hypothesis, which stated that fungal diversity is significantly lower in the subsurface soil than in the surface soil. The result was also in general agreement with the findings of fungal diversity change with soil depth in a local scale study of temperate forest (O' Brien et al., 2005), as well as in a glacier forefield (Rime et al., 2015). However, these trends have not been found in all environments. For example, Mueller et al. (2015) and Xiao et al. (2016) found that fungal richness was significantly higher in the deeper bulk soil relative to the surface soil in arid shrubland and arid grassland ecosystems, respectively. We reasoned that the deeper soil in those arid regions might provide more suitable micro-habitats for fungi, compared to the strong solar radiation influx and rapid drying of the surface parts of the soil. In

addition, a continental scale study of soil fungal diversity in pine forest ecosystem did not reveal any significant difference in fungal richness with soil depth (Talbot et al., 2014), indicating that fungal diversity trends in relation to soil depth are also constrained by spatial scales.

In general, nutrient availability, fungal biomass and enzyme activities decrease with increasing soil depth (Stone et al., 2014; Sinsabaugh et al., 2015; Struecker and Joergensen, 2015; Grishkan and Kidron, 2016). In this study, the DTN (dissolved total nitrogen) content was significantly lower in the subsurface soil than in the surface soil, and a significant positive correlation was observed between fungal richness and DTN for all soil samples (Pearson r= 0.69, *P*=0.001). C:N ratio was negatively related to fungal richness in the surface soil (Pearson r= -0.57, *P*=0.043), which was similar to our recent findings in alpine grasslands across the Tibetan Plateau (Yang et al., 2017) and another study in the maritime Antarctic (Newsham et al., 2016). The observed effects of DTN and C:N ratio appear to suggest that soil nitrogen availability may be a limiting factor on fungal richness in Nagri drylands, which may be different from other arid regions. In addition, we reasoned that high dispersal intensity of fungal spores from other regions and within the sampled region may also enhance the fungal richness in the surface layer. Most of putative dominant fungi we found in the surface soil in our study had previously been observed in other regions located in different continents (Table S2), which was similar to the findings in the study of soil fungal communities in the North American Arctic (Timling et al., 2014). In terms of subsurface-soil fungal communities, it appears that the enclosed microhabitat blocks the immigration of exogenous fungal species, maintaining an ecologically specialized and less diverse local species pool.

Although our study region extended over 900 km, fungal community composition differed significantly more between the two soil layers than between different sites, and no obvious variation of soil fungal communities was found between sites in the surface layer (Fig. 3A, Fig. 5A). This result was in general agreement with the description of soil fungal community composition in black spruce forests in interior Alaska (Taylor et al., 2014), which pointed to the very strong soil-horizon partitioning of fungal communities at the regional scale as well as the very uniform community composition in the surface soil. However, our results for fungi differ from our observations of soil bacterial distribution in the Ngari (Chu et al., 2016). The soil depth-based

differences we observed in fungal communities were much stronger than had been the case for bacterial communities in the same soils (ANOSIM R=0.67 vs R=0.37), and interestingly fungal community dissimilarity between the surface and subsurface soil within each site exceeded that between different sites in the surface soil layer (Fig. 3B), which was also not found in our bacterial study (Chu et al., 2016). Within the same soil layer, different sites were in some cases more than 900 km distant, whereas pairwise soil samples collected from the same soil cores but different depth were less than thirty centimeters apart. This strongly suggests that for soil fungi in this environment, the niche differentiation seen between surface and deep soil in the same core is far greater than for surface samples hundreds of kilometers apart. In our study, soil depth explained 23% of variation in fungal community composition, while space, climate and measured soil properties accumulatively explained 10% of variation (Fig. S2). Similarly, in a study in southern California, Kivlin et al. (2014) found niche filtering rather than dispersal limitation had a dominant role in determining soil fungal community assemblages across a 40 000 km^2 regional landscape. Morrison-Whittle and Goddard (2015) proposed that niche differentiation explained four times more of the variation of fungal communities than geographic location across 1000 km in New Zealand. However, with further increasing geographic distance, it appears likely that broad-scale driving forces such as climate and dispersal limitation would prevail in affecting soil fungal assemblages (Tedersoo et al., 2014; Treseder et al., 2014), and thus the effect of soil depth may then appear relatively small. For example, a study on soil fungal communities across North American forests demonstrated that there was no significant difference in fungal community composition between the organic and mineral soil horizons at the continental scale (Talbot et al., 2014). McGuire et al. (2013) also found that fungal communities were most strongly differentiated by biome, between the boreal and tropical ecosystems, and comparatively weakly segregated by different soil horizons.

Previously in our study of the same region, bacterial community composition in both the surface and subsurface soil was found to be driven by shared environmental variables — total carbon and C:N ratio, and geographic distance contributed equally to the variation in community composition in each of the two soil layers (Chu et al., 2016). In the present study, in the surface soil there was

lack of any significant distance-decay relationship for fungal communities (Fig. 4), and none of the measured soil properties significantly affected fungal community composition (Table S5). Although the primary dispersal vectors should be similar for surface-soil bacterial and fungal communities, the differences in ecology (e.g. colonization capacity) between fungi and bacteria (Peay et al., 2016), and difference in the methodology (Miseq vs 454) might contribute to the different pattern in dispersal between this study and our previous study (Chu et al., 2016). This result also contrasts with the findings in many regional-scale studies, in which soil properties and geographic distance significantly affect fungal community composition (Bahram et al., 2013; Shi et al., 2014; Liu et al., 2015; Chen et al., 2017; Yang et al., 2017). However, other studies demonstrated the absence of a distance effect that might involve dispersal limitation for surface-soil fungal communities on the polar region (Geml et al., 2012; Timling et al., 2014; Cox et al., 2016). In addition, Favet et al. (2013) observed that various fungal species hitchhiked on dry dust in northern Chad to travel long distance across continents, and Itani and Smith (2016) reported that dust rains (i.e. wet deposition) delivered diverse fungal communities from arid regions of North Africa to the Eastern Mediterranean. By contrast, in the subsurface soil in our study, fungal community similarity decreased significantly with increasing geographic distance between sites (R^2=0.43, *P*=0.001, Fig. 4), and soil, climate and spatial distance cumulatively explained 27% of variation in subsurface-soil fungal communities, which was more than four times of the amount of variation explained in surface-soil fungal communities (Fig. S2).

Consistent with our major hypothesis – that different community assemblage processes prevail in the surface and subsurface soil, respectively – we found statistical evidence of stochastic process with high dispersal prevailing in the surface soil, and deterministic process with edaphic, climatic and spatial effects dominant in the subsurface soil. In the surface soil layer, almost all the modified Raup-Crick dissimilarity indexes were -1 (Table 1A), which indicated that the fungal communities compared across sites were significantly more similar to one another than expected by random chance (Chase et al., 2011). Recently, Bahram et al. (2016) used this null-model method to infer a stochastic distribution of soil fungi due to high dispersal and drift in a cool temperate forest in Estonia. In their study, they proposed that values –1 represented the homogenizing effect of high

dispersal. In general, the small-scale studies (e.g. Bahram et al., 2016), have two characteristics – a narrow environmental gradient and short spatial distance between each sample locality. These two characteristics may be expected to cause stochastic processes to overwhelm deterministic processes (Wang et al., 2013). In the case of our study, one feature that was different from these previous studies was the very large spatial distance, over 900 km. However, in our study too, the range of variation in environmental variables in the surface soil was not large (Table S3). The pH range across sites was between 8.2 and 9.0, and the MAP (mean annual precipitation) variation was 156–315 mm/yr. In addition, the sparse vegetation, loose topsoil and frequent dust storms (gale-force winds lasting more than 100 days/yr in Nagri) may mainly contribute to a high dispersal rate of fungal spores in the surface soil (Troll et al., 1972; Chang and Gauch, 1986; Qiu, 2014). Recently, Weil et al. (2017) found that whole microbial communities were transported from the Sahara to the Alps by desert dust storms, which strongly corroborates the hypothesis that extreme meteorological events can efficiently facilitate the long-distance dispersal of fungi and bacteria. In the present study, the surface-soil samples had an unusually large proportion of cosmopolitans, which accounted for 60% of sequence reads (Fig. 5B). The proportion of locally endemic OTUs in the surface soil was significantly smaller than in the subsurface soil (Fig. 5C). In addition, we found a significantly greater distribution range of shared OTUs in the surface layer than in the subsurface layer (Fig. S3).

The Baas-Becking hypothesis that “Everything is everywhere, but, the environment selects” is a famous microbiological tenet (Bass Becking, 1934), but the question has been raised whether microbes are really without dispersal limitation (Martiny et al., 2006; Hanson et al., 2012; Peay et al., 2016). For soil fungi, some studies have supported an effect of dispersal limitation, including examples of apparent endemism (Talbot et al., 2014), OTU-area relationships (Pellissier et al., 2014) and distance–decay relationships (Bahram et al., 2013; Meiser et al., 2014; Liu et al., 2015), whereas others have pointed to the absence of dispersal limitation and the existence of global fungal cosmopolitans (Timing et al., 2014; Davison et al., 2015; Cox et al., 2016; Itani and Smith, 2016). In fact, whether or not fungal community assemblages are predominantly affected by stochastic processes with high dispersal is apparently context-dependent and guild-dependent.

Kilvin et al. (2014) provided evidence that dispersal rate of fungal spores differed between fungal taxa, and was affected by both stochastic and deterministic processes. Both endemic taxa and cosmopolitans should exist simultaneously, but their relative proportions may differ significantly among habitats and ecosystems. High dispersal efficiency has been expected to be more prevalent in extreme environments (Caruso et al., 2011; Favet et al., 2013; Timling et al., 2014; Cox et al., 2016), and (as we found here) in the surface soil layer rather than deeper soil layers.

5. Conclusions

In summary, we report here the striking pattern of community clustering based on soil depth rather than broad scale distance, and find that in this environment dispersal limitation does not structure fungal communities in the surface soil but does in the subsurface soil. Unlike the pattern observed for soil bacteria (Chu et al., 2016), fungal richness was three times greater in the surface soil than in the subsurface soil. Also, community dissimilarity between the surface and subsurface layers within each site (only 30 cm apart) significantly exceeded that between different sites in the surface layer that were between tens and hundreds of km apart. Soil depth explained more variation in community composition than the sum of spatial and environmental variables, which suggested the primary importance of niche differentiation in soil fungal assemblages.

Importantly, it appears that stochastic processes in the context of high dispersal rates predominated in the fungal community assemblages of the surface layer, whereas deterministic processes with respect to the effects of soil, climate and space were more important in subsurface soil fungal community assemblages. These results highlight the importance of stratified soil sampling in barren lands, if one is to form the comprehensive view of soil fungal biodiversity and biogeography. In addition, we suggest that in a windy and dry region with a lack of plant cover, surface-soil fungi with highly evolved dispersal spores may interfere with the emergence of regional endemism.

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Figure legends

Fig. 1. (A) Total fungal diversity (number of observed OTUs) between the surface and subsurface layers and their overlap. (B) The thirteen study sites in Ngari, Tibet. (C) Relative abundance of four fungal phyla for all the 26 samples (All), the surface-soil samples (Surface) and the subsurface-soil samples (Subsurface).

Fig. 2. Fungal alpha diversity between the surface and subsurface soil. The asterisk means the significant difference in fungal alpha diversity between the two soil layers.

Fig. 3. Fungal community composition between the surface and subsurface soil. (A) Two-dimensional ordination using NMDS, based on the average Bray-Curtis dissimilarity matrix, computed from 26 rarefied and hellinger-transformed sites×OTUs matrices. The purple squares represented the subsurface-soil samples, and the green squares represented the surface-soil samples. Two ellipses represented the confidence areas (0.95) of the subsurface and surface layers. (B) The comparison of Bray-Curtis dissimilarity among three groups. “Surface vs Subsurface” means the community dissimilarity between the surface and subsurface soil samples within each site, and “Within Surface” (or “Within Subsurface”) means the community dissimilarity between pairwise sites in the surface (or subsurface) soil layers. Of note, “Within Surface(-1)” means the community dissimilarity between pairwise sites in the surface-soil samples after removing one outlier.

Fig. 4. The distance-decay pattern for fungal community similarity in the surface and subsurface soil. The correlation between fungal community dissimilarity and geographic distance was not significant in the surface soil (Mantel r=0.142, *P*=0.181), and the correlation between fungal

community dissimilarity and geographic distance was significant positive in the subsurface soil (Mantel r= 0.659, *P*=0.001).

Fig. 5. The network analysis on the fungal OTUs in the surface and subsurface soil. Each point represented one independent OTU except that the black points represented soil samples. Red color represented the cosmopolitans, i.e. OTUs shared among all the 13 sites. Green color represented the shared OTUs among two to twelve sites. Dark yellow color represented the endemic OTUs, i.e. the OTUs found exclusively in one site. (A) The network diagram of the surface and subsurface soil fungal communities, (B) The OTUs and sequences proportion for three kinds of OTUs in the two soil layers, (C) The comparison of endemic OTUs and sequences between the surface and subsurface soil. The asterisk means the significant difference between the two layers.

Fig. 1

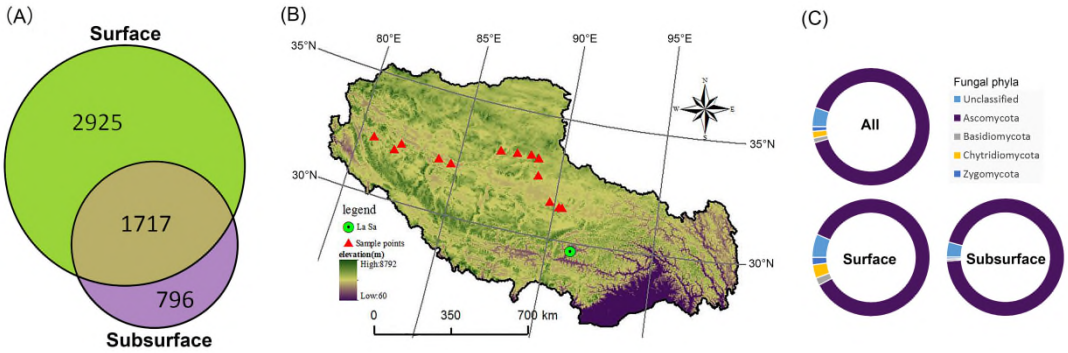
(A)
Surface
2925
1717
796
Subsurface
(B)
80°E
85°E
90°E
95°E
35°N
30°N
legend
La Sa
Sample points
elevation(m)
High:8792
Low:60
0
350
700 km
(C)
All
Fungal phyla
Unclassified
Ascomycota
Basidiomycota
Chytridiomycota
Zygomycota
Surface
Subsurface

Fig. 2

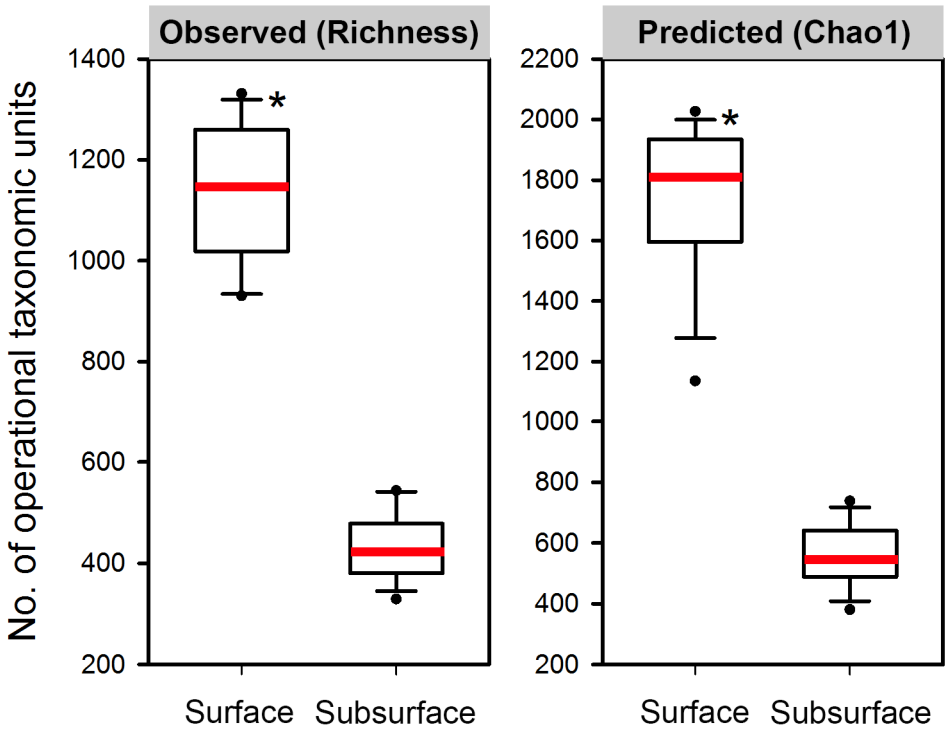
Observed (Richness)
Predicted (Chao1)
No. of operational taxonomic units
1400
1200
1000
800
600
400
200
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
*
*
Surface
Subsurface
Surface
Subsurface

Fig. 3

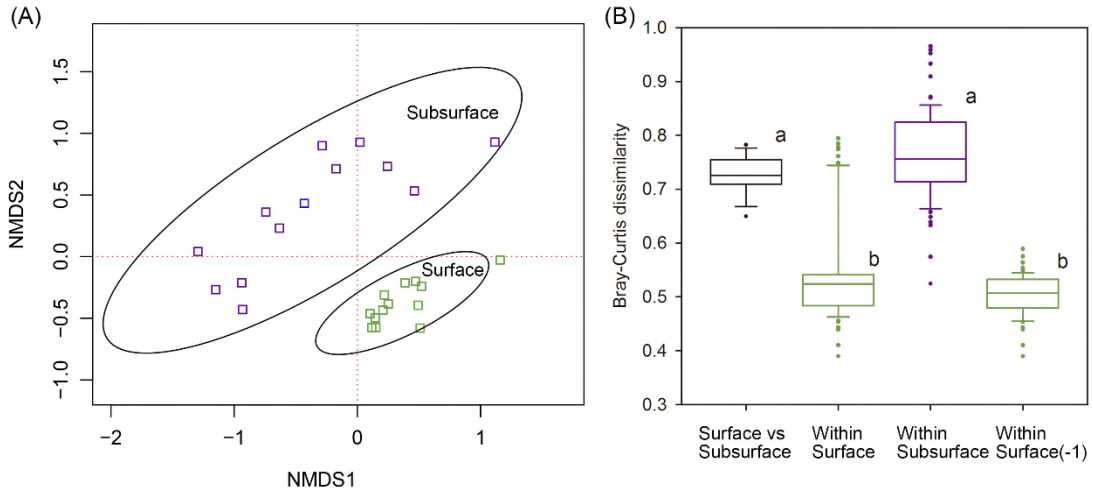
(A)
NMDS2
NMDS1
Subsurface
Surface
(B)
Bray-Curtis dissimilarity
a
b
a
b
Surface vs Subsurface
Within Surface
Within Subsurface
Within Surface(-1)

Fig. 4

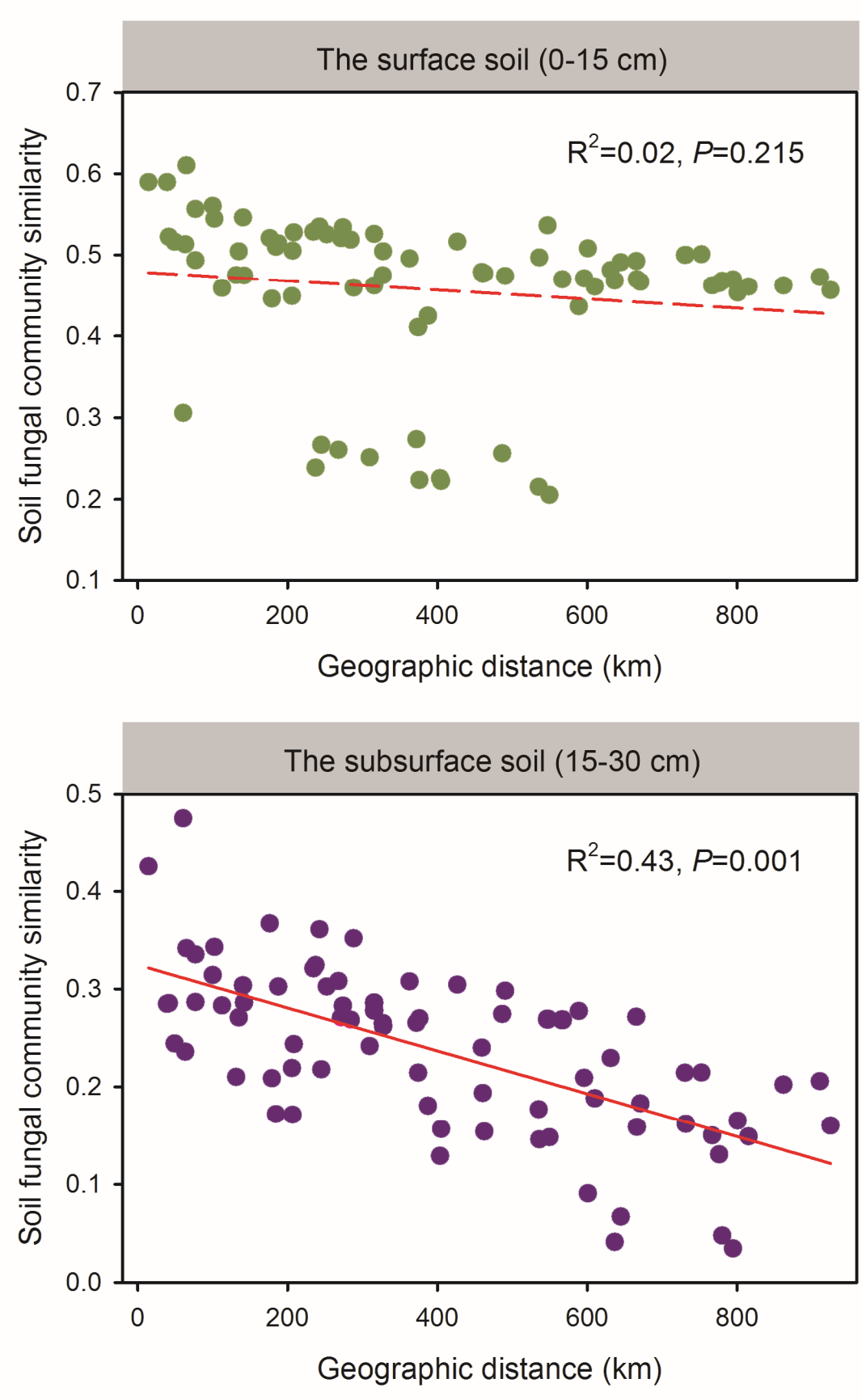
The surface soil (0-15 cm)
R^2=0.02, *P*=0.215
Soil fungal community similarity
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
200
400
600
800
Geographic distance (km)
The subsurface soil (15-30 cm)
R^2=0.43, *P*=0.001
Soil fungal community similarity
0.5
0.4
0.3
0.2
0.1
0.0
0
200
400
600
800
Geographic distance (km)

Fig. 5

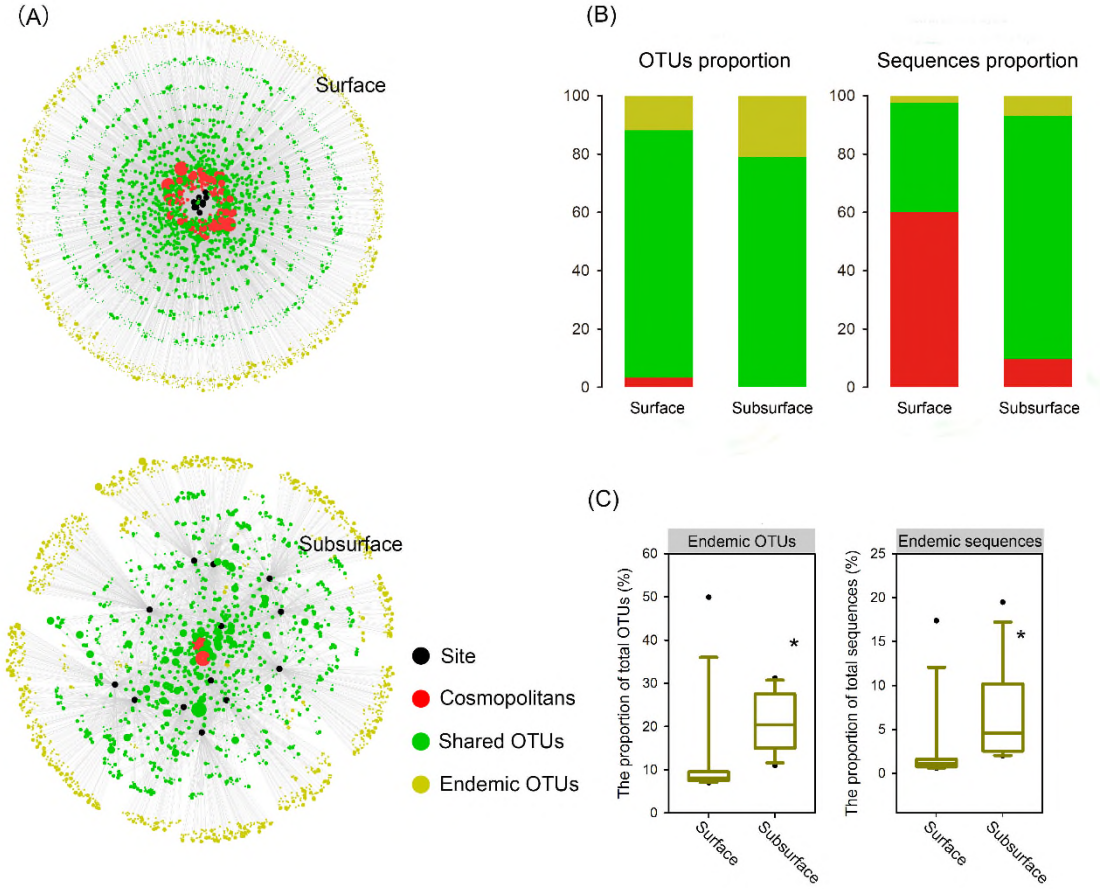
(A)
Surface
Subsurface
Site
Cosmopolitans
Shared OTUs
Endemic OTUs
(B)
OTUs proportion
Sequences proportion
100
80
60
40
20
0
Surface
Subsurface
(C)
Endemic OTUs
Endemic sequences
The proportion of total OTUs (%)
The proportion of total sequences (%)
60
50
40
30
20
10
0
25
20
15
10
5
0
*

Table 1 The modified Raup-Crick dissimilarity matrices between each site in the surface and subsurface soil based on the null-model expectations.

(A) The surface soil (0-15 cm)

Raup-Crick indexes	1A	2A	3A	4A	5A	6A	7A	8A	9A	10A	11A	12A
2A	-1											
3A	-1	-1										
4A	-1	-1	-1									
5A	-1	-1	-1	-1								
6A	-1	-1	-1	-1	-1							
7A	-1	-1	-1	-1	-1	-1						
8A	-1	-1	-1	-1	-1	-1	-1					
9A	1	1	1	1	1	1	1	1				
10A	-1	-1	-1	-1	-1	-1	-1	-1	1			
11A	-1	-1	-1	-1	-1	-1	-1	-1	1	-1		
12A	-1	-1	-1	-1	-1	-1	-1	-1	1	-1	-1	
13A	-1	-1	-1	-1	-1	-1	-1	-1	1	-1	-1	-1

(B) The subsurface soil (0-15 cm)

Raup-Crick indexes	1B	2B	3B	4B	5B	6B	7B	8B	9B	10B	11B	12B
2B	-1											
3B	0.51	0.83										
4B	-1	-1	0.9									
5B	-1	-1	1	-1								
6B	-0.26	-0.1	-0.19	-0.99	-0.97							
7B	-1	-1	0.5	-1	-1	-1						
8B	0.86	0.67	0.73	0.45	0.08	-0.36	-0.81					
9B	1	0.98	-0.84	1	0.99	-0.07	-0.94	-1				
10B	0.04	-0.25	-0.5	1	0.51	-0.96	-1	-1	-1			
11B	1	1	1	1	1	1	0.94	-0.97	-1	0.07		
12B	1	1	1	1	1	1	-0.91	-0.97	-1	-0.73	-1	
13B	1	0.98	1	1	1	1	-0.93	-0.56	-0.93	-0.97	-0.15	-0.65

A represents the 13 samples in the surface soil layer, and B represents the 13 samples in the subsurface soil layer. Positive and negative Raup-Crick indexes represent higher and lower

dissimilarities between each site compared with the null model expectations, respectively. The values near -1 (-0.95 to -1) indicate high dispersal, and the values near 1 (0.95 to 1) indicate environmental selection. The values between -0.95 and 0.95 indicate ecological drift.

Supplementary Tables and Figures

Table S1 – Table S5

Fig. S1 – Fig. S3

References

Table S1 The variation in the relative abundances of fungal phyla between different soil depth.

	Surface (0-15 cm)	Subsurface (15-30 cm)	*P* values of t- test
Ascomycota	85.75±13.34	94.78±3.95	**0.007**
Chytridiomycota	3.72±12.92	0.03±0.10	0.160
Basidiomycota	2.07±2.16	0.51±1.20	**0.001**
Zygomycota	1.99±1.99	0.50±1.23	**<0.001**

Values are the means ± SD, n = 26. The test method was the independent t-test, which was done after the arcsine-sqrt transformation of the original relative abundance data. The significant *P* values were in bold.

Table S2 BLAST-based taxonomic affinity of the ten most abundant OTUs detected in the surface and subsurface soil.

% Abundance	Best blast match to NCBI-nr database				
	GenBank no.	Coverage	% Identity	Definition	Ecological niche
Surface soil (0-15 cm)					
4.68	KT991134	97	99	*Pseudeurotium sp.*	Soil in Fildes Peninsula, Antarctic
4.62	KR261446	100	100	*Penicillium flavigenum*	Soil in Brazil
3.97	KU612322	100	100	*Alternaria sp.*	BSC[a] in an arid grassland, America
3.47	AF216757	100	96	*Spizellomyces acuminatus*	Grassland soil in America
2.79	NR_138294	100	99	*Penicillium elleniae*	Leaf litter in Colombia
2.78	KJ443251	100	100	*Penicillium sp.*	Alkaline soil in Russia
2.31	**KX394541[b]**	**100**	**100**	***Fusarium tricinctum***	**Arthropod cadaver in America**
1.88	LT603041	100	100	*Didymella glomerata*	Surface of volumes from an archive of the University of Milan in Italy
1.66	**KT269499[c]**	**99**	**100**	***Helotiales sp.***	**Surface-sterilized, asymptomatic roots of *Microthlaspi in France***
1.32	**KT269651**	**100**	**99**	***Helotiales sp.***	**Surface-sterilized, asymptomatic roots of *Microthlaspi in France***
Subsurface soil (15-30 cm)					
12.51	KX438348[d]	100	100	*Tetracladium sp.*	Roots of *Quercus robur* in Poland
6.32	**KX394541[b]**	**100**	**100**	***Fusarium tricinctum***	**Arthropod cadaver in America**
3.68	**KT269499[c]**	**99**	**100**	***Helotiales sp.***	**Surface-sterilized, asymptomatic roots of *Microthlaspi in France***
3.24	KX011011	100	100	*Geomyces sp.*	Sub-glacial soil in India
2.74	KT269946	98	98	*Cadophora sp.*	Surface-sterilized, asymptomatic roots of *Microthlaspi* in Greece
2.7	**KT269651**	**100**	**99**	***Helotiales sp.***	**Surface-sterilized, asymptomatic roots of *Microthlaspi in France***
2.69	HG935225	100	100	*Tetracladium sp.*	*Zea mays roots in Germany*
2.65	KP160169	100	97	Uncultured fungus	Orchid roots in Belgium
2.11	KU538619	100	98	Uncultured fungus	The Cacti Microbiome in semi-arid region in Mexico
2.02	KX610348	100	100	*Pseudogymnoascus sp.*	Cave Soils in El Malpais National Monument, New Mexico, America

The three OTUs in bold were the shared dominant OTUs in the surface and subsurface soil. Coverage and similarity were derived from the results of the default megablast in NCBI Basic Local Alignment Search Tool. Every dominant OTU accounted for more than 1% of the sequences.

[a] BSC: biological soil crust.

[b] The seventh dominant OTU in the surface soil was also assigned to uncultured fungus clone (KU536886) from the Cacti Microbiome on semi-arid region in Mexico (Fonseca-Garcia *et al.*, 2016) with the same scores.

[c] The ninth dominant OTU in the surface soil was also assigned to uncultured fungus clone (KU537482) from the Cacti Microbiome on semi-arid region in Mexico (Fonseca-Garcia *et al.*, 2016) with the same scores.

[d] The first dominant OTU in the subsurface soil was also assigned to uncultured fungus (KX776493) from orchid roots in Belgium (Esposito *et al.*, 2016), or uncultured *Tetracladium* (HG935228) from *Zea mays* roots in Germany (Moll *et al.*, 2016).

Table S3 Description of soil physicochemical properties of different soil layers in each site.

Sample code	Soil layer	pH	SC (us/cm)	SM (W/W%)	TC (%)	TN (%)	C:N ratio	DOC (mg/kg)	DTN (mg/kg)
P01-A	Surface	8.51	97.5	10.43	0.95	0.14	6.86	84.8	14.36
P02-A	Surface	8.49	40.3	11	1.49	0.19	7.71	65.1	15.74
P03-A	Surface	9.01	86.6	8.4	2.92	0.07	44.43	63.8	5.55
P04-A	Surface	8.48	72.1	8.72	2.33	0.16	14.27	164.9	9.5
P05-A	Surface	8.62	77.4	6.64	1.88	0.14	13.68	94.5	15.96
P06-A	Surface	8.55	127.2	8.63	2.66	0.21	12.83	152.8	18.31
P07-A	Surface	8.69	86.9	4.53	2.42	0.07	32.65	264.5	8.5
P08-A	Surface	8.92	76.7	1.4	1.13	0.02	56.61	98.4	9.07
P09-A	Surface	8.85	114.6	9.04	3.52	0.1	35.93	140.9	5.68
P10-A	Surface	9.04	148.1	8.11	3.66	0.13	27.37	271.7	11.38
P11-A	Surface	8.21	933	7.72	5.31	0.14	37.06	244.6	12.33
P12-A	Surface	8.66	114	5.31	1.71	0.09	19.87	272.8	5.35
P13-A	Surface	8.41	27.9	3.33	0.37	0.04	8.33	48.5	8.67
P01-B	Subsurface	8.43	59.6	9.9	0.75	0.1	7.29	45.8	9.1
P02-B	Subsurface	8.6	88.1	7.47	0.87	0.08	10.24	75.9	6.05
P03-B	Subsurface	8.95	119.1	8.39	3.1	0.06	48.12	109.9	3.81
P04-B	Subsurface	8.55	95.1	10.68	2.26	0.11	21.51	543	5.6
P05-B	Subsurface	8.58	109.7	11.35	2.85	0.15	19.38	61.7	6.02
P06-B	Subsurface	8.63	121.2	9.91	2.67	0.15	17.61	152.3	8.11
P07-B	Subsurface	8.61	93.1	6.87	3.03	0.07	45.19	125	5.62
P08-B	Subsurface	8.91	115.4	4.25	1.17	0.03	45.21	246.9	3.32
P09-B	Subsurface	8.74	133.1	8.99	3.97	0.11	36.19	272	4.35
P10-B	Subsurface	8.98	120.8	4.19	2.96	0.04	65.89	219.4	3.92
P11-B	Subsurface	8.61	557	6.22	5.92	0.1	57.04	111.6	6.38
P12-B	Subsurface	8.28	124.4	6.05	2.28	0.09	26.64	27.1	4.86
P13-B	Subsurface	8.55	74.9	2.7	0.46	0.06	7.98	119.7	5.27

SC: soil conductivity, SM: soil moisture, TC: soil total carbon, TN: soil total nitrogen, DOC: dissolved organic carbon, DTN: dissolveed total nitrogen.

Table S4 The correlations (r) and significance (P) were determined by Pearson analysis between soil fungal richness and environmental variables.

	All		Surface		Subsurface	
Variables	r	*P*	r	*P*	r	*P*
pH	-0.09	0.658	-0.52	0.067	0.08	0.808
Soil conductivity	-0.01	0.971	-0.09	0.767	-0.47	0.105
SM	-0.02	0.939	0.26	0.399	-0.12	0.700
TC	-0.11	0.579	-0.18	0.556	-0.36	0.230
TN	0.34	0.088	0.40	0.179	-0.14	0.655
C:N ratio	-0.3	0.131	**-0.57**	**0.043**	-0.28	0.361
DOC	-0.06	0.783	-0.16	0.598	0.13	0.681
DTN	**0.69**	**0.001**	0.44	0.135	-0.21	0.500
MAT	-0.11	0.605	-0.52	0.068	-0.18	0.551
MAP	0.03	0.872	0.20	0.519	-0.01	0.271
PET	-0.12	0.548	**-0.59**	**0.026**	-0.24	0.430
Aridity	0.08	0.686	0.41	0.16	0.07	0.823
NPP	0.06	0.786	0.17	0.057	0.31	0.304

All means 26 soil samples including the surface soil and subsurface soil. Surface means the surface soil at the depth of 0-15 cm, and subsurface means the subsurface soil at the depth of 15-30 cm. SM: soil moisture, TC: total carbon, TN: total nitrogen, DOC: dissolved organic carbon, DTN: dissolved total nitrogen, MAT: mean annual temperature, MAP: mean annual precipitation, PET: potential evapo-transpiration, NPP: net primary productivity. The significant *P* values were in bold.

Table S5 The correlations (r) and significance (*P*) were determined by Mantel test between soil fungal community composition and environmental variables.

	All		Surface		Subsurface	
Variables	r	*P*	r	*P*	r	*P*
pH	-0.05	0.679	0.10	0.282	-0.01	0.478
Soil conductivity	-0.01	0.504	-0.06	0.39	**0.52**	**0.002**
SM	0.07	0.236	0.05	0.341	**0.24**	**0.042**
TC	0.16	0.08	0.14	0.167	**0.41**	**0.004**
TN	-0.14	0.938	-0.08	0.57	-0.13	0.818
C:N ratio	**0.17**	**0.029**	0.15	0.105	**0.25**	**0.037**
DOC	0.06	0.357	-0.09	0.683	0.01	0.398
DTN	-0.04	0.608	0.04	0.383	0.08	0.248
MAT	0.09	0.107	0.18	0.1	0.20	0.054
MAP	0.05	0.256	-0.02	0.394	0.19	0.099
PET	0.14	0.052	**0.28**	**0.016**	**0.35**	**0.013**
Aridity	**0.15**	**0.034**	0.15	0.187	**0.50**	**0.002**
NPP	0.01	0.453	0.09	0.208	-0.09	0.742

All means 26 soil samples including the surface soil and subsurface soil. Surface means the surface soil at the depth of 0-15 cm, and subsurface means the subsurface soil at the depth of 15-30 cm. SM: soil moisture, TC: total carbon, TN: total nitrogen, DOC: dissolved organic carbon, DTN: dissolved total nitrogen, MAT: mean annual temperature, MAP: mean annual precipitation, PET: potential evapo-transpiration, NPP: net primary productivity. The significant *P* values were in bold.

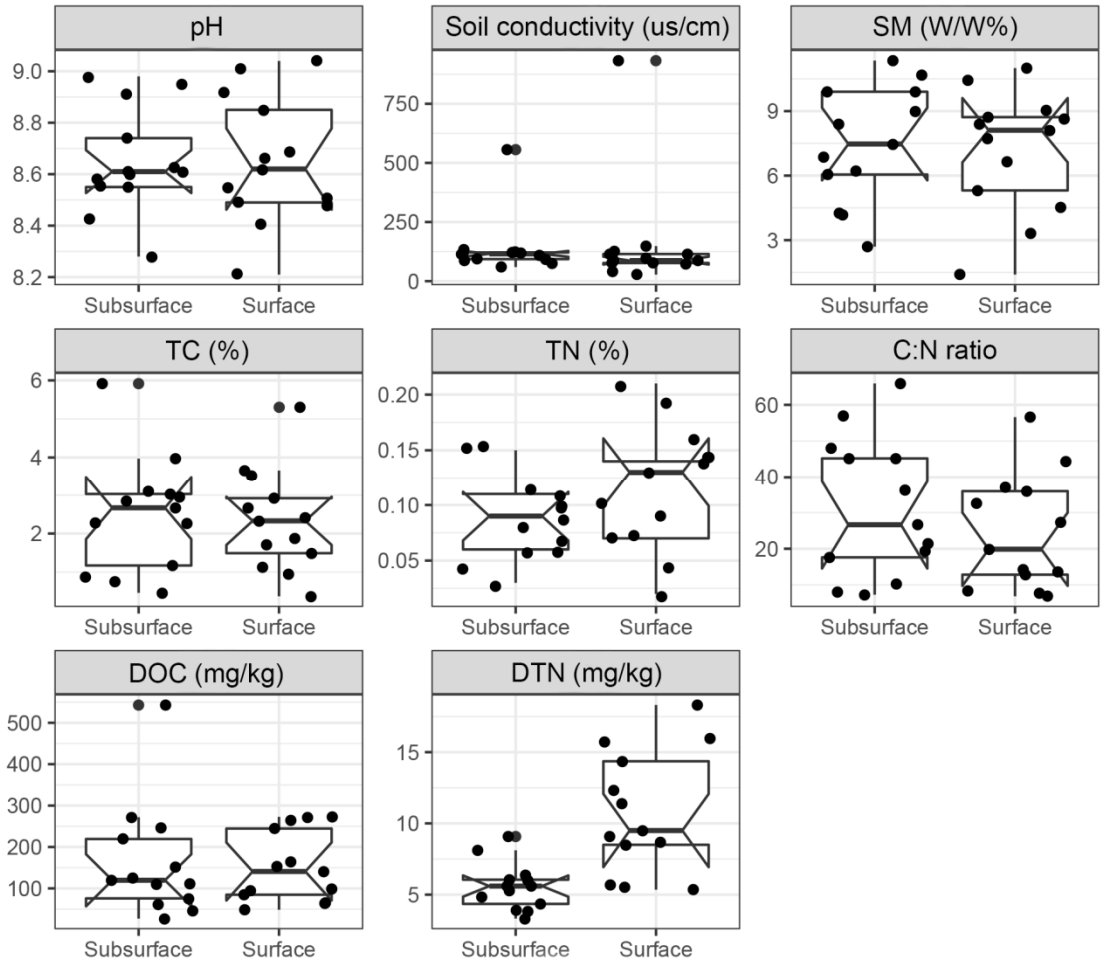

Fig. S1. The variation in soil properties between the surface and subsurface soil layers. Only DTN (dissolved total nitrogen) was significant different between the surface and subsurface soil layers, which was tested by the Independent t-test (*P*=0.001). SM: soil moisture, TC: total carbon, TN: total nitrogen, DOC: dissolved organic carbon.

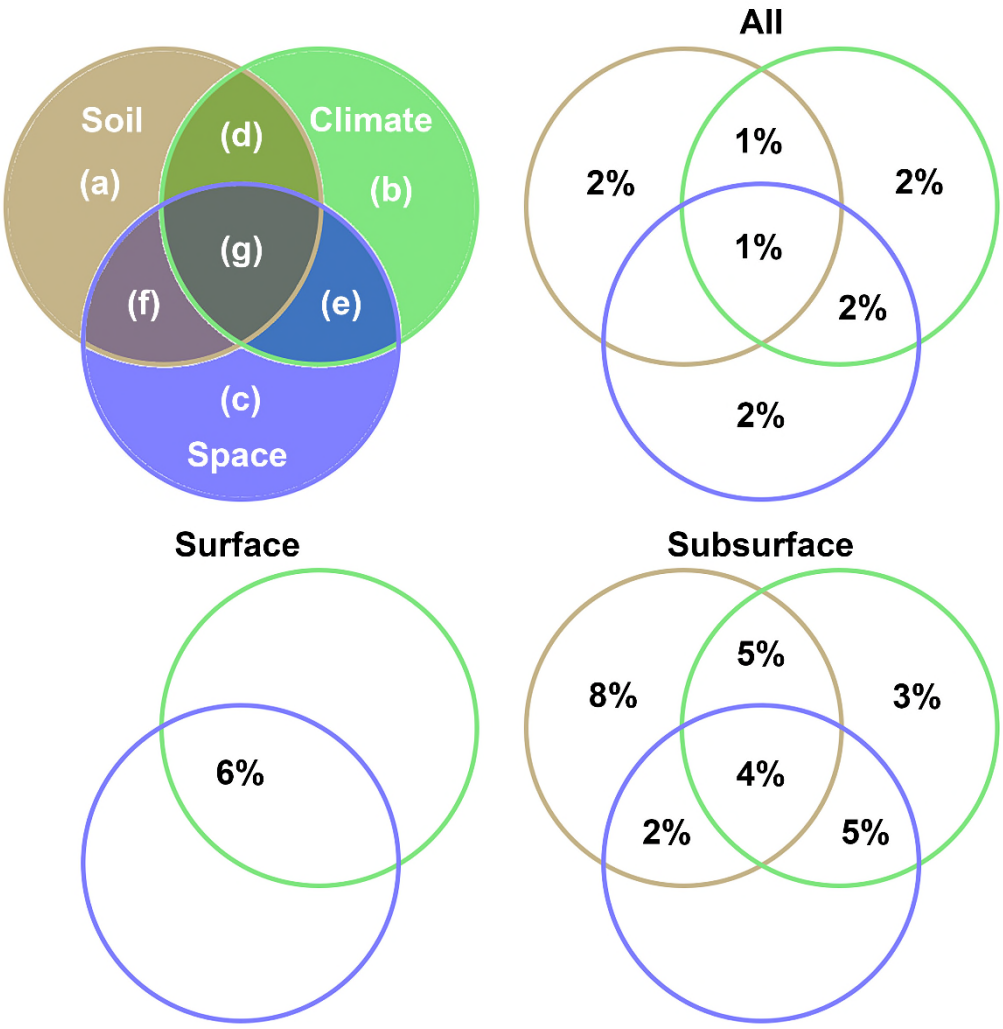

Fig. S2. Venn diagram of variation partitioning analysis, showing the effects of soil, climate and space on fungal community composition in different soil layers. Because soil properties did not significantly affect fungal community composition in the surface soil, only two cycles were shown for the surface-soil Venn diagram.

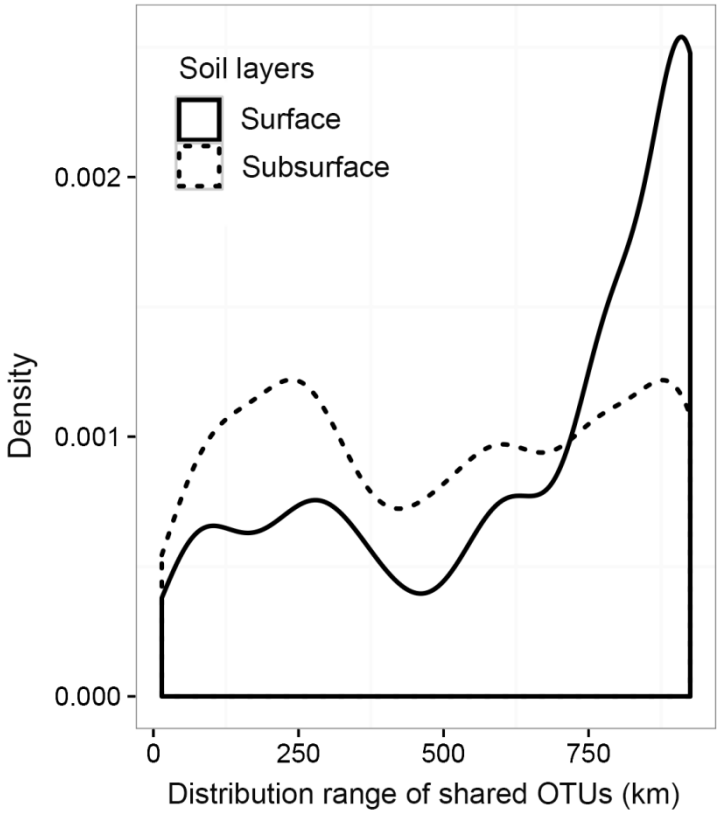

Fig. S3. The density plot of the distribution range of shared OTUs in the surface and subsurface soil. The shared OTUs are the fungal OTUs observed in 2~12 sites in the surface and subsurface soil, respectively.

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Fungal community assemblages in a high elevation desert environment: absence of dispersal limitation and edaphic effects in surface soil

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