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Environmental and spatial variables determine the taxonomic but not functional structure patterns of microbial communities in alpine grasslands

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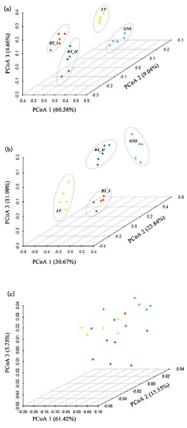
HIGHLIGHTS

- To investigate whether microbial functional structure follows their taxonomic patterns at regional scales.
- The survey of how structure–function relationships hold over large scales remains a major goal for microbial ecologists.
- Using marker genes sequencing and functional gene surveys to evaluate taxonomic and functional structure.
- The patterns of the functional structure did not follow spatial variation in taxonomic diversity.
- We present an issue regarding whether spatial variation in functional structure relates to changing taxonomic diversity.

GRAPHICAL ABSTRACT



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ABSTRACT

There is considerable debate regarding how the taxonomic diversity of microbial communities relates to the functional diversity across space while similar questions have been explored in macro-organism communities. Here, we investigated the taxonomic and functional diversity patterns of soil microbial communities by coupling the data obtained from marker genes sequencing and functional gene surveys. Meanwhile, we evaluated the relative effects of environment and geographic distance on shaping these patterns in alpine grasslands of northern China. Although the taxonomic diversity and composition of microbial communities varied across sites, we found no consistent changes in the functional structure. Both the environmental factors and geographic distance concurrently affected the taxonomic diversity patterns but they had no effects on the spatial variations in functional genes. The functional alpha diversity was weakly correlated to the taxonomic alpha diversity across sites. Moreover, we found no significant relationship between the taxonomic and functional composition similarity among

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microbial communities. Together, our results provide evidence that spatial variation in microbial functions could be independent of their variations in taxonomic diversity. Even the drivers of spatial variations in the functional structure could be totally different from those of taxonomic variations such as environmental differences and dispersal limitation. Our findings suggest that spatial variations of microbial function structure within a community would not follow the variations of taxonomic structures due to different drivers between both of them over space.

1. Introduction

Ecological responses to ongoing global climate change are projected to result in more regionally heterogeneous geographic distributions of various organisms (McCarty, 2001; Walther et al., 2002). The changing patterns in diversity and spatial assembly of ecological communities reflect their intrinsic adaptabilities and flexibilities to external environmental conditions that change over geographic gradients (Chapin et al., 2000; Wittebolle et al., 2009). Such spatial variations in species diversity and composition are often associated with the variations in functional structure (Cadotte et al., 2011; Petchey and Gaston, 2002). These changes have resulting impacts on key ecosystem processes including nutrient cycling rates, productivity, stability and resilience towards disturbance (Strickland et al., 2009; Tilman, 1997). An attempt to investigate the structure–function relationships of ecological communities therefore remains a major goal for ecologists. For example, what on earth are the across-community relationships of taxonomic structure and function? To what extent do the structure–function relationships hold over large spatial scales? Identifying such relationships is particularly critical in microbial systems (Bardgett and Van der Putten, 2014), because microbial organisms such as bacteria and fungi, comprise the majority of the Earth's biodiversity and are recognized as playing important roles in ecosystem functions (Falkowski et al., 2008; Fuhrman, 2009; Zhou et al., 2012). Until now, however, it is still unclear how microbial functions vary across geographic regions, and whether or not the spatial variation of their functional structure is associated with observed taxonomic patterns (Delgado-Baquerizo et al., 2016b; Fierer et al., 2013). Such a knowledge gap limits our ability for the prediction of Earth ecosystem models to global changes, and the understanding of the consequences of biodiversity changes for ecosystem functioning (Widder et al., 2016; Wieder et al., 2013).

Microbial biogeography has been well investigated with the ongoing development in molecular and statistical approaches in recent decades. Most studies were primarily based on taxonomic characterization analyses of ribosomal RNA genes (rDNA). Considerable evidence has demonstrated the restricted distribution of microbial communities within a given spatial scale or habitat type (Martiny et al., 2011; Wang et al., 2017). The spatial distribution of microbial communities has been found to be affected greatly by climatic (Maestre et al., 2015; Tedersoo et al., 2014), edaphic physicochemical (Shen et al., 2014), biotic factors (Prober et al., 2015) and dispersal barriers (Evans et al., 2017). Our knowledge about the phylogenetic and taxonomic biogeography of microbial communities continues to increase. However, we still lack the understanding about the intrinsic associations of spatial changes between microbial community structure and their function: whether the function will concurrently vary with changing taxonomic structure across space, whether the factors that govern the variations in taxonomic and functional structure are different across space. Generally, the structure and function of microbial communities have been frequently found to display coupled variations along an environmental or experimental gradient (Fierer et al., 2012; Marschner et al., 2003; Torsvik and Ovreas, 2002). In some cases, however, such a relationship in microbial communities may not totally be held. For example, distinct phylogenetic taxa can play a similar role in communities whereas closely related taxa may have very different physiologies and environmental tolerances (Philippot et al., 2010). In addition, taxonomic structure of a community is likely to be more sensitive to the changes in environmental conditions relative to their contributions to ecosystem functioning. Owing to high functional redundancy in soil microbes (Allison and Martiny, 2008; Bell et al., 2005; Nielsen et al., 2011), microorganisms enable to function in ecosystems in spite of the significant variation of taxonomic richness to environmental disturbance (Berga et al., 2012).

Researchers have made attempts to investigate the functional characteristics of microbial communities in the past decades. Evidence has emerged that functional characteristics of soil microbial communities, including individual microbial processes (e.g., N_2 fixation, nitrification and denitrification) (Cleveland et al., 1999; Wang et al., 2014), specific functional groups (e.g. nitrogen cycling) (Bru et al., 2011; Petersen et al., 2012), extracellular enzymes activities (Sinsabaugh et al., 2008) and microbial respiration (Delgado-Baquerizo et al., 2016a; Fierer et al., 2006), can vary across environmental gradients. A latest generation of functional gene array (GeoChip 5.0S) (Tu et al., 2014), has also been widely employed to investigate the changes in the functional structure of soil microbial communities (Deng et al., 2016; Xue et al., 2016; Zhou et al., 2012). This microarray-based metagenomics technology sheds a new light on how central functional gene groups in microbial communities involved in nitrogen (N), carbon (C), sulfur (S), and phosphorus (P) cycling, and other processes such as metal resistance, antibiotic resistance and organic remediation etc., act across environmental gradients.

The current study was therefore designed to investigate the spatial variations in taxonomic and functional diversities of soil microbial communities in Chinese alpine grasslands, to reveal potential associations and differences of the spatial variations between microbial taxonomic structure and functions at regional scale, using a combined technique of marker gene sequencing (16S and ITS ribosomal RNA) and functional genes examination (GeoChip 5.0S). Specially, we attempt to address the following two scientific questions: (i) Would the spatial changes in microbial community structure and function be different? If so, would there be any potential associations between both of them? (ii) What factors, such as environmental differences and geographic distance, would be determinants of the spatial variations in microbial taxonomic and functional structures. Meanwhile, we predicted that owing to high functional redundancy in soil microorganisms, they would maintain stable function to mediate their response to changing environmental conditions through altering their taxonomic diversity and composition.

2. Materials and methods

2.1. Region description and sampling strategy

We conducted this study in alpine grasslands, recognized as one of terrestrial ecosystems most sensitive to environmental changes (Huang et al., 2016; Reynolds et al., 2007). The study site is located in the Bayinbuluk (83.45° E to 85.33° E, 42.89° N to 43.09° N), in the southern Tianshan mountains of Central Asia, Xinjiang Uygur Autonomous Region of China (Fig. S1). The Bayinbuluk grassland covers a total area of approximately 23,000 km^2 and mean altitude is 2500 m a.s.l. Mean annual precipitation (MAP) is 265.7 mm with 78% of rain falling during the growing season from May to August, and mean annual temperature (MAT) is −4.8 °C with the highest mean monthly temperature in July

(11.2 °C). The dominant species in the alpine grassland include *Stipa* spp. and *Carex* spp.

The sampling work was carried out at the time of year when aboveground plant biomass is nearly highest. A total of four sampling sites (BY_I, BY_II, GNS and LT) separated by ~30–70 km were selected (Fig. S1). Each site was well representative of the local natural communities. Five 1 m × 1 m quadrats were selected at each site (Fig. S1). For each quadrat, five soil cores (2.5 cm diameter × 10 cm depth) were collected, mixed thoroughly and pooled as one composite sample for subsequent soil chemical and microbial analysis. In each quadrat, we conducted the plant census completely. All living aboveground plants in the quadrat were sorted into species and clipped, and then stored in paper bags for biomass measurement and plant richness (PR) estimation.

2.2. Climate and soil chemistry

For each site, the MAP and MAT were obtained from the WorldClim global climate dataset (Hijmans et al., 2005). Extracted climate data was interpolated using a geographical coordinates system (GIS)-based multiple regression method in ArcGIS version 9.3. Elevations of each site were recorded by a handheld GPS (eTrex Venture, Garmin, USA). Total organic carbon (TOC) and nitrogen (TN) were determined using wet oxidation and a modified Kjeldahl procedure (Wang et al., 2014). Total P (TP) was measured and digested using 1 M H_2SO_4 after ignition at 550 °C in a muffle furnace and by the molybdate colorimetric method at 880 nm on a spectrophotometer (Jiao et al., 2016). Soil pH was measured after creating a 1:2.5 (volume) fresh soil to water slurry. Soil moisture was determined gravimetrically after overnight drying in an oven at 105 °C. Environmental characteristics of each site are shown in Table S1.

2.3. DNA extraction

Soil DNA was extracted from 0.5 g of well-mixed soil for each sample using the MoBio PowerSoil® DNA isolation kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. DNA quality was assessed based on the absorbance ratios of 260 to 280 nm (1.8–2.0) and 260 to 230 nm (>1.7) detected by a NanoDrop ND-1000 Spectrophotometer (NanoDrop Inc., Wilmington, DE, USA). DNA concentration was measured by PicoGreen (Life Technologies, Grand Island, NY, USA) assay (Ahn et al., 1996) using a FLUOstar Optima (BMG Labtech, Jena, Germany).

2.4. PCR amplification and sequencing

An amplicon survey of the ribosomal RNA (rRNA) genes for bacteria and ITS for fungi was performed to provide a higher resolution and more in-depth analysis of the taxonomic composition and diversity of the communities. For bacterial 16S rRNA genes, the V4 region was amplified with the primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011). For fungal ITS, an amplicon of internal transcribed spacer (ITS2) region was targeted using the primers gITS7F (5′-GTGARTCATCGARTCTTTG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Both primers for bacteria and fungi contain Illumina adaptor sequence and a barcode sequence on the reverse primers unique to each sample. PCR amplification was performed in triplicate using a Gene Amp PCR-System 9700 (Applied Biosystems, Foster City, CA, USA) in a 25 μL reaction volume, which contained 2.5 μL of 10 × AccuPrime PCR buffer (including dNTPs) (Invitrogen, Grand Island, NY), 0.5 unit of AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), 10 ng of template DNA and 0.4 μM of both forward and reverse primers. The reaction was carried out under the following conditions: 94 °C for 1 min, followed by 30 cycles at 94 °C for 20 s, 53 °C for 25 s, 68 °C for 45 s, and a final extension at 68 °C for 10 min. PCR products were checked on a 1.5% agarose gel. The triplicate PCR reactions for each sample were combined and quantified with PicoGreen. PCR amplicons from all samples were then pooled together in equimolar concentrations. Final PCR products were purified and recovered using a QIAquick gel extraction kit (Qiagen, Valencia, CA, USA). Sequencing was conducted on an Illumina MiSeq sequencer at the Institute for Environmental Genomics, University of Oklahoma.

2.5. Processing of sequencing data

The raw reads of 16S and ITS genes were collected in Miseq sequencing machine in FASTQ format. Their forward and reverse directions, and barcodes were generated into separated files. First, the spiked PhiX reads were removed using BLAST against PhiX genome sequence with E value < 10^{-5}. Second, the reads were assigned to samples according to the barcodes in the barcode file with up to one mismatch allowed. After reassigning each sequence to its sample based on the unique barcode, bacterial and fungal sequences were quality trimmed using Btrim (Kong, 2011) with threshold of QC > 30 over 5 bp window size. Forward and reverse reads of same sequence with at least 10 bp overlap and <5% mismatches were combined, as single sequence by using FLASH v1.2.5 (Magoc and Salzberg, 2011). Any joined sequences with an ambiguous base, or <240 bp for 16S and <200 bp for ITS were discarded. Thereafter, UChime (Edgar et al., 2011) was used for chimera removal by against "Gold" database (reference database in the Broad Microbiome Utilities, version microbiomeutil-r20110519) for 16S data set, against UNITE/QIIME released ITS reference (http://qiime.wordpress.com/2012/11/27/uniteqiime-12_11-its-reference-otus-now-available-alpha-release/) for ITS data set. Sequences were clustered into operational taxonomic units (OTUs) using the 97% identity threshold with UPARSE (Edgar, 2013) for both 16S and ITS genes and singleton OTUs (with only one read) were removed. Final OTUs were generated based on the clustering results. To correct for sampling effort (number of analyzed sequences per sample), the samples were rarefied at 11,612 sequences for the 16S rRNA genes and 3018 sequences for ITS rRNA genes per sample for subsequent community analysis. The above mentioned steps were performed using an in-house pipeline that was built on the Galaxy platform at the Institute for Environmental Genomics, University of Oklahoma (http://zhoulab5.rccc.ou.edu:8080/).

2.6. Functional structure analysis with GeoChip

To analyze the functional diversity of the soil microbial communities, the latest generation of functional gene array, GeoChip 5.0S (60K arrays) (Agilent Technologies Inc., Santa Clara, CA), was used as described previously by Wang et al. (2014). The GeoChip 5.0S contained >57,000 oligonucleotide probes, covering over 144,000 gene sequences from 393 gene families. In brief, the purified high-quality DNA (0.6 mg) labelled with Cy3 was re-suspended and hybridized on the GeoChip microarray. The hybridization solution (42 μL in total) contained 1 × HI-RPM hybridization buffer, 1 × Acgh blocking, 0.05 μg/μL Cot-1 DNA, 10 pM universal standard DNA and 10% formamide (final concentrations). GeoChip hybridization was carried at 67 °C in Agilent hybridization oven for 24 h. After hybridization, the slides were washed with Agilent Wash Buffers at room temperature. The arrays were then scanned with a NimbleGen MS200 Microarray Scanner (Roche NimbleGen, Inc., Madison, WI, USA) at 633 nm using a laser power of 100% and a photomultiplier tube (PMT) gain of 75%. The images data were extracted by Agilent Feature Extraction program. The raw microarray data were preprocessed for subsequent analysis as described previously (Wang et al., 2014).

2.7. Statistical analysis

We used Shannon-Weiner index to estimate alpha diversity of microbial taxa and functional genes. Correlations between the alpha

diversity and site variables were examined by linear regressions with a Pearson correlation. A one-way analysis of variance (ANOVA) was performed to detect significant differences among sites followed by Tukey's post hoc test. A significance level of $P < 0.05$ was applied for all comparisons. We computed the pairwise taxonomic and functional gene distance matrix (Bray-Curtis), and geographical and environmental distance matrix (Euclidean distance). Mantel tests (Jackson and Somer, 1989) were conducted from the distance matrices (Bray-Curtis) to confirm the significance of correlation between bacterial and fungal communities, and functional gene (groups) with the ade4 package. Patterns of both taxonomic and functional gene composition among samples were determined through principal coordinate analysis (PCoA) (Gower, 1966). A dissimilarity test of the patterns in composition across sites was performed using non-parametric multivariate statistical tests (ANOSIM, 999 permutations) (Clarke, 1993). We also used the principal coordinates score for the first axis (PCoA1) to investigate the relationship between taxonomic and functional gene similarity. Both PCoA and ANOSIM were performed with the vegan and ape package (Oksanen et al., 2013). The distance-decay relationship was estimated by the ordinary least-squares (OLS) regression on the relationship between geographic distance (log transformed) and composition similarity (log transformed) with the ggplot2 package. The significance of the distance-decay relationship was assessed using Mantel test. Partial Mantel tests (Horner-Devine et al., 2004) and multiple regression on matrices (MRM) approach (Martiny et al., 2006) were also used to disentangle the separate influences of environmental variables and geographic distance on the taxonomic and functional composition with the ecodist package. All statistical analyses were carried out with R software version 3.2.5.

3. Result

3.1. Diversity of microbial taxa and functional genes

Across all samples, a total of 687,474 and 240,753 high-quality sequences for 16S and ITS rRNA genes were obtained, which were then clustered into 31,248 and 8643 operational taxonomic units (OTUs) with an identity cutoff of 97%, respectively. After resampling for all samples at an identical sequencing depth (11,612 and 3018 reads per sample), we carried out the subsequent bacterial and fungal community analyses. For the bacterial communities, the soils collected at the GNS site showed significantly lower taxonomic diversity, as estimated by the number of OTUs (with an average of 1578 ± 12 OTUs) and Shannon index (H′), compared with that at the BY_I, BY_II and LT ($P < 0.001$) (Fig. 1a, Table S2). Bacterial diversity was positively correlated with soil pH ($r = 0.75$, $P < 0.01$) and negatively correlated with soil moisture ($r = -0.70$, $P < 0.01$) and TOC ($r = -0.62$, $P < 0.01$) (Table S3). For the fungal communities, the diversity at the BY_I soils was significantly lower than that at the LT soils ($P < 0.05$) (Fig. 1b, Table S2). Of all environmental variables examined, N:P ratio ($r = -0.60$, $P < 0.01$), TP ($r = 0.49$, $P < 0.05$) and PR ($r = -0.47$, $P < 0.05$) showed a strong correlation with fungal diversity (Table S3).

A total of 40,602 gene sequences were detected by GeoChip 5.0. The number of functional genes detected for individual samples ranged from 216 to 354 (with an average of 320 ± 7 genes) (Table S2). The functional diversity, as estimated by Shannon index (H′), was no significant differences among sites (Fig. 1c). There was no significant correlation between all functional genes or functional groups and examined environmental variables (Table S3). The top 100 most abundant functional genes and functional groups were also not significantly different in relative abundance among sites (ANOVA, $P > 0.05$, Tables S4 and S5). The functional gene diversity was weakly correlated with bacterial taxonomic diversity ($r^2 = 0.122$, $P = 0.043$) and fungal diversity ($r^2 = 0.223$, $P = 0.036$) (Fig. 2a, b).

3.2. Microbial taxonomic and functional gene composition

The compositional pattern of bacterial, fungal communities and functional genes across all soils is visualized on the first three coordinates of the PCoA ordination. The close clustering of samples displayed by the PCoA indicated similar bacterial and fungal communities within a single site. The samples among sites were separated distinctly by the first three PCoA axes which explained a large amount of total variance for both bacteria (74.28%) and fungi (66.41%) (Fig. 3a, b). The dissimilarity analysis of bacterial and fungal community composition were both significantly different between groups (ANOSIM: $R = 0.532$ and 0.441, $P < 0.01$, respectively). There was no distinct clustering across all samples for all surveyed functional genes although the first three PCoA

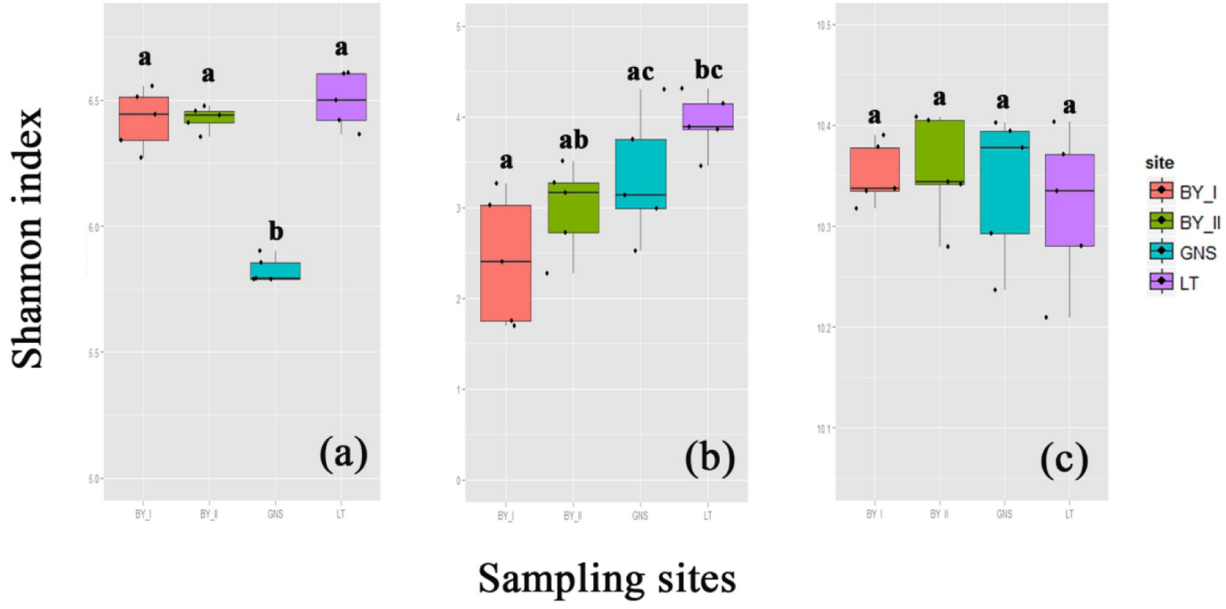

Fig. 1. Alpha diversity estimated by Shannon index across each site for bacterial and fungal communities and functional genes. (a) Bacteria based on 16S rRNA gene; (b) fungi based on ITS; and (c) functional genes examined by GeoChip 5.0. Points represent the samples in each site. The boxplot shows quartile values for each taxon and gene colored by four different sampling sites: BY_I (red), BY_II (green), GNS (blue), and LT (purple). Different letters indicate statistical differences at a P value of <0.05 among sites by Tukey tests. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

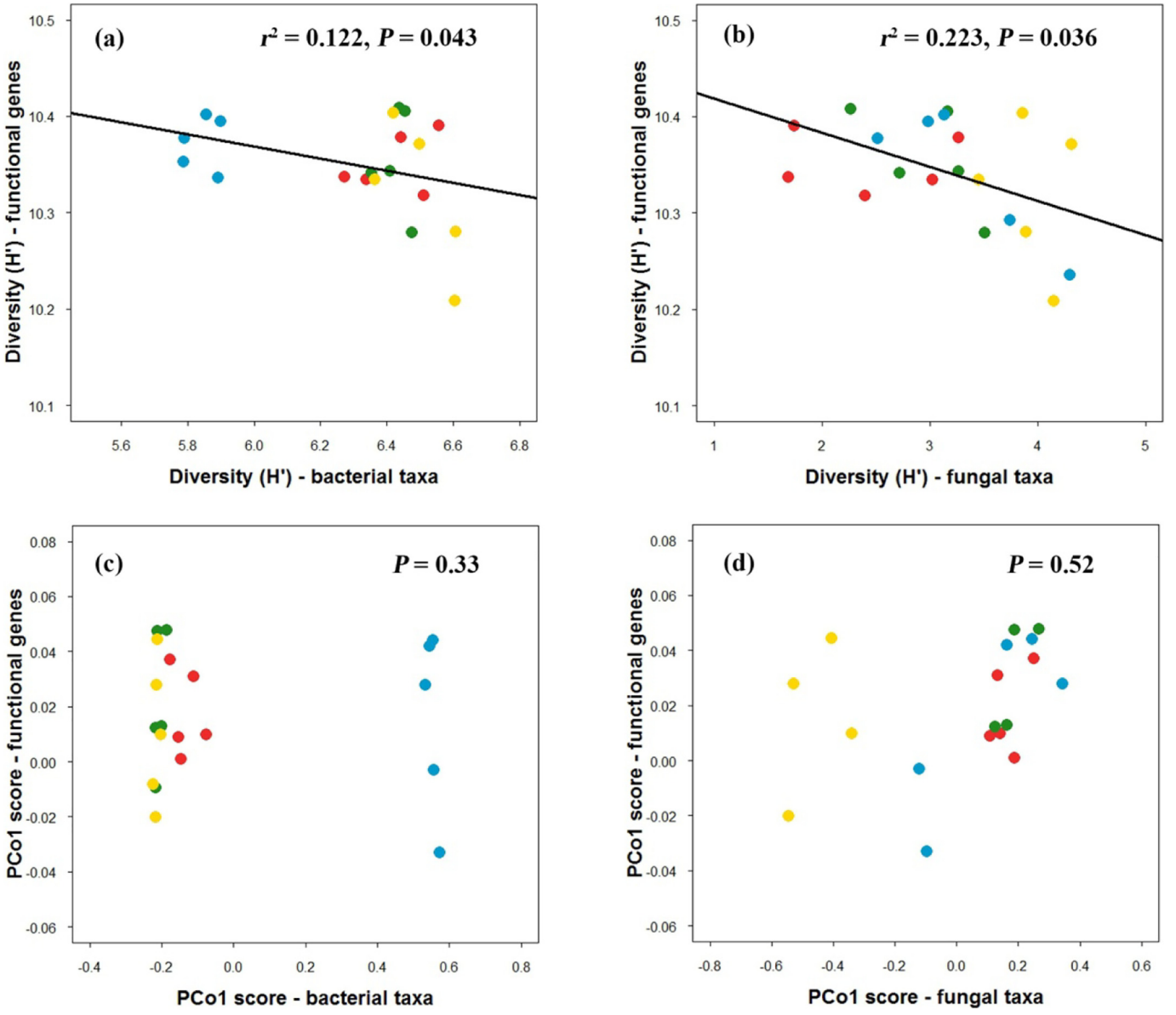

Fig. 2. Relationships between functional and taxonomic diversity (a, b) and community similarity patterns (c, d) across the four sites, with *x* axes showing taxonomic comparisons and *y* axes showing comparisons based on functional genes. Points are colored by the different sites: BY_I (red), BY_II (green), GNS (blue), and LT (yellow). The Shannon index (*H′*) and the Bray-Curtis index of community similarity were used to measure alpha diversity and composition variations of microbial communities, respectively. The compositional variation patterns are shown here using the principal coordinates score for the first axis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

axes explained up to 82.72% of total variance (Fig. 3c). The PCoA1 scores of the bacterial and fungal communities were not significantly correlated with PCoA1 scores of functional genes ($P = 0.33$ and 0.52, respectively) (Fig. 2c, d). There was also no significant correlation based on the Bray-Curtis distance matrices between bacterial and fungal communities, and functional genes (groups) (Tables 1 and S6). Compositional similarity versus geographic distance for each pairwise set of samples clearly displayed a significant distance–decay relationship for bacterial ($r^2 = 0.22$, $P < 0.001$) and fungal communities ($r^2 = 0.11$, $P < 0.001$) (Fig. 4a, b and Table S7). The similarity in functional gene composition was not significantly decreased with increasing geographic distance ($P = 0.28$) (Fig. 4c and Table S7).

3.3. Influences of environmental variables and geographic distance on the taxonomic and functional composition

Across all sites, both environmental factors and geographic distance significantly influenced taxonomic composition. The compositional similarity in bacterial and fungal taxa was strongly correlated with environmental distance ($\rho = -0.587$ and -0.436, $P = 0.001$, respectively), but was weakly related to geographic distance ($\rho = -0.196$, $P = 0.025$; $\rho = -0.115$, $P = 0.044$) (Table 2). In contrast, the compositional variation in functional genes was not significantly related to both environmental distance and geographic distance for all pairwise samples ($P > 0.05$) (Table 2).

MRM model explained a large and significant proportion of the variability in bacterial and fungal community similarity (87% and 67%, $P = 0.0001$, respectively) (Table 3). For the bacterial communities, soil pH was the most important variables explaining community similarity (partial regression coefficient $b = -0.37$, $P < 0.001$). For the fungal communities, soil TP and N/P were the most important variables explaining community similarity (partial regression coefficient $b = 0.47$ and 0.55, respectively, $P < 0.001$). In contrast, the variability in functional gene similarity was not explained by the MRM model ($P = 0.68$), with environmental variables and geographic distance showing no influences on functional similarity pattern (Table 3).

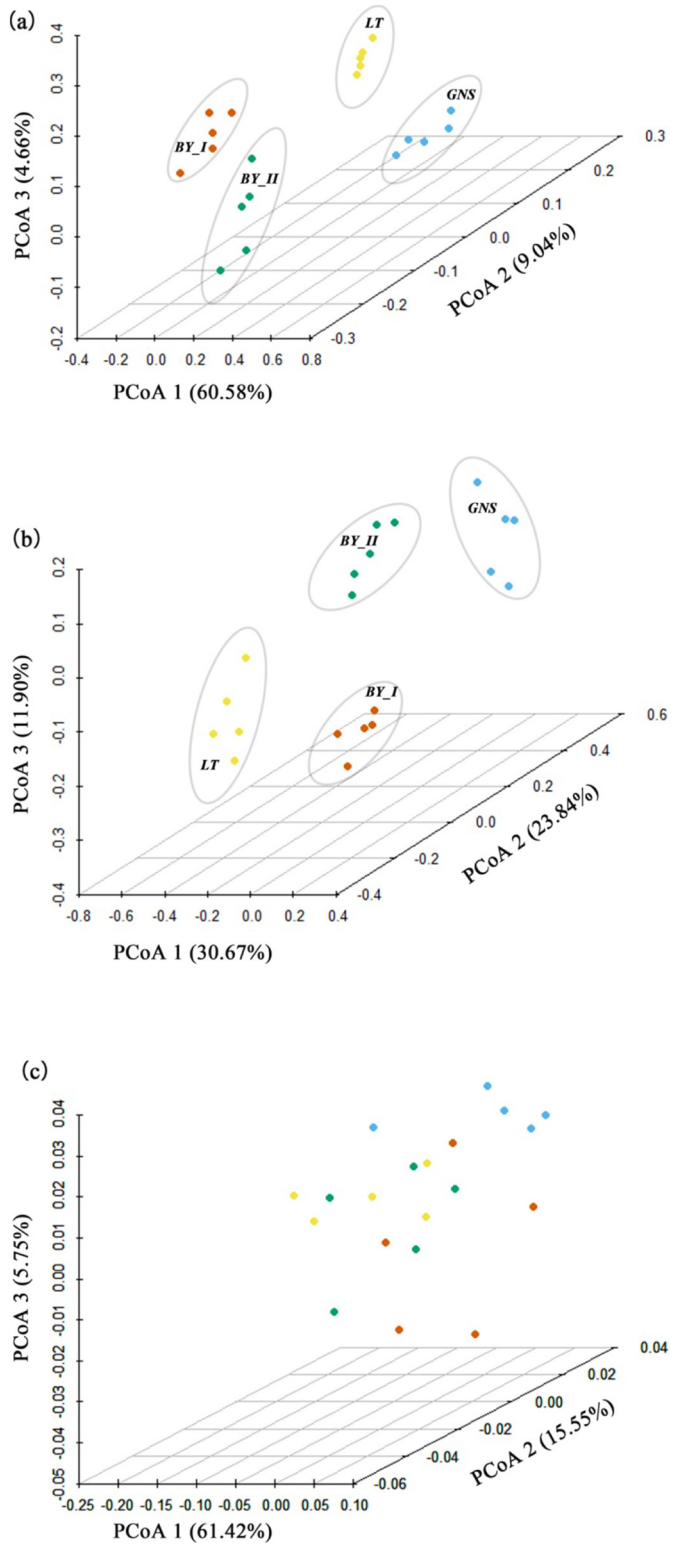

Fig. 3. Principal coordinate analysis (PCoA) with a Bray-Curtis distance matrix of bacterial and fungal communities and functional genes: (a) Bacteria based on 16S rRNA gene; (b) fungi based on ITS; and (c) functional genes examined by GeoChip 5.0. Points are colored by the different sites: BY_I (red), BY_II (green), GNS (blue), and LT (yellow). The percentage of the variation in the samples described by the plotted principal coordinates is indicated on the axes. The figure was generated by the scatterplot3d package in R. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
The correlation based on Bray-Curits distance between bacterial and fungal communities, and functional genes and the major functional groups involved in carbon, nitrogen, phosphorus and sulfur cycling for all pairwise samples. The correlation (r) and significance (P) were determined by Mantel tests based on 9999 permutations. The results for other functional groups were presented in Table S6.

	Bacteria		Fungi	
	r	*P*	*r*	*P*
Functional genes	0.043	0.300	0.159	0.199
Functional groups				
Carbon cycling				
C degradation	0.047	0.294	0.158	0.199
C fixation	0.039	0.295	0.161	0.198
Methane metabolism	0.073	0.282	0.178	0.169
Nitrogen cycling				
Anammox	0.040	0.311	0.169	0.166
Ammonification	0.044	0.305	0.159	0.197
Nitrogen assimilation	0.305	0.054	0.208	0.118
Assimilatory N reduction	0.062	0.280	0.164	0.179
Denitrification	0.041	0.298	0.157	0.193
Dissimilatory N reduction	0.041	0.301	0.151	0.206
Nitrification	−0.029	0.463	0.089	0.272
Nitrogen fixation	0.057	0.284	0.170	0.182
Phosphorus cycling				
Phytic acid hydrolysis	−0.040	0.502	0.030	0.384
Polyphosphate degradation	−0.040	0.511	0.030	0.393
Polyphosphate synthesis	0.011	0.366	0.109	0.253
Sulfur cycling				
Adenylysulfate reductase	0.047	0.303	0.166	0.182
DMSP degradation	−0.006	0.389	0.125	0.236
Sulfide oxidation	0.017	0.339	0.151	0.206
Sulfide reduction	0.060	0.289	0.178	0.166
Sulfur oxidation	0.075	0.273	0.159	0.194
Sulfur reduction	0.077	0.262	0.142	0.218

4. Discussion

The differences of spatial variations in microbial taxonomic and functional structures within a community provide fundamental information for our understanding about how microbes adapt and respond to spatially changing biotic and abiotic environments. In the current study, microbial taxa and functional genes clearly exhibit different regional patterns in alpha diversity and composition across the four sites in alpine grasslands of northern China (Figs. 1, 3 and 4). Moreover, the basic forces that drive changes in taxonomic diversity of microbial communities such as environmental heterogeneity and dispersal limitation did not influence spatial variation in the diversity and composition of functional genes at this regional scale (Tables 2, 3 and S3). These results indicate spatial changes in microbial structure and function may be driven by different factors within a community. In addition, the findings reported here also suggest that functional diversity and composition in free-living microbial communities can be independent of the observed changes in their taxonomic structure along natural environmental gradients. Previous studies have reported the existence of differences in spatial variation between taxonomic structure and function at the fine and biome scales (Talbot et al., 2014; Wertz et al., 2007). The reason for such differences in patterns may relate to relative effects of environmental differences and geographical distance on microbial taxonomic structure and function (Haggerty and Dinsdale, 2017).

It is increasingly common for microbial ecologists to consider the diversity and distributions of functional genes across communities living in a natural habitat (Raes et al., 2011). However, few researchers have attempted to conduct comparable studies between microbial taxonomic and functional diversity relying on the information of functional genes (Gilbert et al., 2010). In this study, the alpha diversity patterns of bacterial and fungal taxa and functional genes were different across the four sampled sites (Fig. 1). It is clear that the soils at the GNS site harbored far lower bacterial diversity than the soils at other sites. The reason for this sharp decrease in bacterial taxonomic diversity may relate to a higher altitude in the GNS site compared with that in other sites

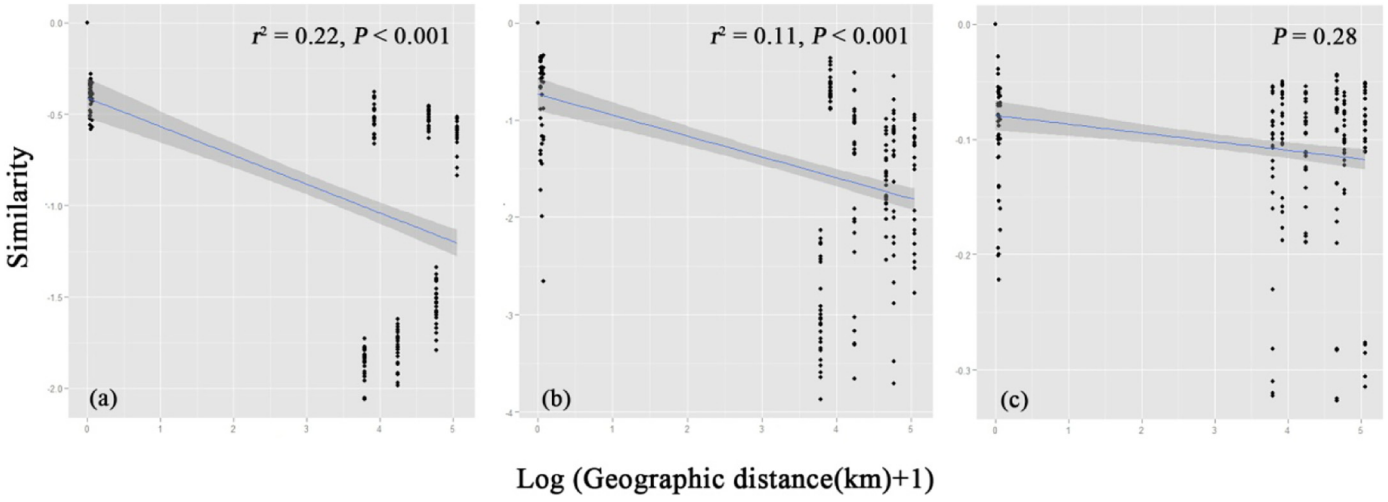

Fig. 4. Distance-decay curves of similarity for the bacterial and fungal communities and functional genes. Line represents least squares regression fit across all samples and the shaded area represents their 95% confidence limits. (a) Bacteria based on 16S rRNA gene; (b) fungi based on ITS; and (c) functional genes examined by GeoChip 5.0. Statistics results derived from regression analysis were summarized in Table S4.

which results in the remarkable alternations of edaphic properties such as decreasing pH and increasing soil moisture (Badía et al., 2016; Kotas et al., 2018; Zhang et al., 2011). Indeed, among all the examined variables, soil pH and moisture were dominant factors affecting bacterial alpha diversity ($r = 0.75$ and -0.70, $P < 0.01$, respectively). Soil pH was also the most important factor influencing the composition variability for bacterial communities ($b = -0.37$, $P < 0.001$) (Table 3). These findings are in accordance with numerous studies showing a strong influence of soil pH on bacterial diversity and community composition at regional scales, with bacterial diversity being generally lower in acidic soils (Fierer and Jackson, 2006; Lauber et al., 2009). Fungal alpha diversity was correlated with N:P ratio, TP and PR (Table S3). The composition variability was also primarily related to N:P ratio for fungal communities ($b = 0.55$, $P < 0.001$) (Table 3). These findings suggest that fungal communities are likely to be subjected by the availability of soil nutrients in the area, as the effects of nutrient limitation on fungal communities have shown to be largely driven by the N:P ratio of nutrients especially in the nutrient-limited grassland ecosystems (Johnson, 2010; Verbruggen et al., 2015). In contrast, we did not find significant variation in the diversity and composition of microbial functional genes across our sites (Figs. 1c and 3c). There were also no significant differences in the abundance of main functional genes (groups) across sites (Tables S4 and S5). This finding are in line with recent studies conducted in soil and other habitats that found stable functional structures despite of high taxonomic variability across microbial communities (Haggerty and Dinsdale, 2017; Louca et al., 2016). We did not find any effects of environmental factors on the diversity and composition patterns of functional genes. This is probably because the region in which the study was conducted was located within a single habitat; thus limited environmental heterogeneity is unlikely to give rise to significant variations at the genomic level in microbial communities (Nunan et al., 2017).

Such a distinct difference in spatial variation between the taxonomic and functional structure of soil microbial communities could have three possible explanations. First, owing to high species diversity and the physiological flexibility within microorganisms, soil microbial communities can function in an identical manner regardless of the diversity and composition of that community in order to adapt rapidly to new environments. This explanation relates to a commonly accepted view that free-living microorganisms are functionally redundant which thus enhances the resistance and resilience to disturbance (Griffiths and Philippot, 2013). This is similar to what we have known about the 'insurance hypothesis' of biodiversity and ecosystem functioning in macro-organisms (Loreau et al., 2001). The lack of correlation found here between taxonomic and functional structure of microbial communities provides further evidence, in that microbial functional diversity and composition similarity in local communities did not increase with increasing taxonomic diversity and composition similarity (Fig. 2 and Tables 1, S6). Such a result also suggests that the overall diversity of functional genes in a given sample cannot be predicted from the taxonomic diversity of a microbial communities and that functional redundancy at the genomic level is widespread. Second, soil microbial functions in a community are likely to be determined by the habitat type (habitat filter) (Nunan et al., 2017; Zak et al., 1994). Our study area is limited to a single alpine grassland. The function of microbial communities could be therefore, hard to reset with insufficient variations in environmental conditions. This is because environmental factors are commonly considered to play important roles in determining

Table 2
The results of correlation between the similarity of bacterial, fungal communities and microbial functional genes, and geographic distance or environmental distance for all pairwise samples using partial mantel test.

		Bacteria		Fungi		Functional genes	
Correlation between composition similarity and:	Controlling for:	ρ	*P*	ρ	*P*	ρ	*P*
Environmental distance	–	−0.624	0.001	−0.530	0.001	−0.080	0.235
Geographic distance	–	−0.204	0.017	−0.387	0.002	−0.023	0.399
Environmental distance	Geographic distance	−0.587	0.001	−0.436	0.001	−0.077	0.255
Geographic distance	Environmental distance	−0.196	0.025	−0.115	0.044	−0.005	0.491

P values are one-tailed tests based on 9999 permutations.

Table 3
Results of the multiple regression analysis on matrices analysis (MRM) for the bacterial and fungal communities, and functional genes.

	Bacteria	Fungi	Functional genes
	$R^2 = 0.87$ $P = 0.0001$	$R^2 = 0.67$ $P = 0.0001$	$P = 0.68$
Ln (Geographic distance (km) + 1)	0.03*	0.14*	0.01
MAP (mm)	−0.12**	−0.14	−0.02
MAT (°C)	−0.09**	−0.25*	−0.05
Altitude (m)	−0.02	−0.14	0.01
TOC (%)	−0.02	−0.15	0.03
TN (%)	0.13**	−0.10	0.01
TP (%)	−0.08**	−0.47**	−0.01
C/N	−0.01	−0.04	0.01
N/P	0.01	−0.55**	0.02
pH	−0.37**	0.13	0.01
SM (%)	−0.15*	−0.11	−0.02
PR	−0.01	−0.02	0.02

Abbreviations: MAP, mean annual precipitation; MAT, mean annual temperature; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; C/N, carbon/nitrogen; N/P, nitrogen/phosphorus; SM, soil moisture, PR, plant richness.
The variation (R^2) of ln composition similarity (1 minus Bray–Curtis distance) that is explained by the remaining variables. The partial regression coefficients (b) and associated P-values of the final model are reported from permutation test (nperm = 9999) if its significance level is <0.05.
* $P \le 0.01$.
** $P \le 0.001$.

the physiology of whole microbial communities (Sinsabaugh, 2010). That was probably why we found no significant effect of environmental variables on the spatial variations in functional diversity and composition among sites (Tables 2, 3 and S3). This finding is similar to the study in macro-organism community ecology in which researchers found that taxonomic composition generally follows regional patterns while functional composition is selected by habitat (Fukami et al., 2005; Hoeinghaus et al., 2007). Furthermore, we found no evidence that the composition similarity in microbial functional genes was affected by geographical distance (Fig. 4c and Tables 2, 3 and S7). Hence, the microbial functional genes in a community may have been initially determined by evolutionary history (Elena and Lenski, 2003), which results in stable functions in microbial communities in spite of the variations in taxonomic diversity with spatially changing environments.

In conclusion, our study presents a fundamental insight into an issue regarding how functional structure of soil microbial communities vary at regional scales, and whether or not the variation in microbial functional structure relates to their taxonomic structure. The findings provide a valuable clue to the understanding of the consequences of biodiversity changes for ecosystem functioning and stability. It appears that the microbial functions in a community would not totally follow the variations in taxonomic structures in the alpine grassland ecosystem we studied. Further across-habitat or -ecosystem investigations at larger spatial scales will be helpful to disentangle the real linkages between microbial structure and function.

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Author contributions

XW, XL, ZW and XH designed the study, XW, XL, XW, and HZ performed the experiment, XW analyzed the data and prepared the manuscript with the help of JY, KL, HL, XH and JZ. All coauthors participated in discussions at the working group meetings and edited the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.11.138.

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