The overlooked dimension: soil depth governs microbial community assembly and enzymatic activity in extreme environments

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Abstract

Soil microbes have a major impact on ecosystem functions in extreme environments, such as deserts, where environmental factors such as salinity and soil moisture act as strong filters in shaping community structure. In fact, environmental filtering in extreme environments can occur at a small scale along soil depths, which is often overlooked, but it is important for an explicit understanding of how microbial community structure relates to microbial functions. Here, we leveraged a 200-km desert salinity gradient to evaluate microbial community structure-function relationships in extreme environments, and to further assess the prime ecological processes controlling the assembly of microbial communities and the activity of carbon, nitrogen, and phosphorus cycling-relevant enzymes along soil depths. We found that differences in soil bacterial and fungal community structure were not linked to differences in enzymatic activity, implying that soil microbial communities have a high degree of functional redundancy among soil depths. We further found that soil depth (at the scale of meters) was just as important as geographic distance (at the scale of kilometers) in shaping bacterial and fungal community structure, while the vertical variability in enzymatic activity along soil depth was largely attributed to the increase in heterogeneity of soil properties, such as soil texture, water content, and pH. Our results suggest that dispersal limitation is particularly important to soil microbial community assembly, while environmental filtering is important to enzymatic activity. Such a mismatch in ecological processes could have disproportionate influences on soil microbial community structure and enzymatic

activity, leading to uncoupled structure-function relationships. Our results further suggest that soil depth governs the assembly and enzymatic activity of soil microbial communities. This work highlights that soil conservation efforts should consider soil depth as a key attribute in the face of ongoing desertification in arid ecosystems.

Keywords: ecosystem functioning; extreme environments; microbial beta-biodiversity; soil microbiome; structure-function relationships; Taklamakan desert

1. Introduction

A major goal of biodiversity-ecosystem functioning (hereafter, BEF) research has been to understand how community structure influences a wide range of ecosystem functions, such as aboveground net primary productivity, soil carbon fluxes, and nutrient cycling (Tilman et al., 2014). Many studies of biodiversity experiment find positive relationships between biodiversity and ecosystem functions, suggesting that enhanced biodiversity would lead to enriched ecosystem functioning (van der Plas, 2019). However, these studies have been largely carried out in constructed communities, in which community structure is often randomly assembled from a species pool (Loreau et al., 2001; Schmid et al., 2002; Jochum et al., 2020). As a result of this human-driven random assembly process, a wide range of naturally occurring biotic and abiotic ecological filters are excluded from the processes of community structure assembly, such as disturbance, environmental heterogeneity or extremes, and

dispersal limitation (Sundqvist et al., 2013; Talbot et al., 2014; Mori et al., 2018). This raises an important and unresolved question: would these positive BEF relationships that we observe in constructed communities hold in natural settings (e.g., natural grasslands) (Wardle, 2016; Manning et al., 2019; Schmid et al., 2022)? Put it another way, can we safely extend our current understanding of BEF relationships from constructed communities to natural communities (Hagan et al., 2021), including belowground microbial communities (Bell et al., 2009; Eisenhauer et al., 2019; Berlinches de Gea et al., 2022)?

Despite the growing recognition that microbial communities in soil are important for numerous ecosystem processes (van der Heijden et al., 2008; Delgado-Baquerizo et al., 2020), extending BEF theory to soil microbial communities is a relatively new endeavor (Bell et al., 2009; Bardgett and van der Putten, 2014; Peay et al., 2016; Berlinches de Gea et al., 2022). One of the challenges is the hyper-diversity of microorganisms that inhabit the soil matrix (Coleman and Whitman, 2005; Nielsen et al., 2015; Díaz and Malhi, 2022), which makes it much difficult to uncover the relationships between biodiversity and functions; there can be 100-9000 microbial species/strains in a small soil sample (Bardgett and van der Putten, 2014) vs. 1~32 species in a constructed grassland biodiversity experiment (Hector et al., 1999).

One strategy to address this challenge is to study BEF in extreme ecosystems, such as desert, glaciers, and hypersaline habitats, where soil microbial diversity is relatively low and environmental constraints are high (Shu

and Huang, 2022). Under extreme environmental conditions, biological community structure, the characteristics of a community that can be described by community diversity and/or composition, is generally simple, providing opportunities to understand how an understandable amount of biodiversity influences ecosystem functions (Wall and Virginia, 1999; Osborne et al., 2020; Ertekin et al., 2021; Shu and Huang, 2022). Moreover, extreme environments provide unique opportunities to predict how biodiversity might respond in an era of environmental extremes and dramatic land-use change (Wall et al., 2015).

Environmental filters such as salinity, soil water, and nutrient availability can shape microbial composition and function in extreme environments (e.g., desert), but these drivers may change with soil depth (Rath et al., 2019a; Rath et al., 2019b). In fact, prior work highlights that soil depth influences microbial community structure, because important drivers of microbial diversity, such as soil organic carbon, nutrient, pH, oxygen concentration, moisture, and salinity, also vary with soil depth (Fierer et al., 2003; Powell et al., 2015; Stone et al., 2015; Sun et al., 2020). However, we don't have as much of an idea of how the vertical patterns in microbial community structure are intimately linked to their functions, even though we know that soil depth is important for microbial functions such as organic matter decomposition and nutrient cycling.

Here, we study the relationships between soil microbial community structure and enzymatic activity relevant to soil carbon, nitrogen, and phosphorus cycling in soils from the Taklamakan desert, the world's 2nd largest shifting sand desert;

a desert area that is roughly the size of Germany. We evaluate BEF relationships across 10 field sites situated along a unique 200-km salinity gradient (the horizontal gradient; Fig. 1a,b). At each site we also examine BEF relationships across a one-meter soil profile (the vertical gradient; Fig. 1a,c), a new dimension for BEF research. We asked whether and how differences in microbial community structure are related to differences in enzymatic activity along these two gradients (Fig. 1a,d). We measured soil bacterial and fungal community structure based on pairwise measures of dissimilarities in community composition between sample units (Anderson et al., 2011), potential soil microbial enzymatic activity, and a range of environmental variables including soil salinity, physical and chemical properties, and nutrient pools.

We hypothesize that differences in soil bacterial and fungal community structure would be positively associated with spatial variability in potential microbial enzymatic activity (H1). This hypothesis is based on the assumption that extreme environments reduce community functional redundancy (Wall and Virginia, 1999), leading to a strong relationship between microbial function and community structure (Peay et al., 2016). However, it remains unclear if this relationship is a positive one (Fig. 1d) (Schimel and Gulledge, 1998), as previous studies have found positive, neutral and negative relationships. Studies that focus on local-scale tend to report positive microbial structure-function relationships (Mori et al., 2016), while studies that span large spatial scale tend

to report neutral or weakly positive relationships (Talbot et al., 2014; Martinez-Almoyna et al., 2019; Jing et al., 2021; Jing et al., 2022).

We also hypothesize that soil depth is as important as horizontal geographic distance in shaping the horizontal and vertical structure-function relationships, but different factors of environmental filtering modify the structure-function relationships across spatial dimensions (H2). Specifically, environmental conditions such as differences in soil salinity would be the main environmental filter driving the horizontal structure-function relationships (Rath et al., 2019a), whereas the heterogeneity of soil properties (Powell et al., 2015) and nutrients (Fierer et al., 2003) along soil depth profiles may drive vertical structure-function relationships.

2. Materials and Methods

2.1. Study sites and data collection

We conducted this study in shrubland vegetation located along the Tarim desert highway, which crosses the Tarim basin of Taklamakan desert (37.1−41.8^{o} N, 82.6−84.3^{o} E) (Fig. S1). The Taklamakan desert is arid, with a mean annual precipitation of 24.6 mm yr^{-1} and a mean annual potential evaporation of 3639 mm yr^{-1}. The mean annual air temperature is 2.4°C, with the coldest monthly mean temperature reaching −8.1°C in December and the warmest monthly mean temperature reaching 28.2°C in July. Most of the desert

is bare land without vegetation cover, except for a few extremely drought- and salt-tolerant shrub species, such as, *Tamarix taklamakanensis* and *Calligonum taklamakanensis*. Soils in the desert are aeolian sandy soil. The top 0□20 cm of soil is composed of 87.3% of sand, 12.4% silt, and 0.3% of clay (Zhang et al., 2016). The soils have extremely low soil organic matter content (0.74 g kg^{-1}) and soil total nitrogen (0.14 g kg^{-1}) in the top 0□10 cm soil depth (Li et al., 2015).

In 2005, the shrubland vegetation was established to prevent shifting sand dunes from damaging the Tarim desert highway. After 12 years of vegetation development the plant community has stabilized, with *Haloxylon ammodendron* dominant the community and *Calligonum aborescens*, *Tamarix ramosissima*, *Nitraria tangutorum*, *Populus euphratica* and *Elaeagnus angustifolia* as much lower abundance. The aboveground plant community did not vary along the 200 km salinity gradient. Moreover, the aboveground biomass of the dominant species *H. ammodendron* did not vary across the studied sites, likely leading to an equivalent amount of carbon inputs into soil. The groundwater level in the Taklamakan desert ranges from 3 to 5 m, and the groundwater is charged by water from the Tianshan and Kunlun Mountains (Fig. S1). The groundwater was pumped for drip irrigation to water shrubs in the vegetation. The pipes for drip irrigation run along the rows of shrubs, and the vegetation was irrigated twice per month from March to May and from September to October, and three times per month from June to August with ~30 L of water per plant per irrigation event (Xu et al., 2006).

We surveyed ten shrubland sites along an extreme gradient of irrigation water salinity that varied from 3.2 to 38.0 g L^{-1} of dissolved salts (the normal range of sea water salinity is between 33-37 g L^{-1} of dissolved salts) from the south (site 34) to the north sampling site (site 1) (Figs. 1b and S1). We collected irrigation water and soil samples in early October of 2017, which marks the end of the growing season in the desert. At each study site, irrigation water was collected from running water pumped from wells with three replicates at each site. Irrigation water samples were stored in 500 ml plastic bottles, sealed, and taken to the laboratory for water salinity analysis. We sampled soils using soil cores (5 cm in diameter) at three randomly selected plots, which were located within the two rows of shrubs and 30□50 cm away from a shrub. We collected soils to the depth of 100 cm with sampling intervals of 20 cm, except that we segmented the topsoils to 0□10 and 10□20 cm. We collected 180 soil samples (10 sites × 6 soil layers × 3 replicates). Using plastic bags and coolers, a subsample (~30 g) of each soil was kept cool and immediately returned to the laboratory. We sieved all soils to 2 mm removing rocks and roots by hand. We stored soils for microbial sequencing at □20 ^{o}C, and soils used for assays of soil enzymatic activity at 4 ^{o}C. We air-dried the remaining soils to analyze soil properties, including water content, texture, salinity, pH, total organic carbon, total nitrogen, and available phosphorus.

We analyzed the salinity of irrigation water in the following ways. We analyzed the concentration of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} using a spectrometer

(iCAP 6300-ICP-OES) with a CID detector (Thermo Scientific, MA, USA). Using titration, we analyzed the concentration of Cl^- with $AgNO_3$, SO_4^{2-} with ethylenediaminetetraacetic acid (EDTA), and CO_3^{2-} and HCO_3^- with sulfuric acid until all bicarbonate was neutralized. Next, we calculated irrigation water salinity as the sum of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, CO_3^{2-} and HCO_3^- concentrations.

We measured soil salinity and pH in a 1:5 suspension of soil to water. We analyzed soil salinity using the methods described above for irrigation water salinity. We analyzed soil pH using the Mettler Toledo FiveEasy FE28 (Greifensee, Switzerland). We measured soil gravimetric water content by oven drying 10 g fresh soils at 105°C for 24 h. We analyzed soil organic carbon using the Walkley and Black method of oxidizing organic carbon with $K_2Cr_2O_7$-H_2SO_4 (Nelson and Sommers, 1983). We analyzed soil total nitrogen using an AutoKjeldahl Unit model K370 (BUCHI, Flawil, Switzerland). We analyzed soil available phosphorus using the sodium bicarbonate extraction with Mo-Sb Anti-spectrophotometer method. We analyzed soil texture using the laser diffraction method (Sympatec GmbH, System-PartikelTechnik, Clausthal-Zellerfeld, Germany) after removing the organic material by 10% hydrogen peroxide and removing carbonates by 0.2% hydrochloric acid.

To analyze the soil bacterial and fungal diversity, we extracted soil DNA using the PowerSoil DNA extraction kit (Qiagen, Carlsbad, CA, USA) following the manufacturer's protocol. We amplified the V4 region of 16S rRNA gene for soil bacteria using the primer pairs 515F/806R and the ITS1 region for soil fungi

using the primer pairs ITS5-1737F/ITS2-2043R. We performed the PCR using the BioRad S1000 thermal cycler (Bio-Rad Laboratory, CA, USA) after mixing 25 µl of 2x Premix Taq polymerase, 2 µl of 10 mM primer pairs, 3 µl of 20 ng μl^{-1} DNA template and 20 µl nuclease-free water. Next, we ran the thermal cycling as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 30 s, with a final step of 72°C for 10 mins. We repeated this PCR procedure three times for each sample. We next mixed the PCR products using GeneTools Analysis Software (Version 4.03.05.1, SynGene). The process was followed by purifying the DNA products using the EZNA Gel extraction kit (Omega Bio-Tek, GA, USA). Following the manufacturer's instruction, we generated sequencing libraries using the NEBNext ® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) and we sequenced the libraries on an Illumina HiSeq2500 platform (Illumina PE250, CA, USA).

We processed raw DNA sequences using the Trimmomatic software (V0.33, http://www.usadellab.org/cms/?page=trimmomatic). Specifically, we excluded the raw DNA sequences by removing paired-end reads with N, read quality <20, and read length <100 bp. Next, we allocated the paired-end reads to corresponding soil samples using the Mothur pipeline (V1.35.1, http://www.mothur.org). We removed barcode and primers to derive the paired-end clean reads, which were merged by FLASH (V1.2.11, https://ccb.jhu.edu/software/FLASH). The minimum length of overlap was 10 bp and the maximum ratio of number of mismatches to

overlap length was 0.1. Next, we assigned these clean tags to OTUs with 97% similarity using the USEARCH software (V8.0.1517, http://www.drive5.com/usearch). We used representative sequences for taxonomic classification using RDP classifier according to Greengenes database (http://greengenes.secondgenome.com) for bacteria and Unite database (http://unite.ut.ee/index.php) for fungi in the QIIME pipeline (version 2; http://qiime.org/scripts/assign_taxonomy.html).

To explore microbial function, we assayed the potential activity of four hydrolytic enzymes (β-1, 4-glucosidase, BG; β-$_D$- cellobiohydrolase, CB; β-1, 4-N-acetyl-glucosaminidase, NAG; alkaline phosphatase, ALP) using the 96-well micro-plate and the fluorometric technique (German et al., 2011; Bell et al., 2013). We selected these enzymes because their potential activities are commonly measured in soils and are used in the acquisition of carbon (BG and CB), nitrogen (NAG) and phosphorus (ALP) (Sinsabaugh et al., 2008) and can be considered as proxies for fast ecosystem processes to define ecosystem multifunctionality (Garland et al., 2021). Briefly, sieved fresh soil (1.5 g) was suspended in 150 ml of 50 mM Tris buffer (pH = 8.0). Soil slurries (200 µl) of each sample were transferred to eight wells of the 96-well micro-plate and mixed with 50 µl of 200 µM standard fluorometric substrates of each enzyme. The micro-plates were incubated for 2.5 h in dark at 25°C. The quantity of fluorescence was determined at 360 nm excitation and 460 nm emission in a 96-well micro-plate reader (Biotek Synergy 2, Winooski, VT, USA). Multiple-point

calibration curve was used to calculate enzymatic activities (Bell et al., 2013). The units were expressed as nmol g dry weight^{-1} h^{-1}.

To measure the spatial variability in soil microbial community structure between assemblages, we used the Podani family of Sorensen indices (Podani and Schmera, 2011; Legendre, 2014; Schmera et al., 2020), including the Sorensen dissimilarity index (β_{sor}), species replacement (β_{repl}) and richness difference (β_{rich}). The Sorensen dissimilarity index quantifies the total differences in species composition between assemblages. It can be partitioned into two components, species replacement and richness difference. The former quantifies species in one assemblage that are replaced by different species in another assemblage and the latter quantifies differences in the number of species between assemblages. We used the following equations:

$$\beta_{sor} = \frac{b + c}{2a + b + c} \qquad \text{Eqn. 1}$$

$$\beta_{repl} = \frac{2 \times min\ (b, c)}{2a + b + c} \qquad \text{Eqn. 2}$$

$$\beta_{rich} = \frac{|b - c|}{2a + b + c} \qquad \text{Eqn. 3}$$

where *a* denotes the number of species present in both assemblages, *b* denotes the number of species present in assemblage 1 but not in assemblage 2, and *c* denotes the number of species present in assemblage 2 but not in assemblage 1.

We considered potential enzymatic activity of BG, CB, NAG and ALP as proxy for microbial function (Burns et al., 2013; Talbot et al., 2014; Fanin et al., 2022). We used the Euclidean distances as the measures of spatial variability in microbial function between soil microbial assemblages (Mori et al., 2016; Martinez-Almoyna et al., 2019; Jing et al., 2021; Jing et al., 2022) as follows:

$$D(x, y) = \sqrt{\sum_{i=1}^{n} (x_i - y_i)^2} \qquad \text{Eqn. 4}$$

where *D(x, y)* denotes the Euclidean distance, *n* is the number of enzymes (*n* = 4), x_i and y_i denote enzymatic activity in assemblage *x* and assemblage *y*, respectively. Before calculating the Euclidean distance, we standardized all the variables with z-score (Peters et al., 2019).

We calculated the Euclidean distances as the measures of geographic distance and differences in soil depth, salinity, properties, and nutrient pools (Table S1). Specifically, the geographic distance was calculated using the geographic coordinates of sampling sites, and it does not account for differences in soil depth. We calculated differences in soil depth using the mean depth of each sampling layer, and it does not account for geographic distance. We calculated differences in soil salinity using the concentrations of soil Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, CO_3^{2-} and HCO_3^{2-}. Heterogeneity of soil properties was calculated using soil clay content, sand content, silt content, pH, and moisture. Differences in nutrient pools were calculated using the data of soil organic

carbon, soil total nitrogen, and available phosphorus (Jing et al., 2022). The variables used for calculating the Euclidean distances of soil salinity, properties, and nutrient pools were standardized with z-score.

Widespread in global drylands, soil salinity varies among sites (horizontally) as well as between soil layers (vertically) within a site (Fig. 1a). Here, we developed a dimension partitioning approach to quantify the impacts of vertical and horizontal differences in soil salinity and other abiotic factors on the spatial variability in the structure and activity of soil bacterial and fungal communities. We applied this approach to assess the relative importance of pure horizontal, pure vertical, and combined vertical and horizontal differences in soil depth, salinity, properties, nutrient pools, and spatial geographic distance on soil microbial community structure and function in a desert system, where a gradient of soil salinity was created by 12-years of saline water irrigation (Fig. 1a-c).

Specifically, we calculated the pair-wise dissimilarity/distance matrix between soil microbial assemblages for all the combinations of sampling site and soil layer (n = 60). The lower triangle of the pair-wise dissimilarity/distance matrix was next converted into a single vector, which resulted in 1770 observations (60 × 59 / 2). We further partitioned the single vector into three spatial dimensions, including the horizontal, vertical, and horizontal & vertical dimensions. Differences in structure/function within a soil layer between sampling sites were defined as the horizontal dimension that has 270 observations (10 × 9 / 2 × 6), between soil layers within a sampling site were defined as the vertical dimension that has 150

observations (6 × 5 / 2 × 10), and between soil layers and between sampling sites were defined as the horizontal & vertical dimension that has 1350 observations. The dimension partitioning was carried out for all the variables, including soil microbial community structure and function, geographic distance, depth difference, salinity difference, and soil heterogeneity.

2.2. Statistical analyses

To test how soil microbial community structure is related to enzymatic activity across spatial dimensions of soil depth profiles, we inspected the strength and direction of bivariate relationships between differences in soil microbial community structure and differences in enzymatic activity using the Spearman's rank correlation test. Next, we used path models and hierarchical variance partitioning analysis to estimate the relative importance of environmental variables on the spatial variability in soil microbial community structure-function relationships.

First, we conducted the path analysis to estimate the relative importance of dispersal limitation and environmental filtering on soil microbial community structure (Fig. S2). In terms of soil bacterial and fungal community structure, we considered geographic distance and differences in soil depth as proxies for dispersal limitation (Powell et al., 2015) and differences in soil salinity, properties, and nutrient pools as proxies for environmental filtering (Martiny et al., 2006; Jing et al., 2022). Using the path analysis, we also estimated the relative importance of environmental variables and soil bacterial and fungal community structure on

differences in microbial enzymatic activity at each spatial dimension. We used the P value of χ^2 statistic test (P >0.05), comparative fit index (CFI >0.90), root mean square error of approximation (RMSEA <0.05), and standardized root mean square residual (SRMR <0.10) as measures of path model evaluation and selection (Grace, 2020). Because a large sample size (*n* > 100) may lead to the P value of χ^2 statistic test that was less than 0.05 (Grace, 2020), we only used CFI, RMSEA, and SRMR to evaluate and select models for overall and horizontal and vertical spatial dimensions. All path models fitted the data well (Table S2). We used 9 999 bootstrapped sampling iterations for significance tests of the path coefficients.

Second, we conducted hierarchical variance partitioning to estimate the proportion of spatial variability in microbial community structure that was independently explained by the individual effects of geographic distance, differences in soil depth, soil salinity, soil properties, and soil nutrient pools. We also included differences in microbial community structure, soil salinity, properties, and nutrient pools to estimate the proportion of spatial variability in enzymatic activity that independently explained by these biotic and abiotic factors. All combinations of explanatory variables were fitted into regression models following the hierarchical partitioning algorithm of Chevan and Sutherland (1991). The independent effects of each explanatory variable were calculated based on the goodness-of-fit measures (i.e., R^2) of regression models with the target variables (microbial community structure or enzymatic activity) as the

dependent variable. We used 9 999 bootstrapped sampling iterations to calculate the 95% confidence interval of the independent effects of each explanatory variable.

All statistical analyses were performed in R version 3.6.1 (R Development Core Team, 2019) using the packages ‘lavaan’ (Rosseel, 2012) and ‘hier.part’ (Mac Nally and Walsh, 2004). Prior to the data analyses, we removed singleton OTUs, which are present only once across all microbial assemblages, from soil microbial community data and pooled all the data to the level of site × soil layer to remove the non-independent influences of sampling replicates.

3. Results

3.1. Soil microbial community structure-function relationships

Overall, differences in soil bacterial and fungal community structure were associated with differences in enzymatic activity, but these relationships were weak (Spearman correlation coefficient $\rho = 0.15$, $P < 0.001$; Fig. 2a,b). The weak associations of soil microbial community structure with enzymatic activity were consistently observed across the horizontal and vertical dimensions of soil depth profiles ($\rho < 0.16$ in all cases; Fig. S3). Moreover, differences in replacement and richness of soil bacterial and fungal community structure were weakly associated with differences in enzymatic activity ($\rho < 0.16$ in all cases; Figs. S4 and S5).

When we controlled for geographic distance and differences in soil depth, salinity, properties, and nutrient pools, differences in enzymatic activity were not explained by differences in soil microbial community structure (standardized path coefficients β_{std} <0.13 in all spatial dimensions; Fig. 3 and Table S3). However, these variables separately explained between 14% and 27% of the variance in differences in enzymatic activity, between 29% and 39% of the variance in differences in soil bacterial community structure, and between 27% and 33% of the variance in differences in soil fungal community structure (Fig. 3a-d). Specifically, we found that differences in soil bacterial and fungal community structure were most strongly associated with geographic distance (Fig. 3a,b,d), while the effects of differences in soil depth (at the scale of meters; Fig. 3c) along the vertical dimension were just as important as the effects of geographic distance (on the scale of kilometers; Fig. 3a,b,d) along the horizontal dimension on soil bacterial and fungal community structure (Appendix A). In addition, the effects of heterogeneity in soil properties on differences in enzymatic activity along the vertical dimension were as important as those effects at the horizontal dimension (Appendix A).

3.2. Factors mediating microbial community structure and enzymatic activity

Geographic distance and differences in soil depth had the largest independent effects on differences in soil bacterial and fungal community structure along soil depth profiles (Fig. 4a,b). Geographic distance explained 20.7-25.8% of variance in differences in soil bacterial community structure and

24.1-31.9% of variance in differences in soil fungal community structure (Fig. 4a,b). Along the vertical dimension, differences in soil depth explained 21.9% (95% confidence interval = [12.6, 32.6]) of variance in differences in soil bacterial community structure (Fig. 4a and Table S4) and 23.8% (95% confidence interval = [12.6, 35.6]) of variance in differences in soil fungal community structure (Fig. 4b and Table S4). The heterogeneity of soil properties further explained 11.0% (95% confidence interval = [4.6, 21.0]) and 5.9% (95% confidence interval = [1.3, 14.3]) of variance in differences in soil bacterial and fungal community structure, respectively (Fig. 4a,b and Table S4).

The heterogeneity of soil properties had the largest independent effects on differences in enzymatic activity at both horizontal and vertical dimensions (Fig. 4c). The heterogeneity of soil properties explained 8.4-17.2% of variance in differences in enzymatic activity along the overall, horizontal, and horizontal & vertical dimensions, and explained 11.9% of variance in differences in enzymatic activity along the vertical dimension (95% confidence interval = [3.9, 23.3]) (Table S4). Differences in soil bacterial and fungal community structure had the least independent effects on differences in enzymatic activity (Fig. 4c).

4. Discussion

Much of our understanding of BEF relationships was developed in temperate ecosystems with a focus on vascular plants. We may expect these relationships to be even stronger in extreme environments with strong environmental gradients (Forrester and Bauhus, 2016). Along strong salinity gradients, both horizontally

and vertically, we predict that soil salinity would define structure-function relationships horizontally among sites, whereas the heterogeneity in soil properties and nutrients would define the structure-function relationships vertically with soil depth. Here, the most salient result of this study is that soil microbial community structure was decoupled from enzymatic activity relevant to carbon, nitrogen, and phosphorus cycling along both the horizontal and vertical dimension of soil depth profiles. This result runs counter to previous work exploring these relationships in microbial systems (Mori et al., 2016; Delgado-Baquerizo et al., 2020) and does not support our first hypothesis that differences in soil microbial community structure would be positively associated with spatial variability in enzymatic activity in extreme environments. The results of spatial dimension partitioning partially support our second hypothesis that soil depth is as important as horizontal geographic distance in shaping bacterial and fungal community structure. However, our results show that the heterogeneity of soil properties (i.e., soil texture, pH, and water) rather than soil salinity or nutrient pools, is most strongly associated with differences in enzymatic activity across all spatial dimensions of soil depth profiles. Our findings suggest that soil depth governs the assembly of soil microbial community structure and the activity of carbon, nitrogen, and phosphorus cycling enzymes. Soil depth profile thus can serve as a test bed for predicting changes in soil microbial community structure and enzymatic activity in real-world extreme environments.

4.1. Uncoupled microbial community structure-function relationships

Differences in soil microbial community structure did not lead to differences in enzymatic activity in the studied hyper-saline environments. The uncoupled relationships between soil microbial structure and function were further verified across the horizontal and vertical dimensions of soil depth profiles. Our findings, thus, support the idea of functional redundancy (Coleman and Whitman, 2005; Peay et al., 2016), implying that soil microbial communities – even ones that are in extreme environments – differ, but function in the same manner. As a result, the losses/gains of certain species may have limited effects on the spatial variability in enzymatic activity. This means that maintaining functionally redundant communities is crucial to safeguard microbial function against the negative influences of extreme environmental changes (Allison and Martiny, 2008), and thus could promote ecosystem stability and the provisioning of multiple ecosystem services.

There are two explanations for the uncoupled structure-function relationships. First, soil bacterial and fungal communities have likely adapted to the harsh desert conditions, where abiotic stress is high (Jansson and Hofmockel, 2020). As soil becomes saltier and drier, soil microorganisms become inactive because soil carbon and nutrients availability declines. In addition, soil microorganisms tend to minimize their activity, even becoming dormant, to conserve energy under harsh conditions. In either case, we would observe the uncoupled relationships between soil microbial community structure and enzymatic activity. Second, the uncoupled structure-function relationship is likely due to the

mismatch of dominant driving factors responsible for microbial community structure and enzymatic activity. Our results show that geographic distance and differences in soil depth have the most strongly independent effects on microbial community structure, while the heterogeneity in soil properties has the most strongly independent effects on enzymatic activity. Such a mismatch in driving factors could have disproportionate influences on soil microbial community structure and enzymatic activity, leading to uncoupled structure-function relationships.

4.2. Soil depth profile as a new frontier for BEF study

We found that the spatial variability in soil microbial community structure was strongly associated with geographic distance and differences in soil depth. This result suggests that soil bacterial and fungal communities show dissimilar community structure with increased spatial distance both horizontally and vertically along soil depth profiles. Moreover, our work indicates that soil depth is as important as geographic distance in shaping soil bacterial and fungal community structure (Fig. 4a,b). The importance of soil depth is supported by an earlier study showing that the random assembly processes of soil microbial communities (e.g., dispersal limitation) intensified with soil depth because soil microorganisms disperse passively (Powell et al., 2015). The observation that soil depth and geographic distance are equally important emerges only when the overall spatial variability in microbial structure is partitioned into the pure vertical and horizontal dimensions (Fig. 4a,b). Without the spatial dimension partitioning,

we would underestimate the contribution of soil depth to the assembly processes of soil microbial communities.

Overall, our work highlights that dispersal limitation in soil microbes is particularly important to the assembly of soil microbial communities in the vertical and horizontal dimensions of soil depth profiles in extreme environments. To maintain the essential and irreplaceable ecosystem functions and services provided by belowground biodiversity in the extreme conditions or severely disturbed conditions, a workable plan is to preserve habitats along both horizontal and vertical habitats along soil depth profiles.

Our study further advances the understanding that the spatial variability in soil enzymatic activity is best explained by the heterogeneity in soil texture, pH, and water, rather than differences in soil salinity and nutrient pools across soil depth. In the extreme environments, environmental filters (e.g., salt stress) and resource levels (e.g., soil organic matter and microbial biomass) are expected to be important drivers of spatial variability in soil microbial community structure and function (Martiny et al., 2006; Rath and Rousk, 2015; Rath et al., 2019a; Rath et al., 2019b). By contrast, our results suggest that differences in soil salinity and nutrient pools do not explain the activity of carbon, nitrogen, and phosphorus cycling enzymes. One possible explanation is that soil salinity is too high in our study system and the first priority of soil microbes is to survive rather than to thrive in the extreme saline environments, which leads soil microbes to allocate few resources to produce enzymes (Rath and Rousk, 2015). As a result,

changes in soil salinity and nutrient pools did not alter enzymatic activity because soil salinity and resources are not limiting factors of enzymatic activity. However, our result indicates that reduced heterogeneity in soil properties, which could be induced by land degradation, soil erosion, and desertification, might result in the loss of soil microbial activity and soil functionality, soil fertility, and plant growth. Given that the variability in soil physical and chemical properties is created by human activities and/or by nature ubiquitously, this work highlights the significance of analyzing the overlooked dimension of microbial structure-function relationships at the vertical dimension along soil depth profiles, which represent a valuable step towards soil conservation efforts in dealing with ongoing desertification in arid ecosystems.

In recent years, microbial ecologists have focused primarily on surface communities because they have the highest biomass density and measured nutrient cycling activities. This study is unique in that it provides a new approach to assess soil microbial community structure-function relationships both horizontally and vertically along soil depths with similar range of salinity variability in one of the extreme biomes, i.e., the Taklamakan desert. However, large-scale studies remain the key to verify whether these findings of this study hold true across other biomes with wide range of horizontal and vertical environmental gradients that are created either by human activities or by nature. Further, it is important to note that the measurements of four hydrolytic enzyme activities capture only a few microbial potential functions that are relevant to carbon,

nitrogen, and phosphorus cycling. Future work that uses shotgun metagenomics could quantify broad shifts in microbial community functionality, e.g., those functions related to organotrophs and perhaps autotrophs (both by phototrophs and chemolithotrophs such as hydrogen oxidizers), and thus advance the understanding of structure-function relation of microbial communities in xeric or arid soils.

5. Conclusion

This study advances the understanding of biodiversity-functioning relationships into naturally assembled soil microbial communities in extreme environments through partitioning soil depth profiles into the horizontal and vertical dimensions. We demonstrate that soil microbial community structure is decoupled from enzymatic activity along both the horizontal and vertical dimensions of soil depth profiles. Moreover, we show that soil depth is as important as horizontal geographic/environmental gradient in shaping soil microbial community structure and enzymatic activity. These findings highlight the need to analyze the new dimension of biodiversity and ecosystem functioning, i.e., the vertical dimension. Such information is crucial to predict the influences of global change drivers on real-world naturally assembled community structure-function relationships across horizontal and vertical spatial scales. Moreover, this study highlights that considering increasing desertification in arid ecosystems, soil conservation efforts should take soil depth into consideration as

a critical factor governing the assembly of microbial communities and the activity of enzymes.

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Author Contributions: XJ, WF and YW designed the study. YW and WF conducted the field- and lab-work. XJ analyzed the data. XJ and WF wrote the first draft of the manuscript. All authors contributed to the conceptualization, review and editing of the manuscript.

Competing Interest Statement: The authors declare no competing interests.

Data availability: Sequence data were deposited in the European Nucleotide Archive with accession number PRJEB52395 (http://www.ebi.ac.uk/ena/data/view/OW744067-OW763241) for soil bacteria, and with accession number PRJEB46878 (http://www.ebi.ac.uk/ena/data/view/OU495976-OU501470) for soil fungi. Data and code will be publicly available in the Zenodo repository prior to publication.

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Figure legend

Fig. 1 A conceptual framework for testing the relationships between community structure and function along the horizontal and vertical dimensions of soil depth profiles

Fig. 2 Bivariate relationships between differences in soil microbial community structure and differences in enzymatic activity

Fig. 3 Uncoupled microbial community structure and function relationships along soil depth profiles

Fig. 4 Independent effects of explanatory variables on differences in soil microbial community structure and enzymatic activity

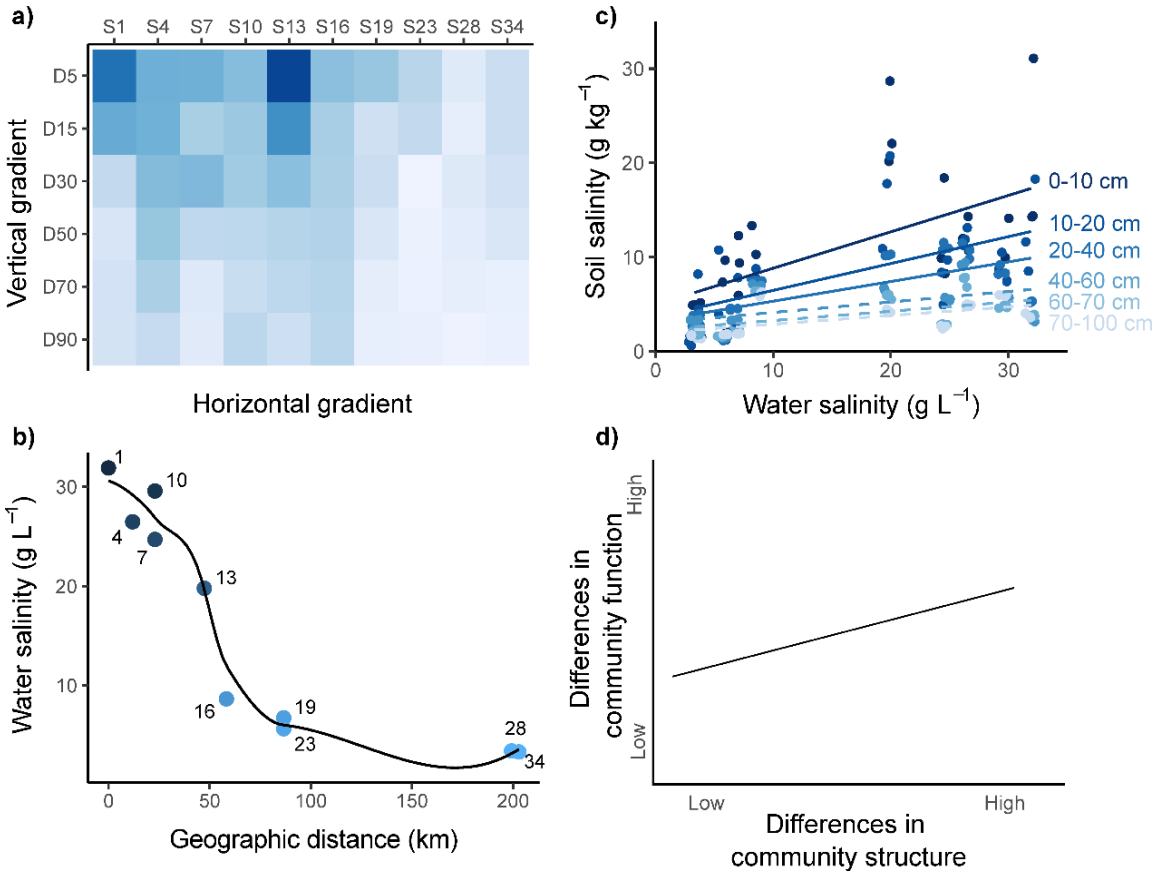

Fig. 1 A conceptual framework for testing the relationships between community structure and function along the horizontal and vertical dimensions of soil depth profiles. a) Heatmap illustrating changes in soil salinity along the horizontal and vertical dimension of soil depth profiles. Codes of the sampling sites are shown at the x-axis, and the average sampling depth for each soil layer is shown at the y-axis. b) The variability in irrigation water salinity along the horizontal gradient (geographic distance). Sampling sites are given next to the points. Site 1 with the highest water salinity is selected as the reference site for calculating the geographic distance (km). c) Impacts of irrigation water salinity on soil salinity across soil layers of the vertical dimension.

Linear mixed-effects models are used for significance tests. Solid lines denote significant effects of irrigation water salinity on soil salinity and dashed lines denote insignificant effects. d) If there is low functional redundancy in extreme environments, we predict that community structure is positively associated with microbial function. The levels of soil/water salinity in a-c) are represented by colors from dark-blue (high soil/water salinity) to light-blue (low soil/water salinity).

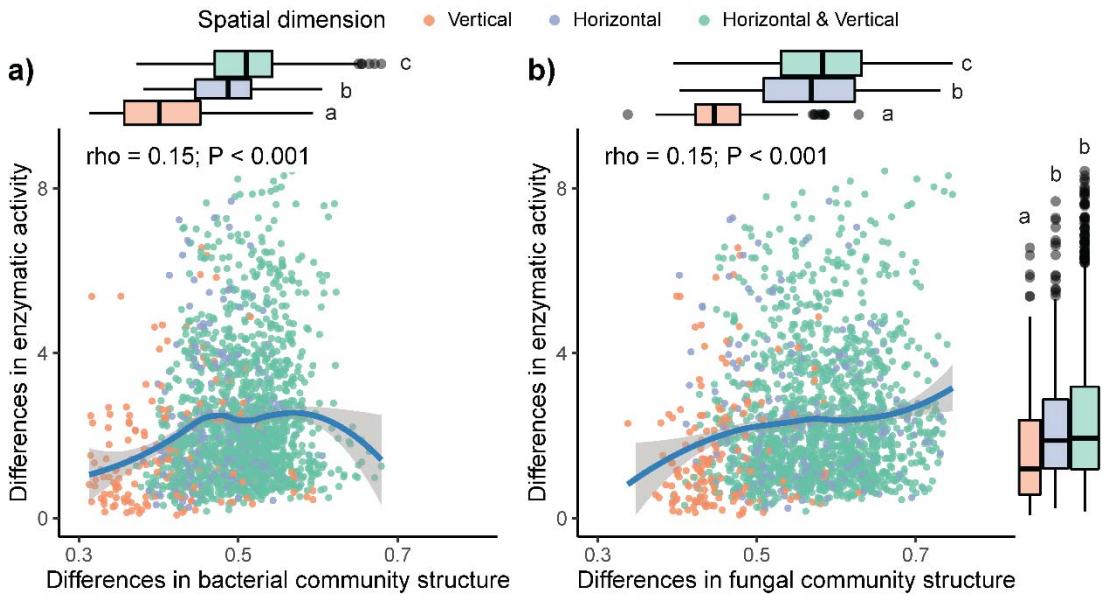

Fig. 2 Bivariate relationships between differences in soil microbial community structure and differences in enzymatic activity. a) bacterial community structure-enzymatic activity relationships; b) fungal community structure-enzymatic activity relationships. Lines denote the loess fitted curves showing the direction of the bivariate relationships. Shaded areas denote the 95% confidence interval. Spearman correlation coefficients are used to show the strength of the bivariate relationships. Boxplots with median, quartiles, and extreme values denote the data distributions of differences in community structure (top) and differences in enzymatic activity (right). Different letters denote significant differences among spatial dimensions.

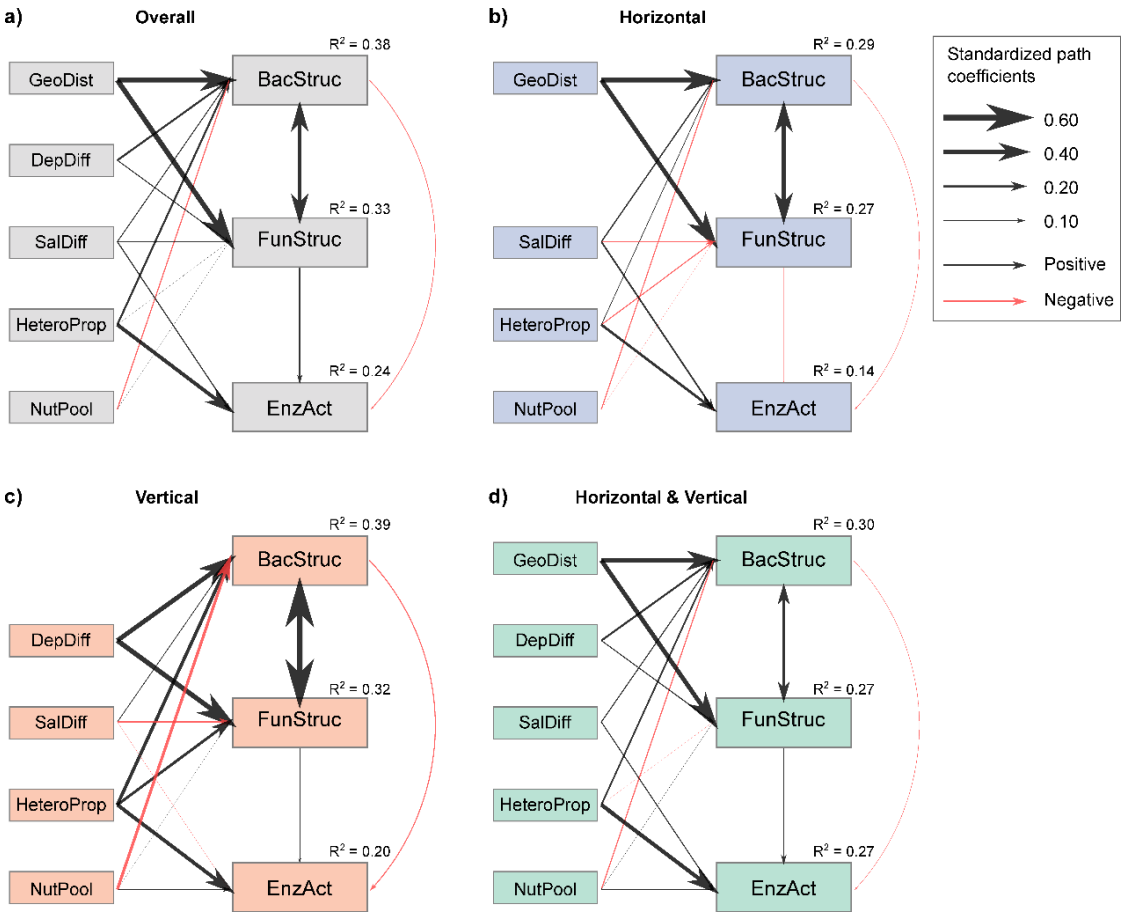

Fig. 3 Uncoupled microbial community structure and function relationships along soil depth profiles. Four path models are shown based on spatial dimensions, including a) all dimensions; b) pure horizontal dimension; c) pure vertical dimension; d) combined horizontal & vertical dimension. Arrow width is proportional to the standardized path coefficients. Black arrows denote positive path coefficients and red arrows denote negative path coefficients. Bootstraps are used for the significance test of path coefficients (see Table S3 for details). R^2 denote the variance of community structure and enzymatic activity explained by environmental factors, including geographic distance (GeoDist), differences in

soil depth (DepDiff), differences in soil salinity (SalDiff), heterogeneity of soil properties (HeteroProp), and differences in nutrient pools (NutPool). BacStruc, FunStruc, and EnzAct denote differences in bacterial community structure, fungal community structure, and enzymatic activity, respectively.

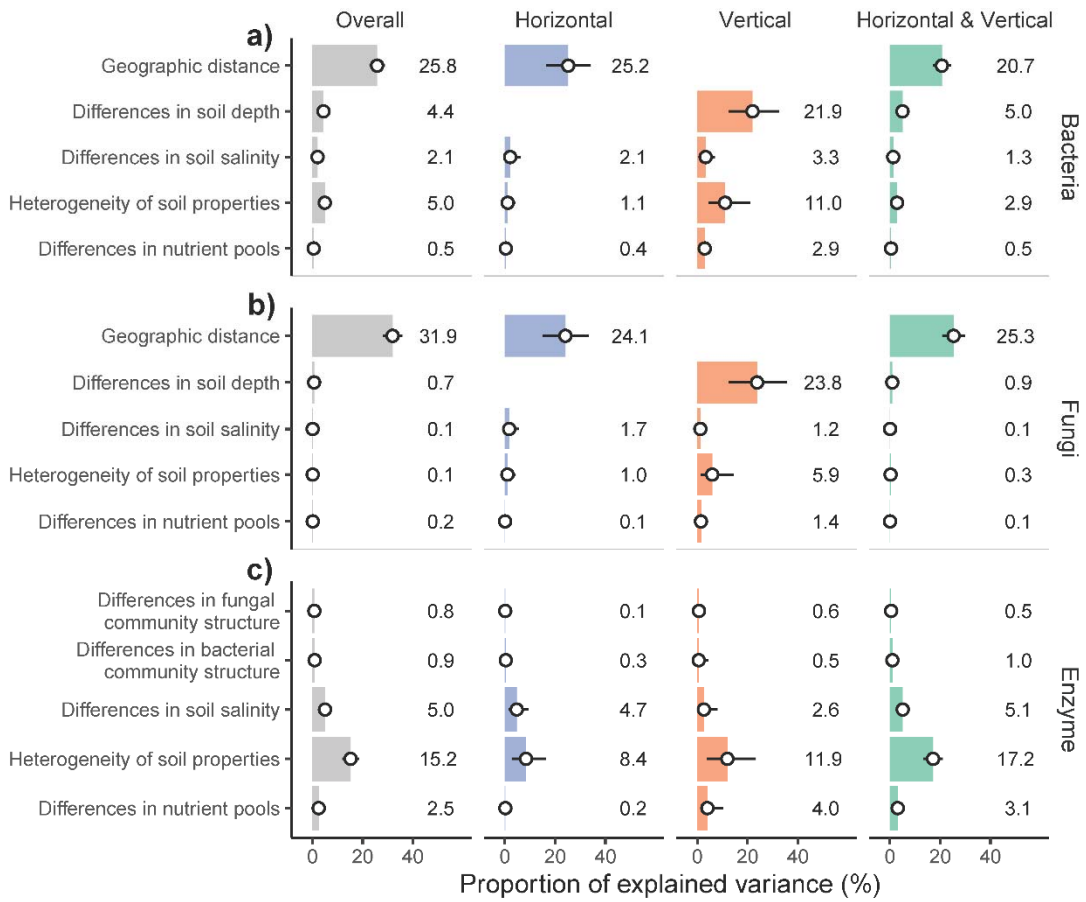

Fig. 4 Independent effects of explanatory variables on differences in soil microbial community structure and enzymatic activity at each spatial dimension. a) differences in bacterial community structure; b) differences in fungal community structure; c) differences in enzymatic activity. Bars represent the pure variance explained by the explanatory variables, and the values are shown along the bars. Points with 95% confidence interval denote the variability of explained variance for each explanatory variable (see Table S4 for details).