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Research article

Unraveling microbial community structure–function relationships in the horizontal and vertical spatial dimensions in extreme environments

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A fundamental challenge in soil macroecology is to understand how microbial community structure shapes ecosystem function along environmental gradients of the land surface at broad spatial scales (i.e. the horizontal dimension). However, little is known about microbial community structure–function relationships in extreme environments along environmental gradients of soil depth at finer spatial scales (i.e. the vertical dimension). Here, we propose a general spatial dimension partitioning approach for assessing the patterns and drivers of soil microbial community structure–function relationships across horizontal and vertical spatial gradients simultaneously. We leveraged a 200-km desert soil salinity gradient created by a 12-year saline-water irrigation in the Tarim basin of Taklamakan Desert. Specifically, using a general linear model, hierarchical variance partitioning, and a path model, we assessed the patterns and key ecological processes controlling spatial turnover in microbial community structure (i.e. β-diversity) and enzymatic activity relevant to carbon, nitrogen, and phosphorus cycling along soil salinity gradients across study sites (horizontal dimension) and soil depths (vertical dimension). We found a decoupled relationship between soil microbial β-diversity and enzymatic activity. Differences in soil depth (on the scale of meters) were as important as geographic distance (on the scale of kilometers) in shaping bacterial and fungal β-diversity. However, the vertical and horizontal turnover in enzymatic activity was largely attributed to an increase in the heterogeneity of soil properties,

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such as soil texture, water content, and pH. Our findings suggest that dispersal limitation controls microbial community β-diversity and that environmental heterogeneity, rather than soil salinization, controls enzymatic activity. Taken together, this work highlights that in the face of ongoing environmental alterations, soil depth is an under-explored spatial dimension that must be considered in soil conservation efforts as a critical factor in determining microbial community structure and function in extreme environments.

Keywords: ecosystem function, extreme environments, microbial beta-biodiversity, soil macroecology, structure-function relationships, Taklamakan desert

Introduction

Human-induced global change has accelerated a biodiversity change worldwide (Wardle et al. 2011, Díaz and Malhi 2022). However, we know little about how global change affects soil microbes (Shangguan et al. 2023) and how it will affect many ecosystem functions on the land surface (i.e. the horizontal spatial dimension) (Jing et al. 2015, Wall et al. 2015, Delgado-Baquerizo et al. 2020, Berlinches de Gea et al. 2023). Of particular interest is how soil microbial community structure, i.e. species diversity and community composition, shapes ecosystem function, which is a fundamental challenge in the field of soil macroecology (Chu et al. 2020, White et al. 2020, Xu et al. 2020, Eisenhauer et al. 2021). Furthermore, it is a relatively new endeavor to investigate the influences of spatial turnover in soil microbial community composition (i.e. β-diversity) on spatial turnover in ecosystem function across broad spatial scales (Mori et al. 2018, van der Plas et al. 2023). Recent research on how β-diversity affects spatial turnover in ecosystem function has yielded mixed results, with both positive and neutral effects reported (Talbot et al. 2014, Mori et al. 2016, Martinez-Almoyna et al. 2019, Jing et al. 2021, 2022). One of the major challenges in this field is that the soil matrix is inhabited by an immense variety of microbes (Coleman and Whitman 2005, Nielsen et al. 2015, Díaz and Malhi 2022). For example, a tiny small soil sample may contain 100–9000 microbial species/strains (Bardgett and van der Putten 2014), whereas a grassland biodiversity experiment would only contain one to 32 plant species (Hector et al. 1999). This makes it much more difficult to reveal microbial community structure–function relationships in response to global change (Peay et al. 2016, Zhou et al. 2020, Yang et al. 2022).

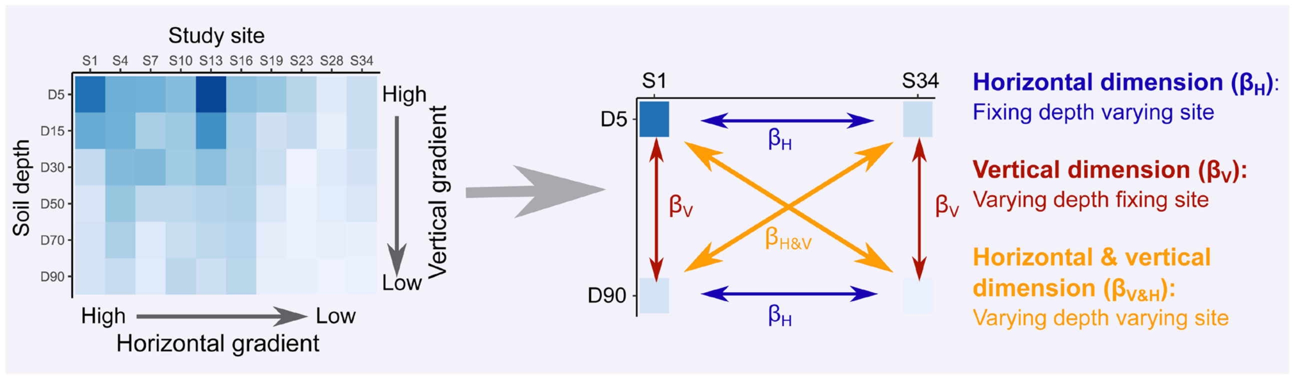
Investigating structure–function relationships in extreme environments could provide insights into the aforementioned challenge at broad spatial scales (Wall and Virginia 1999, Maček et al. 2016, Osborne et al. 2020, Ertekin et al. 2021, Shu and Huang 2022). In extreme environments, for example, intense environmental constraints may impose strong environmental filtering effects on community composition (Shu and Huang 2022). In other words, when environmental conditions differ, such filtering effects will promote spatial turnover in soil microbial community composition, e.g. in extremely arid and saline deserts (Zhang et al. 2019). However, in global change experiments in grasslands and forests, the filtering effects may not be observed or may take decades to develop (Melillo et al. 2017, Shangguan et al. 2023). Therefore, studying extreme environments can provide us with a long-term natural experimental system in which we can investigate how current and future alterations in environmental conditions shape the spatial turnover of soil microbial community composition. In addition, the structure of biological communities in extreme environments is relatively simple, with little or no functional redundancy (Wall and Virginia 1999). It means that simplified communities tracking the effects of changes in community composition of soil microbial communities on ecosystem function is easier than in complex communities. As a result, studying extreme environments provides unique opportunities to better understand how the structure and function of soil microbial communities will respond to accelerating global change (Fierer et al. 2012, Shu and Huang 2022).

Environmental filters such as soil salinity, water, nutrient availability, and geographic distance can impact spatial turnover in soil microbial community composition (Talbot et al. 2014, Powell et al. 2015, Zhang et al. 2019). However, previous studies have shown how these environmental filters affect structure–function relationships in soil microbial communities that vary with spatial scales. For example, after accounting for the influences of environmental filters, one study observed a positive relationship between soil fungal β-diversity and spatial turnover in multiple ecosystem functions at the landscape scale (Mori et al. 2016). Another study reported a positive relationship between soil bacterial, but not fungal, β-diversity and spatial turnover in multiple ecosystem functions at the regional scale (Jing et al. 2022). In extreme environments (e.g. extremely arid and saline deserts), environmental filters can change at relatively small spatial scales through soil depth profiles. Indeed, soil depth, as a proxy for environmental filters, is a spatial dimension that is often under explored in the field of soil macroecology (White et al. 2020, Eisenhauer et al. 2021), but is critical for an explicit understanding of how microbial community structure relates to ecosystem function (Fierer et al. 2003). Previous work finds that soil depth influences microbial community biodiversity because soil properties that affect microbial growth and activity, such as organic carbon, nutrient, salinity, oxygen concentration, and moisture, vary with soil depth (Fierer et al. 2003, Powell et al. 2015, Stone et al. 2015, Sun et al. 2020). However, although soil depth regulates microbial functions such as organic matter decomposition and nutrient cycling, little is known about how vertical patterns of species turnover in microbial communities relate to spatial turnover in ecosystem function along soil profiles.

Here, we address the question of how microbial community structure affects spatial turnover in ecosystem function across spatial scales in extremely saline environments. We use an experimental gradient of soil salinization, that was created by a 12-year saline-water irrigation infrastructure in the Taklamakan Desert, the World's 2nd largest shifting sand desert, covering an area roughly the size of Germany (Supporting information). The potential consequences of soil salinization on microbial physiology, growth and community composition have been extensively studied (Rath and Rousk 2015). However, the impacts of salinization on soil microbial community structure and enzymatic activity have rarely been investigated across spatial scales. We, therefore, propose an approach to partition spatial scales into a purely horizontal dimension, a purely vertical dimension, and a combined horizontal and vertical dimension (Fig. 1a–b). Specifically, we assess soil bacterial and fungal community structure and enzymatic activity relevant to soil carbon, nitrogen, and phosphorus cycling along a unique 200-km salinity gradient, i.e. the horizontal spatial dimension (Supporting information).

(a) Dimension of environmental gradients

(b) Spatial dimension partitioning of β-diversity



(c) Soil salinization would increase soil microbial species turnover in the horizontal and vertical dimensions

(d) Soil salinization would enhance spatial turnover in enzymatic activity indirectly through changes in microbial community structure

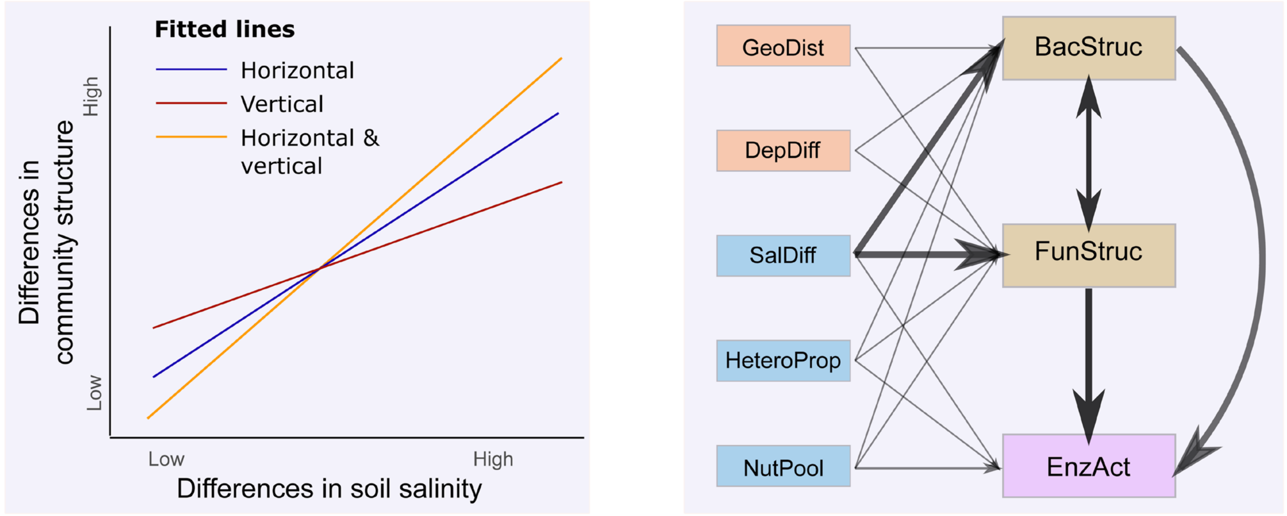


Figure 1. Conceptual depictions of the spatial dimension partitioning approach. (a) Heatmap denotes the horizontal and vertical dimensions of environmental gradients, i.e. soil salinity. The soil salinity gradient was induced by a 12-year saline-water irrigation in the Taklamakan desert. (b) A framework for illustrating the spatial dimension partitioning of β-diversity. β_H, β_V and $\beta_{V\&H}$ denote species turnover along the horizontal, vertical, and horizontal and vertical soil salinity gradients, respectively. (c) The spatial dimension partitioning approach can be applied to analyze the associations between soil salinization and soil microbial species turnover, i.e. β-diversity. (d) A path model shows direct and indirect effects of soil salinity via soil bacterial and fungal community structure when accounting for other environmental filters, including geographic distance (GeoDist), differences in soil depth (DepDiff), differences in soil salinity (SalDiff), differences in soil properties (PropDiff), and differences in nutrient pools (NutPool). BacStruc, FunStruc and EnzAct denote differences in bacterial community structure, fungal community structure and enzymatic activity, respectively.

At each site we also examine bacterial and fungal community structure and enzymatic activity across a one-meter salinity gradient along the soil profile, i.e. the vertical spatial dimension (Supporting information), an under-explored dimension of soil macroecology. A number of studies have examined how microbial community structure varies from place to place on the planet (Fierer and Jackson 2006, Thompson et al. 2017), few have examined how they vary along a vertical depth gradient at a particular place. We, thus, examine a combined horizontal and vertical spatial dimension. In addition to the salinity gradient patterns, we assess a variety of environmental filters, i.e. soil physical and chemical properties, nutrient pools, geographic distance, and soil depth distance that may influence microbial community structure–enzymatic activity relationships (Fig. 1, Supporting information).

We test two hypotheses:

(H1) soil salinization increases soil microbial species turnover not only in the horizontal spatial dimension, but also in the vertical spatial dimension (Fig. 1c). Moreover, if the effects of soil salinization outweigh the effects of other environmental filters (Rath et al. 2019a, b, Zhang et al. 2019, Lin et al. 2022), soil salinization would enhance soil microbial species turnover when accounting for other environmental filters (Fig. 1d).

(H2) if soil microbes have little or no functional redundancy (Delgado-Baquerizo et al. 2016, Mori et al. 2016), soil salinization indirectly enhances spatial turnover in enzymatic activity via soil bacterial and fungal species turnover (Fig. 1d).

By addressing these hypotheses, we aim to improve our understanding of microbial community structure–function relationships in extreme environments and across broad spatial scales. In this context, we first examine how environmental filtering shapes microbial community structure–function relationships in extreme saline environments. Furthermore, inspired by a previous framework for partitioning variation in community composition (i.e. β-diversity) across spatiotemporal scales (Khattar et al. 2021, He et al. 2023), we introduce a general approach to partition community structure–function relationships into a purely horizontal dimension, a purely vertical dimension, and a combined horizontal and vertical dimension. The spatial partitioning approach we present can be used to assess the patterns and drivers of community structure and function across spatial scales ranging from a fine scale of soil depth profile to a broad scale of regional land surface.

Material and methods

Study sites

We conducted this study in shrubland vegetation located along the Tarim desert highway, which crosses the Tarim basin of Taklamakan desert (37°10’–41°80’N, 82°60’–84°30’E) (Supporting information). The Taklamakan desert is arid, with a mean annual precipitation of 24.6 mm year^{-1} and a mean annual potential evaporation of 3639 mm year^{-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 soil is composed of 87.3% of sand, 12.4% of silt and 0.3% of clay (Zhang et al. 2016). The soils have an extremely low organic matter content (0.74 g kg^{-1}) and 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* being dominant the community and *Calligonum aborescens*, *Tamarix ramosissima*, *Nitraria tangutorum*, *Populus euphratica* and *Elaeagnus angustifolia* with much lower abundance. The aboveground plant community did not vary along the 200 km salinity gradient of study region. Additionally, the aboveground biomass of dominant species *H. ammodendron* did not vary across the studied sites, leading to comparable amount of carbon inputs into the 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 (Supporting information). The groundwater was pumped for drip irrigation to water shrubs in the vegetation. The drip irrigation pipes 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 litres of water per plant per irrigation event (Xu et al. 2006).

Soil sampling

We surveyed ten shrubland sites along an extreme gradient of irrigation water salinity using a regression design of field experiment (Pelini et al. 2011 for an example of the regression design). The regression design used a gradient of water salinity that varied from 3.2 to 38.0 g l^{-1} of total dissolved salts (the normal range of seawater salinity is between 33–37 g l^{-1} of dissolved salts) from the south to the north sampling site (Supporting information). We collected irrigation water and soil samples in early October 2017, which is the end of the growing season in the desert. At each study site, three replicates of irrigation water were collected from running water that pumped from wells. 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 between 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 and removed rocks and roots by hand. We stored soils at −20°C for microbial sequencing and at 4°C for the assays of soil enzymatic activity. We air-dried the remaining soil to analyze soil properties, including salinity, pH, water content, texture, total organic carbon, total nitrogen and available phosphorus (see the Supporting information for detailed summary of the soil characteristics).

Soil measurements

We analyzed the soluble salts of irrigation water in the following ways. We determined the concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} using a spectrometer with a CID detector (Thermo Scientific, iCAP 6300-ICP-OES). 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 the total soluble salts from irrigation water as the sum of the concentrations of these soluble salts (Lu 1999). Since salinity is a result of a variety of soluble cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, SO_4^{2-}, CO_3^{2-} and HCO_3^-) (Hardie and Doyle 2012), we used total soluble salts as a proxy for irrigation water salinity.

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). We measured soil gravimetric water content by oven drying 10 g of fresh soils at 105°C for 24 h. We measured soil texture using the laser diffraction method (Sympatec GmbH, System-PartikelTechnik) after removing the organic material by 10% hydrogen peroxide and removing carbonates by 0.2% hydrochloric acid. 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 (BUCHId) and soil available phosphorus using the sodium bicarbonate extraction with Mo-Sb Anti-spectrophotometer method.

Soil bacterial and fungal community composition

To analyze the composition of soil bacterial and fungal communities, 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 PCR using the BioRad S1000 thermal cycler (Bio-Rad Laboratory) 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 min. We repeated this PCR procedure three times for each sample. We next mixed the PCR products using GeneTools Analysis Software (ver. 4.03.05.1, SynGene). The process was followed by purifying the DNA products using the EZNA Gel extraction kit (Omega Bio-Tek). Following the manufacturer's instruction, we generated sequencing libraries using the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs) and sequenced the libraries on an Illumina HiSeq2500 platform (Illumina PE250).

We processed raw DNA sequences using the Trimmomatic software (ver, 0.33, 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 reads on the paired end to the corresponding soil samples using the Mothur pipeline (ver. 1.35.1, www.mothur.org). We removed the barcode and primers to derive the paired-end clean reads, which were merged with FLASH (ver. 1.2.11, 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 USEARCH software (ver. 8.0.1517, www.drive5.com/usearch). We used representative sequences for taxonomic classification using the RDP classifier according to the Greengenes database (http://greengenes.secondgenome.com) for bacteria and the Unite database (http://unite.ut.ee/index.php) for fungi in the QIIME pipeline (ver. 2; http://qiime.org/scripts/assign_taxonomy.html). Details on the taxonomic composition profiling of soil bacteria and fungi at the phylum level can be found in the Supporting information.

Enzymatic activity measurements

To explore microbial functions, we assayed the potential activities 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 microplate 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 used as proxies for the acquisition of carbon compounds (BG and CB), nitrogen (NAG), and phosphorus (ALP) (Sinsabaugh et al. 2008). 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 microplate and mixed with 50 μl of 200 μM standard fluorometric substrates of each enzyme. The microplates were incubated for 2.5 h in the dark at 25°C. In a 96-well microplate reader, the amount of fluorescence was measured at 360 nm excitation and 460 nm emission (Biotek Synergy 2). A 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}.

Statistical analyses

We used a two-step approach to examine how soil salinization affects soil microbial community structure and enzymatic activity.

Step 1, to evaluate whether increasing soil salinity enhances species turnover (hypothesis 1), we developed a dimension partitioning approach (Khattar et al. 2021, He et al. 2023) to quantify the impacts of vertical and horizontal differences in soil salinity on spatial turnover in the structure of bacterial and fungal communities. We calculated the pair-wise dissimilarity matrix between soil microbial assemblages for all combinations of study sites and soil layers ($n = 60$). The lower triangle of the pair-wise dissimilarity matrix was converted into a single vector, resulting in 1770 observations (60×59 / 2). We further partitioned the single vector into three spatial dimensions, including the horizontal, vertical, and horizontal and vertical dimensions. The horizontal and vertical dimensions were combined to represent how variation in microbial assemblages change across sites and soil depths simultaneously. Differences in community structure of the same soil layer between study sites were defined as the horizontal dimension ($10 \times 9 / 2 \times 6 = 270$ observations), between soil layers within a study site were defined as the vertical dimension ($6 \times 5 / 2 \times 10 = 150$ observations), and between soil layers and between sampling sites were defined as the horizontal and vertical dimension (1350 observations).

To measure the spatial turnover in soil microbial community structure between assemblages, i.e. β-diversity, 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 differences in richness (β_{rich}). Specifically, the Sorensen dissimilarity index quantifies the total differences in species composition between assemblages. It was further partitioned into two additive components, i.e. species replacement and differences in richness. The former quantifies the species in one assemblage that are replaced by distinct species in another assemblage, which is also called as turnover (Legendre 2014).The latter estimates differences in the number of species between assemblages, which is a result of species gain or loss (Carvalho et al. 2012). We used the following equations:

$$\beta_{sor} = \frac{b + c}{2a + b + c} \tag{1}$$

$$\beta_{repl} = \frac{2 \times \min(b, c)}{2a + b + c} \tag{2}$$

$$\beta_{rich} = \frac{|b - c|}{2a + b + c} \tag{3}$$

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

To compare the species turnover rate with differences in soil salinity, we calculated the Euclidean distances as the measure of differences in soil salinity. We calculated differences in soil salinity using the concentrations of soil Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} and HCO_3^{2-} as follows:

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

where $D(x, y)$ denotes the Euclidean distance, n is the number of salt ions ($n = 7$), x_i and y_i denote the concentration of salt ions in the sample x and sample y, respectively. We regressed pair-wise compositional dissimilarity (i.e. β-diversity) against differences in soil salinity across the horizontal and vertical spatial dimensions. We used the standardized regression slopes to compare the rate of species turnover among spatial dimensions and the permutation test to examine the significance of regression slopes.

Step 2, to evaluate whether increasing soil salinity mediate microbial community structure–enzymatic activity relationships (hypothesis 2), we used hierarchical variance partitioning and path model. Before the analysis, we first calculated a suite of explanatory variables. We used the Euclidean distances to calculate geographic distance and differences in soil depth, salinity, properties, and nutrient pools (Supporting information). Specifically, the geographic distance was calculated using the geographic coordinates of the sampling sites. Differences in soil depth were calculated using the mean depth of each sampling layer. Differences in soil properties were calculated using soil texture (clay content, silt content, sand content), pH and moisture. Differences in soil nutrient pools were calculated using soil organic carbon, total nitrogen and available phosphorus (Peters et al. 2019, Jing et al. 2022). We acknowledged that the variables used for quantifying differences in soil salinity, properties and nutrient pools, were often combined into one integrative proxy for environmental factors or habitat characteristics (Powell et al. 2015). However, we classified them into groups of soil salinity, properties, and nutrient pools because they provided an opportunity to compare the relative importance of environmental filters in shaping microbial community structure–function relationships. Specifically, our study system had a unique gradient of soil salinity generated by saline-water irrigation (Supporting information), allowing us to assess the influences of soil salinization (Lin et al. 2022, 2023, Feng et al. 2023). Meanwhile, physiochemical properties, such as soil clay content, silt content, sand content, pH, and moisture were often used to predict soil microbial community structure (Fierer and Jackson 2006). The indicators of soil nutrients pools were often linked with changes in community biodiversity and enzymatic activity (Lange et al. 2015, Oelmann et al. 2021, Jing et al. 2022).

We conducted hierarchical variance partitioning to estimate the proportion of spatial turnover in the structure of microbial communities (hypothesis 2a). 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 determined 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 9999 bootstrapped sampling iterations to calculate the 95% confidence interval of the independent effects of each explanatory variable.

We used the path model to estimate the indirect effects of soil salinity on enzymatic turnover via bacterial and fungal species turnover (hypothesis 2b). We considered the potential enzymatic activities of BG, CB, NAG and ALP as a proxy for ecosystem function (Burns et al. 2013, Talbot et al. 2014, Garland et al. 2021, Fanin et al. 2022). We used the Euclidean distances as the measures of spatial turnover in microbial functions, i.e. enzymatic turnover or differences in enzymatic activity (Mori et al. 2016, Martinez-Almoyna et al. 2019, Jing et al. 2021, 2022). The path model was also used to assess the relative importance of dispersal limitation and environmental filtering on the structure of soil microbial communities (Jing et al. 2021, 2022). For soil bacterial and fungal community structure, we considered geographic distance and differences in soil depth as the proxies for dispersal filters (Talbot et al. 2014, Powell et al. 2015) and differences in soil salinity, properties, and nutrient pools as the proxies for environmental selection filters (Martiny et al. 2006, Jing et al. 2022). Using path model, we also estimated the relative importance of environmental filters and community structure of soil bacteria and fungi on differences in microbial enzymatic activity. 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). Since a large sample size ($n > 100$) can 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. We used 9999 bootstrapped sampling iterations to assess the significance of the path coefficients. All path models fitted the data well (Supporting information).

The dimension partitioning was conducted for all the variables, including soil microbial community structure and enzymatic activity, geographic distance, depth difference, salinity difference, property difference, and nutrient pool difference. Prior to data analyses, we removed singleton OTUs, which were present only once across all microbial assemblages, from the soil microbial community data and pooled all the data to the level of site × soil layer to remove the non-independent influences of sample replicates. All statistical analyses were performed in R ver. 3.6.1 (www.r-project.org) using the packages 'lmPerm' (Wheeler et al. 2016), 'hier.part' (Nally and Walsh 2004), and 'lavaan' (Rosseel 2012).

Results

Bacterial total β-diversity (β_{sor}) significantly increased with an increase of differences in soil salinity (standardized regression slopes $\beta_{st} = 0.005$, 0.011 and 0.004 in the horizontal, vertical, and horizontal and vertical spatial dimensions, respectively; permutation p values < 0.05; Fig. 2a, c, e). Differences in bacterial richness (β_{rich}) showed weakly positive relations with differences in soil salinity ($\beta_{st} = 0.009$, 0.010 and 0.009 in the horizontal, vertical, and horizontal and vertical spatial dimensions), while bacterial replacement was low at high level of differences in soil salinity across the horizontal and horizontal and vertical spatial dimensions ($\beta_{st} < 0$; Fig. 2a, e). Fungal β_{sor} significantly decreased with an increase of differences in soil salinity in the horizontal spatial dimension ($\beta_{st} = -0.006$; Fig. 2b). Fungal β_{repl} decreased with an increase of differences in soil salinity in the horizontal spatial dimension ($\beta_{st} = -0.013$; Fig. 2b) but increased with an increase of differences in soil salinity in the vertical spatial dimension ($\beta_{st} = 0.005$; Fig. 2d). Fungal β_{rich} increased with an increase of differences in soil salinity in the horizontal spatial dimension ($\beta_{st} = 0.007$; Fig. 2b), while had no relations with differences in soil salinity in other spatial dimensions (Fig. 2d, f).

Differences in soil salinity did not exert significant independent effects on soil microbial β_{sor}, β_{repl} and β_{rich} (variance explained by differences in soil salinity < 3.3%; Fig. 3, Supporting information). By contrast, geographic distance and differences in soil depth had the largest independent effects on differences in soil bacterial and fungal β_{sor} in the horizontal or vertical spatial dimensions (Fig. 3a–f). Geographic distance explained 20.7–25.2% of the variance in differences in soil bacterial β_{sor} and 24.1–25.3% of the variance in differences in soil fungal β_{sor} (Fig. 3a, c, d, f, Supporting information). Throughout the vertical dimension, the differences in soil depth explained 21.9% (95% confidence interval [12.4, 32.7]) of the variance in differences in soil bacterial β_{sor} (Fig. 3b; Supporting information) and 23.7% [12.7, 35.8] of the variance in differences in soil fungal community structure (Fig. 3b, e, Supporting information).

Path analysis showed that geographic distance and differences in soil depth, salinity, properties and nutrient pools separately explained 14–27% of the variance in differences in enzymatic activity, 29–39% of the variance in differences in soil bacterial community structure, and 27–32% of the variance in differences in soil fungal community structure (Fig. 4a–c). However, differences in enzymatic activity were not explained by differences in soil microbial community structure (standardized path coefficients $\beta_{std} < 0.13$ in all spatial dimensions; Fig. 4, Supporting information). Differences in soil properties but not soil salinity had the largest effects on differences in enzymatic activity in the vertical dimension, which were as important as the effects in the horizontal dimension (Fig. 4). In addition, differences in soil bacterial and fungal community structure were weakly associated with differences in soil salinity (Fig. 4). The effects of differences

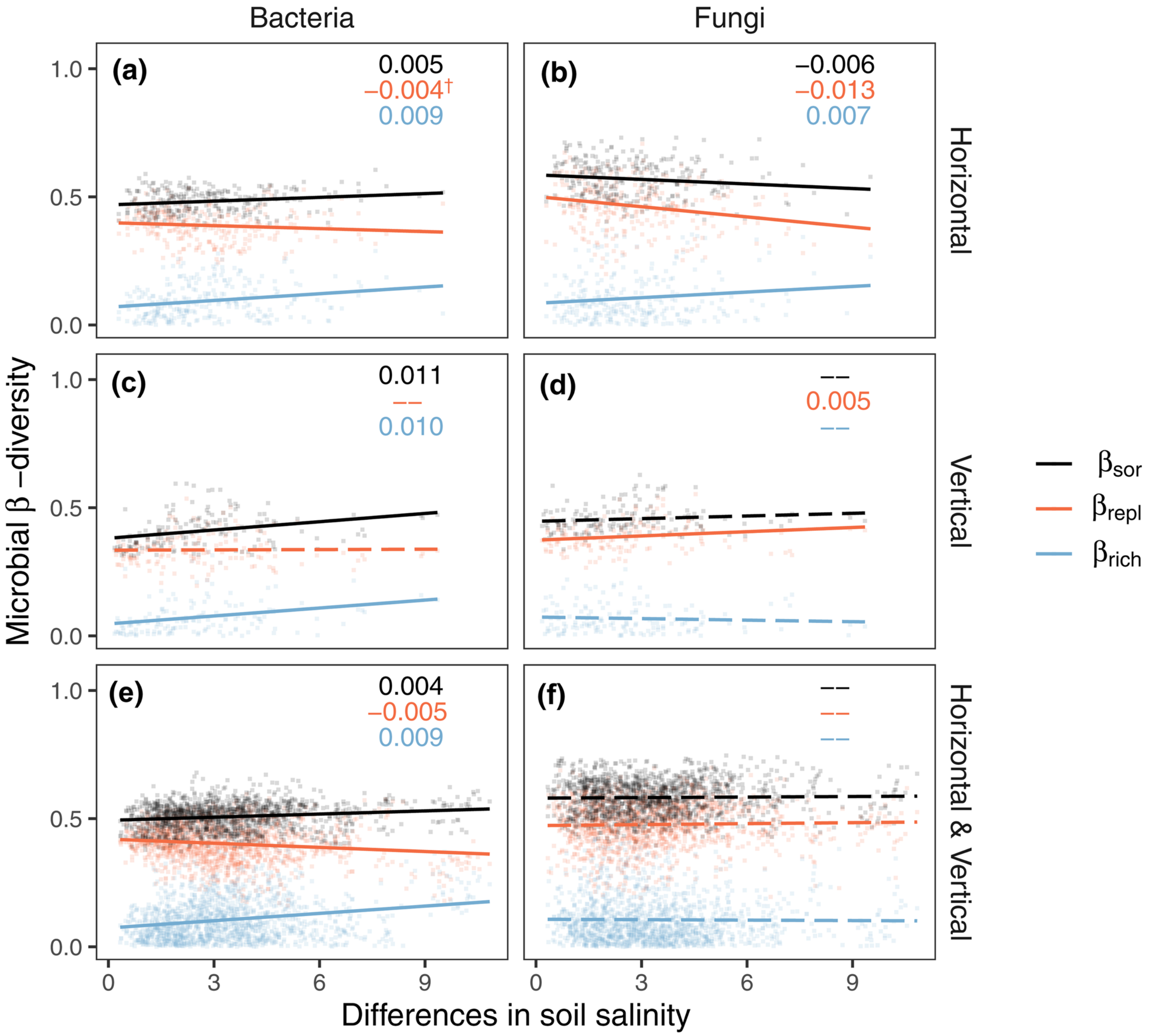


Figure 2. Relationships between microbial β-diversity and differences in soil salinity across the horizontal and vertical spatial dimensions. Solid lines denote significant regressions and dashed lines denote nonsignificant regressions ($p > 0.10$). Significant standardized regression coefficients are shown at the top right side of each panel (if †, $0.05 < \text{permutation } p < 0.10$, otherwise permutation $p < 0.05$) and nonsignificant standardized regression coefficients are shown as '–'. β_{sor} = Sorensen dissimilarity index; β_{repl} = the replacement component of β_{sor}; β_{rich} = the richness difference component of β_{sor}.

in soil depth (Fig. 4b) on soil bacterial and fungal β_{sor} along the vertical dimension were as important as the effects of geographic distance (Fig. 4a, c) along the horizontal dimension on soil bacterial and fungal β_{sor}.

Discussion

Here, our team used a unique environmental gradient (i.e. soil salinity) that generates spatial turnover in a horizonal and a vertical dimension. Using a salinity gradient, we were able to separate the influences of horizontal environmental filters (on the scale of kilometers) on microbial community β-diversity and enzymatic activity from the influences of vertical environmental filters (on the scale of meters), which is an underexplored dimension of soil macroecology. Our first hypothesis that, increasing soil salinity would lead to enhanced microbial species turnover, was supported only weakly by fungal species turnover, i.e. the species replacement component of total β-diversity in the vertical spatial dimension (Fig. 2). Our second hypothesis was rejected, because geographic distance in the horizontal dimension and the difference in soil

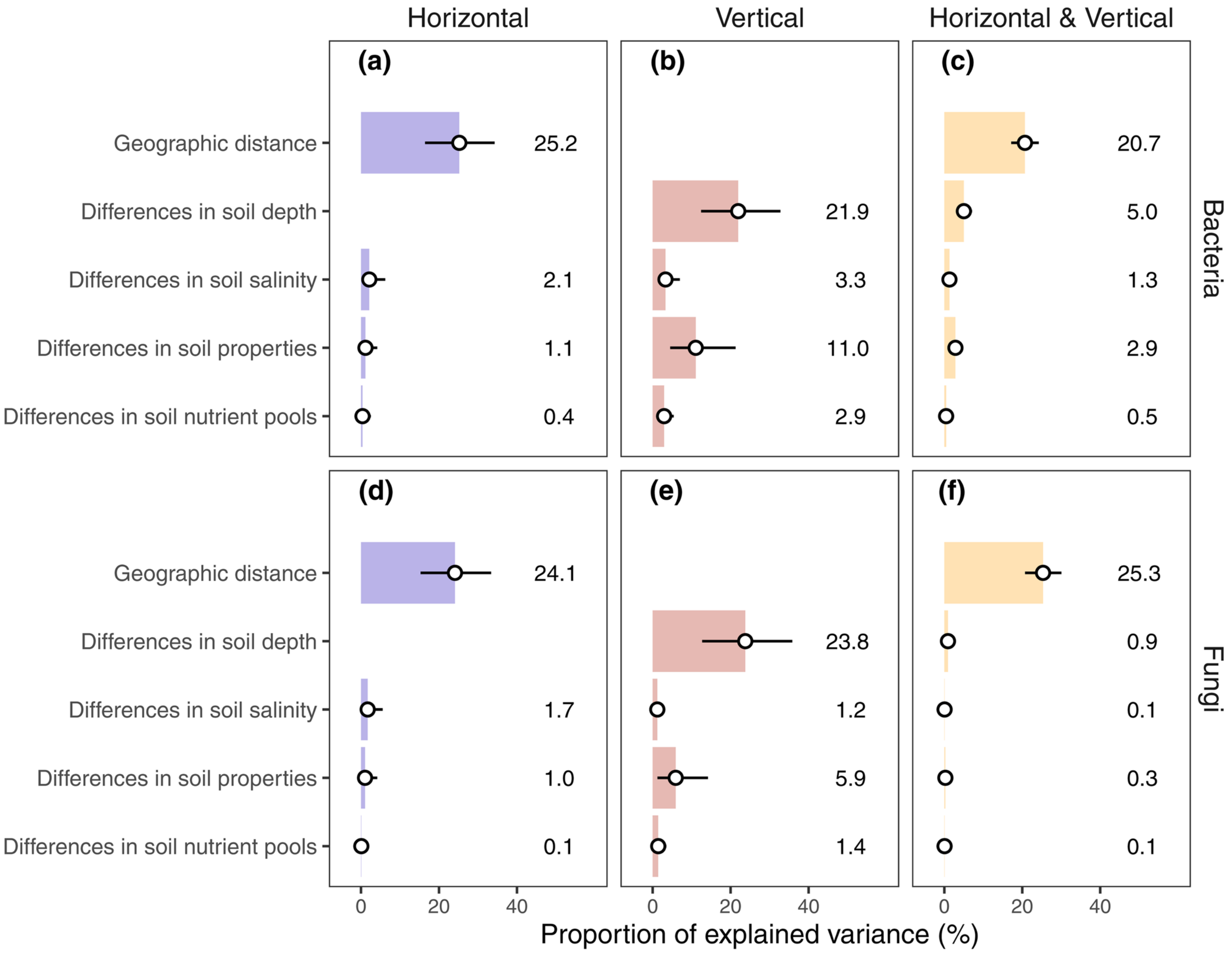


Figure 3. Independent effects of explanatory variables on microbial β-diversity across the horizontal and vertical spatial dimensions by hierarchical variance partitioning. Bars represent the pure variance explained by the explanatory variables, and the proportion of explained variance is shown along the bars. The 95% confidence interval is given. We show only the Sorensen dissimilarity index, see the Supporting information for the summary of the replacement and richness difference component of Sorensen dissimilarity index.

depth in the vertical dimension, not soil salinity, consistently determined turnover in soil bacterial and fungal β-diversity (Fig. 3). Differences in soil properties rather than soil salinity enhanced spatial turnover in enzymatic activity (Fig. 4). Our findings suggest that soil depth serves as a test bed for predicting changes in soil microbial community structure and enzymatic activity in extreme environments.

Soil depth as a new frontier for studies of soil macroecology

We found that spatial turnover in the structure of soil microbial communities was strongly associated with geographic distance and differences in soil depth rather than differences in soil salinity. This result suggests that soil bacterial and fungal communities are resistant to changes in soil salinity (Supporting information), but exhibit a dissimilar community structure with an increase in spatial distance both horizontally and vertically along the soil depth profile. Our finding that soil microbial community structure is resistant to changes in soil salinity contradicts previous work (Van Horn et al. 2014, Rath et al. 2019a, Zhang et al. 2019). One reason our work might differ from prior work is that species replacement and differences in richness are two additive components of total β-diversity (Legendre 2014). Increases in differences in richness can counteract decreases in species replacement, leading to a weak relationship between differences in soil salinity and soil microbial total β-diversity (Fig. 2). Another reason our work might differ from prior work is that soil salinity in our study was extremely high after 12-year of saline-water irrigation, resulting in a long-lasting harsh environmental press condition (Schradin et al. 2023). Soil microbes may have adapted well to the harsh environment, as low species turnover was associated with differences in soil salinity (Fig. 2). However, our work indicates that soil depth is as important as geographic distance in shaping the

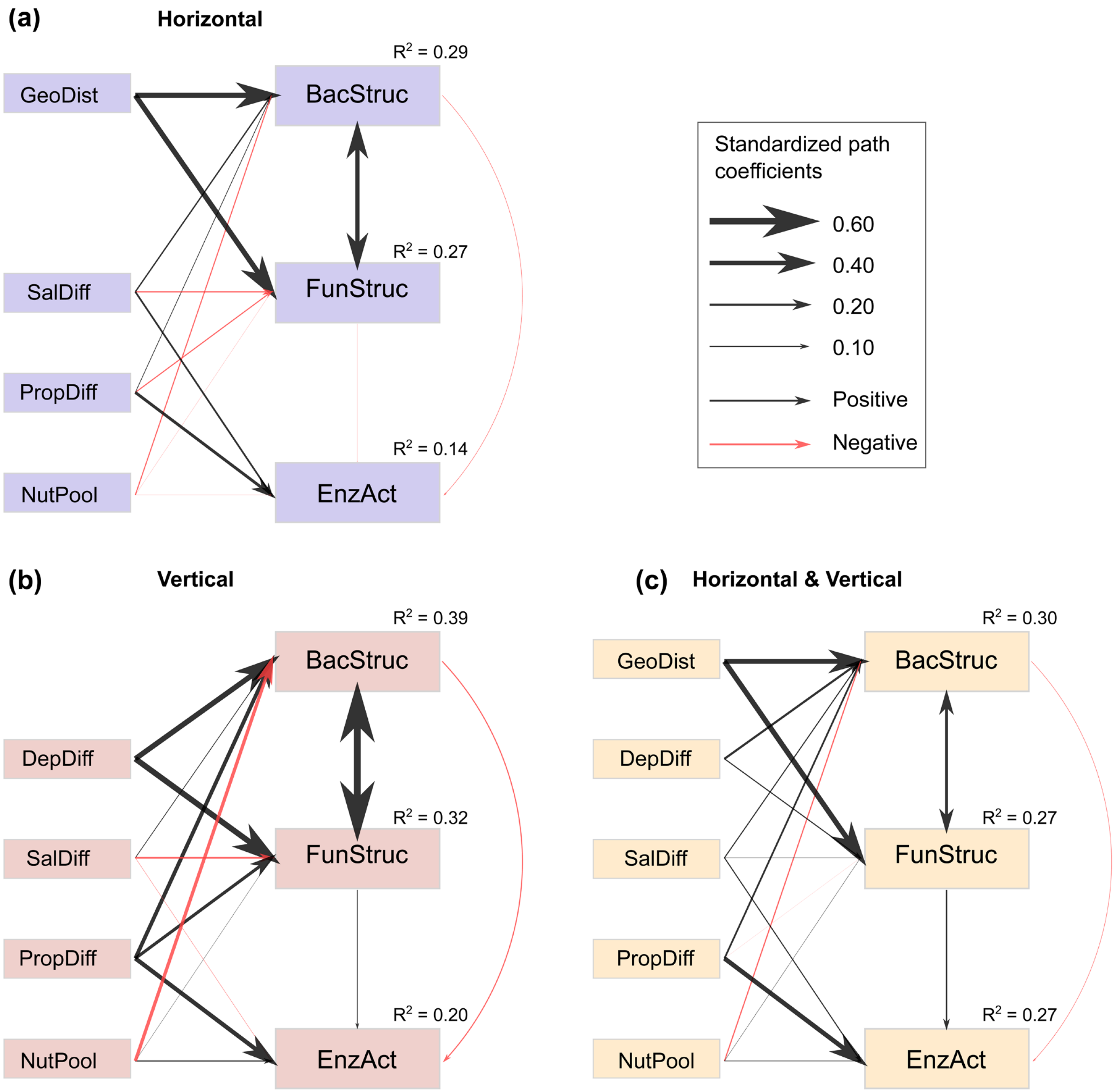


Figure 4. The path model shows the direct and indirect effects of soil salinity on microbial community structure and enzymatic activity across the horizontal and vertical spatial dimensions. Three path models are presented, and they are based on spatial dimensions, including (a) purely horizontal dimension, (b) purely vertical dimension, (c) combined horizontal and vertical dimension. The width of the arrows 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 the Supporting information for details). R^2 denotes the variance in community structure and enzymatic activity explained by environmental filters, including geographic distance (GeoDist), differences in soil depth (DepDiff), differences in soil salinity (SalDiff), differences in soil properties (PropDiff), and differences in nutrient pools (NutPool). BacStruc, FunStruc, and EnzAct denote differences in bacterial community structure, fungal community structure, and enzymatic activity, respectively. We show only the Sorensen dissimilarity index, see the Supporting information for summary of the replacement and richness difference component of Sorensen dissimilarity index.

structure of bacterial and fungal communities in the saline desert soil (Fig. 3–4). The importance of soil depth is supported by an early study, which found that random assembly processes of soil microbial communities (e.g. dispersal limitation) intensified with soil depth because soil microbes disperse passively (Powell et al. 2015). When the overall spatial turnover in microbial structure is partitioned into purely vertical, purely horizontal and combined horizontal and vertical spatial dimensions, our results indicate that soil depth and geographic distance are equally important in driving microbial community composition (Fig. 3a–b, 4a–b). Without partitioning space both horizontally and vertically, we would underestimate the contribution of soil depth to spatial turnover in soil microbial community composition when we only consider the horizontal and vertical dimensions simultaneously (Fig. 3c, f, 4c). Taken together, our work highlights that dispersal limitation in soil microbes is particularly important to the assembly of microbial communities in the vertical and horizontal dimensions of soil depth profiles in extreme environments. To maintain essential and irreplaceable ecosystem functions and services provided by belowground biodiversity in extreme environmental conditions or severely disturbed conditions, a workable plan is to preserve habitats along both the horizontal and vertical dimensions of soil depth profiles.

Our study further indicates that the spatial turnover 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 depths. In extreme environments, environmental filters (e.g. salt stress) and resource levels (e.g. soil organic matter and microbial biomass) are expected to be the drivers of spatial turnover in soil microbial community structure and function (Martiny et al. 2006, Rath and Rousk 2015, Rath et al. 2019a, b). On the contrary, our results suggest that differences in soil salinity and nutrient pools do not explain the microbial activity of carbon, nitrogen, and phosphorus cycling related enzymes. It could be that soil salinity is too high in our study system and the priority of soil microbes is to survive rather than to thrive in such extreme saline environments, preventing soil microbes from producing energy-expensive enzymes (Rath and Rousk 2015). As a result, neither soil salinity nor resources are limiting factors of enzymatic activity. Changes in soil salinity (Supporting information) and nutrient pools did not alter enzymatic activity. However, our result indicates that reduced heterogeneity in soil properties, which could be induced by land degradation, soil erosion, and desertification, may result in a loss of soil microbial activity and soil functionality, soil fertility, and plant growth. Because the variability in soil physical and chemical properties is caused by human activities and nature ubiquitously, we should examine the under-explored dimension of soil macroecology in the vertical dimension along soil depth profiles. This is a valuable step towards soil conservation efforts in the face of ongoing environmental alterations in arid ecosystems.

Uncoupled microbial community structure-function relationships

The most salient result of this study is that differences in soil microbial community structure did not lead to differences in enzymatic activity in the hyper-saline environments. This result runs counter to previous work exploring these relationships in microbial systems with a focus on surface soils (Mori et al. 2016, Delgado-Baquerizo et al. 2020, Jing et al. 2022). The uncoupled relationships between microbial structure and function were further confirmed across the purely horizontal, purely vertical and combined horizontal and vertical dimensions of soil depth profile. Thus, our findings support the mechanism of functional redundancy (Coleman and Whitman 2005, Peay et al. 2016), implying that the structure of soil microbial communities differ, but their functions remain in the same even in extreme environments. As a result, the losses/gains of certain species may have limited effects on the spatial turnover in enzymatic activity. This means that functionally redundant communities may safeguard ecosystem functions against negative impacts of extreme environmental changes (Allison and Martiny 2008).

There are two explanations for the uncoupled structure–function relationships under soil salinization. First, soil bacterial and fungal communities have likely adapted to the extreme conditions in the desert, where abiotic stress is high (Jansson and Hofmockel 2020). As the soil becomes saltier and drier, soil microbes become inactive because the availability of carbon and nutrients decreases in the soil. In addition, soil microbes 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 to be the result of a mismatch between the dominant driving factors responsible for microbial community structure and enzymatic activity. Our results show that geographic distance and differences in soil depth have the strongest independent effects on the structure of microbial communities, while differences in soil properties have the strongest independent effects on enzymatic activity. Different driving factors could have disproportionate influences on soil microbial community structure and enzymatic activity, leading to uncoupled structure–function relationships.

In recent years, soil macroecologists have focused on microbial communities in surface soils because they have high biomass density and nutrient cycling activities (Fierer et al. 2009, Xu et al. 2020). The Taklamakan Desert is a typical extreme biome, and this study is unique in that it provides a novel approach to assess microbial community structure–function relationships both horizontally and vertically along soil depth profiles with the same range of salinity variation. However, large-scale studies will be required to confirm whether the findings hold true in other biomes, which may have a broader range of horizontal and vertical gradients induced by human activities or by nature in the context of soil degradation, e.g. soil salinization, acidification and erosion. Further, it is important to note that the measure of four hydrolytic enzyme activities captures only a few microbial functions that are relevant to carbon, nitrogen and phosphorus cycling. Future work using shotgun metagenomics could quantify broad shifts in microbial community functionality, e.g. the functions related to organotrophs and autotrophs (both by phototrophs and chemolithotrophs), advancing our understanding of the structure–function relationships of microbial communities in xeric or arid soils.

Conclusions

We find that environmental gradients along soil depth profile are as important as horizontal geographic distance in shaping microbial community structure and enzymatic activity in extreme environments. These findings highlight the need to examine the under-explored dimension of soil macroecology, i.e. the vertical spatial dimension (White et al. 2020, Eisenhauer et al. 2021). Such information is crucial to predict the influences of global change drivers on real-world community structure–function relationships across different spatial scales through the purely vertical, purely horizontal and combined horizontal and vertical dimensions. Moreover, this study highlights that given the increase in desertification in arid ecosystems, soil conservation efforts should take soil depth into account as a critical factor governing the β-diversity of microbial communities and the activity of enzymes. Here the spatial dimension partitioning framework we proposed has two important implications for understanding microbial community structure and ecosystem function. First, it demonstrates that approaches based on solely on unidimensional assessments cannot fully elucidate the importance of dispersal limitation on microbial community structure at a fine spatial scale (Khattar et al. 2021, He et al. 2023). Second, the framework can be used to compare the relative strengths of environmental filters on microbial community structure–function relationships across different spatiotemporal scales in soil macroecology (White et al. 2020, Xu et al. 2020, Eisenhauer et al. 2021).

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Author contributions

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Data and R script are available from the Zenodo Digital Repository: https://doi.org/10.5281/zenodo.10657898. (Jing 2024)

Supporting information

The Supporting information associated with this article is available with the online version.

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