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Abstract:

Aim: Climate is widely understood to determine elevational patterns of soil microbial communities, whereas the effects of parental material are uncertain. Changes in the composition of parental materials along elevational transects could also affect soil microbial communities by influencing soil pH and nutrient availability. Here, we aim to illustrate the combined effects of climate and parental material on the biomass and composition of soil microbial communities along an elevational transect.

Location: A subtropical forest on a karst mountain (Mt. Jinfo), China.

Taxon: Bacteria and Fungi.

Methods: We use phospholipid fatty acid analysis (PLFA) and DNA amplicon high-throughput sequencing to determine biomass and diversity patterns of soil microbial communities along a subtropical elevational gradient with contrasting parental materials (limestone and clasolite).

Results: We observed that the microbial communities were more diverse (α-diversity) and productive (biomass) on limestone than on clasolite. Additionally, we found that parental material played a role in shaping the composition (β-diversity) of soil microbial communities along the elevational gradient. The impact of climate on soil microbial communities was found to be significant, albeit relatively weak. Structural equation models provided evidence for both direct and indirect effects of climate and parental material on microbial biomass and α-diversity along the elevational gradient. Notably, the changes in soil pH, influenced by both parental material and climate, were identified as a key factor driving these effects.

Main Conclusions: Our results underline the importance of both climate and parental material variations in space-for-time studies investigating soil microbial communities along elevational gradients.

Keywords: altitude; bedrock; clasolite; climate; karst mountains; limestone; soil biogeography.

1. Introduction

A central goal of modern ecology is a mechanistic understanding of global biodiversity (Fierer and Jackson, 2006; Martiny et al., 2006; Fierer et al., 2009; Bahram et al., 2018; Delgado-Baquerizo et al., 2018). Much of this biodiversity is made up of soil microorganisms, which are linked directly to soil properties such as pH (Griffiths et al., 2011; Tripathi et al., 2018), organic matter content (Smith et al., 2021), nutrient content (Delgado-Baquerizo et al., 2017), and texture (Seaton et al., 2020). More generally, soil properties are determined by multiple factors (Jenny, 1941) such as climate, parental material, topography, and plants, and together these factors influence the spatial distribution of soil microbial organisms. Parental materials affect the soil formation process and the resulting soil physicochemical properties (Jenny, 1941; Wardle et al., 2004; Doetterl et al., 2015; Gu et al., 2020). Previous biogeographical studies of soil microbial communities have focused on the effects of climatic and biotic factors (Tedersoo et al., 2014; Zhou et al., 2016; Zheng et al., 2020; Ma et al., 2022), with the underlying mechanisms and indirect effects of parental material on microbial communities being relatively understudied (Hu et al., 2020; Weemstra et al., 2020). Clarifying the mechanisms by which parental material can influence the generation and maintenance of soil microbial communities through changes in soil conditions is fundamental for predicting the distribution of soil microbial communities in terrestrial ecosystems.

Several studies conducted at regional spatial scales have provided evidence for the significant influence of parental material on soil microbial communities. For example, an incubation experiment in tropical montane forests found that, despite the advanced weathering degree of soils and similar stand age of vegetation, microbial biomass was higher in soils developed from the mafic parent material than from mixed sediment (Kidinda et al., 2022). A study in the Antarctic revealed that bacterial

communities were distinguished by the parent material type; soils derived from gneiss were dominated by Acidobacteria and Actinobacteria, whereas granite derived soils were dominated by Proteobacteria and Cyanobacteria (Tytgat et al., 2016). Another study in subtropical agricultural soils found that, after 30 years of artificial management, a significant effect of parental material on soil microbial diversity persisted (Sun et al., 2016). However, these studies have concentrated primarily on regional scales, overlooking the effects of parental material at local scales. For instance, when examining the spatial pattern of soil microbial communities along an elevational transect, researchers often fail to consider the potential impact of changes in the parental material within the transect.

Elevational gradients in mountainous regions are invaluable as a natural laboratory for the empirical testing of biodiversity patterns (Sanders, 2002; Sundqvist et al., 2013; Mayor et al., 2017; Steinbauer et al., 2018; McCain et al., 2021). Elevational gradients usually cover small horizontal distances, and researchers generally assume that the parental material underlying all plots along the elevational gradient are homogeneous (Frindte et al., 2019; Ma et al., 2022; Zhu et al., 2022). Consequently, elevational patterns are usually attributed to climatic effects, and thenceforth used to predict the consequences of climate change for microbial biodiversity (Körner, 2007; Fierer et al., 2011; Shen et al., 2014). However, mountain ecosystems are usually characterized by complex geological conditions (Hahm et al., 2014; He et al., 2021), and parental materials can change dramatically along an elevational gradient (Lanzén et al., 2016; Hu et al., 2020). Neglecting the effects of parental materials may therefore mislead attempts to explain the distribution of soil microbial organisms (Hu et al., 2020), or even lead to incorrect conclusions that the variation is driven solely by climatic gradients (Lanzén et al., 2016).

Many studies have examined the elevational patterns of soil microbial communities (Hendershot et al., 2017; Wang et al., 2017; He et al., 2020; Chen et al., 2023; Huang et al., 2023), but few have considered the importance of underlying parental materials (Lanzén et al., 2016; Hu et al., 2021). The α-diversity of soil

119 microbial communities on two parent materials in the Pyrenees was significantly
120 correlated with parental material but not elevation (Lanzén et al., 2016). Further
121 evidence of the importance of parental material on the elevational pattern of soil
122 microbial diversity can be found in two recent studies which revealed elevational
123 breakpoints of bacterial α-diversity coinciding with geological faults (Li et al., 2018;
124 Hu et al., 2020). Elsewhere, the inclusion of geological parental material substantially
125 increased the explained variation of bacterial α-diversity (Hu et al., 2020), suggesting
126 that parent material and climatic gradients jointly controlled soil bacterial α-diversity.
127 However, these limited studies confirm that different types of parental material can
128 affect the alpha-diversity of soil microbial communities. The mechanisms underlying
129 the influence of parental material on soil microbial communities remain poorly
130 understood, and there is a notable absence of systematic investigations examining
131 multiple indicators of microbial communities, such as biomass, alpha diversity, and
132 beta diversity.

133 Here, we address the question of how the biomass and diversity of soil microbial
134 communities vary along a subtropical elevational gradient, and the mechanism by
135 which parental material and climate jointly influence the elevational patterns of soil
136 microbial communities. The selected elevational gradient includes two different
137 parental materials, i.e. limestone and clasolite (Fig. 1). It is well-known that climate
138 significantly affects soil physical and chemical properties (Sanders, 2002; Sundqvist
139 et al., 2013; Mayor et al., 2017; Zeng et al., 2023). However, despite the changes in
140 elevation along the transect, the average climate did not differ significantly, allowing
141 us to effectively control for climate whilst isolating the effects of parental material.
142 Soils derived from limestone and clasolite usually differ (Jiang et al., 2020; Zhong et
143 al., 2022; Zeng et al., 2023), including soil pH, SOC, and nutrient content, which are
144 known to influence soil microbial communities greatly (Fierer and Jackson, 2006;
145 Delgado-Baquerizo et al., 2016; Smith et al., 2021). We therefore hypothesize that: 1)
146 the elevational patterns of soil microbial communities are controlled jointly by
147 parental material and climate; and 2) parental materials and climate indirectly affect

soil microbial communities by influencing soil properties such as soil pH and SOC concentration.

2. Materials and methods

2.1 Study sites

We worked in Jinfo Mountain (28°50′–29°20′N, 107°00′–107°20′E) which is known as a karst mountain located in the Nanchuan District of Chongqing city, southwestern China. This area experiences a subtropical humid monsoon climate with a mean annual air temperature of 8.2°C and a mean annual precipitation of 1395.5 mm. The vegetation type is subtropical evergreen broad-leaved forest, and is mainly composed of *Quercus glauca*, *Castanopsis fargesii*, *Sorbus folgneri*, *Cyclobalanopsis sessilifolia*, *Carpinus turczaninowii*, *Polyspora speciosa*, *Cinnamomum wilsonii*, and *Albizia julibrissin* (Zhu et al., 2022). Our transect covered an elevation range of 800 to 2100 m.a.s.l. A total of 14 sites were established along the transect, with five sites on the limestone and nine sites on the clasolite parental materials (Figure 1). Sites were situated at different heights (approximately 100 m) along the transect (determined by GPS). To reduce the influence of aspect, sites were located on the sunny side of any topographical features.

2.2. Sampling and analytical methods

All sites were sampled in May 2021. We created 20 m × 20 m plots at each site, in which we surveyed all trees with a diameter at breast height (DBH) above 1 cm and calculated their Shannon index as a measure of plant diversity. We estimated the forest above-ground biomass (AGB) using the DBH of each tree (Réjou-Méchain et al., 2017). Each plot was divided into four 10 m × 10 m subplots. We used a stainless-steel soil corer (inner diameter 3.5 cm; depth 0 – 15 cm) to collect six soil cores at random from each subplot, and homogenized the cores into composite sample for each subplot. A total of 56 soil samples were collected, which were transported on ice directly to the laboratory. We sampled fresh parental materials using sledgehammers or drills (Hahm et al., 2014). We calculated mean annual soil temperature (MAT) and

mean annual soil moisture (MAM) from measurements of soil temperature and soil moisture taken throughout the year from May 7th 2020 to May 7th 2021 at each site using 14 HOBO dataloggers (HOBO Pro v2 Temp/RH Logger onset computer corporation, Pocasset, USA).

All soil samples were sieved (2 mm) to remove visible roots and other plant material. Each of the 56 soil samples was divided into two subsamples: one was stored at −80°C for the PLFA and HTL analysis, and one was air-dried at room temperature for the measurement of soil physicochemical properties in the laboratory.

Soil pH was determined using a PHS-3C pH acidometer (soil-water ratio of 1:5). Soil total organic C and total N concentrations were determined by dry combustion with an elemental analyser (Perkin Elmer 2400 Series II); Total P concentration was measured using a nitric acid–perchloric acid digestion, followed by a colourimetric analysis (Murphy and Riley, 1962) using a UV-Vis spectrophotometer (UV1800; Shimadzu, Kyoto, Japan). Particle size distribution was measured using a laser particle analyzer based on the laser diffraction technique operating over a range of 0.02-2000 μm (Mastersizer 2000 particle size analyzer, Malvern Instruments, Ltd., UK).

Bacterial and fungal biomass was determined using a modified PLFA analysis (Frostegård and Bååth, 1996). The abundance of individual fatty acids was determined as μg per g of dry soil. Concentrations of each PLFA were calculated based on the 19:0 internal standard concentrations. We chose a set of fatty acids to represent bacterial PLFAs. Microbial biomass was expressed as the sum of identifiable PLFAs. Bacterial PLFAs were obtained by summing the phospholipid fatty acid 14:00, 15:00, 16:00, 18:00, 13:0 anteiso, 13:0 iso, 14:0 iso, 14:1 w5c, 15:0 anteiso, 15:0 iso, 15:1 w6c, 16:0 iso, 16:1 w5c, 16:1 w7c, 17:0 anteiso, 17:0 cyclo w7c, 17:0 iso, 18:1 w7c, 18:1 w9c, 19:0 cyclo w7c, and 19:0 cyclo w9c contents. The sum of 18:2ω6c and 18:3 w6c represented fungal PLFAs.

Soil DNA was extracted from composite soil samples using the FastDNA SPIN Kit for Soil (MP Biomedicals, Heidelberg, Germany) and purified by agarose gel

electrophoresis. The quality of the DNA samples was checked on a spectrophotometer (NanoDrop, ND2000, ThermoScientific, USA). Total DNA was used for high-throughput sequencing on an Illumina MiSeq platform (San Diego, CA, USA). The bacterial V4 hypervariable region of the 16S rRNA gene and fungal internal transcribed spacer (ITS) region was amplified using the primer pair 505F/816R (5′-GTGCCAGCMGCCGCGG-3′/5′-GGACTACHVGGGTWTCTA AT-3′) (Caporaso et al., 2011) and ITS1F/ITS2 (5′-GGAAGTAAAAGTCGTAACAAGG-3′/5′-GCTGCGTTCTTCATCGATGC-3′) (Shen et al., 2020) along with the Illumina adaptor sequence and barcode sequences, respectively.

The raw sequence data were processed and analyzed using QIIME Pipeline (Caporaso et al., 2011). Briefly, sequencing reads with an average quality value ≤20, with ambiguous nucleotides in barcodes, homopolymer reads longer than 8 bp and shorter than 150 bp were removed to improve sequence quality and paired ends were joined with FLASH (Magoc and Salzberg, 2011). Chimeric sequences were detected and eliminated using the Uchime algorithm (Edgar, 2013). All sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold. Finally, the representative sequences of each OTU were classified against the RDP 16S rRNA database for bacteria and UNITE Fungal ITS database for fungi with an 80% confidence threshold. The resultant OTU abundance tables from these analyses were rarefied to an even number of sequences per sample to ensure equal sampling depth (40,851 and 64,923 for 16S rDNA and ITS, respectively). We calculated Shannon diversity index and Bray–Curtis dissimilarities based on rarefied OTU abundance matrices to analyse microbial diversity. The raw reads have been deposited into the National Centre for Biotechnology Information (NCBI) Sequence Read Archive database (Accession Number: PRJNA936849).

2.3 Statistical analyses

We calculated the Shannon diversity index as α-diversity indices of soil microbial and plant communities. We used a Wilcoxon test to compare the climatic, plant, and soil measures between the two parental materials. To examine possible trends along

the environmental gradient (elevation, temperature, or soil pH gradient), we regressed these response variables on the environmental gradient using univariate linear regression models.

We identified the most important predictors of soil microbial biomass and α-diversity using a random forest regression analysis (Breiman, 2001). The predictors include soil mean annual temperature (MAT), soil mean annual moisture (MAM), soil pH, SOC, TN, TP, Clay, Sand, above-ground biomass (AGB), and Plant Shannon diversity index. The importance of each predictor variable is determined by the percentage increase in the mean square error (%IncMSE) between observations and predictions, and the decrease is averaged over all the trees to produce the final estimation for importance (Liaw and Wiener, 2002). Greater values of %IncMSE denote higher variable importance. In this study, the importance measure was calculated for each tree and averaged over the forest (500 trees). These variable importance analyses were conducted using the randomForest R package (Liaw and Wiener, 2002).

We used the Bray-Curtis-dissimilarities-based Principal Coordinates Analysis (PCoA) to assess differences (β-diversity) in microbial communities in different sites and parental materials. We transformed the data of relative abundances of OTUs by square root before the PCoA. We used the Bray-Curt distance metric to compute a dissimilarity matrix between the parental materials, followed by, a PERMANOVA (Permutational Multivariate Analysis of Variance) to test for significant differences between the different parental materials. We performed redundancy analysis (RDA) of the correlation between predictor variables and microbial composition. These ordination analyses were conducted using the *vegan* R package (Oksanen et al., 2020).

We used a structural equation modelling (SEM) framework to explore the direct and indirect effects of climate and parental material type on soil microbial biomass and α-diversity. We allowed climate and parental material to affect soil microbial community parameters both directly, and indirectly through various soil physical-

chemical properties. Temperature was the only climate variable which correlated significantly with soil microbial community parameters, and we thus used temperature to represent climate in the SEM analysis. As for the soil physical-chemical properties, we selected the top two soil predictor variables affecting soil microbial communities in the random forest regression model results. Parental material was a categorial variable and thus treated as a regular numeric covariate (e.g. 1, 2) in the SEM analysis (Wang et al., 2019).

All statistical analyses were performed using R (R Core Team, 2018) and graphs were generated with the ggplot2 package (Wickham, 2016).

3. Results

3.1 Temperature, vegetation, and soil on different parental materials

Mean annual soil temperature (MAT), mean annual soil moisture (MAM) and vegetation (both Shannon diversity and above-ground biomass) were not significantly different between clasolite and limestone sites (Table 1). However, soil pH, SOC, TN, TP, and clay content in the surface soil (0–10 cm) were significantly higher on limestone sites than on the clasolite sites (Table 1). Parent material exerted significant effects on soil physical and chemical properties, whereas temperature, soil moisture, and vegetation were not significantly affected.

Elevation caused significant linear increases in soil pH and AGB on both parental materials (Fig. S1 & 2). Significant linear correlations also existed between elevation and temperature, some edaphic properties, and plant community composition, regardless of parental material, and MAT, SOC, and silt content (Fig. S1 & 2). Elevation correlated significantly with some variables on one parent material only. For example, soil clay and total nitrogen content decreased with elevation on Clasolite, while no significant trend was observed on Limestone (Fig. S1 & 2). Elevation caused a significant decrease in the Shannon diversity of plant communities on Limestone, while no significant elevational trend was observed on Clasolite (Fig. S1). Soil total P concentration showed no significant elevational pattern on either of

the parent material types (Fig. S2).

3.2 Soil microbial biomass and α-diversity on different parental materials

Microbial biomass and Shannon diversity of both bacteria and fungi were significantly higher on limestone sites than on clasolite sites (Table 1). The biomass ratios of bacteria to fungi were significantly higher on limestone sites than on the clasolite sites (Table 1). Across all samples, the dominant soil bacterial phyla were Proteobacteria, Acidobacteria, Chloroflexi, Rokubacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, and Gemmatimonadetes, which collectively accounted for 91.69% of all taxon sequences (Fig. S3). The relative abundance of Acidobacteria and Actinobacteria was significantly higher on the clasolite than on the limestone, while there was significantly less Chloroflexi on the clasolite than on the limestone (Fig. S3). Soil fungi mainly belonged to three phyla: Ascomycota, Basidiomycota, and Mortierellomycota, which altogether account for 86.67% of all taxon sequences. We found significantly more Mortierellomycota on the clasolite than on the limestone, and significantly less Ascomycota on the clasolite than on the limestone (Fig. S3).

We found significant albeit relatively weak effects of MAT on soil microbial community parameters. MAT exerted no significant impact on soil bacterial biomass or fungal Shannon diversity on either parental material. (Fig. 2a & d). MAT caused a significant linear reduction in soil fungal biomass on clasolite, whereas we observed no significant trend with MAT on limestone (Fig. 2b). Shannon diversity of soil bacteria increased with MAT on clasolite, but there was no significant trend on limestone (Fig. 2c).

Random forest regression models revealed that ten selected variables could explain 77.82%, 55.64%, 81.64%, and 13.25% variation of soil bacterial biomass, fungal biomass, bacterial Shannon diversity, and fungal Shannon diversity along the subtropical elevational gradient. These models indicated that soil pH was the most important predictor to explain the variation of these four soil microbial community parameters (Fig. 3).

We found significantly positive correlations between soil pH and soil microbial community parameters, including bacterial biomass, fungal biomass, bacterial Shannon diversity, and fungal Shannon diversity, along the subtropical elevational gradient (Fig. 4). However, the positive correlations were found only in fungal biomass and Shannon diversity on the limestone, and bacterial Shannon diversity on the clasolite. We even found a significantly negative correlation between soil pH and bacterial Shannon diversity on the limestone (Fig. 4). These results suggest that it was the significant difference in soil pH on the two parental materials that led to the positive correlations between soil pH and microbial communities.

The SEM results supported the notion that parental material and climate jointly controlled the soil microbial biomass and Shannon diversity (Fig. 5 & 6). Soil pH and SOC directly explained the variation of soil microbial biomass and diversity. And the variance in soil pH and SOC were jointly influenced by climate and parental material. Soil bacterial community biomass and α-diversity were more susceptible to parental material and climate variation than the soil fungal communities, with soil fungal Shannon diversity explaining just 15% of the total variation in the SEM.

3.3 Soil microbial β-diversity on different parental materials

The first two axes of a PCoA based on Bray–Curtis dissimilarity explained 60.6% of the variation in the bacterial community structure (Fig. 7a). PERMANOVA based on Bray–Curtis dissimilarity showed that the bacterial communities differed significantly between these two parental materials (R^2=0.39, F=33.90, p=0.001). It is particularly noteworthy that bacterial communities at 1200m and 2100m on limestone were highly similar, even though they were at very different altitudes (Fig. 7A). Whilst just 14.8 % of the variation of the fungal community structure could be explained by the first two axes of the PCoA ordination (Fig. 7c), PERMANOVA showed that the fungal communities differed significantly between these two parental materials (R^2=0.07, F=3.85, p=0.001). The RDA results indicated that soil pH is the most important environmental factor for controlling bacterial community composition

(β-diversity) (Fig. 7a & b), although SOC, TN, MAT, and others were also significant. SOC exerted the most influence on fungal communities, although other factors were also significant e.g., TN, MAT, AGB etc. (Fig. 7c & d).

4. Discussion

Climate is thought to structure soil microbial communities along elevational gradients, and the potential role of parental material variation is not well understood. We have revealed how parental material (specifically limestone and clasolite) and climate jointly structure soil microbial communities. Whilst climate and vegetation did not differ significantly between limestone and clasolite sites, microbial biomass and diversity differed significantly on different soils (pH, clay, and SOC etc.). Soil microbial communities showed marginal elevational trends on both parental materials, further undermining the effects of climate on soil microbial communities. Instead, our results highlight the importance of bottom-up control of parental material on microbial biomass and diversity.

4.1 Effects of parental material on soil microbial communities

Our results indicate that parental material influenced soil microbial communities along a subtropical elevational gradient, which aligns with previous studies revealing the significant effects of parental material on soil microbial biomass and diversity (Deng et al., 2015; Sun et al., 2016; Xiao et al., 2022). In a similar subtropical monsoon climate, Deng et al. (2015) found that microbial biomass and α-diversity in soil derived from granite were significantly higher than from quaternary red earth and tertiary red sandstone, and that parental material explained more variation in microbial biomass and α-diversity than land use types. Sun et al. (2016) showed that agricultural soils derived from granite supported more microbial biomass than quaternary red clay soil and purple sandy shale, even after 40 years of agricultural use (Sun et al., 2016). Karst mature forests in southwest China have been shown to be significantly higher in diazotroph richness and Shannon index than non-karst soils

(Xiao et al., 2022). Together these results provide compelling evidence that soil parental materials drive the spatial variation of soil microbial biomass and α-diversity.

Parental material affected the composition (β-diversity) of soil microbial communities in our study, which is consistent with previous studies (Ulrich and Becker, 2006; Lamarche et al., 2007; Eskelinen et al., 2009; Kooijman et al., 2020). We found that soil microbial communities on the same parental material were very similar, even when the climatic conditions differed between distant sites. For example, soil microbial communities (including bacteria and fungi) on limestone grouped in a significant cluster (Fig. 7), even though the sites were at 1200 m and 2100 m. This result can be explained by the differing soil conditions between limestone sites and clasolite sites, including soil pH, SOC concentration, and soil clay content, which corresponds with the fact that differences in mineral and element composition or soil texture are important in shaping microbial community composition (Barton et al., 2007; Tytgat et al., 2016).

Our results suggest that it was soil pH resulting from different parental materials that affected biomass, α- and β- diversity of soil microbial communities. The importance of soil pH for structuring soil microbial communities has been elucidated by various studies (Fierer and Jackson, 2006; Lamarche et al., 2007; Chu et al., 2010; Wang et al., 2017; Tripathi et al., 2018; Shen et al., 2020; Ni et al., 2021). Soil pH is particularly important for microorganisms, largely because the intracellular pH of most microorganisms is usually within 1 pH unit of neutral, and any significant deviation in environmental (extracellular) pH stresses the microorganisms, especially single-celled prokaryotes (Fierer and Jackson, 2006). Moreover, the strong correlation between soil pH and microbial communities could result from the integration of soil pH with other soil variables. For example, soil pH is an efficient integrator of soil nutrient availability since differences in hydrogen ion concentrations affect the capacity to hold charged ions in soils (Glassman et al., 2017). Typically, a broad range of soil pH values, especially in the range of acidic to neutral, is a necessary condition for the strong correlation between soil pH and microbial communities (Fierer and

Jackson, 2006; Fierer, 2017). Soils derived from limestone are neutral or weakly alkaline, producing a broad range of soil pH in acidic subtropical soils (Lazzaro et al., 2009). This explains why parental material influences soil pH and thus soil microbial community biomass and diversity. We found that the effect of soil pH for structuring fungal community diversity (both α- and β-diversity) is weaker compared to bacterial communities. This may also be a reason for the smaller variation in fungal community diversity along the elevational gradient in this study, as well as the lower explanatory power of the models for its variation. The strong influence of pH on bacterial communities is thought to be due to the narrow pH ranges for optimal growth of bacteria, as opposed to the weaker influence on fungi, which generally exhibit a wider pH tolerance (Rousk et al., 2010). This has been supported by numerous studies, for instance, Acidobacteria, as a dominant phylum in bacterial communities, are commonly found in soils with low pH (Jones et al., 2009).

4.2 Effects of climate on soil microbial communities

Contrary to our hypothesis, we found that soil bacterial biomass showed no significant trend with soil MAT on either parental material type. This finding is inconsistent with previous studies reporting that soil bacterial biomass significantly increased with decreasing MAT in subtropical elevational gradients (He et al., 2020). This inconsistency is probably due to contrasting trends of soil pH and SOC along this elevational gradient, both of which usually have positive effects on soil bacterial biomass. The fact that soil MAT had no effect on fungal α-diversity on either parental material is consistent with previous studies (Ji et al., 2022; Ma et al., 2022). Although it should be noted that temperature has been found to play an important role in controlling soil fungal α-diversity (Looby and Martin, 2020; Shen et al., 2020). Evidently, further study is needed to understand how soil fungal communities respond to temperature gradients.

We did observe some effects of MAT gradients on soil microbial community parameters, but these differed on the different parental materials. Soil fungal biomass

correlated positively with MAT on clasolite but showed no significant correlation on limestone. Given the importance of SOC in controlling soil fungal biomass, this could be caused by the significant increase of SOC with MAT on the clasolite. The fact that SOC accumulated on the clasolite but not on the limestone suggests that SOC on clasolite is more sensitive to climate change. We also found soil bacterial α-diversity was significantly negatively correlated with MAT on clasolite, but showed no significant correlation on limestone. This is because soil bacterial α-diversity is correlated with soil pH in acid soils (Fierer and Jackson, 2006). These differing effects of temperature on soil fungal biomass and bacterial α-diversity on contrasting parental materials suggest that models of soil microbial responses to climate change should include parental material as an important mediator.

We acknowledge that the ideal sampling design to study the influence of parental material differences on the elevational pattern of soil microbial communities would be to have two independent elevational transects located on each of the different parental materials, with matching elevations for paired comparisons. In this study, our confidence in our ability to separate the impact of parental material on soil microbial communities from the influence of climate is mainly based on two results: Firstly, we found that there were no significant differences in the average values of climate factors between the two parental materials. We nevertheless found significant differences in multiple characteristics of soil microbial communities and soil physicochemical properties between the two parental materials. Secondly, it is possible for soil microbial community compositions from the same parental material under differing climates to be very similar. These results confirm the significant influence of parental material on soil microbial communities. However, our site design does have certain limitations, such as the difficulty in verifying whether there is a significant interaction between parental material and climate on soil microbial communities. Therefore, future research should focus on more comprehensive site designs to systematically study the interaction of multiple soil-forming factors, such

as parental material and climate, on soil microbial communities.

5. Conclusion

Our goal was to clarify that climate and parental material jointly control the biomass and composition of soil microbial communities using microbial lipid biomarkers and high-throughput amplicon sequencing. Parental material exerted significant effects on soil microbial biomass, α-diversity, and β-diversity. Although climate exerted weaker effects, it was jointly responsible for structuring the soil microbial communities along a subtropical karst elevational gradient. Soil pH was the most important factor affecting soil microbial biomass, α-diversity, and even β-diversity of bacterial communities directly, and soil pH varied with parental material and climate, which underlines the need to consider both parental material and climatic variation in space-for-time studies of soil microbial communities along elevational gradients.

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Table 1 Comparisons of climate, soil, and vegetation on contrasting parental materials (mean±se). Different characters after the values indicate significant differences between two parental materials in Wilcoxon test ($p < 0.05$), while the same character indicates no significant difference ($p > 0.05$). Measures with significant differences are in bold font.

	Clasolite	Limestone
MAT (℃)	11.30 ± 0.33 a	11.91 ± 0.43 a
MAM (%)	21.15 ± 0.49 a	20.79 ± 0.95 a
Above ground biomass (Mg ha^{-1})	142.55 ± 6.70 a	152.76 ± 9.05 a
Plant Shannon diversity	3.11 ± 0.04 a	2.95 ± 0.08 a
Soil pH	3.94 ± 0.13 a	6.08 ± 0.21 b
SOC (g kg^{-1})	45.84 ± 4.59 a	65.99 ± 3.12 b
TN (g kg^{-1})	1.11 ± 0.08 a	1.69 ± 0.08 b
TP (g kg^{-1})	0.65 ± 0.03 a	0.84 ± 0.06 b
Clay (%)	34.34 ± 1.19 a	39.24 ± 1.11 b
Silt (%)	30.26±0.87 a	30.41±0.37 a
Sand (%)	35.39±1.78 a	30.34±1.24 a
Bacterial biomass (ug g^{-1})	14.46 ± 0.68 a	30.19 ± 1.45 b
Fungal biomass (ug g^{-1})	0.38 ± 0.02 a	0.60 ± 0.03 b
Bacteria to Fungi biomass ratio	4.11 ± 0.05 a	4.33 ± 0.08 b
Bacterial Shannon diversity	5.95 ± 0.05 a	6.19 ± 0.04 b
Fungal Shannon diversity	3.56 ± 0.10 a	3.97 ± 0.13 b

MAT: mean annual temperature; MAM: mean annual soil moisture; SOC: soil organic carbon concentration; TN: soil total nitrogen concentration; TP: soil total phosphorus concentration.

Figure 1 Distribution of sampling sites along the subtropical elevational transect. Red points indicate limestone sites and blue points indicate clasolite sites. Numbers near the dots indicate the elevation values. The base map is a coloured DEM map derived from SRTM 90 m data. [This figure is 2/3rd column].

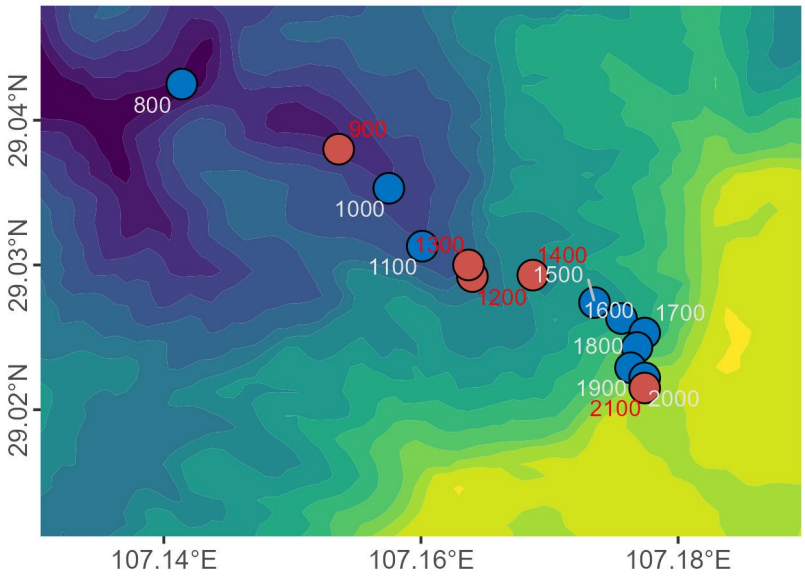


Figure 2 Effects of temperature on soil microbial parameters on clasolite and limestone, respectively. (a) soil bacterial biomass; (b) soil fungal biomass; (c) soil bacterial Shannon index; (d) soil fungal Shannon index. Solid and dashed lines indicate significant ($p < 0.05$) and insignificant ($p > 0.05$) linear regression relationships, respectively. [This figure is double column.]

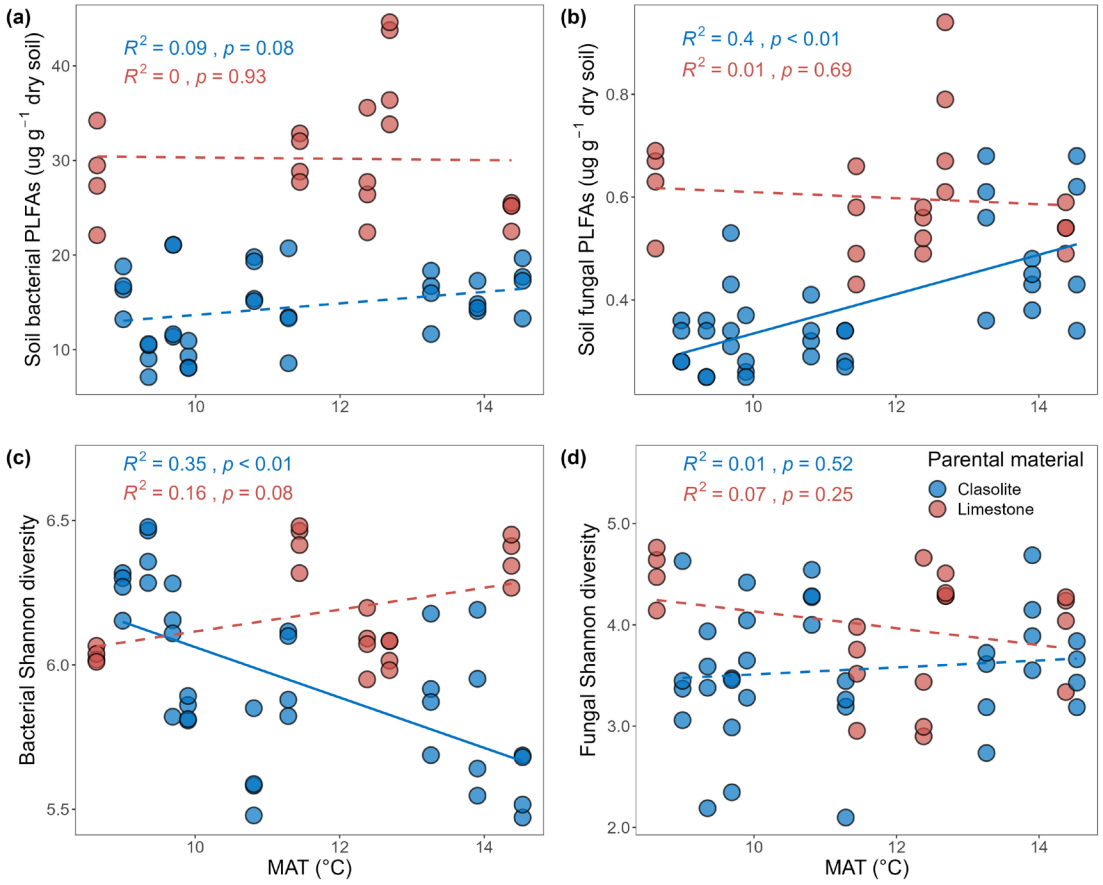
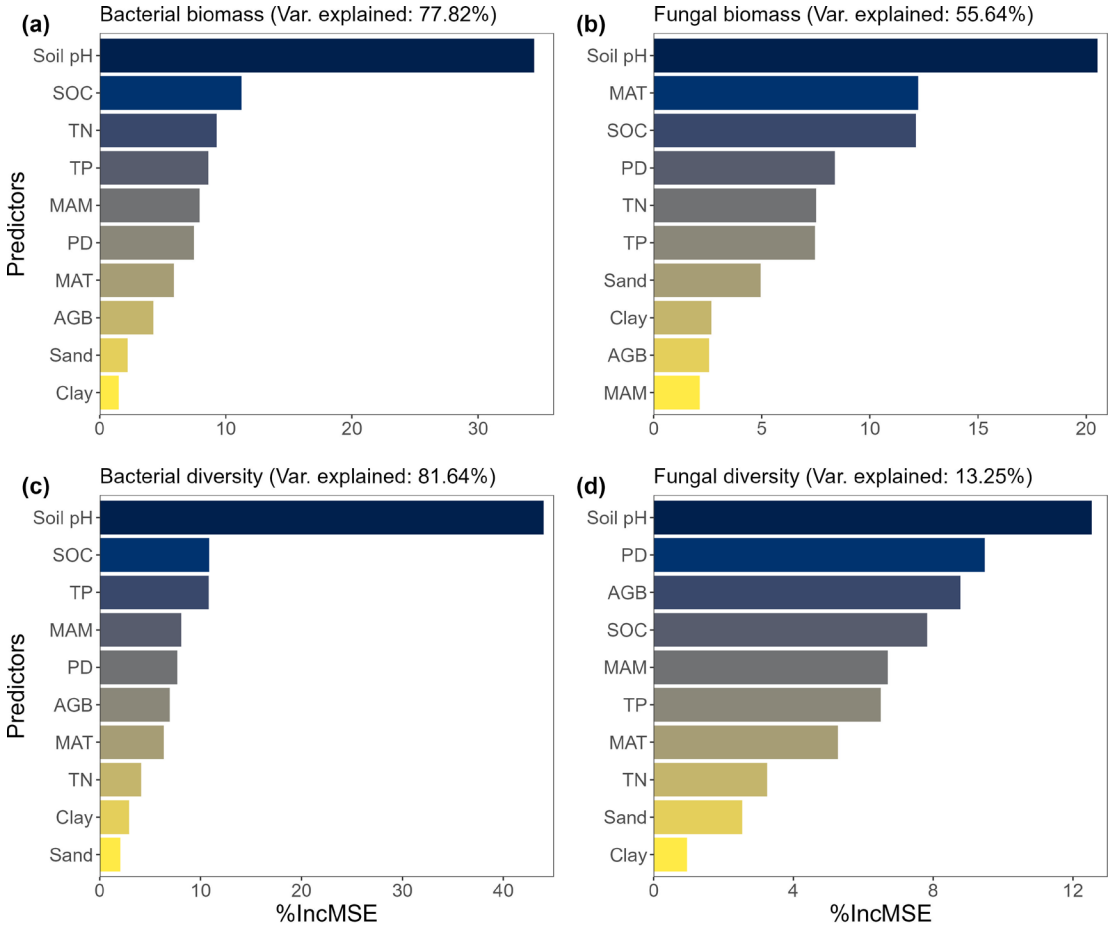


Figure 3 Relative importance of predictors of soil microbial community parameters quantified using random forest models. [This figure is double column.]



MAT: mean annual soil temperature; MAM: mean annual soil moisture; SOC: soil organic carbon concentration; TN: soil total nitrogen concentration; TP: soil total phosphorus concentration.

Figure 4 Relationship between soil pH and soil microbial community parameters. (a) soil bacterial biomass; (b) soil fungal biomass; (c) soil bacterial Shannon index; (d) soil fungal Shannon index. Solid and dashed lines indicate significant ($p < 0.05$) and insignificant ($p > 0.05$) linear regression relationships, respectively. Red lines indicate relationship on limestone; blue lines indicate relationship on clasolite; and black lines indicate relationship on all sites. [This figure is double column.]

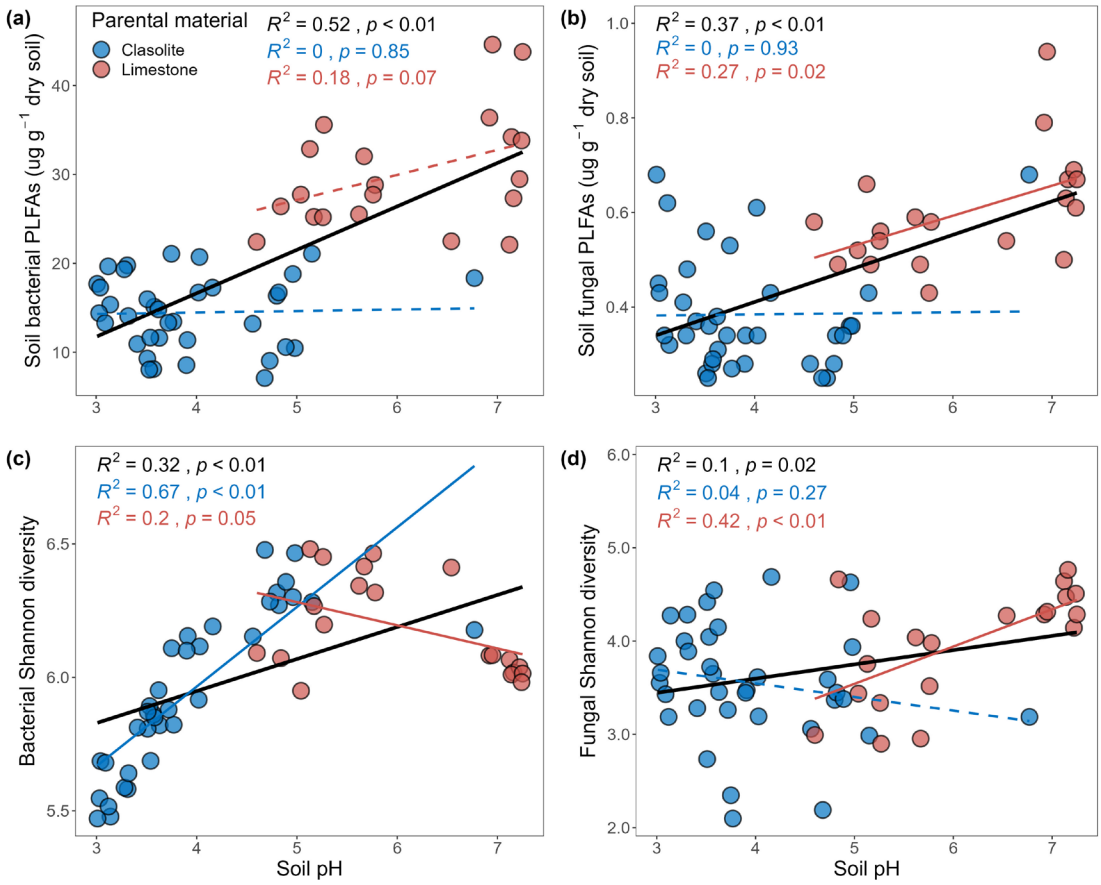


Figure 5 Structural equation modelling evidencing direct and indirect effects of climate and parental material on soil bacterial community biomass and α-diversity, respectively. In (a) and (b), the thickness of the arrows indicates the strength of the causal relationship, supplemented by a path coefficient. R^2 values denote the amount of variance explained by the model for the response variables. ***, **** indicates significance at the 99.9%, and 99.99% levels, respectively. (c) and (d): standardized total effects of climate, parental material and soil properties on soil microbial biomass and α-diversity, respectively. [This is figure is double column.]

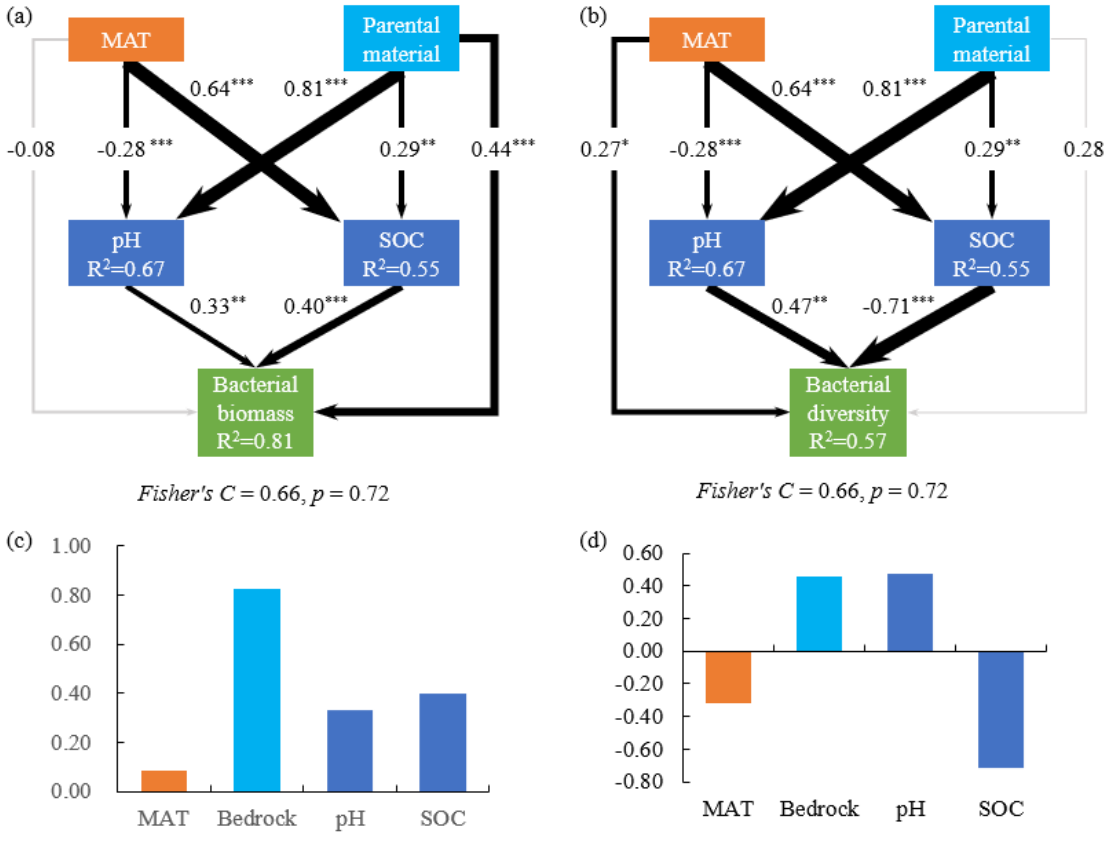


Figure 6 Structural equation modelling evidencing direct and indirect effects of climate and parental material on soil fungal community biomass and α-diversity, respectively. In (a) and (b), the thickness of the arrows indicates the strength of the causal relationship, supplemented by a path coefficient. R^2 values denote the amount of variance explained by the model for the response variables. ***, **** indicates significance at the 99.9%, and 99.99% level, respectively. (c) and (d): standardized total effects of climate, parental material, and soil properties on soil microbial biomass and α-diversity, respectively. PD: plant diversity. [This figure is double column.]

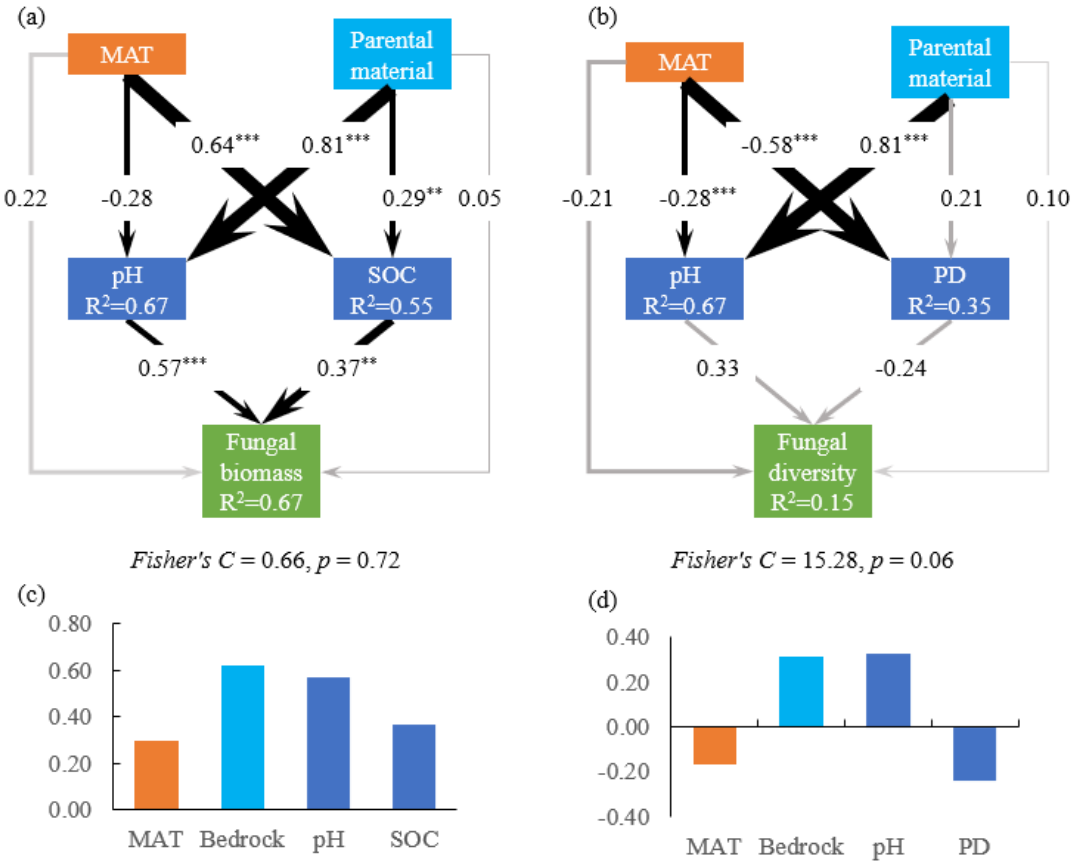


Figure 7 Redundancy analysis (RDA) of the relationship between predictor variables and the Bray–Curtis dissimilarity distance between microbial communities. In (a) and (c), dots indicate individual samples; blue dots indicate clasolite sites, red dots indicate limestone sites; the arrow lengths and directions correspond to the variance explained by the individual variables; only the variables with significant effects are shown in the ordinary plots. R^2 in (b) and (d) indicate the proportion of variation of soil bacterial and fungal communities explained by the predictor variables, respectively; star above a bar indicates it is statistically significant ($p < 0.05$). [This figure is double column.]