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Contrasting patterns of microbial community and enzyme activity between rhizosphere and bulk soil along an elevation gradient

Chengjie Ren[a,b,c], Zhenghu Zhou[b], Yaoxin Guo[d], Gaihe Yang[a,c,*], Fazhu Zhao[e,*], Gehong Wei[f], Xinhui Han[a,c], Lun Feng[g], Yongzhong Feng[c], Guangxin Ren[c]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, Shaanxi, China
[b] Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, School of Forestry, Northeast Forestry University, Harbin 150040, Heilongjiang, China
[c] College of Agronomy, Northwest A&F University, Yangling 712100, Shaanxi, China
[d] College of Life Sciences, Northwest University, Xi'an 710072, Shaanxi, China
[e] Shaanxi Key Laboratory of Earth Surface System and Environmental Carrying Capacity, Northwest University, Xi'an 710072, Shaanxi, China
[f] College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China
[g] Forest Ecosystem Positioning Research Station of Huanglong Mountain, Yan'an 716000, Shaanxi, China

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ABSTRACT

The distribution patterns of the microbial community and enzyme activity in soil systems along an elevation gradient have attracted considerable attention; however, the differences in microbial diversity and enzyme activity between the rhizosphere and bulk soil and their drivers are still unclear. Here, we used an elevation gradient that covered six elevation levels and ranged from 1308 to 2600 m above sea level. Illumina MiSeq sequencing of the 16S rRNA gene and ITS-1 gene was used to analyze the community of bacteria, total fungi, ectomycorrhizal (EcM) fungi, and saprotrophic fungi in both rhizosphere and bulk soil; in addition, the soil enzyme activity (β-glucosidase, *N*-acetyl-glucosaminidase, leucine aminopeptidase, and acid phosphatase) was investigated. The results revealed that the elevation significantly affected the diversity of the bacterial, total fungal, EcM, and saprotrophic fungal community, as well as the enzyme activity dynamics. The difference in the microbial diversity and enzyme activity between rhizosphere and bulk soil diminished as the elevation increased, except for the saprotrophic fungal diversity. Similarly, the dominant phyla from the compositions of bacteria, fungi, EcM fungi, and saprotrophic fungi, such as *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Basidiomycota*, and *Ascomycota*, also changed with elevation and rhizosphere. In addition, the elevation-dependent differences in the microbial community and enzyme activity between the rhizosphere and bulk soil were affected mainly by climatic factors (mean annual temperature and precipitation) and soil properties, such as the bulk density, ammonium nitrogen, and total phosphorus. The effects of the climatic factors were greater than those of the soil properties along the elevation gradient. These results suggest that changes in climatic factors, such as temperature, with elevation may affect the microbial interaction between roots and the soil. The result highlight the importance of the ecological roles of the microbial community in climate change.

1. Introduction

Soil microbes have a significant impact on terrestrial biogeochemical cycles (van der Heijden et al., 2008), and their responses to temperature play a key role in the self-regulating feedback associated with climate warming (Bardgett et al., 2008; Zhou et al., 2012; Truong et al., 2019). For example, a study on the elevation gradient in tropical regions predicted future changes using the microbial activity responses over a wide temperature range (Nottingham et al., 2019). Earth system models that include microbial process have also suggested that the nature of the temperature-adaptive responses in soil microbial growth and enzyme activity may significantly affect soil C losses, and can be used to more accurately project climate change feedbacks (Wieder et al., 2013). However, climate change has a more complex impact on the microbial diversity and community composition at the genetic level (van der Heijden et al., 2008). Because the drivers of the community

* Corresponding authors at: State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, Shaanxi, China (G. Yang).
E-mail addresses: ygh@nwsuaf.edu.cn (G. Yang), zhaofazhu@nwu.edu.cn (F. Zhao).

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composition are variable and thus less predictable, the effects of climate change on microbial diversity and functions are unclear (Burns et al., 2013; Karhu et al., 2014; Cairney, 2011)

Elevation experiments have been used in several studies to examine the effects of climate change on soil microbes (Mayor et al., 2017; Nottingham et al., 2015); these experiments showed that the microbial diversity and enzyme activity exhibit inconsistent patterns (e.g. monotonically decreasing, hump-backed or none) with elevation (Lanzén et al., 2016; Ren et al., 2018b; Wang et al., 2015; Yasir et al., 2015). This is because environmental conditions can vary with elevation, thus creating complex conditions for microbes (Körner, 2007). In addition, although several studies reported the elevation distribution of microbial communities, most of them considered the bulk soil, and few studies have considered the rhizosphere; in particular, the differences between the rhizosphere and bulk soil along an elevation gradient have not been well considered. The rhizosphere generally has higher rates of nutrient transformation than bulk soil (Ai et al., 2012; Pascual et al., 2018). Thus, the microbial species in the rhizosphere soil differ from those in bulk soil owing to plant root selectivity (Ai et al., 2012; Kuzyakov et al., 2007). For example, Fierer et al. (2007) reported that the carbon utilization preferences of *Proteobacteria*, a phylum of bacteria, represent the carbon availability preferences of this domain, and that this phylum may typically be very abundant in rhizosphere soil owing to its higher nutrient content. Van Der Heijden et al. (2015) also observed that mycorrhizal fungi play a critical role in the survival of terrestrial plants root, and predicted that the rhizosphere may contain more ectomycorrhizal (EcM) fungi than the bulk soil. A recent study also found that the microbial nutrient limitation varies significantly between rhizosphere and bulk soil at high elevations, which may constrain the nutrient transport in the root – soil system and further decrease the differences in microbial enzyme activity (Cui et al., 2019a). Therefore, this result suggests that the differences in microbial diversity and enzyme activity between rhizosphere and bulk soil may depend on the elevation; specifically, they may decrease with increasing elevation.

Microbial diversity in rhizosphere and bulk soil may depend on environmental variables such as plant diversity and plant and soil nutrients, as well as climatic factors (Mayor et al., 2017; Zhao et al., 2020). For instance, Safford et al. (2001) revealed that the distribution of the plant diversity with elevation has a humpback shape; however, the microbial diversity does not follow the same pattern (Fierer et al., 2011; Ren et al., 2018b). Mayor et al. (2017) demonstrated that elevation-dependent changes in plant nutrients can select for certain microbial communities in the root–soil system. Further, soil nutrients may determine the growth of microbes and ultimately affect the microbial diversity of the root–soil system (Cui et al., 2019b). Members of *Proteobacteria* prefer soil with high carbon availability, but members of *Actinobacteria* are adapted to harsh environmental conditions (resource-limited and higher temperature) (Fierer et al., 2007). Particularly, changes in vegetation along the elevation gradient will greatly influence belowground fungal communities, not only depending on their evolutionary groups but also their trophic modes (Truong et al., 2019). For example, the strong correlation between the diversity of co-occurring host plants and their EcM fungi could be the main factor controlling EcM fungal community assembly (Saitta et al., 2018; Tedersoo et al., 2012). Thus, some microbial species may differ in both the rhizosphere and bulk soil because of changing nutrient conditions. Additionally, soil enzymes are synthesized by specific groups of microorganisms; thus, the responses of microbial enzyme activity to elevation may also differ between rhizosphere and bulk soil. Cui et al. (2019a) reported that microbial enzymes reveal the carbon and phosphorus limitations of microbial metabolism in the rhizosphere and bulk soil in alpine ecosystems. Thus, identifying the microbial diversity and enzyme activity in root–soil systems along an elevation gradient can help predict the soil nutrient status and climate change. However, the exact manner in which the plant diversity, plant–soil nutrients, and climatic factors together affect the differences in microbial diversity and enzyme activity between rhizosphere and bulk soil, as well as the underlying mechanisms, remain unclear.

To understand the contrasting patterns of microbial diversity and enzyme activity between rhizosphere and bulk soil along an elevation gradient, we performed an extensive elevation gradient study on Taibai Mountain, the highest mountain in the Qinling Range of east China (Tang and Fang, 2006). Taibai Mountain is an ideal model system for evaluating the effects of elevation on plant characteristics, soil nutrients, and microbial community dynamics (Qiao et al., 2015; Ren et al., 2018a; Tang et al., 2012). Illumina MiSeq sequencing was used to examine the elevation-dependent changes in the bacterial, fungal, EcM fungal, and saprotrophic fungal community in both rhizosphere and bulk soil. The enzyme activity along an elevation gradient was also determined. Because of lower nutrient transport in root-soil system at high elevations (Cui et al., 2019a), we predicted that the differences in microbial diversity and enzyme activity between the rhizosphere and bulk soil may diminish with increasing elevation, even though they change significantly with elevation. In addition, several studies have demonstrated that the soil microbial diversity and enzymes are mainly affected by the composition of the soil organic matter, with no or indirect effect from elevation or temperature (Truong et al., 2019), thus, we hypothesized that the differences in microbial diversity and enzyme activity between the rhizosphere and bulk soil were affected by soil properties, such as the soil organic carbon, rather than plant characteristics and climatic factors. The objectives were (1) to reveal the differences in microbial community and enzyme activity between the rhizosphere and bulk soil and (2) to propose an explanation for how climatic factors and plant–soil conditions affect the differences along the elevation gradient.

2. Method and materials

2.1. Study area and experimental design

The field experiment was conducted in the Taibai Mountain (107°19′-107°58′E and 33°45′-34°10′N), which is the highest mountain in the Qinling Range of China. The Qinling Mountains has high elevation in central China and critically affect the north and south movement of air masses. Thus, this range (1200–3767 m a.s.l.) greatly influence the distribution of climate zone in the eastern China (Ren et al., 2018b). The mean annual temperature and mean annual precipitation (which mainly occurs between July and September).

The experiment was carried out on July 2018, six elevations covered three vegetation types: *Q aliena* var. acutiserrata community (QVA) (1308, 1603 m a.s.l.), *Quercus wutaishanica* community (QW) (1915, 2292 m a.s.l.), and *Betula albosinensis* community (BA) (2405, 2600 m a.s.l.). These three co-occurring tree species typically associated with ectomycorrhizal (EcM) fungi, which dominate the soil microbial communities in these forests (Rong et al., 2016). At each elevation, three independent replicated sites (50 × 50 m each) were selected, totaling 18 sites (six elevation × three replicated sites).

2.2. Plant and soil sampling

For description of plant community *in situ*, three 10 × 10 m quadrants, five 5 × 5 m quadrants, and ten 1 × 1 m quadrants were randomly selected in each site to determine the richness and Shannon diversity for tree, shrub, and herb, respectively. Tree leaves were collected from the tree dominated trees species (*Q aliena* var. acutiserrata community, *Quercus wutaishanica* community, and *Betula albosinensis* community) to determine carbon, nitrogen, and phosphorus. Tree fine roots were taken at the depth of 0–10 cm and separated from the soil via water bath extraction and with the help of a fine meshed sieve.

For soil sampling, the rhizosphere soil was defined as soil tightly adhering to the root surface, which were collected with a sterile and soft-bristled paintbrush, while the bulk soil was collected from the un-

vegetated soil at the depth of 0–10 cm and was not directly attached to the root systems. In order to get enough rhizosphere soil for determination, ten tree species were selected from each site, and the rhizosphere soils were homogenized to one composite sample. Consequently, the bulk soil consisting of ten cores that was adjacent to the trees were also collected from each site. Before sieving (< 2 mm), a portion of bulk soil was taken for soil moisture (samples were weighed in the field, and then weighed again in the laboratory after drying at 105 °C till constant weight), and then other bulk soil and all rhizosphere soil samples were stored in an insulated container with ice and immediately transported to the laboratory. All collected samples were divided into three subsamples: portions of fresh soil were stored at −80 °C for DNA extraction and at 4 °C for microbial biomass and enzyme activity analyses, while the remainder was air-dried and stored at room temperature prior to physical and chemical analysis.

2.3. Analysis of plant characteristics and soil physicochemical properties

The C, N, and P in tree leaf and tree root were determined after grinding finely. The C content in both tree leaf and tree root were determined using the $K_2Cr_2O_7$ oxidation method, and the N and P contents of the digested solution were determined using the Kjeldahl and colorimetric (UV spectrophotometer) methods, respectively. Soil pH was measured by a pH meter after shaking a soil-water (1:5 w/v) suspension for 30 min. Soil total carbon (TC) was determined by a TOC-TN analyzer (TOC-L CPH, Shimadzu Corp., Kyoto, Japan). Total nitrogen (TN) and total phosphorus (TP) were analyzed by a continuous flow analyzer (AA3; Germany) after wet digestion (Bao. 2000). Soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were estimated from fresh soil samples using a chloroform fumigation-extraction method as previously described (Brookes et al., 1985; Vance et al., 1987). The concentration of soil ammonium (NH_4^+) and nitrate nitrogen (NO_3^-) were colorimetrically analyzed using a continuous flow analyzer. Soil bulk density (BD) was determined from the gravimetric weight of the core after and before oven drying at 105 °C for 24 h and corrected with the individual core volume (De Vos et al., 2005).

2.4. Analysis of soil enzyme activity

The potential activities of β-glucosidase (BG), N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP) in rhizosphere and bulk soil were determined by the following methods (Cui et al., 2019a). Generally, the BG, NAG + LAP, and AP were considered as C-acquiring, N-acquiring, and P-acquiring enzymes, respectively (Sinsabaugh et al., 2008).

2.5. Soil DNA extraction, PCR amplification, and Illumina MiSeq sequencing

According to the manufacturer's instructions, DNA was extracted in triplicate from fresh soil using FastDNA spin kit (MP Biomedicals, Cleveland, USA). The quality and size of the extracted DNAs were determined by a spectrophotometer (NanoDrop2000, Thermo Scientific, Wilmington, DE, USA). The extracted soil DNA was stored at −80 °C until PCR amplification and analysis.

PCR amplification of bacterial 16S rRNA targeting the V4 region was conducted by using primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTC AATTCMTTTRAGTTT-3′) (Biddle et al., 2008). The fungal ITS-1 region was amplified by using primers ITS1F (50-ACTTGGTCATTTAGAG-GAAGTAA-30) and ITS2 (50-BGCTGCGTTCT-TCATCGATGC-30) (Mukherjee et al., 2014). The protocol of 16S rRNA gene PCR amplification was described previously (Ren et al., 2017b). Specifically, for bacteria, the amplification solution contained two primers (0.4 μl), FastPfu polymerase (0.4 μl) and template DNA (1.25 μl). These samples were denatured at 95 °C for 3 min and then amplified by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by an extension at 72 °C for 10 min. For fungi, the amplification solution contained 0.5 uL of each primer at 30 μmol L^{-1}, 1.5 μL of template DNA, and 22.5 μL of Platinum PCR SuperMix (Invitrogen, Shanghai, China). These samples of fungal amplification solution were denatured at 95 °C for 2 min and then amplified by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and an extension at 72 °C for 5 min. Each sample was amplified three times and then the relative amplicons were mixed to provide one final PCR product. The PCR products were extracted from 2% agarose gels and concentrated using the AxyPrep DNA Gel Extraction Kit. Finally, an equal amount of PCR product (2 × 250 bp) from each sample was sent to pyrosequencing by Illumina's MiSeq platform at Personal Biotechnology Co., Ltd. Shanghai, China.

Sequences were performed using QIIME according to the following three criterions (Caporaso et al., 2012). First, the 300-bp reads at any site were truncate with an average quality score < 20 over a 50-bp sliding window, then the truncated reads shorter than 50 bp were discarded; second, the exact barcode matching, two-nucleotide mismatches in the primer matching, and reads that could not be assembled were removed. Third, only sequences that overlapped longer than 10 bp were assembled based on their overlap sequence. The sequence analysis was performed using the USEARCH v5.2.32 to cluster Operational Units (OTUs) with less than 3% dissimilarity cutoff. Quantitative Insights Into Microbial Ecology pipeline software (QIME) was also used to analyze the taxonomy of each 16S rRNA and fungal ITS-1genes sequences by combining reads of clustered OTUs with 97% similarity. Finally, the gene sequences have been sent to the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) under the accession number of SRP223550 for bacteria and SRP223554 for fungi.

2.6. Statistical analyses

The EcM and saprotrophic fungi species were identified through uploading fungal OTUs to FUNGuild (http://www.stbates.org/guilds/app.php) (Nguyen et al., 2016). A total of 3090 OTUs were found across all the soils, but only half of them (1419 OTUs) were assigned by FUNGuild. In addition, some fungi did not fall exclusively into a single guild because their presence depend on life stage and environmental conditions (Nguyen et al., 2016). Therefore, the EcM and saprotrophic fungi in the current analysis were the species that were highly probable in FUNGuild.

The Shapiro–Wilk test was used to check the distributions of all data, and all variables followed a normal distribution and met the assumption for further variance analysis. Shannon index was used to reflect microbial (bacterial, total fungal, EcM, and saprotrophic fungal) alpha diversity. We generated a non-metrical multidimensional scaling (NMDS) plot using unweighted UniFrac distance to illustrate the clustering of the different samples and further suggested microbial beta diversity (NMDS1 and NMDS2). In order to reveal microbial diversity differences between rhizosphere soil and bulk soil along the elevation gradient (rhizosphere effects), we used the following formant for microbial diversity (alpha and beta) and dominant phyla:

(1) Differences in microbial alpha diversity:

$$Alpha_D = |(Rhi_{alpha} - Bulk_{alpha)}| \quad (1)$$

(2) Differences in microbial of beta diversity:

$$Beta_D = ((Rhi_{NMDS1} - Bulk_{NMDS1})^2 + (Rhi_{NMDS2} - Bulk_{NMDS2})^2)^{0.5} \quad (2)$$

(3) Differences in microbial dominant phyla:

$$Phyla_D = Rhi_{phyla} - Bulk_{phylm} \quad (3)$$

Furthermore, the partial least squares path modeling (PLS-PM) was

used to reveal which environmental variables have a greater impact on the microbial diversity and enzyme activity along the elevation gradient (Li et al., 2018). Through PLS-PM analysis, the observed variables can be explained by the latent variables. In our study, the latent variables included elevation, climatic factors, plant properties, soil properties, microbial biomass, microbial diversity, and enzyme activity. Each latent variable could include at least one observed variables. In the PLS-PM, the path coefficients represented the directions and strength of linear relationships among latent variables, and the explained variability (R^2) were also estimated. The effective model was evaluated by the Average variance extracted (AVE) and the Composite reliability (CR). When the AVE and CR are higher than 0.5 and 0.7, respectively, this model is the acceptable. This analysis was conducted using PLS, provided by the SmartPLS 2.0 M3 software. In our study, both the AVE and CR fitted these two standards (AVE > 0.5; CR > 0.7) (Vanalle et al., 2017).

Moreover, the Redundancy analysis (RDA) was used to analysis the relationship between microbial phyla and the environmental variables including climatic factors, plant properties, soil properties. The one-way analysis of variation (ANOVA) was used to test the effect of elevation gradient on climatic factors, plant properties, soil properties, and soil microbial biomass, microbial diversity, dominant phyla, and enzyme activity. Pearson's correlation analysis were used to determine the relationship between microbial (bacterial and fungal) phyla and the climatic factors, plant properties, soil properties, and soil microbial biomass. Pearson's correlation analysis, RDA, and ANOVA were conducted using the R v.3.4.3 program. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Soil properties and plant characteristics

Table S1 shows the changes in the climatic variables (climatic factors and plant-soil properties) along the elevation gradient. The dominant species along the elevation were *Q. aliena* var. *acutiserrata*, *Quercus wutaishansea*, and *Betula albosinensis*. The richness and Shannon diversity first increased and then decreased with increasing elevation. The highest leaf and root nutrient levels (C, N and P) were found at the 1915 m site, except for the root C, which was highest at 2292 m. The soil properties exhibited similar trends; MBC, MBN, MBP, TN, SOC, and NH_4^+ were highest at the 1915 m site.

3.2. Differences in microbial community between the rhizosphere and bulk soil

Microbial alpha diversity followed a hump-backed curve with increasing elevation in both the rhizosphere and bulk soil (Fig. 1a, d, g, j); specifically, the alpha diversity in the bacterial, fungal, and assigned fungal (EcM and saprotrophic) communities peaked at 1915 m a.s.l. and then significantly decreased at the highest elevation. Notably, the differences in microbial alpha diversity between the rhizosphere and bulk soil decreased greatly with increasing elevation, except for the saprotrophic alpha diversity (Fig. 1c, f, i, l).

Nonmetric multidimensional scaling (NMDS) analysis was used to investigate the microbial beta diversity (Fig. 1b, e, h, k). The bacterial beta diversity values of the rhizosphere and bulk soil were significantly different. The fungal beta diversity showed significant differences between the rhizosphere and bulk soil at lower elevations (1308 and 310 1603 m a.s.l), but the differences were smaller at higher elevations (2400 and 2600 m a.s.l). This pattern was observed for both EcM and saprotrophic fungal guilds. NMDS analysis also revealed an overlapping pattern for the bacterial, fungal, and EcM fungal communities between rhizosphere and bulk soil at higher elevations (2405 and 2600 m a.s.l), with significantly lower beta-diversity at the highest elevation.

The dominant bacterial phyla responded differently to elevation; they also varied between the rhizosphere and bulk soil (Fig. 2, Table S3). Specifically, *Proteobacteria* were significantly more abundant at mid-elevations, whereas the abundance of both *Acidobacteria* and *Actinobacteria* significantly decreased with increasing elevation. *Nitrospirae* and *Chloroflexi* were significantly more abundant at higher elevations (2405 and 2600 m a.s.l.). With regard to the differences between the rhizosphere and bulk soil (Fig. 2c), both *Proteobacteria* and *Actinobacteria* were significantly more abundant in rhizosphere soil and showed a large difference at mid-elevations (1915 and 2292 m a.s.l), but *Acidobacteria* showed the opposite trend. The abundance of other phyla, such as *Nitrospirae* and *Chloroflexi*, differed between rhizosphere and bulk soil.

Among the dominant fungal phyla (Fig. 2, Table S4), the abundance of *Basidiomycota* significantly decreased, whereas that of *Ascomycota* significantly increased with increasing elevation. Moreover, the abundance of these two phyla differed greatly between rhizosphere and bulk soil, but the trends in the differences varied with elevation. Almost all the assigned EcM fungi were from the phylum *Basidiomycota* (> 98%; Fig. 2e, f, Table S5), which was significantly less abundant in the rhizosphere than in bulk soil at lower elevations. By contrast, almost all of the assigned saprotrophic fungi were from the phylum *Ascomycota* (> 83%; Fig. 2g, Table S5), which was significantly more abundant in the rhizosphere than in the bulk soil and became significantly more abundant with increasing elevation. However, the difference in the dominant assigned fungi (EcM fungi: *Basidiomycota*; saprotrophic fungi: *Ascomycota*) between rhizosphere and bulk soil diminished was smaller at higher elevations.

3.3. Difference in enzyme activity between the rhizosphere and bulk soil

Fig. 3 shows that the elevation significantly affected soil enzyme activity. Specifically, the activity of C-acquiring enzyme (BG), N-acquiring enzyme (NAG + LAP), and P-acquiring enzyme (AP) significantly decreased with increasing elevation, and their activities were significantly higher in the rhizosphere soil than in the bulk soil (Fig. 3a, b, c). Like with microbial diversity response, the differences in the C-, N-, and P-acquiring enzymes between rhizosphere and bulk soil significantly decreased with increasing elevation (Fig. 3d, e, f).

3.4. Factors influencing the differences in the microbial community and enzyme activity between the rhizosphere and bulk soil

PLS-PM was used to reveal the possible pathways by which environmental variability (i.e., climatic factors, plant characteristics, soil properties, and soil microbial biomass) explains the microbial (bacterial and fungal) diversity and enzyme activity along the elevation gradient in both rhizosphere and bulk soil (Fig. 4). The results showed that 90.1, 62.4, and 82.3% of the variation in the microbial diversity was accounted for the elevation gradients in rhizosphere, bulk soils, and their difference, respectively (Fig. 4a, b, c). Particularly, the elevation significantly influenced the climatic factors (MAT, MAP), accounting for 95.3, 94.4, and 95.1% of the variation, respectively. Moreover, in addition to the microbial response to climatic factors, microbial diversity in rhizosphere soil (path coefficient = 0.786) was mainly directly affected by plant properties, whereas the microbial diversity in bulk soil (path coefficient = −1.072) and the difference (path coefficient = −1.122) were mainly directly affected by the soil properties, especially for bulk BD, NH_4^+, and TP. We found that the elevation gradient accounted for 96.3, 95.2, and 91.2% of the variation in the soil enzyme activity in the rhizosphere, that in the bulk soil, and their difference, respectively (Fig. 4 d, e, f). Similarly, the climatic factors also changed significantly along the elevation gradient, and the direct effects of climatic factors on the enzyme activity (path coefficient = 0.999, 0.721, and 1.219) were greater than those of the plant characteristics, soil properties, and soil microbial biomass in the rhizosphere, the bulk soil, and their difference.

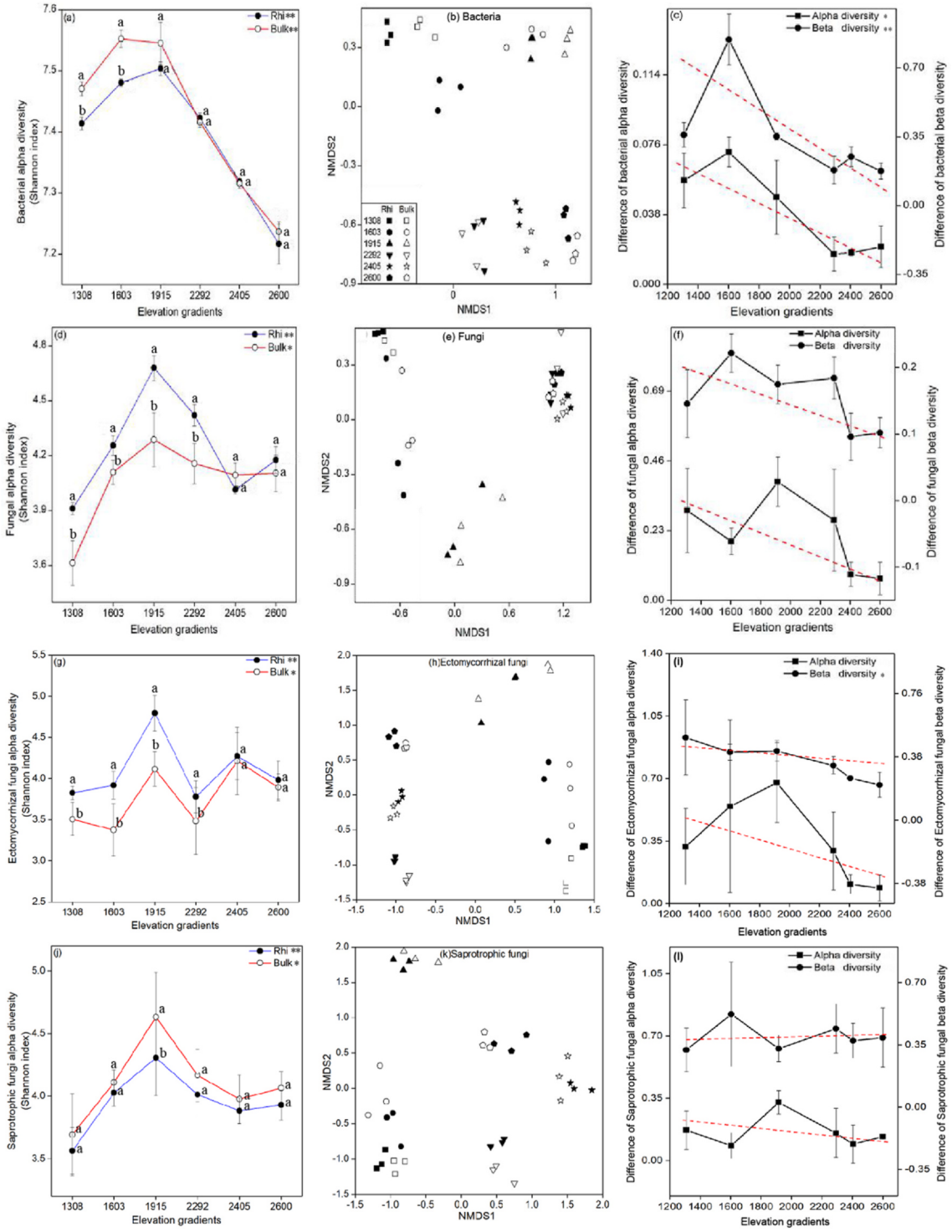

Fig. 1. Differential patterns of the bacterial, total fungal, ectomycorrhizal (EcM) fungal, and saprotrophic fungal diversity between rhizosphere and bulk soil along the elevation gradient: (a) changes of bacterial alpha diversity (Shannon index) in rhizosphere and bulk soil; (b) changes of bacterial beta diversity (Non-metrical multidimensional scaling; NMDS); (c) differences in bacterial diversity between rhizosphere and bulk soil; (d) changes of fungal alpha diversity (Shannon index) in rhizosphere and bulk soil; (e) changes of fungal beta diversity (Non-metrical multidimensional scaling; NMDS); (f) differences in fungal diversity between rhizosphere and bulk soil; (g) changes of ectomycorrhizal (EcM) fungal alpha diversity (Shannon index) in rhizosphere and bulk soil; (h) changes of ectomycorrhizal (EcM) fungal beta diversity (Non-metrical multidimensional scaling; NMDS); (i) differences in ectomycorrhizal (EcM) fungal diversity between rhizosphere and bulk soil; (j) changes of saprotrophic fungal alpha diversity (Shannon index) in rhizosphere and bulk soil; (k) changes of saprotrophic fungal beta diversity (Non-metrical multidimensional scaling; NMDS); (l) differences in saprotrophic fungal diversity between rhizosphere and bulk soil. Note: **effects of elevation on microbial alpha diversity and their differences are significant at the 0.01 level; *effects of elevation on microbial alpha diversity and their differences are significant at the 0.05level; the lower case letters indicate the significant effect between rhizosphere and bulk soil in the same site.

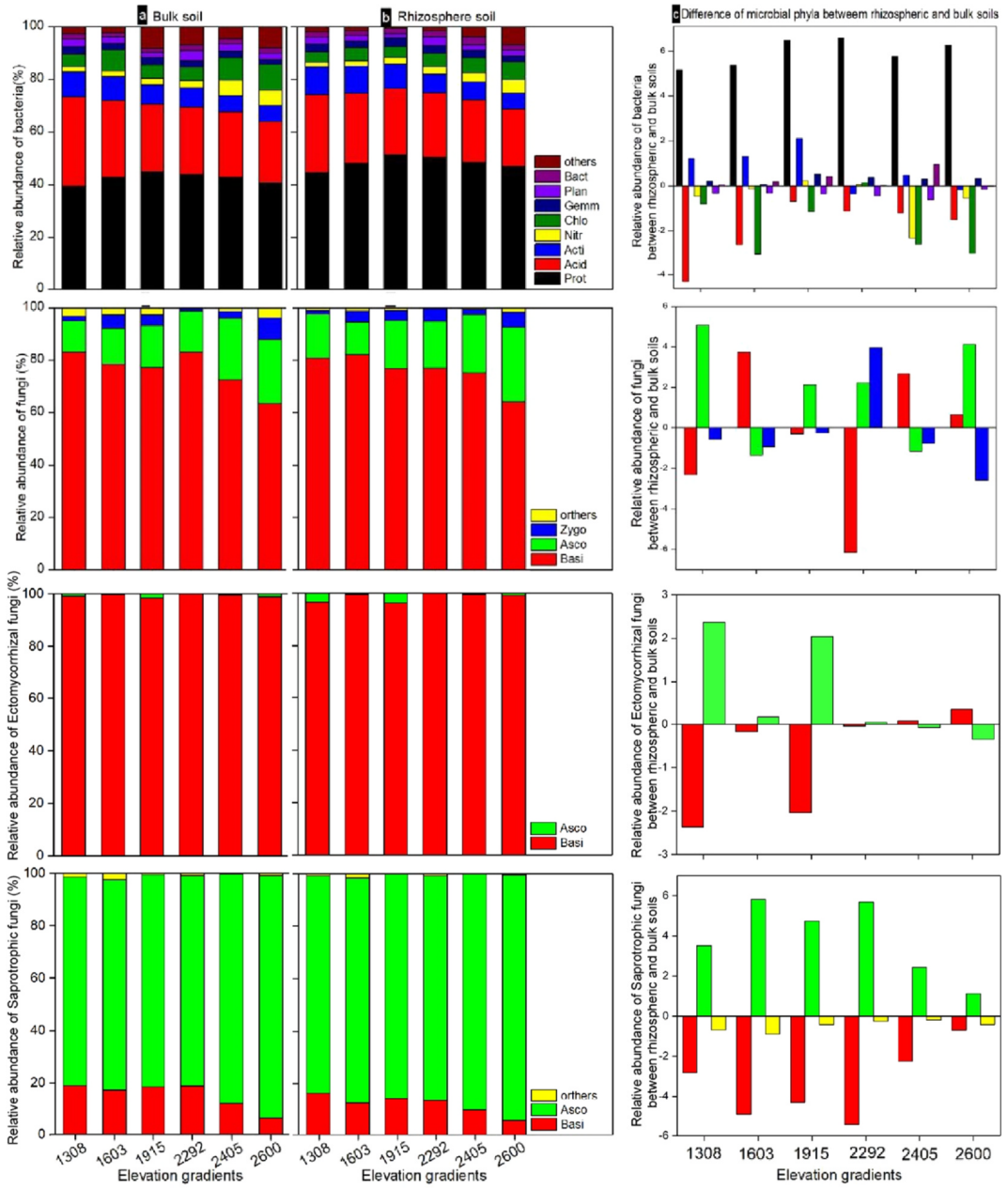

Fig. 2. Differential patterns of the bacterial, total fungal, ectomycorrhizal (EcM) fungal, and saprotrophic fungal dominant phyla between rhizosphere and bulk soil along the elevation gradient: (a) changes of bacterial, total fungal, ectomycorrhizal (EcM) fungal, and saprotrophic fungal in bulk soil; (b) changes of bacterial, total fungal, ectomycorrhizal (EcM) fungal, and saprotrophic fungal in rhizosphere soil; (c) differences in the bacterial, total fungal, ectomycorrhizal (EcM) fungal, and saprotrophic fungal between rhizosphere and bulk soil: These are the abbreviations of microbial taxa: *Proteobacteria (Prot), Acidobacteria (Acid), Actinobacteria (Acti), Nitrospirae (Nitr), Chloroflexi (Chlo), Gemmatimonadetes (Gemm), Planctomycetes (Plan), Bacteroidetes (Bact), Basidiomycota (Basi), Ascomycota (Asco), Zygomycota (Zygo)*.

RDA and Pearson's correlation analysis showed that the microbial (bacterial and fungal) taxa in both rhizosphere and bulk soil responded differently to environmental factors (ie., climatic factors, plant properties, soil properties, and microbial biomass) (Fig. 5a, b, Table S5, S6, and S7). Particularly, the climatic factors (MAT and MAP), BD, pH, TP and NH_4^+ explained more variation in the bacterial and fungal taxa in rhizosphere and bulk soil (Fig. 5c, d). In the rhizosphere soil, MAT, MAP, BD, pH, TP, and NH_4^+ were more positively correlated with changes in *Nitrospirae*, *Chloroflexi*, *Ascomycota*, *Acidobacteria*, Actinobacteria, and *Basidiomycota* abundance. While in the bulk soil, these selected factors were highly related to changes in *Nitrospirae*, *Ascomycota*, *Acidobacteria*, *Actinobacteria*, and *Basidiomycota*.

4. Discussion

4.1. Differences in microbial community and enzyme activity between the rhizosphere and bulk soil

The results of this study indicated that the microbial community diversity varied significantly with elevation, and the differences between rhizosphere and bulk soil greatly decreased with increasing elevation, which supported our first hypothesis (Fig. 1). It is likely that the spatial attributes, climatic factors, plant community, and other biotic and abiotic variables induced by elevation may explain the soil microbial diversity dynamics (Mayor et al., 2017; Ren et al., 2018b). Similar observations have been made in many other plant rhizospheres,

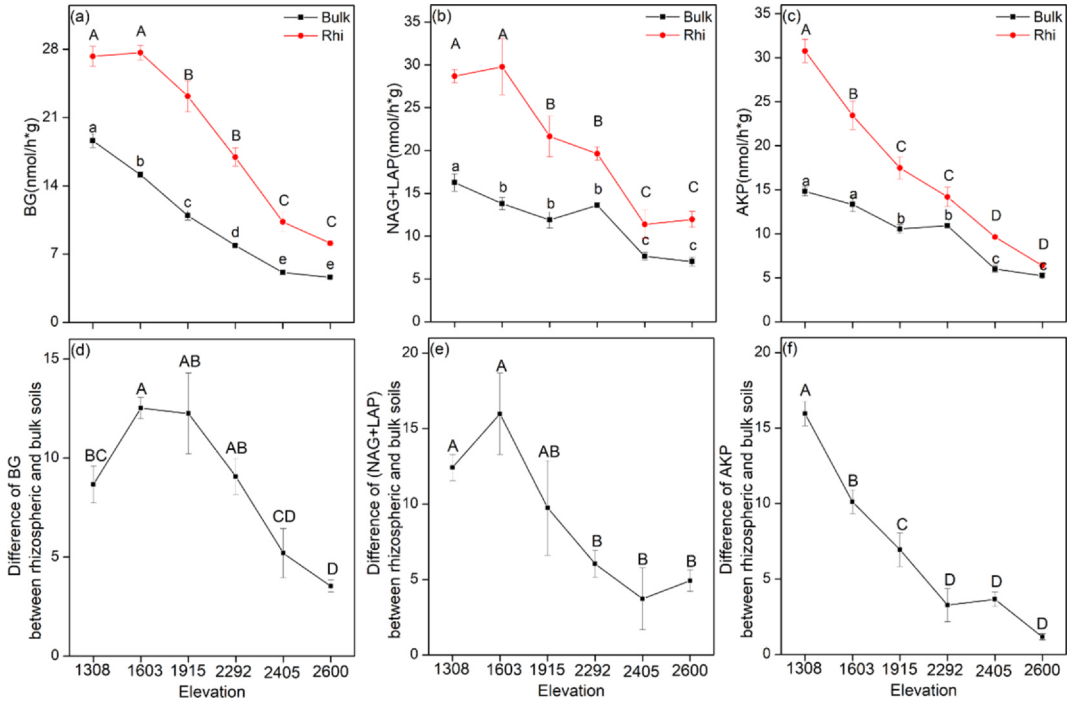

Fig. 3. Differential patterns of enzyme activity between rhizosphere and bulk soil along the elevation gradient: (a) trends of BG in rhizosphere and bulk soil; (b) trends of NAG + LAP in rhizosphere and bulk soil; (c) trends of AP in rhizosphere and bulk soil; (d) difference in BG between rhizosphere and bulk soil; (e) difference in NAG + LAP between rhizosphere and bulk soil; (f) difference in AP between rhizosphere and bulk soil.

suggesting the importance of the rhizosphere effect on the microbial diversity (Cui et al., 2019a; Kumar et al., 2017; Pascual et al., 2018). Additionally, the higher microbial diversity in the rhizosphere soil than in the bulk soil is mainly due to the flow of nutrients released by plants as root exudates to the surrounding soil, and higher temperature can accelerate nutrient release (Bulgarelli et al., 2013; Thion et al., 2016) (Fig. 1). Thus, elevation-dependent decreases in soil temperature can lead to lower nutrient fluctuation (Cui et al., 2019a); consequently, similar microbes appear in rhizosphere and bulk soil, and the differences decrease with increasing elevation. In particular, among the assigned fungal communities, the rhizosphere soil contains more EcM fungi and fewer saprotrophic fungi than the bulk soil, and have major effect on EcM fungi but minor effect on saprotrophic fungi (Fig. 1). One of the primary reasons might be related to the competition between EcM and saprophytic fungi in root-soil system, a phenomenon known as the 'Gadgil effect'. In detail, EcM fungi are more sensitive to the plants and the certain EM fungi may suppress soil saprotrophs when acquiring resources (Fernandez and Kennedy, 2016). It was also suggested in previous studies that the EcM fungi, unlike saprophytic fungi, physically provide more effective protection to the woody plants against root pathogens, nutrient limitation and climatic disturbance (Phillips et al., 2013; Bennett et al., 2017; Liang et al., 2020).

The patterns of microbial diversity in the rhizosphere and bulk soil along the elevation gradient were found to be dependent on the

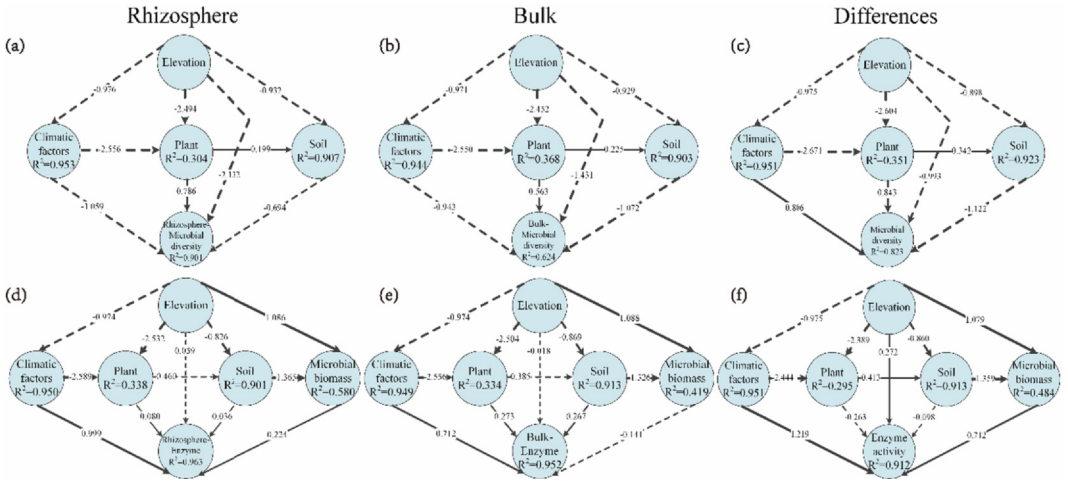

Fig. 4. Direct and indirect effects of elevation, climatic factors, plant, soil properties, and soil microbial biomass attributes on the microbial diversity and enzyme activity in rhizosphere and bulk soil. (a) PLS-PM was performed for rhizosphere microbial diversity; (b) PLS-PM was performed for bulk microbial diversity; (c) PLS-PM was performed for the difference in the microbial diversity between rhizosphere and bulk soil; (d) PLS-PM was performed for rhizosphere enzyme activity; (e) PLS-PM was performed for bulk enzyme activity; (f) PLS-PM was performed for the difference in the enzyme activity between rhizosphere and bulk soil. Continuous and dashed arrows indicate positive significant and negative significant relationships, respectively. The width of continuous and dashed arrows is proportional to the strength of path coefficients. R^2 denotes the proportion of variance explained.

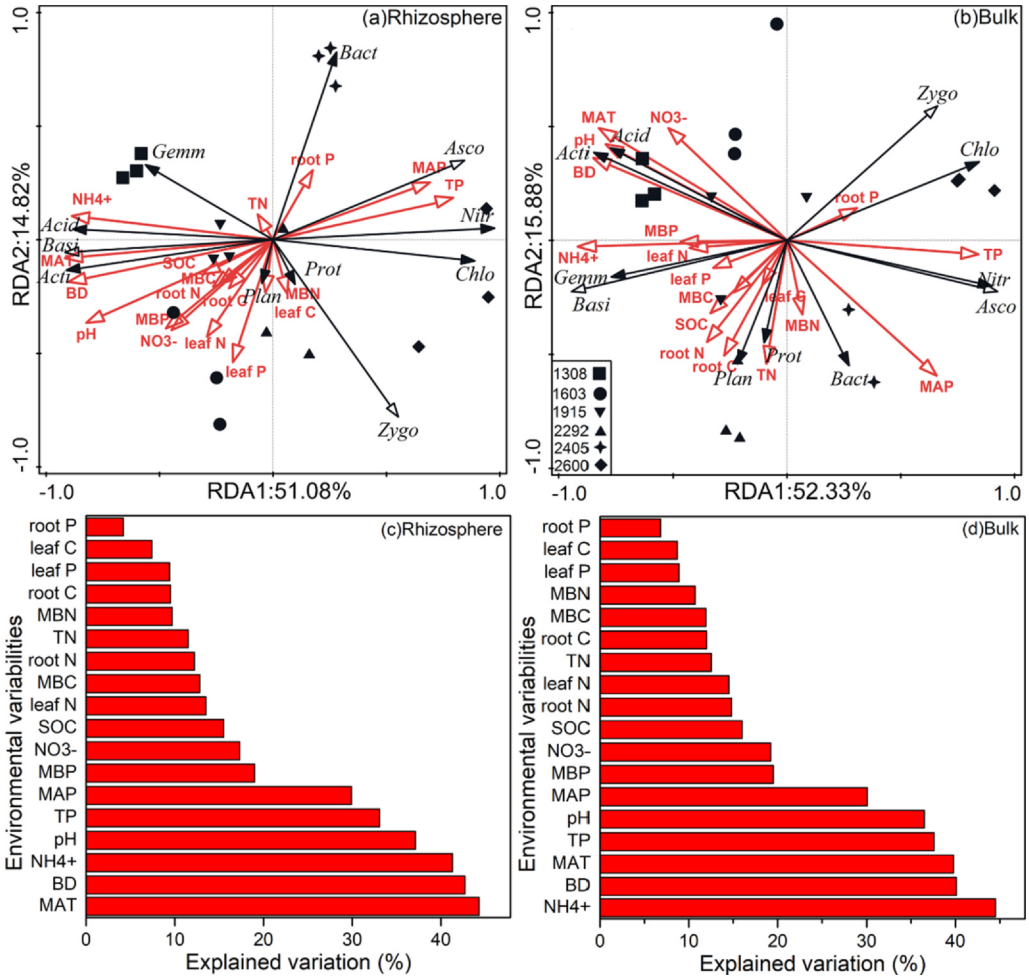

Fig. 5. Ordination plots of the results from the redundancy analysis (RDA) to identify the relationships among the microbial phyla (Black solid arrows: bacteria; Black hollow arrows: fungi) and the climatic variabilities (climatic factors, plant and soil properties). (a) RDA was performed between rhizosphere microbial phyla and climatic variabilities; (b) RDA was performed between bulk microbial phyla and climatic variabilities; (c) the explained variation (%) of environmental variability contribute to rhizosphere microbial (bacterial and fungal) phyla dynamics; (d) the explained variation (%) of environmental variability contribute to bulk microbial (bacterial and fungal) phyla dynamics. Mean annual temperature (MAT), mean annual precipitation (MAP), Bulk density (BD), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP).

taxonomic composition of the communities (Fig. 2, Table S3, S4, and S5). In the present study, the dominant microbial phyla (i.e., *Proteobacteria, Acidobacteria, Actinobacteria, Basidiomycota, and Ascomycota*) have been frequently discussed in elevation studies, with regard to their changing abundance under different environmental conditions (Pascual et al., 2018; Ren et al., 2018b; Zhao et al., 2020). However, due to nutrient differences between rhizosphere and bulk soil, the abundance of the microbial dominant phyla varies (Fig. 2). For instance, *Proteobacteria* have been described as fast growing copiotrophs that favor soil with high C availability (Fierer et al., 2007); thus, their abundance can be enhanced by high nutrient concentrations in the rhizosphere soil. In particular, high nutrient concentrations were observed at mid-elevations and may enlarge the differences in *Proteobacteria* abundance. By contrast, *Actinobacteria* is an oligotrophic group that prefers nutrient-poor environments (Wieder et al., 2013); thus, this phylum may be more abundant in bulk soil, which has lower nutrient levels than rhizosphere soil. *Acidobacteria* is a dominant phylum that can tolerate high pH conditions (Rodrigues et al., 2013; Shen et al., 2013), and elevation-dependent decreases in the pH might decrease the *Acidobacteria* abundance in both the rhizosphere and bulk soil (Ren et al., 2018b). In terms of fungal composition, *Basidiomycota* and *Ascomycota* accounted for more than 90% of the relative percentage of microbes in rhizosphere and bulk soil. This is in agreement with the reported findings for temperate forests globally (Tedersoo et al., 2014). The abundance of these two phyla regularly increased or decreased with elevation; the differences between the rhizosphere and bulk soil changed greatly and not in the same direction. It is plausible that these phyla changed owing to changes in the plant species and root exudate compositions (Clemmensen et al., 2013; Kuzyakov et al., 2007). Altogether, these results indicate that shifts in the soil nutrient level may account for the differences in the microbial dominant phyla and result in the changes in the microbial diversity of the rhizosphere and bulk soil along the elevation gradient.

Furthermore, we found that the activities of β-glucosidase N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP) significantly decreased with increasing elevation, consistently with the microbial alpha diversity in our study. Our result was not consistent with those of recent studies that reported no significant response of the soil enzyme activity to elevation gradients in southern Patagonia (Truong et al., 2019). This difference can probably be attributed to the different transect sample sites used in the two studies. In this study, the sample sites covered 1308–2600 m, whereas those of the other study covered only 130–640 m. The different transects would have different vegetation types, resulting in different responses of the soil microbial community and soil enzyme activity. Soil enzymes are expressed and released into the environment by microbes in response to environmental signals (Burns et al., 2013; Sinsabaugh et al., 2009). Several studies have explained that elevation-dependent changes in soil microbial composition cause differential responses in soil enzyme activity (Bardgett et al., 2008; DeAngelis et al., 2015). In addition, soil enzyme activity may also depend on complex conditions along the elevation gradient, such as soil temperature, moisture (Mayor et al., 2017), and soil nutrient availability (Cui et al., 2019a, b). In our study, we found that soil enzyme activity was not correlated with the soil properties, in particular with the C and N contents (Table S6). Our results contradict those of previous study (Truong et al., 2019), which reported that soil enzyme activity is strongly affected by the soil properties, especially the total C. Notably, we found that the enzyme

activities in the rhizosphere and bulk soil were very similar at higher elevations, indicating that the harsher environment at high elevations would increase the similarity of the nutrient-acquiring enzymes in the root–soil system. Previous studies demonstrated that the low soil temperatures at higher elevations could create a relatively stable habitat and lead to low microbial metabolic activity, resulting in decreased differences between the rhizosphere and bulk soil along the elevation gradient (Cui et al., 2019a; Pascual et al., 2018). Overall, these results further prove the hypothesis, that the soil enzyme activity decreases with increasing elevation, and that the differences in enzyme activities between the rhizosphere and bulk soil decrease significantly along the elevation gradient.

4.2. Environmental conditions co-mediate the differences in the microbial community and enzyme activity in the rhizosphere and bulk soil

Our study suggests that climatic factors induced by elevation play a critical role in shaping microbial communities (Fig. 1). The low temperature and increased physiological stress at high elevation could limit microbial growth and ultimately reduce microbial diversity (McCain, 2007). Zhou et al. (2016) reported that climatic factor (temperature) cause the microbial diversity to vary over broad geographic scales, mainly by changing microbial metabolism rate and growth. Therefore, changes in the climatic factors with elevation are the key ecological factors affecting the microbial diversity in both rhizosphere and bulk soil. However, we found that differences in plant and soil characteristics can also explain the soil bacterial and fungal communities characterizing each elevation site because of the high correlation observed between the soil and climate variables (Table S7, S8). The result was consistent with that of our previous study, in which we found that the positive response of the soil microbial diversity to an elevation gradient resulted from changes in the plant and soil characteristics along the elevation gradient (Ren et al., 2018b). For example, pH was determined to be a key ecological factor affecting the microbial diversity. The result was consistent with previous study (Coince et al., 2014; Jarvis et al., 2015; Rincón et al., 2015; Liimatainen et al., 2020). Furthermore, present study also indicated that rhizosphere microbial diversity was mainly affected by plant characteristics, whereas that in bulk soil was more strongly associated with the soil properties. This is because the rhizosphere microbiome is closer to the roots and may be affected by plant investment in root exudation (Kaštovská et al., 2015), whereas the microbes in the bulk soil may obtain nutrients directly from the substrate (Cui et al., 2019b; van der Heijden et al., 2008). Cui et al. (2019b) also found that the consistency of the microbial communities between the rhizosphere and bulk soil in alpine ecosystems could be attributed to low temperature and high nutrient content. These findings were confirmed in our study, which showed that the differences in microbial diversity were negatively correlated with the soil properties, particularly the BD; this was because the air content of soil, which regulates the nutrient uptake for microbial growth in the rhizosphere and bulk soil, varies with elevation (Celik et al., 2010).

Moreover, microbial communities are highly sensitive to changes in temperature and moisture (Ren et al., 2018a; Zhou et al., 2012). Thus, elevation dependent changes in climatic factors (MAT and MAP) can be important drivers for microbial dominant phyla in both rhizosphere and bulk soil (Fig. 5, Table S7, S8). A primary reasons might be related to the changes in either the ability to withstand osmotic pressure or the ability of hyphae to obtain available nutrients (Evans and Wallenstein, 2014; Nottingham et al., 2019). For instance, *Acidobacteria* were less abundant when the soil was cooler and moister (Evans and Wallenstein, 2014); thus, the elevation dependent change in MAP was negatively correlated with the *Acidobacteria* abundance in both rhizosphere and bulk soil. Similarly, the decrease in *Actinobacteria* with increasing elevation was positively correlated with the MAT, suggesting that temperature may affect the *Actinobacteria* abundance (Evans and Wallenstein, 2014; Ren et al., 2018b). A previous study showed that *Actinobacteria* can be used as a biomarker of disturbance (Hill et al., 2011), and the lower temperature at high elevations may constrain the interaction activity in the root-soil system; and reduce the differences in *Actinobacteria* abundance between rhizosphere and bulk soil (Fig. 5a). In the case of *Basidiomycota* and *Ascomycota* in fungal community, Ren et al. (2018b) confirmed that confirmed that the dominant fungal phyla were more strongly associated with the soil moisture than with the soil nutrients, even though *Basidiomycota* and *Ascomycota* metabolize the organic substrates produced by rhizodeposition (Clemmensen et al., 2013).

Additionally, the soil properties, specifically the N and P availability, are known to affect the occurrence of microbe–plant symbioses worldwide. Thus, the soil N and P may affect the soil microbial community in roots and soil; this hypothesis was also confirmed by the current study, which showed that TP, NH_4^+, and BD were significantly correlated with the microbial dominant phyla in both rhizosphere and bulk soil. We found that the *Nitrospirae* abundance was related to changes in NH_4^+, because *Nitrospirae* can contribute to N accumulation (DeLuca et al., 2002). Meanwhile, according to the growth rate hypothesis (Sterner and Elser, 2002), most microbes need P for the synthesis of ribosomal RNA; thus, the soil TP explains the higher variation of the microbial dominant phyla in rhizosphere and bulk soil along the elevation gradient. Overall, these results suggest that the changes in climatic factors, TP, NH_4^+, and BD with elevation may affect the microbial dominant phyla, resulting in the differences in microbial composition between rhizosphere and bulk soil.

Climatic variables might make an important contribution to the differences in soil enzyme activity along the elevation gradient, because microbial growth and function are strongly affected by climatic factors (i.e., MAP and MAT). In this study, the direct effects of climatic factors on enzyme activity in both the rhizosphere and bulk soil and the differences in enzyme activity were found to be greater than those of the plant characteristics, soil properties, and microbial biomass (Fig. 4), indicating that climate is the primary and most important environmental factor affecting the differences in enzyme activity between rhizosphere and bulk soil. This reason may be due to the diffusion constraints in the soil (McDaniel et al., 2013; Sinsabaugh et al., 2008), and the temperature and moisture in soil may determine either the enzyme production or the diffusion of enzymes and their contact with the substrate (Burns et al., 2013; McDaniel et al., 2013). This suggestion is consistent with a recent meta-analysis, which showed that experimental warming or precipitation can directly affect the soil microbial activity and microbial enzyme activities by altering the soil moisture and temperature (Chen et al., 2018; Ren et al., 2017a; Sinsabaugh et al., 2008; Xiao et al., 2018).

5. Conclusion

This study explored the distributional patterns and drivers of microbial diversity and enzyme activities in the rhizosphere and bulk soil along an elevation gradient. The results showed that the diversity of bacterial, total fungal, EcM, and saprotrophic fungal community, as well as soil enzyme activities changed markedly along the elevation gradient, and the differences between the rhizosphere and bulk soil significantly decreased with increasing elevation. Such differential responses were mainly dependent on the climatic factors, suggesting that climate is critical and the most important ecological factor driving the differences in the microbial diversity and enzyme activities between the rhizosphere and bulk soil. In addition to the climatic factors, the soil properties, particularly for NH_4^+, TP, and BD, also influenced the differences in the microbial diversity and enzyme activities between the rhizosphere and bulk soil with elevation, confirming that soil nutrient levels can account for the differences in the microbial diversity and enzyme activities along the elevation gradient. Altogether, our findings highlighted the importance of the rhizosphere effects represented by the elevation-induced changes of the microbial diversity and enzyme

activities, and further predicting the underlying consequences for climate change.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2020.104921.

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