BMC Plant Biology

RESEARCH

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Distinct roles of bacteria and fungi in driving rhizosphere and bulk soil multifunctionality of *Abies georgei* in an alpine forest

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Abstract

Background Root activity creates a unique microbial hotspot in the rhizosphere, profoundly regulating soil activity and associated soil multifunctionality (SMF), the ability of soil to deliver multiple functions or services simultaneously. However, empirical studies on the characteristics of SMF in the rhizosphere and bulk soil and their microbial regulatory mechanisms remain scarce.

Methods To address this gap, we conducted a field sampling campaign in an alpine forest on the eastern Tibetan Plateau. Soil abiotic and biotic properties, including soil nutrient availability, enzyme activities and microbial attributes were examined to compare the characteristics of SMF in the rhizosphere and bulk soil of *Abies georgei*, and to explore how microbial mechanisms drive SMF in each compartment.

Results We found that the rhizosphere consistently exhibited higher SMF than bulk soil, highlighting its enhanced functional potential regardless of environmental variation. The relationship between microbial diversity and SMF was compartment-specific: bacteria diversity was strongly associated with SMF in the rhizosphere, while fungal diversity was closely linked to SMF in the bulk soil. Furthermore, microbial biomass, particularly fungal biomass, had a strong influence on SMF in both rhizosphere and bulk soils. Structural equation modeling revealed that the relationship between soil diversity and SMF were primarily mediated by variations in soil abiotic properties, including soil pH in the bulk soil, and soil moisture and clay content in the rhizosphere.

Conclusions Our findings demonstrate that microbial contributions to soil multifunctionality are compartment-dependent and emphasize the need to integrate the rhizosphere perspective into biodiversity-multifunctionality frameworks for improving predictions of soil functions in terrestrial ecosystems.

Keywords *Abies georgei*, alpine forest, Bacteria, bulk soil, Fungi, Rhizosphere, Soil multifunctionality

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Introduction

Soils harbors a remarkable diversity of microorganisms, including bacteria and fungi, which are critical for a wide range of soil functions (e.g., nutrient cycling, organic matter decomposition) [1, 2]. Linking soil microbial biodiversity to multiple types of soil functions (i.e., soil multifunctionality, SMF) has led to a surge of studies on soil biodiversity-multifunctionality relationship (soil-SMF) [3, 4]. The continuous input of available resources, such as root exudates, creates distinct soil microhabitats (e.g., rhizosphere, detritusphere, aggregate surfaces and biopores) that support diverse soil communities [5]. However, most of soil-BMF studies have treated soil as a whole [4, 6, 7], often neglecting the structural and functional heterogeneity across different soil compartments, which harbor distinct soil microbial communities [5, 8, 9]. To capture the full scope of belowground diversity in driving ecosystems processes, a comprehensive understanding of the linkages between soil microorganisms and multifunctionality in specific soil microbial compartments is highly required.

The rhizosphere, the narrow zone of soil that is surrounded by plant roots, is considered the soil's most bioactive "hotspot" with distinct properties [5, 10]. Growing empirical evidence has demonstrated that the rhizosphere receives labile carbon inputs from roots (e.g., root exudates, plant litter, plant secondary metabolites) and exerts selective effects on microbial communities, thereby resulting in significant alternations in soil microbial assemblages and activities compared to those in bulk soil [11–13]. A meta analysis confirms that microbial biomass is consistently higher in the rhizosphere than in bulk soil [14]. In addition, recent studies have shown that soil microbes in the rhizosphere exhibit more complex co-occurrence networks than those in bulk soil, indicating stronger microbial interactions in the rhizosphere [15, 16]. Given the different roles of the soil microbes in driving ecosystem functioning [6, 17], the discrepancy in microbial attributes between rhizosphere and bulk soil are likely to result in significant divergence in the SMF between rhizosphere and bulk soil [9, 18]. Yet, our current understanding of soil-SMF in the rhizosphere remains limited, due to the inherent complexity of studying in-situ rhizosphere processes under field conditions, overlooking the uniqueness of the rhizosphere as a bioactive hotspot. Adopting a rhizosphere perspective in soil-SMF studies would substantially improve predictions of microbial-mediated functions and provide valuable insights for optimizing the management of soil microorganisms.

As the biological engine of terrestrial ecosystems, soil microbial communities, particularly bacteria and fungi, constitute major components of soil biodiversity and play essential roles in driving SMF [2]. Bacteria and fungi differ in their strategies for nutrient acquisition and organic matter decomposition, which may lead to distinct impacts on soil multifunctionality SMF [19]. Additionally, microbial properties (e.g., alpha diversity, community composition and biomass) vary markedly between rhizosphere and bulk soils due to niche differentiation driven by resource availability and microhabitat conditions, potentially resulting in compartment-specific relationships between microbial communities and SMF [18, 20]. For example, a recent study in an undisturbed bamboo forest found that bacterial diversity was more strongly associated with SMF in bulk soil, while fungal diversity played a more significant role in the rhizosphere [18]. Despite these findings, most research on the soil microbial community–SMF relationship has focused primarily on bulk soils, with limited attention given to the rhizosphere, especially in studies that directly compare both compartments.

Soil in alpine forest ecosystem harbor diverse soil microorganisms, and their diversity is essential for sustaining ecosystem functions [21, 22]. The alpine forest is often characterized by the continuous distribution of single coniferous species across a wide elevation range. This gradient imposes significant variations in environmental conditions over short geographical distances, providing a natural laboratory to investigate how soil biodiversity and ecosystem functions respond to dramatic environmental variation [23]. In this study, we conducted a field sampling campaign in an alpine coniferous forest (*Abies georgei*) along an elevation gradient from 3,700 to 4,200 m on the eastern Tibetan Plateau. We examined shifts in soil microbial diversity and SMF across the rhizosphere and bulk soil of *A. georgei*, a dominant species in alpine coniferous forest. The aim of this study was to assess the differences of SMF between the bulk soil and rhizosphere and explore the role and importance of bacteria and fungi in mediating soil functions in an alpine forest. We hypothesized that: (H1) the rhizosphere would exhibit greater multifunctionality to that in the bulk soil due to greater microbial interactions and activity in the rhizosphere. Specially, SMF would decline with increasing elevation as a result of harsher environmental conditions (e.g., low temperature and nitrogen availability) limiting microbial processes [24–26]. We expected that (H2) the relationship between microbial properties and SMF would vary across soil compartments. Moreover, we hypothesized that (H3) the dependence of SMF on soil microbial properties would be regulated by edaphic factors via their effects on the capacity of living and adaptation of microbial communities [27].

Materials and methods

Study site and sampling

Soil sampling was conducted at an elevation gradient of the eastern Tibetan Plateau consisting of six study sites along an elevational gradient from 3,700 to 4,200 m on Mount Sejila (Mt. SJL, 29°39′N, 94°42′E) (Figure S1). At each designated site (representing a specific elevation), the five random plots were scattered within a defined area, ensuring a minimum spatial separation of 10 m between adjacent plots. Detailed site descriptions and sampling protocols are available in a previous study by Deng et al. [28]. Briefly, mean annual temperature (MAT) decreases with elevation, declining from 3.69 to 1.73 °C. The mean annual precipitation (MAP) shows minimal variation along elevation, decreasing slightly from 651 to 638 mm, with approximately 80% of precipitation occurring during the growing season (May to September) under the influence of the Indian Ocean monsoon. The transect, spanning ~ 1.5 km in horizontal distance, encompasses a core distribution of alpine coniferous forest, dominated by *A. georgei*. The typical soil types are Luvisols and Cambisols according to IUSS classification and these are representative soil types of Tibetan Plateau.

Sampling was performed in July 2021 during the growing season. At each of the six elevation sites (3,700 m–4,200 m, spaced at 100 m intervals), five randomly located 10 × 10 m plots were established, with a minimum distance of 10 m between plots. Within each plot, three *A. georgei* trees (35–60 cm in diameter at breast height) were selected for rhizosphere and bulk soil collection. The species was identified by T. Cheng, and a voucher specimen of *A. georgei* has been deposited in the Herbarium of Tibet Agriculture & Animal Husbandry University in Nyingchi. Collection of wild plant material was conducted in accordance with local legislation, as authorized by the Tibet Agriculture & Animal Husbandry University. Following removal of the surface organic layer, 6–10 soil cores (15 cm depth, 8 cm diameter) were taken from the root zone of each tree and composited to ensure adequate rhizosphere soil. Samples were immediately sealed in zip-lock bags to prevent contamination. Fine roots (< 2 mm) were isolated using forceps based on surface color and elasticity. Rhizosphere soil was defined as soil tightly adhering to roots after gentle shaking, while remaining soil was classified as bulk soil [29]. This procedure yielded a total of 60 composite samples (6 sites × 5 plots × 2 soil compartments). All samples were transported on ice to the laboratory. Subsamples were allocated for different analyses: one portion was freeze-dried for microbial community profiling and amino sugar quantification, another air-dried for physicochemical characterization, and the remainder stored at − 20 °C for microbial trait measurements.

Climate and soil physicochemical properties

Mean annual temperature (MAT) and mean annual precipitation (MAP) for each site were obtained from the WorldClim database (https://www.worldclim.org) based on the geographic coordinates (latitude and longitude) of the sampling locations. Soil pH was measured using a pH meter (UV-1800, Shimadzu) after shaking a soil deionized water suspension (1:2.5, w/v) for 30 min. Gravimetric soil moisture content was determined by oven-drying samples at 105 °C for 24 h. Soil texture (percent sand, silt, and clay) was analyzed following the method described by Kettler et al. [30]. Sand and silt contents were calculated as the proportion of the original dry mass after oven-drying to a constant weight at 105 °C. Soil organic carbon (SOC) concentration was determined using the potassium dichromate ($K_2Cr_2O_7$) oxidation method. Total nitrogen (TN) was measured with an elemental analyzer (Vario MACRO, Elementar Analysensysteme GmbH, Hanau, Germany), and total phosphorus (TP) was determined via acid digestion. Inorganic nitrogen (NH_4^+-N and NO_3^--N) and available phosphorus (APho) were extracted using 2 M KCl and 0.5 M $NaHCO_3$, respectively, and quantified with a continuous flow analyzer (AutoAnalyzer 3 SEAL, Bran + Luebbe).

Analysis of soil enzyme activities

Activities of seven key hydrolytic enzymes involved in carbon, nitrogen, and phosphorus cycling—β–1,4-glucosidase (BG), cellobiohydrolase (CBH), N-acetyl-β-D-glucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (ACP) were measured using a fluorometric method, following the protocol of German et al. (2011). Briefly, 1.0 g of fresh soil was suspended in 40 mL of acetate buffer (pH 5.0, adjusted to reflect the acidic conditions of the study soils) and vortexed for 1 min. Subsequently, 200 µL of the resulting suspension was dispensed into black 96-well microplates, and 50 µL of a 200 µM fluorogenic substrate solution was added to each well. All enzyme assays were performed in triplicate. To account for fluorescence quenching, standard curves were generated using either 4-methylumbelliferone (MUB; for BG, CBH, NAG, and ACP) or 7-amino-4-methylcoumarin (AMC; for LAP) at concentrations of 0, 5, 10, 25, 50, and 100 µM. The plates were incubated at 25 °C for 2 h (CBH, NAG, ACP) or 4 h (BG, LAP), and fluorescence was measured using a microplate reader (Tecan Spark, Männedorf, Switzerland) at 360 nm excitation and 460 nm emission. Enzyme activities were expressed as nmol g^{-1} dry soil h^{-1}. To assess lignin-degrading enzyme activity, phenol oxidase (PPO) and peroxidase (PER) activities were measured using L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate following the method of DeForest [31]. For each assay, 800 µL of soil suspension was mixed with 200 µL of a

reaction solution (25 mM L-DOPA, 50 mM EDTA) in 2 mL microcentrifuge tubes and incubated in the dark at 25 °C for 4 h. For PER assays, an additional 40 µL of 0.3% H_2O_2 was included. After incubation, samples were vortexed for 10 s, centrifuged at 10,000 rpm for 2 min, and 250 µL of the supernatant was transferred to a microplate. Absorbance was recorded at 450 nm using the Tecan Spark reader. Additionally, microbial biomass carbon (MBC) and nitrogen (MBN) were determined via the chloroform fumigation–extraction method [32], using conversion factors of 0.45 for MBC and 0.54 for MBN as recommended by Fan et al. [33]. MBC values were calculated based on the difference between fumigated and non-fumigated samples.

Soil microbial biomass analysis

Soil microbial biomass was quantified using phospholipid fatty acid (PLFA) analysis, following the method described by [34]. In short, 8 g of fresh soil was homogenized in an extract of chloroform: methanol: phosphate buffer solution (1:2:0.8 v/v/v). Phospholipids were separated and extracted, and derivatized to fatty acid methyl esters through methanol. The contents of various fatty acids were then analyzed by gas chromatography. Bacterial biomass was estimated based on the abundance of selected groups of fatty acids, including 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, 16:1 w7t, 17:0 anteiso, 17:0 iso, 17:0 cyclo, 18:1 w7c, 18:1 w7t, 18:1 w9c, and 19:0 cyclo [35, 36]. Fungal biomass was represented by the sum of 18:2 w6c, 18:3w6c, and 18:3w3c [37]. The total microbial biomass was estimated by summing all the extracted PLFAs.

Assessment of soil microbial diversity

Soil DNA was extracted from soil samples using the OMEGA Soil DNA Kit (D5635-02; Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's protocol. To assess microbial community diversity, the bacterial 16 S rRNA gene and fungal internal transcribed spacer (ITS) region were amplified using primer pairs 515 F/907R and ITS1F/ITS2, respectively, each modified with platform-specific adapter and barcode sequences. PCR amplicons were purified using VAHTS™ DNA Clean Beads (Vazyme, Nanjing, China) and sequenced on the Illumina NovaSeq platform using a NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). Bioinformatic processing was conducted using QIIME2 (version 2019.4). Sequence reads were quality-filtered, denoised, merged, clustered and checked for chimeras using the DADA2 plugin. Shannon diversity indices were calculated using the diversity plugin, with amplicon sequence variants (ASVs) rarefied to 48,700 sequences per sample for bacteria and 46,135 for fungi. Taxonomic assignments were performed using the classify-sklearn naïve Bayes classifier in the feature-classifier plugin, referencing the SILVA 138 database for bacterial ASVs and the UNITE Release 8.0 database for fungal ASVs.

Table 1 Soil functions were used to evaluate soil multifunctionality

Individual function	Indicator
Nutrient cycling	total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), available phosphorus (APho)
Carbon stocks	soil organic carbon (SOC)
Decomposition	β−1,4-glucosidase (BG), cellobiohydrolase (CBH), Nacetyl-β-D-glucosaminidase (NAG), leucine aminopeptidase (LAP), acid phosphatase (AP), phenoloxidase (PPO) and peroxidase (PER)
Microbial productivity	microbial biomass carbon (MBC), microbial biomass nitrogen (MBN)

Assessment of soil multifunctionality

In this study, ecosystem multifunctionality was assessed based on the following parameters: soil nutrient cycling: total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), available phosphorus (APho); soil carbon stocks: soil organic carbon (SOC); decomposition: β−1,4-glucosidase (BG), cellobiohydrolase (CBH), Nacetyl-β-D-glucosaminidase (NAG), leucine aminopeptidase (LAP), acid phosphatase (AP), phenoloxidase (PPO) and peroxidase (PER); microbial productivity: microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) (Table 1). These variables serve as robust proxies for assessing soil microorganisms associated functions and processes in mountain ecosystems [22, 38]. To obtain a weighted ecosystem multifunctionality for each site, we initially normalized (log-transformed when needed) and standardized each of the 15 functions measured using the 0–1 transformation. These standardized ecosystem functions were then averaged to obtain an ecosystem multifunctionality index. This method is widely used in ecosystem multifunctionality literature [6, 7]. We also calculated an SMF index based on the scores from the first principal component of the 15 functions. Since the PC1 scores were significantly correlated with the averaged index (Figure S2), we proceeded with the averaged SMF index for all subsequent analyses.

Assessment of soil microbial co-occurrence network complexity

To analyze interactions among soil microbial taxa, we employed co-occurrence network analysis, a method widely used in microbial ecology [39, 40]. Soil microbial networks were built using bacterial and fungal amplicon sequence variants (ASVs) from both bulk and

rhizosphere soils. Only ASVs with a cumulative relative abundance exceeding 0.1% across all samples were included. Additionally, to ensure robust connections, we retained ASVs detected in at least five sampling sites, while all soil invertebrates were incorporated due to their limited ASV representation. Network construction relied on Spearman's correlation matrices, implemented via the "igraph" package, with stringent thresholds (adjusted $P < 0.05$, correlation coefficient $R > 0.6$) to identify co-occurrence patterns. Several topological metrics were then computed to assess network complexity using r function "sub_graph" [41]. Average path length refers to the average network distance between all pairs of nodes; network diameter refers to the greatest distance between the nodes that exist in the network; average degree refers to the average connections of each node with another unique node in the network; clustering coefficient represents the degree to which the nodes tend to cluster together; and graph density refers to the intensity of connections among node. Therefore, higher values for nodes, edges, average degree, clustering coefficient, and graph density as well as shorter path lengths and diameters, suggesting a more interconnected and complex network [38, 42]. To quantify multitrophic network complexity, we integrated these metrics into a composite index using multidimensional scaling [41]. Notably, path length and diameter were inverted (×−1) prior to index calculation to align with connectivity interpretations.

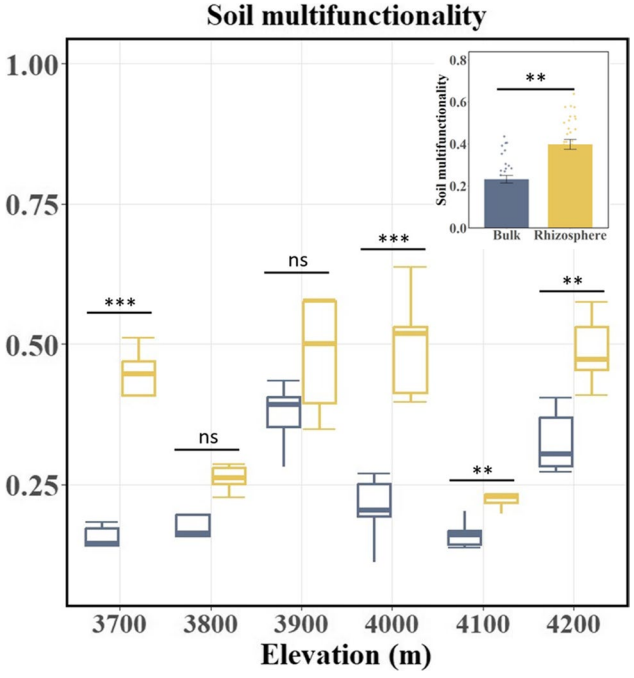

Fig. 1 Soil multifunctionality along an elevation gradient in bulk and rhizosphere soil at Mt. Sejila (mean ± SE). The upper-right plot indicate the differences of soil multifunctionality between the rhizosphere and bulk soil across all sites. The blue and yellow bars denote bulk soil and rhizosphere soil, respectively. Different letters indicate statistically significant differences among treatments based on a Tukey HSD test ($p < 0.05$). ns $p > 0.05$; * $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $p < 0.001$. $n = 5$ replicates for each site for bulk soil and rhizosphere soil separately

Statistical analysis

We used one-way ANOVA to assess differences in individual soil functions and soil multifunctionality (SMF) between rhizosphere and bulk soils. We selected ASVs richness (observed ASV numbers per sample) and Shannon diversity index to investigate changes in alpha diversity of soil microorganisms. The relationships between microbial attributes, including richness, Shannon diversity, co-occurrence network complexity, and microbial biomass and both SMF and individual soil functions were examined separately for rhizosphere and bulk soils using Spearman correlation analysis. Random forest (RF) modeling was performed using the "rfPermute" package to identify the key factors contributing to soil multifunctionality. The importance of each variable was assessed by the percentage increase in mean squared error (%IncMSE), with higher %Inc-MSE values indicating more important variables. The significance of the model was evaluated with 5,000 random permutations of the response variable using the "A3" package in R [43]. Variation partitioning analysis (VPA), implemented via the "vegan" package in R, was used to quantify the unique and shared contributions of bacterial and fungal attributes to the variation in SMF. Additionally, partial least squares path modeling (PLS-PM) was conducted using the "plspm" package to disentangle the direct and indirect effects of abiotic factors and microbial properties on SMF. The best model was selected based on goodness of fit. All statistical analyses were performed in R (v. 4.2.3).

Results

Variations in soil multifunctionality and individual functions in the rhizosphere and bulk soil

Soil multifunctionality differed significantly between rhizosphere and bulk soil along the elevation gradient (Fig. 1). Rhizosphere soil multifunctionality was 71% greater than that from the bulk soil, and similar trends were observed for most individual soil functions. However, rhizosphere decomposition was significantly greater than the bulk soil only at 3,700 m (Figure S3). Changes in soil multifunctionality and individual functions were not consistent along the elevation gradient (Fig. 1; Figure S3). For example, soil multifunctionality, nutrient cycling, carbon stocks, and decomposition of both bulk and rhizosphere soils increased from 3,700 to 3,900 m, declined from 3,900 to 4,100 m and then increased again at 4,200 m.

Links between soil multifunctionality and soil microbial properties

Bacterial and fungal community composition exhibited significant compartment-specific shifts between bulk soil and the rhizosphere across the elevational gradient (Figure S4). These compositional changes were primarily driven by key edaphic properties, including pH, moisture, and clay content (Figure S5). Bacterial properties were positively and significantly correlated with individual soil functions and multifunctionality along the elevation gradient (Fig. 2, Figure S6). In the bulk soil, bacterial diversity showed few significant associations with soil functions and multifunctionality. In contrast, in the rhizosphere, both bacterial richness ($R^2 = 0.48$, $p < 0.01$) and Shannon diversity ($R^2 = 0.58$, $p < 0.01$) were positively associated with soil multifunctionality (Fig. 2). Fungal diversity exhibited contrasting patterns between the two soil compartments. Fungal richness was significantly correlated with soil multifunctionality ($R^2 = 0.50$, $p < 0.01$) in bulk soil (Fig. 2), while fungal Shannon diversity was linked to decomposition and microbial productivity. Notably, fungal network complexity was negatively associated with SMF in the rhizosphere, particularly with decomposition. Compared to microbial alpha diversity and network complexity, bacterial and fungal biomass emerged as stronger predictors of individual soil functions and multifunctionality in both bulk and rhizosphere soils (Fig. 2).

The relative importance of drivers on SMF and their impacting pathways

The relative importance of soil abiotic properties and microbial attributes in driving SMF varied between the rhizosphere and bulk soil (Fig. 3). Soil abiotic properties, including soil moisture, pH, and structure, emerged as the strongest explanatory factors and explained a substantial portion of the variance in soil multifunctionality in bulk (45.6% unique variation) and rhizosphere soil (19.3% unique variation) (Fig. 3 & Figure S7). Fungal biomass was a significant predictor of SMF across both compartments (Fig. 3). Bacterial biomass was the dominant driver of SMF variations in bulk soil, and bacterial Shannon diversity was key predictor of SMF in bulk soil (Fig. 3). Compared to fungi, bacterial attributes were a weaker predictor in explaining soil multifunctionality, particularly in the rhizosphere (Figure S7). The PL-SEM model analysis was conducted to explore the direct and indirect processes by which regulatory factors affected SMF. In line with the VPA results, microbial biomass emerged as the most influential determinant of SMF in both soil compartments (Fig. 4). In bulk soil, soil pH indirectly influenced SMF by negatively affecting bacterial and fungal biomass (Fig. 4a and c). This pattern, however, was not consistent in the rhizosphere. Soil moisture had indirect impacts on SMF by positively affecting bacterial biomass, whereas soil pH and clay structure affected SMF

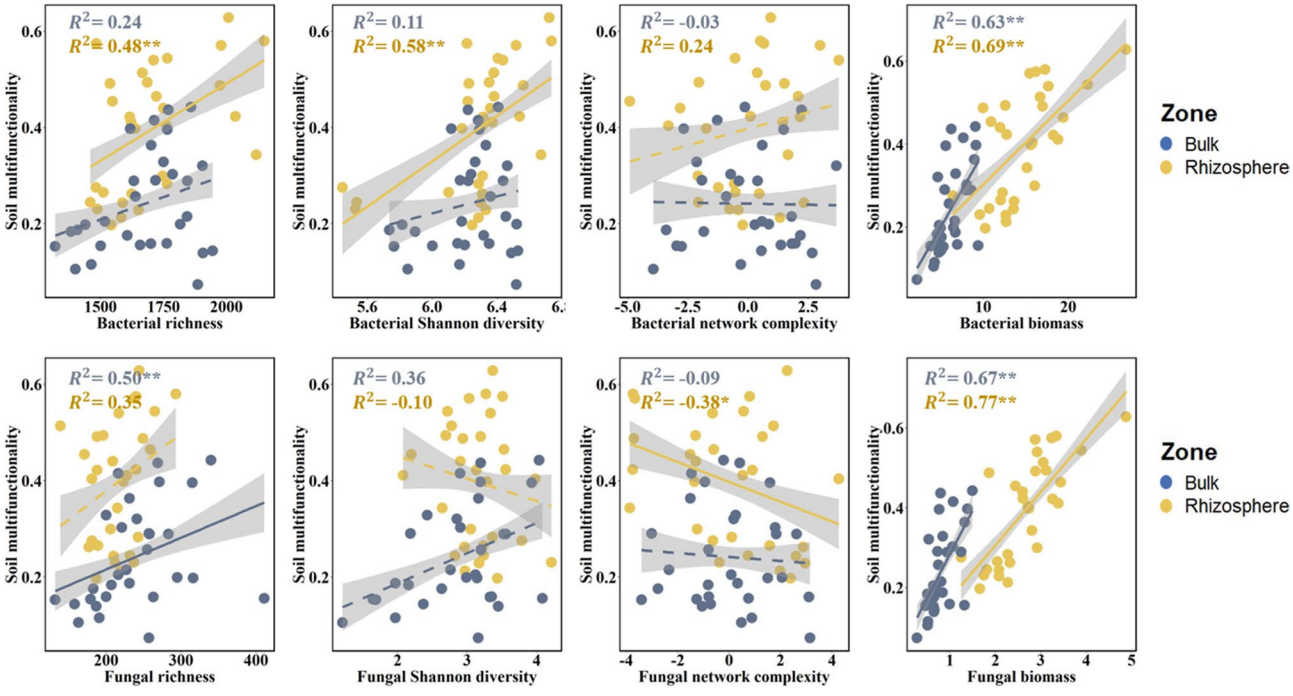

Fig. 2 Linkage of soil multifunctionality with microbial properties (alpha diversity, network complexity and microbial biomass) in the rhizosphere and bulk soil. The blue and yellow dots denote bulk soil ($n = 30$) and rhizosphere soil ($n = 30$), respectively. Solid lines are fitted by ordinary least-squares regressions, and the shadow areas correspond to 95% confidence intervals. *$p < 0.05$; **$p < 0.01$

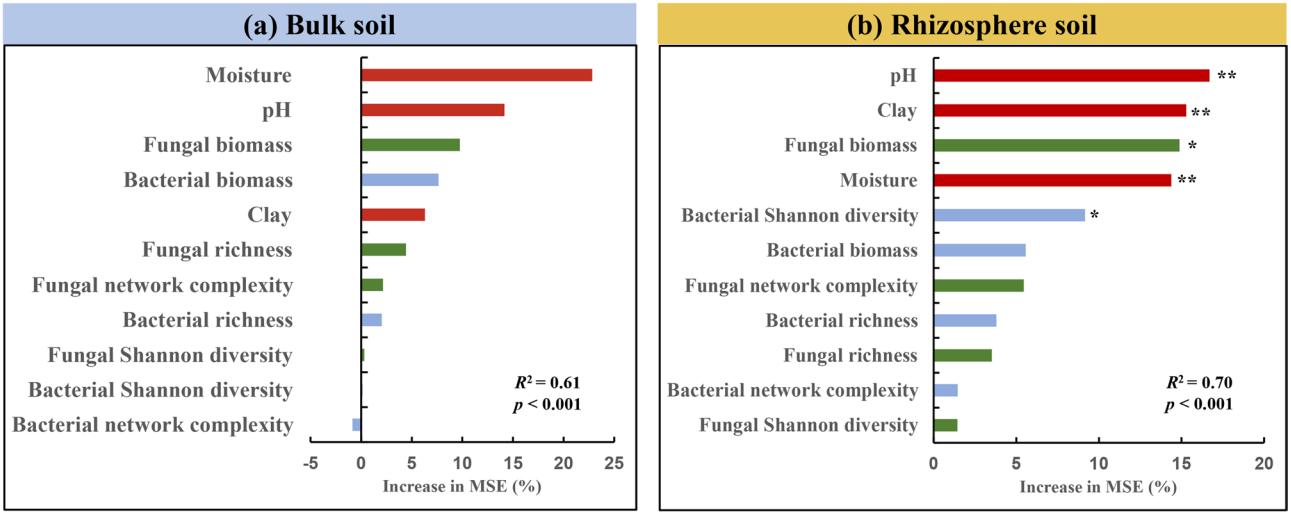

Fig. 3 Random forests showing mean predictor importance of soil abiotic properties (red bars), bacterial attributes (blue bars) and fungal attributes (red bars) on soil multifunctionality. Significance levels of each predictor are: * $p < 0.05$ and ** $p < 0.01$

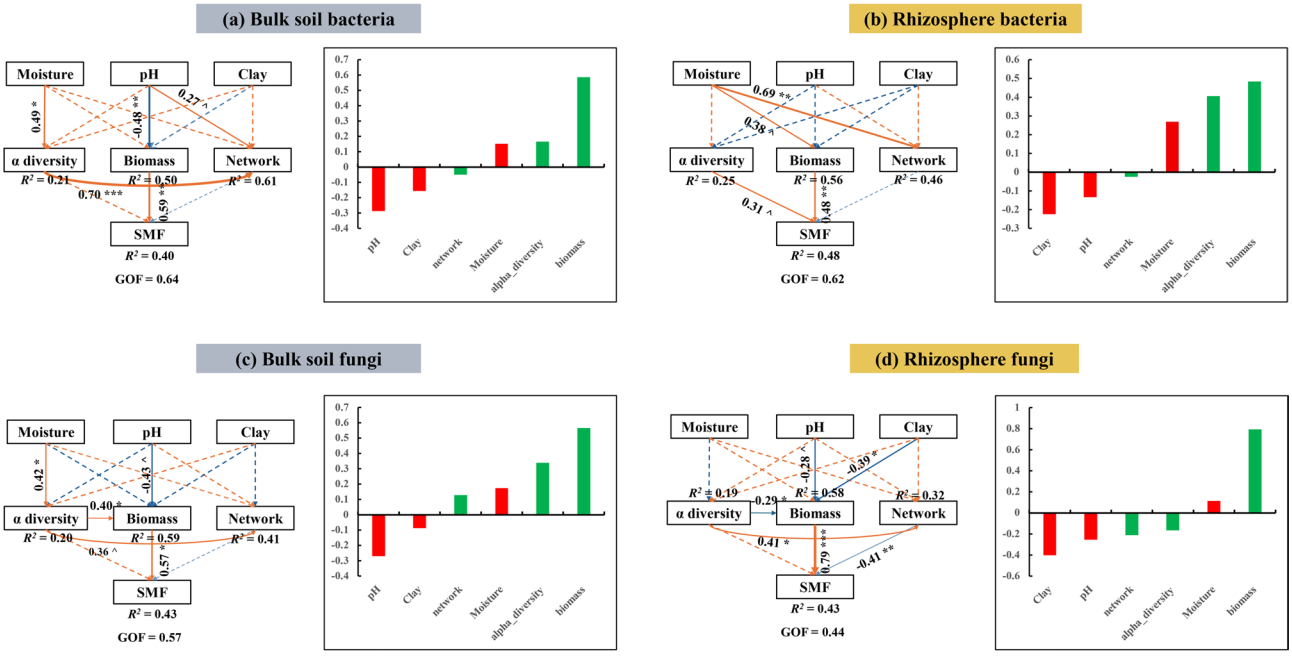

Fig. 4 Direct and indirect effects of MAT, soil abiotic properties on soil multifunctionality by regulating bacterial (**a**, **c**) and fungal (**b**, **d**) properties in the bulk soil and rhizosphere. Standardized effects of predictors sourced from models on SMF were displayed. Models were analyzed using goodness of fit (GOF) statistics. SMF: soil multifunctionality. Significant effects ($p < 0.05$) are plotted by solid lines, and nonsignificant effects are plotted by dash lines. Arrow thickness represents the strength of the relationships. The red and blue arrows indicate positive and negative flows of causality, respectively. R^2 indicates the proportion of variance explained

by negatively affecting fungal biomass in the rhizosphere (Fig. 4d).

Discussion

Soil multifunctionality varies in the rhizosphere and bulk soil

We observed that the rhizosphere generally exhibited higher soil multifunctionality than bulk soil, supporting our first hypothesis (Fig. 1). The higher soil multifunctionality in rhizosphere is likely driven by intensified biological and biochemical processes associated with root activity [9, 11], as also reflected in the elevated levels of individual soil functions (Figure S3). The rhizosphere is enriched with root exudates, such as sugars, amino acids, and organic acids that serve as readily available substrates, stimulating microbial biomass, activity [11, 44, 45]. This leads to enhanced microbial metabolism and higher abundance of functional genes related

to key ecosystem processes, including nitrogen cycling, phosphorus mobilization, and carbon decomposition [20, 46]. In addition, the rhizosphere's high spatial and temporal variability creates diverse microhabitats that promote microbial niche differentiation and functional complementarity [47, 48]. These synergistic effects collectively enhance multiple ecosystem functions, distinguishing the rhizosphere as a hotspot for soil multifunctionality.

Soil multifunctionality, and individual functions, showed inconsistent responses along the elevation gradient, suggesting that the variation in soil multifunctionality along the gradient may not necessarily adhere to a linear pattern (Fig. 1). This is not in line with previous studies that reported either linearly increasing or decreasing patterns in multifunctionality along an elevation gradient [22, 38]. One possible explanation lies in the specific elevational range examined in our study. Limited elevational coverage may have missed broader ecological transitions, which could explain the absence of a clear directional pattern [22, 49]. A recent study across alpine grasslands of the Tibetan Plateau found that ecosystem multifunctionality initially increased and then decreased significantly with increasing elevation with a changing point occurring at 3,900 m [50]. Similarly, we observed changepoints at 3,900 m and 4,200 m, associated with significant transitions in soil multifunctionality and individual functions, such as nutrient cycling and decomposition (Figure S3). These findings highlight the importance of examining broader elevation gradients to better capture the complex and non-linear dynamics of ecosystem functioning in mountainous environments.

Compartment-specific relationship between soil microbial diversity and SMF

Soil bacterial alpha diversity showed positive relationships with soil functions in bulk soil and rhizosphere (Fig. 2). Bacterial diversity–function relationships were more pronounced in the rhizosphere compared to bulk soil. This difference is probably due to the adaptive life strategies characteristic of bacteria. Bacteria generally exhibit great metabolic flexibility [19], which allows them to rapidly respond to the dynamic nutrient environment of the rhizosphere by exploiting labile carbon and other exudate-derived resources [11, 51]. As a result, bacterial diversity is directly linked to key soil functions, particularly those involved in nitrogen and carbon cycling (Figure S6). By contrast, we observed weaker correlations between fungal diversity and SMF in the rhizosphere. Fungi, especially saprotrophic fungi, are generally less competitive for labile carbon but highly efficient at decomposing complex polymers (e.g., lignin, cellulose) in bulk soil [52]. This may explain the stronger association between saprotrophic fungal diversity and SMF in bulk soil (Figure S8). Furthermore, fungi in bulk soil are less directly influenced by root-derived inputs and instead rely on extensive hyphal networks and mycelial growth to access spatially dispersed resources [53], which may promote higher diversity and enhances their contributions to soil microbial functioning. Future research should aim to disentangle the roles of specific microbial groups across soil compartments and under varying environmental conditions, ultimately advancing our understanding of how soil biodiversity supports ecosystem functioning and informing targeted conservation strategies [11].

Relative role of bacterial and fungal properties in driving soil multifunctionality across soil compartments

Random forest and variation partitioning analysis revealed fungi, rather than bacteria, play a more important role in determining SMF in the bulk soil and rhizosphere (Fig. 3, Figure S7), which partly supported our second hypothesis. This finding align with recent studies demonstrating the more dominant role of fungal communities in driving soil functions in alpine forest ecosystems [54, 55]. Alpine forests are generally characterized by low temperatures, short growing seasons and nutrient-poor soils, where soil fungi, particularly mycorrhizal and saprotrophic guilds, play crucial roles in driving multifunctionality [56, 57]. For instance, saprotrophic fungi can decompose accumulated recalcitrant organic matter, whereas bacteria are predominantly linked to the degradation of readily decomposable substrates [58] and they are expected to be secondary decomposers by utilizing dead fungal biomass [59]. Moreover, the widespread symbiotic interactions between mycorrhizal fungi and alpine plants allow the fungi to more efficiently obtain nutrients, which is crucial in the nutrient-deficient soils of alpine regions [60].

Notably, we also observed that both fungal and bacterial biomass, rather than alpha diversity and community composition, were more important in contributing to SMF in the rhizosphere and bulk soil. These results suggest that in high-stress environments, such as those found in alpine forest, ecosystem functioning maybe more closely tied to the quantity of active microbial communities and prevailing abiotic conditions than to the counted number of species or diversity [61]. One potential explanation is the high degree of functional redundancy in soil microbial communities, where multiple taxa can perform similar ecological roles [62]. The loss of specific groups may have little or no effect on ecosystem functioning because other groups can take their place [61, 63]. As a result, increases in the alpha diversity may not necessarily translate to greater ecosystem functioning [64]. By contrast, microbial community structure and biomass reflects the active pool driving key processes like nutrient cycling and organic matter decomposition [65, 66]. Although previous studies have reported broadly

positive effects of microbial network complexity on ecosystem multifunctionality [38, 67], we found limited evidence for such relationships across soil compartments. However, when analyzing specific functions, we observed that the complexity of bacterial and fungal co-occurrence networks was significantly correlated with decomposition processes in the rhizosphere, especially those related to carbon cycling (Figure S6). This pattern underscores the ecological importance of microbial interactions in the rhizosphere, where root exudation and organic inputs likely foster cooperative or complementary metabolic interactions among microbial taxa [68, 69]. Taken together, these microbial attributes may therefore serve as more reliable indicators of functional potential, particularly in dynamic or stressful environments. To enhance predictions of microbial contributions to SMF, future research should move beyond traditional diversity metrics and integrate a broader range of microbial attributes.

The dependence of soil multifunctionality on soil microbial properties was driven by soil edaphic properties

In line with our third hypothesis, the elevation-induced variations in soil properties, particularly soil pH, moisture and clay content, were indirectly related to SMF through their associations with soil microbial properties (Fig. 4). This finding aligns with recent studies in alpine forests, which have identified pH and moisture as critical regulators of SMF by shaping microbial communities [22, 70, 71]. Interestingly, however, our data revealed a diminished effect of soil pH on microbial properties in rhizosphere soils compared to bulk soils, suggesting that the rhizosphere as a distinct microbial niche that is buffered against broader edaphic constraints. This discrepancy could be attributed to the distinct microenvironment created by root activity [72]. Root exudates provide a pulse of readily utilizable carbon and nutrients that selects for copiotrophic, fast-growing taxa [73], whose competitive success may depend more on resource availability than on pH. In addition, these exudate-driven processes also create highly dynamic conditions in the rhizosphere, where pH and other chemical properties fluctuate at fine scales [74]. As a result, rhizosphere microbes are often more adapted to rapid shifts in environmental conditions [75], potentially making pH a comparatively weak predictor of rhizosphere microbial structure and function.

Conclusions

Taking a rhizosphere perspective, we empirically assessed soil microbial communities and their roles in driving soil multifunctionality (SMF) in the rhizosphere and bulk soils of *Abies georgei* in an alpine forest. Our results show that the rhizosphere consistently supports higher SMF than bulk soil, regardless of environmental variation. The relationship between microbial diversity and SMF is compartment-specific. Bacteria diversity is associated with SMF in the rhizosphere, whereas fungal diversity is closely linked to SMF in the bulk soil. Microbial biomass, particularly fungal biomass, had a strong influence on SMF in both rhizosphere and bulk soils. Furthermore, the linkages between soil microbial properties and SMF are mediated by different abiotic factors in the rhizosphere and bulk soil, soil pH in the bulk soil, and soil moisture and clay content in the rhizosphere. Together, our study provides novel insights into how the coupling between microbial drivers and soil functions shifts across soil compartments and highlights the importance of incorporating a rhizosphere perspective into soil biodiversity–function research.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-07637-w.

Supplementary Material 1.

Authors' contributions

C. G. and H. Y. conceived the ideas and designed methodology; S. D. and D. W. collected the data; C. G. analyzed the data; C. G. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Funding

The study was financially supported by the National Natural Science Foundation of China (U23A2051, 32171757, 32201529, 32501448), the Sichuan Science and Technology Program (2023NSFSC0012, 2023NSFSC1192), the Science and technology program of Tibet Autonomous Region (XZ202301YD0028C, XZ202301ZR0047G) and the Open Research Fund from the Key Laboratory of Forest Ecology in Tibet Plateau (Tibet Agriculture & Animal Husbandry University), Ministry of Education of China (XZAJYBSYS-202403).

Data availability

All gene sequences were deposited in the BIG Data Center (https://ngdc.cncb.ac.cn/gsa/), Beijing Institute of Genomic (BIG), Chinese Academy of Science, under the accession number CRA021432.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals. The collection materials of the plants complies the relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 7 August 2025 / Accepted: 23 October 2025
Published online: 19 November 2025

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