

RESEARCH ARTICLE

Different facets of bacterial and fungal communities drive soil multifunctionality in grasslands spanning a 3500 km transect

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Abstract

1. Soil microbial communities are essential in regulating ecosystem functions and services. However, the importance of bacterial and fungal communities as predictors of multiple soil functions (i.e. soil multifunctionality) in grassland ecosystems has not been studied systematically.
2. Here, we measured soil microbial diversity, community composition, biomass and multiple soil functions of 41 sites in five grassland ecosystems spanning a 3500 km northeast-southwest transect. The random forest algorithm was adopted to determine the importance of geographical location, climatic, altitude, edaphic, plant and microbial predictors in driving a proxy of soil multifunctionality (seven soil functions in this study). Moreover, structural equation models were employed to examine the direct and indirect effects of those predictors on soil multifunctionality.
3. Our results demonstrated that soil multifunctionality was positively driven by soil fungal diversity but not by bacterial diversity. Fungal phylogenetic diversity (presence of different evolutionary lineages) showed stronger positive relationships with soil multifunctionality than taxonomic diversity (richness of species). Dominant bacterial taxa, particularly of phyla Actinobacteria and Proteobacteria, were positively associated with soil multifunctionality, while none of the fungal taxa were found to regulate soil multifunctionality. Furthermore, both fungal and bacterial biomass had significant effects on soil multifunctionality, while the effect of microbial biomass was weaker than that of fungal diversity and bacterial taxa. Importantly, the direct positive effects of soil fungal diversity, dominant bacterial taxa, and fungal and bacterial biomass were maintained after accounting for multiple predictors in grassland ecosystems.
4. This study provided strong empirical evidence that soil multifunctionality was driven by different facets of the bacterial and fungal communities in the grassland ecosystems. Our results also highlighted that any loss of fungal diversity, dominant bacterial taxa and microbial biomass might reduce soil multifunctionality,

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exacerbating ecosystem functions and services such as soil fertility, primary production and climate mitigation in grassland ecosystems.

KEYWORDS

bacteria, fungi, grassland, microbial diversity, multifunctionality, Tibetan Plateau

1 | INTRODUCTION

The worldwide loss of biodiversity has stimulated research into biodiversity and its implication for sustaining ecosystem functions and services (IPBES, 2019; Pörtner et al., 2021). Biodiversity is a complex term that involves a multitude of metrics, including species diversity and community composition (i.e. the relative abundance of organisms in a community). Most previous studies have focused on the effects of plant diversity and community composition on multifunctionality (simultaneously providing multiple ecosystem functions and services) in terrestrial ecosystems (Isbell et al., 2011; Le Bagousse-Pinguet et al., 2019; Zavaleta et al., 2010). In contrast, soil microbial communities, which have not been fully characterized (Wagg et al., 2014), contain the most diverse, ubiquitous and abundant organisms on Earth (Locey & Lennon, 2016). Recent studies have shown positive relationships between microbial diversity (and specific bacterial taxa) and ecosystem multifunctionality, including soil organic matter (SOM) decomposition, biogeochemical cycles and climate regulation (Bender et al., 2016; Chen et al., 2020; Delgado-Baquerizo et al., 2016, 2017). By contrast, bacteria and fungi differentially contribute to C and nutrient cycles (He et al., 2020; Rousk & Bååth, 2007; Six et al., 2006); however, a complete distinguishing the roles of bacterial and fungal communities (diversity, composition and biomass) in regulating soil multifunctionality in terrestrial ecosystems remains under appreciated.

Previous experimental and observational studies of microbial taxonomic diversity (richness and abundance of species) and multiple ecosystem functions in natural and manipulated ecosystems provided insights to broaden our understanding of microbial regulation of ecosystem functions and services (Delgado-Baquerizo et al., 2016; Jing et al., 2015; Torsvik & Øvreås, 2002). A recent regional-scale observational study demonstrated a positive relationship between bacterial diversity and ecosystem multifunctionality in the Tibetan Plateau (Jing et al., 2015). Furthermore, Delgado-Baquerizo et al. (2016) reported that microbial diversity, especially fungal diversity is as important as or more important than climate, soil properties and plant diversity in controlling ecosystem multifunctionality across global drylands. Thus, the association between soil microbial diversity and multifunctionality depends on the ecosystem and the geographic scale (Yang et al., 2017). Although taxonomic diversity has been commonly used to characterize microbial diversity (De Vries & Shade, 2013; Jing et al., 2015), several recent studies have investigated phylogenetic

diversity (presence of different evolutionary lineages) as an essential predictor of soil functions at local and regional scales, since a common evolutionary history defines shared functional abilities (Wang et al., 2021). Therefore, consideration of phylogenetic diversity would provide more insights into how microbial diversity influences soil multifunctionality.

Unlike soil microbial diversity, we have limited knowledge of the effects of microbial community composition (i.e. fungal and bacterial taxa abundance) and biomass (i.e. fungal and bacterial biomass) on soil multifunctionality in terrestrial environments. Recently, a study showed that globally dominant bacterial taxa from the phyla Proteobacteria, Bacteroidetes and Actinobacteria are important predictors of soil multifunctionality in the field and microcosm experiments in drylands (Delgado-Baquerizo et al., 2016). Furthermore, Wang et al. (2022) demonstrated that members of the fungal phyla Ascomycota, Basidiomycota and Glomeromycota are potentially essential in regulating nutrient cycling and SOM decomposition and formation in temperate grasslands. In contrast, a recent experimental study has revealed that the positive effect of plant species diversity on microbial respiration was mostly driven by enhanced microbial biomass and indicated that microbial biomass was more important than diversity in controlling soil C dynamics in a forest ecosystem (Beugnon et al., 2021). However, these studies have not comprehensively investigated how bacterial and fungal composition and biomass regulate soil multifunctionality. This hampers predictions of soil multifunctionality under ongoing anthropogenic activities and climate changes and impedes the formulation of conservation and sustainable management policies.

As one of the most widespread vegetation types, grasslands account for 46% of the world's terrestrial surface (Ni, 2004). Chinese grasslands, the third largest in the world, cover nearly one-fourth of Chinese territory. Chinese grasslands mainly include temperate grasslands and alpine grasslands (approximately 80%; Ni, 2002), which are in arid and semi-arid regions and the Tibetan Plateau, respectively. During the past half-century, approximately 80%–90% of Chinese grasslands have been degraded due to anthropogenic activities and climate change (Lu et al., 2006). These changes potentially threaten the above-ground and below-ground biodiversity in natural grassland ecosystems (Bardgett et al., 2021). Hence, evaluating the roles of microbial diversity and community composition in regulating soil multifunctionality in grassland ecosystems is of significance for predicting the dynamics of terrestrial ecosystems.

Here, we assess how geographical location, altitude, climatic, edaphic, plant and microbial predictors regulate a proxy of soil multifunctionality, including variables related to soil C and nutrient stocks and cycling, at 41 sites spanning a 3500 km transect in temperate and alpine grasslands of China (Figure 1). We hypothesized that (i) soil bacterial and fungal diversity are both important predictors of variation in soil multifunctionality based on theoretical frameworks predicting that complex soil processes require diverse microbial interactions (Schimel et al., 2005). In addition, soil fungal diversity has higher linkages with soil multifunctionality compared with bacterial diversity as fungal community plays a more important role in priming effect, SOM dynamics and N mineralization than bacterial community (Fontaine et al., 2011). (ii) Soil bacterial and fungal community composition regulates soil multifunctionality in grassland ecosystems given that different microbial taxa yield distinguishing impacts on soil processes such as SOM decomposition according to previous studies with whole genome data (Romaní et al., 2006; Trivedi et al., 2013). (iii) Soil bacterial and fungal biomass are dominant drivers of soil multifunctionality, even stronger than microbial diversity, based on the fact that microbial biomass regulates C and nutrient cycles and is essential in the supply and conversion of nutrient in terrestrial ecosystems (Berg & Smalla, 2009; Beugnon et al., 2021).

2 | MATERIALS AND METHODS

2.1 | Study sites

Field data were collected from 41 sites across a northeast–southwest grassland transect across the temperate and alpine grassland regions in China (Figure 1; Figure S1). The transect is approximately 3500 km long, spanning longitudes from 92.1°E to 122.5°E and latitudes from 29.7°N to 49.5°N. The climate of the transect shows a strong temperature seasonality and an apparent precipitation gradient (Table S1). The altitude ranges from 157 to 5418 m, the mean annual precipitation ranges from 147 to 472 mm and the mean annual temperature ranges from −4.0 to 7.8°C (1980–2010; <http://data.cma.cn>). The temperate grasslands have a continental, dry climate and the alpine grasslands have a continental, dry and cold climate. The aridity of each site was calculated as $1 - \text{aridity index}$ (AI; Hu et al., 2021). The AI (defined as the ratio of precipitation to potential evapotranspiration) was obtained from the Global Aridity Index and PET database (<https://cgiarcsi.community/>).

Natural grassland types across the transect mainly include temperate meadow steppe (dominated by *Stipa baicalensis* Roshev. and *Leymus chinensis* (Trin.) Tzvel.), temperate typical steppe (dominated by *Stipa grandis* P.A. Smirn., *Leymus chinensis* (Trin.) Tzvel. and *Stipa krylovii* Roshev.), temperate desert steppe (dominated by *Stipa*

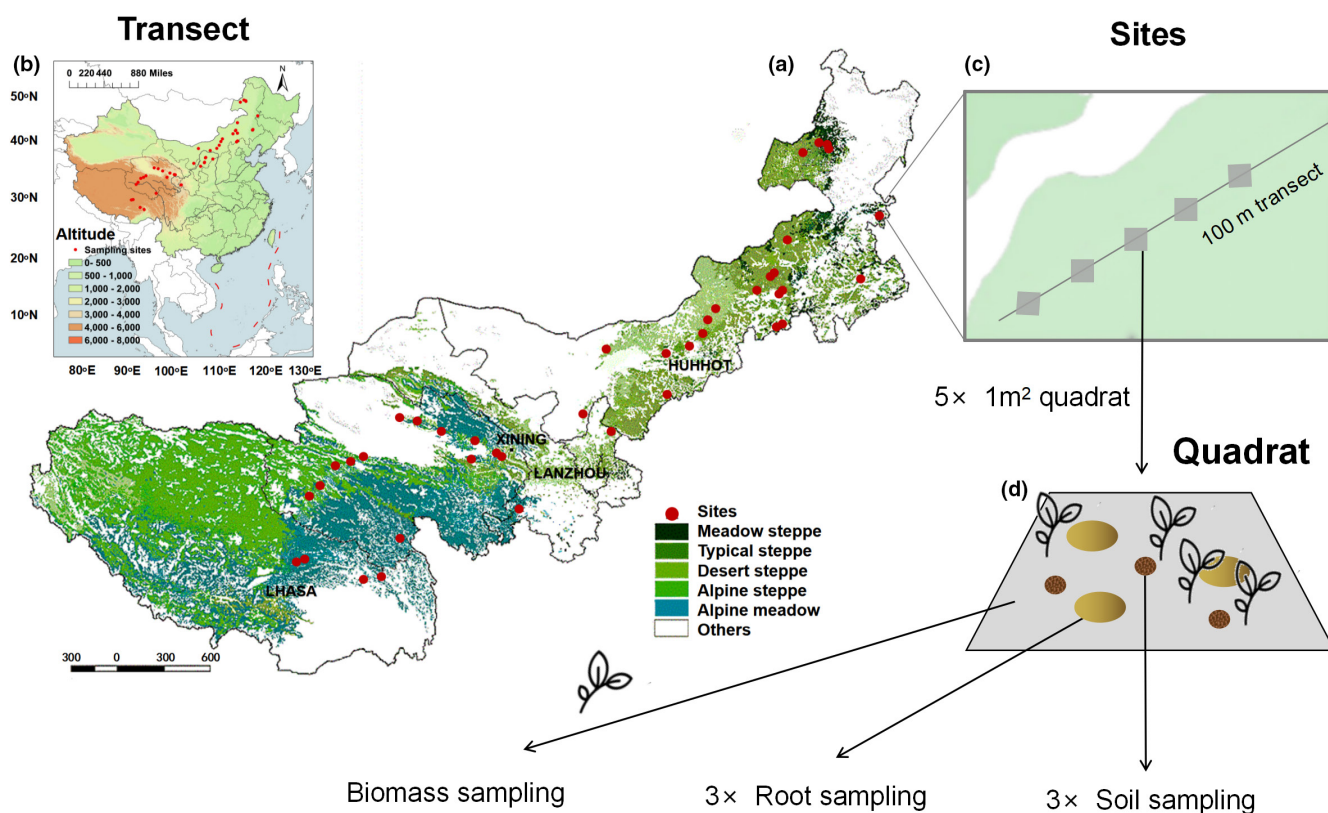


FIGURE 1 Transect geographical distribution (a, b) and sampling design of field sites and a quadrat (c, d) in grassland ecosystems in China. The 41 sites represent five typical grassland ecosystems (i.e. meadow steppe, typical steppe, desert steppe, alpine meadow and alpine steppe).

klemenzi Roshev. Norl. and *Stipa breviflora* Griseb.), alpine meadow (dominated by *Kobresia pygmaea* C.B. Clarke and *Kobresia tibetica* Maxim.) and alpine steppe (dominated by *Stipa purpurea* Griseb. and *Festuca ovina* Linn.) across Inner Mongolia, Ningxia and Gansu provinces and the Tibetan Plateau of China (Ma et al., 2010). According to the Genetic Soil Classification of China, soils can be classified as brown pedocals, castanozems and chernozems, Leptosols, Gleysols, Cambisols and Phaeozems.

2.2 | Sampling and processing

We collected a total of 205 soil samples from the 41 sites (five samples per site; each site was visited once over this period) from late July to mid-August during the peak growing season in 2018 and 2020, respectively. The distances between adjacent sites were approximately 50–80 km. The selected sites varied remarkably in soil properties (Table S1) and covered major grassland types including meadow steppe (4 sites), typical steppe (8 sites), desert steppe (11 sites), alpine meadow (10 sites) and alpine steppe (8 sites). All the field samplings were licensed by local township governments. At each site, we established a 100-m transect and randomly placed five quadrats (1 × 1 m²), with the stipulation that the quadrats were at least 15-m apart. Within each quadrat, three soil cores (5 cm in diameter) were collected at 15 cm depth, bulked and homogenized in the field, and then immediately preserved at 4°C in a cooler to be transported to the laboratory within 7 days. The fresh soil samples were manually screened with any visible roots removed; the soils were then processed using a 2-mm mesh sieve. Soil subsamples for soil organic C, total N, total P and pH analyses were air-dried and ground into a fine powder. Subsamples for soil dissolved organic C, available P and phosphatase activity analyses were immediately stored at –80°C until processing (<7 days before measurements).

The plant richness was recorded as the occurrence of the number of species in each quadrat. To estimate plant productivity, all vascular plants were harvested in each quadrat to measure standing aboveground biomass. Root biomass was measured by soil coring sampling to a depth of 30 cm using a cylindrical root sampler (10 cm inner diameter; 3 soil cores per quadrat). All samples of plant tissues were oven-dried at 65°C to achieve constant weight.

2.3 | Measurements of soil physicochemical properties and functions

Soil pH was determined by a pH meter (PB-10). Soil clay content was determined by an optical size analyser (Mastersizer 2000). Soil organic C was determined using a total organic carbon analyser (Analytik Jena Multi N/C 3100). Soil total N was determined using an elemental analyser (Elementar GmbH). Soil samples were digested in a HClO₄–HNO₃–HF mixture and the digested solution was analysed for soil total P by inductively coupled plasma atomic absorption spectrometry (ICAP6300). Soil available P was measured following

a 0.5 M NaHCO₃ extraction and assayed spectrophotometrically (UV-2550). Soil phosphatase activity was estimated by the phenol release after incubation of samples with p-nitrophenyl phosphate (0.5%) for 1 h at 37°C (Tabatabai, 1994). Soil inorganic N was measured with a flow injection auto-analyser (FIAstar 5000 Analyser). The potential N mineralization rate was estimated as the difference between initial and final inorganic N levels before and after 7-day incubations at 25°C (Allen, 1989). Detailed information on the soil properties is presented in Figure S4.

2.4 | Assessing microbial diversity, community composition and biomass

Soil DNA was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories). The purity and quality of the genomic DNA were checked on 0.8% agarose gels. The extracted DNA was analysed using the Illumina MiSeq PE300 platform (Illumina). To assess soil bacterial and fungal diversity and composition, the V3–4 hypervariable regions of the bacterial 16S rRNA gene were amplified with the primers 806R (5'-GGACTACHVGGGTWTCTAAT-3') and 338F (5'-ACTCCTACGGGAGGCAGCAG-3'). The region of fungal ITS was amplified with the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-TGCCTTCTTCATCGATGC-3'; Caporaso et al., 2012).

For each soil sample, a 10-digit barcode sequence was added to the 5' end of the forward and reverse primers. PCR was performed on a Mastercycler Gradient (Eppendorf). PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN), quantified using real-time PCR, and sequenced at Allwegene Company. More details of the PCR process were described by Wang et al. (2022).

The raw data were first screened, and sequences were removed if they had a low-quality score (≤20), contained ambiguous bases, were shorter than 200 bp, or did not precisely match primer sequences and barcode tags. Then the dataset was assessed by QIIME (version 1.9.0). The sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97% to analyse the microbial diversity indices (Edgar, 2013). The Ribosomal Database Project Classifier tool was applied to categorize all sequences into different taxonomic groups (Cole et al., 2009). Low-abundance OTUs (fewer than two reads) were removed from the subsequent analyses. To assess the soil bacterial and fungal diversity at the same sequencing depth, datasets of OTUs were subsampled to 13,182 sequences for bacteria and 17,642 sequences for fungi. The number of OTUs in the soil samples represented the soil microbial taxonomic diversity (Wang et al., 2022). We also calculated the bacterial and fungal phylogenetic diversity for temperate and alpine grasslands. Representative sequences from each OTU were aligned using PyNAST and filtered to remove uninformative regions (Caporaso et al., 2010). The phylogenetic diversity was calculated using Faith and Baker's metric according to the total branch length of the tree (Faith & Baker, 2006).

We used microbial taxonomic phyla to assess the effects of microbial composition on soil multifunctionality because (i) microbial

functional potential has become increasingly available according to taxonomy (Trivedi et al., 2013); (ii) high microbial taxonomic ranks have been broadly recommended to predict ecosystem functions (Delgado-Baquerizo et al., 2017); and (iii) dominant microbial phyla are widely distributed across grassland ecosystems (e.g. Wang et al., 2022; Figures S2 and S3).

Microbial biomass was measured using phospholipid fatty acids (PLFAs) analysis (Bossio & Scow, 1998). Briefly, PLFAs were extracted from 8.0 g soil subsamples. Separation and identification of the PLFAs were performed using a gas chromatograph (Agilent 6850, Hewlett-Packard). The fatty acids a13: 0, i14: 0, i15: 0, i16: 0, i17: 0, a17: 0, 16: 1 ω 7c, 17: 1 ω 8c, 18: 1 ω 5c, 18: 1 ω 9t, 17: 0cy and 19: 0cy were chosen to represent the bacterial group, and two fatty acids (18: 1 ω 9c and 18: 2 ω 6, 9c) were chosen to represent the fungal group (Zelles et al., 1997). The bacteria:fungi biomass ratio was calculated as the ratio of the sum of all bacterial PLFAs to the sum of all fungal PLFAs.

2.5 | Assessing soil multifunctionality

We assessed soil multifunctionality using seven variables that provide a balanced and comprehensive evaluation of soil C, N and P cycling and sequestration: soil organic C, dissolved organic C, potential N mineralization rate, total N, total P, available P and phosphatase activity (Figure S5; Table S2). These variables are the most widely used indicators for soil multifunctionality studies and act as important determinants of soil functions in drylands or grasslands (Garland et al., 2021; Le Bagousse-Pinguet et al., 2019; Maestre et al., 2016). These variables reflect multiple soil functions involving soil C sequestration, soil fertility and nutrient cycling, which regulate and support ecosystem services (Hu et al., 2021). Soil organic C and dissolved organic C are often used as good indicators of C sequestration. Soil total N and P are build-ups of soil nutrient pools that most frequently limit the plant and microbial biomass, and ultimately production, fibre, food and climate regulation in grassland ecosystems (Jing et al., 2015). The potential N mineralization is the critical process through which organic N converts inorganic N (Delgado-Baquerizo et al., 2016). Soil phosphatase activity enables the mineralization of organic P to increase P availability for both plants and soil organisms (Margalef et al., 2017). Soil available P is the fraction of the soil P pool produced by the microbial mineralization process, and that is more readily available for microbial and plant growth (Canfield et al., 2010).

We assessed potential trade-off effects among multiple soil functions by calculating Pearson's correlation coefficients between each pair of single soil functions. Among the 21 combinations, we found 16 significant positive correlations, and none presented a significant negative correlation (Figure S6), indicating no trade-off effects among them. Moreover, only one combination (i.e. soil total N vs. available P) had *R* values higher than 0.5, suggesting that the functional redundancy was very low.

We used three complementary approaches to evaluate soil multifunctionality: single-function, averaging and multiple-threshold approaches, all of which are commonly applied to assess multifunctionality (Delgado-Baquerizo et al., 2016; Hu et al., 2021). All selected single soil functions significantly and positively correlated with the soil multifunctionality index (Figure S6). For the averaging approach, we normalized and standardized each soil function using the Z-score transformation, and the standardized soil functions were then averaged to acquire a soil multifunctionality index (Hu et al., 2021). This index is widely utilized in multifunctionality studies (Le Bagousse-Pinguet et al., 2019; Lefcheck et al., 2015). However, the averaging approach does not consider potential trade-off effects among the single functions and number of functions. To address these limitations, a multiple-threshold approach was employed to assess whether multiple soil functions are simultaneously performed at high-performance levels (Byrnes et al., 2014; Figures S7–S10). In this approach, each soil function is standardized utilizing the top 5% values of all sites. We considered thresholds from 1 to 99% at 1% intervals, where each threshold reflects a level of functional performance (Delgado-Baquerizo et al., 2016). In contrast, the relationships between microbial diversity (and microbial taxa) and soil multifunctionality obtained using both the single-function and multiple-threshold approaches were comparable to those assessed by the averaging approach. Therefore, this study used the averaged multifunctionality index as the soil multifunctionality index. The multiple-threshold approach also provided evidence that the highest number of maximized soil functions is the same as the number of functions measured (seven, Figures S7 and S9), which suggests that there is no trade-off effect between the soil functions selected in this study.

2.6 | Statistical analyses

To acquire a quantitative soil multifunctionality index for each site, we first standardized each soil function (soil organic C, dissolved organic C, total N, potential N mineralization rate, total P, available P, phosphatase activity) using the Z-score transformation, and then the standardized soil functions were averaged to obtain a multifunctionality index. Linear and quadratic regression fitting regressions were utilized to model the relationships between soil multifunctionality and microbial predictors. The general guideline was to first use linear fitting if the *t*-value was not significantly different between the linear and quadratic fitting. The normality of residuals (obtained from the linear regression models) was tested with the Shapiro–Wilk test ($\log_{10}$ -transformed when necessary; Shapiro & Wilk, 1965; Figures S11 and S12).

We used the random forest algorithm (Liaw & Wiener, 2002) to assess and rank the predictors of soil multifunctionality in the grassland ecosystems: latitude, longitude, altitude, aridity, grassland type, soil clay content, soil pH, plant richness, plant productivity, bacterial diversity and taxa, and fungal diversity and taxa. Random forest is a predominantly used machine learning

algorithm (Breiman, 2001), which integrates the output of multiple decision trees to reach a single result. The algorithm is an extension of the bagging method as it adopts both feature randomness and bagging to build uncorrelated decision trees. When multiple decision trees form an ensemble in the random forest algorithm, they forecast more accurate results, especially when the decision trees are not correlated. All the decision trees are trained with the same parameters but on different training datasets. The error is evaluated internally during the training and is called the out-of-bag (OOB) error rate. The importance of each predictor is then assessed by evaluating the reduction in prediction accuracy (i.e. an increase in the mean square error between observations and OOB predictions). These analyses were performed using the `RANDOM-FOREST` package (Liaw & Wiener, 2002) of the R statistical software (v3.3.1, R Core Team, 2016).

Structural equation models (SEMs) were generated to estimate the direct and indirect effects of geographical location (latitude and longitude), altitude, soil pH, aridity, soil clay content, plant richness, productivity and microbial communities (microbial diversity and composition) on soil multifunctionality based on expectations under an a priori model, which we considered to be reasonable from possible causal relationships (Figure S13; Table S3 and S4). To improve normality, data on latitude, longitude, altitude, soil clay content, plant richness and productivity were $\log_{10}$ -transformed. The SEMs were fitted using IBM SPSS Amos 21 (Amos Development Corporation). The significance level was set at $p < 0.05$ except for the Chi-square test of model fit in the SEMs at $p > 0.05$. R codes used to perform the above analyses are available in <https://doi.org/10.5061/dryad.66t1g1k53> (Ma et al., 2022).

3 | RESULTS

3.1 | Microbial diversity and community composition

After filtering low-quality sequences, high-quality bacterial and fungal sequences were grouped into 20,879 bacterial OTUs and 8943 fungal OTUs (at 97% similarity). Fungal taxonomic and phylogenetic diversity varied 1.3- to 2-fold across all the five grassland types. The highest value appeared in the meadow steppe, and the lowest value appeared in the alpine steppe (Figure S4; $p < 0.001$). However, bacterial taxonomic and phylogenetic diversity showed no significant differences among the grasslands. The dominant soil bacterial phyla were Actinobacteria (abundance c. 15%–30%), Proteobacteria (abundance c. 15%–23%) and Acidobacteria, followed by Chloroflexi (abundance 7%–19%), Bacteroidetes (abundance 7%–9%) and Gemmatimonadetes (abundance c. 4%–7%), while Firmicutes, Verrucomicrobia and Nitrospirae were at lower abundances (cumulative relative abundance $> 85\%$, Figure S2) across the five grassland ecosystems. The dominant fungal phyla were Ascomycota (abundance c. 55%) and Mortierellomycota (abundance c. 32%), followed by Basidiomycota (abundance c. 5%), with Glomeromycota and

Chytridiomycota at lower abundances (cumulative relative abundance $> 90\%$, Figure S3).

3.2 | Microbial communities and soil multifunctionality

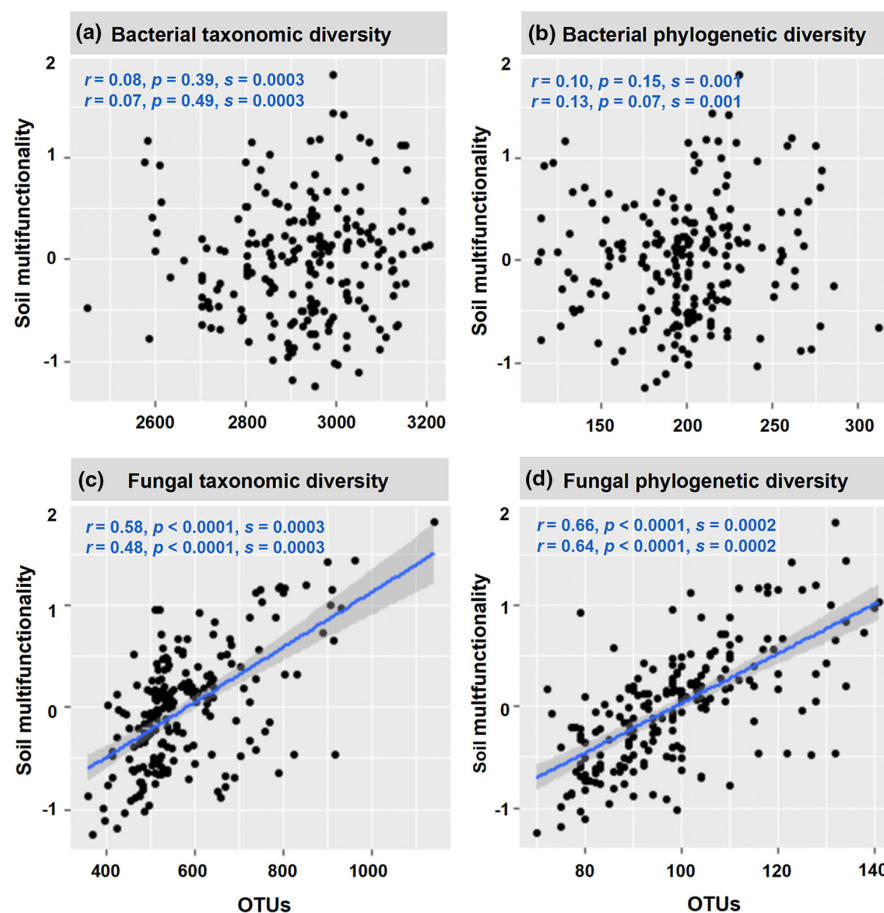
We evaluated the relationship between microbial predictors (i.e. microbial diversity, composition and biomass) and soil multifunctionality with linear and quadratic regression analyses. By contrast, the fitting results (r -value) were less invariably with the quadratic assessment than the linear assessment in each relationship (Figures 2 and 3; Figures S14 and S15). For the bacterial communities, we failed to find any significant relationship between taxonomic and phylogenetic diversity with soil multifunctionality across the grassland ecosystems (Figure 2; Figure S14a,b). We found that the phyla Actinobacteria ($r = 0.62$, $p < 0.001$), Proteobacteria ($r = 0.54$, $p < 0.001$) and bacterial biomass ($r = 0.48$, $p < 0.001$) were positively associated with soil multifunctionality, while the phyla Chloroflexi ($r = 0.44$; $p < 0.001$) and Gemmatimonadetes ($r = 0.44$; $p < 0.001$) were negatively related to soil multifunctionality (Figure 3a,b,d,f,o; Figure S15). With respect to fungal communities, fungal taxonomic diversity ($r = 0.58$; $p < 0.001$), phylogenetic diversity ($r = 0.66$, $p < 0.001$) and fungal biomass ($r = 0.48$, $p < 0.001$) were significantly correlated with soil multifunctionality, while the fungal taxa were not related (Figures 2 and 3; Figures S14 and S15). In contrast, fungal phylogenetic diversity showed a significant higher positive relationship with soil multifunctionality than taxonomic diversity ($p < 0.05$). Furthermore, we also found soil fungal taxonomic and phylogenetic diversity, phyla Actinobacteria and Proteobacteria were significant positively related to most of the individual soil functions (Figure S6).

3.3 | Controls on soil multifunctionality

We first used the random forest model to select the most important predictors (latitude, longitude, altitude, aridity, soil pH, soil clay content, plant richness, plant productivity, grassland type, bacterial and fungal diversity, bacterial and fungal taxa, bacterial and fungal biomass, and bacteria: fungi ratio) of soil multifunctionality (Figure 4). Our random forest model ($r^2 = 0.85$; $p < 0.001$) indicates that aridity, soil pH, dominant bacterial taxa (phyla Actinobacteria and Proteobacteria), fungal phylogenetic diversity and geographical location (latitude and longitude) were the most important predictors in regulating soil multifunctionality ($p < 0.001$), followed by altitude, fungal taxonomic diversity, bacterial and fungal biomass, soil clay content, plant richness and productivity ($p < 0.001$). However, soil bacterial diversity, all fungal taxa, bacteria: fungi ratio and grassland type did not show any significant correlation to soil multifunctionality across the grassland ecosystems (Figure 4).

Finally, we used SEM to test whether the relationship between fungal diversity and biomass (and dominant bacterial taxa and biomass) and soil multifunctionality was maintained when simultaneously accounting for the geographical location (latitude and longitude), aridity,

FIGURE 2 Relationships between soil bacterial taxonomic diversity (a), bacterial phylogenetic diversity (b), fungal taxonomic diversity (c), and fungal phylogenetic diversity (d) and soil multifunctionality. The shaded areas show the 95% confidence interval of the fit. In each panel, the upper r -value and s -value represent the correlation value and slope of the relationship across all data points, and the lower r -value and s -value represents correlation value and slope of the relationship without the extreme data points.



altitude, soil pH and clay content, and plant richness and productivity predictors (see a priori model in Figure S13). The two SEMs, considering either fungal diversity and biomass or dominant bacterial taxa (phyla Actinobacteria and Proteobacteria) and biomass, respectively explained 78% ($\chi^2 = 2.694$, $p = 0.260$, $df = 2$) and 80% ($\chi^2 = 2.639$, $p = 0.214$, $df = 2$) of the variance in the soil multifunctionality (Figure 5). In both SEMs, we found that geographical location, aridity, soil pH and clay content had significant direct and indirect effects on soil multifunctionality, while only altitude indirectly and negatively, through aridity and plant richness, impacted soil multifunctionality. More importantly, soil fungal diversity ($r^2 = 0.42$; $p < 0.001$), dominant bacterial taxa ($r^2 = 0.39$; $p < 0.001$), fungal biomass ($r^2 = 0.29$; $p = 0.009$) and bacterial biomass ($r^2 = 0.33$; $p = 0.034$) significantly and directly drive soil multifunctionality, whereas plant richness indirectly, through microbial communities, impacted soil multifunctionality in the grassland ecosystems (Figure 5a–d).

4 | DISCUSSION

4.1 | Fungal diversity drive soil multifunctionality

We investigated how microbial diversity influenced soil multifunctionality across five grassland ecosystems spanning a 3500 km transect. Partially contrary to our first hypothesis, the data showed that

soil multifunctionality was significantly and positively impacted by soil fungal taxonomic and phylogenetic diversity, but not by bacterial diversity (Figure 2a–d). Our result was consistent with previous experimental studies in boreal forest and agricultural ecosystems, which also demonstrated that soil fungal diversity, but not bacterial diversity, significantly affected soil multifunctionality (Li, Delgado-Baquerizo, et al., 2019; Li, He, et al., 2019). Furthermore, several recent field studies have demonstrated that soil fungal taxonomic diversity showed a stronger positive relationship with multiple ecosystem functions than bacterial diversity across a large-scale gradient in drylands (Delgado-Baquerizo et al., 2016; Hu et al., 2021), grasslands (Wang et al., 2022) and a subtropical forest ecosystem (Beugnon et al., 2021).

There are several potential explanations for this observation. First, soil fungal communities are known to be more resistant to desiccation than bacterial communities in arid and semi-arid grasslands (Ma et al., 2014). Second, fungal community plays more important roles in priming effect and SOM dynamics than bacterial community, which makes them the drivers of C and nutrient cycling (Fontaine et al., 2011). Third, microbial necromass is an important component of SOM, while the contributions of fungal necromass accumulation and use efficiency are higher than that of bacteria in the grassland ecosystems (Buckeridge et al., 2020; Miltner et al., 2012). These mechanisms suggest that multiple soil functions have stronger association with soil fungal diversity than with bacterial diversity. Consequently, our result is in favour of the view that soil fungal

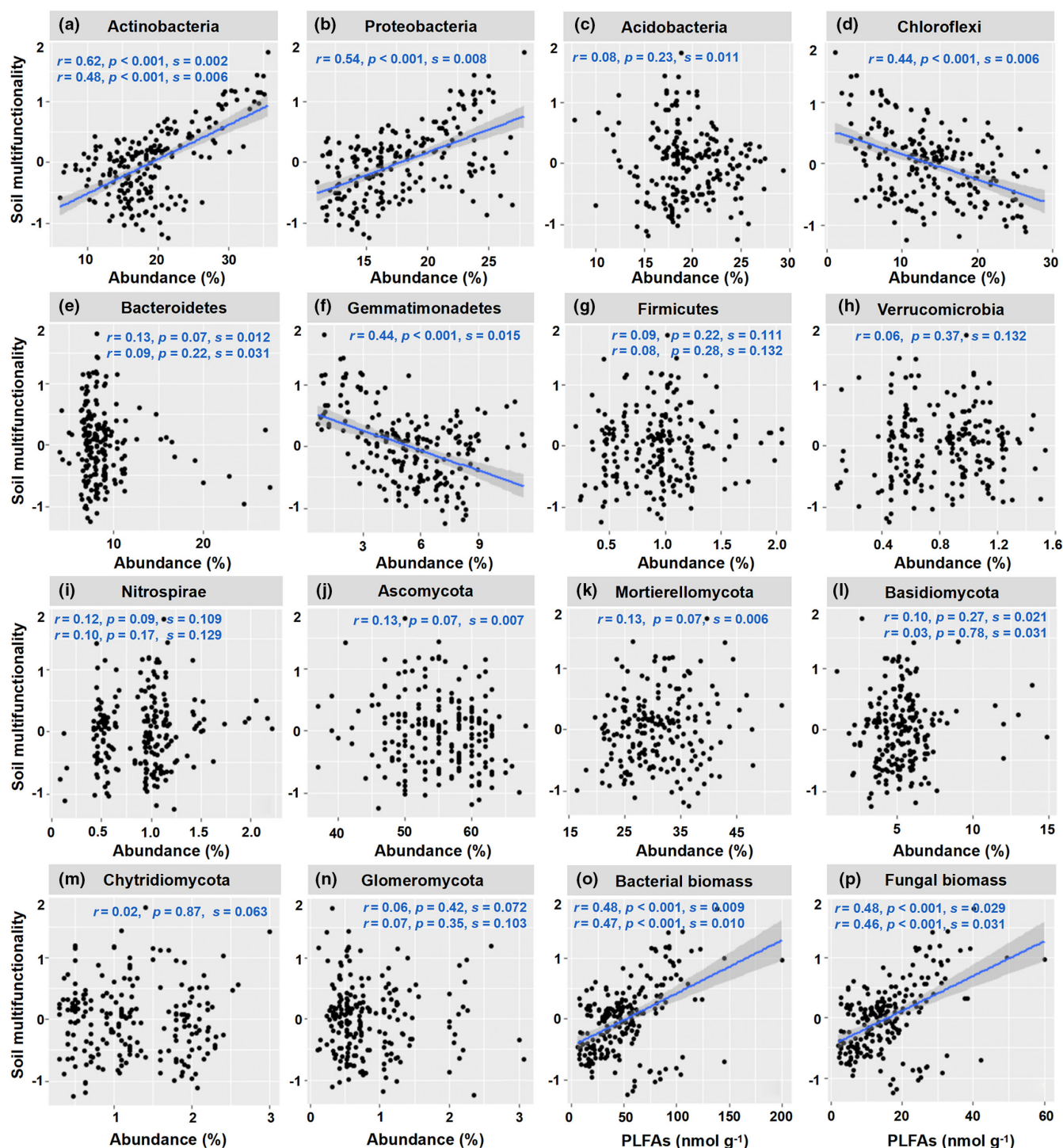
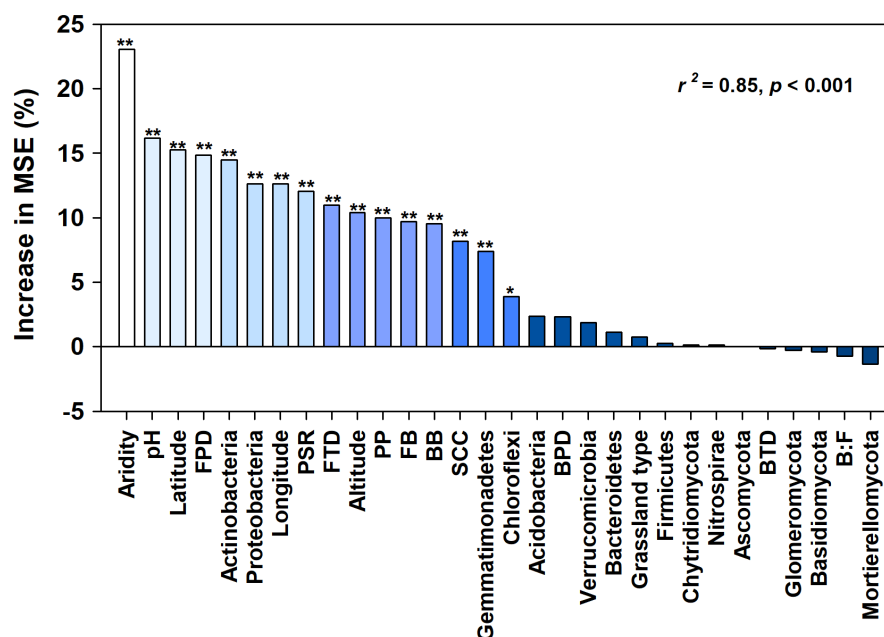


FIGURE 3 Relationships between bacterial community composition (phyla Actinobacteria, Proteobacteria Acidobacteria, Chloroflexi, Bacteroidetes, Gemmatimonadetes, Firmicutes, Verrucomicrobia and Nitrospirae), fungal community composition (phyla Ascomycota, Mortierellomycota, Basidiomycota, Glomeromycota and Chytridiomycota), and bacterial and fungal biomass and soil multifunctionality (a–p). The shaded areas show the 95% confidence interval of the fit. In each panel, the upper r -value and s -value represent correlation value and slope of the relationship across all data points, and the lower r -value and s -value represents correlation value and slope of the relationship without the extreme data points.

diversity is critical to regulating soil multifunctionality (Barberán et al., 2015), suggesting that any loss of fungal diversity will probably reduce the ability of grasslands to support soil functions and services.

More interestingly, we found that soil fungal phylogenetic diversity had a stronger positive relationship with soil multifunctionality than did taxonomic diversity (Figure 2). This suggests that fungal phylogenetic diversity may effectively reflect additional

FIGURE 4 Main predictors of soil multifunctionality. The figure shows the random forest mean predictor importance (% of increase in mean square error) of environmental drivers on soil multifunctionality for grassland ecosystems. Significance levels of each predictor are as follows: * $p < 0.05$ and ** $p < 0.01$. BTD, soil bacterial taxonomic diversity; BPD, soil bacterial phylogenetic diversity; FTD, soil fungal taxonomic diversity; FPD, soil fungal phylogenetic diversity; SCC, soil clay content; PSR, plant richness; PP, plant productivity; FB, fungal biomass; BB, bacterial biomass; B:F, bacteria:fungi ratio.



information specifically relevant to the soil functions of grassland ecosystems, indicating the necessity of incorporating microbial diversity–soil multifunctionality relationships in the CLM-Microbe model (Xu et al., 2014). By contrast, phylogenetic diversity has been proposed to integrate this functional information by assessing genetic diversity among species (Torsvik & Øvreås, 2002). Therefore, our finding is complementary to taxonomic diversity because it allows researchers to evaluate whether soil microbial communities comprise different phylogenetic groups without requiring richness information. This broadens the view of microbial taxonomic diversity to phylogenetic diversity as a critical predictor of soil multifunctionality in grassland ecosystems. Therefore, ignoring the variety of microbial diversity attributes may largely bias the prediction of the biodiversity loss impacts on for ecosystem functions and services.

4.2 | Bacterial community composition drive soil multifunctionality

Partially contrary to our second hypothesis, bacterial community composition (i.e. specific taxa abundance) rather than fungal composition was an important predictor of soil multifunctionality in the grassland ecosystems. The random forest algorithm identified the significant relationships between specific bacterial taxa (phylum level) and soil multifunctionality (Figure 4). These specific taxa are habitat generalists, that is, Actinobacteria and Proteobacteria, which are globally distributed bacteria (Maestre et al., 2016). The importance of bacterial community composition as predictor of soil multifunctionality is supported by multiple small-scale experiments and large-scale observations showing that dominant bacterial taxa control SOM decomposition and formation in both natural and managed

ecosystems (Delgado-Baquerizo et al., 2017; Zhang et al., 2021). Among these bacterial taxa, we found a primary effect of the phyla Actinobacteria in regulating soil multifunctionality.

Actinobacteria taxa had positive relationships with multiple soil functions, such as soil organic C, total N and available P levels, phosphatase activity and potential N mineralization rate (Figure S6). This finding is supported by previous studies that defined Actinobacteria as k-strategists or as having oligotrophic life histories and being more competitive in water- and nutrient-limited environments (Delgado-Baquerizo et al., 2017) such as temperate and alpine grassland ecosystems (Harpole et al., 2007). Importantly, Actinobacteria possess multifunctional traits involved in the decomposition and utilization of recalcitrant SOM such as chitin, cellulose and lignin by releasing extracellular enzymes, the production of different types of bioactive compounds responsible for promoting plant growth and biocontrol of phytopathogens (Trivedi et al., 2013).

Similarly, Proteobacteria taxa were also strongly positively correlated with the most soil functions across the grassland ecosystems (Figure S6). Proteobacteria tend to exhibit r-strategists or copiotrophic life histories, which may promote the greatest soil multifunctionality and support critical processes such as labile and complex SOM decomposition and building in relatively nutrient-rich soils (Pascault et al., 2013). By contrast, the r-strategists would have a greater role in SOM building than decomposition, while the k-strategists would play a stronger role in SOM decomposition than building (Bernard et al., 2022). Our finding highlights that members of the phyla Actinobacteria and Proteobacteria are crucial to cooperatively regulate the supply of soluble nutrients to plants and the building of organic matter reserves. This further indicates that dominant bacterial taxa can be critical for regulating soil multifunctionality, and that changes in these taxa resulting from land disturbance and climate change will likely alter important soil functions.

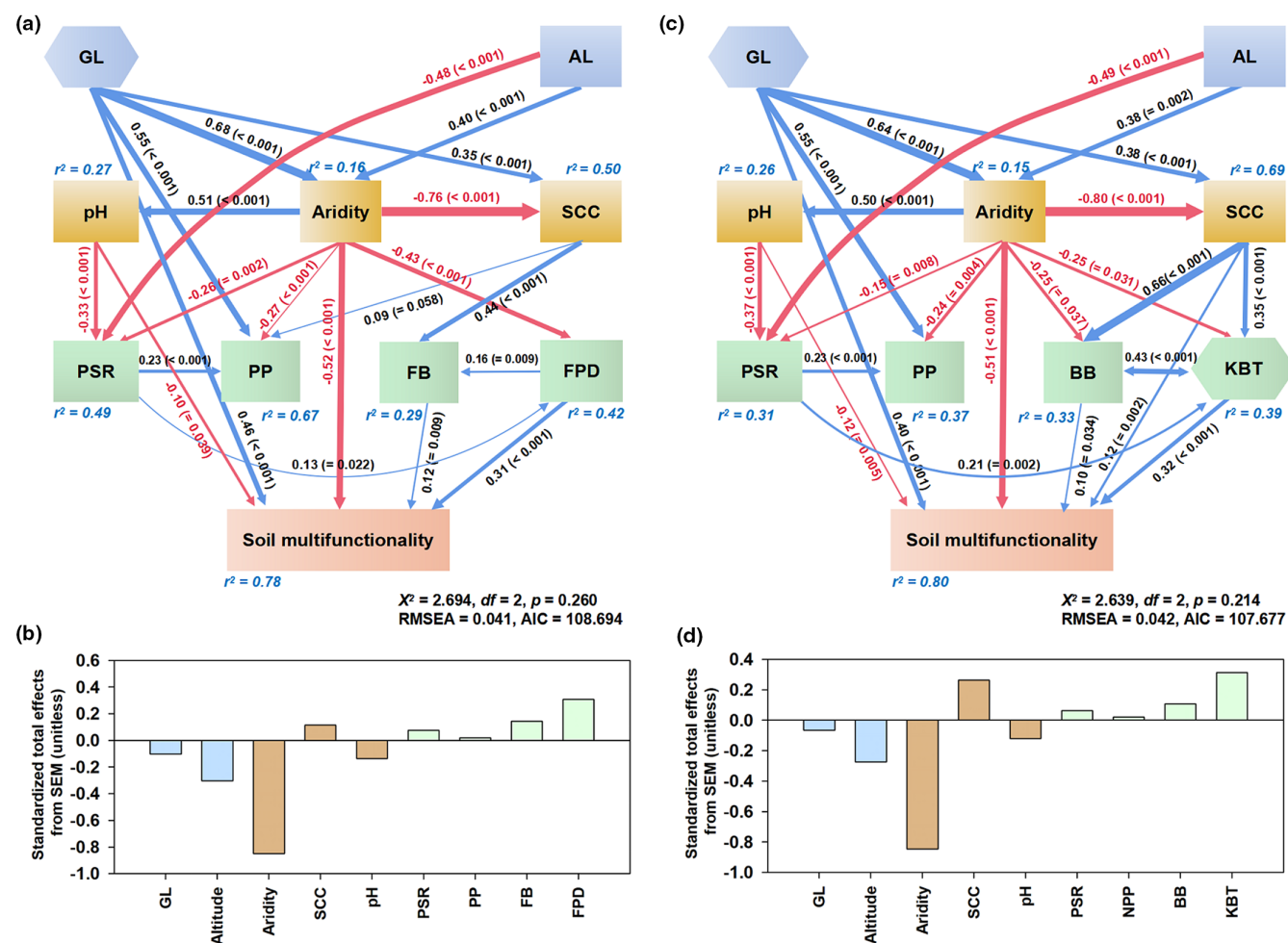


FIGURE 5 Structural equation models (SEMs) are shown for direct and indirect effects of geographical location (latitude, longitude), altitude, aridity, soil pH, soil clay content, plant richness, net primary productivity, fungal phylogenetic diversity (and dominant bacterial taxa), fungal biomass (and bacterial biomass) on soil multifunctionality (a, c). Numbers adjacent to arrows are indicative of the effect-size (bootstrap p -value) of the relationship. Significant positive and negative effects are shown with red and blue arrows, respectively. The width of arrows is proportional to the strength of path coefficients. r^2 denotes the proportion of variance explained. (b, d) standardized total effects (direct plus indirect effects) derived from the SEMs depicted above. GL, geographical location; SCC, soil clay content; PSR, plant richness; PP, plant productivity; FB, fungal biomass; BB, bacterial biomass; FPD, fungal phylogenetic diversity; KBT, dominant bacterial taxa.

4.3 | Fungal and bacterial biomass regulate soil multifunctionality

Partly consistent with our third hypothesis, we found that both soil fungal and bacterial biomass were directly and positively correlated with soil multifunctionality ($p < 0.05$; Figure 5a,b). This is in line with previous findings, showing that microbial biomass is a dominant predictor of multiple soil functions related to microbial respiration, N mineralization and soil C dynamics in forest, ocean and grassland ecosystems (Beugnon et al., 2021; Ma et al., 2014). Beugnon et al. (2021) reported that microbial biomass was more important than diversity in controlling soil C dynamics in a subtropical forest ecosystem. Differently, we found that the effects of fungal and bacterial biomass on soil multifunctionality were weaker than that of fungal diversity and bacterial taxa (Figure 5). This is likely because potential trade-offs may influence the effects of microbial biomass on studied soil functions and prevent grassland ecosystems from providing

high levels of soil multifunctionality. Furthermore, on the one hand, an increase in soil microbial biomass is potentially associated with high soil C mineralization, which may reduce soil C storage (Miltner et al., 2012); on the other hand, high soil microbial biomass can enhance the transformation of plant residues and soil C to microbial necromass, and consequently may increase soil C residency time (Buckeridge et al., 2020).

4.4 | Accounting for multiple soil multifunctionality drivers

In grassland ecosystems, temperature, the amount of precipitation, aridity and soil pH have been highlighted in previous studies as being dominant drivers of productivity and biological activity (Fierer & Jackson, 2006; Hu et al., 2021). Consistent with these findings, our random forest and SEM models (Figures 4 and 5) showed that aridity

(i.e. temperature–precipitation-based aridity level) and soil pH were most the important abiotic drivers of soil multifunctionality. A recent study showed that the biodiversity–soil multifunctionality relationship is aridity dependent (Hu et al., 2021), because plant diversity and soil multifunctionality had a strong positive relationship in less arid regions, while microbial diversity was positively associated with soil multifunctionality in more arid regions along a 4000 km aridity gradient. Furthermore, our result is consistent with previous studies in the grasslands, in which soil pH has been highlighted as being an important driver of multiple soil functions in the Tibetan Plateau and northern China at large scales (Jing et al., 2015; Liu et al., 2022). Recently, Yang et al. (2012) revealed that Chinese grassland soils are experiencing significant acidification. A global meta-analysis indicates a strong negative effect of soil acidification on soil functions, with the potential to inhibit soil C emissions (Meng et al., 2019), which would substantially alter soil C budget and its feedback to climate change.

In this study, we found that the plant diversity–soil multifunctionality relationship was indirect in the grassland ecosystems because only plant richness and fungal diversity (and bacterial taxa) are directly linked but plant richness and soil multifunctionality are not (Figure 5). This result is consistent with an empirical study that reported a positive effect of plant richness on ecosystem multifunctionality was indirect and resulted from the positive effect of plant richness on microbial diversity in the drylands at global scale (Delgado-Baquerizo et al., 2016). This was likely attributed to the competitive interactions among plant species that may have contributed to the weakened relationship of plant diversity with soil multifunctionality in water- and nutrient-limited grasslands (Fanin et al., 2018). Furthermore, trade-offs among different plant species may undermine plant diversity–multifunctionality relationship (Le Bagousse-Pinguet et al., 2019). However, a greater variety of plant species may increase the diversity of substrates and root exudates into soil, providing available niches for soil microbes and then increasing microbial diversity and abundance of specific taxa (Waring et al., 2015). Subsequently, the interaction among greater microbial diversity likely efficiently degrades recalcitrant and complex polymers into more labile monomers (i.e. complementary resource use; Schimel et al., 2005), which would support important soil functions.

Our SEMs revealed direct and significant positive relationships between soil fungal diversity (and dominant bacterial taxa) and soil multifunctionality after accounting for multiple soil multifunctionality predictors (Figure 5), indicating that microbial predictors were as important as or more important than other abiotic and biotic predictors, such as aridity, soil pH, geographical location (latitude and longitude) and plant richness. This result suggests the dominance of top-down effects of soil microbial diversity and community composition in mediating soil multifunctionality by controlling resource inputs and outputs in grasslands (Jackson et al., 2007). However, we did not find support for the significant effects of bacterial diversity, all fungal taxa and bacteria:fungi ratio on soil multifunctionality (Figure 4), suggesting that these

microbial facets, previously suggested to be major predictors of multifunctionality in some ecosystems (De Vries et al., 2012; Jing et al., 2015), may be poor predictors in the grasslands. This is because microbial functional redundancy might contribute to the lack of a visible effect in species-rich microbial communities (Li et al., 2021).

4.5 | Limitations and future work

Although the soil multifunctionality concept is useful in ecological studies, an appropriate assessment of multifunctionality is extremely challenging. Any assessment of soil multifunctionality would contain a subset of all possible functions and so would capture only a part of 'true' multifunctionality (Manning et al., 2018). We identified three limitations in the present study that should be addressed by future work. First, SOM is a legacy from past biogeochemical activities (Ohno et al., 2017) and does not inform on present rate of C sequestration. Second, soil mineral N concentration is extremely variable over time and across space and determined by inner and external soil N fluxes. Furthermore, the results of correlative approaches depend on the types of variables measured in statistical tools. This contributes to the overselling of certain aspects of soil functions. Therefore, further studies are needed to assess which, and how many, soil functions need to be measured to develop a good representation of standardized multifunctionality (Manning et al., 2018). Importantly, the correlative approach needs to be coupled with manipulative experiments to check the causality identified.

5 | CONCLUSIONS

This study carried out an intensive investigation of soil microbial diversity, community composition and multiple soil functions of 41 sites in five grassland ecosystems spanning a 3500 km transect. The findings provide strong empirical evidence that soil fungal diversity, dominant bacterial taxa and microbial biomass are important drivers in maintaining soil multifunctionality of grassland ecosystems. Furthermore, our results extend the view of fungal taxonomic diversity to phylogenetic diversity as an important predictor of soil multifunctionality. These findings imply that any loss in fungal diversity and dominant bacterial taxa resulting from land use and climate change will likely weaken soil multifunctionality, and in turn, exacerbate ecosystem functions and services such as soil fertility, climate regulation and production in grasslands. Therefore, information on soil microbial attributes (i.e. diversity, community composition and biomass) needs to be considered when developing policies for biodiversity conservation and sustainable development (Guerra et al., 2021). Predicting the effects of land use and climate change on ecosystem functions and services pave the way for preserving the soil multifunctionality of grassland ecosystems.

AUTHOR CONTRIBUTIONS

Linna Ma and R.W. conceived the project. Linna Ma, Chaoxue Zhang, Congwen Wang, Guofang Liu, Cunzhu Liang, Xiaolan Zuo, Chengjie Wang and Yixia Lv performed the field experiments. Linna Ma, Chaoxue Zhang and Xiaofeng Xu contributed the datasets. Linna Ma interpreted the results. Linna Ma wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The metadata of biotic and abiotic factors, soil functions, soil bacterial and fungal communities, and R-code in this study are openly available in the Dryad Digital Repository <https://doi.org/10.5061/dryad.66t1g1k53> (Ma et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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