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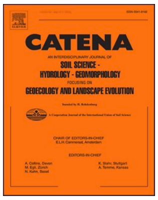

Shrub encroachment enhances soil multifunctionality by promoting interdomain interactions and reducing bacterial network complexity and competition

Yudu Jing[a,b,c,1], Xiaowei Liu[d,1], Junhao Feng[d], Ke Liang[e], Zhiyou Yuan[f], Jianchu Xu[g,h], Changhui Peng[i,j], Qiang Yu[f], Liang Guo[a,b,f,k,*]

[a] *State key Laboratory of Soil and Water Conservation and Desertification Control, The Research Center of Soil and Water Conservation and Ecological Environment, Chinese Academy of Sciences and Ministry of Education, Yangling, Shaanxi 712100, China*
[b] *Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi 712100, China*
[c] *University of Chinese Academy of Sciences, Beijing 100049, China*
[d] *College of Soil and Water Conservation Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China*
[e] *Forestry Institute, Forestry and Grassland Bureau of Longde County, Guyuan, Ningxia 756300, China*
[f] *State Key Laboratory of Soil and Water Conservation and Desertification Control, College of Soil and Water Conservation Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China*
[g] *Center for Mountain Ecosystem Studies, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China*
[h] *World Agroforestry Center, Nairobi 00100, Kenya*
[i] *School of Geographic Sciences, Hunan Normal University, Changsha, Hunan 410081, China*
[j] *Department of Biology Science, Institute of Environment Sciences, University of Quebec at Montreal, Montreal H3C3P8, Canada*
[k] *Ningxia Yunwushan National Nature Reserve Administration, Guyuan, Ningxia 756000, China*

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ABSTRACT

Shrub encroachment significantly alters both above- and below-ground ecological processes in semi-arid grasslands. However, its effects on soil multifunctionality and the underlying microbial mechanisms are not well understood. This study explored the impact of shrub encroachment on soil multifunctionality, microbial diversity, community composition, and microbial networks (both intra- and interdomain) in a typical semi-arid grassland on the Chinese Loess Plateau. Our findings revealed that shrub encroachment profoundly enhanced soil multifunctionality, closely linked to increased microbial diversity, restructured community composition, and altered patterns of microbial interactions. Specifically, bacterial intradomain links—particularly negative links—decreased, resulting in less complex and potentially less competitive bacterial networks. In contrast, the complexity of bacteria-fungi interdomain networks increased significantly, indicating strengthened interdomain connections. This shift from intense intradomain co-exclusion to enhanced interdomain co-occurrence played key roles in enhancing soil multifunctionality, as supported by the Partial least-square pathway model. Collectively, these findings highlight a strategic shift of soil microbial interaction networks under shrub encroachment, illustrating that the adaptive balance between microbial positive and negative interactions plays a pivotal role in regulating soil multifunctionality. This study advances our understanding of the ecological consequences and mechanisms of shrub encroachment in semi-arid grasslands.

1. Introduction

Grasslands, covering approximately 25 % of terrestrial ecosystems (Zhao et al., 2023a), provide vital ecological services, such as carbon sequestration (Bai and Cotrufo, 2022) and nutrient cycling (Sandor et al., 2016; Schimel et al., 1990). However, shrub encroachment—a widespread phenomenon characterized by woody expansion that replaces herbaceous species (Van Auken, 2009) —has increasingly

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[*] Corresponding author at: State key Laboratory of Soil and Water Conservation and Desertification Control, College of Soil and Water Conservation Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China.

E-mail address: guoliang2014@nwafu.edu.cn (L. Guo).

[1] Yudu Jing and Xiaowei Liu are joint first authors.

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affected these ecosystems (Eldridge et al., 2011). This process alters plant community structure (Soliveres et al., 2014), modifies the spatial distribution of carbon and nutrients (Li et al., 2019; Zhao et al., 2023b), and influences soil microbial diversity and interactions (Ding et al., 2020; Hu et al., 2021). While many studies have explored the effects of shrub encroachment on individual ecosystem functions, their results remain inconsistent (Eldridge et al., 2011; Van Auken, 2009). These discrepancies underscore the need for an integrative framework to elucidate how shrub encroachment simultaneously affects multiple ecosystem processes and their underlying mechanisms.

Soil multifunctionality, the capacity of soil to simultaneously support multiple ecological functions, integrates a suite of interconnected soil properties (Byrnes et al., 2014; Emmett Duffy et al., 2003). This concept has emerged as a key framework for evaluating the cumulative effects of global change on ecosystem health (Antiqueira et al., 2018; Feng et al., 2024). However, the influence of shrub encroachment on grassland soil multifunctionality remains unsolved. Some studies propose that shrub encroachment may enhance soil multifunctionality by altering the input of organic matter and energy into soils and promoting belowground biodiversity (Chandregowda et al., 2018), whereas others suggest that it intensifies wind erosion and plant competition, thereby reducing multifunctionality (Wu et al., 2024a; Yue et al., 2025). Such conflicting results highlight the need to identify the biological mechanisms that regulate soil multifunctionality. While climate, human activity, and vegetation traits have been recognized as indirect regulators of soil functions (Van Auken, 2009; Soliveres et al., 2014), advances in omics-based technologies have highlighted the pivotal role of microbial communities in directly mediating ecosystem processes under shrub encroachment (Xiao et al., 2025). Elucidating the structure and dynamics of microbial communities is therefore essential for understanding how shrub encroachment affects ecosystem functioning.

Soil microorganisms, strongly influenced by aboveground vegetation structure (Berg and Smalla, 2009), are central regulators of soil biogeochemical processes and thus represent a crucial link between shrub encroachment and soil multifunctionality (Wu et al., 2024b). Previous studies have primarily focused on how shrub encroachment alters microbial diversity, revealing concurrent increases in microbial richness and soil multifunctionality in semi-arid grasslands (Xiang et al., 2018). Beyond diversity, shrub encroachment also alters microbial community composition, particularly the relative abundance of oligotrophic and copiotrophic taxa, thereby influencing nutrient cycling processes (Guo et al., 2022; Ding et al., 2024). However, neither community diversity nor composition alone can fully capture the complexity of microbial communities, as ecosystem functioning depends not only on which taxa are present but also on how they interact (Barberán et al., 2012). Microbial interactions—often inferred from co-occurrence or co-exclusion networks analyses—thus represent a fundamental dimension of microbial community organization (Faust and Raes, 2012; Guseva et al., 2022). Increasing evidence suggests that microbial interaction network complexity—quantified with metrics such as average degree—serve as a more robust predictor of soil multifunctionality than traditional diversity indices across a range of ecosystems (Chen et al., 2022b; Wang et al., 2024). Despite these advances, the mechanisms through which microbial networks, particularly interdomain interactions between bacteria and fungi, mediate the effects of shrub encroachment on soil multifunctionality remain poorly understood. This represents a critical knowledge gap that limits our mechanistic understanding of how vegetation changes influence ecosystem multifunctionality through belowground microbial coordination.

Understanding these mechanisms is particularly relevant in ecosystems undergoing rapid shrub expansion, such as China's Loess Plateau. This region, characterized by severely eroded soils and degraded semi-arid grasslands (Yu et al., 2020), has experienced notable ecological recovery through long-term enclosure (Wang et al., 2014). Unlike many systems where shrub encroachment is linked to fire suppression or overgrazing (Van Auken, 2009; Eldridge et al., 2011), shrub expansion on the Loess Plateau has emerged as an unintended consequence of enclosure-based restoration, and its functional implications remain unclear (Guo et al., 2022; Zhang et al., 2024). Studying this region provides a unique opportunity to investigate how shrub encroachment affects soil multifunctionality under active restoration scenarios, and to provide guidance for the development of future grassland restoration strategies on the Loess Plateau. To address these questions, we conducted vegetation and soil surveys across shrub-encroached grasslands with the following objectives: 1) to evaluate how shrub encroachment influences soil multifunctionality and microbial community traits, including diversity, composition, and network structures, and 2) to elucidate the regulatory roles of microbial traits, particularly intra- and interdomain interaction networks, in shaping soil multifunctionality. We hypothesized that shrub encroachment enhances soil multifunctionality by increasing microbial network complexity and the portion of positive links (Fig. 1).

2. Materials and methods

2.1. Study site and sampling

The study was conducted in the Yunwushan National Nature Reserve (36.10°–36.17°N, 106.21°–106.27°E; 1800–2100 m a.s.l.) in Ningxia Hui Autonomous Region, China. Situated in the central Loess Plateau, the reserve experiences a temperate semi-arid climate, with a mean annual temperature of 7 °C and an average annual precipitation of 425 mm, most of which falls between June and August (Guo et al., 2017). The vegetation is composed of 313 plant species, with dominant grasses including *Stipa bungeana*, *Stipa grandis*, and *Potentilla acaulis*. The primary shrub species is *Caragana brachypoda*, and the dominant semi-shrub is *Artemisia stechmanniana* (Zhang et al., 2024).

The experiment was conducted within a 500 × 1000 m area of formerly homogeneous *Stipa bungeana* grassland, with uniform aspect, slope, and soil texture across plots. Notably, over recent decades, shrub expansion transformed the landscape into a patch mosaic (Fig. S1). Based on shrub presence and cover (Table S1), we delineated three encroachment conditions: no shrub encroachment (NSE; *Artemisia stechmanniana* and *Caragana brachypoda* cover <5 %), subshrub encroachment (SSE; *A. stechmanniana* cover >50 %), and shrub encroachment (SE; *C. brachypoda* cover >50 %). For each condition, five 5 m × 5 m plots were randomly established. Plots within the same condition were separated by ≥ 20 m, and plots from different conditions by ≥ 100 m. In August 2023, following the vegetation survey, aboveground biomass was harvested by clipping all vegetation within each plot. Simultaneously, topsoil (0–15 cm) was sampled by collecting five subsamples per plot and compositing them. In the laboratory, plant biomass was air-dried and weighed. Soil samples were passed through a 2 mm sieve to remove roots and undecomposed litter and then divided into three portions: one stored at −80 °C for microbial DNA extraction, one air-dried for analysis of soil pH, nutrients, and microbial biomass, and one stored at 4 °C for extracellular enzyme activity assays.

2.2. Soil properties analysis and multifunctionality assessment

A range of soil properties were analyzed, including soil water content (SWC), pH, total and available soil carbon (C), nitrogen (N), and phosphorus (P), microbial biomass and extracellular enzyme activity. SWC was determined by calculating the weight difference between fresh and oven-dried soil samples (Reynolds, 1970). Soil pH was measured using a pH electrode meter (PHS-3C, INESA, China) (Kalra, 1995). Soil organic carbon (SOC) and soil total nitrogen (TN) were analyzed with an elemental analyzer (Vario ELIII, Elementar, Germany) (Avramidis et al., 2015). Dissolved organic carbon (DOC) was extracted with 0.5 M K_2SO_4 and determined with a TOC/TN analyzer (Vario TOC, Elementar, Germany) (Jones and Willett, 2006). Soil nitrate nitrogen (NO_3^-—N) and

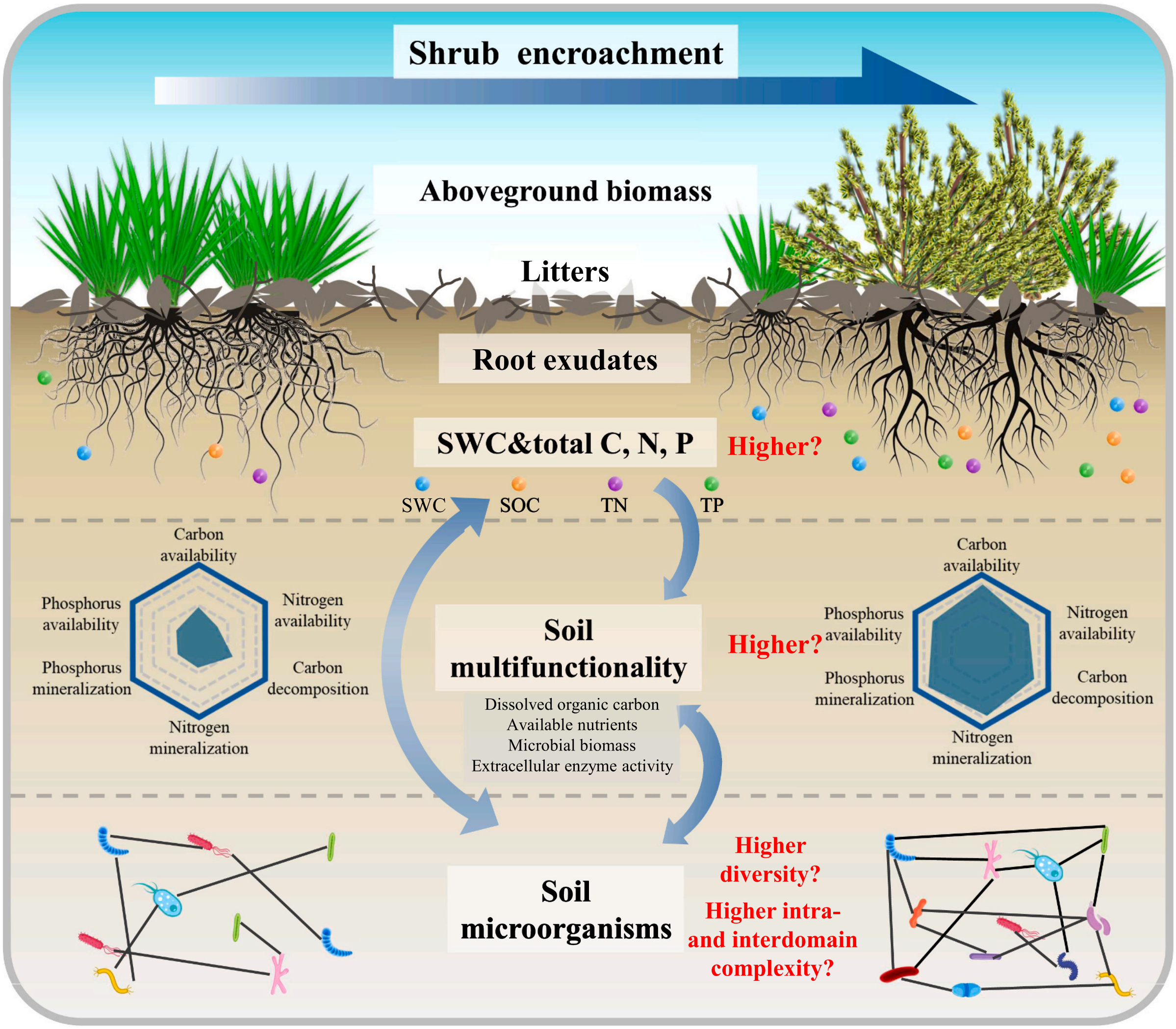

Fig. 1. Potential impacts of shrub encroachment on plant-soil-microorganism interactions in grasslands and the underlying regulatory mechanisms. It is hypothesized that shrub encroachment increases plant biomass, litter, and root exudates, which in turn affect soil properties such as soil water content (SWC), soil organic carbon (SOC), and nutrients including total nitrogen (TN) and total phosphorus (TP). These changes may subsequently influence soil multifunctionality and microbial attributes, such as diversity and the complexity of intra- and interdomain microbial networks.

ammonium nitrogen (NH_4^+—N) was determined with a flow injection analyzer (SAN Plus, Skalar, Netherlands) (Pasquali et al., 2007), which sums to get soil available nitrogen (AN). Soil total phosphorus (TP) and Olsen phosphorus (AP) were measured using the molybdate blue colorimetric method (Wolf and Baker, 1990). Microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were determined using the chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Extracellular enzyme activities, including those of β-1,4-glucosidase (BG), cellobiohydrolase (CBH), leucine aminopeptidase (LAP), *N*-acetyl-β-D-glucosaminidase (NAG), and alkaline phosphatase (AKP), were measured using a plate spectrophotometer (Multiskan FC, Thermo, Finland) (Bell et al., 2013). More details are available in the supplementary materials.

Soil multifunctionality, related to carbon, nitrogen, and phosphorus cycling, was quantified using two complementary indices: an average-based index and a threshold-based index. Both were derived from the soil properties listed in Table S2 that capture availability, sequestration and mineralization of soil C, N, and P (Qiu et al., 2024; Wang et al., 2023). The average approach calculates the means of standardized *Z*-scores for individual soil properties (Hooper and Vitousek, 1998), providing an overall representation of the average performance across multiple soil functions (Byrnes et al., 2014). In contrast, the threshold approach identifies the number of soil properties exceeding specific thresholds, which are set as percentages of the maximum observed value for each property, ranging from 5 to 99 % (Byrnes et al., 2014; Gamfeldt et al., 2008).

2.3. *DNA sequencing and bioinformatic analysis*

Genomic DNA was extracted from soil samples using the FastDNA Spin Kit (MP Biomedicals, USA) according to the manufacturer's instructions and subsequently purified through agarose gel electrophoresis. The V3–V4 region of bacterial 16S rRNA genes was amplified using the primer pair 341F (5'-CCT AYG GGR BGC ASC AG-3′) and 806R (5′-GGA CTA CNN GGG TAT CTA AT-3′). The fungal ITS1-5F region was amplified using the primer pair 1737F (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and 2043R (5'-GCT GCG TTC TTC ATC GAT GC-3′). DNA sequencing was performed on the Illumina Hiseq platform (Hiseq2500, Illumina, America).

Quality control was conducted using vsearch (Rognes et al., 2016) and USEARCH (Edgar, 2010) following the EasyAmplicon pipeline (Liu et al., 2023). Clean amplicons were denoised to generate amplicon sequence variants (ASVs). A total of 8416 bacterial ASVs and 1615 fungal ASVs were identified. Taxonomic classification of bacterial and fungal ASVs were performed using the RDP-16S-v18 (Cole et al., 2014) and UNITE (Abarenkov et al., 2024) database, respectively.

2.4. *Microbial co-occurrence network construction*

Soil bacterial and fungal intradomain networks were constructed to illustrate interactions within soil bacteria and fungi communities, respectively. Prior to network construction, data filtering was performed to ensure reliable correlation calculation. ASVs present in fewer than five samples or with the relative abundance <0.05 % were excluded (Yuan et al., 2021; Deng et al., 2025). To account for the compositional nature of amplicon data, we applied a centered-log-ratio (CLR) transformation after adding a pseudo count of $+1$ to all OTU counts (Faust and Raes, 2012). Comparison of networks constructed from transformed versus untransformed datasets revealed a similar pattern between the two (Table S3), suggesting a negligible compositional bias in this study (Yuan et al., 2021). All co-occurrence networks were built based on Spearman's rank correlation, a robust method for analyzing non-normal microbial data that is particularly suited to detecting monotonic relationships between ASVs (Faust and Raes, 2012). To control for false discoveries from the large number of pairwise correlation tests, Benjamini–Hochberg (BH) false discovery rate (FDR) correction was applied to adjust *P*-values (Benjamini and Hochberg, 1995). Connections with a correlation coefficient (r) greater than 0.8 and statistical significance ($P < 0.05$ after FDR correction) were retained to ensure robust links (Qiu et al., 2024). To evaluate the significance and robustness of the microbial co-occurrence network, we constructed null models by randomly rewiring network edges while maintaining the degree distribution of nodes (Guseva et al., 2022). The network was randomized 100 times to generate a distribution of random networks (Yuan et al., 2021). For further correlation analysis with soil physicochemical properties, subnetworks for each soil sample were generated by extracting sample-specific ASVs from the "aggregate" networks (De Marzio et al., 2023). Interdomain networks were created to represent cross-trophic interactions between bacteria and fungi, based on correlations between bacterial and fungal ASVs. The same methods and parameters for data filtering, compositionality handling, correlation calculation, randomized network generation, and thresholds selection were applied as in the intradomain networks. Topological properties including edge and node number, diameter, average path length, average degree, edge density, average clustering coefficient, relative modularity, module number, and the proportion of negative links were calculated. Furthermore, within-module connectivity (Z_i) and among-module connectivity (P_i) was calculated for each node to identify its topological role: module hubs ($Z_i \geq 2.5$, $P_i < 0.62$), connectors ($Z_i < 2.5$, $P_i \geq 0.62$) and network hubs ($Z_i \geq 2.5$, $P_i \geq 0.62$) (Yuan et al., 2021). Empirical network construction, random and subnetworks generation, and network property calculations were performed using the R package ggClusterNet. For further details of the network analysis, please refer to Wen et al. (2022). Network visualization was conducted with Gephi 0.10.1.

2.5. *Statistical analysis*

Alpha diversity of soil microorganisms was calculated with the vegan package (Dixon, 2003). Analysis of variance (ANOVA) was conducted to determine the impact of shrub encroachment on soil physicochemical properties, multifunctionality, and microbial alpha diversity. Least significant difference (LSD) test at a 5 % statistical probability level was used for post hoc analysis. Principal component analysis (PCA) was used to examine spatial variation in soil functional variables across different shrub encroachment conditions. Additionally, principal coordinates analysis (PCoA) was used to evaluate differences in microbial composition, also employing the vegan package (Dixon, 2003). Microbial composition dissimilarity was further validated with three non-parametric methods: permutational multivariate analysis of variance (Adonis), analysis of similarities (ANOSIM), and multiple response permutation procedure (MRPP). Microbial biomarkers were identified using linear discriminant analysis effect size (LEfSe) on the ImageGP platform (Chen et al., 2022a), with an alpha significance level of 0.01 and an effect size threshold of 3 (Moreno-Arrones et al., 2020). Pearson's correlation was used to explore relationships between soil physicochemical properties, soil multifunctionality (calculating using the average approach), microbial diversity, and network complexity. The relationship between microbial network complexity (indicated by the average degree) and soil multifunctionality (calculated using the threshold approach) was analyzed using a generalized linear model (Byrnes et al., 2014). Partial least-squares path modeling (PLS-PM) was applied to investigate the regulatory mechanism underlying soil multifunctionality, using the plspm package (Sanchez et al., 2024). Model performances were evaluated based on goodness of fit (GOF) statistics. The robustness of the PLS-PM was evaluated via bootstrap resampling with 5000 iterations. The path coefficients were stable, with narrow 95 % confidence intervals and low standard errors (Table S4). The endogenous constructs exhibited high explanatory power (R^2 values ranging from 0.56 to 0.93; Table S5), confirming the model's strong predictive capability. All statistical analyses were conducted using R 4.3.1 programming language (R Core Team, 2023).

3. Results

3.1. *Impacts of shrub encroachment on plant biomass, soil properties, and soil multifunctionality*

Shrub encroachment significantly affected plant biomass and soil properties, including soil pH, water content, organic carbon, nutrients, microbial biomass (Fig. S2), and extracellular enzyme activities (Fig. S3). Most properties were notably higher under shrub encroachment (SE) and subshrub encroachment (SSE) compared to no shrub encroachment (NSE), with the exception of soil pH (Fig. S2b), which exhibited the opposite trend. However, for most properties, no significant differences were observed between SE and SSE (Fig. S2, S3).

Principal component analysis (PCA) revealed a clear separation between the soil functional profiles of NSE and the two encroachment conditions (Fig. 2a), indicating significant differences in soil functions. Soil multifunctionality, which integrates soil carbon, nitrogen, and phosphorus cycling, was assessed by calculating the mean *Z*-scores of the individual soil functions listed in Table S2. Similar to plant biomass and soil properties (Fig. S2), both overall soil multifunctionality and the individual metrics for carbon, nitrogen, and phosphorus cycling were significantly higher in SE and SSE compared to NSE (Fig. 2b-e). However, as with soil properties, no significant differences were detected between SE and SSE.

3.2. *Impacts of shrub encroachment on soil microbial diversity and structure*

Shrub encroachment (SE) and subshrub encroachment (SSE) significantly increased most alpha diversity metrics of soil bacteria compared to no shrub encroachment (NSE) (Fig. 3a, S4a–c). However, no significant differences were observed between SE and SSE. In contrast, the alpha diversity of soil fungi remained relatively stable across NSE, SSE, and SE (Fig. 3b, S4d–f), indicating contrasting responses of bacterial and fungal diversity to shrub encroachment. Principal coordinates analysis (PCoA) revealed significant differences in the community structure of both soil bacteria and fungi across NSE, SSE, and SE (Fig. 3c, d). These results were further supported by non-parametric multivariate statistical tests (Table S6), including permutational multivariate analysis of variance (Adonis), analysis of similarities (ANOSIM), and multiple response permutation procedure (MRPP).

The soil bacterial community was dominated by the phyla *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, and *Firmicutes* (Table S7), while the fungal community primarily

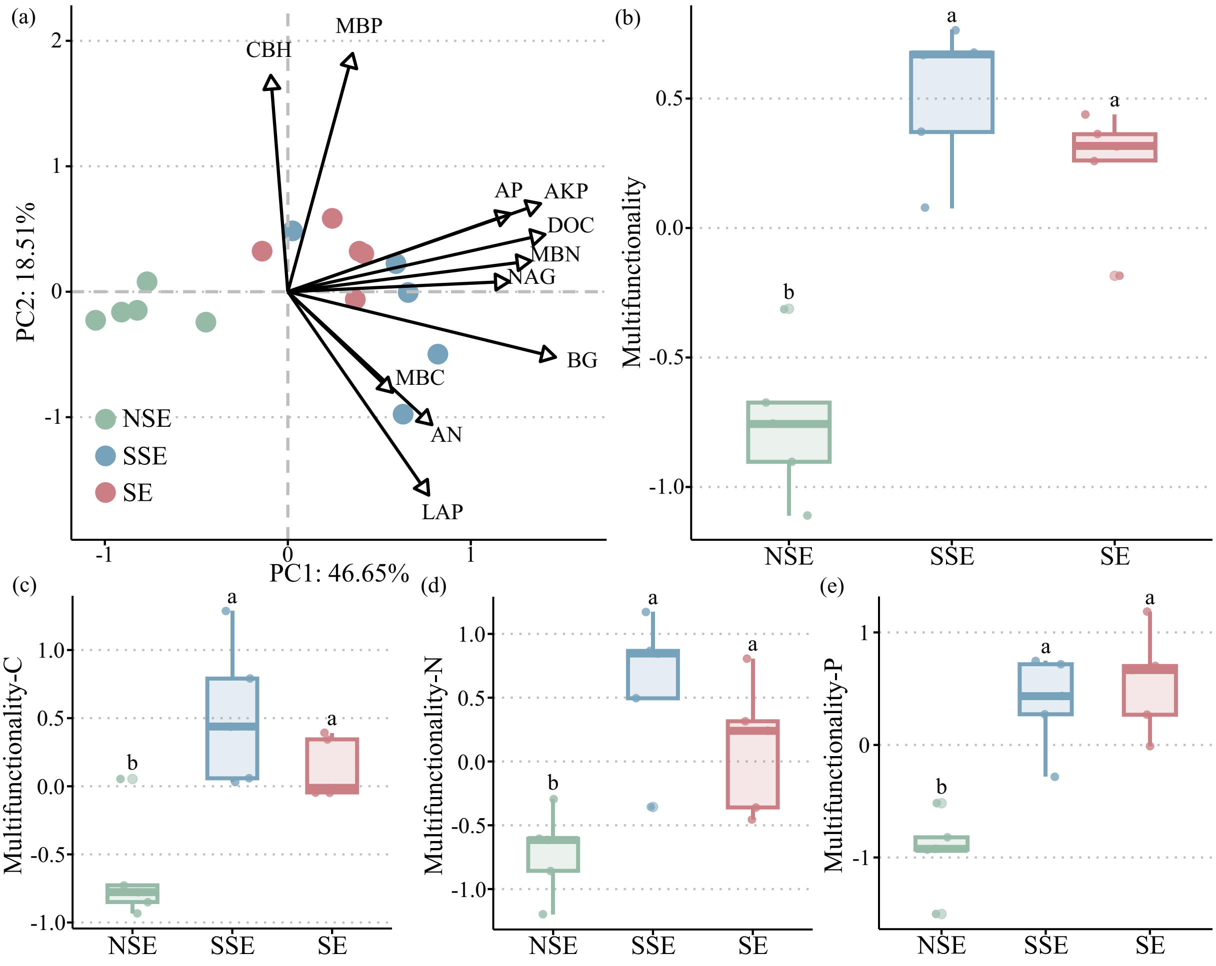

Fig. 2. Structure of soil functions and multifunctionality under no shrub encroachment (NSE), subshrub encroachment (SSE), and shrub encroachment (SE). (a) Principal component analysis (PCA) illustrating the structure of soil functions. (b) Soil multifunctionality related to carbon and nutrient cycling. (c)–(e) Soil multifunctionality related to the cycling of carbon (Multifunctionality-C), nitrogen (Multifunctionality-N), and phosphorus (Multifunctionality-P), respectively. Different letters indicate significant differences among the three shrub encroachment conditions. DOC: soil dissolved organic carbon, AN: available nitrogen, AP: available phosphorus, MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, MBP: microbial biomass phosphorus, BG: β-1,4-glucosidase, CBH: cellobiohydrolase, LAP: leucine aminopeptidase, NAG: *N*-acetyl-β-D-glucosaminidase, AKP: alkaline phosphatase.

consisted of the classes *Agaricomycetes*, *Sordariomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Lecanoromycetes*, and *Glomeromycetes* (Table S8). Shrub encroachment significantly altered the composition of both soil bacteria and fungi. For bacteria, the relative abundances of the phylum *Latescibacteria* were significantly higher in SE and SSE compared to NSE, whereas *Actinobacteria* and *Gemmatimonadetes* showed the opposite trend (Table S7). For fungi, the class *Sordariomycetes* was more abundant in SE and SSE, while *Agaricomycetes* predominated in NSE (Table S8). Microbial biomarkers for each shrub encroachment condition were identified using linear discriminant analysis effect size (LEfSe). Among bacterial biomarkers, the family *Vicinamibacteraceae*, phylum *Bacteroidetes*, and phylum *Rhodothermaeota* were identified as key indicators for NSE, SSE, and SE, respectively (Fig. S5a). For fungi, the family *Clavariaceae*, class *Archaeorhizomycetes*, and class *Leotiomycetes* were identified as biomarkers for NSE, SSE, and SE, respectively (Fig. S5b). These findings highlight the significant impacts of shrub encroachment on the diversity, composition, and structure of soil microbial communities.

3.3. Impacts of shrub encroachment on soil microbial interaction patterns

To evaluate the effects of shrub encroachment on soil microbial interactions, intradomain networks of soil bacteria and fungi were constructed (Fig. 4a, c). All empirical networks exhibited a small-world pattern (small-world coefficient > 1). While the number of nodes in bacteria networks was similar across the three conditions, the number of edges and the average degree was lower in SE and SSE than in NSE (Fig. 4a, b), indicating looser and less complex co-occurrence patterns under the former two conditions. This pattern was further supported by other topological properties of random networks, where average path length and relative modularity were significantly higher in SE than in NSE, whereas the average clustering coefficient showed the opposite trend (Table S9). In contrast, fungi networks exhibited no significant differences in most topological properties among NSE, SSE, and SE (Fig. 4c, d). The proportion of negative links, reflecting the prevalence of negative co-occurrence within communities, showed distinct patterns: in bacterial networks, it decreased significantly under SE and SSE, whereas in fungal networks, it increased (Table S9). These results suggested a reduction in negative co-occurrence intensity within bacteria community and an increase within fungi community under shrub encroachment. Additionally, topological roles of nodes within empirical networks were identified based on Zi (within-module connectivity) and Pi (among-module connectivity). In bacterial community, multiple module hubs were identified under NSE and SE, while only one connector was identified under SSE (Fig. S6 a-c). In contrast, only one connector and one module hub was identified in fungal networks under NSE and SE, respectively (Fig. S6 d-f).

In addition, bacteria-fungi interdomain networks were constructed

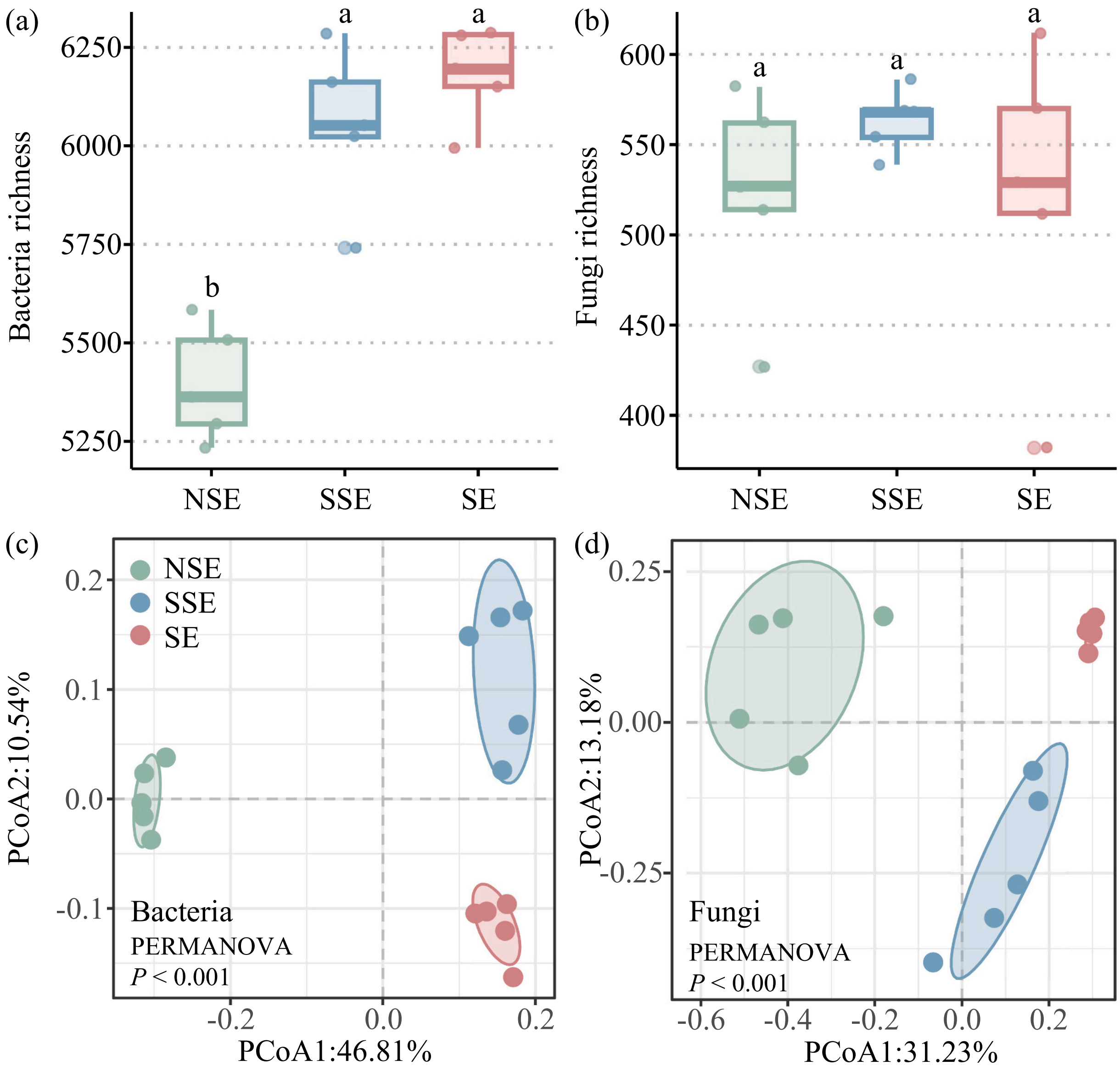

Fig. 3. Diversity and composition of soil microorganisms under no shrub encroachment (NSE), subshrub encroachment (SSE), and shrub encroachment (SE) conditions. (a) and (b) Alpha diversity of soil bacteria and fungi, represented by the richness index, respectively. (c) and (d) Community composition of soil bacteria and fungi, analyzed using principal coordinates analysis (PCoA), respectively. Differences in community composition among the three encroachment conditions were statistically significant ($P < 0.001$), as determined by permutational multivariate analysis of variance (PERMANOVA).

to analyze interactions between the bacterial and fungal communities (Fig. 4e). All interdomain networks exhibited highly modular structures, with fungal nodes predominantly serving as module centers (Fig. 4e). The topological properties of these networks differed significantly across NSE, SSE, and SE. Under SE and SSE, the number of edges, average degree, and relative modularity increased compared to NSE, while the average path length decreased, indicating more complex interdomain interactions between soil bacteria and fungi under SE and SSE (Fig. 4f, Table S9). Furthermore, the proportion of negative links in interdomain networks declined in SE and SSE relative to NSE (Table S9), indicating increased co-occurrence and decreased co-exclusion interactions between soil bacterial and fungal communities under shrub encroachment.

3.4. Correlations between soil physicochemical properties, microbial traits and soil multifunctionality

Pearson's correlations were calculated among soil physicochemical properties (e.g., soil pH, soil water content, soil organic carbon, and soil nutrients), soil multifunctionality, and microbial traits, including microbial diversity and network complexity (Fig. 5a). Soil pH exhibited a strong negative correlation with soil multifunctionality, whereas soil water content (SWC) was positively correlated with soil multifunctionality. Most soil nutrient properties were also significantly and positively correlated with soil multifunctionality. Additionally, bacterial diversity was negatively correlated with soil pH but positively correlated with SWC and most soil nutrient properties. In contrast, fungal diversity showed no significant correlations with soil physicochemical properties, except for a significant association with the first axis of principal coordinates analysis for soil fungal composition (PC1). Moreover, bacterial network complexity was negatively correlated with most soil properties, whereas interdomain network complexity displayed positive correlations. Fungal network complexity showed no significant correlations with most soil properties. These results highlighted the critical role of soil physicochemical properties in shaping soil multifunctionality, bacterial diversity, and bacterial and interdomain network complexity.

Furthermore, the relationship between soil microbial network complexity and soil multifunctionality was analyzed using the threshold approach (Fig. 5b–d). The average degree of soil bacterial networks was

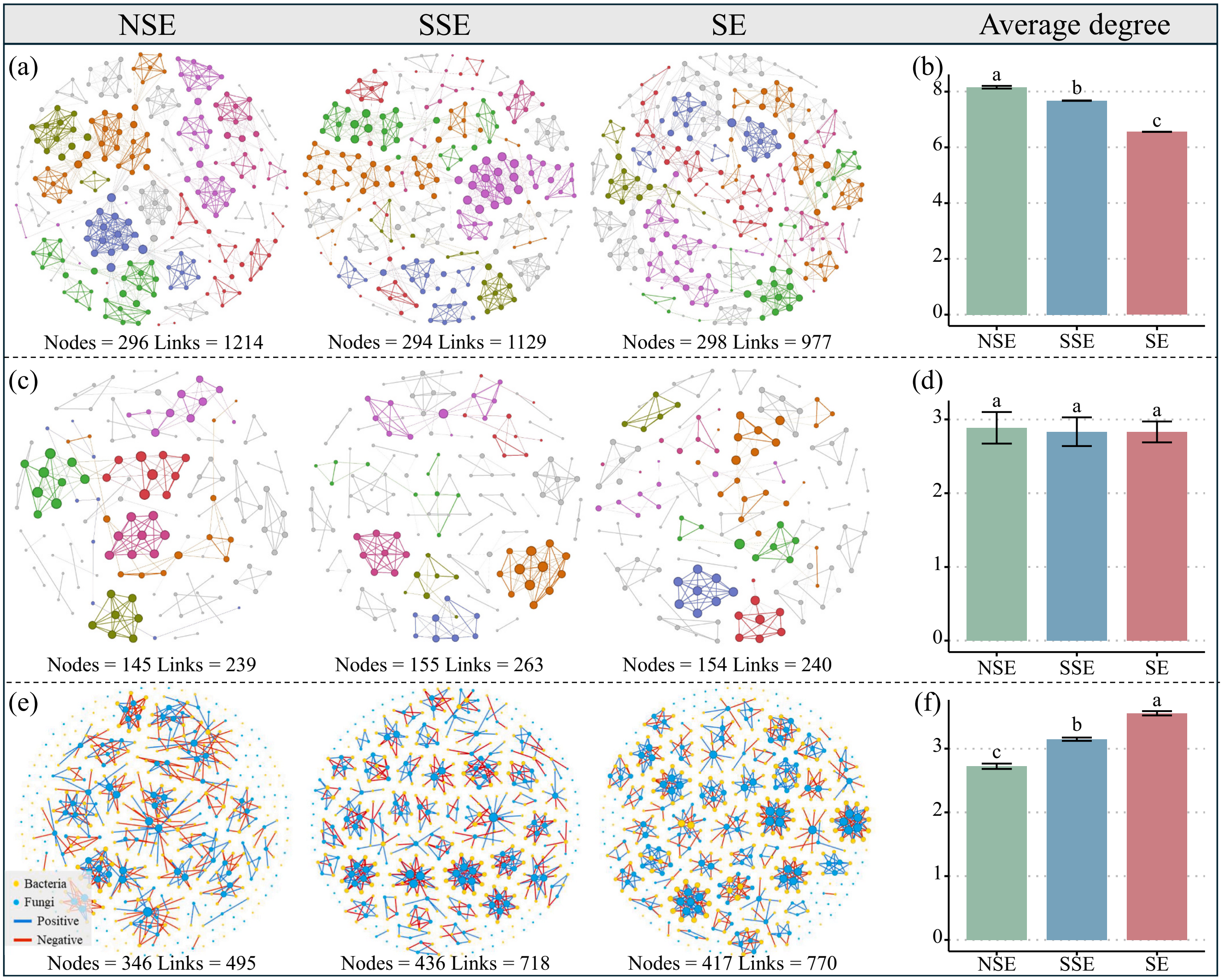

Fig. 4. Intra- and inter-domain co-occurrence networks, as well as their average degree under different shrub encroachment conditions. (a) Bacterial intra-domain networks and (c) fungal intra-domain networks, with different colors indicating different modules. (b) and (d) Average degree of bacterial and fungal intra-domain networks, respectively. (e) Interdomain networks between soil bacteria and fungi, with blue and red lines representing positive and negative links between ASVs, respectively. (f) Average degree of interdomain networks. The degree (number of connections held by a node) of nodes is represented by the size of nodes. Nodes: node number of the network, Links: edge number of the network, NSE: no shrub encroachment, SSE: subshrub encroachment, SE: shrub encroachment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

consistently negatively correlated with soil multifunctionality across various thresholds, ranging from 5 % to 99 % (Fig. 5b). In contrast, the correlation between fungal network complexity and soil multifunctionality varied inconsistently across thresholds (Fig. 5c). Interdomain network complexity, however, was positively correlated with soil multifunctionality (Fig. 5d).

To elucidate how soil microorganisms regulate soil multifunctionality, we applied a partial least-squares path model (PLS-PM; Fig. 6). A conceptual framework was initially formulated from prior literature (Fig. S7) and iteratively refined through model optimization. During this phase, we systematically removed indicators exhibiting high collinearity (variance inflation factor > 5) yet low factor loadings (< 0.7), those with substantial cross-loadings, redundant latent paths, and variables with negligible explanatory power (< 5 % unique variance). This pruning ensured a proper balance between the model's overall explanatory power and statistical reliability, ultimately excluding several bacterial diversity metrics and all fungal-specific indicators. In the final PLS-PM, bacterial network complexity and the proportion of negative links in bacterial networks exerted direct and negative effects on soil multifunctionality, while the number of edges in bacteria-fungi (B—F) interdomain networks influenced multifunctionality indirectly (Fig. 6). Model stability was validated via bootstrap resampling (n = 5000) with all path coefficients and R^2 values of endogenous variables remaining significant (95 % confidence intervals did not cross zero). Overall fit was excellent (goodness-of-fit, GOF = 0.847; Fig. 6), indicating robust explanatory power. Specifically, shrub encroachment enhanced plant biomass, which subsequently increased soil water content (SWC), soil organic carbon (SOC), and total nitrogen (TN). (Fig. 6). These properties, in cascade, elevated bacterial diversity and indirectly reduced bacterial network complexity. Additionally, elevated plant biomass augmented the edge number of B—F interdomain networks, which was negatively correlated with the proportion of negative links in bacterial networks (Fig. 6). Overall, shrub encroachment, plant biomass, bacterial composition, and the edge number of B—F networks yielded positive total effects on soil multifunctionality, while bacterial network complexity and the proportion of negative links in bacterial networks had negative total effects. Collectively, the model explained 83.3 % of the total variation in soil multifunctionality.

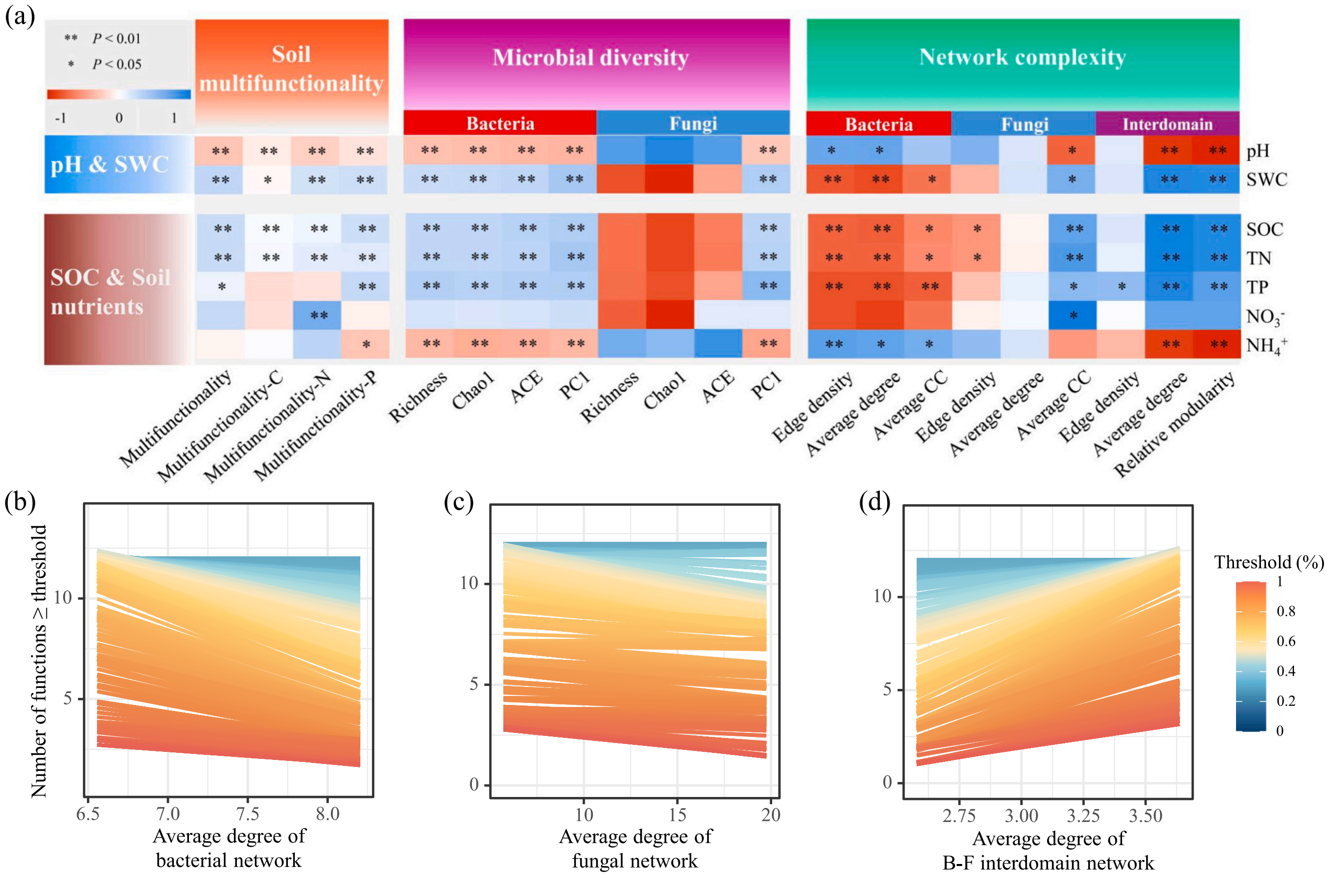

Fig. 5. Correlations between soil physicochemical properties, soil multifunctionality, microbial diversity and network complexity. (a) Pearson's correlations identifying significant factors influencing soil multifunctionality and microbial attributes, including soil pH, soil water content (SWC), and soil nutrients. Statistical significance is denoted by star marks (*: $P < 0.05$, **: $P < 0.01$). (b-d) Relationships between the number of soil functions exceeding threshold and the average degree of bacteria (b), fungi (c), and bacteria-fungi (B—F) interdomain (d) networks. Thresholds represent percentages of the maximum observed value for each function across all samples, with colors indicating different thresholds as shown in the figure legend. SOC: soil organic carbon, TN: soil total nitrogen, TP: soil total phosphorus, NO_3^-: soil nitrate nitrogen, NH_4^+: soil ammonium nitrogen, Multifunctionality-C: soil multifunctionality related to carbon cycling, Multifunctionality-N: soil multifunctionality related to nitrogen cycling, Multifunctionality-P: soil multifunctionality related with phosphorus cycling, PC1: the first axis of principal coordinate analysis for soil microbial composition, Average CC: average clustering coefficient.

4. Discussion

4.1. Shrub encroachment promoted soil multifunctionality

This study investigated the effects of shrub encroachment on plant and soil properties, as well as soil multifunctionality. We found parallel increases in plant biomass, soil water content (SWC), soil organic carbon (SOC), total soil nutrients (Fig. S2), and soil multifunctionality (Fig. 2) under both shrub and subshrub encroachment. These findings are consistent with previous studies reporting concurrent increases in soil physicochemical properties and multifunctionality under shrub encroachment (Chandregowda et al., 2018; Quero et al., 2013; Valencia et al., 2015). We also observed significant increases in soil multifunctionality of carbon, nitrogen, and phosphorus cycling (multifunctionality-C/N/P) following shrub encroachment (Fig.2c-e), aligning with results from the Qinghai-Tibet Plateau, where shrub encroachment enhanced multifunctionality-C/N in alpine steppe (Yang et al., 2024). Because effects on multifunctionality-P have been less frequently reported, our results help to fill this gap.

The observed increase in plant biomass under shrub encroachment (Fig. S2) likely explains part of the enhancement in soil multifunctionality (Quero et al., 2013; Valencia et al., 2015). Compared to grasses, shrubs generally have greater biomass, heights, canopy covers, and basal areas (Eldridge et al., 2013). These traits improve the accumulation of soil water and nutrients, both of which play a crucial role in enhancing soil multifunctionality under shrub encroachment (Valencia et al., 2015). Moreover, increase in SOC and total nutrients under both SE and SSE (Fig. S2) also contributed to higher soil multifunctionality, as demonstrated by their significant positive correlations with the multifunctionality index (Fig. 6). This finding aligns with previous studies that report concurrent increases in SOC, total nitrogen (TN) and total phosphorus (TP), and multifunctionality following shrub encroachment (Chandregowda et al., 2018; Yang et al., 2024). We also observed a decrease in soil pH (Fig. S2) concomitant with higher multifunctionality, consistent with evidence that lower pH in alkaline soils can enhance nutrient availability and microbial activity (Naz et al., 2022).

By contrast, Wu et al. (2024) reported a negative effect of shrub encroachment on soil multifunctionality in the semi-arid grasslands of Inner Mongolia, which differs from our findings on the Loess Plateau and from most studies in mesic or alpine ecosystems (Chandregowda et al., 2018; Yang et al., 2024). This discrepancy may arise from differences in soil water content (SWC) among studies. As SWC decreases, the correlation between shrub encroachment and soil multifunctionality can shift from positive to negative (Soliveres et al., 2014). In our sites, SWC increased with encroachment (Fig. S2), whereas Wu et al. (2024) reported reduced soil water holding capacity, which may explain the contrasting results.

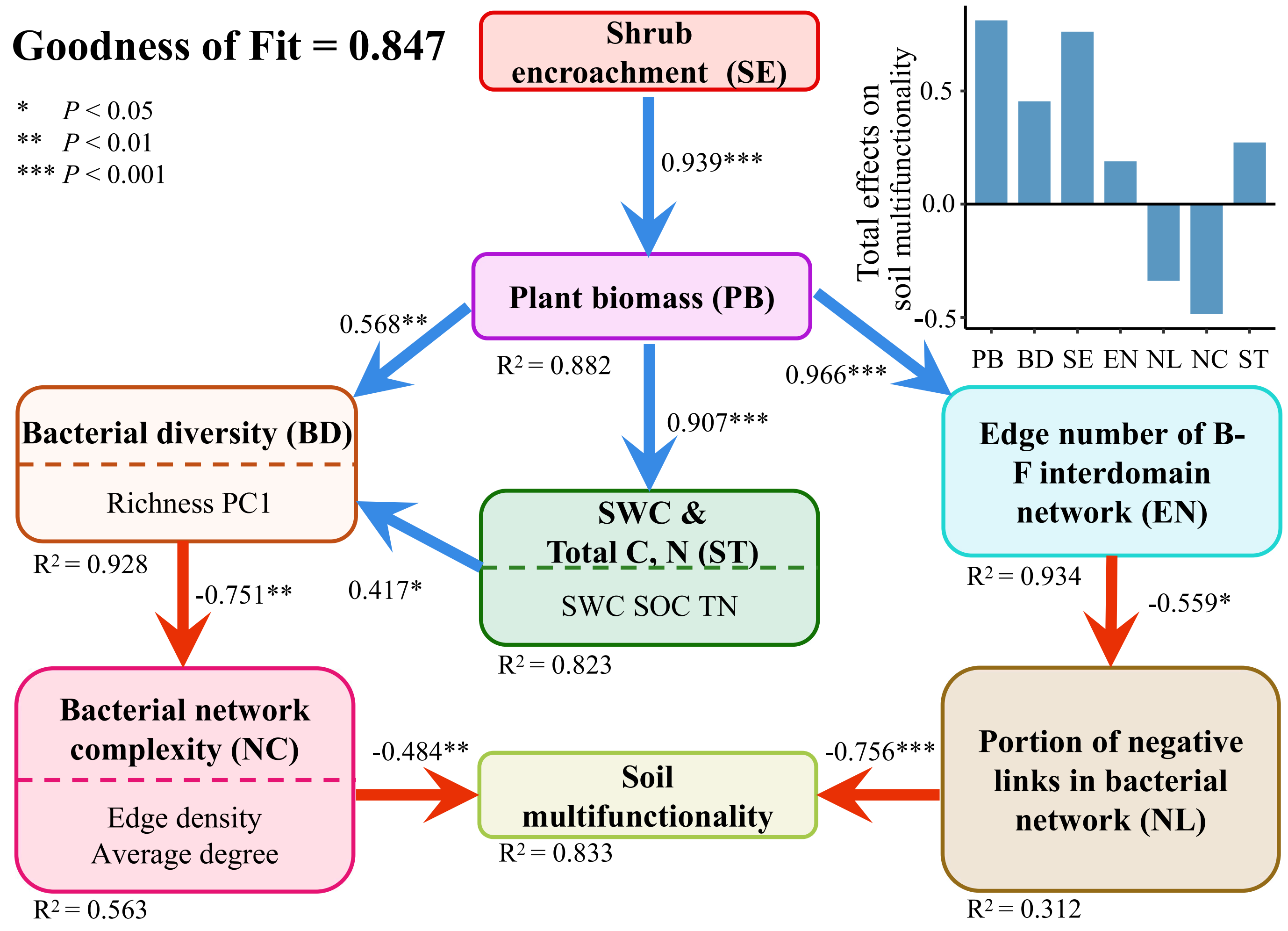

Fig. 6. Partial least-squares path modeling (PLS-PM) demonstrating the regulatory mechanism of soil multifunctionality under shrub encroachment. Blue and red arrows indicate positive and negative correlations between variables, respectively. The variance explained (R^2) for each variable is shown in the lower-left corner of the variable box. Normalized path coefficients are displayed along the arrow, with star marks denoting statistical significance (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). Total effects of variables on soil multifunctionality are summarized in the bar plot inserted in the upper-right corner of the figure. SOC: soil organic carbon, TN: total nitrogen, SWC: soil water content, PC1: the first axis of principal coordinate analysis for soil bacteria composition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. *Different responses of soil bacteria and fungi diversity and composition to shrub encroachment*

Our results revealed that shrub encroachment affected the alpha diversity of soil bacteria (Fig. 3a, S4) and reshaped community composition of both soil bacteria and fungi (Fig. 3c, d, S5). Specifically, soil bacterial diversity increased significantly under shrub encroachment (SE) and subshrub encroachment (SSE) conditions (Fig. 3a, S4a–c). This pattern aligns with studies from Inner Mongolia (Xiang et al., 2018), the Qinghai-Tibet Plateau (Dengzeng et al., 2022), and the Loess Plateau (Guo et al., 2022), which reported positive effects of shrub encroachment on bacterial alpha diversity. The underlying mechanisms remain unclear. In our study area, we hypothesized that the promotion of bacterial alpha diversity under shrub encroachment may be mediated by soil pH, soil water content (SWC), and soil nutrients. Decreased pH in our alkaline study site could alleviate resource limitation and promote bacterial diversity, in line with Naz et al. (2022) and Jiao and Lu (2020). Furthermore, increased SWC under encroachment (Fig. S2) correlated with higher soil bacterial diversity, consistent with evidence that higher SWC can increase bacterial diversity (Li et al., 2021). Additionally, synchronous increase in plant biomass (Fig. S2) and bacterial diversity (Fig. 3) may reflect nutrient enrichment associated with greater plant biomass, creating additional ecological niches for bacterial species (Han et al., 2019). In contrast, fungal diversity was not significantly affected by shrub encroachment in our study (Fig. 3b, S4), differing from reports of strong fungal responses (Li et al., 2024; Marchal et al., 2025). These discrepancies likely reflect the strong context dependency of fungal responses; here, effects were expressed mainly as shifts in community composition rather than richness.

Shrub encroachment also induced significant shifts in microbial community composition (Fig. 3c, d; Table S7, S8). In bacteria, shrub encroachment increased the relative abundance of *Proteobacteria*, *Firmicutes*, *Verrucomicrobia*, and *Latescibacteria* (Table S7). Notably, *Latescibacteria*—often associated with water- and nutrient- rich environments (Farag et al., 2017; Youssef et al., 2015)—increased significantly ($P < 0.05$), consistent with higher SWC and nutrient availability under encroachment (Fig. S2). The increase in *Proteobacteria* under SE and SSE, although not statistically significant (Table S7), aligns with reports that shrub encroachment promotes the proliferation of this phylum (Guo et al., 2022). In contrast, *Actinobacteria* and *Chloroflexi*—typically considered primary oligotrophs (Arocha-Garza et al., 2017; Liu et al., 2024)—decreased under encroachment (Table S7), likely due to improved soil resources conditions. Interestingly, no significant decrease in *Acidobacteria*, another oligotrophic group (Guo et al., 2022), was found under SE and SSE (Table S7), probably because their preference for lower soil pH (Kielak et al., 2016) promoted their growth under the observed pH decreases, offsetting the inhibitory effects of increased nutrients. In fungi, shrub encroachment significantly increased the relative abundance of *Sordariomycetes* (Table S8). As primarily copiotrophic organisms, many members of this class function as saprotrophs (Maharachchikumbura et al., 2016), which may enhance decomposition and nutrient cycling in shrub-encroached grasslands. Taken together, these findings underscore the substantial impacts of shrub encroachment on soil microbial structure, particularly through shifts in diversity and taxonomic composition.

4.3. Soil microbial network structures in response to shrub encroachment and their influence on soil multifunctionality

This study investigated the microbial mechanisms driving enhanced soil multifunctionality in shrub-encroached grasslands, with a particular focus on alternation in microbial intra- and interdomain networks. Specifically, soil bacterial network complexity and the proportion of negative links directly influenced soil multifunctionality, while the edge number of bacteria-fungi interdomain networks had an indirect effect (Fig. 6).

Although prior studies often report that shrub encroachment increases bacterial network complexity (Jiao et al., 2022; Xiang et al., 2018), we observed the opposite pattern (Table S9), contrast to our initial hypothesis. This decline may result from the enrichment of carbon and nutrients associated with shrub encroachment, which redistributes resources and creates new ecological niches (Idbella et al., 2022), thereby reshaping bacterial community composition (Guo et al., 2022). As pre-existing interactions are disrupted and replaced, the bacterial network may become less complex (Chen et al., 2022b). Additionally, complex microbial interactions are often regarded as an adaptive strategy for energy and nutrient acquisition under resource limitation (Zhai et al., 2024). When limitations are alleviated by shrub encroachment (Ding and Eldridge, 2021), the necessity for complex bacteria interactions would diminish, leading to simpler networks. This interpretation is supported by reports of a reversed V-shape relationship between soil bacterial network complexity and nutrient levels (Li et al., 2024).

The relationship between network complexity and soil multifunctionality remains unclear, particularly under shrub encroachment. Our path analysis indicated that lower bacterial network complexity was associated with higher multifunctionality (Fig. 6). Mechanistically, simpler networks may reduce cascade failure risk and dampen antagonistic feedback under disturbance (Chen et al., 2023; Lin et al., 2021), thereby potentially enhancing functional stability (Yonatan et al., 2022). Additionally, the observed negative correlation between bacterial network negative links and soil multifunctionality (Fig. 5) suggests that reduced co-exclusion (putative competitive) interactions within bacterial community can foster microbial diversity and promote soil functioning (Eldridge et al., 2017; Wang et al., 2023).

Furthermore, shrub encroachment increased the number of edges in the bacteria-fungi interdomain networks (Table S9), which indirectly enhanced soil multifunctionality (Fig. 6). This finding aligns with previous studies that emphasize the critical role of microbial interdomain interactions in promoting soil functions (Guseva et al., 2022). Bacteria and fungi play complementary roles in soil nutrient cycling, offering functional compensation (De Menezes et al., 2017). For example, fungi decompose lignocellulosic compounds into soluble sugars and phenolics that fuel bacteria (Tornberg et al., 2003), while bacteria can degrade toxic compounds that facilitate fungal growth (Boer et al., 2005). Such complementarity supports multiple dimensions of soil functioning (De Menezes et al., 2017).

Interestingly, bacterial network complexity and the proportion of negative links responded to shrub encroachment in the opposite manner compared to bacterial-fungal interdomain network complexity (Table S9). Despite this contrast, both bacterial and interdomain networks contributed to regulating soil multifunctionality (Fig. 6). These results suggest that soil bacteria adapted to increased resource availability (SWC, SOC and nutrients; Fig. S2) under shrub encroachment by shifting interaction strategies—weakening within-domain connectivity while strengthening co-occurrence with fungi (Table S9). This shift likely improved bacterial resource acquisition via functional complementarity (De Menezes et al., 2017) and potentially alleviated within-domain competition (Fig. 6). As a result, looser bacterial intradomain interactions coupled with enhanced bacteria–fungi interdomain interactions facilitated soil carbon, nitrogen, and phosphorus cycling (Chen et al., 2023; Zhou et al., 2024), ultimately contributing to higher soil multifunctionality under shrub encroachment.

Limitations should be acknowledged. First, the study was conducted at a single site in the semi-arid grasslands of the Loess Plateau. Although representative, the study site does not capture the Plateau's geomorphic, edaphic, and land-use heterogeneity; therefore, testing generality will require multi-site replication—an effort beyond this study's scope. Second, co-occurrence networks, though widely used to infer potential cooperative and competitive relationships, rely on correlations that do not necessarily reflect actual and direct interactions. Experimental validation—e.g., culture-based assays or pairwise strain-interaction tests—would strengthen network-based inferences. Despite these limitations, our results indicate that shrub encroachment, to some extent, can enhance soil multifunctionality, suggesting potential benefits for ecosystem management on the Loess Plateau. Moreover, the demonstrated microbial contribution to multifunctionality implies that microbial-based interventions (e.g., bioinoculants or microbial fertilizers) may offer practical tools for managing soil multifunctionality and ecosystem health in shrub-encroached grasslands.

5. Conclusions

This study provides comprehensive insights into the effects and mechanisms of shrub encroachment during grassland restoration succession in semi-arid ecosystems. Our findings demonstrate that shrub encroachment enhanced soil multifunctionality, increased soil bacterial diversity, and reshaped the community composition of both soil bacteria and fungi. Additionally, shrub encroachment reduced bacterial network complexity and the proportion of negative links, while increasing the number of edges in bacteria-fungi interdomain networks. These contrasting responses between intra- and interdomain networks suggest a shift in bacterial interaction strategies, which played a vital role in mediating the observed improvements in soil multifunctionality. In conclusion, our findings advance the understanding of the ecological consequences and underlying microbial mechanisms of shrub encroachment in long-term enclosed grasslands. Future research should further explore the critical role of soil microorganisms to inform effective management strategies for grasslands under shrub encroachment.

CRediT authorship contribution statement

Yudu Jing: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Xiaowei Liu:** Methodology, Investigation, Data curation. **Junhao Feng:** Methodology, Investigation. **Ke Liang:** Investigation. **Zhiyou Yuan:** Visualization, Methodology. **Jianchu Xu:** Supervision, Methodology. **Changhui Peng:** Supervision. **Qiang Yu:** Writing – review & editing. **Liang Guo:** Writing – review & editing, Supervision, Conceptualization.

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.2026.109798.

Data availability

NCBI PRJNA1234291 (Original data) (SOL Genomics)

References

Abarenkov, K., Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., May, T.W., Frøslev, T.G., Pawlowska, J., Lindahl, B., Põldmaa, K., Truong, C., Vu, D., Hosoya, T., Niskanen, T., Piirmann, T., Ivanov, F., Zirk, A., Peterson, M., Cheeke, T.E., Ishigami, Y., Jansson, A.T., Jeppesen, T.S., Kristiansson, E., Mikryukov, V., Miller, J.T., Oono, R., Ossandon, F.J., Paupério, J., Saar, I., Schigel, D., Suija, A., Tedersoo, L., Kõljalg, U., 2024. The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. Nucleic Acids Res. 52, D791–D797. https://doi.org/10.1093/nar/gkad1039.

Antiqueira, P.A.P., Petchey, O.L., Romero, G.Q., 2018. Warming and top predator loss drive ecosystem multifunctionality. Ecol. Lett. 21, 72–82. https://doi.org/10.1111/ele.12873.

Arocha-Garza, H.F., Canales-Del Castillo, R., Eguiarte, L.E., Souza, V., De La Torre-Zavala, S., 2017. High diversity and suggested endemicity of culturable Actinobacteria in an extremely oligotrophic desert oasis. PeerJ 5, e3247. https://doi.org/10.7717/peerj.3247.

Avramidis, P., Nikolaou, K., Bekiari, V., 2015. Total organic carbon and total nitrogen in sediments and soils: a comparison of the wet oxidation–titration method with the combustion-infrared method. Agr. & Agr. Sci. Procedia 4, 425–430. https://doi.org/10.1016/j.aaspro.2015.03.048.

Bai, Y., Cotrufo, M.F., 2022. Grassland soil carbon sequestration: current understanding, challenges, and solutions. Science 377, 603–608. https://doi.org/10.1126/science.abo2380.

Barberán, A., Bates, T.S., Casamayor, O.E., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J. 6, 343–351. https://doi.org/10.1038/ismej.2011.119.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. JOVE-J. Vis. Exp. 50961. https://doi.org/10.3791/50961.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B. 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13.

Boer, W.D., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev. 29, 795–811. https://doi.org/10.1016/j.femsre.2004.11.005.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842. https://doi.org/10.1016/0038-0717(85)90144-0.

Byrnes, J.E.K., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., Cardinale, B.J., Hooper, D.U., Dee, L.E., Emmett Duffy, J., 2014. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. Methods Ecol. Evol. 5, 111–124. https://doi.org/10.1111/2041-210X.12143.

Chandregowda, M.H., Murthy, K., Bagchi, S., 2018. Woody shrubs increase soil microbial functions and multifunctionality in a tropical semi-arid grazing ecosystem. J. Arid Environ. 155, 65–72. https://doi.org/10.1016/j.jaridenv.2018.02.006.

Chen, T., Liu, Y., Huang, L., 2022a. ImageGP: an easy-to-use data visualization web server for scientific researchers. iMeta 1, e5. https://doi.org/10.1002/imt2.5.

Chen, W., Wang, J., Chen, X., Meng, Z., Xu, R., Duoji, D., Zhang, J., He, J., Wang, Z., Chen, J., Liu, K., Hu, T., Zhang, Y., 2022b. Soil microbial network complexity predicts ecosystem function along elevation gradients on the Tibetan plateau. Soil Biol. Biochem. 172, 108766. https://doi.org/10.1016/j.soilbio.2022.108766.

Chen, Y., Du, Z., Weng, Z., Han Sun, K., Zhang, Y., Liu, Q., Yang, Y., Li, Y., Wang, Z., Luo, Y., Gao, B., Chen, B., Pan, Z., Van Zwieten, L., 2023. Formation of soil organic carbon pool is regulated by the structure of dissolved organic matter and microbial carbon pump efficacy: a decadal study comparing different carbon management strategies. Glob. Chang. Biol. 29, 5445–5459. https://doi.org/10.1111/gcb.16865.

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42, D633–D642. https://doi.org/10.1093/nar/gkt1244.

De Marzio, M., Glass, K., Kuijjer, M.L., 2023. Single-sample network modeling on omics data. BMC Biol. 21, 296. https://doi.org/10.1186/s12915-023-01783-z.

De Menezes, A.B., Richardson, A.E., Thrall, P.H., 2017. Linking fungal–bacterial co-occurrences to soil ecosystem function. Curr. Opin. Microbiol. 37, 135–141. https://doi.org/10.1016/j.mib.2017.06.006.

Deng, D., Sun, W., Wu, H., Yang, X., Zhu, F., Jiang, Y., Huang, S., Xue, S., Jiang, J., 2025. Plant encroachment increase multifunctionality in bauxite residue by constructing diverse and stable microbial communities. Plant and Soil 509, 105–122. https://doi.org/10.1007/s11104-024-06860-y.

Dengzeng, Z., Ma, W., Wang, C., Tang, S., Zhang, D., 2022. Effect of shrub encroachment on alpine grass soil microbial community assembly. Front. Soil Sci. 2, 829575. https://doi.org/10.3389/fsoil.2022.829575.

Ding, J., Eldridge, D.J., 2021. The fertile island effect varies with aridity and plant patch type across an extensive continental gradient. Plant and Soil 459, 173–183. https://doi.org/10.1007/s11104-020-04731-w.

Ding, L., Shang, Y., Zhang, W., Zhang, Yu, Li, S., Wei, X., Zhang, Yujun, Song, X., Chen, X., Liu, J., Yang, F., Yang, X., Zou, C., Wang, P., 2020. Disentangling the effects of driving forces on soil bacterial and fungal communities under shrub encroachment on the Guizhou plateau of China. Sci. Total Environ. 709, 136207. https://doi.org/10.1016/j.scitotenv.2019.136207.

Ding, L., Chen, H., Wang, M., Wang, P., 2024. Shrub expansion raises both aboveground and underground multifunctionality on a subtropical plateau grassland: coupling multitrophic community assembly to multifunctionality and functional trade-off. Front. Microbiol. 14, 1339125. https://doi.org/10.3389/fmicb.2023.1339125.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Eldridge, D.J., Bowker, M.A., Maestre, F.T., Roger, E., Reynolds, J.F., Whitford, W.G., 2011. Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis: synthesizing shrub encroachment effects. Ecol. Lett. 14, 709–722. https://doi.org/10.1111/j.1461-0248.2011.01630.x.

Eldridge, D.J., Soliveres, S., Bowker, M.A., Val, J., 2013. Grazing dampens the positive effects of shrub encroachment on ecosystem functions in a semi-arid woodland. J. Appl. Ecol. 50, 1028–1038. https://doi.org/10.1111/1365-2664.12105.

Eldridge, D.J., Delgado-Baquerizo, M., Travers, S.K., Val, J., Oliver, I., Hamonts, K., Singh, B.K., 2017. Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores. Ecology 98, 1922–1931. https://doi.org/10.1002/ecy.1879.

Emmett Duffy, J., Paul Richardson, J., Canuel, E.A., 2003. Grazer diversity effects on ecosystem functioning in seagrass beds. Ecol. Lett. 6, 637–645. https://doi.org/10.1046/j.1461-0248.2003.00474.x.

Farag, I.F., Youssef, N.H., Elshahed, M.S., 2017. Global distribution patterns and Pangenomic diversity of the candidate phylum “Latescibacteria” (WS3). Appl. Environ. Microbiol. 83, e00521–e00617. https://doi.org/10.1128/AEM.00521-17.

Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10, 538–550. https://doi.org/10.1038/nrmicro2832.

Feng, B., Liu, Y.Z., Liu, W.T., Lv, W.D., Sun, C.C., Yang, Z.Z., Li, C.D., Zhou, Q.Y., Wang, F.C., Yang, X.X., Dong, Q.M., 2024. Soil physicochemical properties and plant functional traits regulate ecosystem multifunctionality of alpine grassland under different livestock grazing assemblies. Agr Ecosyst Environ 366, 108947. https://doi.org/10.1016/j.agee.2024.108947.

Gamfeldt, L., Hillebrand, H., Jonsson, P.R., 2008. Multiple functions increase the importance of biodiversity for overall ecosystem functioning. Ecology 89, 1223–1231. https://doi.org/10.1890/06-2091.1.

Guo, L., Cheng, J., Luedeling, E., Koerner, S.E., He, J.S., Xu, J., Gang, C., Li, W., Luo, R., Peng, C., 2017. Critical climate periods for grassland productivity on China's loess plateau. Agric. For. Meteorol. 233, 101–109. https://doi.org/10.1016/j.agrformet.2016.11.006.

Guo, Q., Wen, Z., Ghanizadeh, H., Zheng, C., Fan, Y., Yang, X., Yan, X., Li, W., 2022. Shift in microbial communities mediated by vegetation-soil characteristics following subshrub encroachment in a semi-arid grassland. Ecol. Indic. 137, 108768. https://doi.org/10.1016/j.ecolind.2022.108768.

Guseva, K., Darcy, S., Simon, E., Alteio, L.V., Montesinos-Navarro, A., Kaiser, C., 2022. From diversity to complexity: microbial networks in soils. Soil Biol. Biochem. 169, 108604. https://doi.org/10.1016/j.soilbio.2022.108604.

Han, X., Ren, C., Li, B., Yan, S., Fu, S., Gao, D., Zhao, F., Deng, J., Yang, G., 2019. Growing seasonal characteristics of soil and plants control the temporal patterns of bacterial communities following afforestation. Catena 178, 288–297. https://doi.org/10.1016/j.catena.2019.03.021.

Hooper, D.U., Vitousek, P.M., 1998. Effects of plant composition and diversity on nutrient cycling. Ecological monographs 68, 121–149. https://doi.org/10.1890/0012-9615(1998)068.

Hu, X., Li, X.Y., Zhao, Y., Gao, Z., Zhao, S.J., 2021. Changes in soil microbial community during shrub encroachment process in the Inner Mongolia grassland of northern China. Catena 202, 105230. https://doi.org/10.1016/j.catena.2021.105230.

Idbella, M., De Filippis, F., Zotti, M., Sequino, G., Abd-ElGawad, A.M., Fechtali, T., Mazzoleni, S., Bonanomi, G., 2022. Specific microbiome signatures under the canopy of Mediterranean shrubs. Appl. Soil Ecol. 173, 104407. https://doi.org/10.1016/j.apsoil.2022.104407.

Jiao, S., Lu, Y., 2020. Soil pH and temperature regulate assembly processes of abundant and rare bacterial communities in agricultural ecosystems. Environ. Microbiol. 22, 1052–1065. https://doi.org/10.1111/1462-2920.14815.

Jiao, S., Lu, Y., Wei, G., 2022. Soil multitrophic network complexity enhances the link between biodiversity and multifunctionality in agricultural systems. Glob. Chang. Biol. 28, 140–153. https://doi.org/10.1111/gcb.15917.

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991–999. https://doi.org/10.1016/j.soilbio.2005.08.012.

Kalra, Y.P., 1995. Determination of pH of soils by different methods: collaborative study. J. AOAC Int. 78, 310–324. https://doi.org/10.1093/jaoac/78.2.310.

Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., Van Veen, J.A., Kuramae, E.E., 2016. The ecology of Acidobacteria: moving beyond genes and genomes. Front. Microbiol. 7, 744. https://doi.org/10.3389/fmicb.2016.00744.

Li, H., Shen, H., Zhou, L., Zhu, Y., Chen, L., Hu, H., Zhang, P., Fang, J., 2019. Shrub encroachment increases soil carbon and nitrogen stocks in temperate grasslands in China. Land Degrad. Dev. 30, 756–767. https://doi.org/10.1002/ldr.3259.

Li, G., Niu, W., Sun, J., Zhang, W., Zhang, E., Wang, J., 2021. Soil moisture and nitrogen content influence wheat yield through their effects on the root system and soil bacterial diversity under drip irrigation. Land Degrad. Dev. 32, 3062–3076. https://doi.org/10.1002/ldr.3967.

Li, W., Shi, F., Yi, S., Feng, T., Wang, C., Li, Z., Zheng, W., Zhai, B., 2024. Soil multifunctionality predicted by bacterial network complexity explains differences in wheat productivity induced by fertilization management. Eur. J. Agron. 153, 127058. https://doi.org/10.1016/j.eja.2023.127058.

Lin, Q., Dini-Andreote, F., Li, L., Umari, R., Novotny, V., Kukla, J., Heděnec, P., Frouz, J., 2021. Soil microbial interconnections along ecological restoration gradients of lowland forests after slash-and-burn agriculture. FEMS Microbiol. Ecol. 97, fiab063. https://doi.org/10.1093/femsec/fiab063.

Liu, Y., Chen, Lei, Ma, T., Li, X., Zheng, M., Zhou, X., Chen, Liang, Qian, X., Xi, J., Lu, H., Cao, H., Ma, X., Bian, B., Zhang, P., Wu, J., Gan, R., Jia, B., Sun, L., Ju, Z., Gao, Y., Wen, T., Chen, T., 2023. EasyAmplicon: an easy-to-use, open-source, reproducible, and community-based pipeline for amplicon data analysis in microbiome research. iMeta 2, e83. https://doi.org/10.1002/imt2.83.

Liu, J., Peng, Z., Tu, H., Qiu, Y., Liu, Y., Li, X., Gao, H., Pan, H., Chen, B., Liang, C., Chen, S., Qi, J., Wang, Y., Wei, G., Jiao, S., 2024. Oligotrophic microbes are recruited to resist multiple global change factors in agricultural subsoils. Environ. Int. 183, 108429. https://doi.org/10.1016/j.envint.2024.108429.

Maharachchikumbura, S.S.N., Hyde, K.D., Jones, E.B.G., McKenzie, E.H.C., Bhat, J.D., Dayarathne, M.C., Huang, S.K., Norphanphoun, C., Senanayake, I.C., Perera, R.H., Shang, Q.J., Xiao, Y., D'souza, M.J., Hongsanan, S., Jayawardena, R.S., Daranagama, D.A., Konta, S., Goonasekara, I.D., Zhuang, W.Y., Jeewon, R., Phillips, A.J.L., Abdel-Wahab, M.A., Al-Sadi, A.M., Bahkali, A.H., Boonmee, S., Boonyuen, N., Cheewangkoon, R., Dissanayake, A.J., Kang, J., Li, Q.R., Liu, J.K., Liu, X.Z., Liu, Z.Y., Luangsa-ard, J.J., Pang, K.L., Phookamsak, R., Promputtha, I., Suetrong, S., Stadler, M., Wen, T., Wijayawardene, N.N., 2016. Families of Sordariomycetes. Fungal Divers. 79, 1–317. https://doi.org/10.1007/s13225-016-0369-6.

Moreno-Arrones, O.M., Serrano-Villar, S., Perez-Brocal, V., Saceda-Corralo, D., Morales-Raya, C., Rodrigues-Barata, R., Moya, A., Jaen-Olasolo, P., Vano-Galvan, S., 2020. Analysis of the gut microbiota in alopecia areata: identification of bacterial biomarkers. J. Eur. Acad. Dermatol. 34, 400–405. https://doi.org/10.1111/jdv.15885.

Naz, M., Dai, Z., Hussain, S., Tariq, M., Danish, S., Khan, I.U., Qi, S., Du, D., 2022. The soil pH and heavy metals revealed their impact on soil microbial community. J. Environ. Manage. 321, 115770. https://doi.org/10.1016/j.jenvman.2022.115770.

Pasquali, C.L., Hernando, P.F., Alegría, J.D., 2007. Spectrophotometric simultaneous determination of nitrite, nitrate and ammonium in soils by flow injection analysis. Anal. Chim. Acta 600, 177–182. https://doi.org/10.1016/j.aca.2007.03.015.

Qiu, T., Peñuelas, J., Chen, Y., Sardans, J., Yu, J., Xu, Z., Cui, Q., Liu, J., Cui, Y., Zhao, S., Chen, J., Wang, Y., Fang, L., 2024. Arbuscular mycorrhizal fungal interactions bridge the support of root-associated microbiota for slope multifunctionality in an erosion-prone ecosystem. iMeta 3, e187. https://doi.org/10.1002/imt2.187.

Quero, J.L., Maestre, F.T., Ochoa, V., García-Gómez, M., Delgado-Baquerizo, M., 2013. On the importance of shrub encroachment by sprouters, climate, species richness and anthropic factors for ecosystem multifunctionality in semi-arid Mediterranean ecosystems. Ecosystems 16, 1248–1261. https://doi.org/10.1007/s10021-013-9683-y.

Reynolds, S.G., 1970. The gravimetric method of soil moisture determination part IA study of equipment, and methodological problems. J. Hydrol. 11, 258–273. https://doi.org/10.1016/0022-1694(70)90066-1.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.

Sanchez, G., Trinchera, L., Giorgio, R., Bertrand, F., 2024. Partial Least Squares Path Modeling (PLS-PM), R package version 0.5.1. doi:10.32614/CRAN.package.plspm.

Sandor, R., Barcza, Z., Hidy, D., Lellei-Kovács, E., Ma, S., Bellocchi, G., 2016. Modelling of grassland fluxes in Europe: evaluation of two biogeochemical models. Agr Ecosyst Environ 215, 1–19. https://doi.org/10.1016/j.agee.2015.09.001.

Schimel, D.S., Parton, W.J., Kittel, T.G.F., Ojima, D.S., Cole, C.V., 1990. Grassland biogeochemistry: links to atmospheric processes. Clim. Change 17, 13–25. https://doi.org/10.1007/BF00148998.

Soliveres, S., Maestre, F.T., Eldridge, D.J., Delgado-Baquerizo, M., Quero, J.L., Bowker, M.A., Gallardo, A., 2014. Plant diversity and ecosystem multifunctionality peak at intermediate levels of woody cover in global drylands. Glob. Ecol. Biogeogr. 23, 1408–1416. https://doi.org/10.1111/geb.12215.

Tornberg, K., Bååth, E., Olsson, S., 2003. Fungal growth and effects of different wood decomposing fungi on the indigenous bacterial community of polluted and unpolluted soils. Biol. Fert. Soils 37, 190–197. https://doi.org/10.1007/s00374-002-0574-1.

Valencia, E., Maestre, F.T., Le Bagousse-Pinguet, Y., Quero, J.L., Tamme, R., Börger, L., García-Gómez, M., Gross, N., 2015. Functional diversity enhances the resistance of ecosystem multifunctionality to aridity in Mediterranean drylands. New Phytol. 206, 660–671. https://doi.org/10.1111/nph.13268.

Van Auken, O.W., 2009. Causes and consequences of woody plant encroachment into western north American grasslands. J. Environ. Manage. 90, 2931–2942. https://doi.org/10.1016/j.jenvman.2009.04.023.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6.

Wang, D., Wu, G.L., Zhu, Y.J., Shi, Z.H., 2014. Grazing exclusion effects on above- and below-ground C and N pools of typical grassland on the loess plateau (China). Catena 123, 113–120. https://doi.org/10.1016/j.catena.2014.07.018.

Wang, X., Zhang, Q., Zhang, Z., Li, W., Liu, W., Xiao, N., Liu, H., Wang, L., Li, Z., Ma, J., Liu, Q., Ren, C., Yang, G., Zhong, Z., Han, X., 2023. Decreased soil multifunctionality is associated with altered microbial network properties under precipitation reduction in a semiarid grassland. iMeta 2, e106. https://doi.org/10.1002/imt2.106.

Wang, K., Xue, K., Liu, W., Zhang, B., Wu, W., Zhao, R., Cui, L., Wang, Z., Zhou, S., Tang, L., Dong, J., Du, J., Hao, Y., Wang, S., Wang, Y., 2024. Warming decouples associations between microbial network complexity and ecosystem multifunctionality in alpine grasslands. Agr Ecosyst Environ 374, 109189. https://doi.org/10.1016/j.agee.2024.109189.

Wen, T., Xie, P., Yang, S., Niu, G., Liu, X., Ding, Z., Xue, C., Liu, Y., Shen, Q., Yuan, J., 2022. ggClusterNet: an R package for microbiome network analysis and modularity-based multiple network layouts. iMeta 1, e32. https://doi.org/10.1002/imt2.32.

Wolf, A.M., Baker, D.E., 1990. Colorimetric method for phosphorus measurement in ammonium oxalate soil extracts. Commun. Soil Sci. Plan. 21, 2257–2263. https://doi.org/10.1080/00103629009368378.

Wu, H., Cui, H., Fu, C., Li, R., Qi, F., Liu, Z., Yang, G., Xiao, K., Qiao, M., 2024a. Unveiling the crucial role of soil microorganisms in carbon cycling: a review. Sci. Total Environ. 909, 168627. https://doi.org/10.1016/j.scitotenv.2023.168627.

Wu, G.L., Liu, Y., Wang, D., Zhao, J., 2024b. Divergent successions to shrubs- and forbs-dominated meadows decrease ecosystem multifunctionality of hillside alpine meadow. Catena 236, 107718. https://doi.org/10.1016/j.catena.2023.107718.

Xiang, X., Gibbons, S.M., Li, H., Shen, H., Fang, J., Chu, H., 2018. Shrub encroachment is associated with changes in soil bacterial community composition in a temperate grassland ecosystem. Plant and Soil 425, 539–551. https://doi.org/10.1007/s11104-018-3605-x.

Xiao, Y., Wang, J., Wang, B., Fan, B., Zhou, G., 2025. Soil microbial network complexity predicts soil multifunctionality better than soil microbial diversity during grassland-farmland-shrubland conversion on the Qinghai-Tibetan plateau. Agr Ecosyst Environ 379, 109356. https://doi.org/10.1016/j.agee.2024.109356.

Yang, W., Qu, G., Kelly, A.R., Wu, G.L., Zhao, J., 2024. Positive effects of leguminous shrub encroachment on multiple ecosystem functions of alpine meadows and steppes greatly depended on increasing soil nutrient. Catena 236, 107745. https://doi.org/10.1016/j.catena.2023.107745.

Yonatan, Y., Amit, G., Friedman, J., Bashan, A., 2022. Complexity–stability trade-off in empirical microbial ecosystems. Nat. Ecol. Evol. 6, 693–700. https://doi.org/10.1038/s41559-022-01745-8.

Youssef, N.H., Farag, I.F., Rinke, C., Hallam, S.J., Woyke, T., Elshahed, M.S., 2015. In silico analysis of the metabolic potential and niche specialization of candidate phylum “Latescibacteria” (WS3). PloS One 10, e0127499. https://doi.org/10.1371/journal.pone.0127499.

Yu, Y., Zhao, W., Martinez-Murillo, J.F., Pereira, P., 2020. Loess plateau: from degradation to restoration. Sci. Total Environ. 738, 140206. https://doi.org/10.1016/j.scitotenv.2020.140206.

Yuan, M.M., Guo, X., Wu, L., Zhang, Y., Xiao, N., Ning, D., Zhou, X., Wu, L., Yang, Y., Tiedje, M.J., Zhou, J., 2021. Climate warming enhances microbial network complexity and stability. Nat. Clim. Chang. 11, 343–348. https://doi.org/10.1038/s41558-021-00989-9.

Yue, Y., Lai, L., Zhou, J., Wang, G., Zhu, Y., Chen, Q., Zheng, Y., 2025. Decoupled response of aboveground and belowground ecosystem multifunctionality to shrub encroachment in a semiarid grassland. J. Environ. Manage. 379, 124827. https://doi.org/10.1016/j.jenvman.2025.124827.

Zhai, C., Han, L., Xiong, C., Ge, A., Yue, X., Li, Y., Zhou, Z., Feng, J., Ru, J., Song, J., Jiang, L., Yang, Y., Zhang, L., Wan, S., 2024. Soil microbial diversity and network complexity drive the ecosystem multifunctionality of temperate grasslands under changing precipitation. Sci. Total Environ. 906, 167217. https://doi.org/10.1016/j.scitotenv.2023.167217.

Zhang, Y., Chang, X., Zhang, Yuwei, Wilkes, A., Wang, G., 2024. Litter accumulation suppresses grass production but facilitates shrub expansion in a long-term fenced grassland. Ecosphere 15, e4844. https://doi.org/10.1002/ecs2.4844.

Zhao, G., Liu, L., Wang, Z.Y., Jin, Z., He, J.S., 2023a. Grassland science in a new era. Fundam. Res. 3, 149–150. https://doi.org/10.1016/j.fmre.2023.02.001.

Zhao, J., Yang, W., JiShi, A., Ma, Y., Tian, L., Li, R., Huang, Z., Liu, Y.F., Leite, P.A.M., Ding, L., Wu, G.L., 2023b. Shrub encroachment increases soil carbon and nitrogen stocks in alpine grassland ecosystems of the central Tibetan plateau. Geoderma 433, 116468. https://doi.org/10.1016/j.geoderma.2023.116468.

Zhou, L., Liu, S., Lin, D., Hu, H.W., He, J.Z., 2024. Divergent changes in diversity and network complexity across different trophic-level organisms drive soil multifunctionality of fire-impacted subtropical forests. For. Ecosyst. 11, 100227. https://doi.org/10.1016/j.fecs.2024.100227.