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Effects of Relative Precipitation Changes on Soil Microbial Community Structure in Two Alpine Grassland Ecosystems

Jianyu Xiao [1,2,†], Zhishu Wang [1,2,†], Fusong Han [3], Shaolin Huang [4], Chengqun Yu [1,*] and Gang Fu [1,*]

[1] Lhasa Plateau Ecosystem Research Station, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China; xiaojianyu0096@igsnrr.ac.cn (J.X.); wangzhishu221@mails.ucas.ac.cn (Z.W.)
[2] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China
[3] College of Urban and Environment Sciences, Hunan University of Technology, Zhuzhou 412007, China; hanfs.17b@ignsrr.ac.cn
[4] College of Tourism, Henan Normal University, Xinxiang 453007, China; huangshaolin@htu.edu.cn
* Correspondence: yucq@igsnrr.ac.cn (C.Y.); fugang@igsnrr.ac.cn (G.F.)
† These authors contributed equally to this work.

Abstract: Precipitation variability profoundly influences soil microbial diversity, community assembly processes, and co-occurrence networks. However, the responses of soil microbial structure to relative precipitation changes in alpine regions remain uncertain. To address this, we conducted a two-year field precipitation manipulation experiment in alpine steppe and alpine desert steppe ecosystems at the source of the Yarlung Zangbo River on the Tibetan Plateau. The experiment simulated 25%, 50%, and 75% increases and decreases in precipitation to examine how soil microbial communities respond to altered precipitation regimes. Our results reveal that microbial responses varied with precipitation magnitude, grassland type, and microbial kingdom. In the alpine steppe, bacterial α-diversity exhibited a negative asymmetric response to altered precipitation at both species and phylogenetic levels. Both bacterial and fungal species α-diversity tended to respond more strongly to changes in precipitation at high gradients in the alpine steppe than in the alpine desert steppe. Microbial co-occurrence networks in the alpine steppe were generally more responsive to altered precipitation than those in the alpine desert steppe. Furthermore, fungal α-diversity at both species and phylogenetic levels, as well as β-diversity, responded more strongly to altered precipitation than bacterial communities. These findings suggest that precipitation-driven shifts in microbial community composition and network structure vary across alpine grassland ecosystems, with fungal communities exhibiting greater sensitivity than bacterial communities. As warming intensifies precipitation variability, these microbial shifts may have cascading effects on soil biogeochemical processes and ecosystem stability, underscoring the necessity for ecosystem-specific conservation frameworks and adaptive management strategies tailored to alpine grasslands.

Keywords: biodiversity; precipitation changes; soil microbes; community assembly; co-occurrence network; alpine ecosystems

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Academic Editor: Juan Antonio López González

Received: 24 February 2025
Revised: 25 March 2025
Accepted: 26 March 2025
Published: 29 March 2025

Citation: Xiao, J.; Wang, Z.; Han, F.; Huang, S.; Yu, C.; Fu, G. Effects of Relative Precipitation Changes on Soil Microbial Community Structure in Two Alpine Grassland Ecosystems. *Agronomy* **2025**, *15*, 851. https://doi.org/10.3390/agronomy15040851

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1. Introduction

Since the onset of the Anthropocene era, global warming has accelerated the hydrological cycle, resulting in significant changes in precipitation patterns that have profoundly impacted biodiversity [1,2]. Soil microorganisms, critical for supporting ecosystem functions, are especially vulnerable to changes in water availability [3–5]. Although numerous studies have explored the response of soil microbial communities in terrestrial ecosystems

to precipitation changes [6–8], several key ambiguities remain unresolved. Firstly, the double asymmetric model indicates that a drastic decrease in precipitation has a stronger impact on terrestrial productivity than an increase, a pattern known as negative asymmetry [9,10]. Although this model has been validated for various soil parameters, including respiration, extracellular enzyme activity, microbial biomass, etc., its applicability to soil microbial diversity remains uncertain [11,12]. Secondly, the biodiversity-stability hypothesis suggests that greater biodiversity enhances ecosystem stability under environmental fluctuations [13,14]. While widely supported in plant ecology, its applicability to microbial communities remains contentious. For instance, microbial communities in low-diversity ecosystems, such as arid or sparsely vegetated regions, demonstrate high resilience due to efficient resource utilization and specialized adaptations to extreme conditions [15,16]. Thirdly, there is ongoing debate regarding the relative sensitivity of soil fungal and bacterial communities to precipitation changes. Fungal communities are often considered more stable under moisture fluctuations, due to their hyphal structures and slower growth rates [17,18]. Conversely, emerging studies reveal that bacterial communities may exhibit higher resistance to environmental changes in certain contexts, attributed to their functional redundancy and metabolic diversity [19–21]. In arid ecosystems, the scarcity of baseline water resources likely amplifies the sensitivity of both groups, complicating efforts to identify distinct response patterns. Consequently, more field studies, particularly in arid regions where ecosystems are vulnerable to moisture fluctuations, are essential to clarify the impacts of precipitation changes on soil microbial community structure.

The Tibetan Plateau, highly sensitive to climate change, experiences significant interannual variability in precipitation, with an increase in extreme events anticipated during the 21st century [22,23]. Field experiments have been established to explore how shifts in precipitation impact alpine ecosystems [24,25]. Nonetheless, several issues persist in current research. One major limitation is that while alpine meadows, steppes, and desert steppes extend from the eastern to the western plateau, most studies have focused on the central and eastern regions. No on-site research has assessed the effects of altered precipitation on soil microbial communities in the alpine desert steppes of this region [26]. This oversight may underestimate the impact on the entire plateau, as grasslands in the western plateau, with lower biodiversity and harsher climatic conditions, could be more vulnerable to external disturbances [27]. Another unresolved issue is the ongoing debate over whether biodiversity in arid or semi-arid areas is primarily influenced by increases or decreases in precipitation [9,28]. While most experiments on the plateau have been centered on increased precipitation, studies exploring variations in precipitation, including both increases and decreases, are very limited [25,29]. These uncertainties hinder the ability to anticipate how soil microbial diversity in alpine ecosystems will react to varying precipitation patterns. Additionally, the resistance of soil microorganisms to drought is influenced by the extent of drought [16,18]. Inconsistencies in precipitation change gradients, experiment duration, and microbial testing methods limit the comparability of soil microbial community responses across various grassland types on the plateau [22,30]. Furthermore, current research on the plateau has primarily focused on species-level responses, with limited attention given to multidimensional microbial responses such as functional and phylogenetic diversity [19,31]. The relative influence of environmental factors and microbial networks on community structure may differ across these dimensions [32,33]. Therefore, it is necessary to explore how these factors contribute to soil microbial community composition across multiple dimensions under changing precipitation scenarios.

The Yarlung Zangbo River originates in the ecotone between arid and semi-arid regions of the western Tibetan Plateau, which encompasses both alpine steppes and desert steppes. This region is critical for the ecological security of the plateau, downstream water

resource security, and maintaining Asia's ecological balance [34]. Compared to the eastern plateau, the grasslands here are situated at a higher altitude, possess poorer and drier soils, and are more sensitive to external disturbances, making them ideal for studying response mechanisms to global change [34,35]. Hence, this study established a single-factor precipitation manipulation experiment separately in an alpine steppe and an alpine desert steppe within this area to test three hypotheses: (i) Soil microbial diversity exhibits a negative asymmetry in response to relative precipitation changes. (ii) Soil microbial diversity and co-occurrence networks in the alpine desert steppe show higher sensitivity to relative precipitation changes than those in the alpine steppe. (iii) Soil bacterial communities are more sensitive to altered precipitation than fungal communities.

2. Materials and Methods

2.1. Study Site

The study was conducted in the source area of the Yarlung Zangbo River, situated in Zhongba County, Tibet Autonomous Region, China. The two experimental sites consist of an alpine steppe (29°37′ N, 84°22′ E, 4750 m above sea level) and an alpine desert steppe (30°07′ N, 83°24′ E, 4620 m above sea level), separated by approximately 110 km (Figure S1). Annual temperatures average 2.60 °C in the alpine steppe and 4.01 °C in the desert steppe, with annual precipitation totals of 391.41 mm and 269.88 mm (Table S1), respectively. Soil composition differs between the two ecosystems. The alpine steppe soil is characterized by Cryosols or Leptosols [36], with a heterogeneous mixture of sand, clay, and loam, and a higher organic carbon content (Table S7) than the alpine desert steppe. In contrast, the alpine desert steppe soil consists predominantly of Leptosols, which are sandy loam with lower water-retention capacity. Vegetation also varies between the sites. The alpine steppe's dominant plant species include *Potentilla bifurca*, along with *Stipa purpurea*, *Microula tibetica*, *Carex atrofusca*, *Heteropappus semiprostratus*, *Chenopodium glaucum*, *Carex thibetica*, *Rhodiola bupleuroides*, and *Oxytropis*. The *Stipa purpurea* community predominates in the alpine desert steppe, with associated plants including *Carex moorcroftii*, *Heteropappus semiprostratus*, *Microula tibetica*, *Oxytropis*, and *Incarvillea younghusbandii*. Soil texture varies slightly between the sites. Livestock grazing has been prohibited at both sites since 2021, the year before the experiment began.

2.2. Experimental Design

The two steppes investigated are integral components of the Zhongba long-term fixed observation experimental platform. Considering prior studies on the eastern plateau and precipitation variability at the study site over the last 20 years [37,38], a precipitation manipulation experiment with seven gradients was conducted at both experimental sites. This experiment manipulated precipitation levels by implementing 25%, 50%, and 75% increases (IP25, IP50, IP75) and decreases (DP25, DP50, DP75) relative to baseline natural precipitation (CK). A completely randomized design was used at each site, where seven precipitation gradients were established to simulate varied rainfall conditions. Within each gradient, five replicates were randomly assigned, resulting in a total of 35 quadrats per site, each measuring 4 m × 4 m. To minimize potential edge effects between plants and to ensure proper water infiltration, a 3 m interval was maintained between adjacent quadrants.

Passive rainfall shelters were set up on each plot in May 2022 to establish the precipitation gradient. The shelters were constructed with metal frames and V-shaped PVC boards that are UV-transparent (>90% light permeability), angled at 10°, and varying in height from 1.5 m to 1.85 m (Figure S2a). They were designed to intercept the rainwater effectively. Decreased precipitation treatments were achieved by covering 25%, 50%, and 75% of the shelter's top area with transparent panels to ensure minimal light blockage for herbs.

The trapped rainwater was collected in 20 cm × 20 cm grooves, channeled via pipes to plots with increased precipitation, and dispersed using small apertures to simulate natural throughfall on a similar scale (Figure S2b). Regular maintenance of shelters, grooves, and pipes was conducted to prevent blockages from litter or debris.

2.3. Vegetation Survey and Soil Sampling

Vegetation assessments for each plot were performed in late August 2023 using a 50 cm × 50 cm quadrat to assess species composition, after two growing seasons since the experiment began. Aboveground plant materials were harvested and dried at 65 °C until reaching a constant weight, after which aboveground biomass (AGB) was measured [39]. Seventy topsoil samples, each from a depth of 0–10 cm, were gathered from the two steppes. Each sample was obtained by combining three soil cores, each with a diameter of 3.8 cm. Subsamples for soil DNA extraction were kept at −80 °C, while those for soil physical and chemical properties analyses were stored at 4 °C.

Soil bulk density (BD) was measured by sampling topsoil with a 100 cm^3 ring sampler, with samples dried at 105 °C. Soil water content (SWC) was assessed by drying field-fresh soils at 105 °C for 48 h. Soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) were analyzed using potassium dichromate oxidation, Kjeldahl, and molybdenum-antimony colorimetry methods, respectively. Nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) were quantified with a LACHAT Quikchem Automated Ion Analyzer, while available phosphorus (AP) was measured using ammonium bicarbonate extraction followed by molybdenum–antimony colorimetry. Dissolved organic carbon (DIC) and nitrogen (DIN) were analyzed using a TOC-L analyzer (Shimadzu Inc., Kyoto, Japan).

2.4. DNA Extraction and Bioinformatics Analysis

DNA was extracted from 0.5 g of soil using a soil DNA isolation kit. The V3–V4 regions of bacterial 16S rRNA genes were amplified with the barcoded primer pair 338F/806R, while fungal ITS1 regions were targeted using the ITS1F/ITS2 primers [40,41]. PCR amplification was performed in a reaction mixture containing 25 ng of template DNA, 2.5 μL of each primer, 12.5 μL of PCR Premix, and PCR-grade water to a final volume of 25 μL. The amplification began with a 5 min denaturation at 95 °C, followed by 25 cycles of 30 s at 95 °C for denaturation, 30 s at 50 °C for annealing, and 40 s at 72 °C for extension, with a final 7 min extension at 72 °C. PCR products were purified using e.Z.N.A Cycle-Pure Kits (Omega Bio-tek Inc., Norcross, GA, USA). Amplicons were sequenced using the Illumina Novaseq 6000 platform (Illumina Inc., San Diego, CA, USA). Initial quality filtering was conducted with Trimmomatic (v0.33) to obtain high-quality clean tags [42]. The paired-end reads were assembled using USEARCH (v10), with chimeric sequences removed by UCHIME (v8.1) [43]. Amplicon sequence variants (ASVs) were generated by denoising sequences with the DADA2 method in QIIME2 (v2020.06) [44,45].

2.5. Statistical Analyses

Microbial α-diversity indices for the species dimension (Sobs: observed species richness, Chao1, ACE, and species Shannon index) and functional dimension (Guild: functional guild numbers, functional Shannon index) were computed with the 'microeco' package [46]. Microbial α-diversity indices for the phylogenetic dimension (PD: Faith's phylogenetic diversity, MNTD: mean nearest taxon distance) were computed with the 'picante' package [46]. Microbial species and functional β-diversity indices were obtained with the 'vegan' package [47], while phylogenetic β-diversity (βMNTD) was obtained with the 'iCAMP' [48]. To evaluate the effect size of altered precipitation on specific variables, the response ratio (R) was computed as R = Pi/P0, where Pi represents the precipitation change treatment and

P0 is the control treatment [7]. Deviations in the effect size from 1 indicate the strength of the response, with larger deviations signifying greater sensitivity to precipitation changes.

The beta Nearest Taxon Index (βNTI) assessed the deviation between observed βMNTD and average βMNTD derived from a null model [34,49]. This metric supports determining whether the formation of microbial communities is driven more by deterministic processes (e.g., homogeneous or heterogeneous selection) or stochastic processes (e.g., dispersal limitation, homogenizing dispersal, drift, and others) [48,50]. The proportions of the five assembly processes in soil microbial communities were determined with the 'iCAMP'.

Co-occurrence network analysis was performed to quantify species interactions across seven distinct precipitation gradients. Spearman's correlation analysis was conducted on the ASV abundance tables with the 'Hmisc' package, producing correlation coefficients and corresponding *p* values. Random matrix theory (RMT) was employed to set the thresholds for R values in network construction [51]. Four comprehensive co-occurrence networks representing the interactions within soil bacterial and fungal communities in the two steppes were constructed with the 'igraph' package [52]. Sub-network properties were analyzed with the 'igraph', while Gephi software (v0.9.7) was used for network visualization.

Tukey's HSD test assessed the effect of altered precipitation on microbial diversity, assembly processes, and network topology attributes across different precipitation gradients for each grassland type. T-tests compared these parameters across the two ecosystems (alpine steppe and alpine desert steppe) and between microbiota types (bacteria and fungi) within each treatment. The influence of each environmental factor on α-diversity was determined using the 'RandomForest' package [53]. The relative effects of environmental variables and co-occurrence networks on β-diversity were determined using variation partitioning analysis from the 'vegan' package. Statistical analyses and bar graph generation for this study were performed using R software (v4.4.1).

3. Results

3.1. α-Diversity

In the alpine steppe, bacterial α-diversity effect sizes at both the species (Sobs, Chao1, and ACE) and phylogenetic levels (PD, MNTD) increased along the precipitation gradient (Figures 1 and 2). This trend aligned with absolute values of bacterial species and phylogenetic α-diversity, which also increased with precipitation (Figures S3 and S4). However, bacterial diversity exhibited a negative asymmetric response to altered precipitation: decreased precipitation treatments (DP50, DP75) resulted in stronger negative effects compared to the weaker positive effects observed under increased precipitation treatments (IP50, IP75). Functional diversity did not exhibit this asymmetry. The absolute values of diversity further confirmed this trend. For example, compared to the CK treatment, Sobs and PD decreased by 7.25% and 10.90%, respectively, under DP75, whereas under IP75, they increased only slightly, by 2.23% and 1.87%, respectively.

Although bacterial α-diversity in the alpine steppe was significantly lower than in the desert steppe only under DP50 treatment (Figure S3), its overall sensitivity to altered precipitation was higher than that of the desert steppe (Figures 1 and 2). For instance, the negative effect sizes of bacterial richness indices (Sobs, Chao1, and ACE) under the DP75 and DP50 treatments were more pronounced in the alpine steppe compared to those in the desert steppe. Similarly, the bacterial functional Shannon index showed a greater negative effect size in the alpine steppe compared to the desert steppe across most treatments (Figure 2).

Fungal species and phylogenetic α-diversity in the alpine steppe were more sensitive to altered precipitation than those of bacteria, as evidenced by stronger negative effect sizes

for the bacterial species Shannon index and MNTD than fungi across most precipitation gradients (Figures S5 and S6). Similarly, soil fungi exhibited greater sensitivity in functional Shannon index than bacteria across some treatments in the alpine desert steppe (Figure S6).

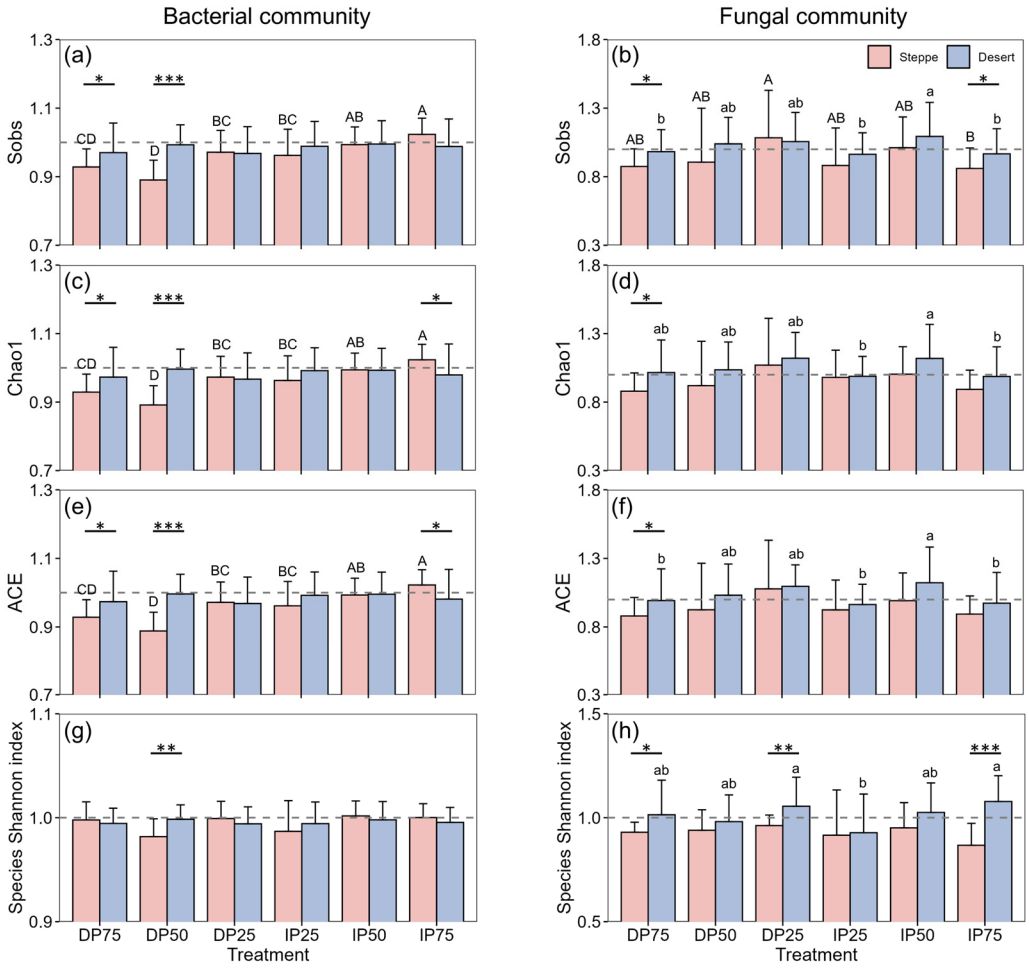


Figure 1. Comparison of effect sizes of altered precipitation on bacterial (**a**,**c**,**e**,**g**) and fungal (**b**,**d**,**f**,**h**) species α-diversity. Significant differences are denoted by uppercase letters for the alpine steppe and lowercase letters for the alpine desert steppe. Differences between the two steppes at each treatment are marked as *, **, and *** for *p*-values of less than 0.05, 0.01, and 0.001, respectively. Sobs: number of observed species; Chao1: species Chao1 index; ACE: species ACE index. DP75, DP50, and DP25 indicate decreases of 75%, 50%, and 25% in natural baseline rainfall, while IP75, IP50, and IP25 indicate corresponding increases.

3.2. β-Diversity

The species and functional β-diversity of the fungal community in the alpine steppe were lowest under the DP75 precipitation gradient (Figure 3a–f). The functional β-diversity of the bacterial community, along with the species and phylogenetic β-diversity of the fungal community in the alpine steppe, was higher in comparison to those observed in the desert steppe across various precipitation gradients. Overall, soil fungi exhibited higher species, functional, and phylogenetic β-diversity than bacteria across all precipitation gradients in both steppes (Figure S7).

Altered precipitation did not impact species, functional, or phylogenetic composition (Table S2). Across the seven treatments, the bacterial community in the alpine steppe was dominated by *Proteobacteria* (28.00%), *Acidobacteriota* (17.85%), *Actinobacteriota* (17.12%), *Bacteroidota* (10.59%), and *Gemmatimonadota* (10.56%) (Figure S8a). In the alpine desert steppe, the main bacterial phyla were Proteobacteria (24.96%), *Actinobacteriota* (22.50%),

Acidobacteriota (16.28%), *Gemmatimonadota* (10.30%), and *Chloroflexi* (7.66%) (Figure S8c). Fungal communities in both ecosystems were predominantly composed of *Ascomycota* and *Basidiomycota*, accounting for 79.69% in the alpine steppe and 84.76% in the desert steppe (Figure S8b,d). The number of common species identified across treatments was 563 for bacterial communities in the alpine steppe, 702 for bacterial communities in the desert steppe, 225 for fungal communities in the alpine steppe, and 184 for fungal communities in the desert steppe (Figure S9). The total number of taxa differing among the seven treatments was 21 for bacterial communities in the alpine steppe, 3 for those in the desert steppe, and 8 for fungal communities in both ecosystems (Tables S3 and S4).

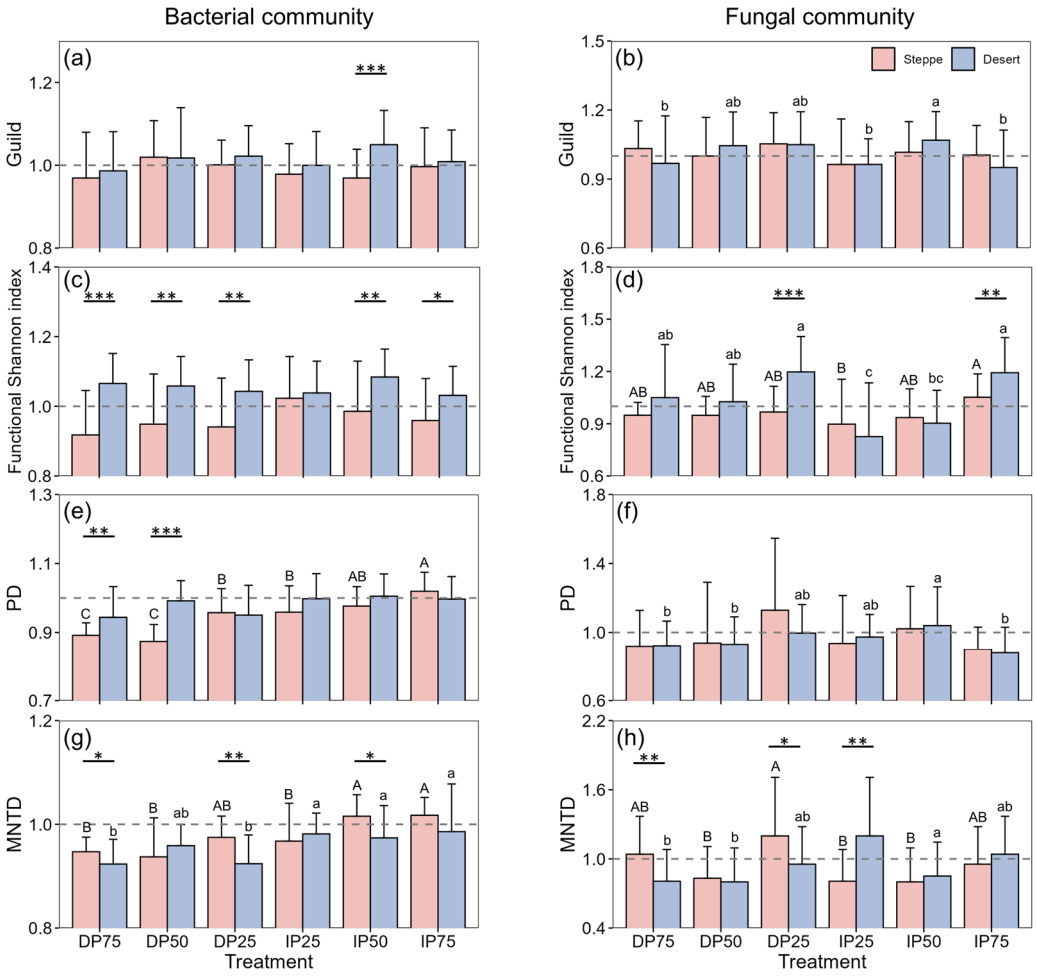


Figure 2. Comparison of effect sizes of altered precipitation on functional (**a**–**d**) and phylogenetic α-diversity (**e**–**h**). Differences between the two steppes at each treatment are marked as *, **, and *** for *p*-values of less than 0.05, 0.01, and 0.001, respectively. Guild: number of functional guilds; PD: Faith's phylogenetic diversity; MNTD: mean nearest taxon distance. DP75, DP50, and DP25 indicate decreases of 75%, 50%, and 25% in natural baseline rainfall, while IP75, IP50, and IP25 indicate corresponding increases. Significant differences are denoted by uppercase letters for the alpine steppe and lowercase letters for the alpine desert steppe.

3.3. Community Assembly

In both steppes, the microbial community assembly across the seven treatments was mainly influenced by stochastic processes, with a greater proportion observed for fungal communities compared to bacterial communities (Figure 4a–d). The sensitivity of stochastic processes to altered precipitation was generally greater in the alpine steppe compared to the desert steppe (Figure S10). For example, the proportion of "dispersal limitation" for bacterial communities under the DP75, IP25, and IP75 treatments, as well as "homogenizing

dispersal" under the DP75 and IP25 treatments, and "dispersal limitation" for the fungal community under the IP25 treatment, was greater in the alpine steppe compared to the desert steppe. Bacteria were more responsive to "homogeneous selection" than fungi, while fungi exhibited a greater proportion of "homogenizing dispersal" than bacteria across all precipitation gradients in both steppes (Figure S11).

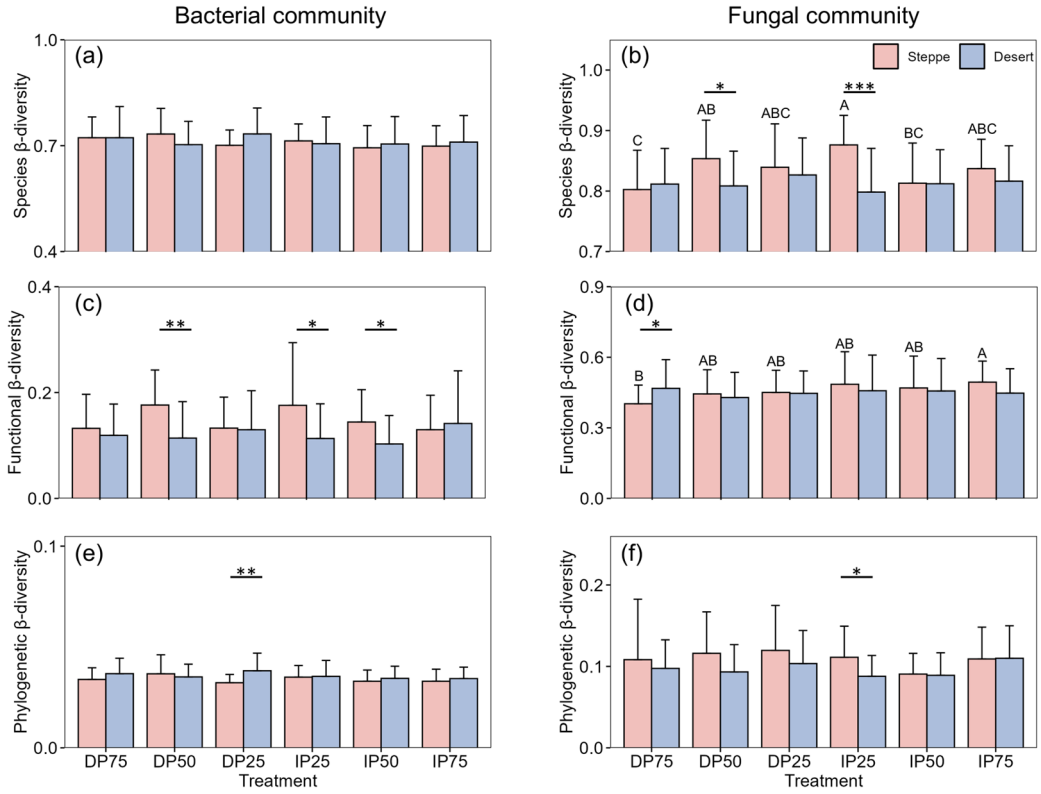


Figure 3. Comparison of effect sizes of altered precipitation on β-diversity across the species (**a**,**b**), functional (**c**,**d**), and phylogenetic dimensions (**e**,**f**). Differences between the two steppes at each treatment are marked as *, **, and *** for *p*-values of less than 0.05, 0.01, and 0.001, respectively. DP75, DP50, and DP25 indicate decreases of 75%, 50%, and 25% in natural baseline rainfall, while IP75, IP50, and IP25 indicate corresponding increases. Significant differences are denoted by uppercase letters for the alpine steppe.

3.4. Co-Occurrence Networks

In the alpine steppe, as the precipitation gradient decreased, there was a general increase in the effect sizes of the average degree, density, and clustering coefficient for bacterial networks, as well as in the density of fungal networks (Figure 5e–r). In the desert steppe, only the effect sizes of betweenness centralization for both bacterial and fungal networks showed a similar pattern. Microbial network topology attributes in the alpine steppe were generally more responsive to altered precipitation, making them less stable than those in the alpine desert steppe (Figures 5e–r and S12). For example, nodes, links, average degree, and clustering coefficient of bacterial networks across most treatments in the alpine steppe showed stronger positive effect sizes than their counterparts in the alpine desert steppe (Figure 5e–r). Additionally, fungal network topology attributes exhibited greater sensitivity to altered precipitation than bacterial networks (Figures S12 and S13). For instance, bacterial network topology attributes in the alpine steppe (nodes, links, average degree, and modularity) and the alpine desert steppe (betweenness centralization and modularity) across most precipitation gradients were generally more responsive than those of fungi (Figure S13).

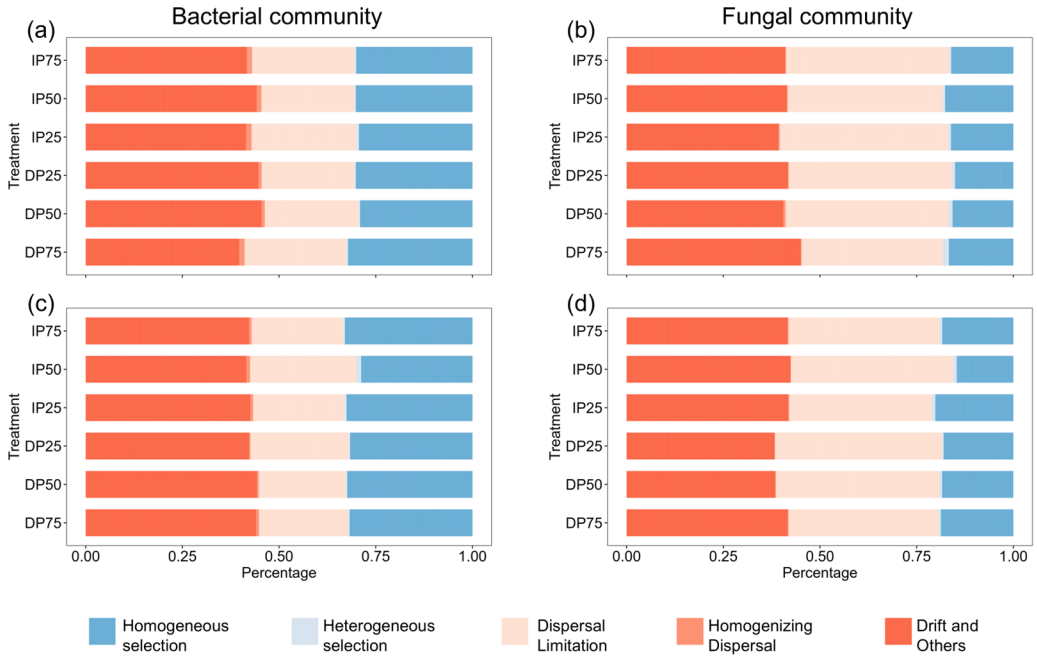


Figure 4. Turnover fractions in microbial community assemblies of the alpine steppe (**a**,**b**) and alpine desert steppe (**c**,**d**) across varying precipitation gradients. DP75, DP50, and DP25 indicate decreases of 75%, 50%, and 25% in natural baseline rainfall, while IP75, IP50, and IP25 indicate corresponding increases.

3.5. Factors Influencing Bacterial and Fungal Community

Microbial α-diversity at species, phylogenetic, and functional dimensions were influenced by distinct predominant environmental variables in the two steppes (Figures 6 and S14). Vegetation characteristics had a greater impact on bacterial α-diversity in the alpine steppe in comparison to the alpine desert steppe (Figure 6a–f). For example, plant richness was the dominant regulator of soil bacterial species, and functional, and phylogenetic α-diversity in the alpine steppe, whereas it had no significant effect in the alpine desert steppe. Furthermore, vegetation characteristics, soil properties, and co-occurrence networks collectively explained a substantial proportion of the variation in species, functional, and phylogenetic β-diversity of bacterial and fungal communities in both steppes (Figure 7). However, this contribution was relatively lower in the fungal communities. The relative contributions of these factors to microbial β-diversity varied across the species, functional, and phylogenetic dimensions. Microbial networks exerted a stronger impact on the variation of bacterial species and phylogenetic β-diversity, as well as fungal species β-diversity, than other variables. Soil properties exerted the strongest exclusive effect in explaining the variation of bacterial and fungal functional β-diversity, along with phylogenetic β-diversity, in the alpine steppe. Overall, soil properties exhibited stronger exclusive effects on bacterial β-diversity and impacted fungal β-diversity to a greater extent than vegetation characteristics in both steppes.

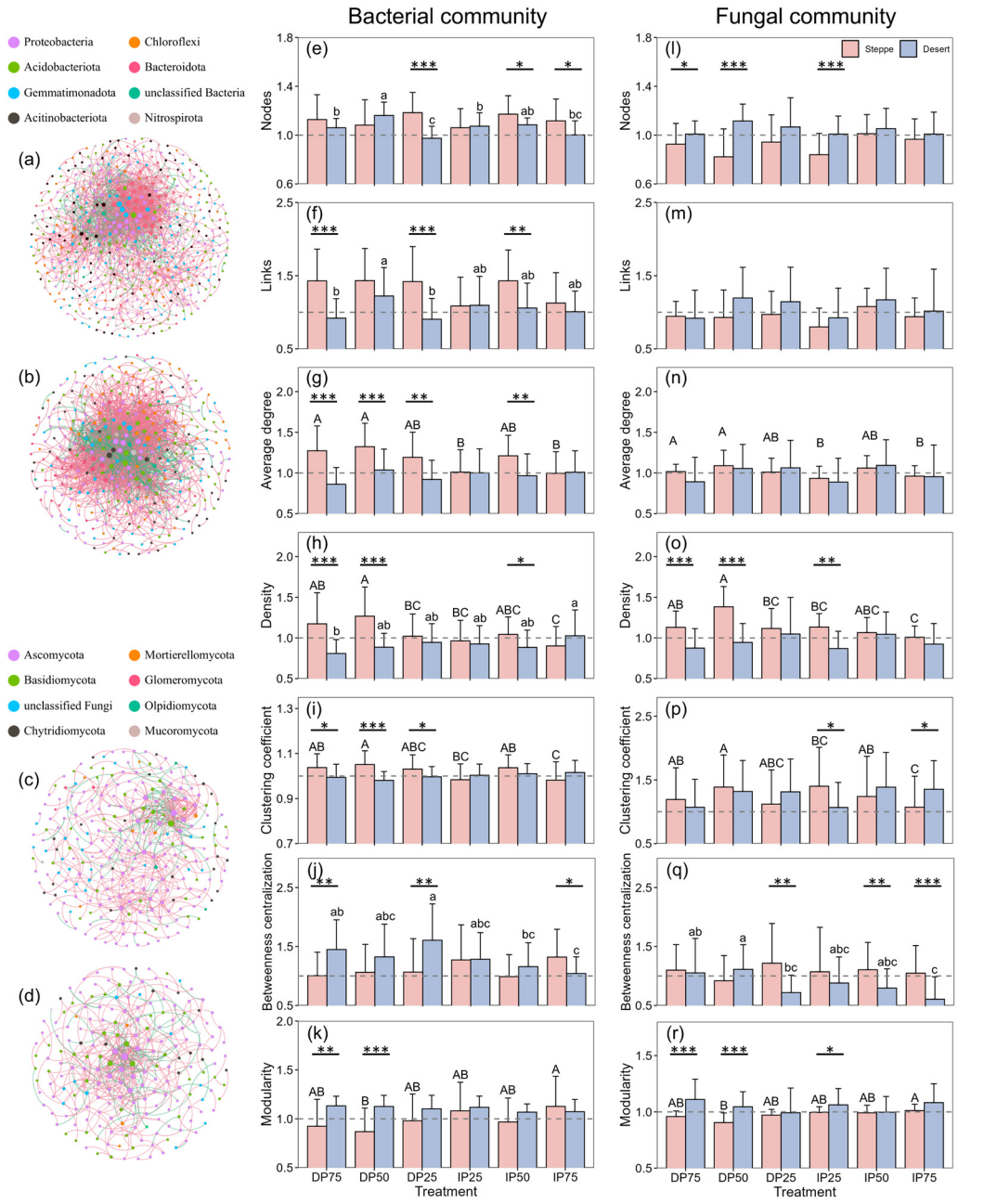


Figure 5. Bacterial comprehensive co-occurrence networks in the alpine steppe (**a**) and alpine desert steppe (**b**), and fungal co-occurrence networks in the alpine steppe (**c**) and alpine desert steppe (**d**). Comparison of effect sizes of altered precipitation on subnetwork topology attributes (**e**–**r**). DP75, DP50, and DP25 indicate decreases of 75%, 50%, and 25% in natural baseline rainfall, while IP75, IP50, and IP25 indicate corresponding increases. Significant differences are denoted by uppercase letters for the alpine steppe and lowercase letters for the alpine desert steppe. Differences between the two steppes at each treatment are marked as *, **, and *** for *p*-values of less than 0.05, 0.01, and 0.001, respectively.

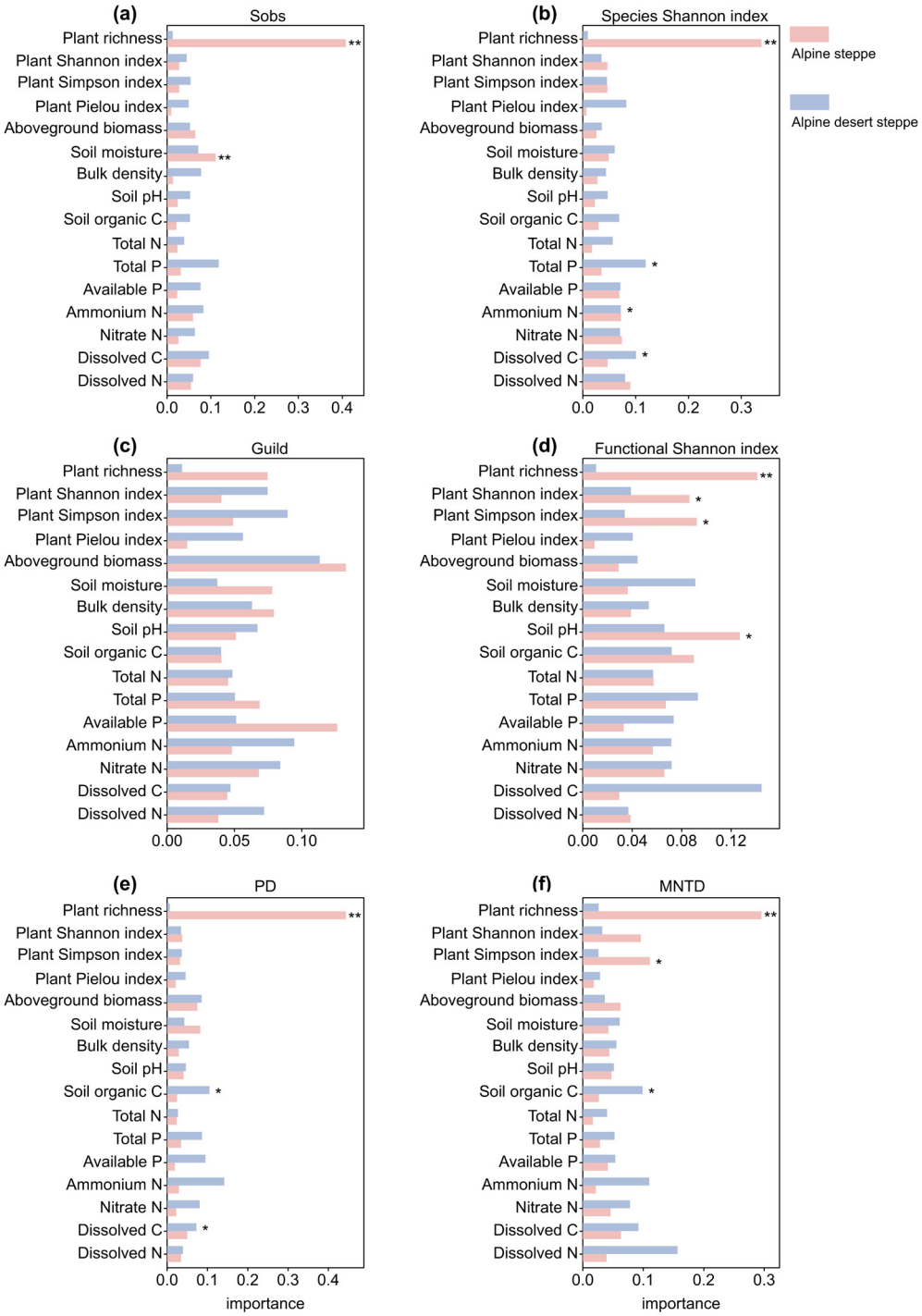


Figure 6. Random Forest analysis determined the influence of environmental variables on the effect size of altered precipitation on soil bacterial α-diversity across species (**a**,**b**), functional (**c**,**d**), and phylogenetic (**e**,**f**) dimensions. The analyzed environmental variables included plant richness, plant Shannon index, plant Simpson index, plant Pielou index, aboveground biomass, soil moisture, bulk density, soil pH, soil organic C content, soil total N content, soil total P content, soil available P content, soil ammonium N content, soil nitrate N content, soil dissolved C content, and soil dissolved N content. Differences between the two steppes at each treatment are marked as *, **, for *p*-values of less than 0.05, 0.01 respectively.

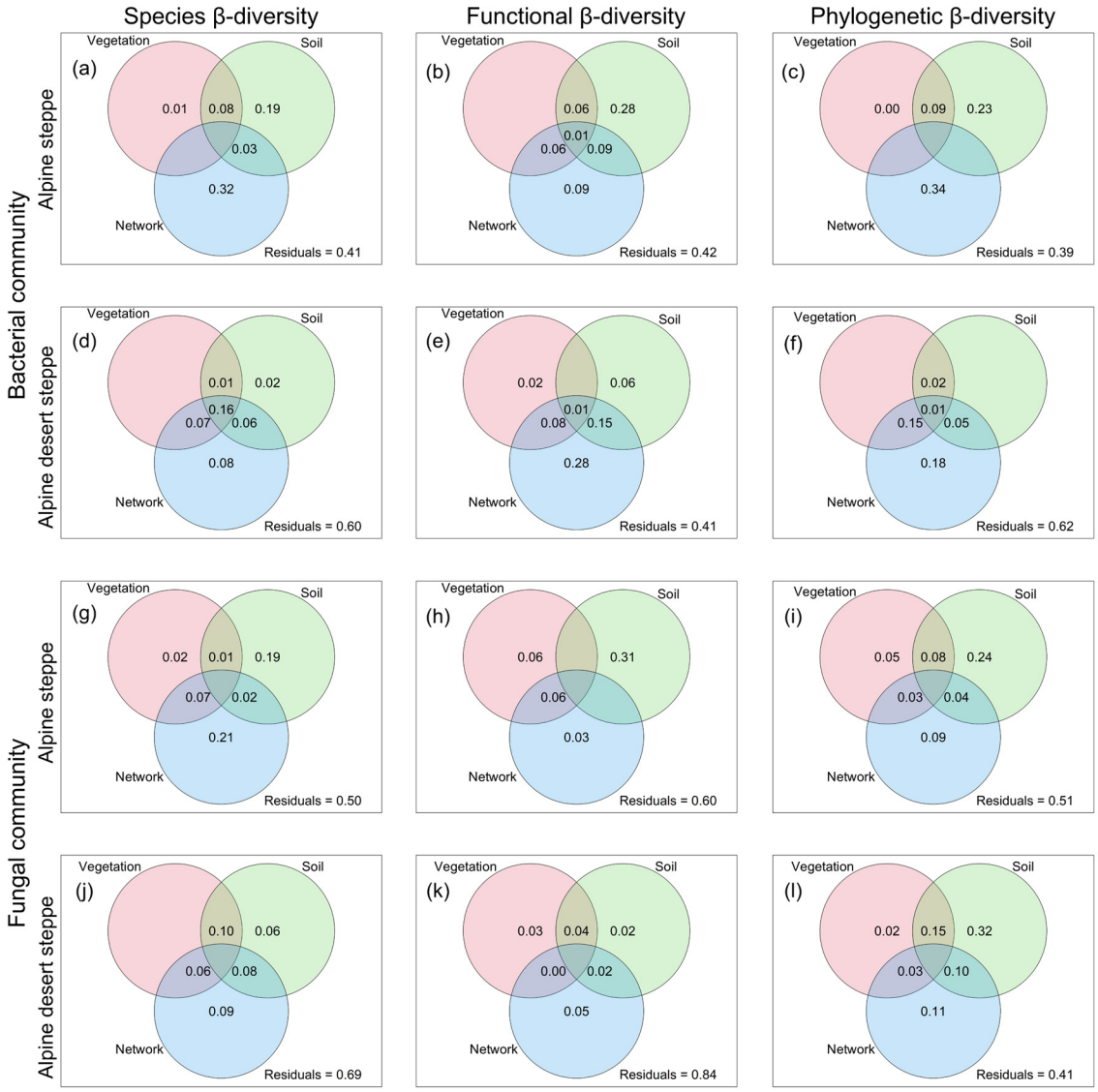


Figure 7. Relative contributions of vegetation characteristics, soil properties, and co-occurrence networks to microbial β-diversity across species, functional, and phylogenetic dimensions in the alpine steppe (**a**–**c**,**g**–**i**) and the alpine desert steppe (**d**–**f**,**j**–**l**).

4. Discussion

4.1. Effects of Relative Precipitation Changes on Soil Microbial Community Assembly

The formation of microbial communities was influenced by both deterministic and stochastic processes, with ongoing debate regarding their relative importance and how they shift with environmental changes [48,54]. In this study, the proportion of deterministic processes in soil bacterial assembly was greater than that in fungal community assembly across two steppes in response to relative precipitation changes (Figure 4). The finding aligns with prior research that emphasizes the importance of deterministic processes in shaping soil bacterial communities [54,55]. Soil bacteria have faster reproduction and transmission rates, enabling them to swiftly adapt to environmental changes and making them more prone to selection pressures, such as homogeneous selection [50,56]. Recent studies further underscored the role of stochastic processes in shaping fungal communities, attributed to their strong regional characteristics and limited dispersal capabilities [57,58].

Stochastic processes of bacterial communities in the alpine steppe were more responsive to relative precipitation changes than those in the alpine desert steppe (Figure S10). This finding suggests that the contributions of stochastic processes can vary depending on

ecosystem type, as confirmed in prior studies [30,50,59]. This variation in sensitivity could be due to higher plant diversity and better nutrient conditions in the alpine steppe compared to the desert steppe (Tables S6 and S7). These factors likely create a more stable and uniform soil environment, offering soil bacteria a broader range of habitats and resources to utilize. This, in turn, could foster increased microbial diversity and activity levels, thereby potentially amplifying the proportion of stochastic processes in assembly [59,60]. Further studies are necessary to confirm these links specifically within these ecosystems.

4.2. Effects of Relative Precipitation Changes on Soil Microbial Co-Occurrence Networks

Microbial co-occurrence networks in the alpine steppe were less stable to relative changes in precipitation than those in the alpine desert steppe (Figure 5e–r), which was contrary to the hypothesis. Possible reasons for this discrepancy include the following. One explanation is that previous studies have reported that microbial networks are closely linked to species diversity, which can influence network stability [5,61]. In the alpine steppe, microbial species α-diversity showed greater sensitivity to altered precipitation compared to the desert steppe (Figure 1). This heightened sensitivity likely leads to increased variability in the microbial network's structure under changing precipitation conditions. Another factor is that precipitation changes significantly altered plant α-diversity in alpine steppes, whereas they had no significant effects on plant α-diversity in alpine desert steppes (Table S6). These shifts in plant community diversity may amplify the effects of precipitation on the microbial co-occurrence network in alpine steppes. Furthermore, the greater resistance of soil microbes in the alpine desert steppe to high-stress environments may contribute to the relatively higher stability of networks [62,63].

Decreased precipitation elevated soil bacterial network complexity in the alpine steppe (Figure 5), which can be attributed to several factors. The sharp decrease in soil available water results in nutrient scarcity, prompting some microorganisms to develop more complex interaction patterns to efficiently utilize limited resources, consequently increasing the co-occurrence network complexity [64,65]. In addition, in the alpine steppe, higher altitude and lower annual temperatures, in conjunction with reduced precipitation, may alter the local soil environment more significantly than in the desert steppe, thereby promoting microbial growth, metabolism, and interactions [66,67].

Fungal co-occurrence networks exhibited greater stability than those of bacteria under altered precipitation conditions (Figure S13), aligning with prior research [68,69]. Multiple factors could explain this observation. Bacterial communities are often more dynamic, with faster reactions to external disturbance due to their shorter life cycles [68,70]. This rapid responsiveness can lead to greater instability in bacterial networks under fluctuating moisture conditions. Conversely, owing to their extensive hyphal networks and symbiotic relationships with plants, most fungi can demonstrate greater resilience to fluctuations in soil water, contributing to the preservation of network stability [15,65]. Moreover, fungi linked to plants, inhabiting both the soil and the rhizosphere or root interiors, can access essential resources during drought periods, thereby mitigating the disruption of interaction networks induced by precipitation changes [71,72].

4.3. Effects of Relative Precipitation Changes on Soil Microbial Diversity

Bacterial α-diversity in the alpine steppe exhibited a negative asymmetric response to altered precipitation at both species and phylogenetic dimensions (Figures 1 and 2), which aligned with the hypothesis. This pattern stems from the soil bacterial communities' adaptability to variations in water availability [10,19]. Decreased precipitation limits soil water content, inhibiting bacterial growth and reducing the number of species [8,15]. While increased precipitation brings higher soil moisture, its effects on microorganisms

in high-altitude environments are complex. Increased precipitation may counteract potential benefits by lowering soil temperature and exacerbating nutrient loss in alpine regions [73,74]. Bacterial α-diversity in the alpine steppe shows higher sensitivity to reduced precipitation compared to increased precipitation, facilitating their survival and reproduction under extreme conditions.

Microbial species α-diversity in the alpine steppe has greater sensitivity to relative precipitation changes than the alpine desert steppe (Figures 1 and 3), which contradicted the hypothesis. This could be attributed to several factors. The alpine steppe exhibited higher plant diversity and aboveground productivity than the alpine desert steppe (Table S6), providing ample organic matter sources and habitats for soil microbes (Table S7). As a result, ecological interactions between soil microorganisms and plant communities make microbial communities in the alpine steppe more influenced by changes in aboveground vegetation. The stronger impact of vegetation characteristics on bacterial α-diversity observed in the alpine steppe in comparison to the alpine desert steppe further underscored the importance of aboveground conditions in shaping microbial responses to altered precipitation (Figure 6). Additionally, soil microbial co-occurrence networks, especially those of bacterial communities, exhibited greater sensitivity to relative changes in precipitation than those in the alpine desert steppe (Figure 5), indicating reduced resistance to external disturbances. More importantly, effect sizes of altered precipitation on soil microbial species α-diversity in both steppes tended to differ under greater gradients of precipitation change (Figures 1 and 2). This variation may result from microbial adaptation exceeding resilience thresholds, limiting their ability to recover under extreme precipitation conditions [69,75]. This underscores the need to consider gradient effects when exploring how soil microbes react to changes in precipitation across different ecosystems.

Fungal diversity generally responded more sensitively to altered precipitation than bacteria (Figures S5 and S6). This observation aligned with previous research and can be attributed to several factors [76,77]. Compared to bacterial communities, fungal communities were influenced by fewer deterministic processes during assembly, experiencing lower environmental selective pressure (Figure 4 and S11). This may result in fungal communities exhibiting lower environmental tolerance and adaptability to changes in water availability than bacterial communities. Furthermore, fungal co-occurrence networks in the two steppes were simpler than those of bacterial communities, characterized by fewer edges and nodes (Figure 5a–d). Key taxa in the fungal network are more important for maintaining stability [78,79]. Precipitation changes can significantly impact these key fungal species, leading to substantial alterations in the network and a more sensitive response in fungal diversity. Thirdly, differences in species pool sizes between soil bacterial and fungal communities might explain the varied responses of their diversity to changes in precipitation [26].

Environmental variables and co-occurrence networks jointly influenced microbial β-diversity at the species, functional, and phylogenetic levels under precipitation changes (Figure 7). This reveals their crucial role in shaping microbial communities, as supported by prior research [80]. Precipitation changes alter microbial community formation by modifying soil properties, vegetation characteristics, and assembly processes, while also impacting stability through changes in co-occurrence network complexity and connectivity. Among these variables, soil properties have a more pronounced impact on β-diversity than vegetation characteristics. This greater impact may stem from the direct effects of soil physical structure and nutrient content on the microbial growth environment, which in turn alters community composition [17,18]. Vegetation affects microbial communities indirectly by altering soil properties, including nutrient levels and organic matter content, with these interactions often influenced by existing soil characteristics [28,81]. Notably, the

explanatory power of these variables for bacterial β-diversity variation was greater than that for fungi, likely due to their distinct ecological niches, lifestyles, and rates of response to water availability [70,82]. In addition, the relative effects of soil properties, vegetation characteristics, and co-occurrence networks on microbial β-diversity varied across different dimensions. This suggests that examining only one dimension of diversity may not fully capture the effects of altered precipitation on overall diversity [32,83]. Functional redundancy and similar phylogenetic traits among microbial species enable some species to compensate for others affected by precipitation changes, leading to varied β-diversity responses [26,79].

4.4. Limitations and Prospects

The interpretation of the results from this study might be constrained by several limitations. Firstly, microbial responses were assessed in two alpine grassland types at a single site, potentially introducing site-specific environmental biases. To enhance the ecological representativeness of microbial responses to precipitation variability, future research should incorporate multi-site studies spanning diverse ecosystem types. Secondly, our experimental design manipulated precipitation changes in relative terms without accounting for absolute precipitation variability. This limitation constrains our ability to comprehensively assess ecosystem water thresholds, particularly in regions with substantial baseline precipitation differences. Future studies should integrate controlled laboratory experiments with long-term field monitoring to provide a more mechanistic understanding of microbial community responses to precipitation dynamics. Thirdly, soil microorganisms occupy diverse trophic levels, which requires further investigation through manipulative experiments to understand how altered precipitation impacts biodiversity at other trophic levels, including protists and viruses [3]. Additionally, while network analysis serves as a valuable tool for identifying species relationships, directly inferring interactions among microorganisms remains a challenge [66,84]. The co-occurrence networks developed in this study rely on static microbial data, underscoring the need for higher sampling frequency to accurately capture the dynamics in microbial interactions. Lastly, observed patterns in microbial diversity and co-occurrence networks due to precipitation changes could be limited by the relatively short duration of the experimental treatments, potentially missing delayed ecosystem responses [85,86]. Longer-term observations are required to fully comprehend how precipitation changes affect soil microbial communities in alpine ecosystems.

5. Conclusions

This study presents one of the first systematic investigations of soil microbial community responses to relative precipitation changes in the alpine steppe and desert steppe ecosystems of the western Tibetan Plateau. Our findings reveal that the effects of altered precipitation are shaped by the magnitude of change, the type of grassland, and the microbial kingdom studied. Bacterial α-diversity exhibited a negative asymmetric response to altered precipitation in the alpine steppe. Microbial α-diversity and co-occurrence networks in the alpine steppe were generally more sensitive to relative precipitation changes compared to those in the desert steppe. Bacterial communities did not consistently show greater sensitivity than bacterial communities under altered precipitation regimes. Furthermore, altered precipitation can influence the dissimilarity of soil microbial communities in both grassland ecosystems by modulating vegetation characteristics, soil properties, and microbial networks. These findings provide valuable insights into ecosystem stability under shifting precipitation regimes and can inform conservation and sustainable management strategies for alpine grasslands. Future research should prioritize long-term monitoring of microbial responses to precipitation fluctuations, explore their interactions with soil

nutrient cycling and plant communities, and assess the broader ecological implications of microbial shifts in the context of climate change.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15040851/s1, Figure S1. Location of the two study sites. Figure S2. The designed rain shelter intercepts rainwater in the decreased precipitation plot (a) and directs the collected water to the corresponding increased precipitation plot (b). Figure S3. Comparison of species α-diversity across seven precipitation change gradients. Figure S4. Comparison of functional (a–d) and phylogenetic α-diversity (e–h) across seven precipitation change gradients. Figure S5. Comparison of effect sizes of altered precipitation on species α-diversity between bacterial and fungal communities. Figure S6. Comparison of effect sizes of altered precipitation on functional (a–d) and phylogenetic α-diversity (e–h) between bacterial and fungal communities. Figure S7. Comparison of effect sizes of altered precipitation on species (a,b), functional (c,d), and phylogenetic β-diversity (e,f) between bacterial and fungal communities. Figure S8. Relative abundance of bacterial and fungal phyla in the alpine steppe (a,b) and the alpine desert steppe (c,d) across different precipitation gradients. Figure S9. Venn plot for species of bacterial and fungal communities in the alpine steppe (a,b) and the alpine desert steppe (c,d) across different precipitation gradients. Figure S10. Comparison of effect sizes of altered precipitation on community assembly processes. Figure S11. Comparison of effect sizes of altered precipitation on community assembly processes between bacterial and fungal communities. Figure S12. Comparison of microbial subnetwork topology attributes across seven precipitation change gradients. Figure S13. Comparison of effect sizes of altered precipitation on co-occurrence network topology attributes between bacterial and fungal communities. Figure S14. Random Forest analysis determined the influence of environmental variables on the effect size of altered precipitation on soil fungal α-diversity across species (a,b), functional (c,d), and phylogenetic (e,f) dimensions. Table S1. Annual precipitation during the experimental period and multi-year average precipitation at the two study sites. Table S2. The permutational multivariate analysis of variance for microbial β-diversity across the seven precipitation gradients. Table S3. Relative abundance of significant soil bacterial taxa determined by linear discriminant analysis across the seven precipitation gradients. Table S4. Relative abundance of significant soil fungal taxa determined by linear discriminant analysis across the seven precipitation gradients. Table S5. Relative abundance of significant FUNGuild taxa determined by linear discriminant analysis across the seven precipitation gradients. Table S6. Vegetation characteristics across different precipitation gradients in the two study sites. Table S7. Soil properties across different precipitation gradients in the two study sites.

Author Contributions: Conceptualization, G.F.; methodology, J.X.; software, Z.W.; validation, Z.W.; formal analysis, Z.W.; investigation, J.X., Z.W. and S.H.; resources, C.Y.; data curation, Z.W.; writing—original draft preparation, J.X.; writing—review and editing, J.X., G.F., F.H. and S.H.; visualization, J.X. and Z.W.; supervision, C.Y. and G.F.; project administration, C.Y. and F.H.; funding acquisition, C.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Pilot Project of the Chinese Academy of Sciences (XDA26050501), the Tibet Autonomous Region Science and Technology Project (XZ202101ZD0003N, XZ202401JD0029, XZ202201ZY0003N, XZ202501ZY0056), and the Construction of Zhongba County Fixed Observation and Experimental Station of First Support System for Agriculture Green Development.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

Acknowledgments: We thank the editors and reviewers for their valuable comments.

Conflicts of Interest: The authors declare no conflicts of interest.

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