nature communications

Article

https://doi.org/10.1038/s41467-025-58080-2

Core microbes regulate plant-soil resilience by maintaining network resilience during long-term restoration of alpine grasslands

Received: 16 April 2024

Accepted: 6 March 2025

Yao Du[1,2,3,5], Yan Yang[3,5], Shengnan Wu[1,5], Xiaoxia Gao[4,5], Xiaoqing He[2,3] & Shikui Dong[1,2]

The alpine grasslands of the Qinghai-Tibetan Plateau (QTP), the world's highest plateau, have been severely degraded. To address this degradation, human-involved restoration efforts, including grassland cultivation, have been implemented. However, the impact of these practices on soil microbial community stability and its relationship with plant-soil system resilience has not been explored. In this study, we evaluate the effects of grassland restoration on microbial communities. We show that bacteria demonstrate higher composition resistance and resilience during the restoration process, when compared to fungi. The changes we observe in microbial community interactions support the stress gradient hypothesis. Our results emphasize the synergistic role of network resilience and the restoration of the plant-soil system. Importantly, we find that core microbial species significantly influence the resilience of the plant-soil system by sustaining the co-occurrence networks. These insights underscore the critical roles of microbial communities in grassland restoration and suggest new strategies for boosting grassland resilience by safeguarding core microbes.

The grasslands of the Qinghai-Tibetan Plateau (QTP), the highest plateau in the world, are crucial for food supply, environmental conservation, and social development[1]. However, these alpine grassland ecosystems have undergone significant degradation in recent decades due to various drivers, including climate change, overgrazing, and anthropogenic interventions[2]. Soil microorganisms, which encompass a diverse and intricate array of biological communities that play a crucial role in organic matter decomposition, nutrient cycling and maintaining soil functionality in grassland ecosystems[3]. Notably, soil bacteria and fungi respond differently to environmental filtering, thereby influencing the distribution and diversity of the soil microbial community[4,5]. Mean annual temperature and aboveground net primary productivity are determining factors for changes in fungal diversity, soil pH, and N:P ratio are determining factors for changes in bacterial diversity[5]. While the significance of microorganisms in restoring degraded grasslands is well-recognized, there remains a notable gap in research regarding the disparities in soil microbial diversity, the relationship between bacteria and fungi, as well as the complexity and stability of the microbiome. Addressing these knowledge gaps is crucial for developing effective strategies to restore and preserve the fragile alpine grassland ecosystems of the QTP.

The stability of an ecosystem in the face of disruption hinges on its resistance and resilience[6]. Referring to the concept of Griffiths[7] and Gao[8] et al., stability of microbial community can be defined as the ability of a microbial community's composition and network to resist environmental disturbances (resistance) and to recover to its original state after the disturbance is eliminated (resilience). Such stability is influenced not only by the composition of its community, but also by

---

[1]School of Grassland Science, Beijing Forestry University, Beijing, China. [2]State Key Laboratory of Efficient Production of Forest Resources, Beijing Forestry University, Beijing, China. [3]College of Biological Sciences and Technology, Beijing Forestry University, Beijing, China. [4]School of Ecology and Nature Conservation, Beijing Forestry University, Beijing, China. [5]These authors contributed equally: Yao Du, Yan Yang, Shengnan Wu, Xiaoxia Gao.
e-mail: lenahe@bjfu.edu.cn; dongshikui@bjfu.edu.cn

the myriad interactions among the co-existing microbial communities. These interactions span a spectrum from negative to positive and weak to strong, encompassing both significant to non-significant relationships among bacteria, fungi, or both[9]. Investigating these correlations sheds light on the dynamics of resistance and resilience within ecosystems. The stress gradient hypothesis (SGH) suggests that the occurrence of cooperation and competitive interactions will vary inversely across abiotic stress gradients. Cooperation is more common in high-stress conditions and competition is more favorable in low-stress environments[10–12]. With ecosystems facing increasing anthropogenic disturbances, there is a potential for microbial communities to become destabilized. Gao et al.[9] bolstered the stress gradient hypothesis by demonstrating an increase in positive associations among microbial communities in response to drought conditions. This underscores the significance of investigating how microbial communities adapt to stressful environments. Despite these insights, there remains a notable gap in research specifically addressing how the stress gradient hypothesis applies to microbial communities during the process of grassland restoration. This area of study presents a valuable opportunity for further exploration to understand the resilience and adaptation mechanisms of microbial ecosystems under restoration actions.

In a recent study, Qiao et al. utilized core amplicon sequence variants (ASVs), pinpoint key microbial species, distinguishing core species on the basis of shared ASVs, specialist ASVs, and keystones[13]. A notable outcome of their study was the discovery that the relative abundance of each core species exhibited a positive correlation with network properties (number of links, relative modularity and positive cohesion). These pivotal findings underscore the essential influence of these species in augmenting network complexity and bolstering plant health. Despite the progress made, researchers are still struggling to fully understand the specific functions of microbial core taxa in promoting microbial stability. This area remains ripe for further exploration to fully unravel the mechanisms through which core microbial species contribute to the resilience and functionality of their ecosystems.

This study was carried out in the alpine grasslands of Maqin county, located in Qinghai Province, China. We utilized a space-for-time substitution approach to delve into the impacts of grassland restoration efforts. Our restoration efforts for degraded grasslands involved reseeding natural grasslands and establishing cultivated grasslands to boost grassland productivity. The study objectives are: (1) to unravel the pattern of soil microbial diversity and the intricacies of network complexity throughout the restoration of the alpine grassland, (2) to assess the resistance and resilience of fungal and bacterial communities during the period of ecological recovery, (3) to elucidate the pivotal role played by soil microbes in mediating the interactions within the plant-soil system over the course of the long-term restoration process. This comprehensive investigation was designed to shed light on the critical contributions of microbial communities to the ecological restoration and sustainability of alpine grasslands. Here, we show that core microbial species significantly contribute to the resilience of the plant-soil system by supporting co-occurrence networks.

Results

Pattern of soil microbial diversity and network complexity

During the process of grassland restoration, both bacterial (Statistics = 21.959, Degrees of freedom = 10, $P = 0.015$) and fungal (Statistics = 24.095, Degrees of freedom = 10, $P = 0.007$) diversities exhibited significant variations (Fig. 1A, Supplementary Fig. 1A–D). According to the Bray-Curtis dissimilarity index, bacteria demonstrated higher composition resistance and resilience (Fig. 1B, Statistics = 412.715, Degrees of freedom = 19, $P = 0.0E0$). Lower Bray-Curtis dissimilarity values showed lower changes (i.e., higher composition resistance) and lower variability (i.e., higher composition resilience). PCoA analysis showed that communities of bacteria and fungi were significantly different among restoration processes (Supplementary Fig. 1E, F). The effect of restoration on bacteria ($F = 4.511$, $P = 0.01$) was greater than that of fungi ($F = 1.189$, $P = 0.31$) by PERMDISP analysis, which was also confirmed by PERMANOVA analysis ($R^2 = 0.48$, $P < 0.01$; $R^2 = 0.43$, $P < 0.01$). The dominant bacterial phyla identified were *Proteobacteria, Actinobacteria*, and *Acidobacteria*, whereas the fungal community was predominantly composed of *Ascomycota, Zygomycota*, and *Basidiomycota* (Supplementary Fig. 1G, H). Furthermore, at the genus level, the relative abundance of *Balneimonas* and *Flavisolibacte* decreased in response to restoration efforts (Supplementary Fig. 1I, J and Supplementary Fig. 2).

The soil microbial community networks exhibited unique co-occurrence patterns at each stage of grassland restoration (Fig. 1C, Supplementary Fig. 3). The network of non-degraded samples contains a greater number of edges compared to severely degraded samples (Fig. 1C). There is no significant difference in network characteristics such as average degree and clustering coefficient (Fig. 1D, E, $t = 1.834$, $P = 0.09$; $t = 0.453$, $P = 0.66$), while the network complexity represented by network connections has a significant difference (Fig. 1F, $t = -8.748$, $P = 0.000005$), which indicates a more complex network throughout the restoration processes. Analysis of network eigenvalues showed a pattern of fluctuations: a decrease in the grassland after 4 years of restoration, an increase in the grassland after 6 years, then decreases in the grasslands after 8 years and 9 years, followed by an increase from the grassland from year 12 onwards, reaching a peak at year 16, and finally decreasing after 18 years of restoration (Fig. 1D–F). The network complexity, as evidenced by the number of connections, was higher in non-degraded sample (Fig. 1G). The recovery was divided into early (first to ninth year) and late (twelfth to eighteenth year) stages for analysis (Fig. 1H). Upon comparing network connections between these two periods and calculating the resilience index, the late stage demonstrated a more significant recovery than the early stage (Fig. 1H).

Overall cohesion, encompassing both positive and negative interactions, after restoration (including early stage and late stage) and of non-degraded samples is no significant difference than that of severely degraded samples (Fig. 1I, Statistics = 0.041, Degrees of freedom = 3, $P = 0.99$). The ratio of negative to positive cohesion increased as the recovery period extended, with non-degraded samples showing the highest ratio (Fig. 1J, Statistics = 13.332, Degrees of freedom = 3, $P = 0.004$). This pattern suggests increased competition within the microbial community in non-degraded samples (low stress) stages and more cooperation in the severely degraded samples (high stress) network, aligning with the stress gradient hypothesis.

Network stability of fungi and bacteria

We analyzed the interactions among soil microorganisms including interactions within fungi (F-F), within bacteria (B-B), between bacteria and fungi (B-F), across 11 networks (Fig. 2A, Supplementary Fig. 4). Both the interactions of B-F and B-B were found to be more abundant in the non-degraded samples compared with the severely degraded samples. The resilience of these interactions increased in late stages than early stages except bacteria (Fig. 2B). The quality of B-F interactions showed similar trends of change and plant-soil system quality index (PSQI) (Fig. 2C, $t = -9.7$, $P < 0.0001$). B-F resilience and B-F(negative) resilience paralleled the pattern of the resilience index of plant-soil systems (PSQIR), unlike composition resilience, which did not strongly correlate with PSQIR trend (Fig. 2D; Supplementary Table 1).

Bacteria nodes demonstrated higher constancy (Statistics = 932.118, Degrees of freedom = 1, $P = 0$) and persistence than fungal nodes (Fig. 3A, B). Bacteria also exhibited a broader ecological niche compared to fungi (Fig. 3C, Statistics = 5076.602, Degrees of freedom = 1, $P = 0$). The assembly mechanisms for both groups indicated a dominance of heterogeneous selection for bacteria and diffusion limitation for fungi, suggesting distinct microbial community

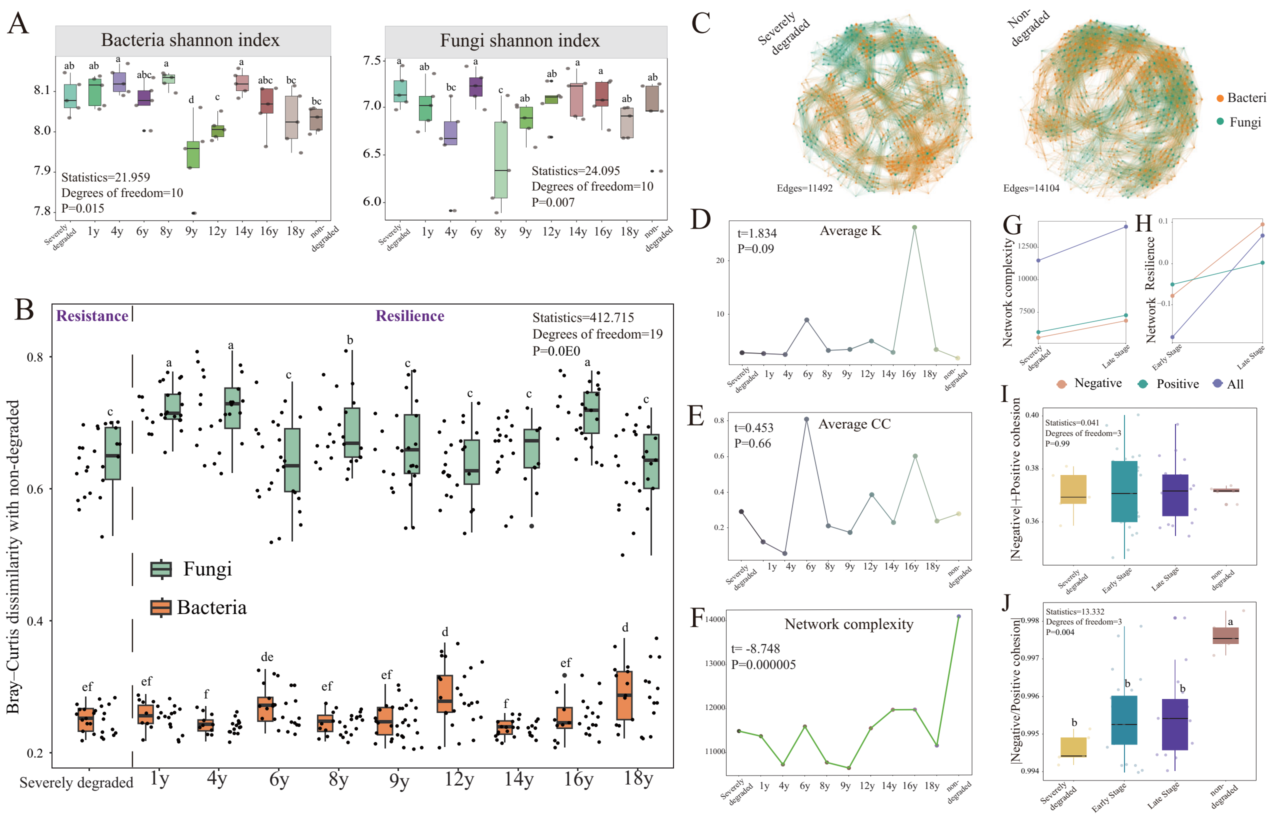

Fig. 1 | Changes in soil microbial diversity pattern and network with grassland restoration. A Shannon index of bacterial and fungal communities (Kruskal-Wallis test, two-sided; Duncan's test, Lowercase letters show significant differences between restoring stages at the $P < 0.05$ level). **B** The Bray-Curtis dissimilarities of bacteria and fungi indicated the resistance and resilience stability (Kruskal–Wallis test, two-sided; Duncan's test, Lowercase letters show significant differences between restoring stages at the $P < 0.05$ level). **C** The networks of non-degraded, severely degraded samples. The networks variation of feature values in different networks: **D** Average K (T-test, two-sided), **E** Average CC (T-test, two-sided), **F** Network complexity (T-test, two-sided). **G** Changes in the number of network edges for non-degraded and severely degraded, **H** as well as the recovery of edge numbers in the early and late stages, early recovery stages and late recovery stages. **I** Change of Negative+Positive cohesion (Kruskal-Wallis test, two-sided). **J** Change of |Negative/Positive cohesion| (Kruskal-Wallis test, two-sided; Duncan's test, Lowercase letters show significant differences between restoring stages at the $P < 0.05$ level). Non-degraded ($n = 5$), severely degraded ($n = 5$), and restored alpine grasslands at different years: 1 year (1 y, $n = 5$), 4 years (4 y, $n = 5$), 6 years (6 y, $n = 5$), 8 years (8 y, $n = 5$), 9 years (9 y, $n = 5$), 12 years (12 y, $n = 5$), 14 years (14 y, $n = 5$), 16 years (16 y, $n = 5$), and 18 years (18 y, $n = 5$), early recovery stages ($n = 25$, from the first year to the ninth year) and late recovery stages ($n = 20$, from the 12th to the 18th year). Box plots: Centerline, median; upper and lower quartiles; whiskers, 1.5× interquartile range; points, outliers. Source data are provided as a Source data file.

assembly responses to grassland restoration (Fig. 3D). Fungal community aligned more closely with a neutral community model than bacterial community (Supplementary Fig. 5A, B). Environmental adaptability analysis showed fungi were more adaptable to nutrients such as nitrogen, phosphorus, and potassium, whereas bacteria showed greater adaptability to TS (Total sulfur) and TN (Total Nitrogen) (Fig. 3E). Additionally, bacteria demonstrated stronger phylogenetic conservatism than fungi (Fig. 3F).

Relationship between core species and network complexity

After calculating the within-module connectivity (Z_i) and among-module connectivity (P_i) values across 11 networks, we identified keystone central node microorganisms within each network (Fig. 4A, Supplementary Fig. 6). Notably, the peak presence of keystone microorganisms occurred 16 years post-recovery (Fig. 4B). A significant negative correlation was observed between abundance of keystone species and network resilience (R = −0.38, $p = 0.015$), underscored the importance of these organisms in network resilience. In order to further investigate the contribution of core groups to network resilience, fungi and bacteria were categorized as either specialists or generalists based on their niche width (Fig. 4C). Through this analysis, five generalist core OTUs were identified, distinguished by their broad ecological niches and consistent presence throughout the 18-year restoration period, highlighting their roles as stable components of the ecosystem (Fig. 4D). In contrast, 190 specialist core OTUs were pinpointed, characterized by their narrower niche and fluctuating abundance during the recovery process. Their variability suggests their specialized adaptation to certain environmental conditions, which may shift throughout the years of recovery.

The resilience of co-occurrence network, including both B-F positive and negative interaction, was positively associated with the abundance of the 5 generalist core OTUs. Conversely, a strong negative correlation was observed between the 190 specialist core OTUs and positive B-F resilience (Fig. 4E). This indicates while generalists contribute to network resilience, specialists may play a different role, potentially destabilizing positive interactions under certain conditions. Moreover, the 190 specialist core microorganisms demonstrated a negative correlation with fungi AVD (average variation degree, Supplementary Fig. 7), indicating that lower AVD, indicative of less variability within the fungal community, contributes to greater microbial community stability.

Relationship between network resilience and plant-soil system resilience

A comparative analysis was conducted on the Spearman correlation among various factors, revealing a significant positive correlation

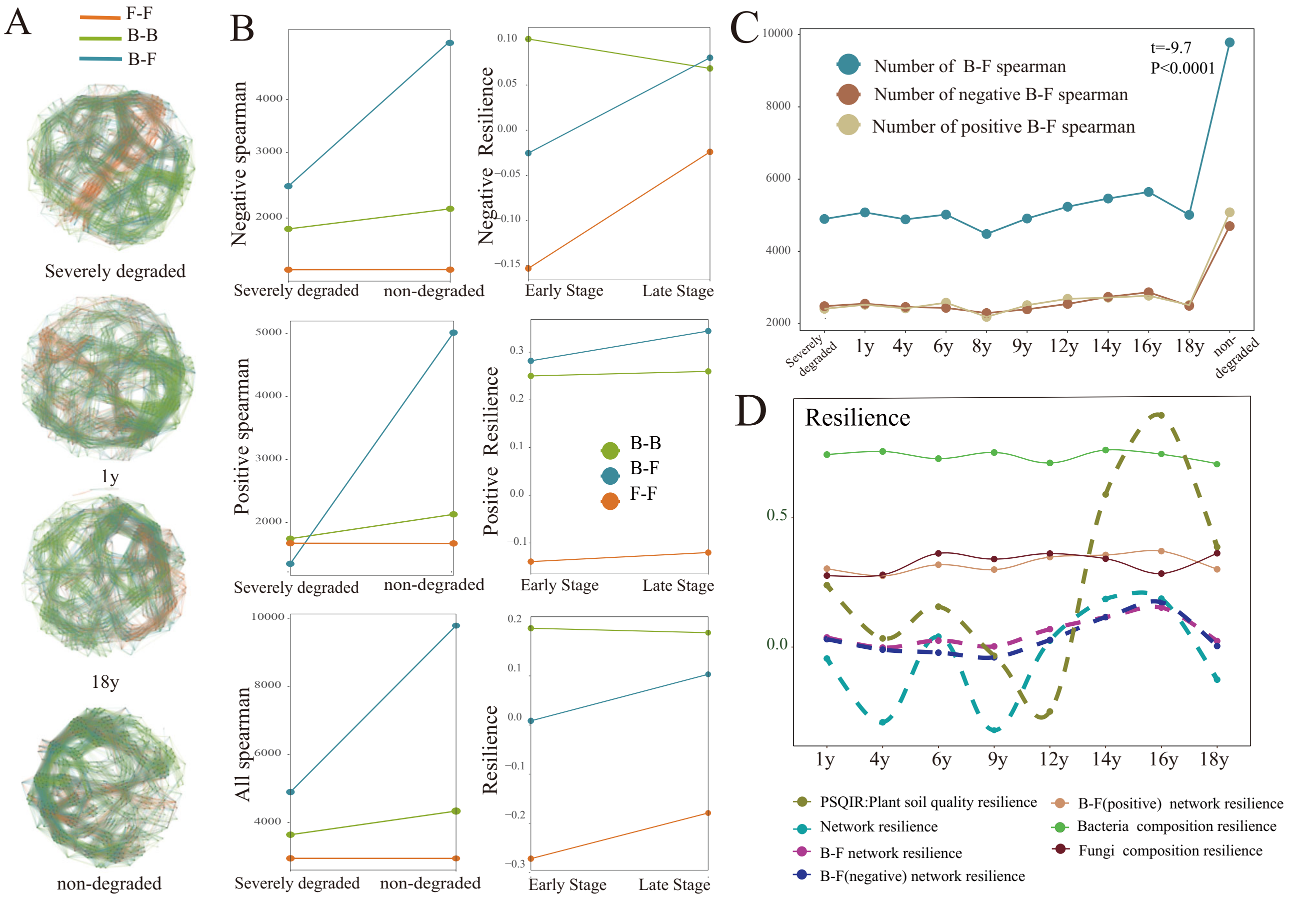

Fig. 2 | Network complexity and stability of fungi and bacteria. A Network visualization of bacterial-fungal networks over a restore stage. The colors of different colors edges represent different types of interactions, positive and negative correlation. **B** The positive and negative correlation between non-degraded and severely degraded samples, as well as the changes in early and late recovery. **C** The variation of B-F positive and negative connections (T-test, two-sided). **D** Compared the trend of network resilience, resilience stability and PSQIR (Matched samples T-test, two-sided). Non-degraded ($n = 5$), severely degraded ($n = 5$), early recovery stages ($n = 25$, from the first year to the ninth year) and late recovery stages ($n = 20$, from the 12th to the 18th year), Kruskal–Wallis, two-sided; Duncan's test, Lowercase letters show significant differences between restoring stages at the $P < 0.05$ level. Source data are provided as a Source data file.

between PSQIR and network resilience. Conversely, a notable negative correlation was observed between the stability and complexity of the network. Distinct different correlations were identified between the core species of generalist and specialist with most factors (Fig. 5A). Regression analysis also further confirmed a significant positive correlation between network resilience (including negative, positive and overall B-F resilience) and PSQIR, aligning with the Spearman correlation results presented in Fig. 5A, B.

To delve deeper into these relationships, we employed structural equation modeling to analyze the impact of core species, network resilience and PSQIR (CMIN/DF = 1.075, CFI > 0.9, NFI > 0.9, GFI > 0.9, RMSEA < 0.05, $P = 0.341$). The analysis indicated that the presence of generalist core species enhanced the resilience of the network, particularly in the negative interactions between fungi and bacteria, potentially leading to an improvement in PSQIR. The presence of core species significantly influenced the recovery of fungal and bacterial communities, which in turn affects the overall network recovery and, ultimately, influenced PSQIR. Collectively, these factors accounted for 47% of the variance in PSQIR (Fig. 5C).

Discussion

The pivotal role of environmental factors in shaping microbial communities and their subsequent consequent impact on ecosystem functions is well-recognized[14]. The extensive positive correlations between soil organisms underscore their vital contribution to maintaining global soil biodiversity and the integrity of ecological network[15]. Previous study has established a strong connection between the stability of plant and microbial community in degraded grasslands, highlighting the mutual dependence essential for community stability[16]. The space-for-time substitution method has been widely used in ecological studies to generate a chronosequence[17]. The space-for-time substitution method was used to study the steady-state transition of ecosystems in the temperate steppe of Inner Mongolia, China[18]. A previous study in North America also supported the use of the space-for-time substitution method in predicting community responses to climate change[19]. Similarly, we used the space-for-time substitution for examining the complexity and stability of microbial community during long-term restoration of alpine grasslands. We recognize the limitations associated with using space-for-time substitution methods[18,20], as they involve assuming that plant, soil, and microbial processes will not be affected by a series of environmental covariates (e.g., precipitation, vegetation type). The approach was also compromised when the effects of variables such as soil type and landform were not minimized[21]. Our results supported that space-for-time substitution in this study was accountable. Previous studies have demonstrated a trade-off between resistance and resilience[22,23]. However, our findings suggested that bacteria exhibited greater composition resistance and resilience during degradation compared to fungi, without displaying a trade-off relationship between the two. Likewise, two studies conducted in Europe observed no trade-off between microbial resistance and resilience[24,25]. Due to human-involved grassland restoration efforts, the complexity of microbial networks had

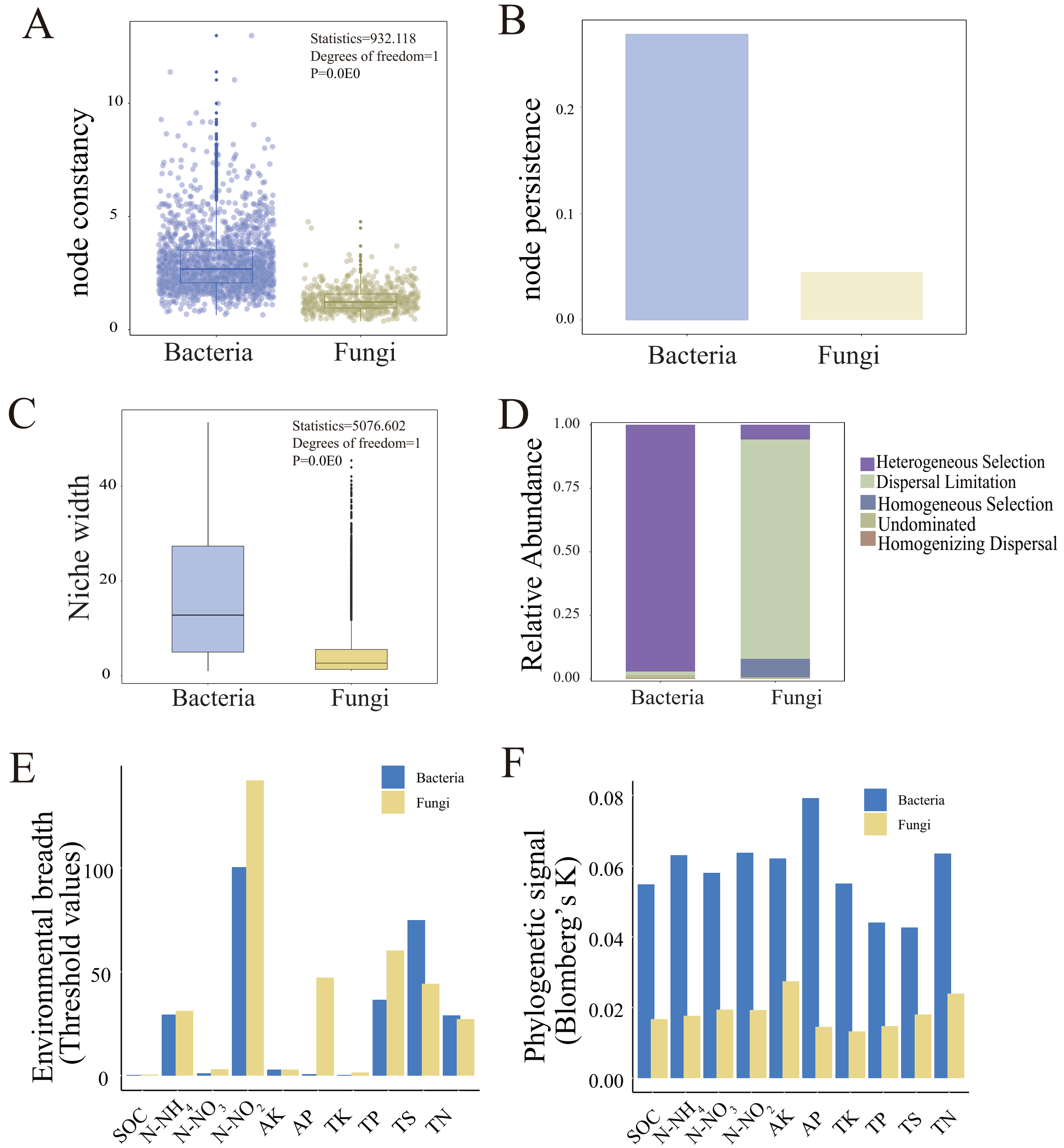

Fig. 3 | Community assembly and environmental adaptability of fungi and bacteria. Compared the **A** node constancy (*n* = 55) and **B** node persistence (*n* = 55) between fungi and bacteria. **C** Compared the niche width between fungi and bacteria (*n* = 55). **D** The relatively explained ecological processes of bacterial and fungal communities (*n* = 55) relied on the βNTI and RC_{bray}. **E** Environmental breadth is determined by the environmental threshold values of bacteria and fungi (*n* = 55). **F** Based on Blomberg's K, the Phylogenetic signal illustrates the conservatism for environmental preferences of the bacterial and fungal community (*n* = 55). Box plots: Centerline, median; upper and lower quartiles; whiskers, 1.5× interquartile range; points, outliers. Source data are provided as a Source data file.

increased, and the interactions between microorganisms had become more intricate and complex, underscoring the importance of core species in connecting network restoration with the plant-soil system.

The characteristics of bacterial-fungal networks during the restoration process showed improvement, reflecting a non-linear trend in the restoration capacity of grassland vegetation, soil, and vegetation-soil system. During the restoration of grasslands, the complexity of microbial network decreased after 4 years of restoration, possibly due to competition for nutrients among early plant growth. After 6 years of restoration, complexity had increased as more stable plants grew. However, after 8–9 years, there was a "secondary degradation" and a drop in complexity observed. Interestingly, after 12–16 years, began to rise again, indicating a trend towards recovery. Previous studies have demonstrated the intertwined nature of plant communities' diversity and soil microorganisms, both of which are crucial for providing multiple ecosystem functions[6,26–28].

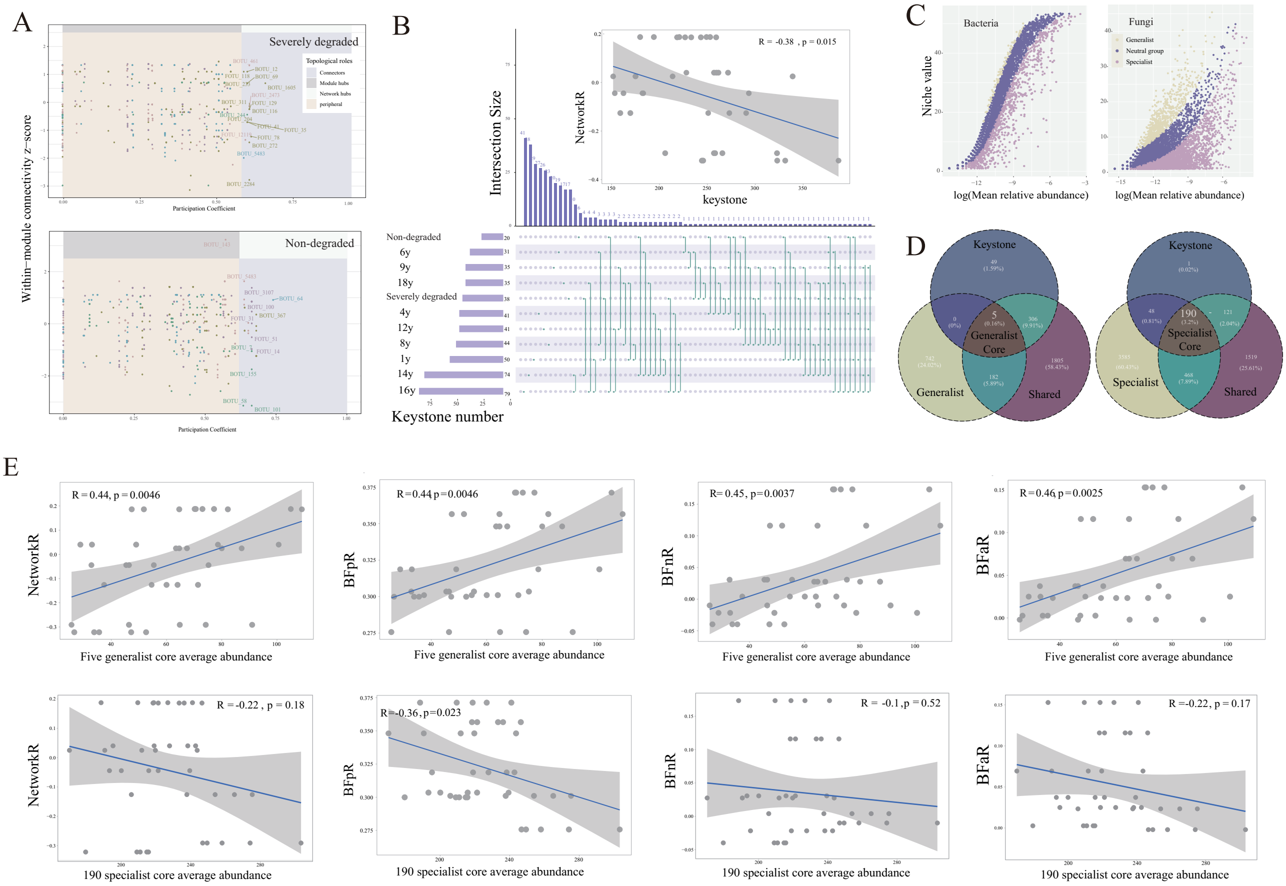

Fig. 4 | The relationship between core species and network complexity. **A** Calculating the ZIPI values for 11 networks dividing the Keystone central node microorganisms in each network. **B** The screening of overlap keystone and the relationship between keystone and PSQIR (Pearson, two side, Shaded bands are 95% CIs). **C** Screening of generalists and specialists in bacteria and fungi, **D** Screening of core microorganisms. **E** Correlation analysis of core microorganisms and network resilience (Pearson, two side, Shaded bands are 95% CIs). NetworkR network resilience, BFaR B-F(all) resilience, BFnR B-F (negative) resilience, BFpR B-F (positive) resilience. Source data are provided as a Source data file.

Microbes mainly enhance plant health through improved nutrient acquisition and disease resistance[29]. The intricate web of positive and negative correlations among microbial species plays a significant role in this dynamic, as demonstrated by Gao et al.[9], highlighting the complexities of microbial co-occurrence networks. The network complexity and the negative connection interactions between bacteria and fungi both played a role in regulating soil multifunctionality, with the former promoting and the latter potentially hindering this multifunctionality[30]. Although our results clearly link microbial diversity and composition resilience, the connection to the recovery of plant soil system is less pronounced. Nevertheless, we observed a significant correlation between the degree of network recovery and plant-soil system restoration, suggesting that microbial interactions may play a pivotal role in the functioning of microorganisms. These interactions facilitate soil colonization by microorganisms[31], indicating that the trend in microbial network resilience aligns with previous findings on the resilience of plant-soil systems, which peaked at the 16th year of recovery[32].

The growth of microbial communities is influenced by the soil environment and plants. We have directly evaluated soil nutrient factors related to abiotic stress (Supplementary Table 2). The average values of these factors provide a clear indication of nutrient conditions and stress levels to categorize severely degraded samples as high stress samples, non-degraded samples as low stress samples, and the after restoration (including early stage and late stage) as a stress situation falling between high and low stress. The abundance of N-NH_4, TP, and TS increases with the increase of stress gradient, while the other nutrients show no consistent pattern. Hernandez et al. found that total cohesion decreases with increasing stress[10], attributed to a reduction in both positive and negative cohesion along the pressure gradient. Our results, where the overall cohesion of severely degraded samples showed no significant difference compared to non-degraded samples. The relationship between network complexity and stability is complex[33]. This suggests that increased network recovery may not always correspond to microbial community stability. Hernandez et al. also found that the ratio of negative to positive cohesion decreases with increasing stress, indicating a dominance of cooperative behaviors in high stress environments[10]. Our findings, supporting the stress gradient hypothesis, suggest that microbial species exhibit more cooperative rather than competitive behavior during recovery stages and non-degraded environments.

The longer the restoration period of the interconnection between fungi and bacteria in the network, the greater it grows. The resilience between fungi and bacteria best represents the network resilience. Fungi demonstrate greater resilience compared to bacteria, with a more significant increase in connections, suggesting that fungi are more volatile while bacteria maintain greater stability. Bacteria nodes demonstrated higher constancy and persistence than fungal node, indicating greater stability and longevity of bacteria within the community[34]. The higher niche width of bacteria indicates stronger environmental adaptability[35]. Fungal taxa being dominated by dispersal limitation and stochastic processes, consistent with a weaker phylogenetic signal. This indicates a more stochastic assembly process for fungi, contributing to their flexibility in metabolic functions and

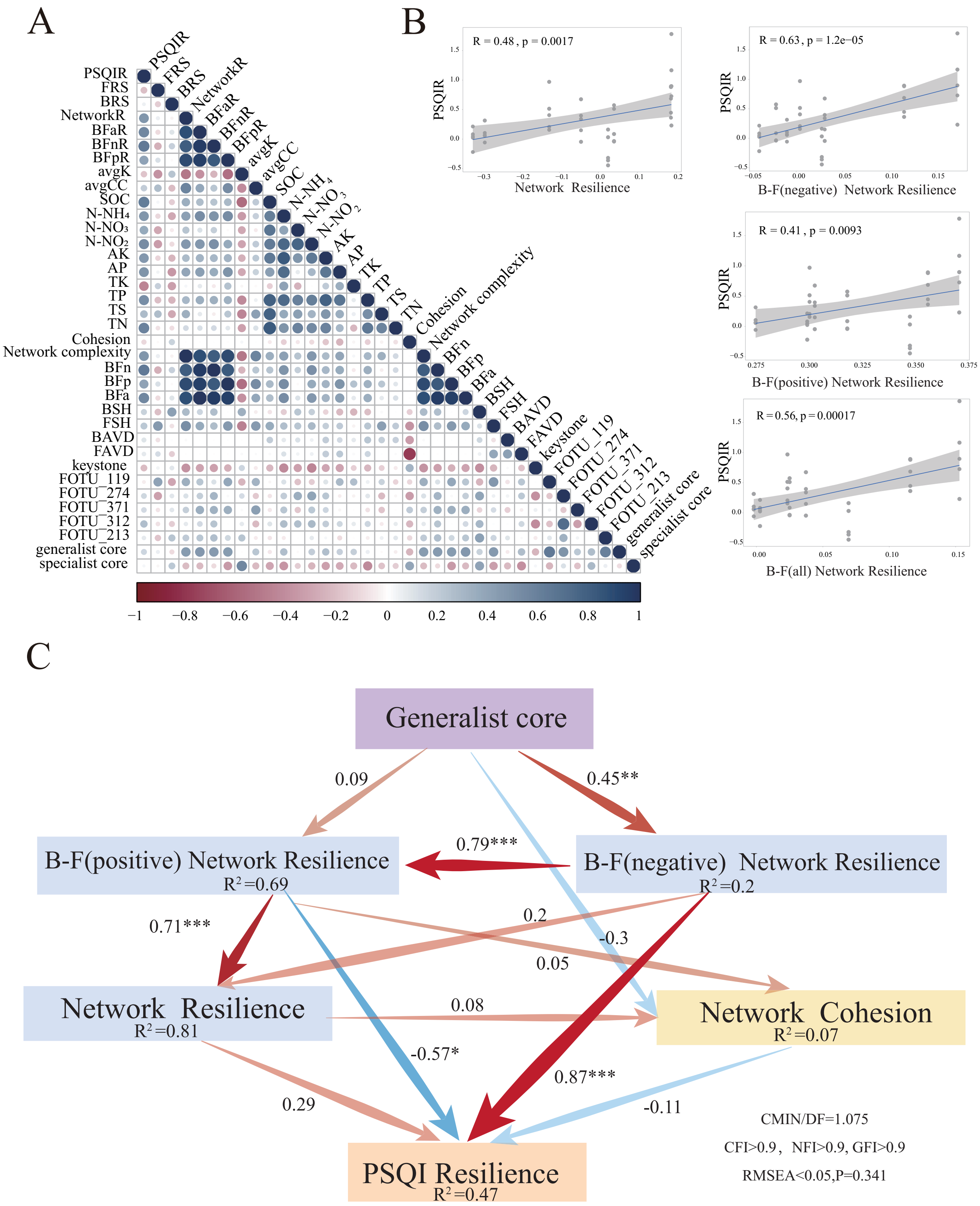

Fig. 5 | The relationship between plant-soil system resilience and microbial network resilience. A Pearson correlations analysis of soil physical and chemical properties, network stability, complexity resilience, core microorganisms and PSQIR. **B** Regression analysis of network resilience and PSQIR (Pearson, two side, Shaded bands are 95% CIs). **C** Structural Equation Modeling of Core species, complexity resilience and PSQIR (CMIN/DF = 1.075, CFI > 0.9, NFI > 0.9, GFI > 0.9, RMSEA < 0.05, P = 0.341). PSQIR plant soil quality resilience, FRS Fungi resilience stability, BRS Bacteria resilience stability, NetworkR network resilience, BFaR B-F(all) resilience, BFnR B-F (negative) resilience, BFpR B-F (positive) resilience, BFa B-F all spearman correlation, BFn B-F negative spearman correlation, BFp B-F positive spearman correlation, BSH Bacteria Shannon, FSH Fungi Shannon. Source data are provided as a Source data file.

ability to maintain community structure under fluctuating environmental conditions[35]. In contrast, bacterial taxa are governed by heterogeneous selection, explained by a stronger phylogenetic signal, which aids their adaptation to extreme environments and favors deterministic heterogeneous selection mechanisms, leading to greater structural stability. This comprehensive analysis may explain why bacteria demonstrated higher resistance and resilience stability, and underscores the complex and distinct interactions between bacteria and fungi within soil microbial communities, highlighting the importance of considering microbial dynamics in efforts to restore degraded grasslands.

The restoration of connections between fungi and bacteria closely correlates with the trend of PSQIR. The interplay between these microbial groups is crucial for the overall network recovery process. Investigation into specialized taxa have further emphasized their roles in maintaining community stability[4,13,36]. In previous research, dominant bacterial and fungal taxonomic groups exhibit strategies for drought resistanc[37]. Wang et al. have shown that elevated precipitation leads to soil C loss through interactions between microbes-plants-soil[38]. Ma et al. highlight the pivotal role of core microorganisms in enhancing network resilience during restoration and, consequently, in the restoration of plant soil health[39]. Our research builds on this by emphasizing the critical role of microorganisms in restoring plant-soil systems and exploring the impact of core species on the microorganism-plant-soil relationship. Generalist core species, which exhibit higher adaptability and play a crucial role in networks resilience, helped to rebuild the relationship between bacteria and fungi, thereby aiding in the restoration of the overall network and further promoting the restoration of the ecosystem. The significance of core species in maintaining network resilience throughout grassland restoration was a key finding of this study, revealing synergistic effect among plants, soil, and microorganisms. These findings highlight the intricate balance between specialist and generalist microorganisms in shaping the resilience and cohesion of soil microbial communities during ecological recovery processes. Understanding the roles and dynamics of these core OTUs offers valuable insights into ecosystem restoration and management strategies. Microorganisms play a crucial role in soil ecological processes, with their resistance and resilience determining the sustainability of ecosystem functionalities. Microbes can optimize nutrient cycles, improve soil structure, and increase resistance to disturbances. By introducing key microorganisms during restoration, or by managing the balance between bacteria and fungi, we can implement effective strategies for soil and plant restoration. Monitoring the health of soil microbes can serve as a valuable indicator of the progress and success of restoration efforts. These insights into the resistance and resilience of soil microbial communities are essential for assessing soil health and predicting the effects of perturbations on ecosystem functions in the face of future global change scenarios. This has profound implications in developing new strategies to enhance ecosystem resilience, particularly through the protection of core microbes in alpine grassland and other similar ecosystems globally.

Methods

Sampling sites and data collection

The restoration treatments in the experimental site, Dawu town of Maqin country which is a typical alpine area with a mean annual temperature (MAT) is 0.28 °C, changing from −15.9 °C to 13.0 °C and average annual rainfall is 516 mm, were undertaken since 2000 when the severely degraded alpine grasslands were replanted with the native perennial grass, *Elymus nutans, Elymus sibiricus, Festuca sinensis, Poa crymophila*[40]. To improve the grassland, a mixture of four native grass species was seeded. The seed are mixed well and evenly distributed. The seeding rates were 6 kg/hm^2 for *Elymus nutans*, 6 kg/hm^2 for *Elymus sibiricus*, 4.5 kg/hm^2 for *Festuca sinensis* and 1.5 kg/hm^2 for *Poa crymophila* according to their seedling density ratios of 1:1:1:1. The site was plowed to remove poisonous plants and annual weeds before planting the grass mixture. The site was plowed to remove poisonous plants and annual weeds before planting the grass mixture. After planting the sites received no further management. All reseeded sites were fenced to reduce disturbance and progressive succession. The samplings were conducted in 2017 to measure the soil microbe and chemistry of the restored alpine grasslands at different years: 1 year(1 y), 4 year(4 y), 6 year(6 y), 8 year(8 y), 9 year(9 y),12 year(12 y), 14 year(14 y), 16 year(16 y) and 18 year(18 y). Meanwhile, the non-degraded alpine grasslands and unrestored severely degraded alpine grasslands were sampled as the controls. All the repeated samples of 3 soil cores using an auger boring in each quadrat were randomly collected and mixed from each of 5 separate patches with the same treatments (the restored grasslands in the same successional years or the controlled grasslands) across the space. The five separate sampling patches were situated within independently restored fields, with a sampling quadrat randomly placed within each of the five patches. All the restored/reseeded fields from 1-year to 18-year were fenced to get rid of disturbance to guard the progressive succession. Therefore, five patches across the larger field were sampled actually from the fenced/undisturbed field or habitats. In total, we collected 55 samples for the subsequent analyses. Supplementary Fig. 8 showed the sampling sites. The size of the patches varied a bit due to the difference of their ownership, as they belong to different households. However, the topography or field conditions were not much different, they were flat fields with similar soil and vegetation conditions.

Soil physicochemical measurement

The physicochemical properties of the soil samples, including total nitrogen (TN), total phosphorus (TP), total potassium (TK), total sulfur (TS), soil organic carbon (SOC), ammonia-nitrogen (N-NH$_4$), nitrate nitrogen (N-NO$_3$), nitrite nitrogen (N-NO$_2$), available potassium (AK) and available phosphorus (AP), were measured as previously reported[8]. All the soil physicochemical quantification were in triplicate.

Soil DNA extraction and amplicon sequencing

Total microbial DNA was extracted from 0.5 g soils using FastDNA®SPIN Kit for Soil (MP Biomedicals, CA, USA) according to the standardized protocol. DNA extracts were dissolved with DES buffer, quantified, and qualified using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Germany). The V4-V5 regions of the bacterial 16S rRNA gene were amplified using the primers 515F (GTGCCAGCMGCCGCGG) and 907R(CCGTCAATTCMTTTRAGTTT). ITS1F (5-CTTGGTCATTTA-GAGGAAGTAA-3′) and ITS2R (5-GCTG CGTTCTTCATCGATGC-3′) were applied to amply fungal ITS gene at the ITS2 region[41]. The PCR amplification cycles were set as follows: 95 °C for 2 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. The PCR products were purified and quantified as previously demonstrated[8].

The PCR products were purified in identical quantities and sequenced on the Illumina HiSeq2500 platform (Illumina Inc., San Diego, CA, USA). The raw paired-end reads were denoised and assembled by using DADA2 v1.1.3. USEARCH tool was applied to detect and remove chimera. The raw reads were assigned to distinguish samples according to the barcodes with Lima. FLASH was used to combine forward with reverse sequences. 16S rRNA and ITS taxonomic annotation were determined based on the SILVA database (version 132) and the UNITE fungal. Operational taxonomic units (OTUs) with a 97% similarity cutoff were clustered using UPARSE[42]. The OTU table was used for subsequent analyses.

Microbial diversity patterns analysis

To test whether diversity patterns differed along the stage of grassland restoration succession, alpha diversity indices of bacteria and fungi were evaluated with the vegan package in R.4.2.1. Kruskal-Wallis test was applied to analyze the effects of grassland restoration on α-diversity. Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity was applied to explore the pattern of the microbial community. The influences of grassland restoration on bacteria and fungi were assessed by permutational multivariate analysis of variance (PERMANOVA).

Co-occurrence network construction

Microbial co-occurrence networks were independently created for every year using the OTUs table. Correlations with Spearman's correlation coefficients (ρ) greater than 0.6 and corresponding *P*-values less than 0.01 were considered significant. The '*rcorr*' function from the '*Hmisc*' package was used to perform pairwise comparisons based on OTUs, with *p*-values adjusted accordingly. Co-occurrence networks were constructed using the '*igraph*' package, where each node represented one OTU and each edge represented a strong and significant correlation. The resulting networks were visualized using the interactive platform Gephi (0.9.7). We classified the nodes into four categories based on their within-module connectivity (Z_i) and among-module connectivity (P_i) to identify the topological roles of taxa in the networks. To identify keystones in the network, we calculated the within-module connectivity (Z_i) and among-module connectivity (P_i) of each node. According to corresponding criteria, we identified module hubs ($Z_i \geq 2.5$, $P_i < 0.62$), connectors ($Z_i < 2.5$, $P_i \geq 0.62$) and network hubs ($Z_i \geq 2.5$, $P_i \geq 0.62$), which are referred to as keystone.

Analysis of the habitat specialists and generalists and core species

To determine the habitat specialization of microorganisms, we employed Levin's niche breadth[43]. Briefly, the significance of the habitat specialization index was tested using the *Ecolutils* in R for 1000 permutations. When the habitat specialization values exceed the upper 95% confidence interval or fall below the lower 95% confidence interval of 1000 permutations, they are identified as habitat generalists or specialists. All statistical analyses were conducted using R version 4.3.0.

We took the intersection between specialists, Keystone, and shared microorganisms to obtain core specialists[13]. Similarly, we took the intersection between generalists, Keystone, and shared microorganisms to obtain core generalists.

Quantification of community assembly

The null model was implemented to determine deterministic and processes and stochastic processes of both bacteria and fungi[44]. Bray-Curtis-based Raup-Crick (RC_{bray}) and β nearest taxon index (βNTI) were calculated to identify the community assembly of bacteria and fungi. The partial Mantel test was conducted to quantify the relationship between the environmental variables and βNTI. The correlations between βNTI and the vital physicochemical factors were assessed by linear regression models.

Phylogenetic conservation and environmental threshold

Environmental threshold values of bacteria and fungi were calculated by threshold indicator taxa analysis (TITAN2) to unravel microbial response to environmental factors[45]. TITAN2 categorizes the community into two groups: Z^- taxa that negatively respond to increased environmental gradient, and Z^+ taxa that positively respond to increased gradient. Taxa without any response to the environmental gradient were not considered. TITAN then tracks the cumulative responses of declining taxa (sum(Z^-)) and increasing taxa (sum(Z^+)) in the community. Ecological thresholds are defined as the points where the maximum aggregate change in the frequency and relative abundance of responding taxa occurs. When the environmental values reach and exceed the ecological thresholds, the abundance and occurrence frequency of species will decrease in the Z^- group while increasing in the Z^+ group. Therefore, the range of niche optima for the community is defined as the gradient below sum(Z^-) and above sum(Z^+).

Phylogenetic signals were instrumental in testing whether the environmental preference was related to the phylogeny[46,47]. Spearman correlations between environmental parameters and the relative abundance of OTUs were calculated to identify the ecological preference[48]. Blomberg's K statistic was performed to excavate phylogenetic signals for the environmental adaption of bacteria and fungi.

Assessing microbial community stability and quality of plant-soil system

Community stability in response to disturbance includes two components: resistance and resilience. In this study, to measure composition resistance, we used Bray-Curtis dissimilarity values to compare non-degraded grassland samples with severely degraded ones. Similarly, for composition resilience, we compared non-degraded grassland samples with restored ones using Bray-Curtis dissimilarity values. We also used the resilience index measuring the extent of recovery by determining the spearman correlation between species. It calculates the ratio of the difference in Spearman correlation between each sample and severely degraded samples to the difference in non-degraded and severely degraded samples in order to characterize the network resilience. Moreover, we also utilize the AVD index[36,49] and Cohesion index[13,33] to assess the stability of microbial communities. AVD (average variation degree) is defined as the degree to which microbial communities bear environmental changes, implying the overall stability of the microbial community. Lower AVD suggested higher stability of the microbial community. The index plant-soil systems quality index (PSQI) and the resilience index of plant-soil systems (PSQIR) were quantified in previous research[32]. We carefully selected from nine plants indicators and sixteen soil indicators to create the plant-soil system quality index (PSQI) for assessing ecosystem changes during restoration efforts. The ultimate ecological goal for the restored sites is to establish a thriving natural grassland with optimal plant and soil quality. The specific goals for plant community composition is to have a coverage rate of 100%, with the proportion of edible plants exceeding 70%, and reaching the height of edible plants over 25 cm, based on the standards outlined by Ma et al.[50]. The recovery index in this article is calculated based on this goal.

Statistics and reproducibility

We used 5 biological replicates in each year: non-degraded ($n = 5$), severely degraded ($n = 5$), and restored alpine grasslands at different years: 1 year (1 y, $n = 5$), 4 years (4 y, $n = 5$), 6 years (6 y, $n = 5$), 8 years (8 y, $n = 5$), 9 years (9 y, $n = 5$), 12 years (12 y, $n = 5$), 14 years (14 y, $n = 5$), 16 years (16 y, $n = 5$), and 18 years (18 y, $n = 5$). There were early recovery stages ($n = 25$, from 1 y to 9 y) and late recovery stages ($n = 20$, from 12 y to 18 y). The diversity calculation and co-occurrence network indicators for each treatment were based on 5 biological replicates from each treatment. The total sample size for calculating these parameters (Node constancy, Node persistence, Niche width, Phylogenetic conservation and Environmental threshold, Quantification of community assembly) is all samples ($n = 55$). No statistical method was used to predetermine sample size. Our experiment does not have any subjective bias towards the experimental observation results, so we did not use blind experiments. No data was excluded except for outliers, which are defined as observations that are lower than or higher than the whiskers on the box plot. The interquartile range is the difference between the third quartile and the first quartile in the data.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data used in this study have been deposited in the Sequence Read Archive database under accession codes PRJNA1199960 and RJNA1199985. All other data generated in this study are provided in the Source data file. Source data are provided with this paper.

Code availability

The code for implementing the analyses is available on Zenodo: https://doi.org/10.5281/zenodo.14881382[51].

References

1. Dong, S., Shang, Z., Gao, J. & Boone, R. B. Enhancing sustainability of grassland ecosystems through ecological restoration and grazing management in an era of climate change on Qinghai-Tibetan Plateau. *Agric. Ecosyst. Environ.* **287**, 106684 (2020).
2. Bardgett, R. D. et al. Combatting global grassland degradation. *Nat. Rev. Earth Environ.* **2**, 720–735 (2021).
3. Wang, Y. et al. Meadow degradation increases spatial turnover rates of the fungal community through both niche selection and dispersal limitation. *Sci. Total Environ.* **798**, 149362 (2021).
4. Jiao, S. et al. Linking soil fungi to bacterial community assembly in arid ecosystems. *iMeta* **1**, e2 (2022).
5. Liu, S. et al. Decoupled diversity patterns in bacteria and fungi across continental forest ecosystems. *Soil Biol. Biochem.* **144**, 107763 (2020).
6. de Vries, F. T. et al. Soil bacterial networks are less stable under drought than fungal networks. *Nat. Commun.* **9**, 3033 (2018).
7. Griffiths, B. S. & Philippot, L. Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiol. Rev.* **37**, 112–129 (2013).
8. Gao, X. et al. Revegetation significantly increased the bacterial-fungal interactions in different successional stages of alpine grasslands on the Qinghai-Tibetan Plateau. *Catena* **205**, 105385 (2021).
9. Gao, C. et al. Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. *Nat. Commun.* **13**, 3867 (2022).
10. Hernandez, D. J., David, A. S., Menges, E. S., Searcy, C. A. & Afkhami, M. E. Environmental stress destabilizes microbial networks. *ISME J.* **15**, 1722–1734 (2021).
11. Maestre, F. T., Callaway, R. M., Valladares, F. & Lortie, C. J. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J. Ecol.* **97**, 199–205 (2009).
12. Forrester, D. I. & Bauhus, J. A review of processes behind diversity—productivity relationships in forests. *Curr. For. Rep.* **2**, 45–61 (2016).
13. Qiao, Y. et al. Core species impact plant health by enhancing soil microbial cooperation and network complexity during community coalescence. *Soil Biol. Biochem.* **188**, 109231 (2024).
14. Yan, L. et al. Environmental selection shapes the formation of near-surface groundwater microbiomes. *Water Res.* **170**, 115341 (2020).
15. Liu, X. et al. Positive associations fuel soil biodiversity and ecological networks worldwide. *Proc. Natl Acad. Sci. USA* **121**, e2308769121 (2024).
16. Zhang, H., Chen, W., Dong, L. & Wang, W. Grassland degradation amplifies the negative effect of nitrogen enrichment on soil microbial community stability. *Glob. Change Biol.* **30**, e17217 (2024).
17. Pickett, S. T. A. in *Long-Term Studies in Ecology: Approaches and Alternatives* (ed. Likens, G. E.) 110–135 (Springer New York, 1989).
18. Hu, Z. et al. Shifts in the dynamics of productivity signal ecosystem state transitions at the biome-scale. *Ecol. Lett.* **21**, 1457–1466 (2018).
19. Blois, J. L., Williams, J. W., Fitzpatrick, M. C., Jackson, S. T. & Ferrier, S. Space can substitute for time in predicting climate-change effects on biodiversity. *Proc. Natl Acad. Sci. USA* **110**, 9374–9379 (2013).
20. Lovell, R. S. L., Collins, S., Martin, S. H., Pigot, A. L. & Phillimore, A. B. Space-for-time substitutions in climate change ecology and evolution. *Biol. Rev.* **98**, 2243–2270 (2023).
21. Wang, M. et al. Global soil profiles indicate depth-dependent soil carbon losses under a warmer climate. *Nat. Commun.* **13**, 5514 (2022).
22. Orwin, K. H., Wardle, D. A. & Greenfield, L. G. Context-dependent changes in the resistance and resilience of soil microbes to an experimental disturbance for three primary plant chronosequences. *Oikos* **112**, 196–208 (2006).
23. de Vries, F. T. et al. Land use alters the resistance and resilience of soil food webs to drought. *Nat. Clim. Change* **2**, 276–280 (2012).
24. Winterfeldt, S., Cruz-Paredes, C., Rousk, J. & Leizeaga, A. Microbial resistance and resilience to drought across a European climate gradient. *Soil Biol. Biochem.* **199**, 109574 (2024).
25. Tang, Y., Winterfeldt, S., Brangarí, A. C., Hicks, L. C. & Rousk, J. Higher resistance and resilience of bacterial growth to drought in grasslands with historically lower precipitation. *Soil Biol. Biochem.* **177**, 108889 (2023).
26. Chen, K. et al. Plant community and soil available nutrients drive arbuscular mycorrhizal fungal community shifts during alpine meadow degradation. *Fungal Ecol.* **62**, 101211 (2023).
27. Chen, K. et al. Long-term fencing can't benefit plant and microbial network stability of alpine meadow and alpine steppe in Three-River-Source National Park. *Sci. Total Environ.* **902**, 166076 (2023).
28. Shen, C. et al. Plant diversity enhances soil fungal network stability indirectly through the increase of soil carbon and fungal keystone taxa richness. *Sci. Total Environ.* **818**, 151737 (2022).
29. Noman, M., Ahmed, T., Wang, J. & White, J. C. Micronutrient–microbiome interplay: a critical regulator of soil–plant health. *Trends Microbiol.* **32**, 319–320 (2024).
30. Wang, X. et al. Decreased soil multifunctionality is associated with altered microbial network properties under precipitation reduction in a semiarid grassland. *iMeta* **2**, e106 (2023).
31. Wu, D. et al. Microbial interactions within beneficial consortia promote soil health. *Sci. Total Environ.* **900**, 165801 (2023).
32. Gao, X. et al. Resilience of revegetated grassland for restoring severely degraded alpine meadows is driven by plant and soil quality along recovery time: a case study from the Three-river Headwater Area of Qinghai-Tibetan Plateau. *Agric. Ecosyst. Environ.* **279**, 169–177 (2019).
33. Yuan, M. M. et al. Climate warming enhances microbial network complexity and stability. *Nat. Clim. Change* **11**, 343–348 (2021).
34. Cornell, C. R. et al. Land use conversion increases network complexity and stability of soil microbial communities in a temperate grassland. *ISME J.* **17**, 2210–2220 (2023).
35. Yang, Y., Cheng, K., Li, K., Jin, Y. & He, X. Deciphering the diversity patterns and community assembly of rare and abundant bacterial communities in a wetland system. *Sci. Total Environ.* **838**, 156334 (2022).
36. Xun, W. et al. Specialized metabolic functions of keystone taxa sustain soil microbiome stability. *Microbiome* **9**, 35 (2021).
37. Lavallee, J. M. et al. Land management shapes drought responses of dominant soil microbial taxa across grasslands. *Nat. Commun.* **15**, 29 (2024).
38. Wang, M. et al. Long-term elevated precipitation induces grassland soil carbon loss via microbe-plant–soil interplay. *Glob. Change Biol.* **29**, 5429–5444 (2023).
39. Ma, L. et al. Long-term conservation tillage enhances microbial carbon use efficiency by altering multitrophic interactions in soil. *Sci. Total Environ.* **915**, 170018 (2024).
40. Li, J. et al. Application of design theory for restoring the "black beach" degraded rangeland at the headwater areas of the Qinghai-Tibetan Plateau. *Afr. J. Agric. Res.* **5**, 3542–3552 (2010).
41. Zhang, Y. et al. Climate change and human activities altered the diversity and composition of soil microbial community in alpine grasslands of the Qinghai-Tibetan Plateau. *Sci. Total Environ.* **562**, 353–363 (2016).

42. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).
43. Yan, Q. et al. Distinct strategies of the habitat generalists and specialists in sediment of Tibetan lakes. *Environ. Microbiol.* **24**, 4153–4166 (2022).
44. Stegen, J. C., Lin, X., Konopka, A. E. & Fredrickson, J. K. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **6**, 1653–1664 (2012).
45. Baker, M. E. & King, R. S. A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods Ecol. Evol.* **1**, 25–37 (2010).
46. Martiny, J. B. H., Jones, S. E., Lennon, J. T. & Martiny, A. C. Microbiomes in light of traits: a phylogenetic perspective. *Science* **350**, aac9323 (2015).
47. Jiao, S. & Lu, Y. Abundant fungi adapt to broader environmental gradients than rare fungi in agricultural fields. *Glob. Change Biol.* **26**, 4506–4520 (2020).
48. Oliverio, A. M., Bradford, M. A. & Fierer, N. Identifying the microbial taxa that consistently respond to soil warming across time and space. *Glob. Change Biol.* **23**, 2117–2129 (2017).
49. Shade, A. et al. Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* **3**, 417 (2012).
50. Ma, Y., LANG, B., LI, Q., SHI, J. & DONG, Q. Study on rehabilitating and rebuilding technologies for degenerated alpine meadow in the Changjiang and Yellow river source region. *Pratacultural Sci.* **19**, 1–5 (2002).
51. He, X. Core microbes regulate plant-soil resilience by maintaining network resilience during long-term restoration of alpine grasslands. *Znodo*. https://doi.org/10.5281/zenodo.14881382 (2025).

Acknowledgements

This research was financially supported by grants from the National Key Research and Development Program of China (2021YFE0112400, Dong; 2023YFF1304302, He), National Natural Science Foundation of China (32361143870, Dong), the Second Tibetan Plateau Scientific Expedition and Research Program (2019QZKK0307, Dong).

Author contributions

Y.D. contributed to methodology, data analysis, software, paper-writing. Y.Y. contributed to data preprocessing and writing—original draft. S.W. and X.G. contributed to experiment implementation and material collection. X.H. contributed to writing—reviewing. S.D. contributed to study designing.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-025-58080-2.

Correspondence and requests for materials should be addressed to Xiaoqing He or Shikui Dong.

Peer review information *Nature Communications* thanks Elizabeth Bach and the other anonymous reviewer(s) for their contribution to the peer review of this work. A peer review file is available.

Reprints and permissions information is available at http://www.nature.com/reprints