Network classification reveals the variation of soil bacterial diversity among plant species

Zijian Ding
dingzj0828@163.com
Horticultural College, Shenyang Agricultural University

Tianze Li
15640411053@163.com
Horticultural College, Shenyang Agricultural University

Baihui Ren
bhren@syau.edu.cn
Horticultural College, Shenyang Agricultural University

Jiyun Yang
yangjiyun@syau.edu.cn
Horticultural College, Shenyang Agricultural University

Long Bai
bailong2018@syau.edu.cn
Horticultural College, Shenyang Agricultural University

Jiahuan Li
LiJHecol@163.com
Horticultural College, Shenyang Agricultural University

Lizhu Guo
ellenguo@sina.cn
Institute of Grassland Research

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Zijian Ding[1], Tianze Li[1], Baihui Ren[1], Jiyun Yang[1], Long Bai[1], Jiahuan Li[1*], Lizhu Guo[2*]

[1] Horticultural College, Shenyang Agricultural University, Shenyang, 110866, China;

[2]Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Hohhot, 010010, China.

Author information

Zijian Ding : dingzj0828@163.com

Tianze Li: 15640411053@163.com

Baihui Ren: bhren@syau.edu.cn

Jiyun Yang: yangjiyun@syau.edu.cn

Long Bai: bailong2018@syau.edu.cn

***Corresponding author**

Jiahuan Li: LiJHecol@163.com, Shenyang Agricultural University, 120 Dongling Road, Shenyang, Liaoning province, 110866, China

Lizhu Guo: ellenguo@sina.cn, Ulanqab East Street NO. 120, Saihan District, Hohhot, 010010, China

Abstract

Soil bacterial diversity often shows different trends due to changes in dominant plant species. However, the potential key drivers of processes that reveal bacterial diversity *per se* have not been clearly elucidated. We established a field experiment with 9 native grassland species and assessed the relationship between soil bacterial diversity and ecological taxa classified by network construction. A co-occurrence network of 1065 points and 10023 edges, among 9 native grasses, was established to classify microbial ecological taxa. The results showed that the relative abundance of ecological taxa classified as peripherals, which is influenced by soil urease activity, inhibited bacterial diversity. Conversely, the relative abundance of specific taxa directly controlled by plants was positively related to bacterial diversity. Further, the composition of peripherals was not affected by soil physicochemical properties, while the composition of specific taxa was affected by NO_3^-, TP, AP, SU, TC and AK. The composition of peripherals and specific taxa have different responses to soil properties due to their sensitivity to environmental changes. Our findings reveal that plant-dominated bacterial diversity is closely linked to the abundance of peripheral and specific taxa. Understanding these mechanisms may provide a

new insight in to the management of grassland soil microbes through the selection of grass species following disturbance and subsequent restoration.

Keywords: Plant–microbial interactions; Microbial diversity; Co-occurrence network; Soil physicochemical properties

INTRODUCTION

Soils harbor a remarkably diverse range of microbes [1], which play crucial roles in ecosystem functions and services [2]. High diversity of microbes was always related with higher carbon use efficiency [3], plant health [4] and increased drought tolerance [5]. Top-down framework provides a credible suggestion that soil microbial diversity, like microbial community [6], is generally directly affected by aboveground plants through root exudates [7], symbiotic strategies [8], and litter decomposition [9]. It means that plant distinctness lead to differences in microbial diversity [10]. For instance, a recent study suggested that higher sugar levels in root exudates decreased overall microbial diversity through benefited certain microbial members [11]. In addition, the impacts of plants on soil properties, such as nitrate levels and urease activity, are deemed essential in shaping microbial diversity [12,13]. Aboveground revegetation can boost soil nitrogen availability, which eases microbial nutrient constraints and enhances microbial diversity [14].

Nevertheless, plants remarkably shape soil microbial community and mediate its diversity, but the underlying processes remain poorly understood.

Different microbial species show strongly positive or negative relationships with each other and these inter-species interactions potentially regulate soil microbial diversity [15–17]. In recent years, microbial network construction, an efficient method to reflect microbe-microbe interactions has aroused extensive attention [18–20]. Highly connected species can form complex network structures that regulate various ecosystem functions [21], while the impact of microbial diversity on ecosystem functions are indirectly driven by microbial network complexity [22]. Further, the whole microbial network could be divided into multiple ecological clusters [23], where microbes within the same cluster usually perform similar ecological functions [24,25]. Based on connectivity of species in a certain cluster or between clusters, the ecological taxa in a microbial network can be classified into four parts: peripherals (with only a few connections, mostly within their own modules), connectors (connecting to multiple modules), module hubs (connecting to many nodes within their own modules), and network hubs (serving as both module hubs and connectors) [26]. Network hubs, connectors, and module hubs are usually identified as keystone taxa in ecological

networks [27], playing a crucial role in shaping the structure and function of microbial community [28]. Similarly, the abundance of keystone species is considered an important driver of diversity patterns in classical ecological studies[29,30]. Nevertheless, the identification of keystone species in microbial networks is inherently ambiguous and serves merely as a potential candidate for key species, given the influence of indirect effects and niche overlap[31]. Thus, we speculate that the effect of plant species on soil microbial diversity is mediated by the relative abundance of microbe species with high connectivity in the network. In addition, numerous specific microbial species which are frequently excluded before constructing the network since their lower survival probability in most treatments [32]. This may neglect the role of these species in ecological processes [33] and lead to biotic homogenization [34].

Cropland abandonment, one of the most common changes in land use patterns, always causes soil degradation, such as microbial diversity loss [35]. Natural revegetation usually takes a long time and result in soil nutrient loss [36]. The utilization of native grass species in ecological restoration can help restore the ecosystem to a near-natural state [37]. Reestablishing grassland following cropland not only mitigates soil degradation [38] but also regulates soil microbial diversity [39]. We therefore surveyed 163 local grasslands

and counted the frequency of plants, selecting 9 representative native grass species. In addition, long-term ploughing of conventional farmland tends to destroy the soil aggregates[40], distribute nutrients evenly[41] and make microorganisms highly homogeneous[42]. Therefore, we assumed that the influence of soil factors on microbes was the same and negligible before grasses planting. In this study, we planted a variety of plant species on uncultivated cropland and employed the ecological taxa with different interactions as a foundation for investigating the relationship between plants and soil microbial diversity, as well as assessing the influence of soil physicochemical properties on this relationship.

MATERIALS AND METHODS

Study area

This study was conducted at the Fuxin Mongolian Autonomous County (42°11’53’’N, 121°41’53’’E) which is located in northwest of Liaoning Province, China. It has a typical temperate semi-arid continental seasonal climate. Spring (March to May) has a long cold period and is dry; Summer (June to August) is hot, and the precipitation is concentrated; Autumn (September to November) cools down quickly and the rainfall drops sharply; Winter (December to February) is cold and dry, with four distinct seasons. The terrain

is dominated by low mountains and hills, with altitudes ranging from 45.8 m to 831.4 m. The soil is classified as brown soil (Dystric Cambisols, FAO) with a sandy and stony structure. The main agricultural crop is corn, which mainly grows after grasslands and forests are reclaimed. In this area, an increasing amount of cropland is being abandoned due to dry weather conditions and poor soil quality. The soil physicochemical properties of cropland and abandoned land were shown in Table S1.

Experimental design and soil sampling

We surveyed 163 natural grasslands in Liaoning Province and randomly designed 3 plots (1 m×1 m) for each grassland to count the frequency of natural grass species. In total, 9 common native grass species were selected and their wild seeds were collected and preserved in autumn 2020 (Table 1). In May 2021, we germinated 9 native grass species in nutrition bowls (The trapezoid has a bottom side length of 2 cm, a top side length of 3 cm, and a height of 4 cm, with 15g of peat soil inside.) at the Scientific Research Base of Shenyang Agricultural University (Liaoning, Shenyang, 41 ° 49 '24' 'N, 123 ° 33' 40 ''E) in order to make sure plant germination rate and initial growth remain consistent and transplanted the seedlings to the field (farming stopped after 2020) respectively in June 2021 when the average plant height is about 15-20 cm. The same grass

species were evenly distributed with the density of 9 plant per square meter. Each grass species covering an area of 600 m^2 (20 m × 30 m), with an interval of more than 1 m between the species.

Tabel 1 Species name, family names, abbreviation, type and frequency of 9 native grass species. Frequency was calculated based on the number of plant occurrences in 163 grasslands.

Species name	Family	Abbreviation	Type	Frequency (%)
Leymus chinensis	Gramineae	*L. chin*	perennial	22.09
Arundinella hirta	Gramineae	*A. hirt*	perennial	58.90
Elymus kamoji	Gramineae	*E. kamo*	perennial	60.12
Astragalus laxmannii	Leguminosae	*A. laxm*	perennial	3.07
Lespedeza bicolor	Leguminosae	*L. bico*	perennial	98.77
Artemisia gmelinii	Compositae	*A. gmel*	perennial	56.44
Artemisia frigida	Compositae	*A. frig*	perennial	6.75
Sanguisorba officinalis	Rosaceae	*S. offi*	perennial	12.27
Potentilla chinensis	Rosaceae	*P. chin*	perennial	28.22

In July 2022 (after 2 growing seasons), aboveground biomass of plants and topsoil (0-20 cm) were collected. For each grass species, 3 plots were randomly selected and 3 repetitions in each plot were mixed as a sample. In total, 27 soil samples (3 samples × 9 grass species) were obtained. Subsequently, samples were immediately

transported to the laboratory. 200 grams of soil were air-dried and sieved through a 2 mm sieve to analyze soil properties and 10 grams were stored in an -80 °C refrigerator for studying the microbial community. The vegetation biomass for 2021 is displayed in Table S2.

Measurement of soil physicochemical properties

The aggregate stability of the soil samples was assessed utilizing a wet sieve technique [43] with a 20-minute duration and an oscillation magnitude of 3 cm. Soil aggregate was represented by the size of > 0.25 mm water-stable aggregate (WSA), mean weight diameter (MWD), geometric mean diameter (GMD) and fractal dimension (Dm). Soil pH and soil electrical conductivity (EC) were determined with a pH and a EC meter using soil: water (w/v) = 1:5 [44]. Total nitrogen (TN) and total carbon (TC) are determined by combustion method with a carbon and nitrogen analyzer (Elementar Vario El Cube, Hanau, Germany) [45]. Soil organic carbon (SOC) was determined by dichromate oxidation Method [46]. Total phosphorus (TP) was determined by the $HClO_4$-H_2SO_4 method, and available phosphorus (AP) was determined by the $NaHCO_3$ extraction method [47]. Nitrogen availability ($NH4^+$ and $NO3^-$) was determined through KCl (2 mol/L) extraction using a chemical analyzer (Smartchem140 ，AMS ，Italy) [48]. Soil potassium content was

188 determined using the Soil Available Potassium Assay Kit [49]. Soil
189 phosphatase activity (SALP) was determined using the Soil Alkaline
190 Phosphatase (S-AKP/ALP) Activity Assay Kit [50]. Soil urease activity
191 (SU) was determined using the Soil Urease (S-UE) Activity Assay Kit
192 [51,52]. All Assay Kit were provided by Beijing Solarbio Science and
193 Technology Co., Ltd.

194 **High throughput sequencing**

195 The soil microbial community was determined through high-
196 throughput sequencing. Soil microbial DNA was extracted from each
197 soil sample using the Omega Mag-Bind DNA Kit for Soil (Omega Bio-
198 Tek, Norcross, GA, USA). The V4-V5 region of the bacterial 16S
199 rRNA gene was amplified via polymerase chain reaction (PCR) using
200 the following conditions: 50 s at 94 °C, 30 s at 40 °C, 35 cycles of 60
201 s at 72 °C, followed by 5 min at 72 °C. The universal primers F
202 (ACTCCTACGGGAGGCAGCA) and R (GGACTACHVGGGTWTCTAAT)
203 were used for this extension. The PCR products were then purified
204 using the VAHTS DNA Clean Beads (Vazyme Biotech, NJ, JS, CN) and
205 quantified with the Microplate Reader FLx800 (Bio-Tek, Winooski,
206 Vermont, US). Subsequently, a mixture of amplicons was subjected
207 to sequencing on the Illumina MiSeq platform. In brief, the raw
208 sequence data were demultiplexed using the demux plugin, followed
209 by primer trimming using the cutadapt plugin [53]. The sequences

were then subjected to quality filtering, denoising, merging, and chimera removal using the DADA2 plugin [54] and QIIME2 (V.2019.4) [55]. After denoising all libraries, the ASV feature sequences and ASV table were merged, and singletons ASVs (i.e., ASVs with a total sequence count of only 1 across all samples, default operation) were removed [56]. Eventually, an R script was used to statistically analyze the length distribution of high-quality sequences contained in all samples. ASV taxonomic classification was conducted by the silva_138_1 database. The whole sequencing process was completed by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

Bioinformatic analysis

Before bioinformatic analysis, all ASV sequences were rarefied using a rarefaction method to ensure that they were analysed at the same sequencing depth. Network analysis was performed to classify soil ecological taxa affected by different grass species. In the process of network analysis, the number of bacterial ASV occurrence was filtrate with the 1/5 of the sample size (i.e. number of ASV occurrences > 6) [32]. The P value for the network was <0.05. The r value for the network was 0.6. The co-occurrence networks were constructed with R package “picante”, “reshape2” and “dplyr” based on a Spearman correlation matrix. Gephi was used to plot and analysis the nodes and edges, ecological clusters, average degree,

average weighted degree, network diameter, modularity, statistical inference, graph density, clustering coefficient and average path length. The topological role of each node was calculated, based on Zi degree (within-module connectivity) and Pi degree (among-module connectivity) in R with “reshape2” and “ggrepel”. We selected nodes with Zi = 2.5 and Pi =0.62 as threshold. ZIPI was plotted with R package “ggplot”.

Statistical Analysis

The Chao1, Observed species, Shannon, Simpson and Pielou’s evenness were used to assess bacterial diversity with R package “vegan”. To analyze the differences among grass species, we performed an analysis of variance (ANOVA) and conducted a post-hoc Duncan's test ($p < 0.05$) using (R, “car” package). The linear discriminant analysis Effect Size (LEfSe) was performed with non-parametric Kruskal-Wallis and Wilcoxon tests at different phylogenetic levels (from kingdom to genus) to explore microbial community. The heatmap utilizes the Pearson algorithm for conducting correlation analysis. Mantel test was engaged to identified the relationship between the microbial community and soil physicochemical properties with the “linkET” package in R.

RESULTS

General information of bacterial diversity and community

In total, 14968 sequences were identified across 27 soil samples based on high-throughput sequencing. The unique sequences of *L. chin, A. hirt, E. kamo, A. laxm, L. bico, A. gmel, A. frig, S. offi* and *P. chin* were 1885, 1323, 1903, 1993, 1765, 1205, 1722, 1193 and 1256 respectively, and the shared sequences among these grasses were 723 (Fig. S1). In this experiment, bacterial diversity was represented by the Observed species, Chao1, Shannon, Simpson and Pielou's evenness (Table 2). The highest Chao1, Observed species, Shannon and Simpson were found in *L. chin*, while the highest Pielou's evenness was found in *A. frig*. The lowest Chao1 and Observed species were found in *S. offi*, while the lowest Shannon, Simpson and Pielou's evenness were found in *P. chin*. The correlation heatmap showed that Chao1, Observed species, Shannon, and Simpson were significantly positively correlated with TN (Fig. S2). Additionally, Observed species and Chao1 were significantly positively correlated with TP, and Observed species was significantly negatively correlated with SU. At the phyla level, Actinobacteriota (~29.91%), Proteobacteria (~25.55%) and Acidobacteriota (~14.45%) were dominant, accounting for >50% of community composition (Fig. S3). *L.chin* significantly increased 1 phylum, 1 order, 2 families, and 9 genera, *A.laxm* significantly increased 1 genus, *L.bico* significantly increased 3 genera, and *A.gmel* significantly increased 1 genus (Fig.

S4).

Table 2 Soil bacterial diversity of 9 grass species (means ± SE). Lowercase letters indicate significant difference ($p < 0.05$).

Species	Observed species	Shannon	Simpson	Pielou's evenness
L. chin	2892.33±43.80^{a}	10.61±0.03^{a}	0.9988±0.0000^{a}	0.92±0.00^{ab}
A. hirt	2247.83±43.19^{b}	10.18±0.03^{bc}	0.9983±0.0000^{bcd}	0.91±0.00^{abc}
E. kamo	2428.40±223.50^{b}	10.35±0.20^{abc}	0.9985±0.0002^{abc}	0.92±0.01^{ab}
A. laxm	2507.40±42.74^{b}	10.25±0.05^{bc}	0.9982±0.0001^{cd}	0.91±0.00^{cd}
L. bico	2562.00±131.93^{ab}	10.30±0.12^{abc}	0.9982±0.0002^{cd}	0.91±0.01^{bcd}
A. gmel	2278.90±102.92^{b}	10.19±0.07^{bc}	0.9983±0.0001^{bcd}	0.91±0.00^{abc}
A. frig	2573.37±67.10^{ab}	10.49±0.08^{ab}	0.9987±0.0001^{ab}	0.93±0.00^{a}
S. offi	2208.90±95.05^{b}	10.15±0.08^{c}	0.9983±0.0001^{bcd}	0.91±0.00^{abc}
P. chin	2363.67±128.79^{b}	10.06±0.13^{c}	0.9979±0.0003^{d}	0.90±0.01^{d}

General information of the network

We identified 12 ecological clusters within a bacterial network consisting of 1065 nodes and 10023 edges (Fig. 1a). We classified all microbial species in the soil bacterial community into six ecological taxa. Nodes (species) in the network were classified as network hubs, connectors, module hubs and peripherals according to their roles (connectivity within and between modules) (Fig. 1b). In the process of bacterial network construction, there are usually two main filter conditions: frequency and correlation. Based on this, we classified

288 microbes with a sample size of less than 6 (＜1/5 of the total) as
289 specific taxa, and the rest, which are not correlated ($p>0.05$), as
290 others. In total，0.4% bacterial species were classified in network
291 hubs, 9.7% were connectors, 4.6% were module hubs, 62.8% were
292 peripherals, 21% were specific taxa, and 1.4% were others (Fig. 1c).
293 Further, we counted the sources of six ecological taxa of ASV at the
294 phylum level. The results indicated that network hubs had the
295 highest proportions of ASVs belonging to Actinobacteriota,
296 Firmicutes, and Gemmatimonadota. Connectors exhibited the
297 highest proportion of Acidobacteriota ASVs, while module hubs
298 showed the highest proportion of Proteobacteria ASVs. Specific taxa
299 had the highest proportions of Myxococcota, Bacteroidota,
300 Verrucomicrobiota, and Patescibacteria ASVs, while others had the
301 highest proportion of Chloroflexi ASVs (Fig. 1d).

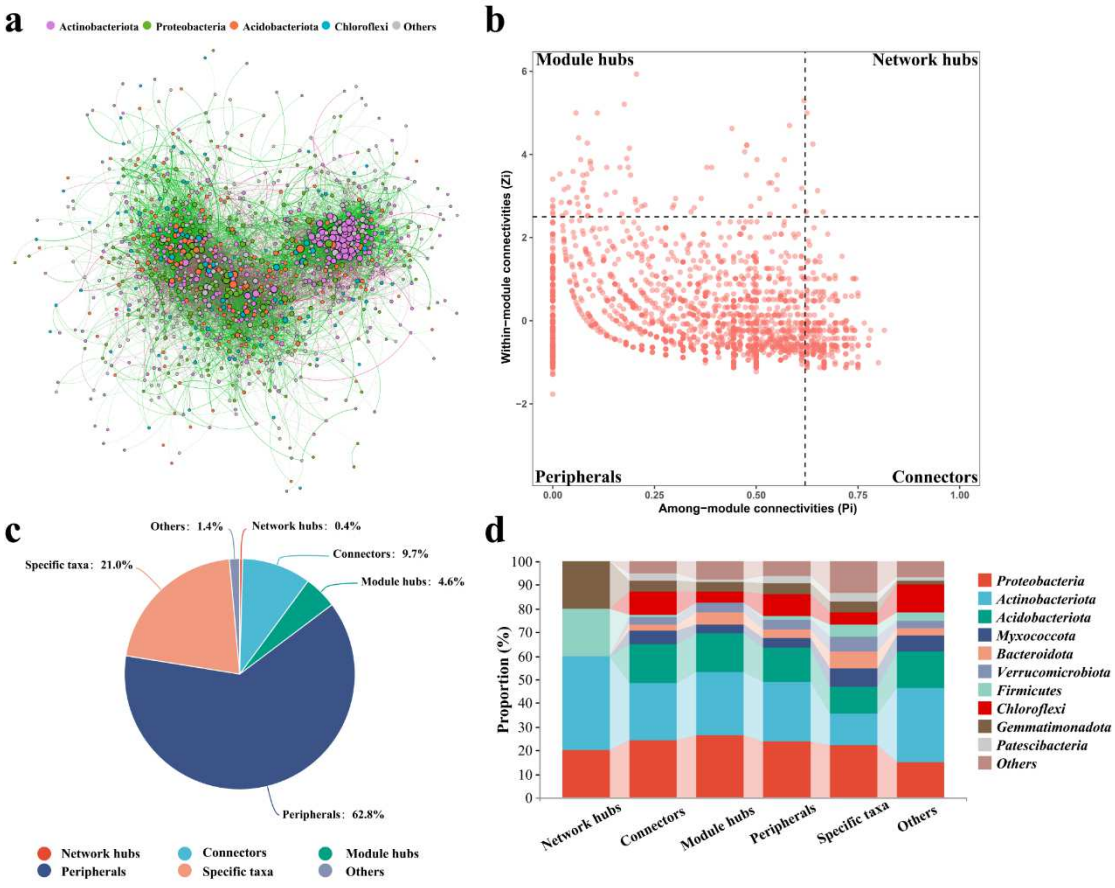

Fig. 1 Ecological taxa based on microbial networks. a Soil bacterial co-occurrence network from 9 grass species. Only three main modules are presented. **b** Within-module (Zi) and among-module (Pi) connectivity of all network ASVs. Network hubs, Connectors, Module hubs and Peripherals represent 4 ecological taxa based on within- and among- module connectivity. **c** Relative abundance of ecological taxa within bacterial community. **d** Bacterial taxonomic proportion of ecological taxa at the Phylum level.

Relationship between the relative abundance of ecological taxa, bacterial diversity, and soil physicochemical properties

Our results revealed a significant negative correlation between the

relative abundance of taxa classified as peripherals and bacterial diversity, while specific taxa showed a significant positive correlation with bacterial diversity (Fig. 2a) (soil physicochemical properties were shown in Table S3). Other ecological taxa did not significantly correlate with microbial diversity. Pearson correlation analysis revealed distinct relationships between different ecological taxa and soil properties. Specifically, we found that the relative abundance of network hubs was significantly positively correlated with AK, connectors showed a significant positive correlation with NO_3^- and a significant negative correlation with SALP, module hubs exhibited significant negative correlations with EC, NO_3^-, TP, and AP, peripherals were significantly positively correlated with SU, and others were significantly positively correlated with TC. Interestingly, we did not find any significant correlations between specific taxa and soil physicochemical properties (Fig. 2b).

Relationship between the community of ecological taxa and soil properties

The community composition of network hubs was significantly positively correlated with TP and AK. The community composition of connectors was significantly positively correlated with TC. The community composition of module hubs was significantly positively correlated with NO_3^-, AP, and AK. The community composition of

specific taxa was significantly positively correlated with NO_3^-, TP, AP, SU, TC, and AK. The community composition of others was positively correlated with TC (Fig. 2c, Table S4).

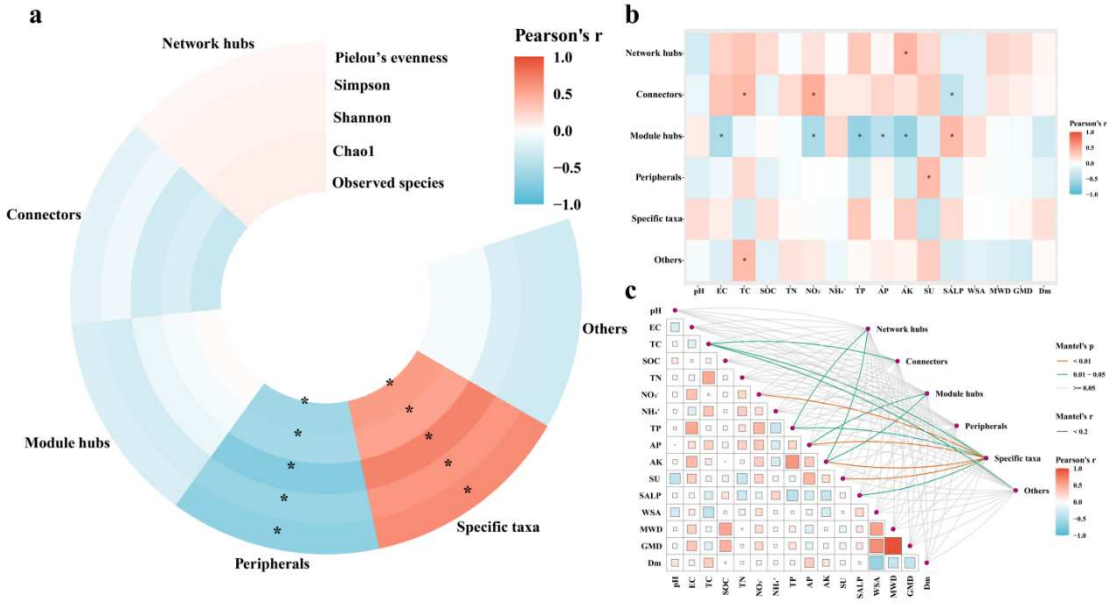

Fig. 2 Relationship among ecological taxa, bacterial diversity and soil physicochemical properties. a Relationship between the relative abundance of ecological taxa and bacterial diversity. **b** Relationship between the relative abundance of ecological taxa and soil physicochemical properties. **c** Relationship between the community composition of ecological taxa and soil physicochemical properties. Pearson was used for correlation analysis. Significant (p <0.05) are indicated by an "*".

DISCUSSION

Based on the interaction relationships identified by microbial network analysis, we classified the soil bacterial community into six ecological taxa. Our results showed that bacterial diversity had a

positive correlation with the relative abundance of specific taxa and a negative correlation with peripherals, while other ecological taxa did not display any significant correlation with diversity. The relative abundance of peripherals was positively correlated with soil urease activity, but specific taxa were not correlated with any soil physicochemical properties. In turn, the community composition of peripheral microbes is not influenced by soil physicochemical properties, but specific taxa are affected by NO_3^-, TP, AP, SU, TC and AK. Our results indicate that different ecological taxa play distinct roles in regulating bacterial diversity and soil physicochemical properties serve different functions in influencing the composition and abundance of ecological taxa.

The relative abundance of ecological taxa contributing to bacterial diversity

Microbial community exhibits complex interactions, establishing connections amongst themselves while also maintaining their independent existence within their respective kingdoms of life [57]. A widely used method for studying interactions within microbial community is microbial network analysis [58–60]. It attempts to assess network topology indices to shift the focus of the problem from simply identifying presence ("who is there") to understanding co-occurrence patterns ("who co-occurs with whom, and why?") [61].

Previous studies generally identified nodes with high connectivity in the network as keystone species, which play an important role in ecosystem functions [62–64]. For example, Shi et al. (2020) showed that the relative abundance of network hub (kinless hubs) was closely related to soil carbon, nitrogen, phosphorus and sulfur cycle [65]. However, contrary to our assumptions, the relative abundance of high connectivity ecological taxa, whether network hub, connectors or module hubs, did not showed a significant correlation with microbial diversity. Considering that ecological taxa with high connectivity may have overlapping ecological niches and functional redundancy [66], it seems understandable that their relative abundance is not related to microbial diversity. From a molecular ecological network perspective, peripherals are widely found across almost all grasslands, but they have weak connectivity [26]. These microbes may occupy the majority of ecological niches in soil, leading to intense competition for nutrients that hinders the establishment of new species [67]. In addition, according to Verdú et al [68], we suppose that peripherals resemble the transitive competition model, favoring the linear structure of the competition winner, which may not promote community equity.

Microbes excluded from the co-occurrence network are often considered as errors [69]. However, we argue that, the role of

specific taxa in soil ecological network has been ignored. The top-down effects significantly contribute to the recruitment and succession of soil microbial community by facilitating root growth, metabolic activities, and the addition of detritus [70]. These reactions are usually based on the characteristics of plants, and different plant species can affect the microbial environment in unique ways because of plant distinctness [71]. Various plants may offer different habitats for soil microbes because of plant distinctness, leading to diverse recruitment strategies and specific taxa [72]. Therefore, we speculate that after planting, plants may affect bacterial diversity by regulating the relative abundance of these specific taxa. In other words, changes in the relative abundance of specific taxa may directly demonstrate how plant distinctness affects soil bacterial community.

The role of soil properties in regulating ecological taxa

Vegetation types and soil properties are two major factors controlling soil microbes [73,74]. Plants can regulate microbial diversity through soil physicochemical properties[75]. For example, it is generally believed that soil nutrients influenced bacterial diversity in the study of forest and grassland ecosystems [76,77]. In our study, soil nutrients, especially nitrogen and nitrogen cycle, also contribute to bacterial diversity. We speculate that this may be

related to the nutrient preference of bacterial species in peripherals. Urease can promote the cycling of nitrogen in the soil, making it easier for plants to absorb and utilize [78]. Given that the positive relationship between peripherals and urease activity, peripherals may benefit from urease activity and restrict the survival of other microbes, thus hindering microbial diversity. However, the relative abundance of specific taxa, significantly positively correlated with soil bacterial diversity, were not affected by soil physicochemical properties. This is consistent with our previous speculation that specific taxa are directly regulated by plants. Plants may affect the content of a certain element or other components in the soil due to their distinctness (such as root exudates, detritus, etc.), resulting in heterogeneity of soil resources and regulating the relative abundance of specific taxa. Unfortunately, these may not be involved in this experiment. Nevertheless, we argue that the relative abundance of specific taxa is indeed regulated in some unknown way. Further, we found that the community composition of peripherals was not affected by soil physicochemical properties, while specific taxa were affected by NO_3^-, TP, AP, SU, TC and AK. Microbes are usually recognized as generalists with a wide niche and specialists with a narrower niche [79]. Peripherals, similar to generalists, may have a broader niche and strong adaptability to the environment,

leading to a relatively stable community composition, while specific taxa, similar to specialists, have a narrower niche that is more sensitive to environmental changes [80]. Simultaneously, the peripherals belong to inactive species within networks, which can be considered as conservative species in microbial interactions [27]. Due to their low connectivity strategy, they may avoid fierce competition with specific taxa under limited resources and spatial conditions [81]. Therefore, we argue that soil physicochemical properties have different roles in regulating the two ecological taxa: soil properties control the relative abundance of peripheral microbes, while they influence the community composition of specific taxa (Fig. 3).

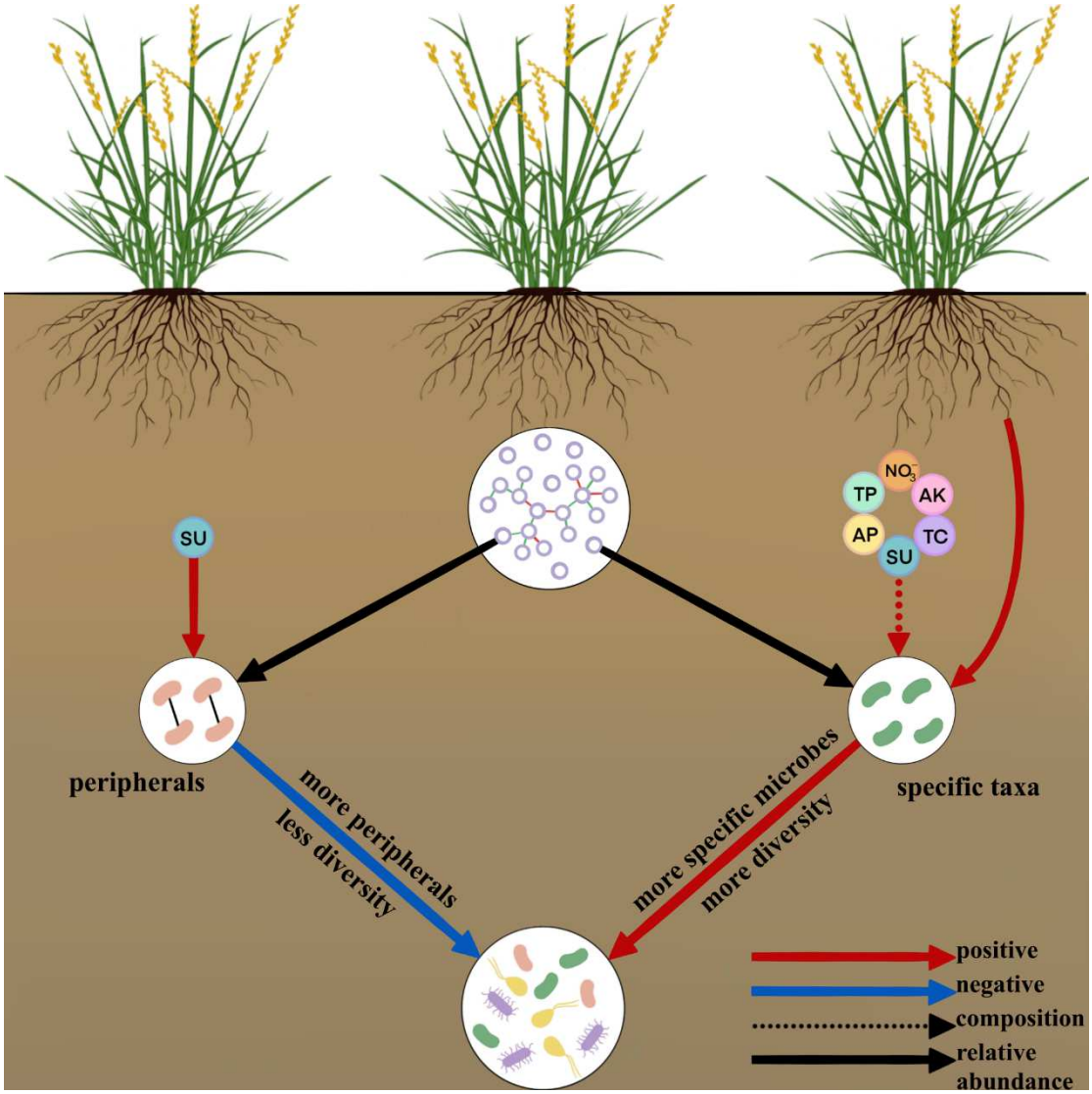

Fig. 3 A conceptual model showing the potential relationship between grass species and bacterial diversity.

Based on ecological taxa explored by network analysis, we illustrated that plants regulate bacterial diversity through direct effects on the relative abundance of specific taxa and peripherals in short term, while soil physicochemical properties play a secondary role. Plants with higher relative abundance of specific taxa and lower relative abundance of peripheral microbes can markedly enhance bacterial diversity, as evidenced by *A.frig, L.chin* and *L.bico* in our

study, which exhibit robust soil bacterial diversity. In future practice, we should pay attention to the performance of these ecological taxa, especially in the neglected parts of the co-occurrence network, which will help us better understand the interaction mechanism between plants and microbial diversity.

CONCLUSIONS

Overall, our results suggested that plants regulated the relative abundance of peripherals by soil urease activity, which inhibited bacterial diversity. Plants directly controlled the relative abundance of specific taxa, which enhanced bacterial diversity. Furthermore, we found that the composition of peripherals was not affected by soil physicochemical properties, while the composition of specific taxa was affected by NO_3^-, TP, AP, SU, TC and AK. These results help us further understand plant-dominated microbial diversity evolution process. If a plant species has the ability to alter the relative abundance of specific taxa and peripherals, it should be carefully considered as a potential resource for restoring soil microbial diversity. This is worth in grassland management and ecological restoration.

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Data availability: 16S rRNA gene sequences were deposited to the Sequence Read Archive (SRA) under the project accession number PRJNA1127545. R code was displayed with the identifier https://zenodo.org/doi/10.5281/zenodo.12526004.

Author contributions：The research was designed by JL, LB, LG, and TL. ZD and JL collected the samples. ZD performed laboratory work, performed the analyses, and wrote the manuscript with the help from all co-authors. Sequence processing, data curation and data analyses were done by LG, TL, and JL. BR and JY supervised entire research. All authors approved the final manuscript.

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754 Do Organic Substrates Drive Microbial Community Interactions in
755 Arctic Snow? Front Microbiol. 2019;10.

756

757 Tabel 1 Species name, family names, abbreviation, type and frequency of
758 9 native grass species. Frequency was calculated based on the number of
759 plant occurrences in 163 grasslands.

Species name	Family	Abbreviation	Type	Frequency (%)
Leymus chinensis	Gramineae	*L. chin*	perennial	22.09
Arundinella hirta	Gramineae	*A. hirt*	perennial	58.90
Elymus kamoji	Gramineae	*E. kamo*	perennial	60.12
Astragalus laxmannii	Leguminosae	*A. laxm*	perennial	3.07
Lespedeza bicolor	Leguminosae	*L. bico*	perennial	98.77
Artemisia gmelinii	Compositae	*A. gmel*	perennial	56.44
Artemisia frigida	Compositae	*A. frig*	perennial	6.75
Sanguisorba officinalis	Rosaceae	*S. offi*	perennial	12.27

Potentilla chinensis	Rosaceae	*P. chin*	perennial	28.22

760 Table 2 Soil bacterial diversity of 9 grass species (means ± SE).

761 Lowercase letters indicate significant difference ($p < 0.05$).

Species	Observed species	Shannon	Simpson	Pielou's evenness
L. chin	2892.33±43.80^{a}	10.61±0.03^{a}	0.9988±0.0000^{a}	0.92±0.00^{ab}
A. hirt	2247.83±43.19^{b}	10.18±0.03^{bc}	0.9983±0.0000^{bcd}	0.91±0.00^{abc}
E. kamo	2428.40±223.50^{b}	10.35±0.20^{abc}	0.9985±0.0002^{abc}	0.92±0.01^{ab}
A. laxm	2507.40±42.74^{b}	10.25±0.05^{bc}	0.9982±0.0001^{cd}	0.91±0.00^{cd}
L. bico	2562.00±131.93^{ab}	10.30±0.12^{abc}	0.9982±0.0002^{cd}	0.91±0.01^{bcd}
A. gmel	2278.90±102.92^{b}	10.19±0.07^{bc}	0.9983±0.0001^{bcd}	0.91±0.00^{abc}
A. frig	2573.37±67.10^{ab}	10.49±0.08^{ab}	0.9987±0.0001^{ab}	0.93±0.00^{a}
S. offi	2208.90±95.05^{b}	10.15±0.08^{c}	0.9983±0.0001^{bcd}	0.91±0.00^{abc}
P. chin	2363.67±128.79^{b}	10.06±0.13^{c}	0.9979±0.0003^{d}	0.90±0.01^{d}

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763

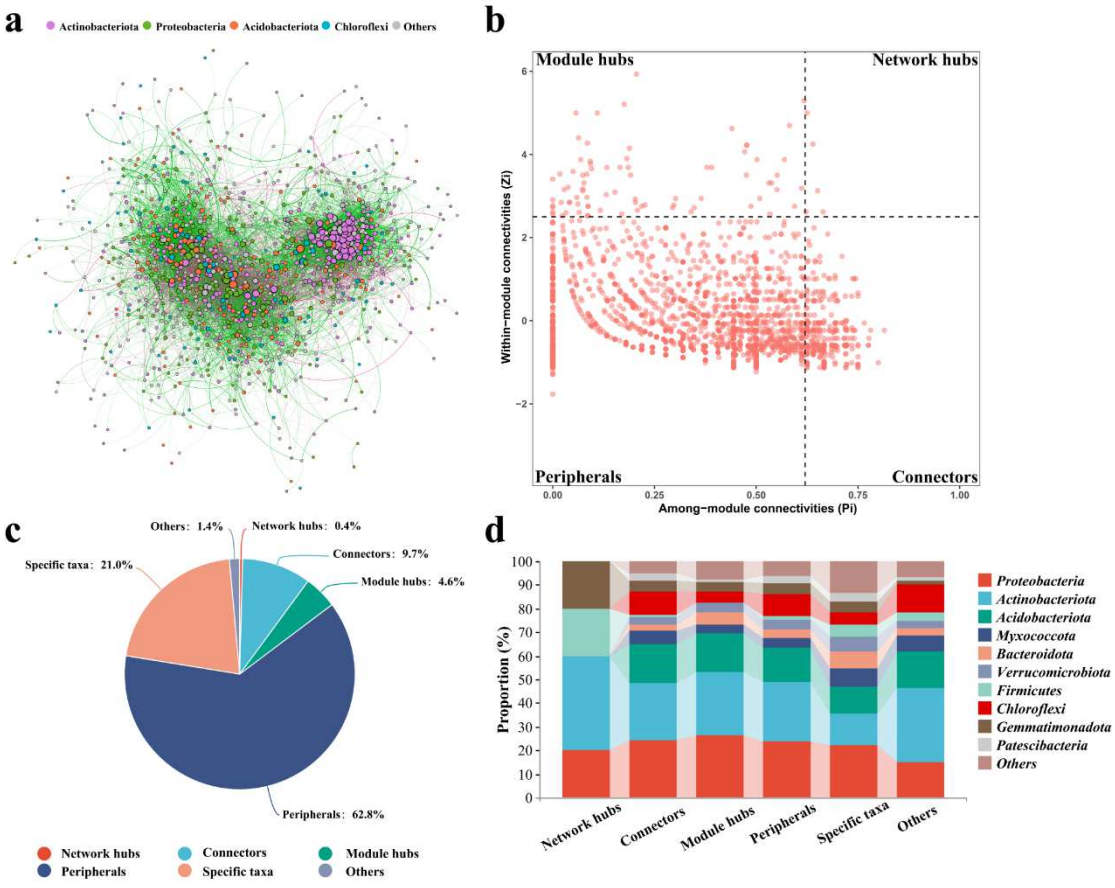

Fig. 1 Ecological taxa based on microbial networks. a Soil bacterial co-occurrence network from 9 grass species. Only three main modules are presented. **b** Within-module (Zi) and among-module (Pi) connectivity of all network ASVs. Network hubs, Connectors, Module hubs and Peripherals represent 4 ecological taxa based on within- and among- module connectivity. **c** Relative abundance of ecological taxa within bacterial community. **d** Bacterial taxonomic proportion of ecological taxa at the Phylum level.

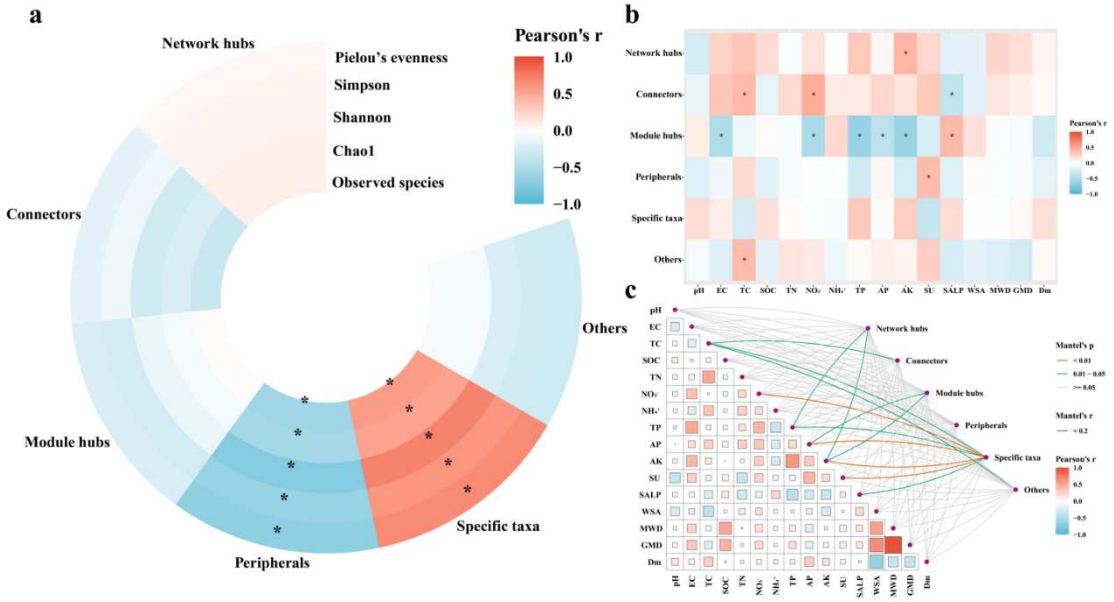

Fig. 2 Relationship among ecological taxa, bacterial diversity and soil physicochemical properties. a Relationship between the relative abundance of ecological taxa and bacterial diversity. **b** Relationship between the relative abundance of ecological taxa and soil physicochemical properties. **c** Relationship between the community composition of ecological taxa and soil physicochemical properties. Pearson was used for correlation analysis. Significant (p <0.05) are indicated by an “*”.

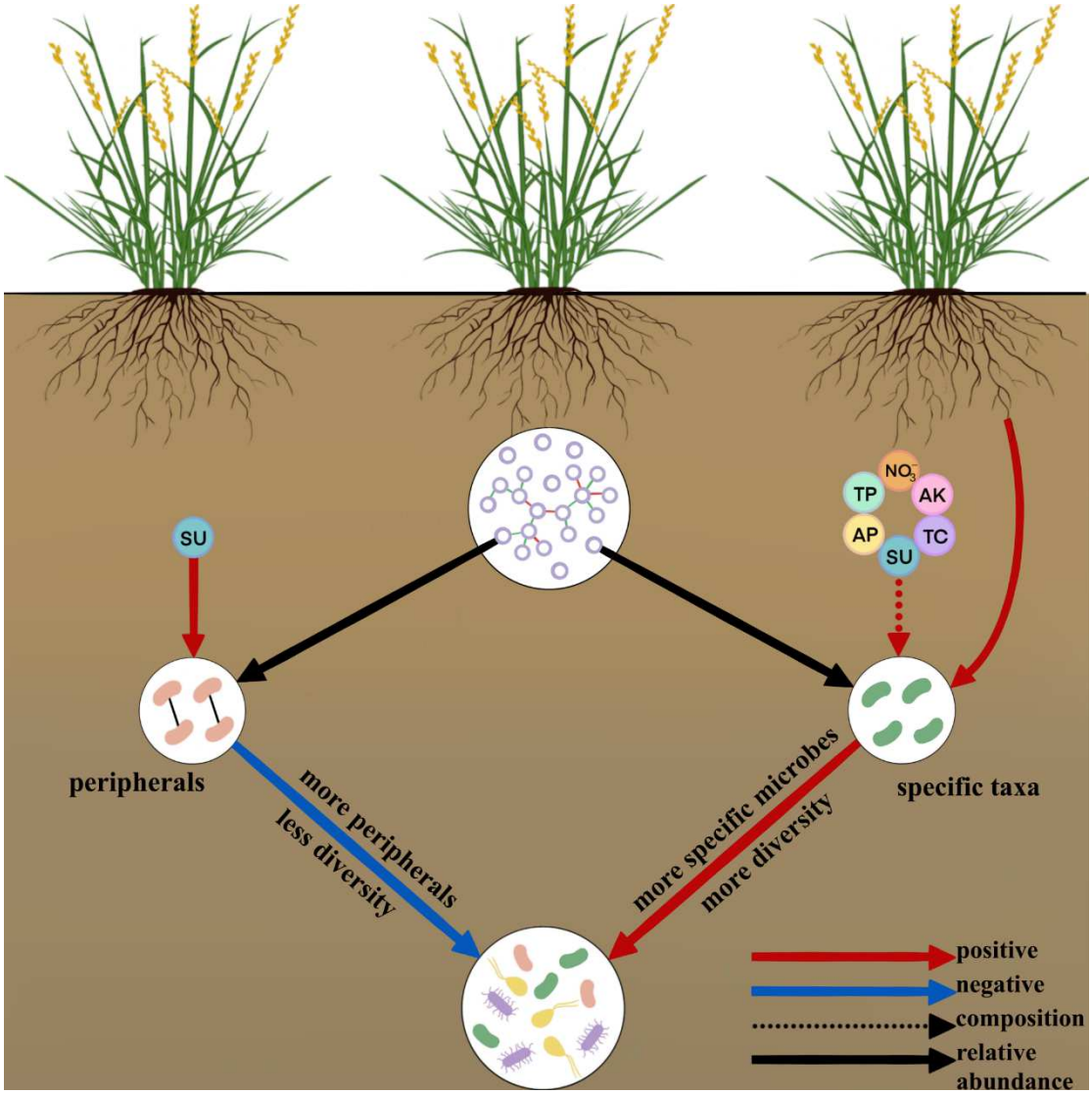

Fig. 3 A conceptual model showing the potential relationship between grass species and bacterial diversity.

Figures

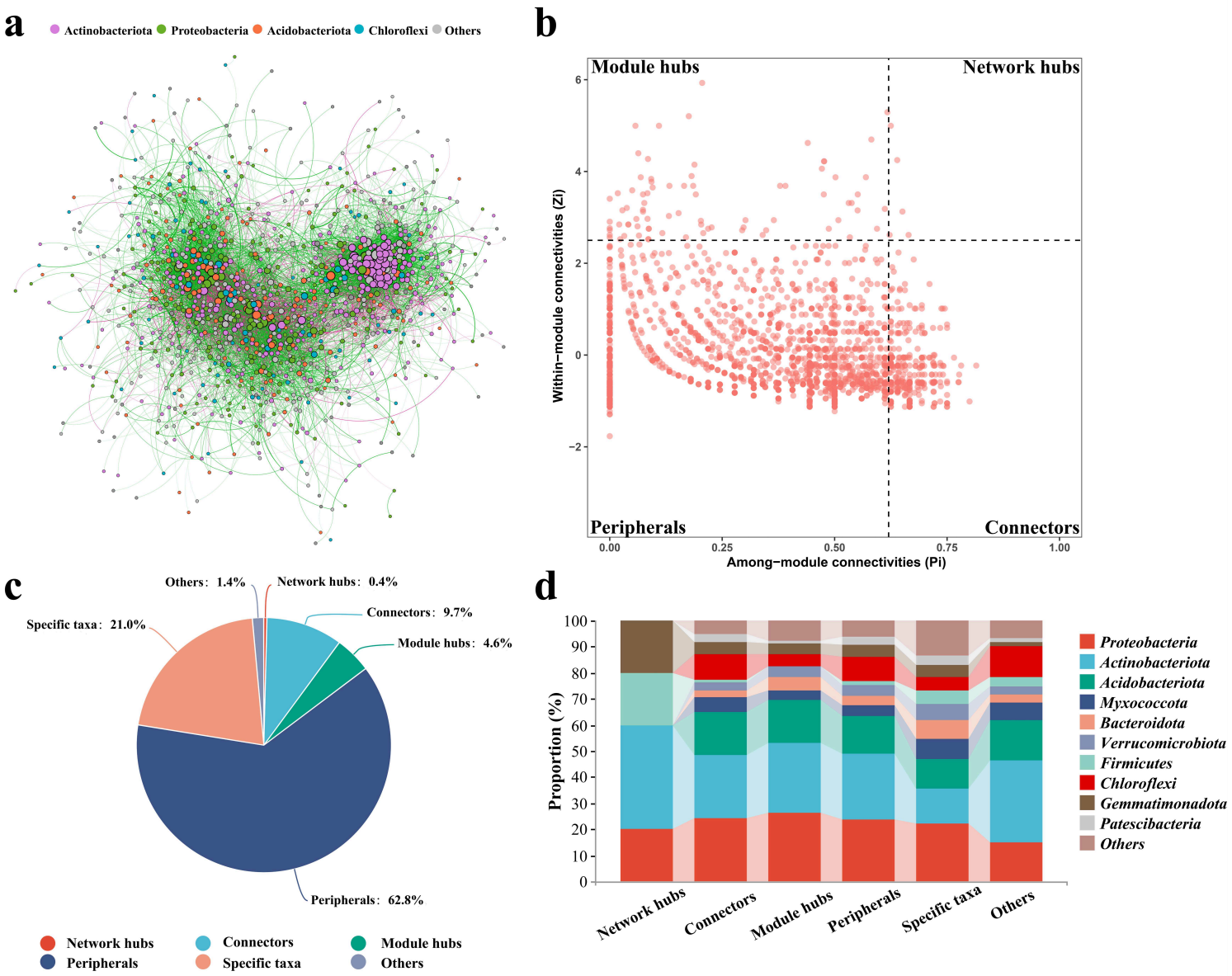

Figure 1

Ecological taxa based on microbial networks. a Soil bacterial co-occurrence network from 9 grass species. Only three main modules are presented. **b** Within-module (Zi) and among-module (Pi) connectivity of all network ASVs. Network hubs, Connectors, Module hubs and Peripherals represent 4 ecological taxa based on within- and among- module connectivity. **c** Relative abundance of ecological taxa within bacterial community. **d** Bacterial taxonomic proportion of ecological taxa at the Phylum level.

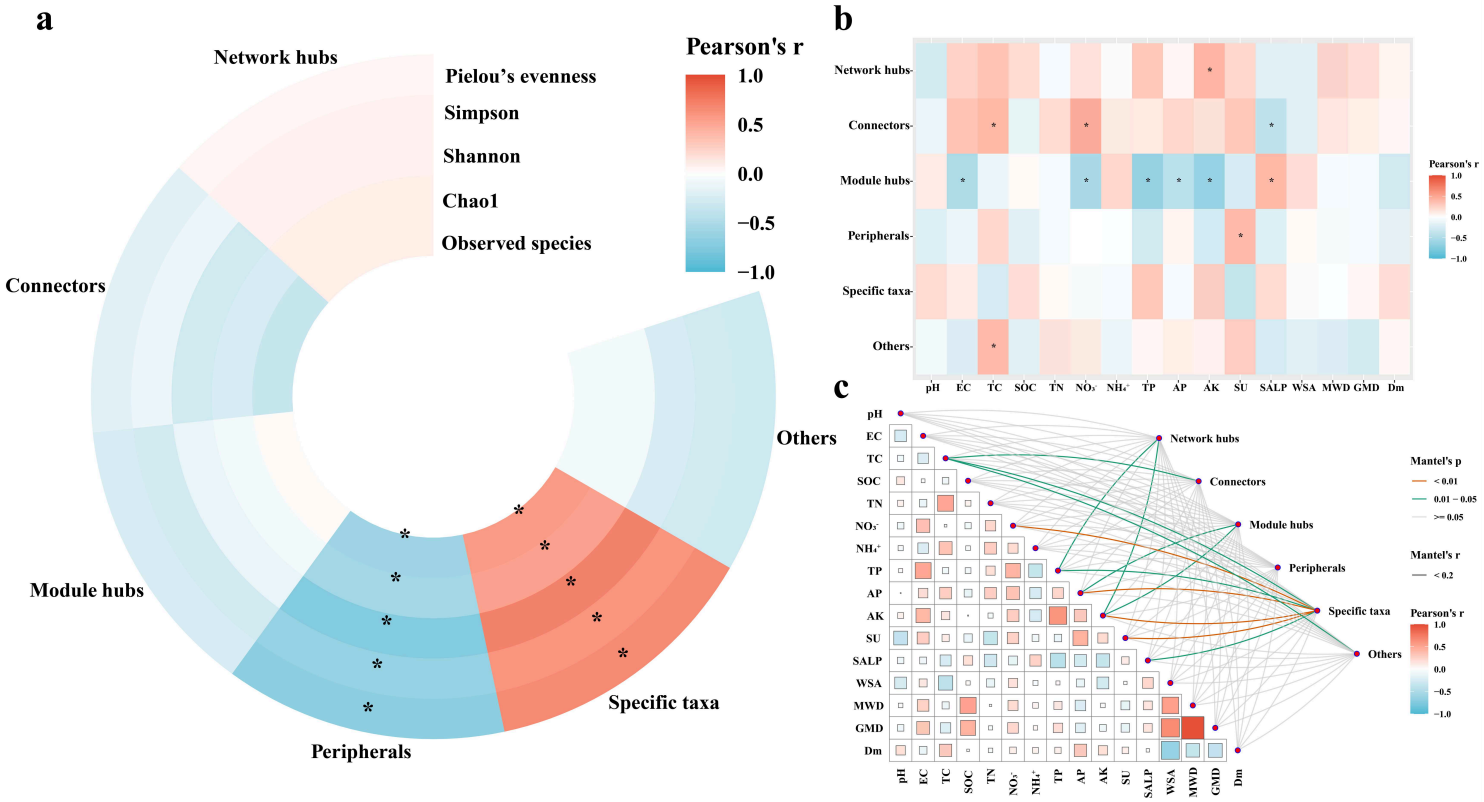

Figure 2

Relationship among ecological taxa, bacterial diversity and soil physicochemical properties. a Relationship between the relative abundance of ecological taxa and bacterial diversity. **b** Relationship between the relative abundance of ecological taxa and soil physicochemical properties. **c** Relationship between the community composition of ecological taxa and soil physicochemical properties. Pearson was used for correlation analysis. Significant (p 0.05) are indicated by an "*".

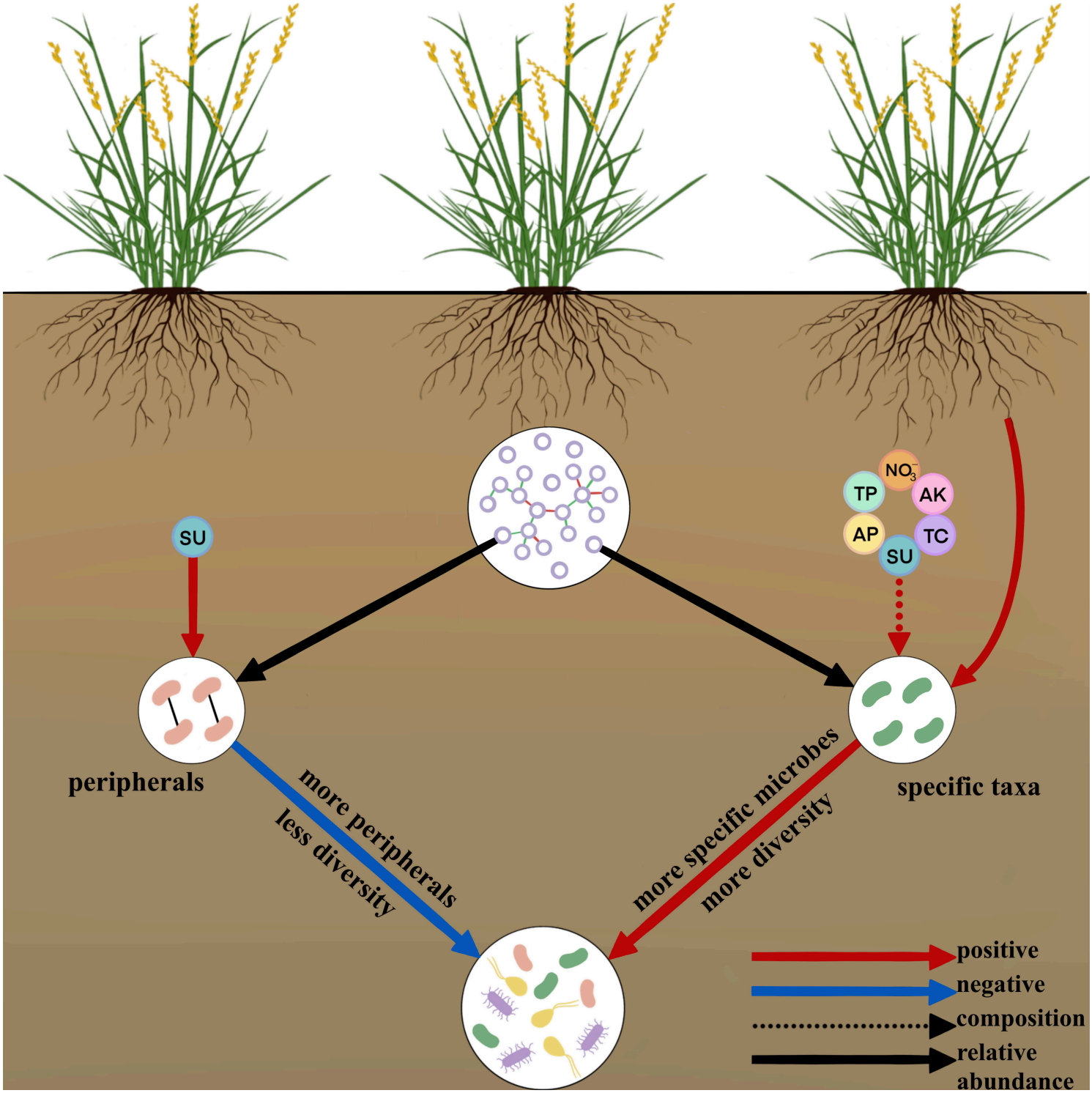

Figure 3

A conceptual model showing the potential relationship between grass species and bacterial diversity.

Supplementary Files

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