scientific reports

Check for updates

OPEN

Generalists and keystone species drive rhizosphere microbial diversity and stability in feral *Brassica napus*

Yingshun Cui[1,8], Jihoon Kim[2,3,8], Mengqi Sun[4], Mirye Park[5], Kyong-Hee Nam[2], Jun-Woo Lee[2], Chang Soo Lee[6], An Suk Lim[7] & Seong-Jun Chun[2]✉

Brassica napus **(rapeseed) is a globally important crop, primarily valued for its oil production. However, feral *B. napus* in non-agricultural areas remains under-researched. This study aims to examine the roles of microbial generalists and network keystone species in shaping microbial diversity and network stability of feral *B. napus*. We analyzed prokaryotic, fungal, and eukaryotic communities in the rhizosphere and bulk soil from five grassland sites. The rhizosphere microbial communities differed significantly from those in adjacent bulk soil, showing lower diversity and richness. *Pseudomonas brassicacearum* (bacterium), *Olpidium brassicae* (fungus), and *Glissomonadida* (eukaryote) were predominantly found in the rhizosphere. Inter- and intra-kingdom association occurred almost exclusively within the rhizosphere, with low interconnectivity compared to the bulk soil, and network keystone species served to bridge these connections. Furthermore, structural equation modeling highlighted the role of generalists and network keystone species in maintaining microbial diversity and stability. Feral *B. napus* selectively influenced rhizospheric generalists, which, along with keystone species, played key roles in determining microbial diversity and network stability, controlling community structure and interspecies interactions.**

Keywords Feral *Brassica napus*, Rhizosphere, Microbial communities, Ecological network, Rhizosphere generalist

Brassica napus, commonly known as oilseed rape or canola, is of significant agricultural and economic value globally[1]. This versatile plant is prized for its oil-rich seeds, which are a useful resource in various industries, including production of cooking oil and biodiesel, and in animal feed. Natural ecosystems harbor feral populations of *B. napus*, which grow in habitats that are largely distinct from conventional cultivation environments, even though *B. napus* is believed to lack native habitats[2–4]. The ecological implications of canola growth outside managed agricultural settings deserves consideration. While cultivated canola is well-studied in controlled environments, the ecological dynamics of feral *B. napus* in natural ecosystems remain relatively unexplored[5]. Understanding the ecological behavior and value of feral canola may offer insights into interactions with native flora and fauna, ecosystem dynamics, and potential ecological services.

The composition of the rhizospheric microbial communities at broad phylogenetic levels (e.g. phylum, class level) exhibits largely similar patterns across a range of geographical regions and environments[6,7], but specific plant species or even species genotypes are characterized by relatively unique microbial community structures in their rhizosphere[8–10]. Plants rely on a diverse and functional rhizosphere microbiome for improved nutrient uptake, pathogen suppression, and modulations in their immunity[11]. As an integral component of the plant root system, the rhizosphere selectively recruits microbes from the adjacent bulk soil, thereby establishing a distinct microbiome that contributes to plant health and growth[11]. Plant interactions with other species are diverse and

[1]National Ecosystem Survey Team, National Institute of Ecology, 1210 Geumgang-ro, Maseo- myeon, Seocheon 33657, Republic of Korea. [2]LMO Team, National Institute of Ecology, 1210 Geumgang-ro, Maseo-myeon, Seocheon, Republic of Korea. [3]Department of Biology, Wonkwang University, 460 Iksan-daero, Iksan, Republic of Korea. [4]College of life science, Changchun Sci-Tech University, Changchun 130600, China. [5]Protist Research Division, Nakdonggang National Institute of Biological Resources, Sangju 7242, Republic of Korea. [6]Fungi Research Division, Nakdonggang National Institute of Biological Resources, Sangju 7242, Republic of Korea. [7]Division of Life Science and Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 52828, Republic of Korea. [8]Yingshun Cui and Jihoon Kim have contributed equally to this work. ✉email: sjchun@nie.re.kr

intricate, and depend on specific compartments[12]. Investigating the interactions between microbial communities in bulk soil and the rhizosphere is therefore essential to better understand the broader ecological role of *B. napus* in diverse environments[13]. In the context of soil ecosystems, key biological components such as archaea, bacteria, and eukaryotes (including fungi) interact synergistically to maintain diversity and functionality[14]. For instance, filamentous fungi, known for their thread-like structures, play a crucial role in nutrient cycling and organic matter decomposition in both bulk soil and the rhizosphere[15]. These fungi interact with bacteria and other eukaryotic organisms, contributing to the overall stability and functionality of the soil ecosystem[16]. Microeukaryotes, represented by protists such as amoebas and flagellates, are known predators that contribute to nutrient cycling and organic matter, and are pivotal components in soil ecosystems[17,18]. Their significance in the rhizosphere underscores their influence on plant–microbe interactions, shaping the ecological dynamics of the plant holobiont. Therefore, unraveling inter- and intra-kingdom interactions, particularly between communities in bulk soil and rhizosphere, is of paramount importance.

Interactions between plants and microorganisms are more intense in natural ecosystems than in cultivated fields[5,19,20]. In this study, we collected 154 rhizosphere and adjacent bulk soil samples of feral *B. napus* from five sites to investigate ecological relationships with three biological kingdoms: prokaryotes, fungi, and eukaryotes. We used network analysis and structural equation modeling (SEM) to evaluate intra- and inter-kingdom biological associations among amplicon sequence variants (ASVs) assigned to the different niche-based categories in two closely related microenvironments: the rhizosphere of feral *B. napus* and nearby bulk soil. The aims of this study were to (1) investigate differences in microbial communities and their ecological relationship within and between the natural rhizosphere of feral *B. napus* and surrounding bulk soil; (2) identify microbial variations and the roles of different niche-based ecological categories (generalists, common taxa, and specialists) in the rhizosphere and bulk soil; (3) detect network keystone species in the rhizosphere of feral *B. napus* and their potential contribution to the ecological network and microbial diversity; and (4) reveal factors that potentially affect the stability of the rhizosphere network and influence the microbial diversity of this microecosystem. By addressing these questions, we strived to provide a comprehensive understanding of the ecological dynamics of the feral *B. napus* rhizosphere in natural ecosystems and a reference for potential environmental risk assessments of genetically modified *B. napus* if released into the environment in the future.

Methods

Study site and sample collections

We collected 77 rhizosphere samples of feral *B. napus* plants and 77 nearby bulk soil samples from five different grassland ecosystems in South Korea during the flowering season in April 2021. Sampling locations were chosen to largely avoid human disturbance and to cover the natural habitats of feral *B. napus* in South Korea. Details regarding the sampling sites have been presented in our previous report[21]. Briefly, the distance between sampling sites ranged from 70 to 200 km, and between 16 and 39 rhizosphere samples were collected at the different locations.

To minimize root damage, we first excavated the plants using an ethanol-sterilized shovel and gently shook to obtain the dislodged soil; we defined this as the bulk soil. The remaining soil tightly attached to the roots of feral *B. napus* was defined as the rhizosphere. To reduce contamination, we rinsed the shovel, forceps, and blades with 70% ethanol and sterile water after each sample collection. The collected samples were preserved at − 80 °C until DNA extraction.

Bulk soil biogeochemical analyses

We determined the characteristics of bulk soil by analyzing ten samples from each site. The levels of soil organic matter, total nitrogen, and available phosphorus, the exchangeable cation composition (K, Ca, Mg, and Na), cation-exchange capacity, pH, electrical conductivity, NaCl concentration, and the relative percentages of sand, silt, and clay were measured at the AT Analysis Center Co., Ltd. in Incheon, Korea, according the methods established by the National Institute of Agricultural Science and Technology (NIAST, 2000).

DNA extraction and sequencing

DNA extraction was carried out using DNeasy PowerMax® soil kits (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed on a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). To comprehensively capture sample biodiversity, the analysis encompassed prokaryotic, eukaryotic, and fungal sequences. The prokaryotic 16 S rRNA gene was amplified using a universal prokaryotic primer set, with overhang adapter sequences, which target both bacterial and archaeal 16 S rRNA gene (342 F: 5′-CTACGGGGGGCAGCAG-3′ and 806R: 5′- GGACTACCGGGGTATCT − 3′)[22]. The eukaryotic 18 S rRNA gene was amplified using a universal primer set with overhang adapter sequences, which targets the V4 region (TAReuk454FWD1: 5′-CAGCASCYGCGGTAATTCC-3′ and TAReukREV3: 5′-ACTTTCGTTCTTGATYRA-3′)[23]. Due to the limitations of the eukaryotic 18 S rRNA gene in capturing the diversity of complex fungal communities[24], we employed a universal primer set for the fungal internal transcribed spacer (ITS) gene region; specifically, ITS1F_KYO1 (5′-CTHGGTCATTTAGAGGAASTAA-3′) and ITS2_KYO2 (5′-TTYRCTRCGTTCTTCATC-3′)[25]. Consequently, from this point forward, the term 'eukaryotes' refers specifically to non-fungal taxa, including protists and other small eukaryotic microbes, as fungal communities were analyzed separately using the ITS gene region to capture their diversity more accurately. Amplicon libraries were constructed according to the method described in our previous report[21]. Briefly, TaKaRa Ex Taq™ Hot Start Version (TaKaRa Bio, Shiga, Japan) was used for amplification. For prokaryotic sequencing, antimitochondrial peptide nucleic acid (mPNA: 5′-GGCAA GTGTTCTTCGGA-3′) was added to reduce the amplification of host DNA[26]. Purification was conducted using

a 1:1 ratio of AmpureXP beads (Beckman Coulter, IN, USA), and quantification was performed with Quant-iT™ PicoGreen® dsDNA detection kits (Invitrogen, San Diego, CA, USA). The final products were used for paired-end read sequencing reactions and sequenced on a MiSeq platform (2 × 300 bp reads) at Macrogen Corporation (Seoul, South Korea).

Bioinformatics analysis

For high-resolution clustering, ASV analysis was performed using DADA2 (version 1.16), according to the pipeline tutorial v.1.16 for the 16 S and 18 S rRNA genes and v.1.8 for the ITS gene region (accessed date: March 2023; https://benjjneb.github.io/dada2/tutorial.html and https://benjjneb.github.io/dada2/ITS_workflow.html) in R[27].

The latest Silva database (release 138) was used to align and classify the sequences of the 16 S and 18 rRNA genes[28]. After classification, the chloroplast, mitochondrial, and eukaryotic sequences were removed from the 16 S rRNA gene dataset. For 18 S rRNA datasets, ASVs assigned to Archaeplastida and labeled as "NA" at the phylum level, as well as those categorized under Fungi at the order level, were excluded from subsequent analyses. The UNITE database (UNITE general FASTA release for Fungi 2. Version 10.05.2021.) was used to align and classify the sequences of the fungal ITS gene region[29]. ASVs that comprised only singletons, doubletons, or tripletons were removed from further analyses. The raw sequences, along with the accompanying metadata and detailed parameters used in the pipelines, are accessible in the Sequence Read Archive of NCBI under the project accession numbers PRJNA821335 and PRJNA816676, and published in *Data in Brief*[21].

All statistical analyses were performed using the R software (version 3.4.0)[30]. We used non-metric multidimensional scaling (NMDS) with Bray–Curtis distances to order the samples in the microbial community based on dissimilarities, using the "metaMDS" function in Vegan[31]. The NMDS results were quantitatively evaluated using analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) using the "anosim" and "adonis" functions in Vegan, respectively (permutation 999).

To categorize ASVs into generalist, common taxa, and specialist groups, we employed the normalized niche breadth index[32,33], with values assigned as follows: generalist (> 0.6), common taxa (0.4–0.6), and specialist (< 0.4)[33]. Next, the averaged proportions and observed frequencies of each ASV in both the rhizosphere and bulk soil were calculated and represented using box plots (Fig. S1). The Chao1 and Shannon diversity indices were calculated using the "diversity" functions in Vegan. The reads were normalized to the lowest number of reads in the "rrarefy" function of Vegan[31]. The lowest read counts were 22,434 for bacteria, 11,743 for fungi, and 2,389 for eukaryotes in bulk soil. However, due to the very low diversity of eukaryotic ASVs observed in rhizosphere samples, rarefying was not performed for those samples. A Venn diagram was constructed to show the distribution of ASVs among different niche-based categories in the rhizosphere and bulk soil, using the webtool developed by Bioinformatics & Evolutionary Genomics (https://bioinformatics.psb.ugent.be/webtools/Venn).

Cross Kingdom ecological network analysis

To investigate inter- and intra-kingdom microbial interactions, as well as potential microbial connections between the two microenvironments, a network was constructed with ASVs (described below) from prokaryotes, eukaryotes, and fungi using the SpiecEasi (Sparse inverse covariance estimation for Ecological association inference) package[34,35]. The parameters were configured as follows: method = "mb," sel.criterion = "bstars," lambda.min.ratio = 0.001, nlambda = 100, and thresh = 0.005. The original ASV tables were categorized into six groups, prokaryotic, eukaryotic, and fungal ASVs in bulk soil and rhizosphere samples. To reduce inclusion of rare ASVs in each dataset, only those meeting the following thresholds were considered: (1) detected in at least 25% of the samples and (2) at a relative proportion of > 0.01% for at least one sample. ASVs that did not meet the criteria were consolidated and included in a category labeled "Others" and excluded from further analyses. The network was visualized using the Compound Spring Embedder algorithm in the open-source software Cytoscape 3.10.1[36]. Network topological parameters, including average number of neighbors, density, degree of heterogeneity, centralization, average clustering coefficient, characteristic path length, and small-world coefficient, were calculated using the Networkanalyzer plugin in Cytoscape[37]. Network modules based on the Louvain algorithm were computed using the "cluster_louvain" function in the igraph package[38,39]. Erdös–Rényi random networks with the same numbers of nodes and edges of the real networks were generated using the Network Randomizer plugin in Cytoscape and used as a null model to compare network topological features[40]. To ascertain the topological roles of each node in the microbial network, we computed the within-module connectivity (Z_i), which describes how well a node is connected to other nodes in the same module, and the among-module connectivity (P_i), which indicates how well a node is connected to nodes in other modules[41]. Nodes were categorized into four groups: peripherals ($Z_i \leq 2.5$, $P_i \leq 0.62$), module hubs ($Z_i > 2.5$, $P_i \leq 0.62$), connectors ($Z_i \leq 2.5$, $P_i > 0.62$), or network hubs ($Z_i > 2.5$, $P_i > 0.62$), based on threshold values according to Olesen, et al.[42].

Structural equation modeling (SEM) analysis

We used SEM to estimate the relationships among different niche-categories, network stability, diversity, and environmental parameters. To eliminate multicollinear variables, Spearman rank correlation coefficients (ρ) were calculated before SEM analysis. The model was constructed using the "sem" function in the Lavvan package[43]. Conceptually, the hypothetical relationships were that (i) rhizosphere generalists and microbial network stability influence the rhizosphere microbial diversity and (ii) ecological network keystone species influence the rhizosphere network stability and microbial diversity. Considering that Z_i and P_i values represent the relatedness of a specific node (or ASV) with other nodes (or ASVs) in the same or other modules, respectively, we assumed

that these two values could be useful as metrics of the stability of an ecological network. Additionally, the presence or absence of each ASV across samples was considered to contribute to network stability. Hence, latent variables representing network stabilities were computed by summing the product of Z_i and P_i values for each ASV, multiplied by the corresponding occurrence data for each vertex. All variables were normalized using min–max feature scaling[44]. Given that the Shannon index of each biological kingdom in the rhizosphere may represent the level of microbial diversity within each kingdom, we utilized this index to create another latent variable, Rhizosphere diversity. As over 98.5% of the observed ASVs in the rhizosphere were from bacteria and fungi, we employed data from these two biological kingdoms to represent the rhizosphere microbiome to simplify the model. We calculated the first component of the NMDS of the rhizosphere generalist and network keystone species (rhizosphere ASVs only) and used them as representatives of rhizosphere generalist and network keystone species, respectively. Indices of fitness were calculated using the "model_performance" function in the performance package[45]. The fitness indices, as described by Schermelleh-Engel et al.[46], included the following:

Chi-square test (χ^2: The model has an acceptable fit when $\chi^2/df \leq 3$.
Comparative Fit Index (CFI): $0.95 \leq CFI < 1.0$.
Goodness-of-Fit Index (GFI): $0.90 \leq GFI < 1.0$.
Tucker-Lewis Index (TLI): $0.95 \leq TLI < 1.0$.
Standardized Root Mean Square Residual (SRMR): $0 \leq SRMR \leq 0.1$.
Root Mean Square Error of Approximation (RMSEA): $0 \leq RMSEA \leq 0.08$.

Results

Physiochemical characteristics of bulk soil

The variability in the physiochemical characteristics of bulk soil samples can be attributed to inherent features of the natural grassland sampling sites, which are characterized by a diverse community of wild plants. The characteristics of bulk soil from each sampling site are summarized in Table S1. Pairwise *t*-tests indicated that no significant differences were observed across the sampling sites, although organic matter levels were slightly higher in Gurye and Naju (2.5% and 3.3%, respectively) compared to other regions. The relatively uniform soil properties measured in this study suggest that their influence on microbial community variation was likely limited.

Microbial community composition and diversity index in the rhizosphere and bulk soil

The total read counts per bulk soil and rhizosphere were approximately 2,800,000 bacterial reads, 3,700,000 fungal reads, and 24,000 eukaryotic reads (excluding fungi) in the rhizosphere, and 5,200,000 bacterial reads, 3,400,000 fungal reads, and 1,900,000 eukaryotic reads in bulk soil. After removing unnecessary ASVs according to the criteria mentioned above, we identified 123 archaeal, 38,399 bacterial, 5,702 eukaryotic, and 4,564 fungal ASVs. All samples reached saturation in the rarefaction curves (Fig. S2), indicating the validity of comparison across the samples. In the bulk soil, bacterial ASVs displayed the highest diversity, with 31,934 ASVs, followed by eukaryotic (5,469 ASVs), fungal (4,162 ASVs), and archaeal ASVs (131). In contrast, a much limited number of ASVs were detected in the rhizosphere. Similar to the pattern in bulk soil, bacterial ASVs dominated with 17,973 ASVs, followed by Fungi (2,425 ASVs), Eukaryota (269 ASVs), and Archaea (37 ASVs). The NMDS ordination plots, constructed using data from prokaryotic, fungal, and eukaryotic communities, showed distinct clustering of samples from the rhizosphere and bulk soil (Fig. 1a, b, and c). Both the ANOSIM and PERMANOVA analyses supported the significant differences between bulk soil and rhizosphere microbial communities (prokaryotes: ANOSIM, $R = 0.59$, $p = 0.001$ and PERMANOVA, pseudo-$F = 20.3$, $p = 0.001$; fungi: ANOSIM, $R = 0.41$, $p = 0.001$ and PERMANOVA, pseudo-$F = 16.9$, $p = 0.001$; and eukaryotes: ANOSIM, $R = 0.54$, $p = 0.001$ and PERMANOVA, pseudo-$F = 44.1$, $p = 0.001$). Compartment-specific PCoA plots (Fig. S3) and PERMANOVA revealed significant site-specific differences in both bulk soil and rhizosphere microbial communities (Table S2, PERMANOVA, $p = 0.001$). Most pairwise comparisons showed significant differences across sites in both compartments and for all biological kingdoms, except for fungi in the rhizosphere (Table S2). For example, fungal communities were not significantly different between Naju and Sangju ($p = 0.516$) and between Gurye and Seosan ($p = 0.242$). These results suggest that spatial heterogeneity exists in both bulk soil and rhizosphere microbial communities, although rhizosphere fungal communities appear more conserved, likely due to plant-mediated selection. Diversity values calculated using the Shannon diversity and Chao1 indices were significantly higher in bulk soil than in rhizosphere samples (*t*-test: $p < 0.001$) (Fig. 1d). Across regions, no significant differences were observed in terms of the diversity indices, except for the prokaryote Chao1 index in the bulk soil (Fig. S4).

Archaea constituted approximately $0.2 \pm 0.3\%$ of the prokaryotic community, and a predominant portion were taxonomically classified as candidatus *Nitrocosmicus* (Crenarchaeota). We identified 44 bacterial phyla, and approximately 40% of the observed sequences belonged to the phylum Proteobacteria (Fig. 2a). The relative abundance of Proteobacteria, Bacteroidota, and Actinobacteriota in the rhizosphere was approximately 1.5-fold higher than that in the bulk soil. The most dominant bacterial ASV in both sample types was identified as *Bradyrhizobium* sp. (ASV_B00001). This was consistently observed in all samples, at an average of 1.3% in the total prokaryotic community. Several ASVs were identified as common microbes in the rhizosphere but were absent (or nearly absent) in the bulk soil: ASV_B00254 (*Polaromonas* sp.), ASV_B00062 (*Devosia* sp.), ASV_B00810 (uncultured *Sandaracinaceae*), and ASV_B00174 (*Pseudomonas brassicacearum*), with a relative abundance of up to 4.6% in the rhizosphere (Fig. S6).

Compared with the prokaryotic profile, the structure of the fungal community, at the class level, differed substantially between the rhizosphere and bulk soil, (Fig. 2b). *Olpidium* sp. (ASV_F0001), which was observed across sampling regions in both bulk soils and the rhizosphere, ubiquitously dominated the rhizosphere of feral *B. napus*. This ASV comprised an average of 60% of the fungal community in the rhizosphere, with an

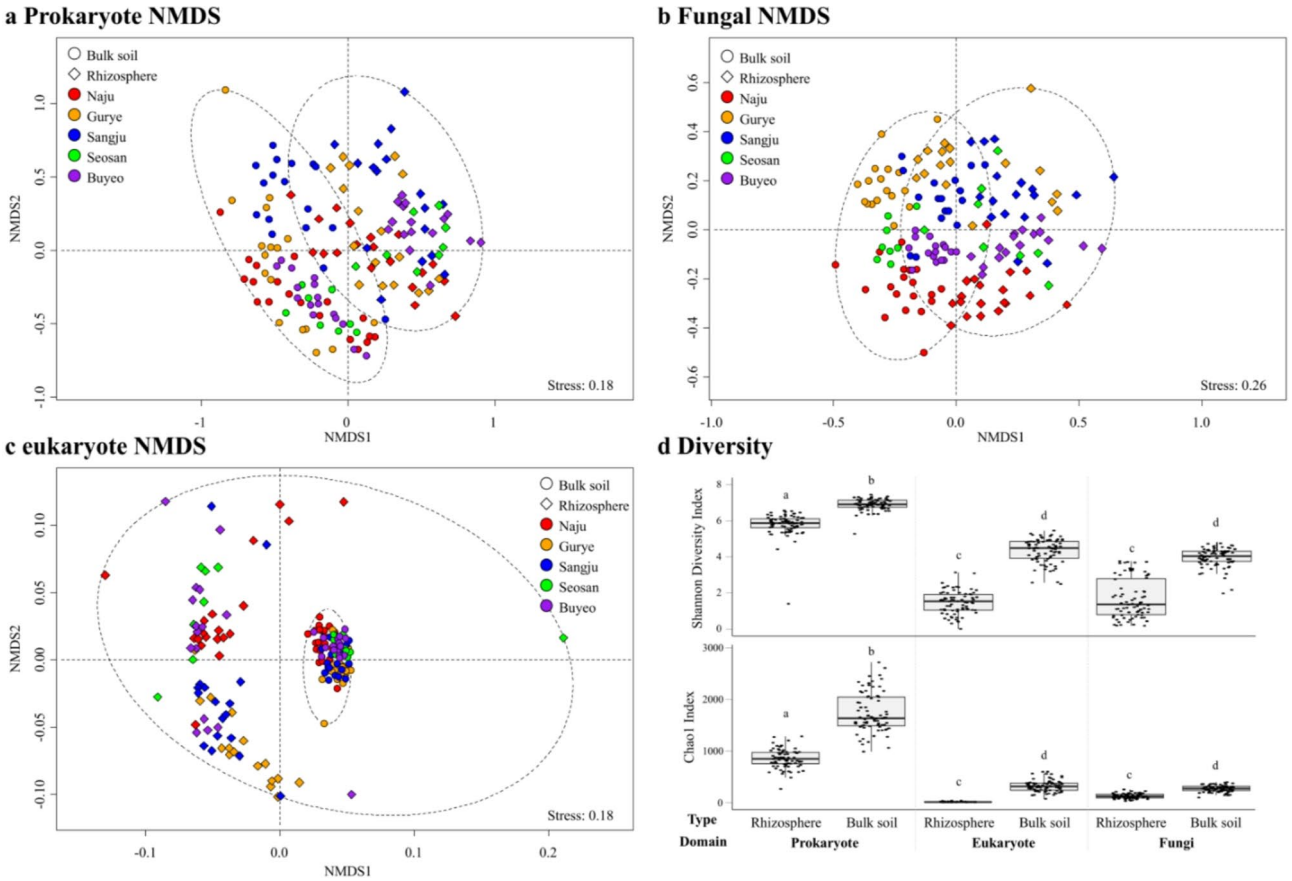


Fig. 1. Non-metric multidimensional scaling (NMDS) ordination plots. The plots were generated based on Bray–Curtis community dissimilarities based on (**a**) prokaryotic, (**b**) fungal, and (**c**) eukaryotic ASVs. (**d**) Shannon diversity index and Chao 1 index of prokaryotic, eukaryotic, and fungal communities. Different letters above the plot indicate statistically significant differences between groups based on *t*-test results ($p < 0.001$).

approximately 10-fold higher abundance than that in the bulk soil. In a few samples from Gurye and Sangju, ASVs belonging to Letiomycetes (mostly *Tetracladium*) dominated instead of ASV_F0001, comprising up to 24% of the total fungal community. Conversely, *Fusarium* sp. (ASV_F0003) and *Mortierella* sp. (ASV_F0004) were an average eight-times more abundant in bulk soil than in rhizosphere samples (Fig. 2d).

Regarding the structure of the eukaryotic community, the SAR supergroup (62%) and Opisthokonta (35%) dominated in both bulk soil and rhizosphere (Fig. 2c). Notably, Peronosporomycetes (formerly Oomycetes) exhibited a five-fold higher abundance in the rhizosphere than in bulk soil, while Ciliophora dominated the bulk soil communities (at a relative average of 8.5%) but accounted for <1% in the rhizosphere. At the ASV level, bacterivorous amoeboflagellate *Glissomonadida* sp. (ASV_E0030), nematode *Acrobeloides* sp. (ASV_E0004), oomycete *Lagena* sp. (ASV_E0017), and cercozoan *Spongospora* sp. (ASV_E0012) constituted approximately one-third of the rhizosphere eukaryotic community (Fig. 1d and Fig. S5).

Niche-based habitat specialization

To explore the ecological roles of each ASV in the bulk soil and rhizosphere, we calculated the normalized niche breadth of each ASV, which were then categorized into generalists, common taxa, and specialists (Fig. 3a and b). Overall, ASVs assigned to generalists were found to be ubiquitous across almost all collected samples and accounted for an average of 0.4% in the bulk soil samples and 5.3% in the rhizosphere samples. ASVs assigned to specialists, on the other hand, were more localized and accounted for either low average proportions or only dominated in a few samples of either origin. In the bulk soil, 22 ASVs were assigned to generalists, with a relative abundance of 3.4%, 2.5%, and 4.1% in the total prokaryotic, eukaryotic, and fungal communities, respectively (Fig. 3c; Table 1). Approximately half of the generalist ASVs (11) in bulk soil belonged to the Rhizobiales group. Specialists consisted of more diverse taxa and were much more abundant, making up to 88.3% of prokaryotes, 91.8% of eukaryotes, and 92.8% of fungi (Fig. 3c). In the rhizosphere samples, 11 prokaryotic ASVs and one fungal ASV (ASV_F0001, assigned to *Olpidium* sp.), but no eukaryotic ASVs, were identified as generalist taxa, constituting 5.9% of the prokaryotes and 58.6% of the fungi (Fig. 3c; Table 1). Interestingly, six generalist ASVs in the rhizosphere were also classified as generalists in the bulk soil, while 13 ASVs were unique to generalists in bulk soil, and three ASVs were unique to rhizosphere generalists (Fig. 3d).

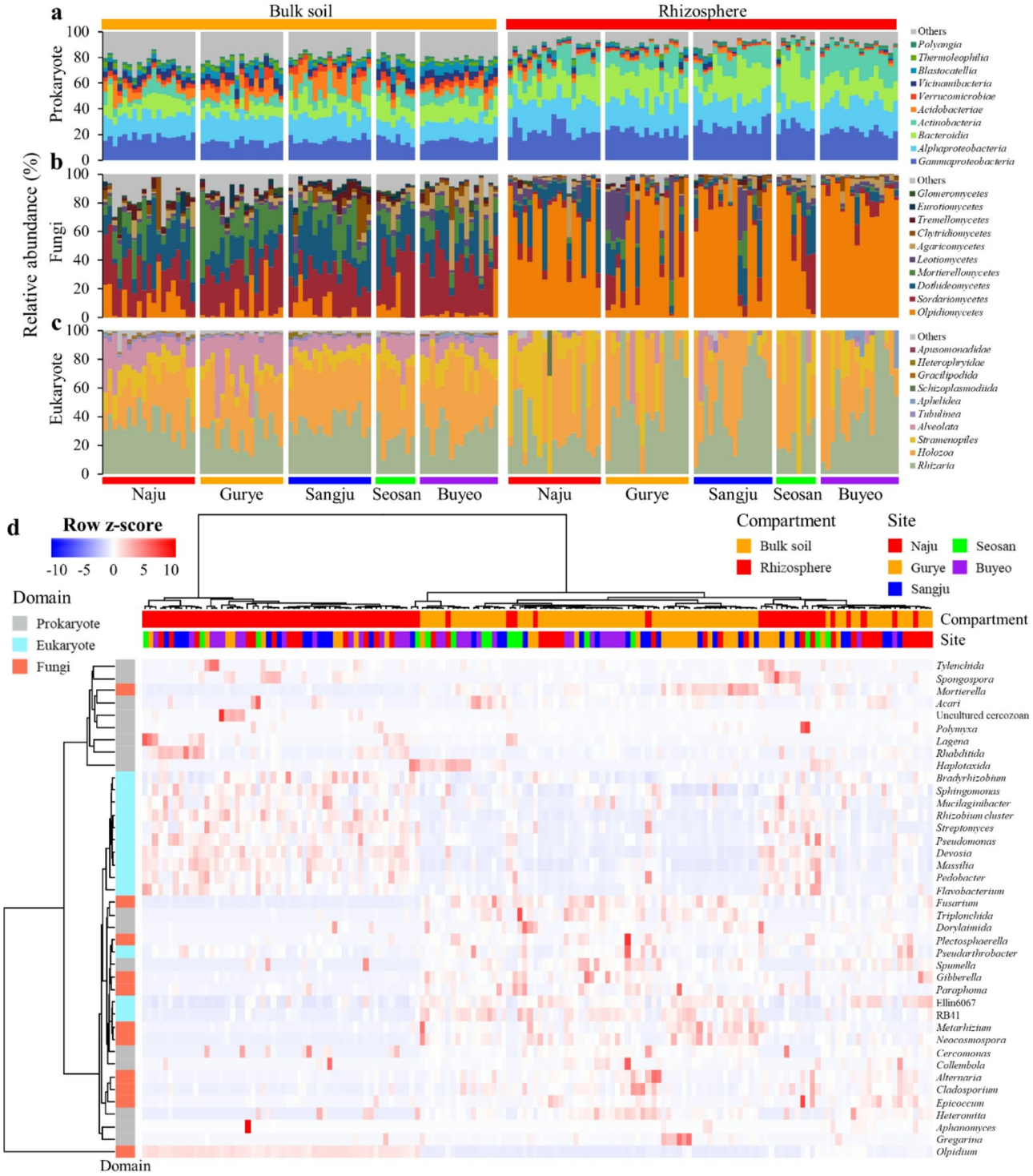


Fig. 2. Diversity of microbial communities. (**a**–**c**) Relative abundance of prokaryotes, fungi, and eukaryotes, respectively, at the class level in the bulk soil and rhizosphere. (**d**) Dendrogram and heatmap obtained via hierarchical clustering analysis based on observations from prokaryotes, fungi, and eukaryotes at the genus level.

Inter- and intra-kingdom association network

To explore inter- and intra-kingdom biological associations in the bulk soil and rhizosphere, we constructed SpiecEasi networks to identify keystone species and investigate their roles in shaping community structure and stability (Fig. 4a). The networks consisted of 2,664 nodes with 5,589 positive and 1,066 negative edges, displaying an average neighbor count of 5.036. A characteristic path length of 5.621 suggested efficient communication, while a clustering coefficient of 0.065 and a low network density of 0.002 indicated a relatively sparse and

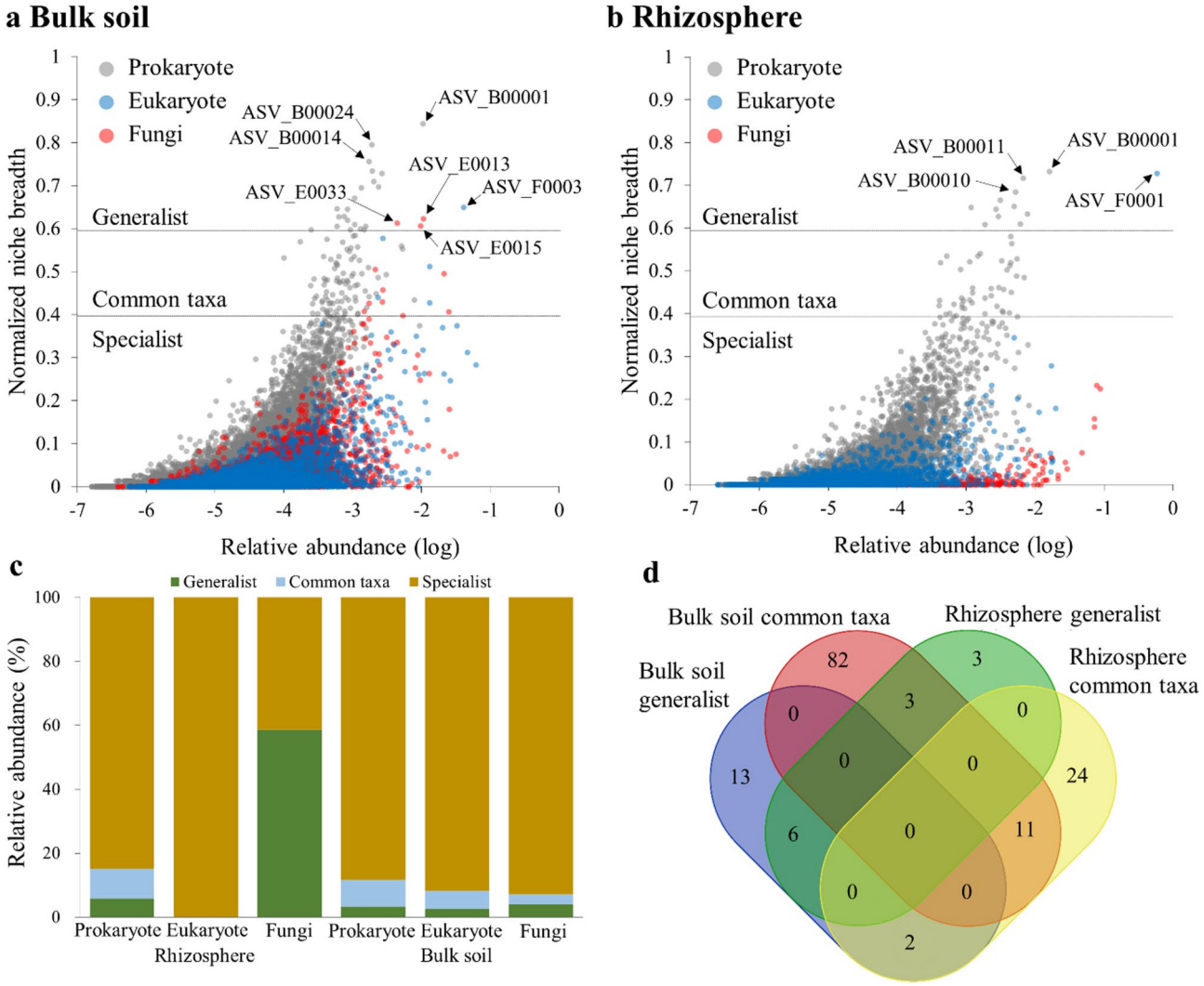


Fig. 3. Normalized niche breadth (B). The metric allowed assignment into specialists ($B < 0.4$), common taxa ($0.4 \le B \le 0.6$), and generalists ($B > 0.6$) in (**a**) the bulk soil and (**b**) rhizosphere. The x-axis indicates the log-transformed relative abundance of each ASV. Gray, blue, and red dots represent prokaryotic, eukaryotic, and fungal ASVs, respectively. Few representative generalists are arrow-marked with their ASV IDs. (**c**) The accumulated relative abundance of generalists, common taxa, and specialists in the rhizosphere and bulk soil. (**d**) Venn diagram constructed using data from generalists and common taxa in the rhizosphere and bulk soil.

decentralized organization, with a degree of network heterogeneity of 0.696 and centralization of 0.008. Network features (degree of heterogeneity, centralization, and average clustering coefficient) surpassed those observed in Erdős–Rényi random networks, indicating that rhizosphere and bulk soil microbial networks had small-world properties (that is, microbial interconnections are closer than those predicted in a random network with similar size) and modular structures. Therefore, the loss of keystone species in a small-world network may weaken network integrity and potentially lead to dysbiosis or disruption of the microbial community structure. Microbial interconnections were clearly differentiated in bulk soil and rhizosphere, with interactions within each microenvironment found to be more pronounced than those between the two microenvironments (Fig. 4a). Furthermore, within the network, a majority of eukaryotic and fungal ASVs were identified as originating from bulk soil ASVs (Fig. 4b). Approximately 75% of the connections of the rhizosphere microbes occurred within the rhizosphere, and, accordingly, approximately 70% of the rhizosphere microbes formed their own modules, with a lower average node degree (1.40) than that in the bulk soil (2.28). Among the rhizosphere microbes, we observed 33 network keystone species, including 22 module hubs and 11 connectors. Most connections of these rhizosphere module hubs occurred within the rhizosphere microbes, with only 4% representing connections with the bulk soil microbes (Fig. 5). In contrast, connectors showed 26% of connections with the bulk soil microbes. Taxonomically, over 60% of these network keystone species were assigned to Chitinophagaceae, Rhizobiaceae, Comamonadaceae, Xanthobacteraceae, Mycobacteriaceae, Flavobacteriaceae, and Rhodanobacteraceae.

To investigate the level of microbial associations between and within microenvironments and niche-based categories, we assessed the ratio of observed connections among theoretically possible connections, defining

ASVs	Niche-based category		Relative abundance (%)		Appearance ratio (%)		Taxa (Phylum; Order; Genus)
	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	
ASV_B00001	Gen.	Gen.	1.05	1.62	100	100	Bac.; Rhizobiales; *Bradyrhizobium*
ASV_B00002	Spe.	Gen.	0.94	0.62	90.9	94.8	Bac.; Micrococcales; *Pseudarthrobacter*
ASV_B00009	Com.	Gen.	0.06	0.78	53.2	92.2	Bac.; Rhizobiales; *Shinella*
ASV_B00010	Com.	Gen.	0.17	0.52	62.3	89.6	Bac.; Burkholderiales; *Massilia*
ASV_B00011	Com.	Gen.	0.12	0.68	77.9	97.4	Bac.; Rhizobiales; *Devosia*
ASV_B00014	Gen.	Gen.	0.17	0.50	88.3	94.8	Bac.; Streptomycetales; *Streptomyces*
ASV_B00024	Gen.	Gen.	0.19	0.31	98.7	97.4	Bac.; Rhizobiales; *Bradyrhizobium*
ASV_B00033	Gen.	Gen.	0.16	0.27	92.2	90.9	Bac.; Rhizobiales; *Devosia*
ASV_B00046	Gen.	Gen.	0.13	0.19	84.4	80.5	Bac.; Rhizobiales; *Devosia*
ASV_B00055	Spe.	Gen.	0.04	0.29	24.7	81.8	Bac.; Burkholderiales; *Massilia*
ASV_B00068	Gen.	Gen.	0.11	0.12	70.1	81.8	Bac.; Caulobacterales; *Phenylobacterium*
ASV_F0001	Spe.	Gen.	6.20	58.57	80.5	92.2	Fun.; Olpidiales; *Olpidium*
ASV_B00039	Gen.	Com.	0.20	0.09	97.4	74.0	Bac.; Nitrospirales; *Nitrospira*
ASV_B00114	Gen.	Com.	0.07	0.08	75.3	68.8	Bac.; Rhizobiales; *Nordella*
ASV_B00018	Gen.	Spe.	0.27	0.29	97.4	70.1	Bac.; Rhizobiales; *Bradyrhizobium*
ASV_B00031	Gen.	Spe.	0.24	0.09	85.7	58.4	Bac.; NA; NA
ASV_B00048	Gen.	Spe.	0.19	0.08	96.1	49.4	Bac.; Rhizobiales; NA
ASV_B00063	Gen.	Spe.	0.13	0.09	70.1	45.5	Bac.; Rhizobiales; NA
ASV_B00113	Gen.	Spe.	0.11	0.02	85.7	22.1	Bac.; Rhizobiales; *Rhodoplanes*
ASV_B00117	Gen.	Spe.	0.08	0.05	76.6	39.0	Bac.; Corynebacteriales; *Mycobacterium*
ASV_B00137	Gen.	Spe.	0.08	0.03	80.5	41.6	Bac.; Rhizobiales; *Rhodoplanes*
ASV_B00200	Gen.	Spe.	0.06	0.03	67.5	35.1	Bac.; Polyangiales; *Pajaroellobacter*
ASV_B00233	Gen.	Spe.	0.06	0.03	87.0	45.5	Bac.; Burkholderiales; mle1-7
ASV_B00299	Gen.	Spe.	0.06	0.02	79.2	18.2	Bac.; Rhizobiales; *Labrys*
ASV_E0013	Gen.	Spe.	1.07	0.30	89.6	5.2	Euk.; Ochrophyta; *Spumella*
ASV_E0015	Gen.	Spe.	0.97	0.15	92.2	6.5	Euk.; Ciliophora; NA
ASV_E0033	Gen.	Spe.	0.44	0.23	88.3	3.9	Euk.; Cercozoa; *Heteromita*
ASV_F0003	Gen.	Spe.	4.10	0.50	100	92.2	Fun.; Hypocreales; *Fusarium*

Table 1. Habitat generalists in the rhizosphere and bulk soil. Average relative abundance, appearance ratio, and taxonomic features of each ASVs were listed. Abbreviations: Gen., Generalist; Spe., Specialist; Com., Common taxa; Bac., Bacteria; Euk., Eukaryote; Fun., Fungi.

it as the connectivity ratio (Fig. 4c and d). Connectivity ratios were highest within prokaryotes and lowest in eukaryotes in both the rhizosphere and bulk soil. Most connections of the rhizosphere generalists occurred within the rhizosphere, and no module hub was assigned to the rhizosphere generalist.

SEM results

We used SEM to estimate the relationships among niche-based categories, network stability, diversity, and environmental parameters (Fig. 6). We compared several candidate models, and selected the best-fitting model. The final model resulted in a χ^2/df ratio of 1.28, CFI of 0.986, GFI of 0.944, TLI of 0.92, RMSEA of 0.073, and SRMR of 0.049, indicating performance within acceptable limits and good fit for the intended purpose. The rhizosphere generalists showed a direct influence on the diversity of the rhizosphere microbial community, suggesting the key roles of these generalists in shaping the microbial community composition. The network keystone species, which were composed of module hubs and connectors, affected both the rhizosphere diversity indices and network stability. Since the role of keystone species was to promote stability and resilience within specific modules by maintaining network structure and function, their presence contributed to enhanced network stability and overall resilience of the rhizosphere microbial community. In addition, rhizosphere network stability positively influenced rhizosphere diversity, suggesting that the more stable the network, the greater its ability to support a diverse microbial community.

Discussion

Differences in the structure of microbial communities between the rhizosphere and bulk soil have been well documented in previous studies. The soil microbiome is highly dynamic and composed of a wide variety of microorganisms, partly due to the highly heterogeneous soil environment, serving as a pool for the rhizosphere and influencing the microbial community structures of the rhizosphere[47]. We found significant differences in the community compositions of three biological kingdoms between rhizosphere and bulk soil, which were particularly remarkable in the eukaryotic communities. Consistent with previous results[48], the proportions of *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* were enriched in the rhizosphere compared with those in

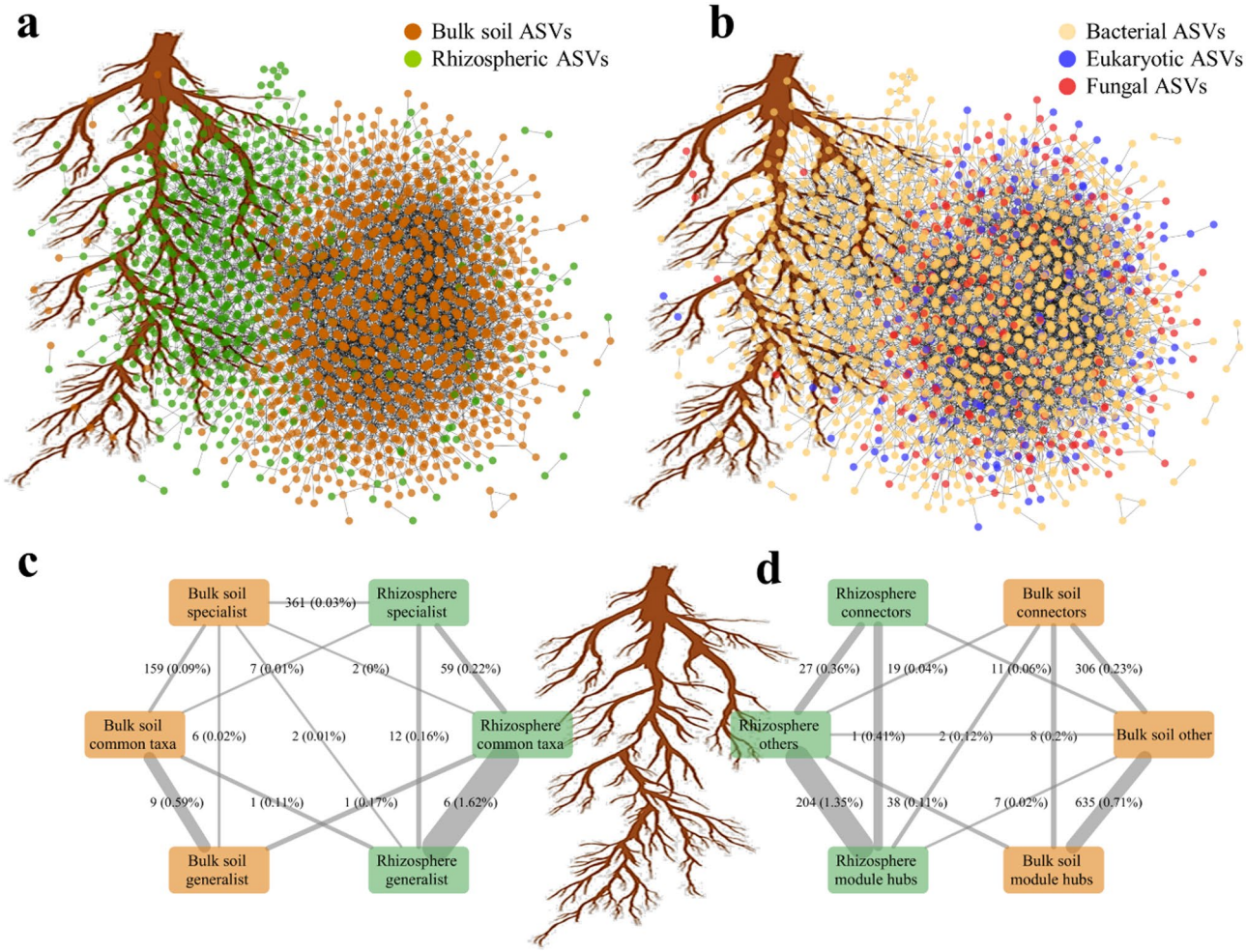


Fig. 4. Microbial association network. The network represents (**a**) the different microenvironment (rhizosphere and bulk soil) and (**b**) the different biological kingdoms. (**c**) Intra- and inter-kingdom associations and connectivity ratios between and within microenvironments. (**d**) Observed interconnections and the connectivity ratios between and within niche-based categories. Numbers at rectangular (parentheses) represent numbers of ASVs. Edge thickness was in proportion to connectivity ratios. The numbers at edges represent the number of connections and connectivity ratios (parentheses). The rectangular size represents the total number of nodes in each module.

the bulk soil samples. Many species in these groups belong to copiotrophs, with multiple rRNA genes in their genomes[49], enabling them to achieve rapid growth rates[50]. Therefore, the plant-secreted photosynthates (*B. napus* in this study) could contribute to the development of a resource-rich environment in the neighboring root zone, potentially facilitating copiotroph enrichment within the plant rhizosphere.

In contrast to the bacterial community structures in the feral *B. napus* rhizosphere, which were composed of relatively evenly distributed ASVs, *Olpidium brassicae* (ASV_F0001) and *Glissomonadida* (ASV_E0030) accounted for up to 97% and 66% of the total rhizospheric fungal and eukaryotic communities, respectively. As one of the dominant fungal groups in the cultivated *B. napus* rhizosphere[51,52], *O. brassicae* was previously misclassified as *O. virulentus*, a well-known vector of viruses of cruciferous plants[53]. The low sequence similarity with *O. virulentus* (< 90%) and ubiquitous presence of *O. brassicae* (ASV_F0001) in healthy feral *B. napus* suggest that this fungal species is unlikely to be a pathogen that mediates plant disease.

Within the rhizospheric microeukaryotic community, E_ASV0030, an uncultured *Glissomonadida* identified as bacterivorous flagellates in a wide variety of environments[54,55], was the dominant group, constituting up to 66% of the eukaryotes. In comparison, E_ASV0030 was depleted in the bulk soil, accounting for only up to 2% in our samples. This discrepancy suggests that these uncultured gliding flagellates may thrive in the microenvironment created within the rhizosphere of *B. napus*, potentially exerting control over the microbial community structure through their bacterivorous properties.

Ecological classifications according to niche breadth can enhance our understanding of how different organisms navigate and contribute to ecological niches, shedding light on their roles in ecosystem dynamics and resilience[44,56,57]. Vegetation functions as a key driver of the distribution of microbial generalists, which in turn contribute to reshaping the remaining microbial community structures in the tundra ecosystem[44]. In

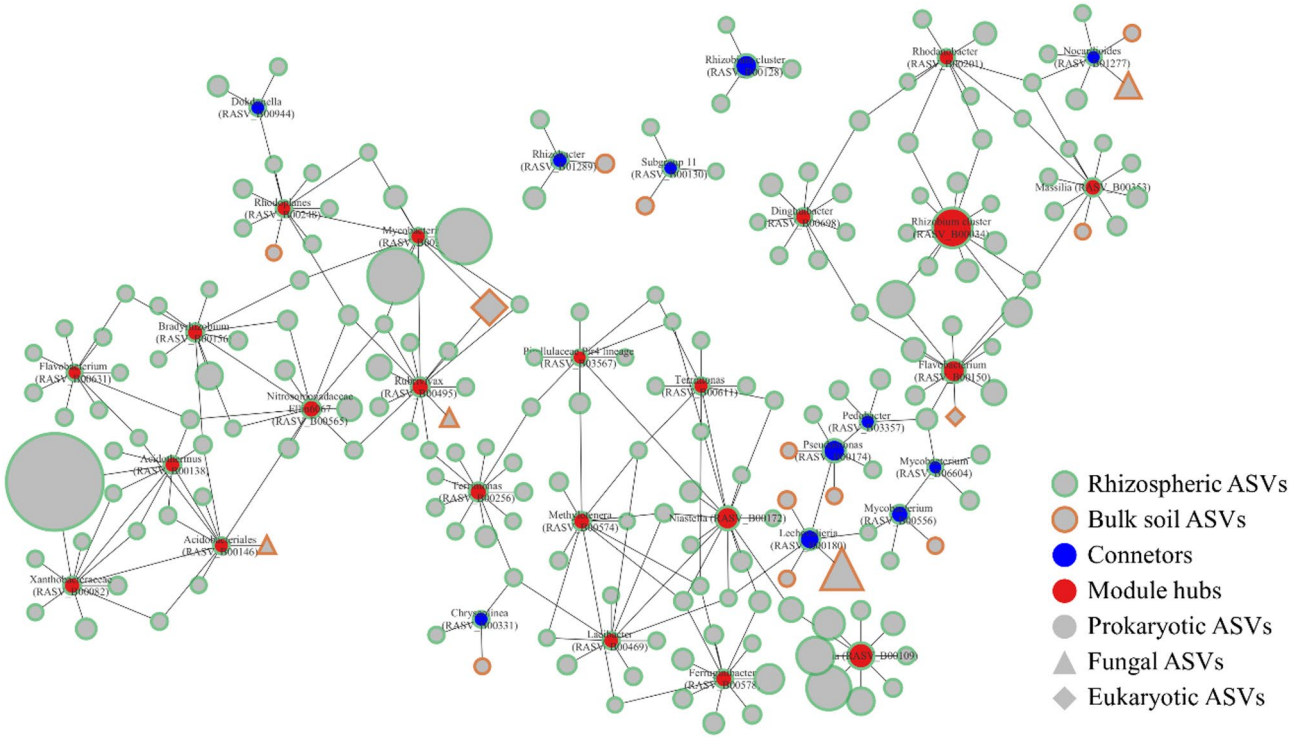
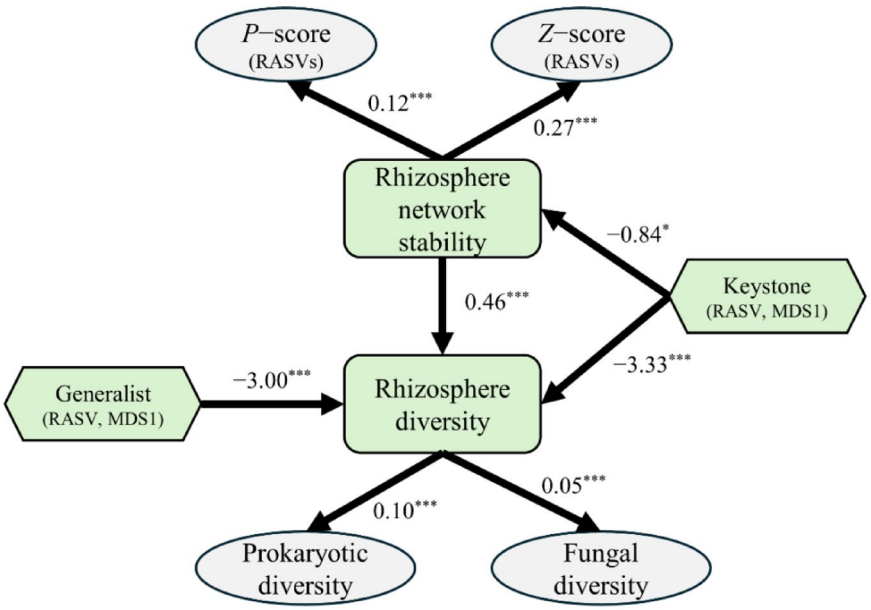


Fig. 5. Subnetwork constructed with rhizosphere microbial keystone species and their directly linked microbes.



Chi −Square/df : 1.3, CFI: 0.99, GFI: 0.94, TLI: 0.97 RMSEA: 0.063, SRMR: 0.080

Fig. 6. Structural equation model (SEM). The model represents the relationships among rhizosphere generalists, microbial diversity index, network keystone species, the network stability. The latent variables Rhizosphere network stability and Rhizosphere diversity were calculated using the P and Z scores and prokaryote and fungal diversity indices, respectively. ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$. CFI, comparative fit index; GFI, Goodness-of Fit Index; TLI, Tucker–Lewis Index; SRMR, standardized root mean square residual; RMSEA, root mean square error of approximation.

this study, *Massilia*, *Phenylobacterium*, *Pseudarthrobacter*, *Streptomyces*, *Devosia*, *Shinella*, and *Bradyrhizobium*, were enriched in the rhizosphere. The genus *Massilia*, which produces heteroauxin and cellulose-degrading enzymes[58], is a well-known plant growth-promoting microorganism that colonizes the rhizosphere and roots of various plant species[58–61]. Our previous study demonstrated that ASVs within the genus *Massilia* exhibit distinct divisions depending on plant holobiont components, such as dead leaf and rhizosphere, suggesting that they mediate different ecological roles within the plant holobiont[5]. Six ASVs assigned to Rhizobiales were identified as rhizosphere generalists in this study, including *Devosia*, *Shinella*, and *Bradyrhizobium*, at the genus level. Previous studies have demonstrated that *Devosia* positively correlate with nitrogen input[62], while *Bradyrhizobium*, along with other rhizospheric beneficial microbes, stimulates plant growth through enhanced mineral nutrient acquisition (nitrogen and phosphorus)[63]. *Streptomyces* has been reported to promote plant growth and tolerance to stressors by inhibiting pathogen growth through the production of antibiotics and antimicrobial peptides, among other agents[64,65]. Therefore, *B. napus* recruited potential beneficial microbes as generalists to support development and immune function, enhancing growth and resilience through improved nutrient uptake and pathogen suppression.

In ecological networks, module hubs and connectors are crucial keystone species, assisting other microbes and maintaining network structure and function[66–68]. We found that keystone species comprised a wide variety of microbes, including Acidothermaceae, Chitinophagaceae, Comamonadaceae, Devosiaceae, Flavobacteriaceae, Mycobacteriaceae, Oxalobacteraceae, Pseudomonadaceae, Rhizobiaceae, and Xanthobacteraceae. Among these groups, *Acidothermus*[69], *Ferruginibacter*[70], *Niastella*[71], *Terrimonas*[72], *Rhizobacter*[73], *Devosia*[62], *Mycobacterium*[74], *Pseudomonas*[69], and *Bradyrhizobium*[73], at the genus level, promote plant growth or immune system development, with a potential to function as core plant root-associated microbes. We found that the rhizosphere keystone species, all of which were bacteria, were almost exclusively connected with rhizosphere microbes, indicating their substantial influence on the overall microbial community structure in the rhizosphere microenvironment. Notably, no keystone species were identified among the rhizosphere generalists or common taxa. We assume that the rhizosphere generalists recruited by the plant interact with the roots to support and/or improve plant growth or immune functions. Since rhizosphere generalists are recruited by the plant, they may primarily interact with the plant itself rather than with other microbes, which could explain why they did not serve as keystone species in the microbial network. However, as rhizosphere keystone species were correlated with rhizosphere generalists, their influence on rhizosphere microbial structure is likely indirect and mediated through keystone species.

Network stability is a crucial facet of complex ecological systems, reflecting the ability of networks to maintain consistent patterns of interactions while minimizing instability[75,76]. High network stability implies resilience to environmental changes, facilitating the seamless functioning of the entire system. This concept is fundamental for understanding and managing the robustness of ecosystems in the face of dynamic perturbations[77]. The SEM results demonstrated that the rhizosphere keystone species directly affected network stability and, together with the rhizosphere generalists, influenced microbial diversity in this microenvironment. These findings are also congruent with our earlier observations on a tundra ecosystem where vegetation-driven macroenvironmental filtering effects were identified as the key factor in shaping the distribution of generalists, which, in turn, controlled the overall microbial community[44]. The rhizospheric generalists in this study are typical plant root-associated beneficial microbes that can improve plant metabolism and enhance the plant immune system, thereby promoting growth. Consequently, plants may secrete more metabolites into the surrounding microenvironment, which can serve as nutrient sources for rhizospheric microbes, thereby affecting microbial diversity and influencing further the overall microbial community structures in the rhizosphere. The keystone species were identified as key drivers of network stability and microbial diversity in the rhizosphere and their loss can lead to the collapse and deterioration of the entire network[78]. As the keystone species assist other microbes and maintain the structure and function of the ecological network, they have the potential to directly influence network stability and microbial diversity. In summary, the microbial generalists and network keystone species in the rhizosphere of feral *B. napus* function as key drivers of microbial diversity and ecological network stabilization.

Conclusions

The present study highlighted the critical roles of rhizosphere microbial generalists and network keystone species in the regulation of microbial communities and interspecies interactions within the root system of feral *B. napus*. Consistent with previous studies[6,13,47,54], the rhizosphere microbial community composition largely differed from that in adjacent bulk soils, exhibiting diminished microbial diversity and richness in all three biological kingdoms. These results suggest that microbial selection by the plant itself from the soil pool is critical in shaping the rhizospheric microbial communities. Microbial correlations were primarily observed among rhizosphere-associated taxa, with fewer correlations detected with bulk soil microbes, further supporting the role of plant-driven niche filtration in shaping rhizosphere microbial communities. No keystone species were detected from the rhizosphere generalists, suggesting the tight coupling of the latter with the plant itself rather than with the rhizosphere microbes. SEM revealed that the rhizosphere generalist and network keystone species functioned as key drivers of microbial diversity and network stability in the rhizosphere. In summary, *B. napus* exerted a selective effect on their rhizospheric generalists, which in turn, together with the rhizosphere keystone species, served as primary determinants of microbial diversity and network stability to control the microbial community structures and interspecies interactions.

Data availability

The raw sequences, along with the accompanying metadata and detailed parameters used in the pipelines, are accessible in the Sequence Read Archive of NCBI under the project accession numbers PRJNA821335 and PRJNA816676, and published in *Data in Brief*[21].

Received: 30 December 2024; Accepted: 14 May 2025
Published online: 20 May 2025

References

1. Friedt, W., Tu, J. & Fu, T. Academic and economic importance of *Brassica napus* rapeseed. *The Brassica napus Genome*, 1–20 (2018).
2. Lu, K. et al. Whole-genome resequencing reveals *Brassica napus* origin and genetic loci involved in its improvement. *Nat. Commun.* **10**, 1154 (2019).
3. Mizuguti, A., Yoshimura, Y., Shibaike, H. & Matsuo, K. Persistence of feral populations of *Brassica napus* originated from spilled seeds around the Kashima seaport in Japan. *Japan Agric. Res. Q.: JARQ.* **45**, 181–185 (2011).
4. Pascher, K. et al. Molecular differentiation of commercial varieties and feral populations of oilseed rape (*Brassica Napus* L). *BMC Evol. Biol.* **10**, 1–13 (2010).
5. Chun, S. J., Cui, Y., Yoo, S. H. & Lee, J. R. Organic connection of holobiont components and the essential roles of core microbes in the holobiont formation of feral *Brassica napus*. *Frontiers Microbiology* 13 (2022).
6. Xu, J. et al. The structure and function of the global citrus rhizosphere Microbiome. *Nat. Commun.* **9**, 4894 (2018).
7. Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T. & Singh, B. K. Plant–microbiome interactions: from community assembly to plant health. *Nat. Rev. Microbiol.* **18**, 607–621 (2020).
8. Li, Y. et al. Rhizobacterial communities of five co-occurring desert halophytes. *PeerJ* **6**, e5508 (2018).
9. Matthews, A., Pierce, S., Hipperson, H. & Raymond, B. Rhizobacterial community assembly patterns vary between crop species. *Front. Microbiol.* **10**, 441189 (2019).
10. Pérez-Jaramillo, J. E., Mendes, R. & Raaijmakers, J. M. Impact of plant domestication on rhizosphere Microbiome assembly and functions. *Plant Mol. Biol.* **90**, 635–644 (2016).
11. Ling, N., Wang, T. & Kuzyakov, Y. Rhizosphere bacteriome structure and functions. *Nat. Commun.* **13**, 836 (2022).
12. Pieterse, C. M. & Dicke, M. Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends Plant Sci.* **12**, 564–569 (2007).
13. Pii, Y. et al. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol. Fertil. Soils.* **51**, 403–415 (2015).
14. Fierer, N. et al. Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Appl. Environ. Microbiol.* **73**, 7059–7066 (2007).
15. Frey, S. D. Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annu. Rev. Ecol. Evol. Syst.* **50**, 237–259 (2019).
16. Powell, J. R. & Rillig, M. C. Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytol.* **220**, 1059–1075 (2018).
17. Seppey, C. V. et al. Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling. *Soil Biol. Biochem.* **112**, 68–76 (2017).
18. Zhang, L. & Lueders, T. Micropredator niche differentiation between bulk soil and rhizosphere of an agricultural soil depends on bacterial prey. *FEMS Microbiol. Ecol.* **93**, fix103 (2017).
19. Arunkumar, N., Rakesh, S., Rajaram, K., Kumar, N. R. & Durairajan, S. S. K. in Plant Microbe Interface, 309–324 (Springer, (2019).
20. Chen, Y. H., Gols, R., Stratton, C. A., Brevik, K. A. & Benrey, B. Complex tritrophic interactions in response to crop domestication: predictions from the wild. *Entomol. Exp. Appl.* **157**, 40–59 (2015).
21. Chun, S. J. Microbiome dataset of eukaryotic and fungal communities in the bulk soil and root of wild *Brassica napus* in South Korea. *Data Brief.* **43**, 108457 (2022).
22. Mori, H. et al. Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes. *DNA Res.* **21**, 217–227 (2014).
23. Stoeck, T. et al. Multiple marker parallel Tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol. Ecol.* **19**, 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x (2010).
24. Lord, N., Kaplan, C. W., Shank, P., Kitts, C. L. & Elrod, S. L. Assessment of fungal diversity using terminal restriction fragment (TRF) pattern analysis: comparison of 18S and ITS ribosomal regions. *FEMS Microbiol. Ecol.* **42**, 327–337 (2002).
25. Toju, H., Tanabe, A. S., Yamamoto, S. & Sato, H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PloS One.* **7**, e40863. https://doi.org/10.1371/journal.pone.0040863 (2012).
26. Fitzpatrick, C. R. et al. Chloroplast sequence variation and the efficacy of peptide nucleic acids for blocking host amplification in plant Microbiome studies. *Microbiome* **6**, 1–10 (2018).
27. Callahan, B. J. et al. DADA2: High-resolution sample inference from illumina amplicon data. *Nat. Methods.* **13**, 581–583 (2016).
28. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596 (2012).
29. Abarenkov, K. et al. UNITE general FASTA release for Fungi 2. *UNITE Community* 761 (2020).
30. Team, R. C. R: A language and environment for statistical computing. (2013).
31. Oksanen, J. et al. The vegan package. *Community Ecol. Package.* **10**, 719 (2007).
32. Levins, R. *Evolution in Changing Environments: some Theoretical Explorations* (Princeton University Press, 1968).
33. Novakowski, G. C., Hahn, N. S. & Fugi, R. Diet seasonality and food overlap of the fish assemblage in a Pantanal pond. *Neotropical Ichthyol.* **6**, 567–576 (2008).
34. Kurtz, Z. D. et al. Sparse and compositionally robust inference of microbial ecological networks. *PLoS Comput. Biol.* **11**, e1004226 (2015).
35. Tipton, L. et al. Fungi stabilize connectivity in the lung and skin microbial ecosystems. *Microbiome* **6**, 1–14 (2018).
36. Shannon, P. et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504 (2003).
37. Doncheva, N. T., Assenov, Y., Domingues, F. S. & Albrecht, M. Topological analysis and interactive visualization of biological networks and protein structures. *Nat. Protoc.* **7**, 670–685 (2012).
38. Blondel, V. D., Guillaume, J. L., Lambiotte, R. & Lefebvre, E. Fast unfolding of communities in large networks. *Journal Stat. Mechanics: Theory Experiment* **2008**, P10008 (2008).
39. Csardi, G. & Nepusz, T. The Igraph software package for complex network research. *InterJournal Complex. Syst.* **1695**, 1–9 (2006).
40. Tosadori, G., Bestvina, I., Spoto, F., Laudanna, C. & Scardoni, G. Creating, generating and comparing random network models with NetworkRandomizer. *F1000Research* 5 (2016).
41. Guimera, R. & Nunes Amaral, L. A. Functional cartography of complex metabolic networks. *nature* 433, 895–900 (2005).
42. Olesen, J. M., Bascompte, J., Dupont, Y. L. & Jordano, P. The modularity of pollination networks. *Proc. Natl. Acad. Sci.* **104**, 19891–19896 (2007).
43. Rosseel, Y. & lavaan An R package for structural equation modeling. *J. Stat. Softw.* **48**, 1–36 (2012).

44. Wong, S. K. et al. Vegetation as a key driver of the distribution of microbial generalists that in turn shapes the overall microbial community structure in the low Arctic tundra. *Environ. Microbiome*. **18**, 1–18 (2023).
45. Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P. & Makowski, D. Performance: an R package for assessment, comparison and testing of statistical models. *Journal Open. Source Software* **6** (2021).
46. Schermelleh-Engel, K., Moosbrugger, H. & Müller, H. Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures. *Methods Psychol. Res. Online*. **8**, 23–74 (2003).
47. Berendsen, R. L., Pieterse, C. M. & Bakker, P. A. The rhizosphere Microbiome and plant health. *Trends Plant Sci.* **17**, 478–486 (2012).
48. Praeg, N. & Illmer, P. Microbial community composition in the rhizosphere of Larix decidua under different light regimes with additional focus on methane cycling microorganisms. *Sci. Rep.* **10**, 22324 (2020).
49. Schöps, R. et al. Land-use intensity rather than plant functional identity shapes bacterial and fungal rhizosphere communities. *Front. Microbiol.* **9**, 394479 (2018).
50. Klappenbach, J. A., Dunbar, J. M. & Schmidt, T. M. rRNA Operon copy number reflects ecological strategies of bacteria. *Appl. Environ. Microbiol.* **66**, 1328–1333 (2000).
51. Bazghaleh, N. et al. From plant genetics to environment of selection: exploring the drivers of fungal communities in the roots and rhizosphere of *Brassica napus*. (2022).
52. Li, Y. et al. Root and rhizosphere fungi associated with the yield of diverse *Brassica napus* genotypes. *Rhizosphere* **25**, 100677 (2023).
53. Lay, C. Y., Hamel, C. & St-Arnaud, M. Taxonomy and pathogenicity of *Olpidium brassicae* and its allied species. *Fungal Biology*. **122**, 837–846 (2018).
54. Fiore-Donno, A. M. et al. Soil compartments (bulk soil, litter, root and rhizosphere) as main drivers of soil Protistan communities distribution in forests with different nitrogen deposition. *Soil Biol. Biochem.* **168**, 108628 (2022).
55. Howe, A. T., Bass, D., Vickerman, K., Chao, E. E. & Cavalier-Smith, T. Phylogeny, taxonomy, and astounding genetic diversity of glissomonadida Ord. Nov., the dominant gliding zooflagellates in soil (Protozoa: Cercozoa). *Protist* **160**, 159–189 (2009).
56. Pandit, S. N., Kolasa, J. & Cottenie, K. Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. *Ecology* **90**, 2253–2262 (2009).
57. Richmond, C. E., Breitburg, D. L. & Rose, K. A. The role of environmental generalist species in ecosystem function. *Ecol. Model.* **188**, 279–295 (2005).
58. Turnbull, A. L., Liu, Y. & Lazarovits, G. Isolation of bacteria from the rhizosphere and rhizoplane of potato (*Solanum tuberosum*) grown in two distinct soils using semi selective media and characterization of their biological properties. *Am. J. Potato Res.* **89**, 294–305 (2012).
59. Krishnamoorthy, R. et al. Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil. *Agric. Ecosyst. Environ.* **231**, 233–239 (2016).
60. Ofek, M., Hadar, Y. & Minz, D. Ecology of root colonizing *Massilia* (Oxalobacteraceae). *PloS One*. **7**, e40117 (2012).
61. Xu, A., Liu, C. & Sun, H. Dynamic distribution of *Massilia* spp. In sewage, substrate, plant rhizosphere/phyllosphere and air of constructed wetland ecosystem. *Front. Microbiol.* **14**, 1211649 (2023).
62. Chen, S. et al. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. *Microbiome* **7**, 1–13 (2019).
63. Meena, R. S., Vijayakumar, V., Yadav, G. S. & Mitran, T. Response and interaction of *Bradyrhizobium japonicum* and arbuscular mycorrhizal fungi in the soybean rhizosphere. *Plant. Growth Regul.* **84**, 207–223 (2018).
64. Li, J. et al. Synergistic effect of co-culture rhizosphere streptomyces: A promising strategy to enhance antimicrobial activity and plant growth-promoting function. *Front. Microbiol.* **13**, 976484 (2022).
65. Yang, Z. et al. *Streptomyces* alleviate abiotic stress in plant by producing pteridic acids. *Nat. Commun.* **14**, 7398 (2023).
66. Chun, S. J., Cui, Y., Baek, S. H., Ahn, C. Y. & Oh, H. M. Seasonal succession of microbes in different size-fractions and their modular structures determined by both macro-and micro-environmental filtering in dynamic coastal waters. *Sci. Total Environ.* **784**, 147046 (2021).
67. Cui, Y. et al. Unique microbial module regulates the harmful algal bloom (*Cochlodinium polykrikoides*) and shifts the microbial community along the Southern Coast of Korea. *Sci. Total Environ.* **721**, 137725 (2020).
68. Chun, S. J., Kim, Y. J., Cui, Y. & Nam, K. H. Ecological network analysis reveals distinctive microbial modules associated with heavy metal contamination of abandoned mine soils in Korea. *Environ. Pollut.* **289**, 117851 (2021).
69. Cipriano, M. A. et al. Lettuce and rhizosphere Microbiome responses to growth promoting Pseudomonas species under field conditions. *FEMS Microbiol. Ecol.* **92**, fiw197 (2016).
70. Lewin, G. R. et al. Cellulose-enriched microbial communities from leaf-cutter ant (*Atta colombica*) refuse dumps vary in taxonomic composition and degradation ability. *PLoS One*. **11**, e0151840 (2016).
71. Yan, Z. F. et al. *Niastella hibisci* Sp. nov., isolated from rhizosphere soil of Mugunghwa, the Korean National flower. *Int. J. Syst. Evol. MicroBiol.* **66**, 5218–5222 (2016).
72. Deng, X. et al. Rhizosphere bacteria assembly derived from fumigation and organic amendment triggers the direct and indirect suppression of tomato bacterial wilt disease. *Appl. Soil. Ecol.* **147**, 103364 (2020).
73. Han, G. et al. Response of pine rhizosphere microbiota to foliar treatment with resistance-inducing bacteria against pine wilt disease. *Microorganisms* **9**, 688 (2021).
74. Bouam, A., Armstrong, N., Levasseur, A. & Drancourt, M. *Mycobacterium terramassiliense*, *Mycobacterium rhizamassiliense* and *Mycobacterium numidiamassiliense* Sp. nov., three new *Mycobacterium simiae* complex Sp.cies cultured from plant roots. *Sci. Rep.* **8**, 9309 (2018).
75. Li, W., Liu, Q., Xie, L. & Yin, C. Interspecific plant-plant interactions increase the soil microbial network stability, shift keystone microbial taxa, and enhance their functions in mixed stands. *For. Ecol. Manag.* **533**, 120851 (2023).
76. Yuan, M. M. et al. Climate warming enhances microbial network complexity and stability. *Nat. Clim. Change*. **11**, 343–348 (2021).
77. Donohue, I. et al. Navigating the complexity of ecological stability. *Ecol. Lett.* **19**, 1172–1185 (2016).
78. Bascompte, J. & Stouffer, D. B. The assembly and disassembly of ecological networks. *Philosophical Trans. Royal Soc. B: Biol. Sci.* **364**, 1781–1787 (2009).

Acknowledgements

This research was supported by the National Institute of Ecology (NIE) funded by the Ministry of Environment (MOE) of the Republic of Korea (NIE-A-2025-10; NIE-A-2025-04; NIE-A-2025-11) and National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2022-NR070837).

Author contributions

YC and JK: Formal analysis, Visualization, Writing-Original draft preparation; MS: Methodology, Writing-Review and Editing; MP and CSL: Visualization, Writing-Review and Editing; N-HN and J-WL: Methodology, Investigation, Writing-Review and Editing; ASL: Writing-Review and Editing; S-JC: Conceptualization, Resources,

Writing-Review and Editing, Supervision, Project administration, Funding acquisition. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-02562-2.

Correspondence and requests for materials should be addressed to S.-J.C.

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

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.