Ecological Processes

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Endophytic bacteria in the periglacial plant *Potentilla fruticosa* var. *albicans* are influenced by habitat type

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Abstract

Background Microbial communities in different plant compartments are relatively independent entities. However, the influence of environmental factors on the microbial community in different compartments of periglacial plants remains unclear. In this study, we quantified the bacterial communities in the rhizosphere soil, as well as root and leaf endosphere compartments of a periglacial plant, *Potentilla fruticosa* var. *albicans*, using high-throughput DNA sequencing. Moreover, we evaluated the impacts of habitat types (glacier terminus zone, moraine ridge, and alpine meadow) on the bacterial community in different plant compartments of *Potentilla fruticosa* var. *albicans*.

Results Our results showed that habitat type had a significant effect on the alpha diversity (Chao1 richness) of endophytic bacteria, but not on the rhizospheric bacteria. The community composition of rhizospheric and endophytic bacteria was significantly different across the three habitats, and habitat type had a greater effect on the endophytic bacteria than on rhizospheric bacteria. The contribution of rhizosphere soil to the root and leaf endophytes decreased with the transformation of habitats from glacier terminus zone to alpine meadow. In contrast, host selection pressure sequentially increased from the glacier terminus zone to the moraine ridge to the alpine meadow. Furthermore, we found that the bacterial co-occurrence network in the alpine meadow was more modular but had lower complexity and connectedness than that in the glacier terminus zone. The bacterial community was governed primarily by stochastic processes regardless of habitat type.

Conclusion This study reveals that the diversity and composition of endophytic bacteria associated with *Potentilla fruticosa* var. *albicans* are more affected by habitat types than that of rhizospheric bacteria. Our study also demonstrates that the assembly patterns and co-occurrence patterns of bacterial communities associated with *Potentilla fruticosa* var. *albicans* vary by habitat type. These results advance the current understanding of community assembly and ecological interactions of microbial communities associated with periglacial plants.

Keywords Bacterial community, Endophytic bacteria, Periglacial plant, *Potentilla fruticosa* var. *albicans*

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Introduction

Plants growing in periglacial environments face several abiotic stressors relating to seasonal and climate variation (Thomas et al. 2014; Given et al. 2020), and the accumulation and recycling of nutrients that are mainly available in inaccessible forms (Given et al. 2020). Nevertheless, previous studies showed that the periglacial environment possesses abundant plant diversity (Shetekauri et al. 2012; Wang and Hong 2020). For example, the periglacial environment of the Qinghai-Tibet Plateau harbors the richest alpine flora in the world (Wu 1988; Li 1993; Xu et al. 2013). However, plants are not standalone entities but holobionts consisting of the hosts and their associated microbiome (Qian et al. 2019; Xiong et al. 2020). The plant microbiome acts as a secondary genome and is linked to host health and adaptation (Turner et al. 2013; Xiong et al. 2020). Thus, a better understanding of the fundamental community assembly processes of periglacial plant-associated microbiomes could provide a novel insight into periglacial plant diversity maintenance and evolution.

Host plants provide microbes with multiple compartments (e.g., rhizosphere, root endosphere, leaf endosphere, and phyllosphere) for the growth and proliferation of diverse microbes that in turn help to maintain plant growth, productivity, and fitness via nutrient acquisition, hormone production, and protection against biotic and abiotic stressors (Edwards et al. 2015; Muller et al. 2016; Ritpitakphong et al. 2016; Vorholt et al. 2017; Álvarez-Pérez et al. 2017; Hassani et al. 2018; Turan et al. 2019; Trivedi et al. 2020; Marian et al. 2022). Microbial communities in different plant compartments are relatively independent entities, because (1) the plant compartment is a highly heterogeneous environment for microbes, e.g., root and leaf having distinct structures and nutrients (Sun et al. 2021); (2) the densities, diversities, compositions, and life history strategies of microbial communities vary significantly depending on the plant compartment (Muller et al. 2016; Bulgarelli et al. 2013; Tkacz et al. 2020; Trivedi et al. 2020; Zhong et al. 2022), and (3) the influence of environmental factors on the microbial community associated with plants exhibits compartment differentiation (Ren et al. 2015; Sun et al. 2021; Zhong et al. 2022; Shao et al. 2023). This suggests that systematic studies considering the microbial community in various plant compartments along the soil–plant continuum (i.e., the microenvironment involved from soil to plant roots and aboveground portions) would provide comprehensive evidence on fundamental community assembly processes of plant-associated microbiomes. In the studies on microbial communities associated with periglacial plants, however, most attention has been dedicated to the effects of elevation, soil properties, and plant species on the diversity and composition of the microbial community in the rhizosphere (Miniaci et al. 2007; Teixeira et al. 2010; Nissinen et al. 2012; Knelman et al. 2012; Fujimura and Egger 2012; Ciccazzo et al. 2014; Massaccesi et al. 2015; Mapelli et al. 2018; Praeg et al. 2019). We still know little about the fundamental community assembly processes of periglacial plant-associated microbiomes along the soil–plant continuum. More importantly, little work was conducted to assess the effects of the environmental conditions on the microbial community in different compartments of periglacial plants.

Here, we quantified the bacterial communities in the rhizosphere soil, as well as root and leaf endosphere compartments of a periglacial plant, *Potentilla fruticosa* var. *albicans* (*P. fva* hereafter) (Additional file 1: Fig. S1), using high-throughput DNA sequencing. Moreover, we evaluated the impacts of habitat types on the bacterial community in different compartments of *P. fva*. Because microbial communities in different plant compartments are relatively independent entities, and the endophytes are subject to host selective pressure except for soil environment and climate (Xiong et al. 2020; Znój et al. 2021), we hypothesize that (1) the influence of habitat types on the bacterial community associated with *P. fva* would exhibit compartment differentiation, and (2) the endophytic bacteria would be more influenced by habitat types in comparison with rhizospheric bacteria. *P. fva* is a perennial pioneer plant that is widely distributed in the alpine meadow and subnival belt of the Qinghai-Tibet Plateau (Li et al. 2003). As a non-nodulated plant species naturally growing on the newly formed moraines following the retreatment of a glacier on the Qinghai-Tibet Plateau, *P. fva* is a good model plant for dissecting the fundamental community assembly processes of periglacial plant-associated microbiomes.

Materials and methods

Study site and sampling

The study was conducted in the forefield of the Qiangyong glacier, which originates from Mt. Kaluxung (28.8°N, 90.3°E, 6674 m above sea level) (Additional file 1: Fig. S2). The mean air temperature and annual mean precipitation are 2.4 °C and 370 mm, respectively. From 1976 to 2006, the Qiangyong glacier has retreated at an average rate of 4 m year^{-1} (Yao et al. 2012). Downstream of the Qiangyong glacier, three sets of latero-frontal moraines can be seen (Additional file 1: Fig. S2b) (Sun et al. 2020). In August 2020, we sampled soil and *P. fva* at the Glacier Terminus Zone (GTZ), Moraine Ridge (MR), and Alpine Meadow (AM) (Additional file 1: Fig. S2b–f). The GTZ is located between the glacier terminus and the west and east glacier runoff confluence,

and its forming time corresponds to the Ice Age Little (0.13 ± 0.02 to 0.36 ± 0.09 ka BP) (Additional file 1: Fig. S2b–d) (Sun et al. 2020). The GTZ with low plant diversity and coverage mainly consists of a shrub of *P. fva* and herbs of *Cavea tanguensis* and *Silene nigrescens*. The MR is the moraine complex that surrounds Daqiangyong Lake, and its forming time corresponds to the Neoglacial (2.09 ± 0.14 to 3.48 ± 0.32 ka BP) (Additional file 1: Fig. S2b–e) (Sun et al. 2020). The MR primarily consists of *P. fva*, *Eriophyton wallichii*, *Rheum spiciforme*, *Lophanthus tibeticus* and *Thalictrum squamiferum*. The AM is located between the Daqiangyong Lake outlet and the outermost moraine complex of Qiangyong valley, and its forming time corresponds to the early Holocene (9.17 ± 0.22 to 10.63 ± 0.26 ka BP) (Additional file 1: Fig. S2b–f) (Sun et al. 2020). The AM is dominated by *P. fva*, *Caragana chumbica*, *Hippophae rhamnoides*, *Astragalus densiflorus*, *Artemisia mattfeldii*, *Swertia hispidicalyx*, *Anaphalis contorta* and *Nepeta coerulescens*. Firstly, a quadrat frame (1 × 1 m) was used to estimate plant density in each habitat, and plant height in the quadrat frame was measured by a ruler. Secondly, in each habitat, we collected seven healthy individuals of *P. fva* (> 20 m apart from each other) that were in anthesis and were about 20 cm tall using sterilized spades. Finally, mature and asymptomatic leaves and intact roots with aggregated soil were collected from each plant. Further, the bulk soil not in contact with the root system and located at least 50 cm from each sampled plant was collected after removing the topsoil. All collected samples were placed in sterilized plastic bags that were frozen (-20 °C) immediately until arrival at the laboratory. To eliminate cross-contamination, the sample collection tools were washed with distilled water and wiped with 70% (*v/v*) ethanol during sampling.

DNA extraction, amplification and Illumina sequencing

Frozen leaves were submerged in sterile cooled TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and then subjected to alternating sonication (45 s) and vortexing (1 min) three times (Yao et al. 2019). The treated leaves were transferred to new 50-mL sterile centrifuge tubes and surface-sterilized by consecutive immersion for 1 min in ethanol (80%, *v/v*), 5 min in sodium hypochlorite (3.25%, *v/v*), and 30 s in ethanol (80% *v/v*), followed by three rinses in sterile distilled water (Yao et al. 2019). To confirm that the disinfection protocol was successful, the final rinse water was plated in Tryptic Soy Agar plates that were incubated in the dark at 28 °C for 7 days. Then, plates were examined to confirm the absence of microbial growth. The soil strongly adhering to the roots and within the space explored by the roots was considered as the rhizosphere soil (Dinesh et al. 2010). The rhizosphere soil was obtained by gently shaking off the loosely bound soil, and the rhizosphere soil adhering to the root system was collected using a brush (Feng et al. 2005). Roots (5.0 g) were placed into 100 mL sterile wild-mouth bottles and washed with PBS buffer (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, pH 7.4) on a shaking table (150 r min^{-1}) for 1 h at room temperature (Qian et al. 2019). The root samples were then surface-sterilized and verified as described above for leaf samples. In total, we collected 63 plant samples (21 individual plants × 3 compartments) for DNA extraction, and 21 bulk soil samples for measuring physicochemical properties, all of which were stored at -80 °C until processing.

The leaves and roots were freeze-dried using liquid nitrogen and homogenized using a sterilized mortar and pestle under aseptic conditions. We extracted total DNA from 500 mg of each rhizosphere soil sample, and 400 mg powder of each root and leaf sample using the DNeasy^R PowerSoil^R Kit (MoBio Laboratories, Carlsbad, CA, United States), following the manufacturer's instructions. The DNA quality and concentration were determined by NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States). The V5–V7 hypervariable region of the bacterial 16S rRNA genes was amplified for high-throughput Illumina MiSeq sequencing using a two-step PCR procedure (Wang et al. 2018). A more detailed description of bacterial 16S rRNA gene amplification and Illumina sequencing is available in Additional file 1.

Bioinformatic analysis

Raw sequences were quality trimmed using FASTP (version 0.20.0) (Chen et al. 2018), and the overlapping paired-end reads were merged into a single sequence using FLASH (version 1.2.7) (Magoč and Salzberg 2011). The sequences were clustered into operational taxonomic units (OTUs) using the UPARSE algorithm in USEARCH (version 11.0.667) (Edgar 2013) with a 97% threshold of sequence similarity. Representative sequences of each OTU were aligned using PyNAST (DeSantis et al. 2006). The taxonomic identity of each phylotype was determined using the SILVA 132 database (Quast et al. 2012) via the RDP classifier (Wang et al. 2007). A detailed description of the bioinformatic analysis is available in Additional file 1.

Measurements of soil physicochemical properties

Soil potential of hydrogen (pH) and electrical conductivity (EC) were determined in a 1:2.5 air-dried soil:deionized water suspension using PHS-3C pH meter and DDS-307A conductivity meter (Shanghai Lida Instrument Factory, Shanghai, China), respectively, after one night of equilibration (Cui et al. 2019; Zhang et al.

2020). Fresh soils were dried at 105 °C for 48 h to determine water content (WC) (Cui et al. 2019). For the measurement of total nitrogen (TN) content, the air-dried soil was digested with the reaction mixture [100:10:1 K_2SO_4:$CuSO_4$:Se (1.85 g), H_2SO_4 (5 mL)], followed by titration using 0.02 M HCl (Cui et al. 2019). Soil nitrogen nitrate (NO_3^--N) and ammonium nitrogen (NH_4^+-N) contents were measured according to the method of Cui et al. (2019) by using a Seal Auto Analyzer after extraction with 2 M KCl. Soil total carbon (TC) content was determined by using TOC-Vcph (Shimadzu Corp., Japan) following the standard methods (Yan et al. 2022).

Data analysis

All the statistical analyses were implemented in R version 4.0.3. More detailed descriptions of data analysis are available in Additional file 1.

Results

Variations in soil properties across habitat types

The pH of the bulk soils presented a significant difference, but the content of NH_4^+-N maintained a similar level across the three habitat types (Table 1). The values of pH and EC of the bulk soils in AM were significantly decreased by 0.04-fold and 0.31-fold, respectively, compared with the bulk soils in GTZ. The WC, NO_3^--N, TC, and TN contents of bulk soils in AM were significantly increased by 1.73-, 4.67-, 1.46-, and 6.29-fold, respectively, compared with the bulk soils in GTZ. There were no significant differences in the WC, NO_3^--N, TC, and TN contents of bulk soils between the GTZ and AM.

Effects of habitat types on the bacterial community diversities

A total of 409,888, 465,871 and 484,910 high quality sequences were obtained from the samples of rhizosphere soil, root endosphere and leaf endosphere, respectively, after quality filtering and the removal of sequences belonging to non-bacteria and singletons. Sequence number was, respectively, normalized to 17,429 (rhizosphere soil), 20,520 (root endosphere) and 21,397 (leaf endosphere), resulting in three normalized datasets, respectively, comprising 39,692 (rhizosphere soil), 17,629 (root endosphere) and 10,524 (leaf endosphere) bacterial OTUs at a 97% sequence similarity level. For the community in the rhizosphere soil, rarefaction curves for the observed OTUs showed no signs of reaching asymptotes (Additional file 1: Fig. S3). In comparison, the rarefaction curves for the observed OTUs in the root and leaf endosphere almost reached a saturation plateau (Additional file 1: Fig. S3). Good's coverage scores of the three compartments ranged from 0.789 ± 0.017 to 0.973 ± 0.010 (Additional file 1: Table S2). In summary, although further sampling would recover additional OTUs in the rhizosphere soil, we sampled most of the diversity in *P. fva* microbiome, and the sequencing depths were sufficient to reliably describe the bacterial microbiome.

One-way ANOVA showed that habitat types did not cause a significant effect on Shannon diversity ($F=0.27$, $P=0.76$) and Chao1 richness ($F=0.67$, $P=0.52$) in the rhizosphere soil. The Chao1 richness in the rhizosphere soil of *P. fva* in AM (11,504 ± 1398 (mean ± SD) and MR (11,688 ± 945) was slightly higher than that in the rhizosphere soil of *P. fva* in GTZ (11,031 ± 853) (Fig. 1a, d). One-way ANOVA also showed that habitat types had a significant effect on the Chao1 richness in the root endosphere ($F=4.49$, $P=0.03$) and leaf endosphere ($F=5.70$, $P=0.01$). The Shannon diversity and Chao1 richness in the root endosphere decreased with the transformation of habitats from GTZ to AM, while the bacteria in the leaf endosphere exhibited an opposite trend (Fig. 1b, c, e, f). In comparison with *P. fva* in GTZ, the Chao1 richness in the root endosphere of *P. fva* in AM significantly decreased by 0.23-fold. The Chao1 richness in the leaf endosphere of *P. fva* in AM significantly increased by 0.72-fold, compared with *P. fva* in GTZ.

Effects of habitat types on the bacterial community compositions

The heatmap revealed that the occurrence of some relatively abundant bacterial OTUs in the rhizosphere soil, root endosphere, and leaf endosphere varied among habitat types (Additional file 1: Fig. S4a, b, c). One-way ANOVA showed that differences in the relative

Table 1 Physicochemical properties of bulk soil collected from three habitat types in the Qiangyong glacier foreland

Habitat	pH	EC ($\mu S\ cm^{-1}$)	WC (%)	NH_4^+-N ($mg\ g^{-1}$)	NO_3^--N ($mg\ g^{-1}$)	TC ($mg\ g^{-1}$)	TN ($mg\ g^{-1}$)
GTZ	8.12 ± 0.05b	135.19 ± 20.38a	9.65 ± 3.56b	0.001 ± 0.0003a	0.003 ± 0.0006b	16.74 ± 2.61b	0.60 ± 0.10b
MR	8.24 ± 0.07a	89.13 ± 14.77b	7.09 ± 4.02b	0.001 ± 0.0003a	0.006 ± 0.006b	23.59 ± 5.09b	1.33 ± 0.61b
AM	7.79 ± 0.10c	94.34 ± 22.83b	26.30 ± 5.47a	0.002 ± 0.0007a	0.017 ± 0.009a	41.13 ± 10.24a	4.34 ± 1.42a

pH represents potential hydrogen, EC represents electrical conductivity, WC represents water content, NH_4^+-N represents ammonium content, NO_3^--N represents nitrate content, TN represents total nitrogen content, TC represents total carbon content. GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively. Each value represents mean ± SD of seven individual replications, and different lowercase letters in each column indicate significant differences (Tukey's HSD test: $P<0.05$)

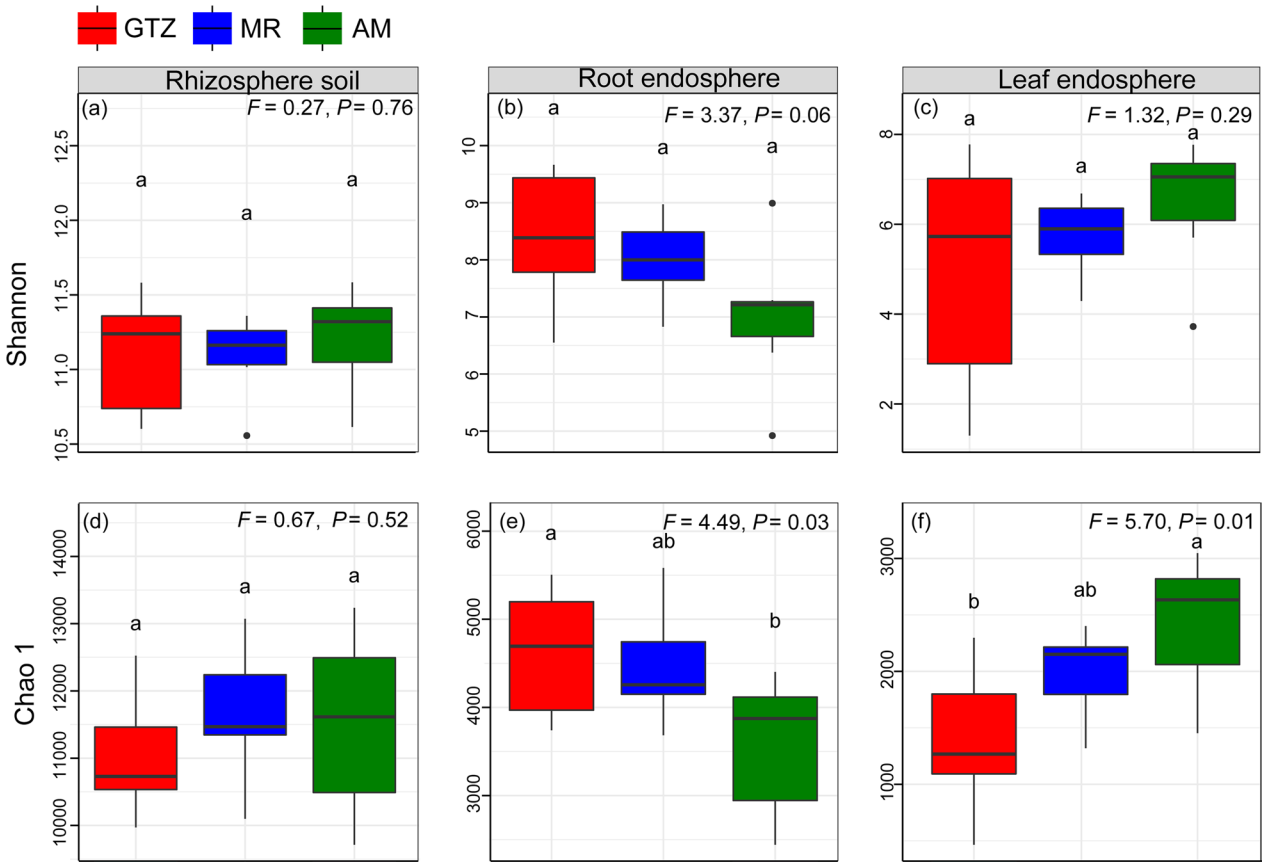

Fig. 1 The differences in Shannon diversity (**a**–**c**) and Chao1 richness (**d**–**f**) among the three habitat types. GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively. Different lowercase letters indicate significant differences in Shannon diversity and Chao1 richness among the three habitats (Tukey's HSD test: $P < 0.05$)

abundance of the most bacterial order in the rhizosphere soil and root endosphere were significantly affected by habitat types ($P < 0.05$) (Additional file 1: Fig. S5a, b). In comparison, only the relative abundance of two orders (Burkholderiales and Actinomycetales) in the leaf endosphere was significantly affected by habitat types ($P < 0.05$) (Additional file 1: Fig. S5c). The taxonomic classification illustrated that the majority of OTUs in the rhizosphere soil belonged to the members of order Actinomycetales, Rhizobiales, and Solirubrobacterales (Fig. 2a). The most abundant OTUs in the root endosphere belonged to the members of order Pseudomonadales, Rhizobiales, and Solirubrobacterales (Fig. 2b). The dominant taxa in the leaf endosphere were Burkholderiales, Sphingomonadales and Pseudomonadales (Fig. 2c). Furthermore, Acidimicrobiales, Gaiellales, and Rhodospirillales exclusively existed in the rhizosphere soil, Xanthomonadales exclusively existed in the root endosphere, Enterobacteriales and Lactobacillales exclusively existed in the leaf endosphere (Fig. 2a–c).

The Venn diagram analysis indicated that the unique OTUs in the rhizosphere soil of *P. fva* in GTZ, MR and AM were fewer than the shared OTUs of the three habitats (Fig. 3d). The unique bacterial OTUs in the root endosphere of *P. fva* in GTZ and MR, and the unique bacterial OTUs in the leaf endosphere of *P. fva* in MR and AM were larger than the shared OTUs of the three habitats (Fig. 3e, f). The principal coordinates analysis (PCoA) further revealed that the bacterial community composition of rhizosphere soil, root endosphere, and leaf endosphere was significantly different across the three habitats (Fig. 3a–c, Additional file 1: Table S3). Notably, the variation explained by habitat types was slightly higher for the endophytic bacterial community than for the bacterial community in the rhizosphere soil (Fig. 3a–c).

Effects of habitat types on the potential sources, enrichment processes, and assembly processes of the bacterial communities

Rhizosphere soil, root endosphere, and leaf endosphere samples shared 4,774 OTUs, which accounted for 9.82%

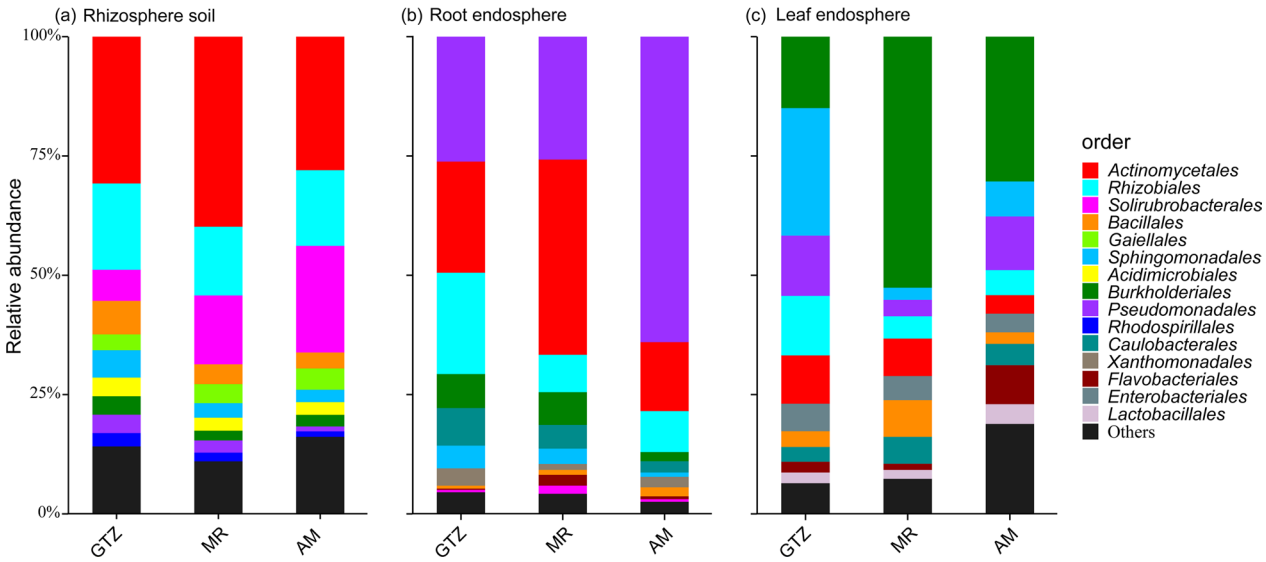

Fig. 2 Taxonomic composition of the bacterial communities at the order level. Except for 10 representative OTUs, the remaining parts were assigned to "Others". GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively

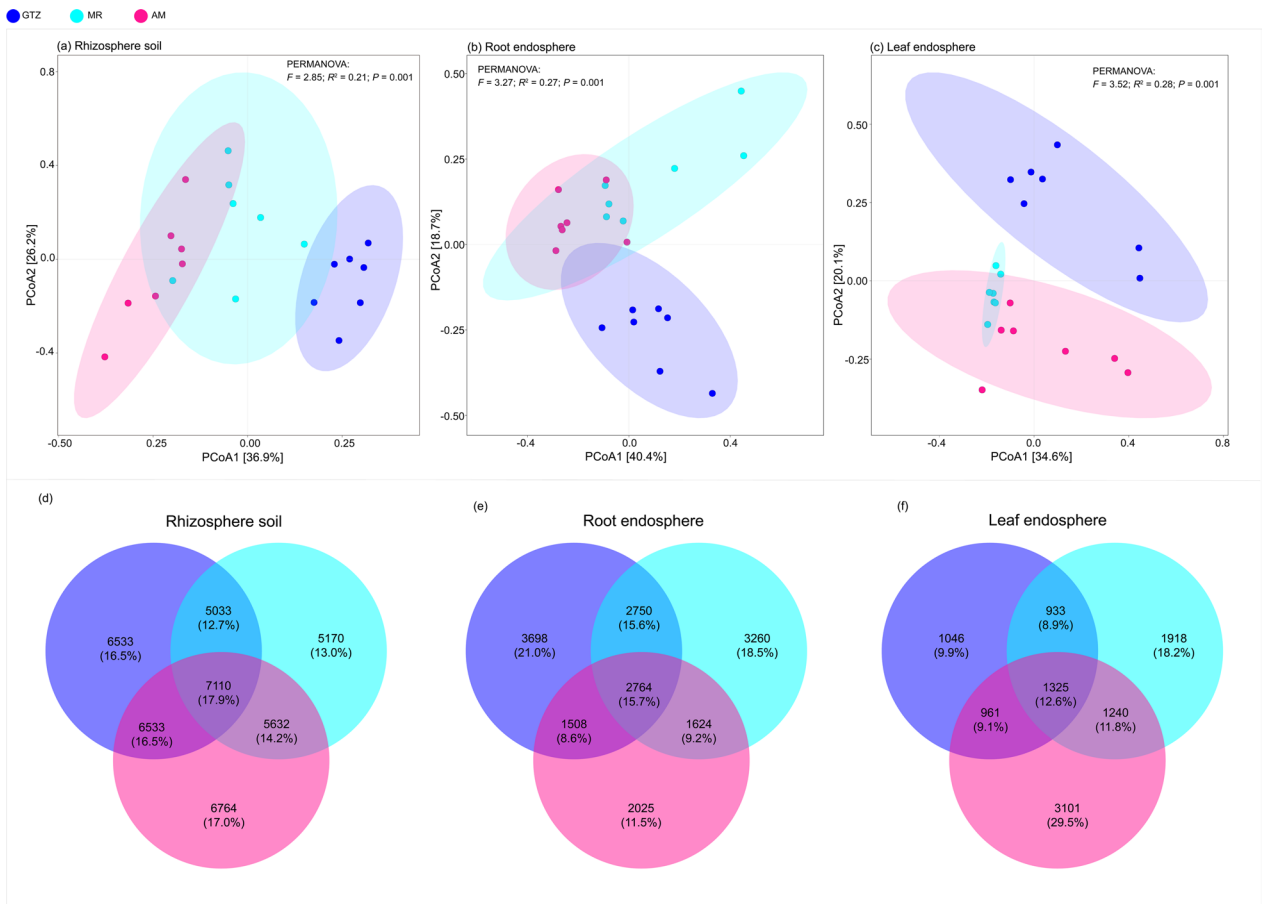

Fig. 3 PCoA ordination plots of bacteria based on the Bray–Curtis distances of the classified 16S rRNA gene sequences (**a**–**c**). Venn diagram showing the numbers of unique and shared OTUs among the three habitats (**d**–**f**), and the percentage of these OTUs accounting for the total number of OTUs shown in parenthesis. GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively

of the total OTUs (Fig. 4a), indicating a potential interconnection of bacteria from the three compartments. The results of source tracking analysis showed that the bacterial community in the root endosphere of *P. fva* in GTZ and MR, respectively, had 41.0% and 39.6% of the community sourced from rhizosphere soil and 25.5% and 7.2% from the respective leaf endosphere (Fig. 4b). Notably, for *P. fva* in AM, the bacterial community in the root endosphere had 39.8% of the community sourced from leaf endosphere, which was obviously higher than that contribution of rhizosphere soil (10.5%) (Fig. 4b). A total of 30.2% of the bacteria in the leaf endosphere of *P. fva* in GTZ originated from the root endosphere (18.4%) and rhizosphere soil (11.8%) (Fig. 4c). The bacterial community in the leaf endosphere of *P. fva* in MR and AM, respectively, had 10.4% and 12.7% of the community sourced from the root endosphere and 3.5% and 1.9% from the respective rhizosphere soil (Fig. 4c).

The values of depletion index (DI), which represent host depleted effect, increased from GTZ (0.51–2.14) to AM (1.94–4.69), suggesting that bacterial community that harbors the root or leaf endosphere of *P. fva* in AM was subjected to higher host selective pressure than those in GTZ (Fig. 5A). Notably, we found that Oxalobacteraceae and Pseudomonadaceae were significantly enriched and overlapped in two plant compartments (endosphere of leaf and root) (Fig. 5B). By contrast, the taxa belonging to Hyphomicrobiaceae, Geodermatophilaceae, and Micrococcaceae were significantly depleted in the endosphere of leaf and root (Fig. 5B).

There was no significant difference in the niche breadth of bacterial communities in rhizosphere soil, root endosphere and leaf endosphere among the GTZ, MR and AM (Fig. 6a). For *P. fva* in GTZ, MR and AM, the bacterial communities in the leaf and root endospheres exhibited significantly lower niche breadths than those in the rhizosphere soil (Fig. 6a). Furthermore, we found that drift and dispersal limitation was the most important process structuring of the bacterial communities in rhizosphere soil, root and leaf endosphere of *P. fva* in GTZ, MR, and AM (Fig. 6b), suggesting that stochastic processes explained a higher proportion of *P. fva*-associated bacterial community variations than deterministic processes.

Effects of habitat types on the bacterial co-occurrence patterns

Construction of correlation-based networks of the bacterial communities resulted in three networks, consisting of 150, 163, and 88 nodes connected by 1220, 1041, and 306 edges, respectively (Table 2). The average path length, average clustering coefficient and modularity of the empirical networks were all significantly higher than those of Erdös–Rényi random networks (Table 2), suggesting the observed networks were non-random and modular structures. Furthermoer, the co-occurrence network of *P. fva*-associated bacteria in AM presented higher modularity but lower complexity (a higher average degree representing a greater network complexity) than those in GTZ and MR (Table 2,

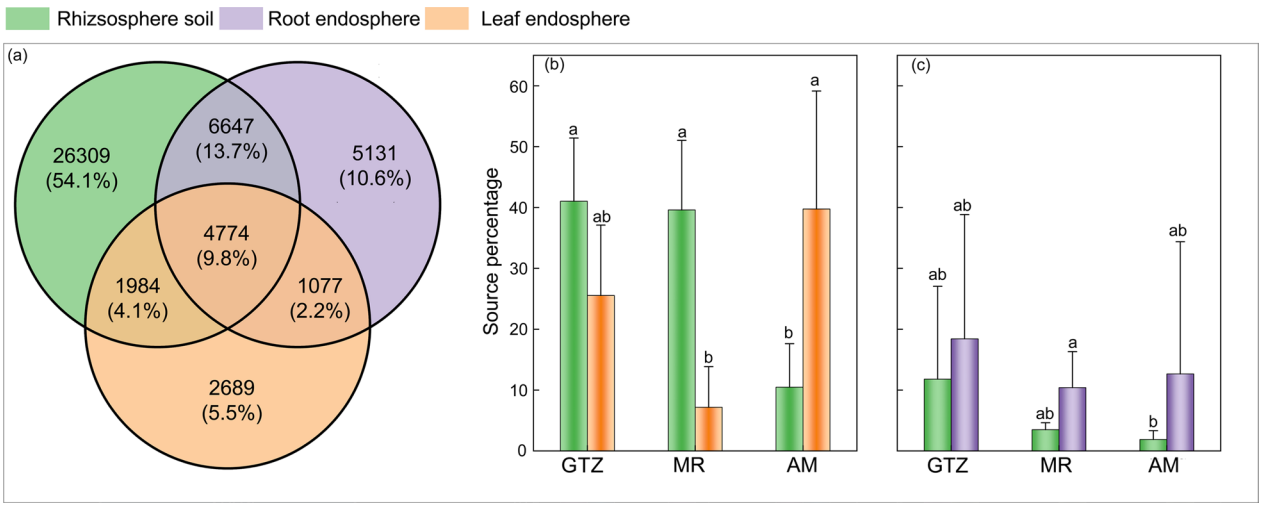

Fig. 4 Venn diagram showing the numbers of unique and shared OTUs between compartment niches, and the percentage of these OTUs accounting for the total number of OTUs shown in parenthesis (**a**). The potential sources of bacterial communities based on OTUs level [**b** rhizosphere soil and leaf endosphere as the potential source of root endosphere; **c** rhizosphere soil and root endosphere as the potential source of leaf endosphere]. GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively. Bars without shared letters indicate significant differences (Kruskal–Wallis test: $P < 0.05$)

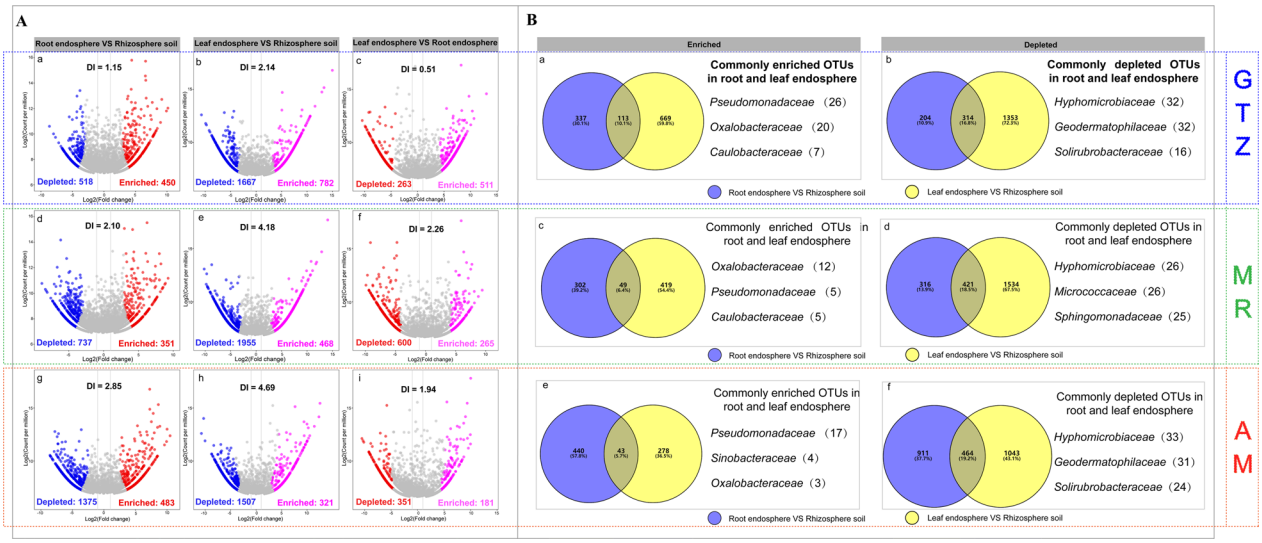

Fig. 5 **A** The enrichment and depletion patterns of the root endophytes and leaf endophytes of *Potentilla fruticosa* var. *albicans* in GTZ (**a**, **b**), MR (**d**, **e**), and AM (**g**, **h**) when using rhizosphere soil as control. Each red and pink point represents an individual enriched OTU, while each blue point represents an individual depleted OTU (a, b, d, e, g, h). The enrichment and depletion patterns of the leaf endophytes of *Potentilla fruticosa* var. *albicans* in GTZ (**c**), MR (**f**), and AM (**i**) when using root endophytes as control. Each pink point represents an individual enriched OTU, while each red point represents an individual depleted OTU. The "Depletion index" (DI = The numbers of depleted OTUs / The numbers of enriched OTUs) defined by Xiong et al. (2020) was used to evaluate the selective effect degree of the plants to their associated bacteria, and higher DI value represents greater depletion effect. **B** Venn diagrams showing the shared and specific bacterial OTUs in different compartment niches within the significantly enriched OTUs and depleted OTUs (**a**–**e**). For these shared differential OTUs, only the top 3 taxonomies were shown. GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively

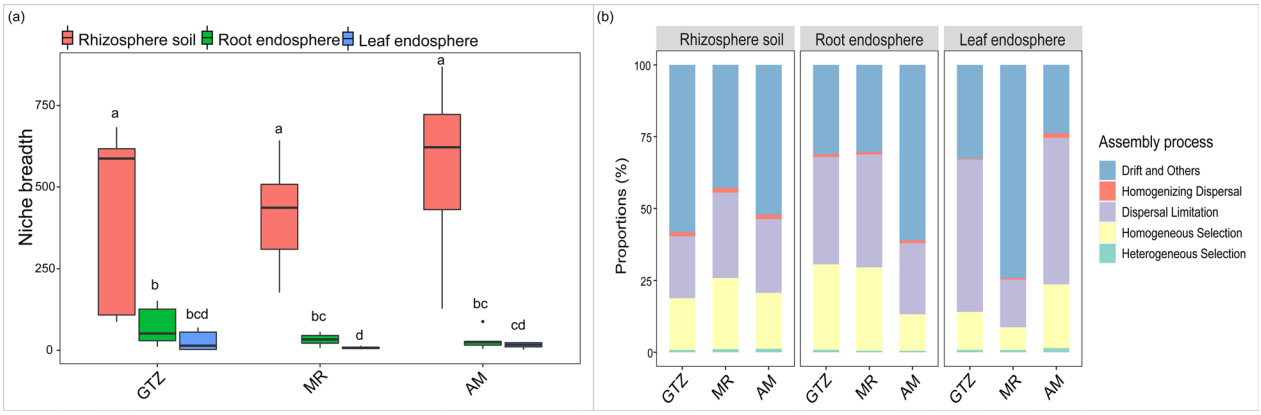

Fig. 6 Niche breadth of the bacterial taxa (**a**). Delineation of the assembly processes underlying the bacterial community (**b**). GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively. Bars without shared letters indicate significant differences (Kruskal–Wallis test: $P < 0.05$)

Fig. 7B). The node degree and betweenness were significantly larger for GTZ networks than for AM networks (Fig. 6B). The taxonomic composition of *P. fva*-associated bacteria co-occurrence networks was similar among the three habitats, with more nodes belonging to Proteobacteria and Actinobacteria (Fig. 7A).

Discussion

Differences in soil properties across habitat types

In this study, we compared the difference in physicochemical properties of soil among the three habitat types. The results showed that soil pH and EC in the AM significantly decreased, while the content of soil WC, TC,

Table 2 Key topological properties of bacterial communities in the rhizosphere soil, root endosphere, and leaf endosphere

Network properties	GTZ	MR	AM
Empirical networks			
Edges	1220	1041	306
zNodes	150	163	88
Average clustering coefficient	0.55	0.61	0.54
Average path length	2.15	3.27	2.91
Modularity	0.38	0.41	0.54
Graph density	0.11	0.08	0.08
Diameter	6.86	12.57	8.99
Average degree	16.27	12.77	6.95
Random networks			
Average path length	2.04 ± 0.004	2.26 ± 0.004	2.50 ± 0.014
Average clustering coefficient	0.11 ± 0.004	0.08 ± 0.004	0.08 ± 0.009
Modularity	0.19 ± 0.006	0.23 ± 0.006	0.31 ± 0.011

GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively

TN and NO_3^--N in the AM significantly increased in comparison with the soil in the GTZ (Table 1). This suggests that the soil in the AM exhibits a higher level of acidification and more efficient cycling of organic matter, compared with the soil in the GTZ. Previous studies showed that plant litter and aboveground biomass play a significant role in influencing the exudation of organic acids by roots, as well as the accumulation of soil carbon (C) and nitrogen (N) pools (Lauber et al. 2009; Jiang et al. 2018). Additionally, the roots produce substantial amounts of labile hydrophilic organic molecules, which help to enhance soil water content by promoting the formation of stable aggregates (Fageria and Stone 2006; Cocco et al. 2013). We also found that plant density and plant height were significantly higher in the AM than in the GTZ (Additional file 1: Fig. S6). Therefore, the significant difference in soil properties between GTZ and AM may be because the AM hosted richer plant diversity and had higher plant coverage than the GTZ.

The diversity and composition of endophytic bacteria are more affected by habitat types than that of rhizospheric bacteria

The rhizosphere is widely recognized as a crucial hotspot for microbial colonization and activity within the soil environment (Lynch et al. 2001; Qu et al. 2020). The microbial community associated with the rhizosphere is susceptible to numerous abiotic factors, such as soil properties (Debnath et al. 2016) and root exudates (Paterson et al. 2007). Endophytes, which are chosen by plants through nutrients within tissues and host immunity responses (Yao et al. 2019, 2020), live inside the root, stem, leaf, or flower tissues for at least part of their lifespan without inducing apparently deleterious effects on the host (Yao et al. 2019, 2020). Endophytes are less affected by exterior disturbance and more dominated by host-genetics (Trivedi et al. 2020; Wagner et al. 2020). Previous studies on sorghum-associated microbiomes also showed that environmental disturbance had more pronounced effects on rhizosphere soil microbiomes than on endophytic microbiomes (Sun et al. 2021). Interestingly and notably, this study showed that the habitat type had a greater effect on the diversity and composition of endophytic bacteria than on that of rhizospheric bacteria (Figs. 1, 3), indicating that bacterial communities in rhizosphere soil are more resistant than bacterial communities in root endosphere and leaf endosphere to environmental change. Such inconsistency may be ascribed to the difference in host plant species, which played a crucial role in shaping the microbial community in terms of a specific compartment (Xiong et al. 2020; Laforest-Lapointe et al. 2016; Walters et al. 2018). For example, Yao et al. (2020) revealed that plant species had a greater effect on the epiphytic than on the endophytic bacteria, and Massaccesi et al. (2015) also showed that the ability to colonize harsh environments of *Silene acaulis* is linked to the shape and functions of its canopy rather than through the rhizosphere effects to recruit and foster growth-promoting microbial communities, while *Helianthemum nummularium* do the opposite. Furthermore, with the exception of soil environment and climate, the host selection effect was found to play a significant role in shaping the microbial community in the plant endosphere (Xiong et al. 2020). Thus, endophytic bacteria associated with *P. fva* are more influenced by habitat types which may be closely related to the host selection effect.

There are clear separations of bacterial community composition among the three habitat types in this study (Fig. 3). Such differences between environments of the habitat in bacterial communities were also found in *Thylacospermum caespitosum* (Wang et al. 2020) and *Oryza sativa* (Xu et al. 2020), suggesting that differences in environments between the habitats are the primary drivers of selection. Meanwhile, we detected that the bacterial communities in the rhizosphere soil were dominated by Actinomycetales (Actinobacteria), Rhizobiales and Solirubrobacterales (Proteobacteria), most abundant OTUs in the root endosphere belonged to Pseudomonadales (Proteobacteria), Actinomycetales and Rhizobiales, and bacterial communities in the leaf endosphere were dominated by Burkholderiales (Proteobacteria), Sphingomonadales (Proteobacteria), Pseudomonadales and Actinomycetales (Fig. 2). In comparison, several studies have documented that Firmicutes was the most abundant

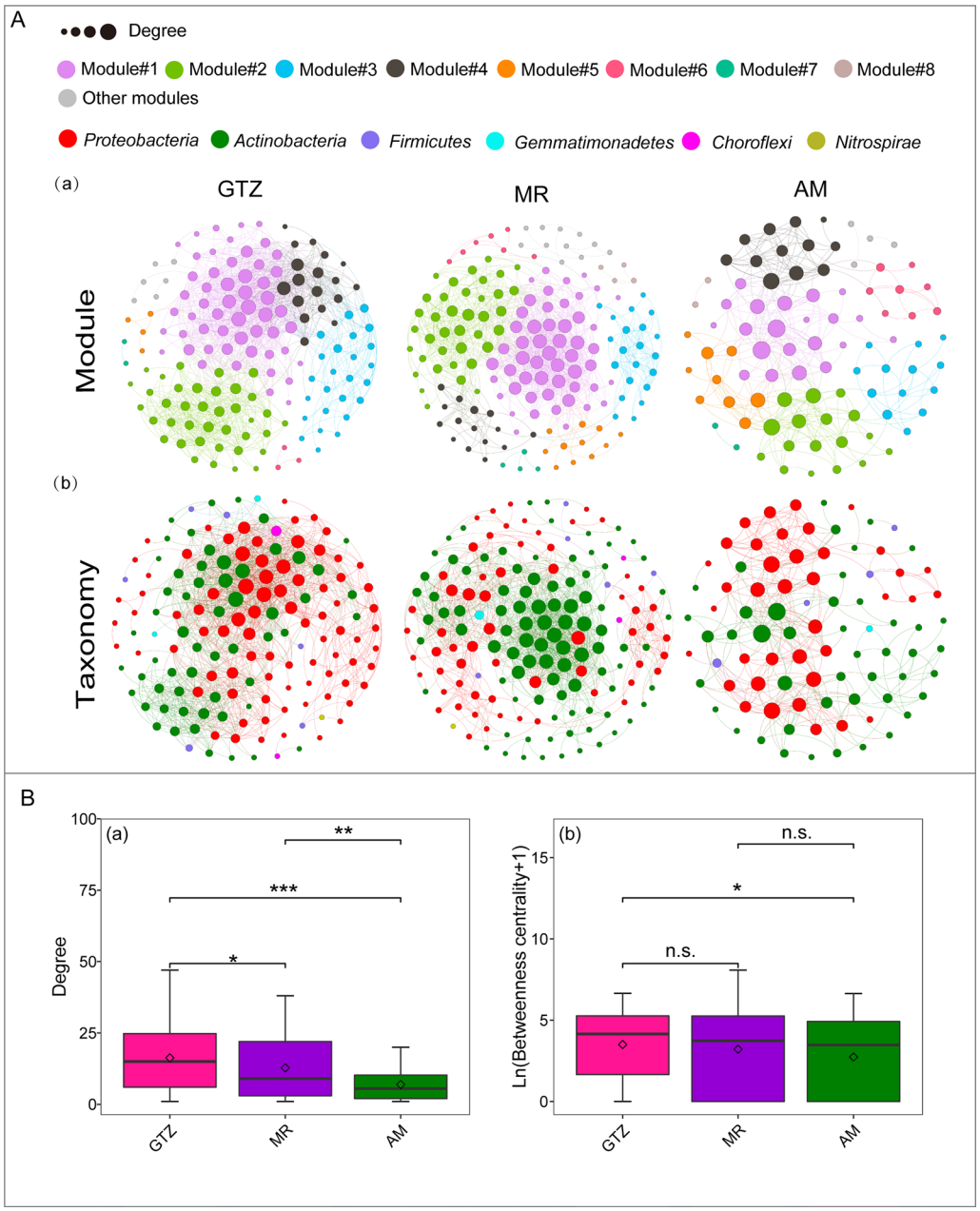

Fig. 7 **A** Correlation-based networks of the bacterial communities. Bacterial OTUs are represented as nodes and significant correlations as edges. The size of each node is proportional to the degree of the OTU and the node color indicates the corresponding module (**a**) or taxonomic assignment at the phylum level (**b**). **B** The Wilcox test was used to compare the node-level degree and betweenness centrality among the three habitats (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. not significant). GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively

phylum in rhizosphere soil from Antarctic vascular plants (Teixeira et al. 2010), Burkholderiales (Proteobacteria) and Rhizobiales (Proteobacteria) were highly abundant in the root endosphere of Arctic pioneer plants (Kumar et al. 2017; Given et al. 2020), and Firmicutes and Proteobacteria (Pseudomonadales and Enterobacteriales) were dominant taxa in leaf endosphere of the pioneer plants (Given et al. 2020). Such inconsistency could be closely related to the differences in host identities and environmental conditions. Our results also indicate that the endophytic communities are unique groups, not opportunistic subdivisions of the rhizosphere (Gottel et al. 2011), which was supported by the results that each compartment of *P. fva* had its own preferred and specific taxa

(Additional file 1: Fig. S4). It is worth noting that the top 10 orders in rhizosphere soil, root endosphere and leaf endosphere had 6, 5, and 2 taxa, respectively, which were significantly influenced by habitat types (Additional file 1: Fig. S5). This result highlights that the rhizosphere is an interface of plants and soils, which are more susceptible to environmental change (York et al. 2016). However, this was contrary to the results of the diversity and structure of the bacterial community in this study. Although the mechanisms of this phenomenon are unclear, one possible explanation may be that the rare taxa, which have a more restricted distribution in comparison with the abundance taxa, play an essential role in community variations (Zhang et al. 2018). Furthermore, we found that some members, including Oxalobacteraceae and Pseudomonadaceae, were significantly enriched and overlapped in root and leaf endosphere, whereas some members belonging to family Hyphomicrobiaceae, Micrococcaceae and Solirubrobacteraceae were significantly depleted in these compartments (Fig. 5). This finding suggested that Oxalobacteraceae and Pseudomonadaceae had capability of colonizing a wide range of compartments of *P. fva*. Oxalobacteraceae have been reported as a major endophyte group in *Oxyria digyna* (Kumar et al. 2017; Given et al. 2020) and *Pinus flexilis* (Carper et al. 2018), and the ability of Oxalobacteraceae to utilize oxalic acid, nitrogen fixation and solubilize inorganic and organic phosphate would make these taxa well adapted to various plant compartments (Given et al. 2020). *Pseudomonas* has been considered the most abundant bacteria inhabiting the roots of numerous plants and plays an important role in promoting the fitness of host plants by stimulating plant growth and/or suppressing pathogens (Zamioudis et al. 2013; Sitaraman 2015). Solirubrobacterales (Thermoleophilia) can produce varieties of extracellular hydrolytic enzymes that can degrade plant polymers such as lignin, cellulose, and other organic compounds (Eisenlord and Zak 2010), and the high abundance of this taxonomic group is closely related to the permanent presence of organic residues from plants (de Araujo et al. 2017). As a result, we speculate that the capacity of bacteria to colonize specific plant compartments is strongly tied to their functional traits.

The potential sources, enrichment processes, assembly patterns, and co-occurrence patterns of bacterial communities vary by habitat types

Soil has been widely considered the seed bank for plant microbiotas (Vorholt 2012; Gopal and Gupta 2016), and the selection pressure produced by the host plays an essential role in structuring microbial communities associated with itself (Zhou et al. 2021). In this study, our results showed that the contribution of rhizosphere soil to root and leaf endophytes decreased with the transformation of habitats from GTZ to AM when using rhizosphere soil as a control (Fig. 4). On the one hand, this indicates the potential sources of *P. fva* in the AM were more complex than those in GTZ and MR, but on the other hand, this also indicates that *P. fva* in a suitable environment exhibited higher selection pressure to endophytic bacteria, which was supported by the results that we observed maximum host depletion index in the AM. Notably, the contribution of the bacterial community in rhizosphere soil to the root endosphere in this study was smaller than that in the studies on crops (wheat, barley, and maize) (Xiong et al. 2020), which demonstrated that the contribution of the bacterial community in rhizosphere soil to root endosphere exceeded 75%. Such inconsistency should be a combined result of both the growth environment and plant identity, although the mechanism needs further exploration. Furthermore, the neighbor compartments accounted for a smaller proportion of the derivation in leaf endophytes in this study (Fig. 4), which verified the viewpoint that the potential sources of phyllosphere microbiome were more complex (Lopez-Velasco et al. 2013; Vacher et al. 2016).

The niche breadth of the taxa in rhizosphere soil, root endosphere and leaf endosphere maintained a similar level among the three habitats of *P. fva* in this study (Fig. 6), indicating that the same kind of process governed the community assembly. The deterministic and stochastic processes are known to play an essential role in assembling microbial community structures (Zhou and Ning 2017; Jiao et al. 2020). In general, the microbial communities successfully colonized in each compartment of plants are combined results of both the environmental filter and host selection. Thus, the deterministic processes should be a dominant assembly process in structuring plant microbiomes. Intriguingly, we found that the stochastic assembly processes (drift and dispersal limitation) were dominant in bacterial communities associated with *P. fva* (Fig. 6), consistent with the findings of Liu et al. (2021) who observed that stochastic processes dominated the assembly of communities among the different compartments of the bean. However, Zhong et al. (2022) found that the bacterial community in the rhizosphere soil of *Stipa* and *Reaumuria* were governed by deterministic processes, while the root endosphere was dominated by stochastic assembly processes. In short, these observations suggest that the community assembly process of plant-associated microbiomes was closely related to plant identity and exhibited compartment differentiations.

Beyond the diversity and composition, studies on the co-occurrence network can offer a novel insight into the interactions between microbial communities associated

with plants and corresponding ecological assembly rules (Xue et al. 2018). A network with a higher modularity value can maintain network stability by mitigating the influence of a lost taxon on the remaining components of the network (Herren and McMahon 2017; Zhong et al. 2022). Additionally, co-occurrence networks with lower complexity and connectedness can enhance the resilience to environmental change by reducing resource competition among species (Yan et al. 2021; Zhong et al. 2022). Previous studies have shown that the properties of co-occurrence networks for microbial taxa in the soil-root continuum differ among habitat types, and the host selection effect significantly influences the construction of microbial community co-occurrence networks associated with plants (Xiong et al. 2020; Zhong et al. 2022). Consequently, the topological characteristics of microbial community co-occurrence networks associated with plants should be a combined result of both the environmental disturbance and host selection. Notably, a harsh environment can destabilize microbial community co-occurrence networks (Hernandez et al. 2021). However, this study found that the network of bacterial communities associated with *P. fva* in the AM had lower complexity and connectedness than that in the GTZ (Fig. 7). Although the mechanism of this phenomenon is unclear, this result indicates that bacterial communities associated with *P. fva* in the AM are more resistant to environmental perturbation. Furthermore, the co-occurrence network in GTZ that exhibited a lowly modular architecture could be due to a lack of keystone taxa, which usually play a crucial role in maintaining network structure relative to the other taxa in the network structure (Qian et al. 2019).

Conclusion

Our results demonstrated that the effects of habitats on the diversity and composition of the bacteria community associated with *P. fva* exhibited a compartment differentiation. Habitat type had greater effects on the diversity and community composition of endophytic bacteria than that of rhizospheric bacteria. Moreover, we found that the potential source, enrichment process, assembly pattern, and co-occurrence pattern of the bacterial community associated with *P. fva* varied by habitat types. In particular, host species had the strongest selection effect on endophytic bacterial community associated with *P. fva* in the AM, and the bacterial community associated with *P. fva* in the AM had a lower complex but more modular network than the bacterial community associated with *P. fva* in the GTZ. These findings significantly advance our current understanding of the bacterial community assembly process associated with periglacial plants. Furthermore, we revealed that Oxalobacteraceae and Pseudomonadaceae were significantly enriched and overlapped in two plant compartments (endosphere of leaf or root). This finding would advance the co-evolutionary theory of mutualism between periglacial plants and microbes. To comprehensively reveal the ecological mechanisms underlying the compartment differntiation of periglacial plants-associated microbes as affected by environments, future studies are expected to establish a connection between the biotic/abiotic factors (i.e., plant age, nutrient content of each plant compartment, and soil physicochemical properties) and microbial community changes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-023-00466-5.

Additional file 1: Table S1. The geographic information and habitat type of *Potentilla fruticosa* var. *albicans* samples collected from Qiangyong glacier foreland. **Table S2.** Good's coverage scores (mean ± sd) of the bacteria in the rhizosphere soil, root endosphere, and leaf endosphere. **Table S3.** Bray–Curtis distance-based dissimilarity tests of the bacterial communities among glacier terminus zone (GTZ), moraine ridge (MR), and alpine meadow (AM). **Fig. S1.** *Potentilla fruticosa* var. *albicans* growing in the Qiangyong glacier terminus. **Fig. S2.** Map of sampling sites. **Fig. S3.** Rarefaction curves of the bacteria operational taxonomic unit (OTU) richness. **Fig. S4.** Heatmap depicting the distribution of relatively abundant bacterial operational taxonomic units (OTUs) of rhizosphere soil, root endosphere, and leaf endosphere, which were biased among habitat types. Cluster analysis was performed based on Euclidean similarities. GTZ, MR, and AM represent the glacier terminus zone, moraine ridge, and alpine meadow, respectively. **Fig. S5.** The effects of habitat type on the relative abundance of bacterial communities associated with *Potentilla fruticosa* var. *albicans*. Bars without shared lowercase letters indicate a significant difference in the relative abundance of bacteria among habitat types (Tukey's HSD test, $P < 0.05$). One-way ANOVA was used to evaluate the significant effect of habitat type on variation in the relative abundance of dominant bacterial taxa (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). **Fig. S6.** Differences in plant density and plant height between the glacier terminus zone (GTZ) and alpine meadow (AM). The Wilcox Test was used to compare the plant density and plant height between the GTZ and AM (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

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Author contributions

WS, YL, and LG conceptualized and planned the study. WS performed the fieldwork, analyzed the data, and prepared the manuscript. YL and LG revised the manuscript. All authors were involved in revising the manuscript critically.

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Data availability

The raw sequence data generated in this study have been deposited in the NCBI Sequence Read Archive under the accession number PRJNA931734.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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