Rhizosphere and endophytic bacterial communities of the endangered alpine modest primrose and their plant growth-promoting potential

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Abstract

Plant microbiomes play critical roles in host growth and stress resilience, yet remain underexplored in alpine ecosystems. We investigated the rhizosphere and endophytic bacterial communities of an endangered alpine modest primrose (*Primula modesta* var. *hannasanensis*), which is endemic to the high-altitude regions of Korea. Using 16S rRNA gene amplicon sequencing, we compared the bacterial communities in wild and cultivated populations. The rhizosphere microbiota exhibited greater diversity than the root endophytes, and cultivated plants harbored more diverse and compositionally distinct endophytic communities than wild plants, suggesting that cultivation influences the microbial structure. We isolated bacterial strains from these communities and identified *Leifsonia lichenia* JBCE310 and *Chryseobacterium piperi* JBCE316 as significantly enhancing seed germination and seedling growth of *Primula malacoides* and *Arabidopsis thaliana*. The co-inoculation yielded synergistic effects that were likely mediated by phytohormone production. These results indicate the functional potential of alpine plant-associated bacteria and provide microbial candidates for conservation and *ex situ* propagation of endangered alpine flora.

Introduction

Alpine ecosystems are increasingly threatened by anthropogenic pressures, including climate change, habitat fragmentation, and human encroachment, which places many endemic high-mountain plant species at risk of decline or extinction. Recent studies have emphasized the critical role of plant-associated microbial communities, particularly those inhabiting the rhizosphere and root endosphere, in enhancing plant resilience and ecological performance under environmental stress (Bhattacharyya and Lee 2016; Jansson and Hofmockel 2020). These microbiomes contribute to key functions such as nutrient acquisition, abiotic stress tolerance, disease resistance, and regulation of plant development, which are essential for plant survival in the harsh, nutrient-poor conditions of alpine habitats. Despite their potential importance, microbial communities associated with rare and endangered alpine plants remain largely uncharacterized. This hinders the development of microbial-assisted conservation strategies that leverage beneficial plant–microbe interactions for species recovery and ecosystem restoration.

Primula modesta var. *hannasannensis* T. Yamaz (hereafter referred to as alpine modest primrose; AMP) is an at-risk species. It is a rare and endemic perennial herb belonging to the family Primulaceae, and is confined to moist rock crevices above 800 m in elevation in the southern mountainous regions of the Korean Peninsula (Chung et al. 2017; Kim et al. 2022). Due to its narrow ecological range, limited habitat availability, and fragmented population structure, AMP has been officially designated as an endangered species by the Korea Forest Service (Korea National Arboretum 2008; Cho and Lee 2017). Conservation efforts are further hindered by the inherent biological constraints of the species, including low seed germination rates due to physiological dormancy and a limited capacity for vegetative propagation (Grigoriadou et al. 2020; Morozowska 2002). These factors underscore the urgent need for innovative and ecologically informed strategies to support the propagation and conservation of AMP.

Among these strategies, microbial-assisted approaches, particularly the application of plant growth-promoting rhizobacteria (PGPR), are gaining attention as sustainable tools to enhance seed germination, seedling vigor, and stress tolerance in rare or propagation-recalcitrant plants (Fatemeh et al. 2014). PGPR have been shown to improve rooting and acclimatization in alpine taxa, including *Primula*, where strains such as *Pseudomonas* spp. enhanced microshoot establishment (Digat et al. 1987). However, the use of native, functionally characterized rhizobacteria in conserving endangered alpine plants remains underexplored, representing a promising yet untapped avenue for biological intervention.

Additionally, comparative analysis of microbial communities between wild and cultivated plant populations provides insights into how *ex situ* cultivation and environmental management influence plant–microbe interactions. Disruptions or shifts in the rhizosphere and endophytic microbiota during cultivation can alter plant fitness and resilience, potentially undermining restoration success (Schemske et al. 1994). Therefore, preserving or restoring native microbial assemblages may be crucial to the success of conservation and reintroduction strategies.

In this study, we examined the structure and diversity of rhizosphere- and root-associated bacterial communities in both wild and cultivated populations of AMP using a combination of culture-independent and culture-dependent approaches. We further isolated culturable bacterial strains from AMP roots and evaluated their plant growth-promoting (PGP) properties using functional assays. Promising isolates were tested for their ability to enhance seed germination and seedling growth in *Primula malacoides* (fairy primrose) and *Arabidopsis thaliana*. Co-inoculation experiments were conducted to assess the synergistic interactions, microbial compatibility, and potential mechanisms of plant benefits with a particular focus on phytohormone production. This integrated approach not only deepens our understanding of plant–microbe interactions in alpine ecosystems but also provides microbial resources that could aid in the propagation and conservation of endangered alpine flora.

Material and Methods

Sample collection and processing

Samples of AMP (*P. modesta* var. *hannasanensis*) was collected as described by Dutta et al. (2021). Two individual AMP plants, spaced approximately 1 m apart, were randomly selected and then excavated with intact root systems and surrounding soil (collected within a 5 cm radius from the root zone). Each plant was placed into a sterile plastic bag and stored at 4°C. All samples were transported to the laboratory and processed within 48 h for microbial isolation and DNA extraction from the bulk soil, rhizosphere soil, and roots (endosphere), following the protocols of Fernández-Gómez et al. (2019) and Zeng et al. (2018).

The loosely attached particles were gently shaken off and passed through a 2-mm sieve to collect the bulk soil. The rhizosphere soil was obtained by vigorously shaking the roots and collecting the soil that remained tightly adhered to the root surface using sterilized brushes. The roots were used as endosphere samples after thoroughly washing with tap water and surface sterilization using 70% (v/v) ethanol for 1 min, 2.5% (w/v) NaOCl for 5 min, and five rinses with sterile distilled water (DW).

Sterilization efficacy was confirmed by plating 100 µL of the final rinse water onto Luria-Bertani (LB) agar plates and checking for microbial growth after incubation at 30°C for 48 h. Samples from three different sites were combined to form one biological replicate, and three replicates were prepared for each compartment (bulk soil, rhizosphere soil, and endosphere). All samples were stored at 4°C and processed for microbial and DNA analysis within 2 days.

Soil physicochemical property analysis

Genomic DNA was extracted using an Exgene Soil DNA Isolation Kit (GeneAll, Korea), according to the manufacturer's protocol. Surface-sterilized roots were homogenized using a sterile pestle before DNA extraction (Dutta et al. 2021; Khanh et al. 2024). DNA quality and purity were assessed using agarose gel electrophoresis and quantified using a BioTek Epoch spectrophotometer (USA). The V3–V4 region of the bacterial 16S rRNA gene was amplified using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'), which included Nextera adapter sequences as described by Fadrosh et al. (2014). A second PCR was performed to add Illumina dual indices and sequencing adapters: i5 (5'-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXX-TCGTCGGCAGCGTC-3') and i7 (5'-CAAGCAGAAGACGGCATACGAGAT-XXXXXXXX-GTCTCGTGGGCTCGG-3'), where “XXXXXXXX” represents the unique index sequences. The PCR cycling parameters were initial denaturation at 94°C for 3 min; 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s; and a final extension at 72°C for 5 min. PCR products were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, USA) and assessed for quality using an Agilent 2100 Bioanalyzer. Libraries were pooled and sequenced on the Illumina MiSeq platform (MiSeq Reagent Kit v2; Illumina, USA) at ChunLab Inc. (Korea). Sequencing data are available under BioProject accession number PRJNA1086577. PRJNA1086581, and PRJNA108644 for the bulk soil, rhizosphere, and roots, respectively.

DNA extraction and 16S rRNA gene sequencing

Genomic DNA was extracted using the Exgene™ Soil DNA Isolation Kit (GeneAll, Korea), following the manufacturer's protocol. Surface-sterilized roots were homogenized using a sterile pestle prior to DNA extraction (Dutta et al. 2021; Khanh et al. 2024). DNA quality and purity were assessed by agarose gel electrophoresis and quantified using a BioTek Epoch™ spectrophotometer (USA). The V3–V4 region of the bacterial 16S rRNA gene was amplified using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'), which included Nextera adapter sequences as described by Fadrosh et al. (2014). A second PCR was performed to add Illumina dual indices and sequencing adapters: i5 (5'-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXX-TCGTCGGCAGCGTC-3') and i7 (5'-CAAGCAGAAGACGGCATACGAGAT-XXXXXXXX-GTCTCGTGGGCTCGG-3'), where “XXXXXXXX” represents the unique index sequences. PCR conditions were as follows: initial denaturation at 94°C for 3 min; 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s; and a final extension at 72°C for 5 min. PCR products were quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, USA) and assessed for quality using an Agilent 2100 Bioanalyzer. Libraries were pooled and sequenced on the Illumina MiSeq platform (MiSeq Reagent

Kit v2; Illumina, USA) at ChunLab Inc. (Korea). Sequencing data are available under BioProject accession number PRJNA1086577. PRJNA1086581, and PRJNA108644 for bulk soil, rhizosphere, and root, respectively.

Bioinformatics and microbial community analysis

Raw sequence data were analyzed using the EzBioCloud bioinformatics pipeline (https://www.ezbiocloud.net) developed by ChunLab Inc. (Yoon et al. 2017). The pipeline performed quality filtering, merging of paired-end reads, assigns taxonomy, and conducts operational taxonomic unit (OTU)-based diversity analyses. Low-quality reads (average quality score < 25) were removed using Trimmomatic v0.32, and the paired-end reads were merged using PandaSeq (Bolger et al. 2014). The primer sequences were trimmed using EzBioCloud tools with a similarity threshold of 0.8. The error correction was performed using DUDE-Seq (Lee et al. 2017). Chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011). Taxonomic classification was performed by aligning the sequences against the EzBioCloud 16S rRNA gene database using USEARCH (Edgar 2013). Sequences with ≤ 3% dissimilarity to the reference sequences were assigned at the species level. Unclassified sequences were clustered using CD-HIT and UCLUST at 97% similarity (Li and Godzik, 2006). OTUs derived from both methods were combined to construct the final OTU set. Taxonomic assignment thresholds followed Yarza et al. (2014): genus (97% >x ≥ 94.5%), family (94.5% >x ≥ 86.5%), order (86.5% >x ≥ 82%), class (82% >x ≥ 78.5%), and phylum (78.5% >x ≥ 75%). A suffix indicating the taxonomic level was added when a taxon could not be fully identified (e.g., *Acidobacteria_c* for an unknown class).

Alpha diversity metrics (abundance-based coverage estimator (ACE), Chao1, Shannon, Simpson, and phylogenetic diversity) and rarefaction curves were calculated using CLcommunity v3.43 (ChunLab Inc., Seoul, Korea) (Yi et al. 2014). Beta diversity was assessed using principal coordinate analysis (PCoA) and unweighted pair group method with arithmetic mean (UPGMA) clustering using Bray–Curtis dissimilarity (Bray and Curtis 1957) at the species level. Differences in OTU richness and community composition among the bulk soil, rhizosphere, and endosphere samples were analyzed at the phylum and species levels.

Enumeration and isolation of culturable bacteria

Culturable microbial populations were quantified by serial dilution plating on low-nutrient 1/10 LB agar. For total bacterial counts, 5 g of soil was suspended in 50 mL of sterile DW, shaken for 5 min, serially diluted, and plated on 1/10 LB agar supplemented with 50 µg/mL cycloheximide to inhibit fungal growth. Endophytic bacteria were isolated from the surface-sterilized roots. The tissues were ground using a sterile mortar and pestle, resuspended in sterile DW (10 mL/g fresh weight), serially diluted, and plated onto 1/10 LB agar. Plates were incubated at 30°C for 3 days, and colonies with distinct morphologies were randomly selected, subcultured in LB broth containing 15% glycerol (v/v), and stored at − 80°C for further analyses.

Selection of PGPR strains and growth promotion assay using Arabidopsis

Bacterial isolates previously stored at − 80°C were screened for growth-promoting effects on *Arabidopsis thaliana* (Col-0). Among them, two isolates, JBCE310 and JBCE316, showed significant growth enhancement and were identified as *Leifsonia lichenia* and *Chryseobacterium piperi*, respectively, using 16S rRNA gene sequencing (Lee et al. 2008). Overnight LB cultures of each strain were harvested, washed, and resuspended in sterile DW (1×10^7 CFU/mL) containing 0.2% carboxymethyl cellulose (CMC). Surface-sterilized *Arabidopsis* seeds (70% ethanol for 90 s and 1% NaOCl for 5 min) were soaked in the bacterial suspension for 30 min at 28°C with gentle shaking (150 rpm), air-dried, and sown on ½-strength MS medium supplemented with 1.5% sucrose and 0.8% agar. Plates were sealed with parafilm and incubated at a 70° angle under a 16 h light/8 h dark photoperiod (100 µmol m^{-2} s^{-1}, 23 ± 1°C). Growth parameters, including root length, shoot height, lateral root number, and fresh weight, were measured 14 days after sowing.

Seed germination and growth assay using Primula malacoides

Seeds of *P. malacoides* (fairy primrose), an AMP congener, were surface-sterilized with 70% ethanol for 1 min, followed by 1% NaOCl for 5 min, and rinsed five times with sterile DW. Seeds were soaked in bacterial suspensions (JBCE310 or JBCE316; 1×10^7 CFU/mL) for 1 h at room temperature. For co-inoculation, equal volumes of both strains were mixed before seed treatment. Treated seeds were sown in pots filled with horticultural nursery soil and incubated in a growth chamber (20 ± 1°C, 65% relative humidity, and 16 h light/8 h dark) for 10 weeks. The germination rate and plant growth parameters were monitored weekly.

Bacterial compatibility assay

The compatibility between JBCE310 and JBCE316 was evaluated using cross-streaking and disk diffusion assays. In the cross-streaking method, JBCE310 was streaked onto LB agar, followed by the streaking of JBCE316 perpendicular to it. The plates were incubated at 30°C for 3 days, and the intersection was examined for inhibition. In the disk diffusion method, LB plates pre-inoculated with JBCE310 (1×10^7 CFU/mL) were spot-inoculated with 20 µL of JBCE316 on sterile paper disks (8 mm diameter). The plates were incubated at 30°C for 2 days. The absence of inhibition zones indicated mutual compatibility. The experiments were conducted twice with three replicates per treatment.

Functional assays for PGP traits

Siderophore production was assessed using a modified chrome azurol S (CAS) agar (Schwyn and Neilands 1987). Bacterial cultures were spot-inoculated on sterile paper discs placed on CAS agar plates and incubated at 30°C for 4 days; orange halos indicated siderophore production. Phosphate solubilization was assessed using Pikovskaya's agar (Pikovskaya 1948) and tricalcium phosphate medium (Rodriguez et al. 2001). Each strain or a 1:1 mixture of JBCE310 and JBCE316 was inoculated on the media and halo zones were measured after 3 days of incubation at 28°C. Protease activity was evaluated on skim milk agar by spotting bacterial cultures on filter paper disks. Clear zones surrounding the disks after 3 days of incubation at 30°C indicated casein degradation.

Phytohormone production assay

Bacterial strains were cultured in indole-3-acetic acid (IAA)-inducing broth (Apine and Jadhav 2011) at 30°C for 48 h in the dark, with shaking at 150 rpm, and the supernatants were mixed with Salkowski reagent (3:2) to determine IAA production. The absorbance was measured at 530 nm after 30 min of incubation in the dark. Cytokinin production was tested in M9 minimal medium supplemented with casamino acids, thiamine, and biotin (Akiyoshi et al. 1987). The cultures were incubated for 4 days at 28°C and 160 rpm. The cytokinin content was determined at 665 nm (Patel and Saraf 2017). Gibberellic acid (GA) production was assessed after 5 days of incubation in the GA production broth. The culture supernatants were acidified (pH 2.5) and extracted with ethyl acetate, and GA was quantified by measuring the absorbance at 254 nm (Pandya and Desai 2014). All assays were conducted in triplicate.

Statistical analysis

Statistical analyses were performed using JMP software (SAS Institute, Cary, NC, USA). The statistical procedures for individual experiments are described in the respective sections. For other variables, data were analyzed using analysis of variance (ANOVA), and mean comparisons were conducted using the least significant difference (LSD) test at $P < 0.05$. Each experiment was independently analyzed.

Results

Samplings and soil physicochemical properties of AMP habitats

We sampled naturally occurring plants from Ungseokbong Mountain (N35.36. xxxx, E127.87.xxxx), in Sancheong-eup, Sancheong-gun, Gyeongsangnam-do Province, South Korea (Online Resource Fig. S1) to investigate the microbial communities associated with different compartments (bulk soil, rhizosphere soil, and root endosphere) of AMP. Collections were made during the flowering season in late May 2022. The plants were located at an elevation of 988 m and grew between rocks in mountain valleys. For a comparative analysis, AMP plants were sampled from cultivated fields maintained by the Baekdudaegan National Arboretum in Chunyang-myeon, Bonghwa-gun, Gyeongsangbuk-do Province.

Previous reports have suggested that *Primula* species typically thrive in moist grasslands or limestone-rich wetland margins, where calcareous substrates are present, and extremely acidic soils often require amendment for optimal growth (Goodwin 1991). Consistent with this, soil analysis revealed that wild habitats were strongly acidic, with pH values ranging from 4.1 to 5.5, whereas cultivated soils were mildly acidic to neutral (pH > 6.4) (Online Resource Table S1). Additionally, the concentrations of exchangeable cations such as potassium, magnesium, and sodium were significantly lower in wild soils than in cultivated soils, which exhibited particularly high calcium levels. The results indicated that AMP plants in the wild persist in nutrient-poor, acidic environments, whereas cultivated plants grow in more nutrient-rich, moderate conditions.

Bacterial 16S rRNA gene sequencing and alpha diversity

A total of 18 samples were collected from the different compartments described above to characterize the bacterial communities associated with AMP plants in wild and cultivated settings. High-throughput 16S rRNA gene sequencing yielded 1,020,466 raw reads (median 56,692 reads per sample). In total, 727,215 high-quality reads, with a median read length of 417.93 bp, remained after quality filtering, merging, and chimera removal. Rarefaction analysis at 3% sequence dissimilarity threshold indicated that the sequencing depth was sufficient to capture the majority of the bacterial diversity in bulk and rhizosphere soils. On average, 3,501 and 3,426 OTUs were observed in the bulk and rhizosphere soils, respectively, from the wild samples, and 3,964 and 4,166 OTUs from the cultivated samples (Online Resource Table S2; Table S3). In contrast, the root endosphere samples yielded substantially fewer OTUs, with averages of 145 (wild) and 541 (cultivated), suggesting lower bacterial richness and potential underrepresentation of rare taxa, particularly in wild plant roots.

Good's coverage exceeded 97% across all sample types, indicating a high level of sequencing completeness. Non-parametric estimators such as ACE, Chao1, and Jackknife confirmed that bulk and rhizosphere soils in both wild and cultivated environments harbored high bacterial diversity. However, the differences between sites were not statistically significant. The alpha diversity indices across all habitats consistently demonstrated higher richness and evenness in the soil compartments (bulk and rhizosphere) than in the root compartments. The Shannon and phylogenetic diversity indices further supported this trend, with both indices indicating greater microbial complexity in the soil than in the roots. Although the total bacterial diversity within the roots was comparable between the wild and cultivated environments, cultivated AMP plants exhibited significantly higher endogenous bacterial richness and abundance. This suggested that cultivation practices or soil nutrient conditions modulate the colonization or survival of endophytic bacterial populations. In summary, alpha diversity analyses revealed that bacterial richness and diversity are generally higher in soil compartments than in roots and that cultivated AMP harbor a more abundant and diverse root-associated microbiome than their wild counterparts

Beta diversity of bacterial community structures

Beta diversity was analyzed using PCoA based on Bray–Curtis dissimilarity to assess the differences in bacterial community composition between the soils and roots of AMP plants grown in wild and cultivated habitats. PCoA revealed a clear separation between the root-associated microbiota and those of bulk and rhizosphere soils across all samples, indicating distinct taxonomic structures between plant compartments (Fig. 1A). Root samples clustered separately from soil samples In both the wild and cultivated environments, forming discrete groups along the principal coordinate axes. This distinct clustering pattern underscores the uniqueness of the root-associated bacterial community compared with the surrounding soil niches (Fig. 1A–C). In contrast, the bacterial communities of the bulk and rhizosphere soils tended to overlap and cluster together within each habitat, suggesting that these two soil niches harbored relatively similar bacterial assemblages. Hierarchical clustering using the UPGMA based on Bray–Curtis distances further supports these findings. Root samples from wild and cultivated

plants formed separate cohesive clusters, reinforcing the conclusion that the root endosphere maintained a specific and selective bacterial composition distinct from that of the surrounding soils.

Taxonomic composition of bacterial communities at the phylum level

We analyzed the relative abundance of bacterial taxa at the phylum level to compare bacterial community structures across different plant compartments and habitats. The bulk and rhizosphere soils from both wild and cultivated areas exhibited similar taxonomic profiles, with the dominant phyla consistently represented across habitats (Fig. 2). Notably, the number of phyla with a relative abundance greater than 1% was higher in the cultivated soil samples than in those from the wild habitat, indicating a more taxonomically diverse soil microbiome in managed environments. Proteobacteria was the most dominant phylum across all soil compartments, comprising 38.9% of the total bacterial community in both the bulk and rhizosphere soils of the wild habitat, and increased to 42.2% and 48.0% in the corresponding compartments of the cultivated area. Acidobacteria was the second most abundant phylum in wild soils, accounting for 31.7% and 31.9% of the bulk and rhizosphere soils, respectively. In contrast, Parcubacteria (OD1) was the second most abundant phylum in the bulk soil of the cultivated habitat with a relative abundance of 11.8%, whereas Acidobacteria represented only 10.3% in the rhizosphere.

The root endosphere bacterial communities exhibited a strikingly different taxonomic profile from that of the soil compartments. Proteobacteria dominated the root microbiome, accounting for 99.6% and 96.0% of the total bacterial abundance in the wild and cultivated plants, respectively. This high dominance contrasts with the more diverse phylum distribution observed in the surrounding soils, highlighting the selective colonization and enrichment of Proteobacteria within the root tissues. These results suggested that, while the overall bacterial community structure in soils is broadly consistent between wild and cultivated habitats, the root endosphere harbors a distinctly less diverse and more specialized bacterial population dominated by Proteobacteria.

Bacterial community compositions at the genus and species level

At the genus level (≥ 1% relative abundance), the bacterial community compositions of bulk soil and rhizosphere were similar within each habitat (wild or cultivated), yet exhibited substantial differences between the two habitats (Fig. 3). In the wild, *Bradyrhizobium*, *Acidibacter*, *Rhizomicrobium*, and *Tepidisphaera* dominated the soil compartments, whereas *Flavobacterium*, *Acidibacter*, *Sphingomonas*, and *Rhizomicrobium* were the most abundant. *Sphingomonas*, *Acidibacter*, *Tepidisphaera*, and *Rhizomicrobium* were common to both habitats (Fig. 4A, B). In total, 20 and 22 genera exceeded 1% of the relative abundance of wild AMP plants in the bulk soil and rhizosphere, respectively, with 17 genera shared between these two compartments (Fig. 4C). Comparing soils and roots, the endophytic community of wild AMP roots was dominated by *Pseudomonas* (89.2%), followed by *Rahnella* (6.1%) and *Serratia* (1.9%). *Rahnella* and *Serratia* were present in < 0.1% of the soil compartments. In total, 15 genera with > 1% abundance were common between the bulk soil and rhizosphere in cultivated plants, and 3 additional genera were found exclusively in the rhizosphere (Fig. 4D).

Pseudomonas (39.6%) was the most abundant genus in the root endosphere of cultivated plants, followed by *Pantoea* (35.4%), *Erwinia* (3.7%), and *Salinicola* (1.1%). Among these, *Pseudomonas* was also enriched in the rhizosphere (3.6%) but remained low in the bulk soil (0.7%), whereas the other endophytes (*Pantoea*, *Rahnella*, and *Erwinia*) were nearly absent in the soil compartments (< 0.1%). *Salinicola* was detected only in the root tissues, suggesting exclusive endophytic colonization. *Herbaspirillum*, *Janthinobacterium*, *Oxalicibacterium*, and *Sphingomonas* were also detected at > 0.1% relative abundance in the roots of wild plants (Online Resource Table S4). Of these, *Sphingomonas* was present in all the compartments. In cultivated plant roots, 22 additional endophytic genera were detected above the 0.1% threshold, including *Rhizobium*, *Achromobacter*, *Aquidulicibacter*, *Opitutus*, *Hyphomicrobium*, *Tepidisphaera*, *Solibacter*, and *Rhizomicrobium*. Fourteen of these were shared across all three compartments (soil, rhizosphere, and roots). These results highlighted that the root endosphere microbiota is distinct from that of the surrounding soils and is more diverse in cultivated AMP plants.

Considering populations > 0.1% relative abundance, *Bradyrhizobium japonicum* (~ 3%) was the most dominant species in both the bulk soil and rhizosphere of wild plants, followed by the unclassified members of *Proteobacteria* and *Acidobacteria* (Fig. 5). In the roots, *Pseudomonas tolaasii* was the most abundant species (76.4%), followed by *Rahnella woolbedingensis* (6.0%) and *Serratia* spp. (1.3%). In the cultivated soils, unclassified species of *Proteobacteria* (~ 5%) and *Verrucomicrobia* were prevalent in the bulk soil, whereas *Pseudomonas corrugata* (1.6%) and *P. fulva* (1.3%) were dominant in the rhizosphere. The root endophytes of the cultivated plants were dominated by *Pantoea agglomerans* (30.3%), *Rahnella aquatilis* (10.6%), and multiple *Pseudomonas* species (*P. flavascens*, *P. fulva*, *P. marginalis*, *P. tolaasii*, *P. amygdali*, and *P. viridiflava*), which together accounted for more than 34% of the total root-associated community. These findings suggested that *R. aquatilis* and various *Pseudomonas* species are core members of the cultivated AMP root microbiota.

Heatmap analysis further confirmed the distinct species-level compositions across the compartments and habitats (Fig. 6). *B. japonicum*, unclassified *Proteobacteria* (e.g., KF956747_s and EF520409_s), and *Verrucomicrobia* (LSTA_s) were prevalent in wild soils. *P. fulva*, *P. corrugata*, and unclassified species (e.g., CP011215_f_us_s, LMNW_s, DQ837262_s, and FNJA_s) were dominant in cultivated soils. The root microbiota of the wild plants was dominated by *P. tolaasii* and *R. woolbedingensis*, whereas the roots of the cultivated plants harbored *P. agglomerans*, *R. aquatilis*, and several *Pseudomonas* spp. *P. putida* was the only species common to both wild and cultivated roots, highlighting the divergence of endophytic bacterial communities between natural and managed habitats.

Culturable bacterial populations

The total number of culturable bacteria was quantified from the bulk soil, rhizosphere, and root samples of AMP plants collected from both wild and cultivated habitats. No significant differences were observed in the culturable bacterial populations among the rhizocompartments within or between the two habitats (Fig. S2). Only a small fraction of soil and rhizosphere microbial communities—typically around 1%—are culturable using standard laboratory media, and this fraction is highly dependent on the composition of

the growth medium (Vierra and Nahas 2005). Consequently, culturable bacteria likely represent only a limited subset of the microbial community and may not accurately reflect broader differences in bacterial diversity and composition across rhizocompartments or environmental conditions.

Growth promoting effects of bacterial strains on A. thaliana

Treatment with JBCE310, JBCE316, or their combination at a concentration of 1×10^7 CFU/mL significantly promoted the growth of Arabidopsis seedlings compared to the untreated control (Fig. S3). Among these treatments, the combined application of both bacterial strains resulted in the most pronounced growth-promoting effect. Specifically, the number of lateral roots and the overall fresh biomass were significantly higher in plants treated with the combined bacterial suspension than in those treated with either strain alone. indicate d a synergistic interaction between JBCE310 and JBCE316, which contributed to improved root system architecture and biomass accumulation. Enhanced root development likely facilitates greater nutrient uptake, thereby promoting the overall plant vigor.

Effect of bacterial inoculation on seed germination and growth of P. malacoides

P. malacoides seeds exhibited delayed germination under natural soil conditions, with only ~ 4.0% germinating after 5 weeks of sowing. Inoculation with either *L. lichenia* JBCE310 or *C. piperi* JBCE316 significantly accelerated germination, with 4.0% and 4.5% of seeds germinating within 2 weeks, respectively (Fig. 7A, B). Remarkably, co-inoculation with both strains led to earlier germination, with 4.0% of the seeds sprouting as early as 10 days after sowing. In addition to enhancing germination time, bacterial treatments also promoted seedling vigor. Plants inoculated with either the strain alone or in combination exhibited significantly increased shoot and root lengths 10 weeks after sowing, along with greater fresh and dry biomass compared to those in the untreated control (Fig. 7C, D; Fig. 8). These results indicated that inoculation with the AMP-associated strains JBCE310 and JBCE316 not only stimulated early germination but also enhanced subsequent seedling growth. The synergistic effect observed in the co-inoculation treatment highlights the potential of these strains as effective PGPR for improving the propagation of *P. malacoides*.

Strain compatibility and PGP traits of bacterial strains

We assessed the compatibility of JBCE310 and JBCE316 cells using cross-streak and dual culture assays to evaluate the feasibility of co-inoculation. Neither strain exhibited antagonistic activity toward the other, indicating mutual compatibility and supporting their concurrent application in plant systems (Online Resource Fig. S4).

We further characterized the functional traits related to nutrient mobilization and phytohormone biosynthesis to gain insight into the mechanisms underlying the effects of PGP. JBCE316 produced siderophores and extracellular proteases, suggesting its potential role in enhancing nutrient availability (Online Resource Table S5). Moreover, JBCE316 synthesizes IAA, a plant hormone that modulates root architecture and promotes vegetative growth. These complementary traits, along with their

demonstrated compatibility, indicated the synergistic potential of JBCE310 and JBCE316 as microbial consortia for the promotion of sustainable plant growth.

Discussion

Microbial communities are integral to plant adaptation and survival, especially in extreme alpine ecosystems characterized by low temperatures, poor nutrient availability, and high abiotic stress (Lembrechts et al. 2019). Owing to their rapid evolutionary dynamics relative to plants, soil microbes provide critical ecological functions, including nutrient mobilization, stress mitigation, and growth promotion (Compant et al. 2019; Philippot et al. 2013). Given their faster evolutionary rates and dynamic responsiveness to environmental changes, microbes can confer adaptive advantages to their plant hosts, often outpacing their intrinsic genetic adaptations (Trivedi et al. 2020). We investigated the rhizosphere and root endosphere bacterial communities associated with *Primula modesta* var. *hannasannensis* (AMP), a rare alpine endemic species in Korea, in this study to elucidate the microbial community dynamics across wild and cultivated habitats and to isolate beneficial taxa for conservation purposes.

16S rRNA gene amplicon profiling revealed clear microbial compartmentalization and habitat-specific structuring. Consistent with findings from other alpine and forest ecosystems (Uroz et al. 2019; Nicol et al. 2024), the rhizosphere exhibited higher bacterial diversity than the root endosphere in both wild and cultivated AMP populations, supporting the role of the rhizosphere as a dynamic microbial reservoir. Although the rhizosphere communities within each habitat were taxonomically similar at the phylum level, they diverged at the genus and species levels between habitats, reflecting the environmental influences on community assembly. Interestingly, cultivated AMP roots showed broader and more compositionally diverse endophytic microbiota than wild roots, which is consistent with the findings that managed environments can reduce host selection pressure and enable the proliferation of generalist and opportunistic microbes (Mendes et al. 2014; Pérez-Jaramillo et al. 2016; Shade et al. 2017).

Root endophytes in wild AMP plants were predominantly composed of oligotrophic and stress-resilient taxa such as *Bradyrhizobium*, *Sphingomonas*, and members of Acidobacteria, which are well-suited to nutrient-poor and acidic alpine soils (Bergkemper et al. 2016; Fierer et al. 2007). In contrast, copiotrophic genera, such as *Pantoea*, *Erwinia*, and *Salinicola*, were prevalent in cultivated roots, likely reflecting increased nutrient input and microbial influx from the surrounding agricultural settings (Zhao et al. 2023). Notably, *P. putida* was the only taxon consistently detected across all compartments and habitats, suggesting its role as a core member of the AMP microbiome with broad functional versatility and stability across environmental gradients (Banerjee et al. 2018; Toju et al. 2018).

Compartmental differentiation was further evidenced by the dominance of Proteobacteria, particularly *Pseudomonas*, in the root endosphere. In contrast, Acidobacteria, Planctomycetes, and Parcubacteria exhibited greater taxonomic richness in the rhizosphere and bulk soils. This pattern corresponds to observations in polar and alpine species, such as *Deschampsia antarctica* and *Colobanthus quitensis*,

where plant roots act as strong microbial filters for selecting functional and stress-tolerant endophytes (Nicol et al. 2024). Such microbiome filtering may reflect the long-term co-evolutionary dynamics between AMP and their core microbiota, particularly under strong selection pressures typical of alpine environments (Vannier et al. 2015).

Although the total culturable bacterial counts did not vary significantly among habitats or compartments, functional screening revealed distinct PGP capacities. Among these, *L. lichenia* JBCE310 and *C. piperi* JBCE316 significantly enhanced the seed germination and growth of *P. malacoides* and *A. thaliana*. JBCE316 exhibited well-known PGPR traits, including IAA production, siderophore secretion, and protease activity. In contrast, JBCE310, despite lacking PGP features, effectively stimulated germination. This suggested that alternative mechanisms, such as modulation of seed dormancy, microbial quorum signals, or microbe-derived small molecules, influence host signaling (Finkel et al. 2017; Truyens et al. 2015). Further studies, including omics analyses, are required to elucidate JBCE310's mode of action, which possibly involves host transcriptional reprogramming or seed coat modification (Lugtenberg and Kamilova 2016; Vishwakarma et al. 2020). Compatibility assays revealed no antagonistic interaction between JBCE310 and JBCE316, enabling their co-inoculation. This co-application resulted in synergistic effects on root architecture and biomass accumulation, supporting the functional complementarity hypothesis underlying synthetic microbial communities (SynComs) (Herrmann and Lesueur 2013; Del Barrio-Duque et al. 2019). SynCom strategies have gained traction in both ecological and agricultural research to enhance plant resilience under environmental constraints (Liu et al. 2022). The ability of these strains to enhance germination without stratification is particularly valuable for the conservation of alpine plants, where dormancy is a significant constraint. Similar effects of PGPR on breaking seed dormancy have been observed in *Crataegus monogyna* and *Picea abies* (Fernández et al. 2014; Schwienbacher et al. 2011).

Our results indicated the functional relevance of alpine plant-associated microbiomes in influencing host physiology and regeneration potential. The identification of AMP-associated PGPR with growth-promoting and dormancy-breaking abilities offers an ecologically compatible strategy for improving *ex situ* propagation of endangered alpine species. However, the long-term success of these strains under field conditions remains to be verified. Their persistence, colonization dynamics, and interactions with native soil microbiota under fluctuating environmental conditions must be rigorously evaluated (Lembrechts et al. 2019; Rousk et al. 2018). Precise molecular approaches are required to understand the germination-promoting activities of JBCE310 and plant responses to its interaction (Lugtenberg and Kamilova 2016; Vishwakarma et al. 2020). Exploring microbial metabolite production, gene expression in plant hosts, and colonization dynamics would yield deeper mechanistic insights (Liu et al. 2022; Vannier et al. 2015).

In summary, this study provided the first detailed characterization of the rhizosphere and root endophytic bacterial communities in *P. m.* var. *hannasannensis* and demonstrated the functional potential of native bacterial isolates for growth promotion and alleviation of dormancy. The results of this study contribute

to a broader understanding of alpine plant–microbiome interactions and offer microbial resources to support conservation, restoration, and sustainable propagation efforts for endangered alpine flora.

Declarations

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Author contributions

SD: Data curation, bioinformatics analysis, Investigation, Methodology, Writing of original draft. NVK: Bioinformatics analysis, Methodology, Investigation. YHL: Conceptualization, Project administration, Supervision, Validation, Visualization, Writing, review and editing, and funding acquisition.

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Data availability

All data supporting the findings of this study are available in the manuscript and Supplementary Materials.

Competing interests

The authors declare no competing interests relevant to the contents of this article.

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Figures

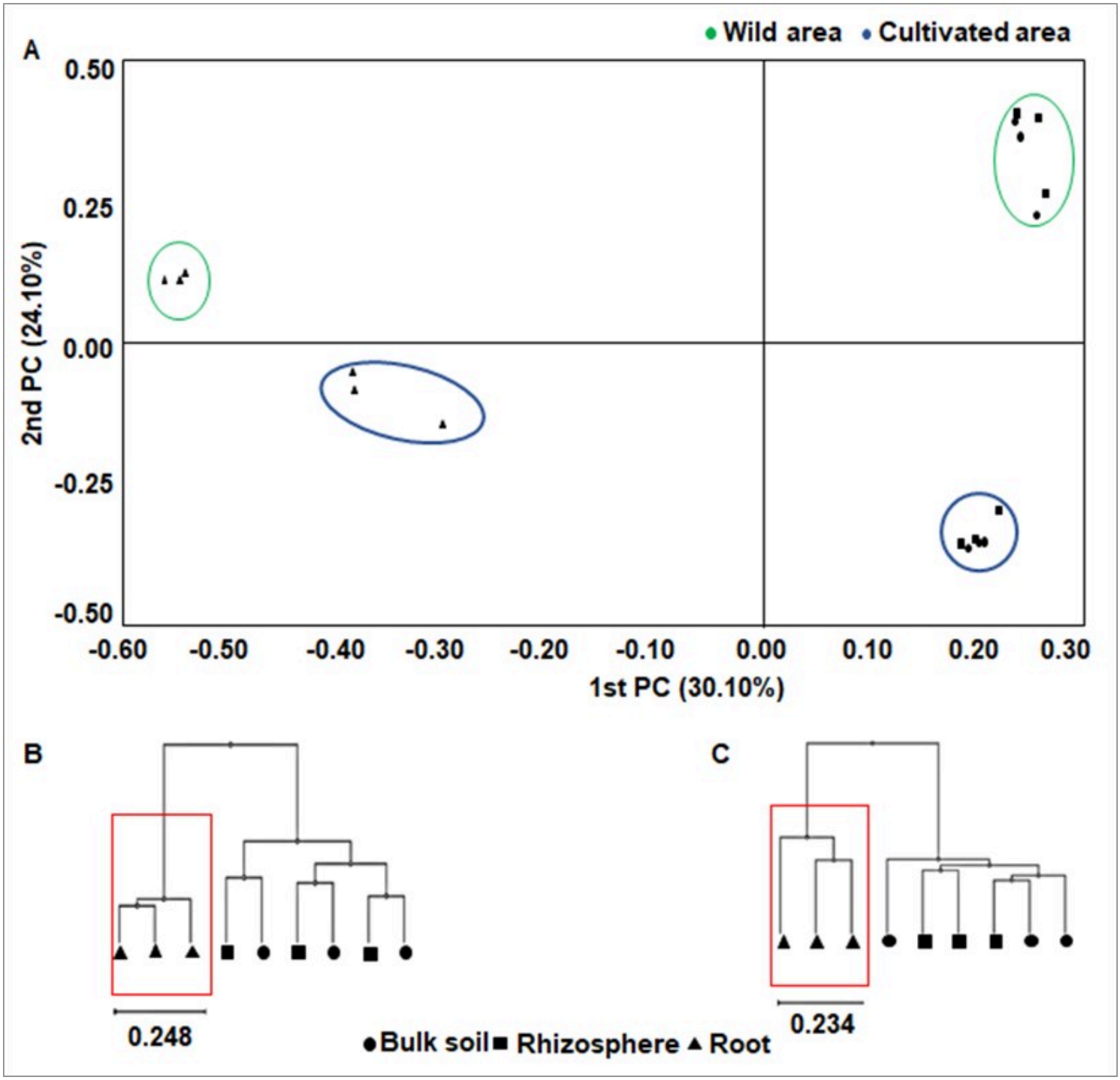

Figure 1

Beta diversity of bacterial communities across compartments and habitats of alpine modest primrose plants. (a) Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity showing separation of microbial communities among bulk soil, rhizosphere, and root compartments. (b, c) Unweighted pair group method with arithmetic mean (UPGMA) clustering based on Bray–Curtis distances for samples from wild (b) and cultivated (c) AMP habitats. Root-associated bacterial communities form distinct clusters (highlighted in red boxes), indicating compositional divergence from bulk and rhizosphere soils

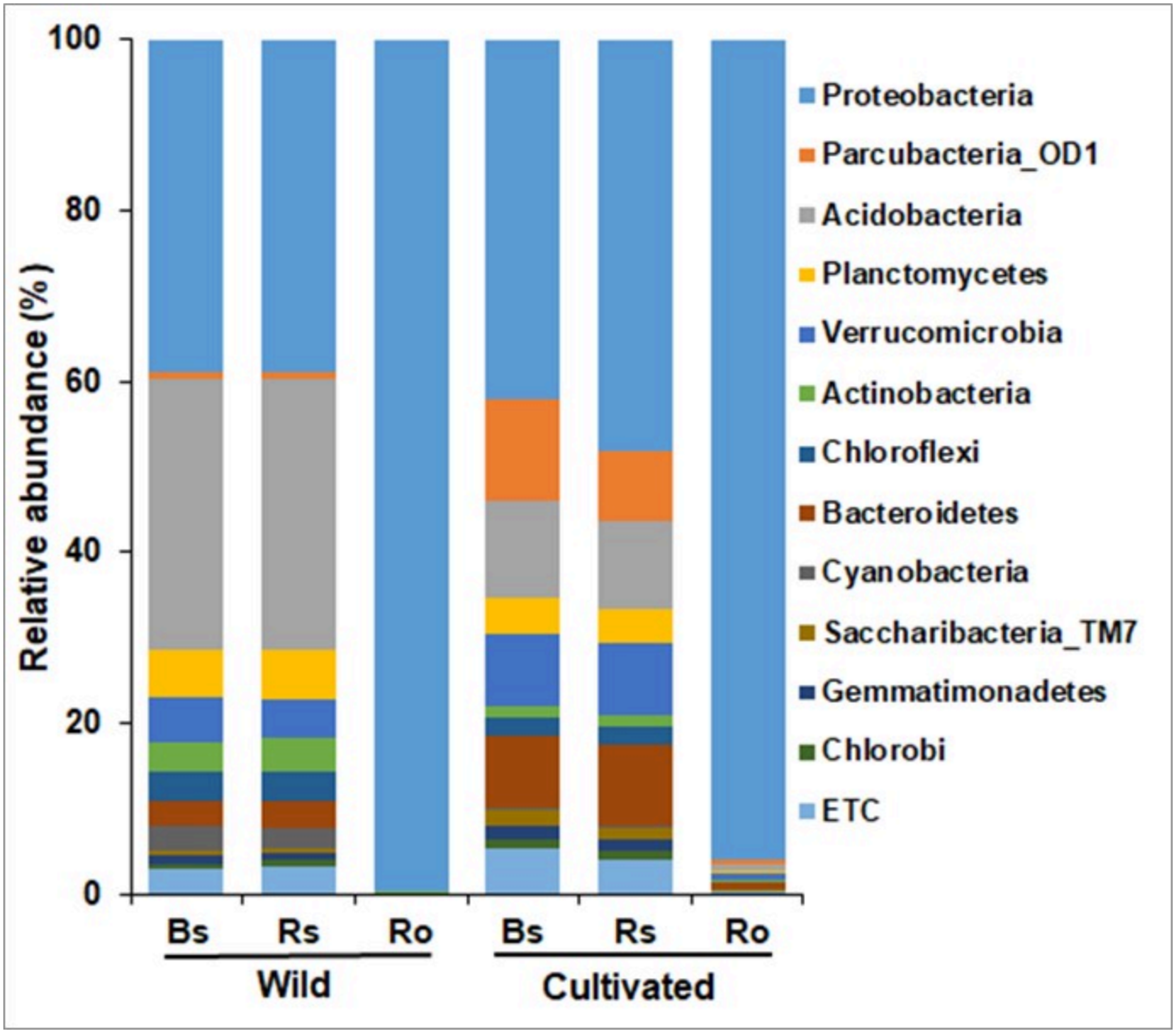

Figure 2

Relative abundance of bacterial phyla in bulk soil (Bs), rhizosphere soil (Rs), and roots (Ro) compartments of alpine modest primrose **plants from wild and cultivated habitats.** Only phyla with relative abundance >1% in at least one sample group are displayed. Phyla with lower relative abundances (<1%) were grouped under the category “ETC.” The taxonomic profiles highlight a conserved soil community across habitats, and a distinct root-associated community dominated by Proteobacteria

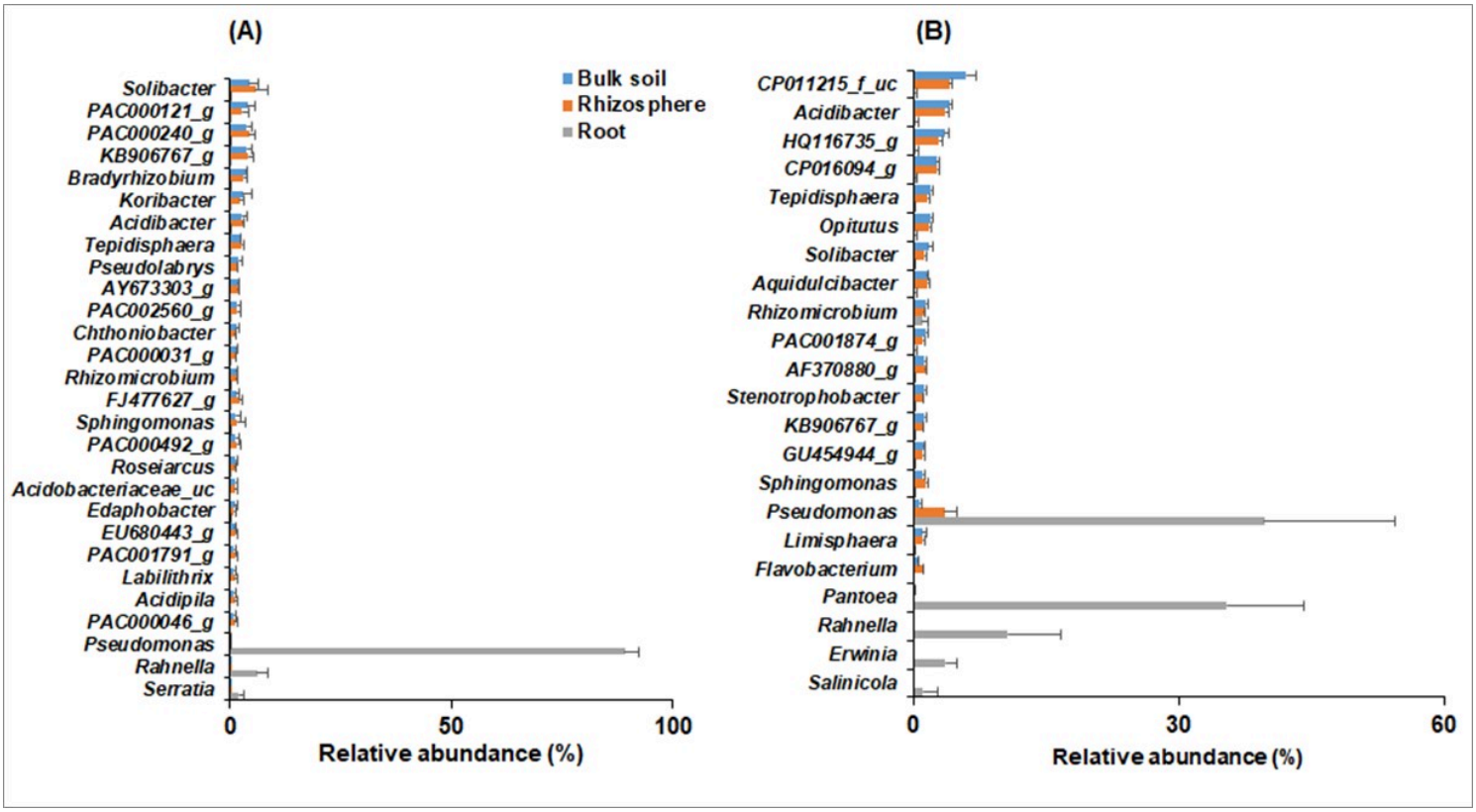

Figure 3

Comparison of the relative abundance of bacteria at the genus level in bulk soil, rhizosphere, and roots of alpine modest primrose plants. The bacterial genera with at least 1% relative abundance in each compartment of wild (a) and cultivated (b) habitats were selected for comparison

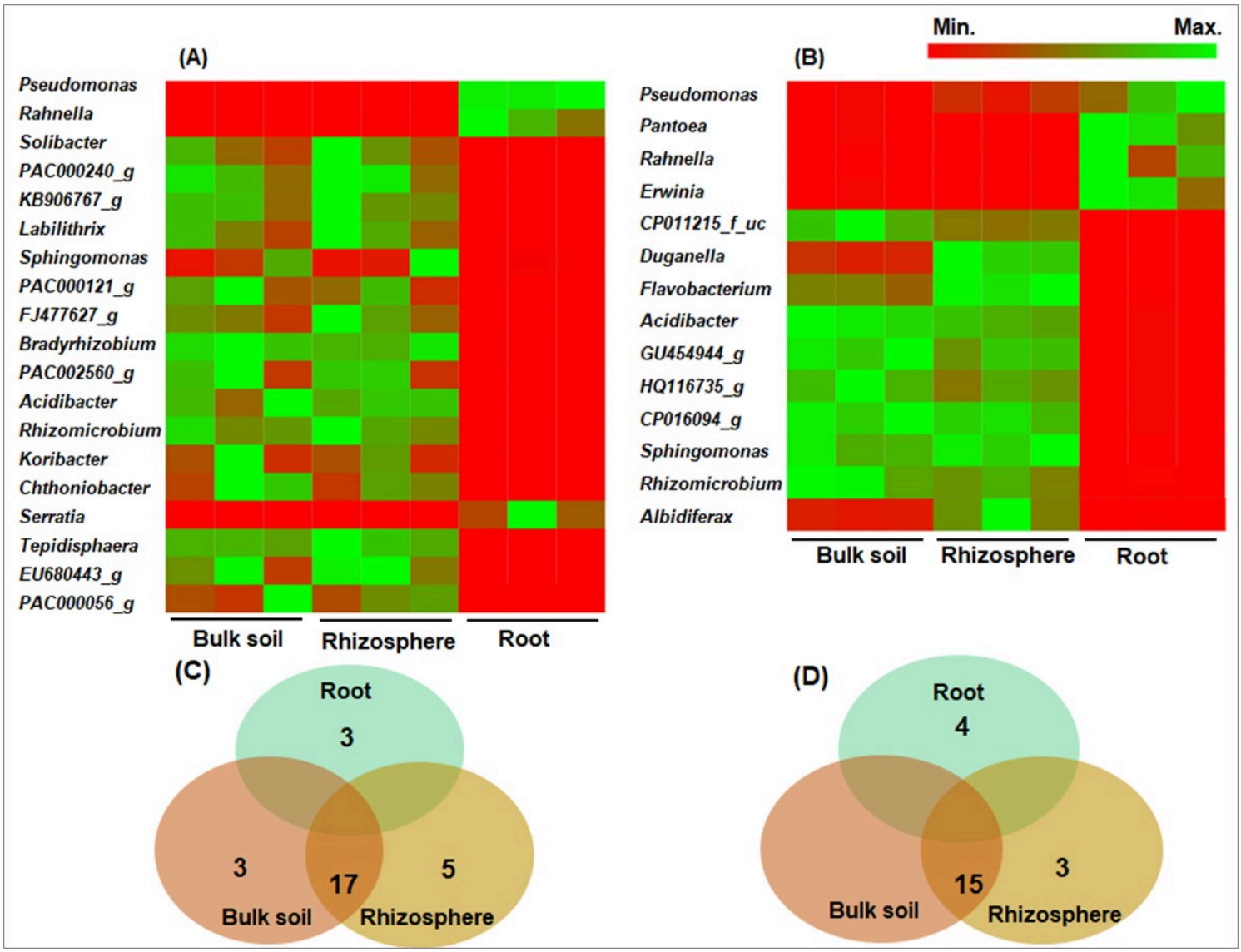

Figure 4

Comparison of the relative abundance of bacteria at the genus level in bulk soil, rhizosphere, and roots of alpine modest primrose plants. The bacterial genera with more than 1% relative abundance in each compartment of wild (a) and cultivated (b) habitats were selected for comparison. The Venn diagrams show unique or shared genera of bacterial microbiota with relative abundance above 1% in each compartment of wild (c) and cultivated (d) areas

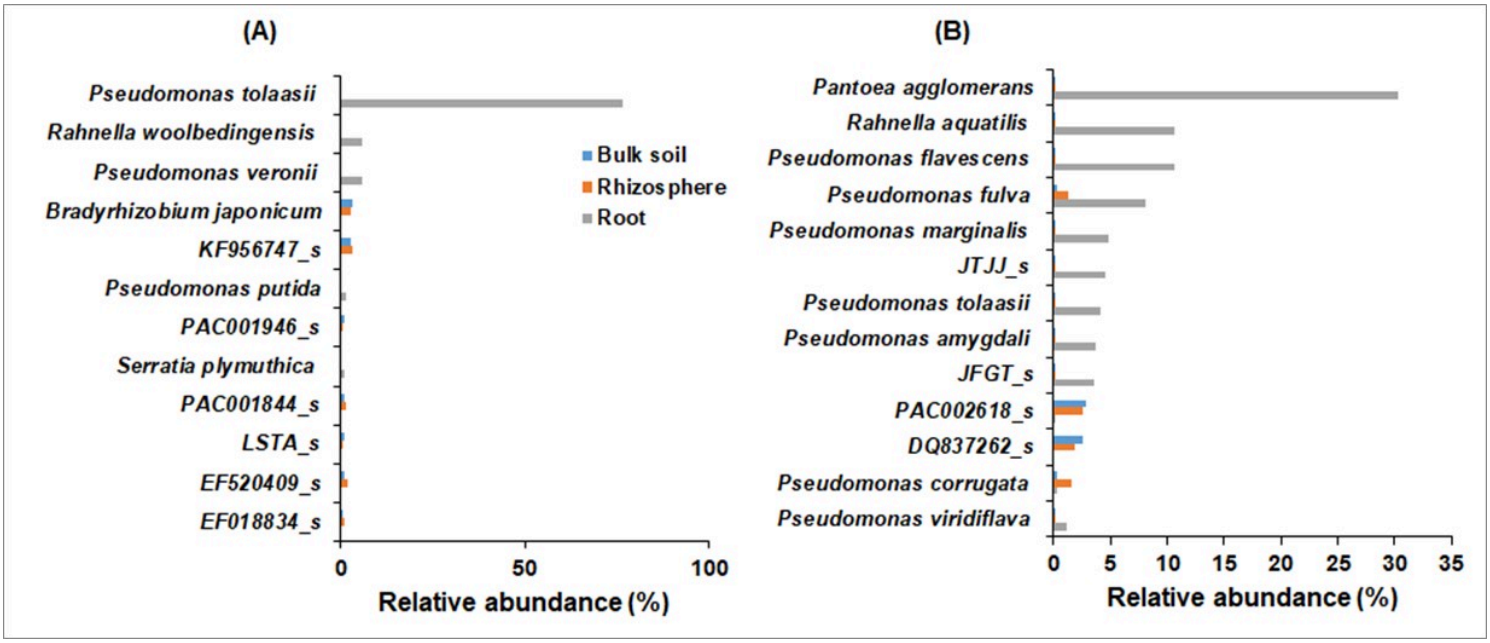

Figure 5

Combined comparison of bacterial genera in bulk soil, rhizosphere, and root of alpine modest primrose plants. The bacterial genera with at least 1% relative abundance in each compartment of wild (a) and cultivated (b) habitats were selected for comparison

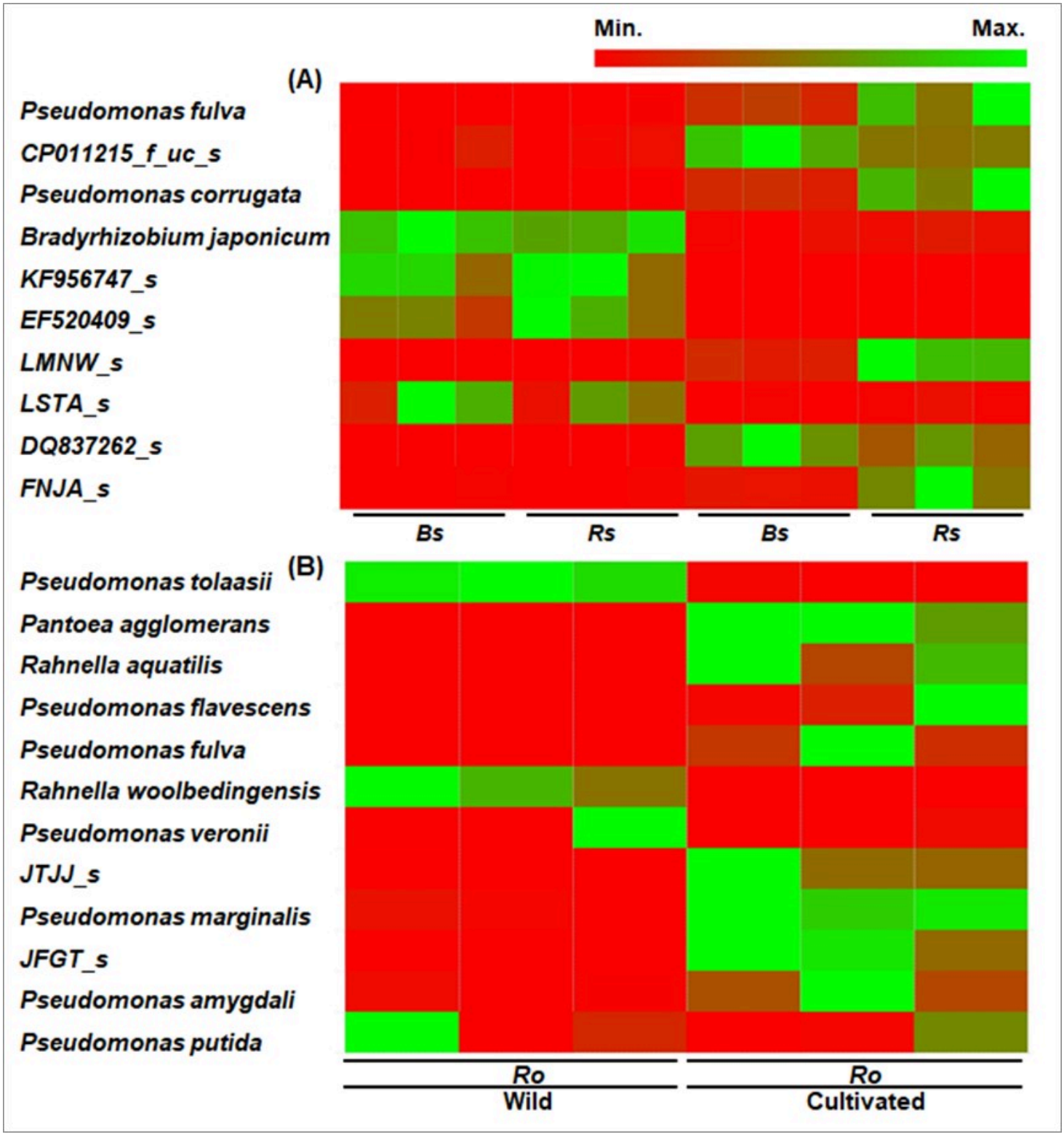

Figure 6

Heatmap of bacterial community composition at the species level in alpine modest primrose. (a) Bulk soil (Bs) and rhizosphere (Rs); (b) roots (Ro) compartments from wild and cultivated habitats. Only species with >0.1% relative abundance are shown

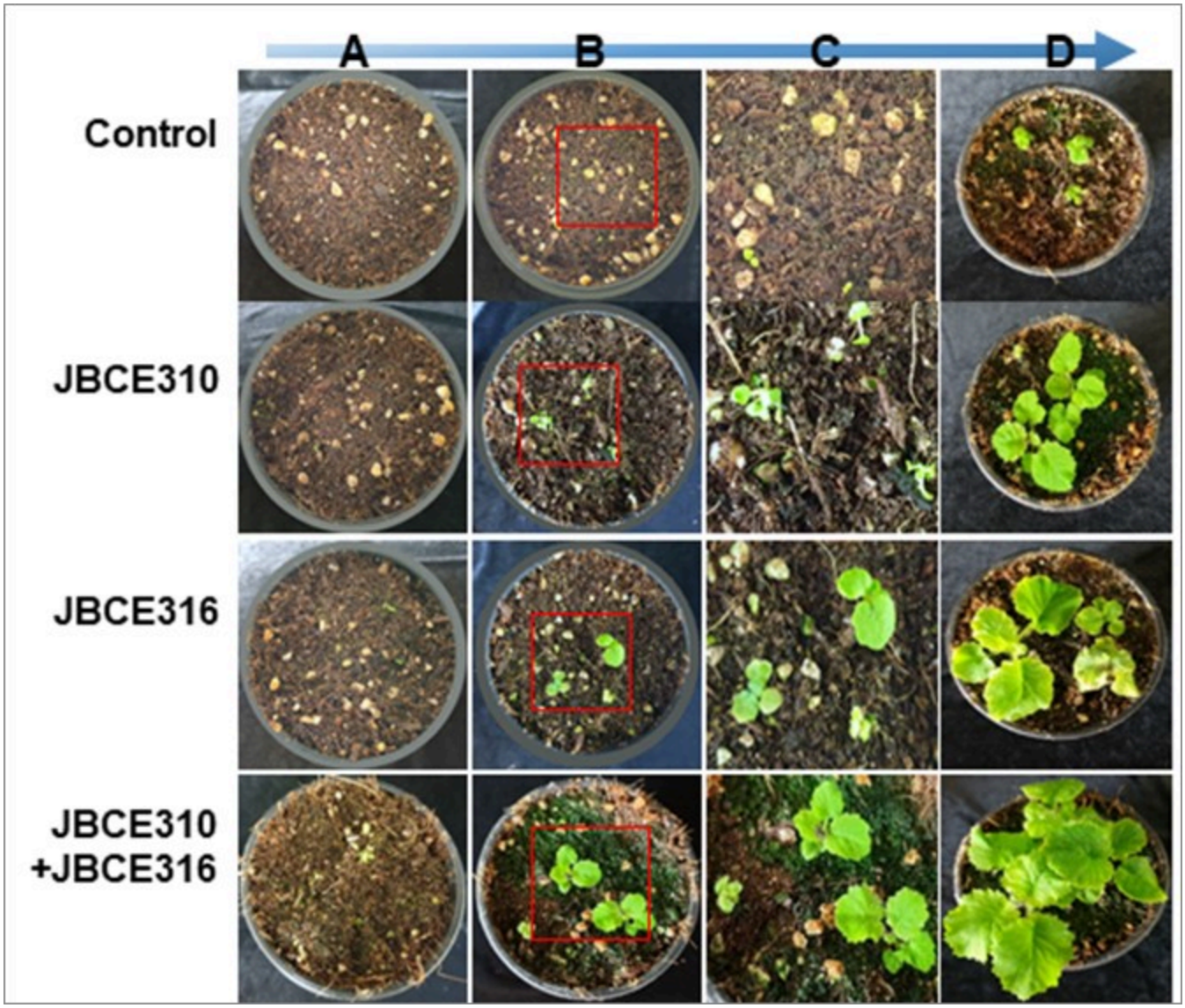

Figure 7

The effects of *Leifsonia lichenia* JBCE310, *Chryseobacterium piperi* JBCE316, and their combination on the germination and growth of *Primula malacoides*. Seeds were coated with each bacterium and sown in pots containing horticultural soil. (a) Germination percentage at 2 weeks after sowing. (b) Germination percentage at 5 weeks after sowing. (c) Magnified view of seedlings at 5 weeks. (d) Seedling growth at 10 weeks after sowing

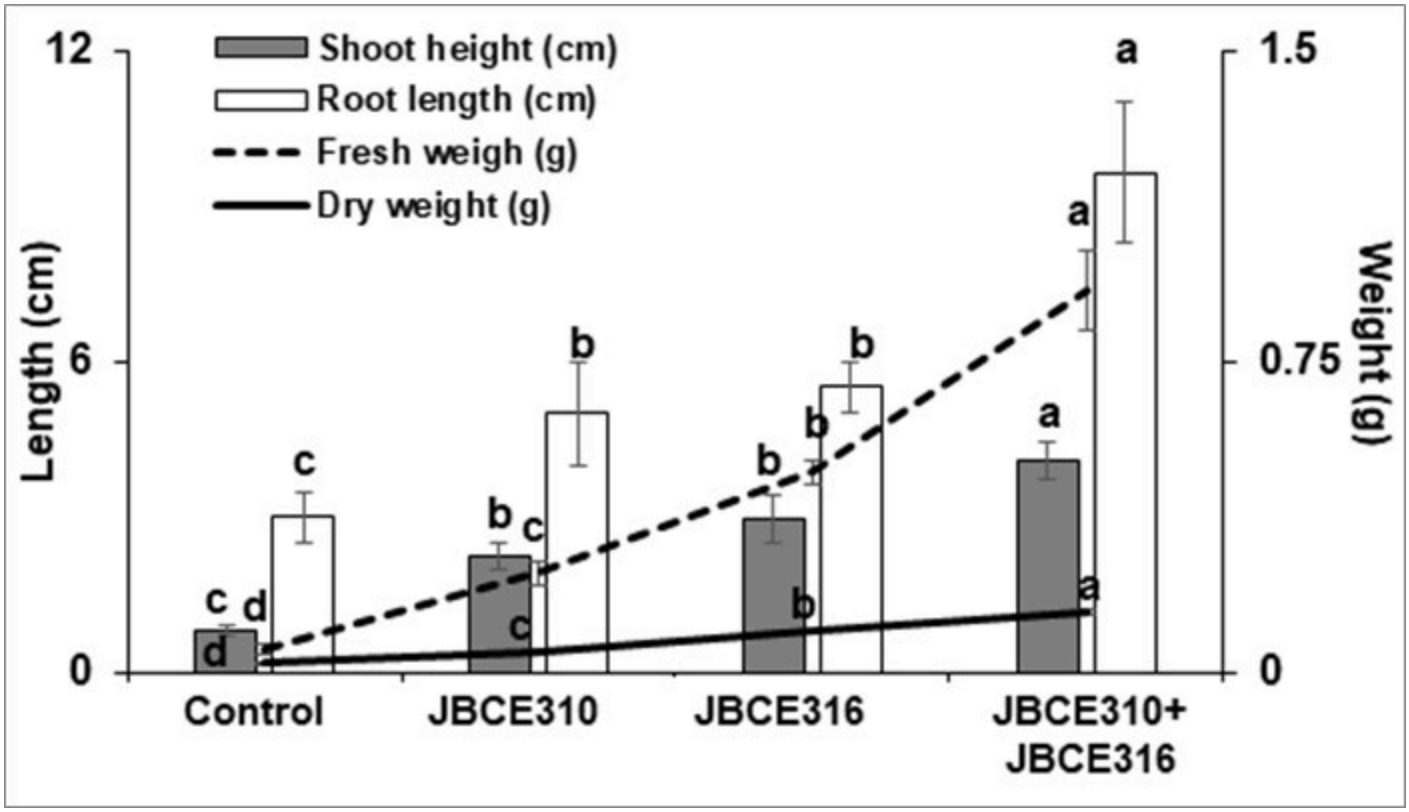

Figure 8

The effects of *Leifsonia lichenia* JBCE310, *Chryseobacterium piperi* JBCE316, and their combination on the growth of *Primula malacoides*. Seeds were treated with each bacterium and sown in horticultural soil. Shoot and root length, as well as fresh and dry weights, were measured 10 weeks after sowing. Values are means ± standard deviation. Different letters above bars indicate statistically significant differences ($P < 0.05$, ANOVA with post hoc test)

Supplementary Files

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