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Ecological niche and plantation composition differentially shape bacterial community composition and assembly across the root-soil continuum in *Populus simonii* plantations

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Abstract

Mixed plantations are widely established to restore degraded sandy landscapes, yet bacterial community patterns across roots and surrounding soils remain poorly understood. This study examined bacterial diversity, composition, inferred assembly patterns, environmental associations, and predicted functional potential in contrasting *Populus simonii* plantations. We analysed 60 composite samples from root (R), rhizosphere (RS), and non-rhizosphere soil (NRS) in four stands in the Kubuqi Desert, northern China. These comprised a *P. simonii* monoculture and mixtures with *Salix psammophila*, *Caragana korshinskii*, and *Pinus sylvestris* var. *Mongolica*, with five plots sampled per stand. Root communities exhibited lower richness and diversity and distinct taxonomic composition compared with the two soil compartments. PERMANOVA confirmed significant effects of both ecological niche (F = 24.995, p = 0.001) than stand type (F = 2.649, p = 0.005) on community composition, with niche showing the stronger association. Null-model classifications varied among sample groups. Homogeneous selection was the most frequently assigned category in most groups, whereas dispersal limitation accounted for a larger proportion of comparisons in the sampled *P. simonii* and *C. korshinskii* mixture. Co-occurrence patterns also varied across ecological niches and plantation types, with the rhizosphere showing the highest connectivity and mixed plantations generally exhibiting greater connectivity than the monoculture. Soil water content was strongly associated with bacterial community variation (r^2=0.815, p = 0.0005). Predicted KEGG and COG profiles varied among niche and plantation combinations, but no consistent functional response to mixed planting was observed. Together, these results reveal that bacterial taxonomic composition and community assembly responded to different ecological dimensions, ecological niche was more strongly associated with compositional differentiation, whereas assembly processes varied more clearly among the four sampled stands. This decoupling highlights the multidimensional organization of bacterial communities across the root-soil continuum in sandy plantations.

1. Introduction

Low organic matter content, weak soil structure, and poor retention of moisture and nutrients constrain plant establishment and ecological functioning in dryland sandy environments [1, 2]. Extensive afforestation programmes in northern China aim to rehabilitate degraded land and curb desertification [3, 4]. Populus simonii is commonly selected for these programmes owing to its fast growth and capacity to tolerate drought [5]. Nevertheless, prolonged cultivation in monospecific stands may impair soil quality, diminish biodiversity, and increase vulnerability to environmental stress, raising concerns about plantation sustainability [6, 7]. Mixed-species plantations represent an alternative strategy for restoration. Variation in plant traits leads to more diverse litter inputs, belowground root distributions, and canopy structures, which in turn affect soil moisture, organic matter storage, and nutrient availability [8–11]. Associated changes in microclimate and soil properties can further modify the habitats available to microorganisms [12]. Such environmental shifts may alter bacterial richness and composition, as well as the processes governing community assembly. Nevertheless, how bacteria respond to mixed planting across different belowground compartments in resource-limited sandy plantations remains poorly understood. Forest soil carbon storage can also reflect interactions between tree functional traits, diversity, and site properties [13]. Evidence from other forest systems indicates that changes in tree distributions can affect soil carbon storage [14]. Long-term ecosystem carbon exchange has also been examined across contrasting terrestrial ecosystems [15].

Mixing woody species with complementary trait profiles represents another route for plantation restoration. Through shifts in canopy architecture, belowground root placement, and the amount and quality of litterfall, mixed stands can regulate soil water status, nutrient supply, and organic matter sequestration [8–11]. Alterations in site climate and edaphic conditions may also reshape the niches available to microbes [12], thereby potentially influencing bacterial richness, taxonomic composition, and assembly processes inferred from community patterns. Yet, whether such bacterial responses differ between root and adjacent soil compartments is still not well understood in sandy plantations where water and nutrients are limiting. Tree diversity has been associated with microbial carbon-use efficiency in other forest settings [16]. Mixed planting can alter microbial carbon and phosphorus limitations [17]. The effects of nitrogen-fixing companion species on soil multifunctionality may depend on climatic context [18].

Microbial assemblages are central to forest ecosystem functioning, driving nutrient cycling, litter decomposition, organic substrate transformation, and plant–soil feedbacks [19, 20, 21]. In sandy habitats, limited water retention and small nutrient pools constrain microbial growth and substrate use, rendering bacterial communities highly responsive to resource availability (Fuentes et al., 2020). Root exudates add carbon and modify local soil chemistry, generating microhabitats that differ from the surrounding bulk soil [22, 23]. Such environmental heterogeneity may be accompanied by pronounced bacterial differentiation between roots and adjacent soil compartments. In stands comprising multiple woody species, shifts in vegetation composition can further affect resource inputs and belowground habitat conditions. Investigating bacterial patterns in these plantations therefore requires an integrated consideration of compartment differences and stand composition. Microorganisms can contribute to terrestrial feedbacks associated with climate change [24]. Microbial nitrogen- and phosphorus-use efficiencies also vary across environmental settings [25]. Substrate stoichiometry has been linked to microbial metabolic pathways and necromass formation [26].

Plant effects and resource availability are spatially heterogeneous across internal root habitats, the rhizosphere, and the surrounding bulk soil, which together constitute a belowground continuum [27, 28]. Bacteria inhabiting these compartments are exposed to contrasting carbon sources and environmental constraints [22]. Because root exudation is continuous, the rhizosphere represents a zone where plant activity and soil properties interact, whereas habitats beyond the immediate influence of roots are more directly shaped by moisture and nutrient conditions [29, 30]. Such contrasts may yield distinct bacterial assemblages; however, taxonomic differentiation alone does not identify the mechanisms responsible for

community change. Assembly frameworks integrate both selective pressures imposed by hosts and environmental conditions and stochastic processes related to dispersal and demographic drift [19]. Evaluating patterns consistent with these processes can aid in interpreting bacterial turnover among belowground compartments, even without direct measurement of the processes themselves. Mixed-species stands may modify such patterns by altering resource distribution and habitat variability, potentially producing different responses depending on the identity of companion species. Nonetheless, research has primarily focused on bacterial richness and taxonomic profiles. How compositional variation corresponds to inferred assembly patterns, bacterial abundance associations, and predicted functional capacities across root and soil habitats remains poorly resolved, especially in plantations established on sandy substrates. Microbial niche construction provides a broader conceptual perspective on habitat-associated metabolism [31]. Nutrient limitation has been associated with microbial niche differentiation during ecological succession [32]. Soil pore structure can influence microbial community composition and metabolism [33]. Spatial heterogeneity in resource availability has been linked to bacterial assembly in other ecosystems [34].

We examined bacterial assemblages associated with *Populus simonii* We examined bacterial assemblages associated with Populus simonii in the Kubuqi Desert, northern China, comparing a monospecific stand with mixtures containing *Salix psammophila*, *Caragana korshinskii*, and *Pinus sylvestris* var. *mongolica* in the Kubuqi Desert of northern China. Sampling encompassed roots and both rhizosphere and non-rhizosphere soils. Soil property measurements and sequencing data were combined with null-model inference, exploratory network analysis, and functional prediction to characterise multiple aspects of bacterial community organisation. Our objectives were to (1) determine how ecological niche and plantation type influence bacterial diversity and community composition; (2) evaluate whether community assembly processes and co-occurrence patterns vary across ecological niches and plantation types; and (3) identify the soil environmental factors associated with bacterial community variation and characterize predicted functional potential. We hypothesized that different dimensions of bacterial community organization would respond differently to ecological niche and plantation composition: (1) ecological niche would be more strongly associated with bacterial community composition because of increasing plant mediated filtering toward the root compartment; (2) plantation composition would more clearly modify community assembly processes and cooccurrence patterns, with responses differing among companion species combinations; and (3) soil moisture and nutrient availability would be closely associated with bacterial community variation across the root soil continuum. By comparing taxonomic patterns with null-model classifications, we sought to assess whether these attributes displayed corresponding variation across belowground habitats and sampled plantation contexts. Because each plantation type was represented by a single stand, the study provides site-specific comparisons and hypotheses for further testing rather than an independently replicated assessment of companion-species effects.

2. Materials and Methods

2.1 Study site

Sampling took place in Dalate Banner, Ordos City, within the Inner Mongolian portion of the Kubuqi Desert (Fig. 1). The site is managed by the Ordos Forestation General Farm and lies at roughly 1043 m above sea level. A temperate continental monsoon regime produces pronounced seasonal variation and dry conditions. Annual temperature averages 7.5–9.8°C, and mean yearly precipitation totals approximately 505 mm. Sunshine duration reaches about 2900–3200 h annually, with 130–140 frost-free days. Sandy loam predominates; its weakly consolidated structure makes it susceptible to erosion. Common native plants include *P. simonii*, *Salix psammophila*, *C. korshinskii*, *P. sylvestris* var. *mongolica*, *Artemisia ordosica*, *Hedysarum scoparium*, *Polygonatum sibiricum*, and *Agriophyllum squarrosum* (The plant species investigated in this study were formally identified by Xinghua Zhao. No voucher specimens were deposited in a publicly accessible herbarium.).

2.2 Sample collection

Sampling was conducted in August 2025 in plantations administered by the Ordos Forestation General Farm, Dalate Banner. According to available records, no other land use had been documented prior to afforestation. Four plantation types were selected for this study, including a poplar (*P. simonii*) monoculture as the control (CK) and three mixed-species, stands-poplar with *S. psammophila* (PS), poplar with *C. korshinskii* (PC), and poplar with *P. sylvestris* (PZ). All stands were initially planted at a density of 1200 individuals ha^{-1}. In each mixture, poplar and its companion species were planted at a 1:1 ratio (600 trees ha^{-1} each). Species-specific planting densities and basal areas were compiled from the farm's afforestation records (Table 1).

Table 1
Basic information for the sample sites.

Type		Stand age/a	Mean height/m	Mean DBH/m	Density (trees/ha)	Understory vegetation coverage /%
P		34	8.01 ± 0.99	30.13 ± 6.58	1200	46
PS	P	36	12.04 ± 2.78	37.27 ± 6.11	600	42
	S	28	3.29 ± 0.33	-	600	
PC	P	36	8.46 ± 0.82	44.55 ± 5.99	600	40
	C	28	1.81 ± 0.17	-	600	
PZ	P	35	9.15 ± 1.03	35.9 ± 2.42	600	51
	Z	20	4.57 ± 0.68	-	600	

Note: DBH represents diameter at breast height. P represents *P. simonii* monoculture plantation, PS represents mixed plantations of *P. simonii* and *S. psammophila*, S represents *S. psammophila* plantations, PC represents mixed plantations of *P. simonii* and *C. korshinskii*, C represents *C. korshinskii* plantations, PZ represents mixed plantations of *P. simonii* and *P. sylvestris*, Z represents *P. sylvestris* plantations. The same below.

For each plantation category, only one stand was available. Within every stand, five plots of 20 m × 20 m were established, resulting in 20 sampling plots in total. This design accounted for spatial variability within stands but did not offer independent replication at the plantation-category level. In each plot, three poplars with similar height and diameter at breast height were selected. These trees provided material that was combined into plot-level composite samples; individual trees were not treated as separate analytical replicates. Only *P. simonii* was targeted for sampling, and bacteria associated with the companion species were not examined. Consequently, the comparisons made here pertain to bacterial communities associated with poplar across the four stands investigated.

Surface litter was first cleared, after which soil was collected from the upper 20 cm within a 1 m radius of each selected poplar trunk. A sterilised trowel was employed to recover non-rhizosphere material (NRS) from the exposed profile, with visible fine roots excluded. Fine roots of P. simonii less than 2 mm in diameter were then carefully excavated. Loosely adhering particles were removed, and the soil closely attached to the roots was collected as the rhizosphere fraction (RS). The remaining fine-root material was designated as the root fraction (R). For each plot, samples from the three selected trees were pooled by compartment, producing separate composite samples for R, RS, and NRS. The final dataset consisted of 60 composites, covering three compartments in each of five plots across four stands. Since compartment samples from a given plot shared the same sampling location, they were not independent; pooling tree subsamples did not generate additional stand-level replicates. All composites were kept chilled during transport to the laboratory. Root and soil portions intended for DNA extraction were stored at -80°C. The remaining soil was air-dried and passed through 1 mm and 0.149 mm sieves before physicochemical determination.

2.3 Soil property determination

Soil physicochemical conditions were characterised using standard analytical procedures. pH was measured with a PHS-3E meter equipped with a combination electrode (INESA, Shanghai, China) in suspensions at a soil-to-water ratio of 1:2.5 (w/v). Gravimetric soil water content (SWC) was obtained from the mass loss of cutting-ring samples after oven drying. Organic matter (SOM) was measured by potassium dichromate oxidation under external heating, while flame photometry was used to quantify total (TK) and available (AK) potassium. After digestion, total phosphorus (TP) was determined colorimetrically using molybdenum-antimony reagents. Available phosphorus (AP) was quantified by the same colour-development method following sodium bicarbonate extraction.

2.4 DNA extraction and sequencing

DNA was isolated from rhizosphere and bulk-soil samples with a PowerSoil® kit supplied by MoBio Laboratories (Carlsbad, CA, USA). Extract quality was checked on 1% agarose gels, while DNA concentration was measured with a NanoDrop 2000 instrument (Thermo Scientific; Wilmington, DE, USA). Amplification targeted the bacterial 16S rRNA V4 region, yielding products approximately 290 bp long with primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [35]. Each PCR mixture had a final volume of 50 μL and comprised 2× Premix Taq (25 μL), each primer at 10 μM (1 μL each), template DNA (50 ng), and sufficient nuclease-free water to reach the required volume. Reactions were run on a Biometra TOne 96G thermocycler manufactured by Analytik Jena. Thermal cycling began with 5 min at 94°C, followed by 30 amplification cycles comprising denaturation for 30 s at 94°C, annealing for 30 s at 52°C, and extension for 30 s at 72°C. A 10 min extension at 72°C preceded a final hold at 4°C. Amplification products were examined on 1.5% agarose gels, and those displaying the expected band size were retained. Band intensities guided equimolar pooling, after which the pooled amplicons were purified with an E.Z.N.A.® gel extraction kit from Omega Bio-Tek (Norcross, GA, USA). Libraries were prepared according to standard procedures. Guangdong Magigene Biotechnology Co., Ltd., based in Guangzhou, China, performed Illumina sequencing with 250 bp paired-end reads (PE250). Raw sequence data were submitted to the NCBI repository under accession PRJNA1435822.

Sequence processing was initiated with quality filtering in fastp version 0.14.1, using a four-base sliding window and an average quality threshold of at least 20. Primers and adapters were then removed from the retained reads with Cutadapt. Read pairs were merged using USEARCH version 10

provided that their overlapping regions were at least 16 bases long and contained no more than five mismatches; pairs failing these criteria were discarded. A second round of fastp filtering was performed to eliminate low-quality merged tags. The retained sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using UPARSE. Taxonomic assignment of OTU representatives was performed against SILVA release 138, with assignments retained at a confidence level of 0.8. Prior to downstream analysis, sequences classified as chloroplasts or mitochondria were removed, as were those without kingdom-level assignment. After discarding low-abundance and spurious entries, the OTU abundance matrix used in subsequent analyses was obtained.

2.5 Statistical analysis

Statistical procedures were carried out in R (version 4.4.1) and SPSS (version 26.0). The sampling campaign generated 60 composite samples, corresponding to four stands, each containing five plots, with three ecological compartments sampled per plot. Values for R, RS, and NRS were matched according to their plot of origin. Because material from three trees was pooled separately within each compartment, individual trees were not treated as independent analytical replicates. Given that each plantation category was represented by a single stand, stand identity was confounded with plantation composition; conclusions were therefore restricted to the sites investigated. Before subsequent analyses, sequencing libraries were rarefied to a common read count to reduce differences attributable to unequal sampling depth.

To ensure comparability, all sequencing libraries were rarefied to an identical number of reads prior to further analyses. Bacterial alpha diversity was assessed with the Chao1 richness estimator and the Shannon index. An initial two-factor ANOVA tested for effects of compartment, sampled stand, and the niche×stand interaction. This model ignored the dependency structure introduced by observations collected from a common plot. Therefore, the resulting *p*-values were considered exploratory and were neither treated as evidence of overall plantation-composition effects nor used to assess the relative contribution of stand and compartment variation.

Bray-Curtis dissimilarities were used as input for hierarchical clustering and two ordination techniques: principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS). An initial PERMANOVA, performed with the vegan package, was based on 999 permutations. Nevertheless, the described workflow did not state how within-plot pairing was retained under permutation, and no assessment of group dispersion was provided. Thus, the obtained *p*-values were not accepted as sufficient evidence for effects attributable to ecological compartment or plantation composition. Clustering and ordination results were used solely to depict community patterns within the sampled dataset. Relative abundances were converted to Z-scores for heatmap visualisation.

To characterise inferred assembly patterns, a null-model framework combining βNTI (the β-nearest taxon index) with RCBray, a Raup-Crick metric based on Bray-Curtis dissimilarity, was employed. Within each compartment and surveyed stand, the proportion of sample pairs assigned to each process category was quantified. These proportions reflect model-derived classifications rather than empirical measurements of process rates or causal relationships. Sample pairs sharing a common sample were treated as dependent observations and therefore not regarded as independent replicates. Consequently, βNTI values and category frequencies were interpreted descriptively only. Direct measurements were not obtained for environmental selection, organism dispersal, or putative plant-associated mechanisms.

Redundancy analysis (RDA) was used to explore how soil physicochemical properties related to bacterial composition. In addition, Mantel tests assessed the concordance between community dissimilarity and variation in soil attributes. Neither approach was intended to infer causality; both served as exploratory evaluations of association. Several constraints imposed by the sampling design limited interpretation: within a given plot, samples from different compartments were not independent, and plantation categories were not replicated across separate stands.

Co-occurrence networks were employed to investigate relationships among bacterial abundances at two levels of grouping. Compartment-specific networks were based on 20 observations pooled across all stands, whereas stand-specific networks comprised 15 observations derived from three niches sampled across five plots. Consequently, the stand-specific networks included compartment observations that were related because they originated from the same plots. An edge was retained provided that the Spearman correlation coefficient met $|r|>0.6$ and $p < 0.05$. Network visualisation and computation of descriptive topological metrics were performed with Gephi version 0.10.1. These metrics included node and edge counts, modularity, mean degree, mean path length, and mean clustering coefficient, as well as the proportions of positive and negative associations.

PICRUSt2 was applied to bacterial 16S rRNA sequences to predict functional capacities, which were grouped into KEGG pathways at level 2 and COG classifications. Retained categories underwent standardisation before being displayed as heatmaps. The resulting estimates reflected inferred gene content, without directly quantifying transcription, enzymatic activities, or ecological process rates. Heatmaps served as descriptive comparisons between sample groups and did not demonstrate improvements in nutrient turnover, persistence of functional performance, or redundancy among microbial functions.

3. Results

3.1 Soil property

The stands sampled differed in soil physicochemical properties (Table 2). Soil pH varied from 7.80 to 7.92, indicating slightly alkaline conditions, with no significant differences detected among groups ($p > 0.05$). Marked variation among stands was observed for SWC, SOM, TP, TK, and AK ($p < 0.05$). The highest SWC and SOM occurred in PC, whereas PZ showed the greatest TP, AP, and AK values ($p < 0.05$). With the exception of TK, nutrient concentrations were generally higher in the mixed poplar stands than in the monoculture.

Table 2
Soil properties of poplar in monoculture and mixed stands.

Plot	pH	SWC/%	SOM/g·kg^{-1}	TP/g·kg^{-1}	TK/ g·kg^{-1}	AP/ mg·kg^{-1}	AK/ mg·kg^{-1}
P	7.80 ± 0.12a	4.10 ± 0.07c	3.75 ± 0.67b	0.15 ± 0.00b	7.82 ± 0.08a	2.68 ± 0.31b	155.4 ± 3.21c
PS	7.92 ± 0.08a	4.35 ± 0.10b	3.61 ± 0.21c	0.14 ± 0.00c	4.88 ± 0.13c	2.74 ± 0.10b	185.6 ± 4.88b
PC	7.82 ± 0.05a	4.87 ± 0.12a	3.92 ± 0.37a	0.12 ± 0.01d	4.26 ± 0.23d	2.53 ± 0.06b	132.2 ± 1.64d
PZ	7.88 ± 0.08a	3.42 ± 0.06d	3.80 ± 0.39b	0.28 ± 0.01a	6.60 ± 0.40b	3.52 ± 0.21a	225.4 ± 7.53a
F	2.495	323.072	15.463	715.694	294.940	21.070	149.471
p	0.114	**1.1624×10^{-13}**	**0.000183**	**2.2299×10^{-16}**	**2.3652×10^{-13}**	**0.000033**	**4.4372×10^{-11}**

Note: Different letters indicate significant differences among treatments ($p < 0.05$), while bold values highlight statistically significant result

3.2 Bacterial diversity and community composition

Sequencing yielded 3,712,655 reads passing quality control, averaging 61,878 ± 3,643 per sample (Table S1). Clustering at a similarity threshold of 97% resolved 12,368 bacterial OTUs. Rhizosphere (RS) and non-rhizosphere (NRS) communities shared more OTUs with one another than either shared with roots (R), and OTU counts were greatest in NRS (Fig. 2A). Across the four stands, 4,867 OTUs occurred in every plantation category; PC and PZ each harboured more stand-specific OTUs than the P monoculture (Fig. 2B). Rhizosphere (RS) and non-rhizosphere (NRS) communities shared more OTUs with one another than either shared with roots (R), and OTU counts were greatest in NRS (Fig. 2A). Across the four stands, 4,867 OTUs occurred in every plantation category; PC and PZ each harboured more stand-specific OTUs than the P monoculture (Fig. 2B). Compartment-related patterns were evident in bacterial α diversity (Fig. 2C, D). The initial two-factor ANOVA reported differences among niches for both Shannon diversity and Chao1 richness ($p < 0.05$). Between-stand variation was detected for Chao1 ($p = 0.033$), whereas Shannon values did not differ significantly ($p > 0.05$, Table S2). Root samples had lower values for both indices than either soil compartment across all stand categories. In NRS, PC exceeded P for both richness and diversity, with the original comparisons reporting $p < 0.05$ (Fig. 2C-D).

Ordinations derived from Bray–Curtis dissimilarities revealed compartment-related patterns in bacterial composition (NMDS and PCoA; Fig. 3A, S1). R occupied a distinct region of ordination space, while RS and NRS samples overlapped more extensively. PCoA dimensions 1 and 2 accounted for 44.78% and 9.20% of overall variation, respectively. The initial PERMANOVA returned $F = 24.995$ and $p = 0.001$ for ecological niche, compared with $F = 2.649$ and $p = 0.005$ for stand type. Clustering likewise separated roots from both soil compartments, with sample groups largely corresponding to niche identity (Fig. 3B).

Taxonomic annotation resolved 37 bacterial phyla and 632 genus-level groups, including unclassified taxa. Actinobacteriota and Proteobacteria predominated, representing 26.48–41.39% and 21.69–56.29% of sequences, respectively, and jointly comprising most reads (Fig. 4A). Actinobacteriota had higher relative representation in RS, while Proteobacteria was more prevalent in NRS. Variation between sampled stands was comparatively modest, although the dominant phylum proportions in PC and PZ differed from those in P (Fig. 4A, B). Variation between sampled stands was comparatively modest, although the dominant phylum proportions in PC and PZ differed from those in P (Fig. 4A, B).

Genus-level profiles also displayed compartment-specific patterns. Both soil fractions contained greater relative abundances of *Bacillus* and *Streptomyces* than R, whereas *Pseudomonas* was relatively enriched in R. Compared with P, the PS and PC stands showed higher proportions of *Bacillus* but lower proportions of *Streptomyces*. *Pseudomonas* decreased in PS and PC but increased in PZ relative to P. Additional prominent genera, including *Arthrobacter*, *Sphingomonas*, and *Nocardioides*, also varied among niches and stand types (Fig. 4C, D).

3.3 Bacterial community assembly and co-occurrence patterns

Null-model outputs showed variation between compartments and surveyed stands in both βNTI values and assembly-category frequencies (Fig. 5). The R, RS, and NRS groups exhibited overlapping βNTI distributions, whereas stand-specific distributions displayed differing patterns. Most groups contained the greatest fraction of sample pairs assigned to homogeneous selection, alongside a sizeable fraction attributed to dispersal limitation. In PC, assignments to dispersal limitation outnumbered those to homogeneous selection, and undominated processes represented a comparatively larger share. These findings characterise model-derived assembly patterns rather than demonstrating variation in empirically measured selection or dispersal.

Descriptive network metrics varied between belowground compartments and surveyed stands (Fig. 6; Table 3). RS comprised 138,381 links, corresponding to a mean degree of 301.812, while R comprised 2,288 links and a mean degree of 210.947. Node counts peaked at 1,147 in NRS.

Networks representing the sampled mixtures displayed greater edge counts and mean degrees than the monoculture network, with PC reaching 150,181 edges, the highest stand-level total. Across all networks, positive correlations represented 62.88–90.18% of detected associations.

Table 3
Descriptive topological properties of bacterial co-occurrence networks across niches and sampled stands.

Topological feature	Ecology niches			Stand types			
	R	RS	NRS	P	PS	PC	PZ
Node	418	917	1147	871	917	893	947
Edge	2288	138381	52357	97024	138381	150181	110377
Modularity	0.561	0.181	0.316	0.315	0.181	0.131	0.281
Average degree	210.947	301.812	227.155	291.294	301.812	336.352	233.109
Average path length	4.043	1.987	2.756	2.054	1.987	1.881	2.064
Average clustering coefficient	0.388	0.705	0.496	0.669	0.705	0.697	0.636
Positive	1585(69.28%)	111881(80.85%)	32922(62.88%)	87496(90.18%)	111881(80.85%)	115895(77.17%)	85619(77.57%)
Negative	703(30.72%)	26500(19.15%)	19435(37.12%)	9528(9.82%)	26500(19.15%)	34286(22.83%)	24758(22.43%)

3.4 Environmental drivers of bacterial community variation

RDA indicated relationships between bacterial composition and soil physicochemical conditions (Fig. 7A, S2). SWC was strongly associated with community variation (r^2=0.815, p = 0.0005), with its vector directed predominantly toward positive RDA1 scores. In contrast, vectors for AP, TP, TK, and AK pointed toward the negative side of this axis (Table S3). SOM corresponded chiefly to the second ordination dimension, whereas pH displayed a weaker association with community patterns. Sample groups occupied distinct regions of ordination space, and the initial PERMANOVA returned F = 1.177 and p = 0.003 (Fig. 7A). Compartment-specific RDA suggested that environmental associations differed in direction and magnitude between niches (Fig. S2).

Mantel tests further showed that bacterial and environment relationships differed among ecological niches (Fig. 7B). The original tests detected significant relationships with several variables in R and RS, while fewer associations were observed in NRS. SWC and nutrient-related variables, particularly P and K availability, showed recurrent associations with bacterial communities across the root-soil continuum. This correspondence with RDA highlighted moisture and nutrient conditions as prominent environmental correlates. Associations also differed between surveyed stands (Fig. 7C); in PZ, significant relationships were reported for SWC, TP, AP, TK, and AK. Together, the two analytical approaches suggested recurring links between bacterial variation and soil resource conditions.

3.5 Predicted functional profiles of bacterial communities

Functional predictions generated by PICRUSt2 displayed variation between compartments and sampled stands (Fig. 8). At the KEGG level, the predicted functions were mainly assigned to Metabolism, Genetic Information Processing, Cellular Processes, and Environmental Information Processing (Fig. 8A). Their standardized relative abundances varied among sample groups, although no single functional category showed a consistent directional pattern across all ecological niches or plantation types. Clustering also revealed contrasting predicted profiles among compartment–stand combinations.

Predicted COG profiles likewise differed between sample groups (Fig. 8B). Assignments covered diverse metabolic and cellular functions, including transport and metabolism involving carbohydrates, amino acids, nucleotides, lipids, inorganic ions, and coenzymes. Additional categories included energy conversion and production, signal transduction, transcription, translation, replication and repair, and biogenesis of cell envelopes, membranes, and walls. Category proportions varied between compartments and surveyed stands, with some functions exhibiting contrasting distributions across groups.

4. Discussions

3.1 Niche differentiation shapes bacterial community structure

Bacterial communities exhibited marked differentiation between root and adjacent soil habitats. Root samples (R) harboured less diverse communities with lower richness than the rhizosphere (RS) and non-rhizosphere soil (NRS), and their taxonomic profiles occupied separate regions in ordination space. Although the initial PERMANOVA produced a higher F statistic for niche than for sampled stand, this comparison cannot establish their relative importance unless within-plot dependence and multivariate dispersion are taken into account. The root-soil continuum

observed here is consistent with progressively stronger selection during microbial recruitment from soil into root tissues [36, 37]. Host defence mechanisms, antimicrobial compounds, and limited resource availability may restrict colonisation and select for bacteria adapted to internal root habitats [38, 39]. The surrounding soils provide a broader spectrum of microhabitats, which may support richer bacterial assemblages. These findings align with the compartment differentiation predicted by our first hypothesis, yet they neither directly demonstrate host filtering nor establish that niche effects are stronger than those of plantation composition.

Taxonomic profiles further reflected this niche differentiation. Actinobacteriota and Proteobacteria dominated the dataset overall, yet their relative proportions differed among belowground habitats. Actinobacteriota were more represented in RS, whereas Proteobacteria were relatively more abundant in NRS. These widespread soil phyla include organisms with diverse capacities for carbon and nutrient acquisition, and their constituent taxa vary considerably in ecology [40, 41]. Shifts in their proportions therefore cannot be reduced to a simple replacement of copiotrophic organisms by oligotrophic ones, or vice versa [42]. At the genus level, *Bacillus*, *Streptomyces*, and *Pseudomonas* also showed distinct distributions among niches. *Bacillus* and *Streptomyces* were more abundant in RS and NRS, whereas *Pseudomonas* was relatively enriched in roots. These distributions are compatible with habitat-associated filtering between roots and surrounding soils. Nevertheless, relative-abundance patterns alone cannot establish either the filtering mechanisms or the ecological roles of particular genera [43, 36]. Variation among the stand types accompanied the nich-related differences in bacterial composition. Dominant taxa differed in their relative representation between PC, PZ, and P, while NRS from PC contained greater bacterial richness than those from P. Differences in litter supply, root distribution, and resource conditions offer potential explanations, as species mixtures may create a wider range of microbial habitats [44, 9]. The leguminous companion *C. korshinskii* could also contribute to nutrient conditions associated with the richness observed in PC [45, 9]. Actinobacteria include taxa adapted to harsh environmental conditions [46]. Rhizosphere-associated bacterial communities have also been investigated in disease-affected crop systems [47].

3.2 Plantation composition modifies bacterial community assembly and cooccurrence patterns

Assessing microbial organisation in dryland forests requires examining vegetation composition alongside variation in belowground habitats. Limited nutrients and fluctuating environmental conditions restrict biological activity on sandy substrates, making microbial contributions to organic matter transformation and nutrient turnover particularly relevant [48, 49]. Although plant identity and local habitat conditions are recognised influences on microbial assemblages, their respective associations with community patterns remain poorly resolved in mixed plantations established for restoration. Our observations revealed compositional separation between root-associated bacteria and communities inhabiting rhizosphere and bulk soils. Differences among surveyed stands also occurred in taxonomic profiles, exploratory abundance associations, and predicted functional capacities. However, these patterns do not establish that compartment effects outweigh stand differences or that species mixing caused the observed variation. The root-soil continuum is compatible with selective recruitment under host-associated conditions, while soil heterogeneity offers a potential explanation for variation outside roots. Neither mechanism was directly tested, so these interpretations provide hypotheses for further investigation.

Differences between root and soil communities are compatible with a sequence of habitat filters, although the relative roles of host selection and environmental conditions were not directly established. Bacterial diversity was lowest within roots, potentially reflecting selective pressures associated with plant defences and access to carbon resources [50]. In root tissues, the restricted availability of easily accessible carbon substrates, together with antimicrobial compounds released by plants, can limit microbial colonization and favor a subset of specialized taxa adapted to host associated conditions [51, 39, 38]. Rhizosphere bacteria, by comparison, encounter root-derived compounds that can create locally enriched resource conditions, offering a possible explanation for their greater observed diversity. Microbial activity was not measured, however, and cannot be inferred from diversity alone. Among the sampled mixtures, PC had the greatest bacterial richness in non-rhizosphere soil. Nitrogen supplied through association with the leguminous shrub *C. korshinskii* could potentially ease resource constraints and support bacterial establishment [45, 9]. This explanation remains untested because nitrogen fixation was not quantified, and companion-species influences could not be distinguished from other stand characteristics. The findings therefore describe bacterial differentiation across belowground habitats and surveyed stands without demonstrating that plant-mediated filtering predominated or that mixed planting caused changes in diversity. Studies of crop establishment under saline conditions likewise report relationships between management and soil bacterial diversity [52]. Long-term warming studies have documented differences between rhizosphere and non-rhizosphere microbial communities [53].

The assembly classifications differed between sampled stands, whereas βNTI values showed considerable overlap across belowground compartments. Homogeneous selection accounted for the largest fraction of comparisons in most groups, while dispersal limitation was more prevalent in PC. These observations can be considered within frameworks that incorporate selective and stochastic components of microbial assembly [19, 54]. However, the assigned fractions quantify outcomes of the null-model procedure; they do not measure process rates or identify the causes of community turnover. Differences in root characteristics, litter deposition, and soil conditions offer possible explanations for variation in local filtering and habitat structure [12]. Establishing these pathways would require measurements beyond those obtained here. Interpretation is further constrained by the model assumptions and the dependence between comparisons involving shared samples. Moreover, the absence of multiple independent stands within each plantation category prevents separation of vegetation composition from stand-specific conditions. Accordingly, the analysis provides a descriptive account of inferred assembly patterns, with companion-species mechanisms remaining hypotheses for subsequent testing. Experimental environmental changes have been associated with distinct microbial community responses [55]. Responses to

nitrogen fertilization can differ across microbial groups [56]. Soil pH and phosphorus have been associated with bacterial assembly in restored mining landscapes [57].

Co-occurrence networks summarised correlations between bacterial abundances across the sampled habitats. Under the chosen inference thresholds, RS had the highest mean degree, R exhibited the fewest connections, and NRS included the most nodes. These contrasting rankings suggest that taxonomic richness and inferred network topology capture different attributes of the dataset. Concentrated root-derived substrates and spatially clustered microorganisms could generate shared abundance responses in the rhizosphere (Bai et al., 2021; Ling et al., 2022), although this explanation was not directly tested. Networks representing the three sampled mixtures contained more connections than the monoculture network, with PC having the greatest edge count and mean degree. Previous work has associated vegetation diversification with changes in microbial network structure [58], but the present comparisons do not establish a mixing effect. Limited sample numbers, variation in node counts, and the absence of robustness assessments restrict interpretation of these descriptive contrasts. Correlated increases in abundance may reflect common habitat preferences or responses to resource conditions, rather than cooperation between organisms [59]. Similarly, a densely connected network does not demonstrate resistance to disturbance or recovery capacity; such properties require temporal observations or perturbation experiments [60, 58]. The observed topology therefore provides hypotheses about bacterial abundance associations, without establishing stronger interactions or improved ecosystem stability in mixed stands. Proteomic studies of individual bacteria examine cellular stress responses at a different biological scale from the community-level correlations analysed here [61]. Previous research in desert plantations has characterised ectomycorrhizal communities associated with Mongolian pine [62].

3.3 Soil environmental factors regulate bacterial community variation and functional responses

Soil conditions provide an important context for interpreting bacterial compositional variation [63]. Moisture, organic matter, and phosphorus and potassium availability may influence microbial habitats through their effects on resource supply [64]. Water scarcity is particularly relevant on sandy substrates, where weak moisture retention can restrict solute transport and microbial metabolism [65]. Changes in hydration may consequently affect substrate access and enzyme-mediated transformations, offering a plausible explanation for moisture-associated community differences [66, 67]. Soil environmental conditions were closely associated with bacterial community variation across plantation types and ecological niches. RDA identified SWC as the strongest measured correlate of bacterial community variation, while phosphorus- and potassium-related variables and SOM were also associated with community distribution. Mantel tests further showed that the strength of these relationships depended on ecological niche and plantation type, with multiple environmental associations detected in R and RS and particularly broad associations observed in PZ. These results support our third hypothesis that soil moisture and nutrient availability are closely linked to bacterial community variation along the root-soil continuum. Water availability is particularly important in sandy soils because their low water-holding capacity can constrain substrate diffusion, microbial growth, and metabolic processes [67, 65, 66]. The strong relationship between SWC and bacterial composition therefore suggests that even relatively small differences in soil moisture may contribute to microbial habitat differentiation in this water-limited ecosystem. Plantation specific changes in soil properties provide additional environmental context for the observed microbial patterns. PC had the highest SWC and SOM, whereas PZ had the highest TP, AP, and AK, demonstrating that different companion species altered the soil environment in distinct ways. These differences parallel the stand-dependent variation observed in bacterial composition and assembly, but should not be interpreted as direct causal links because the RDA and Mantel analyses quantify associations rather than causal pathways. Early ecosystem succession can be accompanied by shifts in soil microbial communities and their functions [68]. Organic-matter composition and bacterial communities have also been examined under biochar amendments [69]. Responses of bacterial communities and nutrient turnover to contamination have been examined in other soils [70].

Variation in nutrient supply offers another possible explanation for bacterial compositional differences. In resource-limited soils, phosphorus scarcity may restrict microbial proliferation and influence nutrient-acquisition strategies, whereas organic matter supplies carbon and participates in nutrient transformations [71, 49, 72]. Maintaining intracellular potassium balance supports enzyme activity and osmotic adjustment, potentially making K availability relevant under dry conditions [73, 74]. The repeated correspondence between bacterial profiles and TP, AP, TK, and AK places nutrient supply alongside moisture as part of the environmental setting of these communities. Nevertheless, the observed associations do not establish that any particular soil variable controls assembly. Relationships identified for taxonomic composition cannot, by themselves, explain βNTI values or null-model category proportions. Evaluating those connections requires analyses that explicitly relate environmental variation to the corresponding assembly metrics. Long-term fertilization can affect diazotrophs and nitrogen fixation [75]. Studies in hyperarid desert soils have also linked physicochemical conditions with microbial community composition [76].

PICRUSt2 estimates potential gene content by combining marker-gene information with reference-genome data; it does not quantify metabolic performance [77]. The predicted KEGG and COG profiles differed between niche-stand groups, yet the sampled mixtures showed no uniform directional response. Because the heatmaps displayed standardised values, their contrasts describe variation under the chosen scaling procedure rather than measured pathway activity or nutrient turnover. Correlations involving soil attributes and bacterial composition also cannot establish whether predicted functions contributed to environmental differences. Similar broad functional assignments across distinct taxonomic assemblages do not, on their own, demonstrate redundancy. Although this concept offers a useful perspective on microbial organisation [78, 79], assessing it requires explicit examination of how functions are distributed among taxa. Temporal stability likewise cannot be evaluated from a single field campaign. Further investigation would benefit from metagenomic characterisation of gene inventories, complemented by expression

measurements, enzyme assays, and ecological process rates appropriate to the proposed mechanisms. The predictions therefore serve as exploratory descriptions of functional potential, without establishing improved nutrient cycling, persistence of functional performance, or redundancy within the communities..

5. Conclusions

Bacterial communities associated with *P. simonii* inthe four sampled stands showed marked differentiation along the root-soil continuum, with root communities exhibiting lower diversity and distinct composition compared with the surrounding soils. Null-model assignments also varied among sample groups. Homogeneous selection was the most frequent category in most groups, whereas dispersal limitation accounted for a larger proportion of comparisons in PC. These classifications describe inferred assembly patterns without directly establishing the underlying mechanisms.

Co-occurrence networks displayed different topological properties among compartments and sampled stands, although these contrasts did not establish stronger microbial interactions in rhizosphere or mixed-stand communities. Bacterial composition was associated with soil water and nutrient conditions, whereas predicted functional profiles lacked a uniform pattern across the sampled mixtures. Taxonomic differentiation and null-model assembly classifications provided complementary descriptions of community variation, but their relative responses to niche and plantation composition could not be established. These observations offer site-specific insights into belowground bacterial organisation in sandy plantations and hypotheses for testing with independent stand replication.

Declarations

a. Ethics approval and consent to participate

Not applicable.

b. Consent for publication

Not applicable.

c. Availability of data and materials

The raw sequencing data generated during the current study are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject accession number PRJNA1435822 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1435822).

d. Competing interests

The authors declare that they have no competing interests.

e. Funding

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f. Authors' contributions

Yue Ren contributed to conceptualization, investigation, formal analysis, data curation, visualization, writing the original draft, and reviewing and editing the manuscript. Guang Yang contributed to conceptualization, methodology, and funding acquisition. Min Su, Xinyu Guo, Shi Qiao, and Jiajia Shang contributed to investigation, formal analysis, and data curation. All authors read and approved the final manuscript.

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Figures

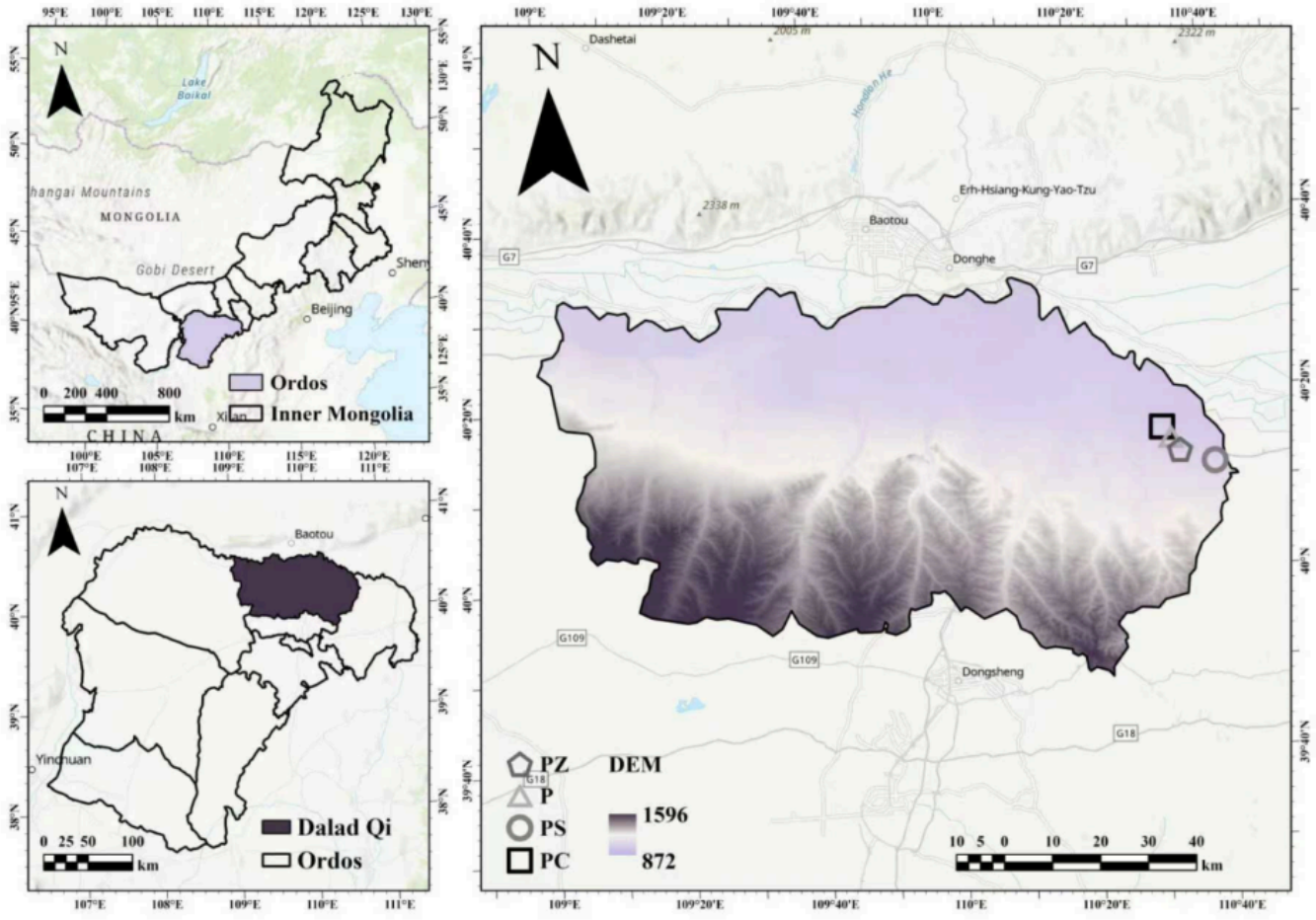


Figure 1

Map of the study site location. Geospatial datasets were obtained from the Geospatial Data Cloud platform (http://www.gscloud.cn), maintained by the Computer Network Information Centre of the Chinese Academy of Sciences. P: *P. simonii* monoculture plantation, PS: the mixed plantations of *P. simonii* and *S. psammophila*, PC: the mixed plantations of *P. simonii* and *C. korshinskii*, PZ: the mixed plantations of *P. simonii* and *P. sylvestris*, the same below.

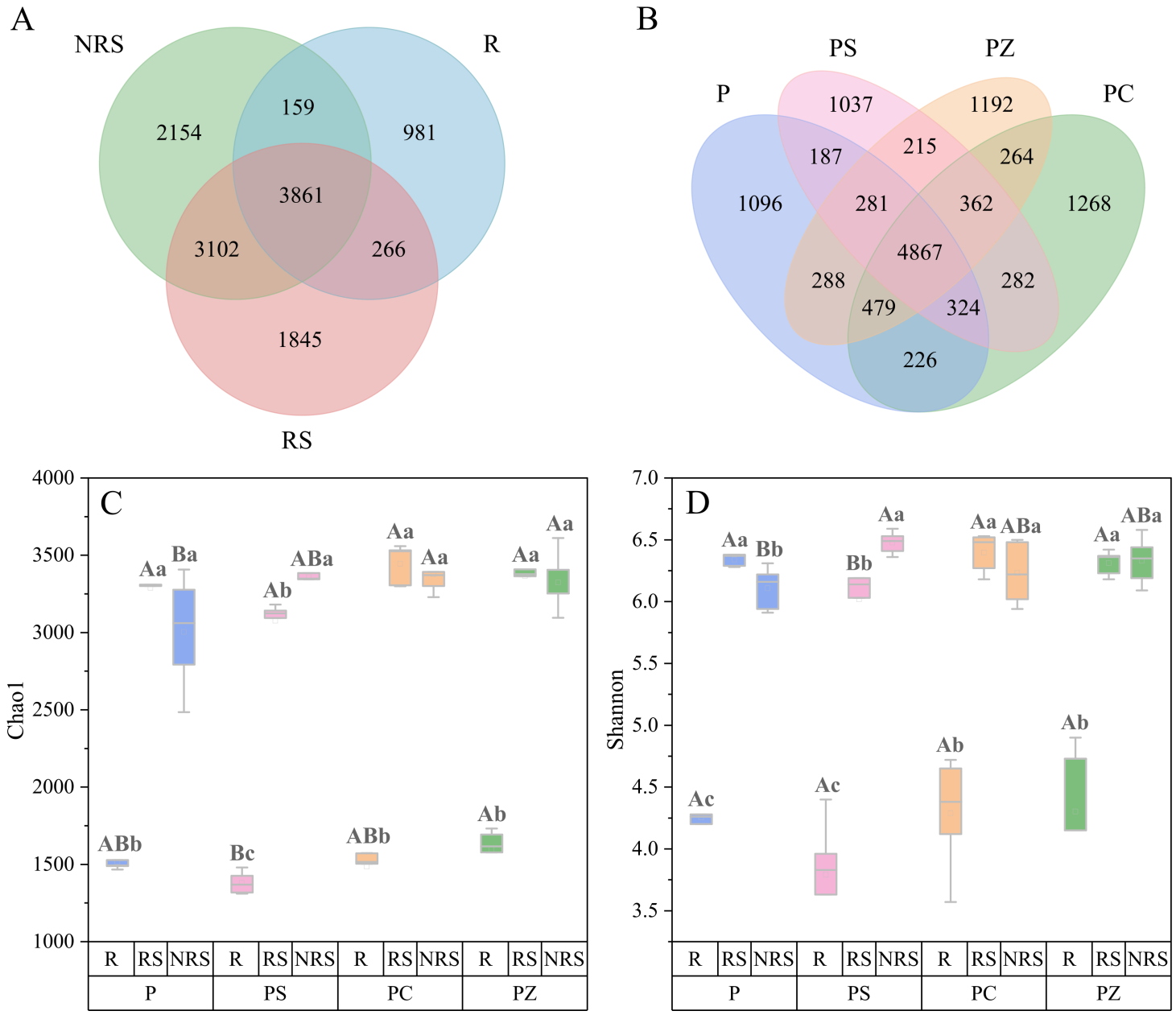


Figure 2

Variations in bacterial diversity and shared OTUs across niches and stand types. (A), Venn diagram showing shared and unique OTUs among niches (R: root, RS: root soil, and NRS: non-rhizosphere soils). (B), Venn diagram illustrating shared and unique OTUs among stand types (P, PS, PC, and PZ). (C), Chao1 index representing bacterial richness across niches and stand types. (D), Shannon index reflecting bacterial diversity across niches and stand types. Different uppercase letters indicate significant differences among stand types within the same niche, whereas different lowercase letters indicate significant differences among niches within the same stand type (α = 0.05).

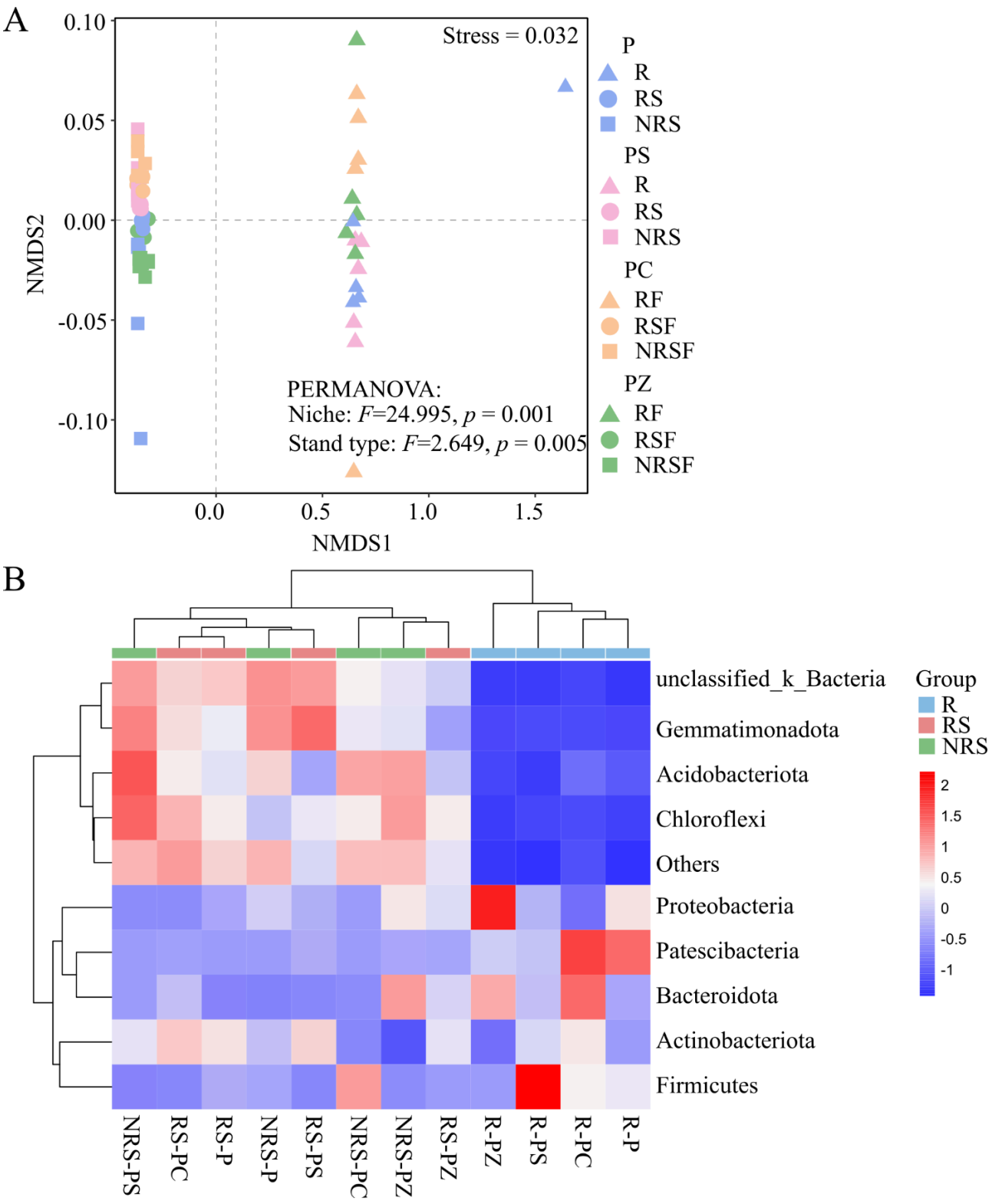


Figure 3

Bacterial community differentiation across ecological niches and plantation types. (A) Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances showing the distribution of bacterial communities across ecological niches (R, RS, and NRS) and plantation types (P, PS, PC, and PZ). (B) Hierarchical clustering heatmap of bacterial community composition at the phylum level. The color scale represents the relative abundance (Z-score transformed), and clustering was performed based on Bray-Curtis distances. PERMANOVA results indicate significant differences in community composition among groups.

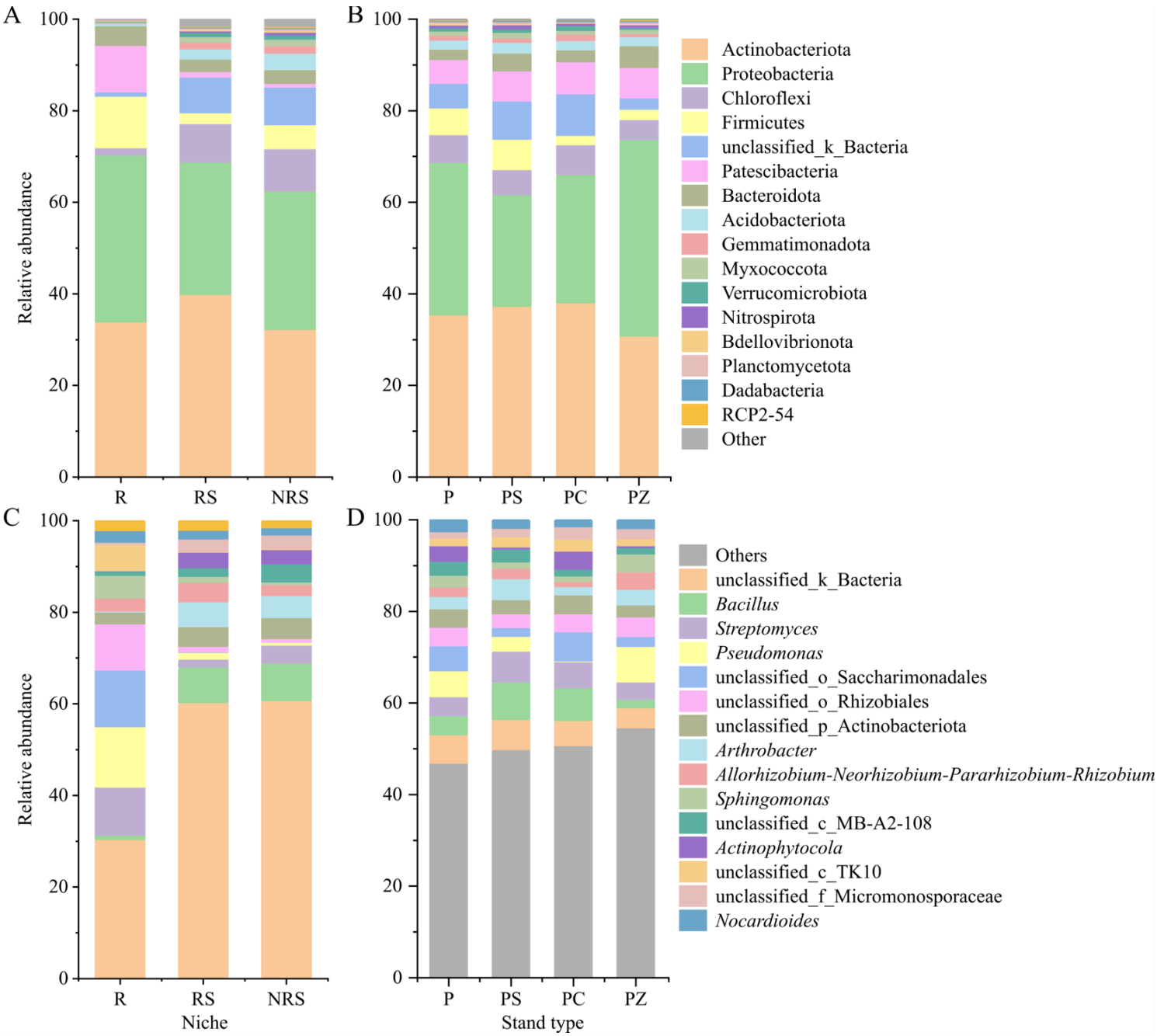


Figure 4

Relative abundance of bacterial communities at the phylum and genus levels across niches and stand types. (A, B), Relative abundance of dominant bacterial phyla across niches (R, RS, and NRS) and stand types (P, PS, PC, and PZ), respectively. Phyla with relative abundance <1% are grouped as "Others". (C, D), Relative abundance of dominant bacterial genera across niches and stand types. Only the top 15 genera are shown, with all remaining genera grouped as "Others".

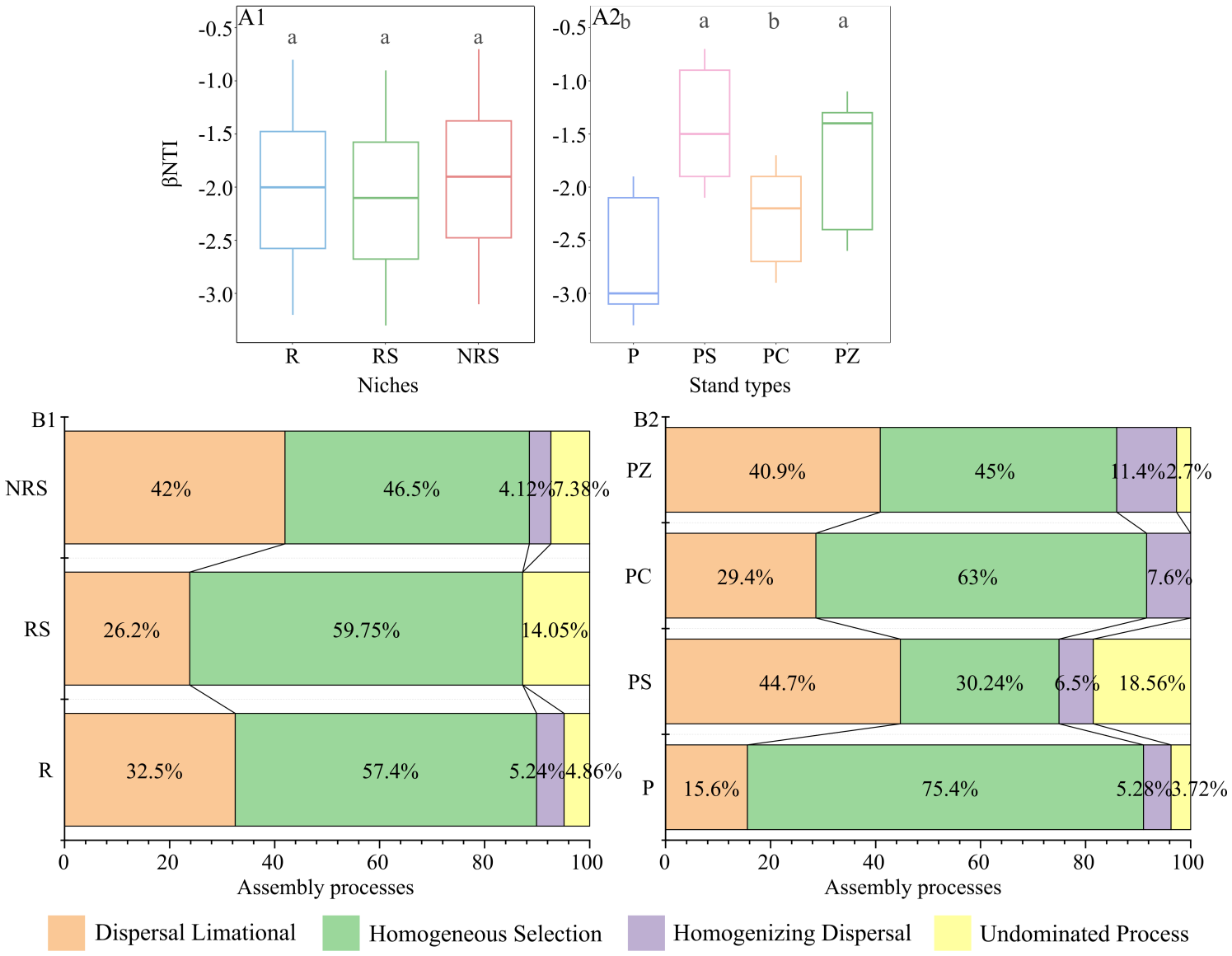


Figure 5

Variations in assembly processes of belowground bacterial community across ecologiccal niches and stand types. A, βNTI of bacterial community based on null model. B, Relative contributions of deterministic and stochastic assembly processes.

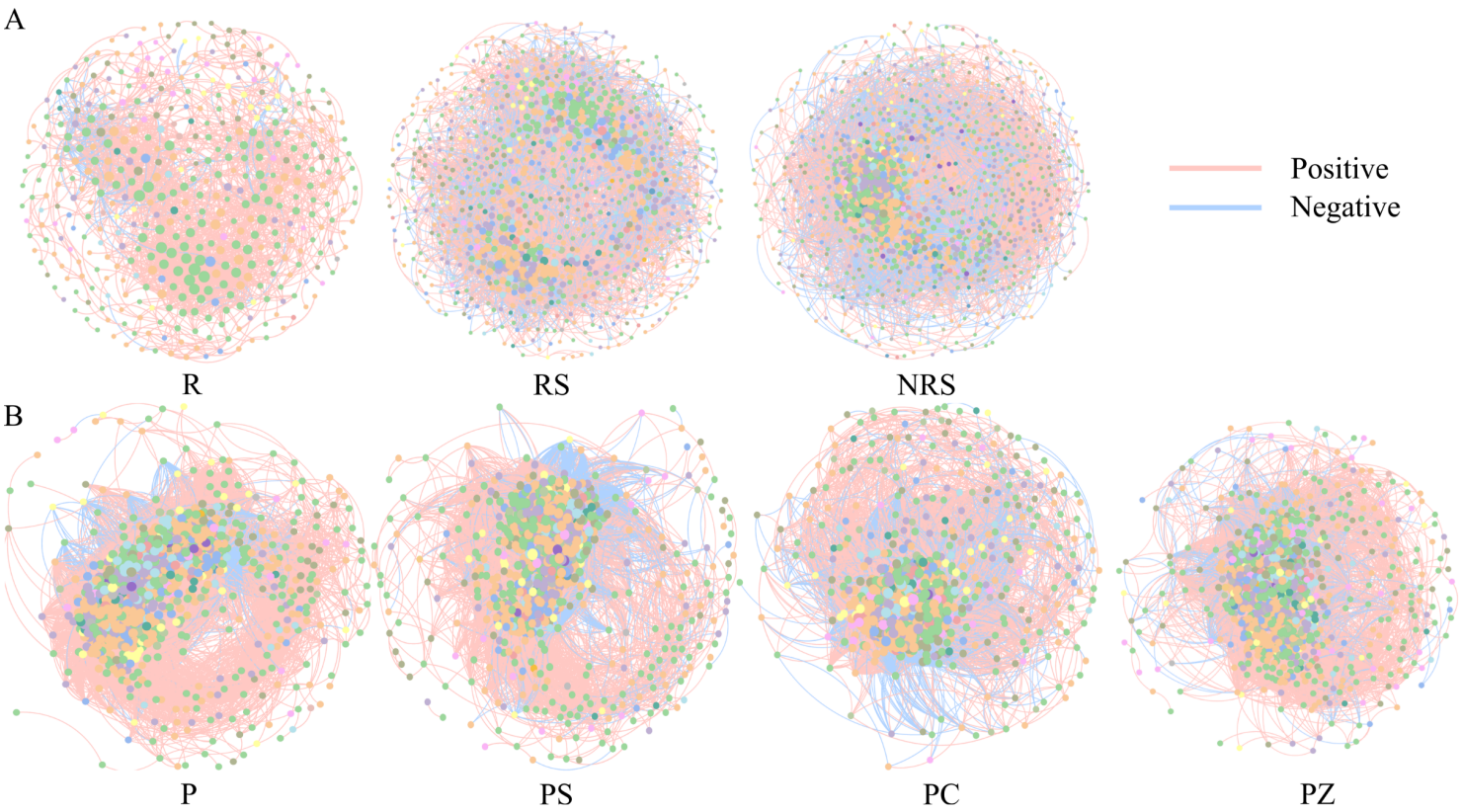


Figure 6

Co-occurrence network analysis between belowground bacterial community across ecologiccal niches and stand types. A, Co-occurrence network analysis between fungal community in different niches. B, Co-occurrence network analysis between fungal community in different stand types. Nodes correspond to individual OTUs and are color-coded by fungal phylum, consistent with the phylum-level colors in Figure 4A. Compartment-level networks included 20 samples each, and stand-level networks included 15 samples each from five plots. Edges represent Spearman correlations satisfying $|r| > 0.6$ and $p < 0.05$. Network metrics are descriptive; neither network stability nor statistical differences in these metrics were evaluated.

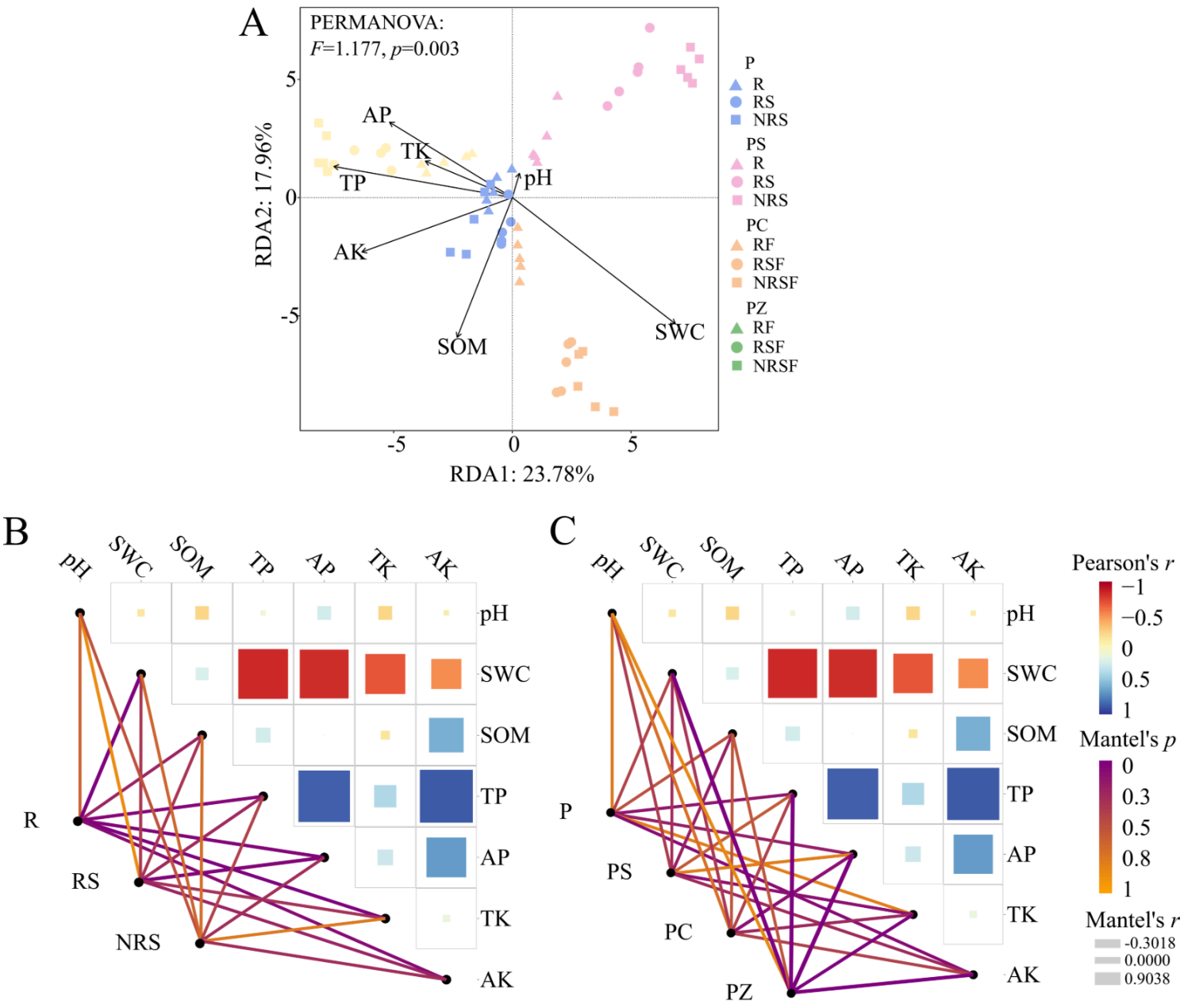


Figure 7

Relationships between bacterial community and environmental variables. A, Redundancy analysis (RDA) showing the effects of soil properties on bacterial community structure. B, C, Mantel tests linking microbial community dissimilarity to individual soil factors, grouped by ecological niche (B) and stand type (C).

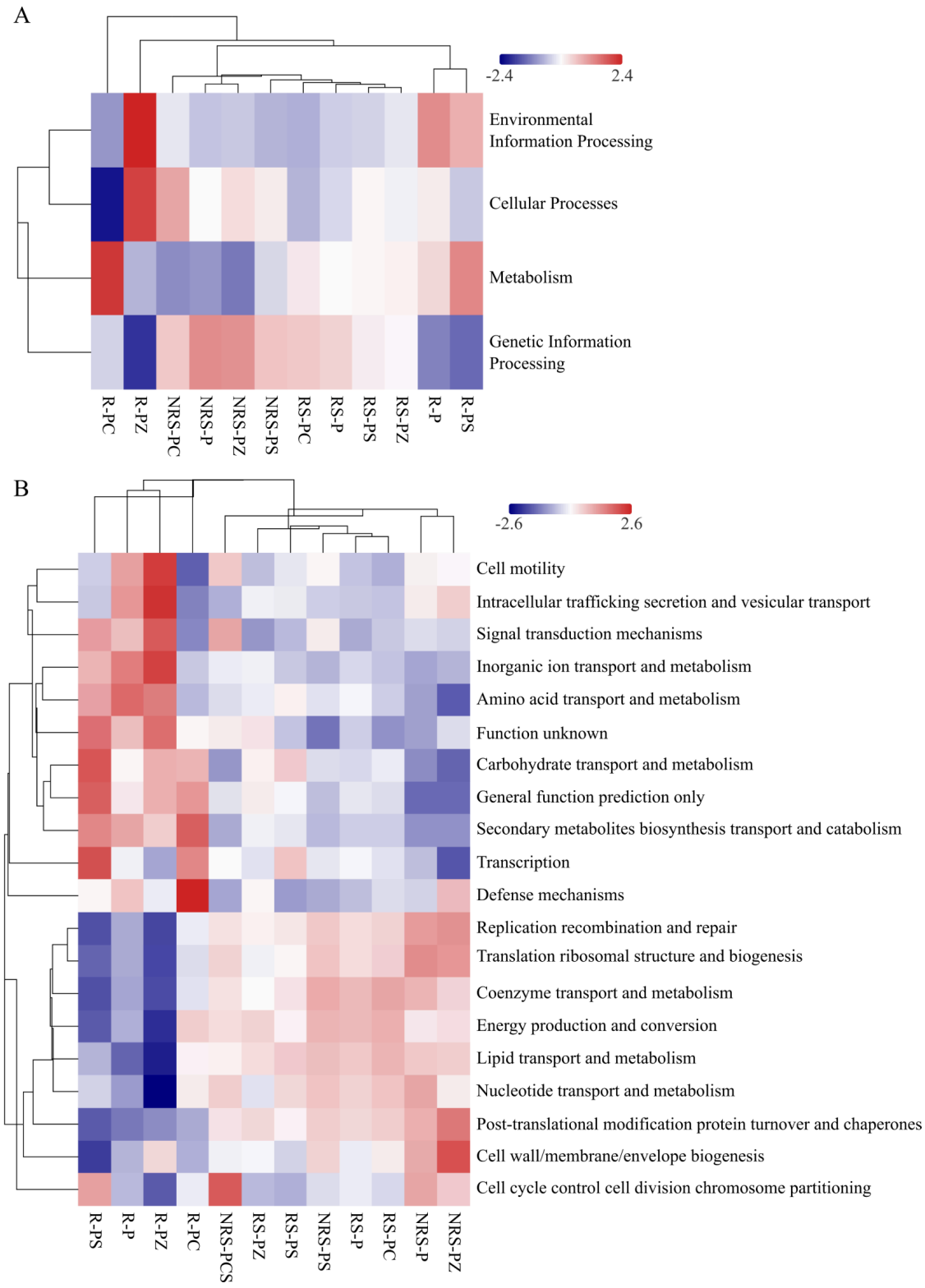


Figure 8

Predicted functional profiles of bacterial communities across ecological niches and plantation types. (A) KEGG and (B) COG functional categories. Heatmaps show the standardized relative abundance of predicted functions, with hierarchical clustering indicating similarities among samples and functional categories.

Supplementary Files

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- Supplementmaterials.docx