The Temporal Structure and Association Networks of Endophytic Bacteria in Pea Roots and Nodules

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Abstract

Background and aims Endophytic bacteria from legume roots and nodules play important roles in regulating plant growth and health. However, little is known about how the endophytic bacteria community changes and how it interacts with each other throughout the plant life cycle.

Methods We collected twenty pea cultivars to study the composition and structure of endophytic bacterial community during the whole developmental period using Illumina sequencing of 16S rRNA gene.

Results Here we show that the development period significantly affected the structure of root endophytic bacterial community and peas recruit different root microbes during different developmental stages. The complexity of microbial community first increased and then decreased with the growth of pea. *Rhizobium* began to accumulate in pea seedling roots, and the content peaked at flowering stage and remained at high levels during the mature stage. In the flowering and mature stage, the relative abundance of *Pseudomonas* increased.

Conclusions These findings can deepen the overall understanding of the community structure and interaction network of endophytic bacteria from pea root and nodules, and provide a detail for the establishment of root endophytic bacteria throughout the plant life cycle.

Introduction

Plants are the major producers of carbohydrates in the ecosystem, and their roots grow in the soil environment rich in microorganisms. As an adaption to the soil, plants have evolved systems for monitoring microbial presence, or invasion and corresponding plant-microbe response strategies (Rafal et al. 2015). Plants also constitute a diverse ecological niche for endophytic organisms (Dudeja et al. 2012) Root microbial community structure within a plant is dynamic and is influenced by abiotic and biotic factors (Gaiero et al. 2013). For example, the diversity of root-associated microbial populations of *Tamarix parviflora* was significantly affected by soil conditions (Polivkova et al. 2018). The diversity of bacterial endophytes in potato (*Solanum tuberosum* L.) stems was increased when plants were infected by the bacterial pathogen *Erwinia carotovora* subsp.*atroseptica* in contrast to healthy plants (Reiter et al. 2002). It has been shown that foliar endophytes participate in controlling stomatal opening and closure, which further indicates that, apart from their other roles, endophytic bacteria could indirectly affect the further colonization of other microorganisms (Hyungmin et al. 2018). Therefore, it was shown that endophytes diversity at both structural and functional levels is crucial to plant growth, but the underlying principles are not well understood. To shed new light on this phenomenon, analysis of the structure and co-occurrence networks of endophytes could be utilized.

Endophytes are located inside plant organs to promote plant growth and yield (Bulgarelli et al. 2018). Specially, nitrogen input and availability are key factors regulating plant growth and yield (Boring et al. 1988). Nitrogen-fixing organisms are responsible for the biological fixation of atmospheric nitrogen

Bradyrhizobium, *Sinorhizobium*,

Mesorhizobium, and *Azorhizobium*, were commonly known as rhizobia (Clémence et al. 2000). Rhizobia, endophytes and mycorrhizae can establish beneficial interactions with legume plants and fix atomosphere N_2 by forming nodule (Dudeja et al. 2012; Mus et al. 2016; Zipfel et al. 2017). Endophytic bacteria in a single plant host are not restricted to a single bacteria species, but comprise several genera and species[2]. For example, several studies showed that the genera *Aerobacter*, *Agrobacterium*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pseudomonas*, *Sphingomonas*, and *Rhizobium* existed within roots and nodules of leguminous crops (Dudeja et al. 2012). However, it was little known how these rhizobia and other endophytic microorganisms inside the plants interact during the whole plant development stage. Ecological networks effectively represent the interactions of various organisms in an ecosystem (Faust et al. 2012). We believe that the wider application of correlation networks in endophytic community analysis will provide valuable insights into the organizational function and evolution of these important communities within plants.

Pea (*Pisum sativum* L.) is an important human and animal legume crop cultivated throughout the world (Cousin et al. 1997). Here, we collected pea roots and nodules of different cultivars to explore the effects of developmental stage and cultivars on endophytic community using the 16S rRNA gene amplicon sequences. We hypothesized that: (1) peas accumulate different endophytic bacteria at different stage and these endophytic bacteria play important roles during pea growth and development; (2) pea root endophyte - endophyte correlation shifted across pea developmental stages, the complexity of endophytic community changed during the whole development stage of pea. In our article, we have proven the two hypotheses and described a detailed characterization of pea root and nodule microbiome composition, and provide interactions across all stages.

Materials And Methods

Study area and sampling

The study site was located in Qingdao academy of agricultural sciences (36 °09 '8.05 " N, 120 °25 '15.77 " E), Shandong Province, China. Twenty pea cultivars were classified into six edible pea types, i.e. dry-grained peas (DGP), fresh peas (FGP), snow pea (SP), sweet crispy pea (SCP), pea tip (SEP), pea sprout (SHP) (Supplement Data Table 1). Each cultivar picked two individuals for further experiment. Thus, 40 individuals were collected from each stage. These pea seeds were planted into flowerpots (39 × 75cm) and placed in plastic houses. The flowerpots were filled with artificial soils of Pindstrup sphagnum moss peat purchased from JingDong shopping center (Beijing, China). Non-planted soils (NS) were collected from the homogenized artificial soil.

The pea seeds were planted into flowerpots on 20 March, 2017. After two weeks cultivation, the roots at pea seedling stage were collected. Similarly, the roots at pea flowering stage were collected when the first flower was bloomed, and the roots of pea mature stage were collected when the pod rate reaches 50%. Root nodules were most thriving during flowering, so nodules were taken only at the flowering stage. Plant samples were kept from the flowerpots in coolers. Most of the soils were removed by slight shaking,

and soils which closely associated with roots were separated by violently eddying in sterile tap water. The roots and nodules were removed from plants aseptically and first surface sterilized separately to remove bacteria from the surface of these tissues. They were washed with sterile tap water and then rinsed 5 to 7 times with double distilled water, and then they were rinsed with 70% ethanol for 3 minutes. After that, they were thoroughly washed with sterile double distilled water to remove surplus ethanol. To test the sterilizing effect, we took 100 µL sterile water from the last rinse to Tryptose Soya Agar (TSA) medium, and cultured it in incubators at 28℃ for 3 days. All plants were packed with Whatman1 filter paper and stored at -20℃ for the following experiments and further use.

DNA extraction and Illumina Miseq sequencing

Total genomic DNA was extracted from roots, nodules, non-planted soil and pea seeds using DNA Extraction Kit (QIAamp 96 PowerFecal QIAcube HT kit (5), (Dusseldorf, Germany)) following the manufacturer's instructions. Illumina sequencing was performed by amplifying the V3-V4 region of the bacterial 16S rRNA gene using individually bar-coded forward primers 343F (5'- TACGGRAGGCAGCAG – 3') and reverse primers 798R (5'- AGGGTATCTAATCCT-3') (Nossa et al. 2010). The PCR reactions were performed in 30 µL mixtures, containing 15 µL of 2 × Gflex PCR Buffer, 1 µL of each primer, 50 ng of template DNA, 0.6 µL of Tks Gflex DNA Polymerase and deionized water to a final volume of 30 µL. The PCR conditions were as follows: 94℃ for 5 min, 26 cycles of 94℃ for 30 s, 56℃ for 30 s and 72℃ for 20 s, and a final elongation step at 72℃ for 5 min. The PCR products were detected by electrophoresis in a 1% agarose gel. The PCR products from every sample run on the Illumina Miseq platform. The Illumina sequence reads deposited at the NCBI under accession number PRJNA677834.

Raw sequencing data were in FASTQ format. Paired-end reads were then preprocessed using Trimmomatic software (Bolger et al. 2014) to detect and cut off ambiguous bases (N). It also cuts off low quality sequences with average quality score below 20 using sliding window trimming approach. After trimming, paired-end reads were assembled using FLASH software (Reyon et al. 2012). Parameters of assembly were: 10 bp of minimal overlapping, 200 bp of maximum overlapping and 20% of maximum mismatch rate. Sequences were performed further denoising as follows: reads with ambiguous, homologous sequences or below 200 bp were abandoned. Reads with 75% of bases above Q 20 were retained. Then, reads with chimera were detected and removed. These two steps were achieved using QIIME software (version 1.8.0) (Caporaso et al. 2010).

Statistical analysis

Alpha-diversity calculations were performed based on the rarefied OTU table. The bacterial Chao 1 richness, Shannon index, Simpson index and Good's coverage were calculated by QIIME (Caporaso et al. 2010). The significance was determined by one-way analysis of variance (ANOVA). Beta diversity was employed to evaluate the differences in bacterial community composition using Bray-Curtis distances. The Principal coordinate analysis (PCoA) was generated from the Bray-Curtis similarity index matrices of all samples, and permutational multivariate analysis of variance (PERMANOVA) was used to measure

significance differences of beta-diversity. All procedures were implemented in MicrobiomeAnalyst cloud platform with the MicrobiomeAnalystR packages (Dhariwal et al. 2017).

Network analysis

Network analyses were performed to gain a better understanding of the microorganism interactions in the seeds, roots, and nodules. We selected the dominant bacterial genera of Top 30 for correlation analysis. To enrich the correlation in the network, Spearman correlation coefficient (r) > 0.4 and the P-value < 0.01 were considered to indicate a valid moderate interactive event (Barberán et al. 2012). To describe the complex pattern of interrelationships of bacterial genera, the topological characteristics of the networks were calculated as follows: average path length (APL), graph density, network diameter, average clustering coefficient (avgCC), average degree (avgK), and modularity (M) (Jiang et al. 2017). Statistical analyses were conducted by psych packages in R, Correlation networks were visualized using Gephi-0.9.2 (https://gephi.org/users/).

Results

Sequence data analysis.

To minimize the effects of random sequencing errors, we eliminated low-quality sequences and blurred sequencing raw data from 40 seeds and 120 roots and 40 nodules of 20 pea cultivars, and 2 non-planted artificial soils. A total of 6 363 185 high-quality 16S rRNA sequence valid tags obtained after quality filtering. The number of sequences per sample ranged from 13 195 to 73 334, with an average of 32 969 valid tags. Vsearch software (Rognes et al. 2016) was used to classify the high-quality sequence valid tags obtained by quality control according to 97% similarity, and a total of 20 361 OTUs were obtained. The sequence with the highest abundance in each OTU was selected as the representative sequence of the OTU, and Ribosomal Database Project (RDP) Classifier Naive Bayesian classification algorithm (Wang et al. 2007) was applied to compare the representative sequence with the database to obtain the species information of each OTU.

Diversity and abundance of endophytic bacterial community.

Good's coverage values were comparatively high and ranged from 94.98–99.85%, which demonstrated that the sequences obtained represented the bacterial communities well. The results of one-way ANOVA revealed that Shannon index and Chao 1 richness considerably varied among different developmental stages of pea (Fig. 1, P < 0.01). The flower root (FR) possessed the highest Shannon index and Chao 1 richness. The flower nodule (FN) and the seed (S) presented the lowest Shannon index and Chao 1 richness, respectively ($P < 0.01$). The whole datasets of Alpha-diversity were displayed in Supplement Data Table 2. The Alpha-diversity was composed by Shannon index, Chao 1 richness, Simpson index, and Good's coverage.

The analysis of group similarities (ANOSIM) ($r = 0.77$, $P < 0.001$) showed the significant difference among different stages of pea. We used principal coordinate analysis (PCoA) to determine the endophytic bacterial community composition based on the Bray-Curtis distances. The seedling root (SR), FR, FN and mature root (MR) were clustered together but well separated from the S samples (Fig. 2A). Furthermore, we added NS data to make Fig. 2B, and results indicate that endophytic bacterial community of pea roots was more similar with that of NS than S. Here, all these data suggest that the soil has a higher influence on the endophytic microbial community structure of pea roots than pea seeds. Moreover, the ANOSIM ($r = -0.0019$, $P > 0.05$) showed that the endophytic bacterial community composition had no significant difference among different edible pea types. Figure 2C and 2D showed that all edible pea types possessed similar root endophytic bacterial community, revealed that the edible types rendered a slight effect on the root endophytic bacterial community composition in the artificial soil.

Endophytic bacterial community composition.

The endophytic bacteria composition of different pea stages at the phylum level (TOP15) was shown in Fig. 3A. Bacteroidetes and Firmicutes were obviously higher in S and NS than that in roots and nodules, whereas Proteobacteria was two-fold higher in the FN than that in S and NS. Firmicutes and Actinobacteria were most abundant in S and NS, respectively. The Fig. 3B demonstrated that different edible pea types had similar bacterial composition. The endophytic bacterial community was dominated by Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria at each development stage in different edible pea types. These endophytic bacteria had similar proportions in these pea cultivars. However, the dissimilar proportions of these bacterial community were shown in NS.

To further investigate the changes in specific taxa throughout the pea developmental cycle, we compared the relative abundance of endophytic bacterial community at the genus level (Fig. 3C). From seedling to the mature stage, the relative abundance of *Pseudomonas* dramatically increased over time, whereas *Bacteroides* remarkably decreased. It was worth noting that the relative abundance of *Rhizobium* dramatically increased from seedling to the flowering stage, and then decreased from flowering to the mature stage. Moreover, the endophytic bacterial community structure during the pea developmental cycle was more similar to that of the soil than that of the seeds. The Fig. 3D demonstrated that different edible pea types have a little effect on endophytic bacterial composition.

Co-occurrence patterns among root endophytic bacteria.

Different microbes strains can co-occur or exclude each other to drive population structure and dynamics. Hence, we used Gephi software to structure the correlation networks in different stage of pea roots and nodules. The network analysis let us explore the co-occurrence patterns of bacteria in the pea seeds, the roots of whole pea development stage, and nodules. The number of edges in bacterial networks, typically increased following the stage. They increased in the order in which they are listed: S < MR < FR < FN < SR (Fig. 4). There were 21 correlations in the S, 308 correlations in the SR, 124 correlations in the FR, 297 correlations in the FN and 85 correlations in the MR stage (Supplement Data Table 3). These results

d nodule are more complex than pea seed.

From S to SR, the complexity of the network was significantly increased, but the complexity of the network gradually decreased from SR to MR (Fig. 4). In addition, the negative correlations among endophytic bacteria in SR were 47.4%. However, the negative correlation decreased significantly in flowering and mature roots, accounting for 12.01% and 11.76%, respectively. This positive and negative relationship may be related to the pea development and growth.

We focused on two taxa, *Rhizobium* and *Mesorhizobium* as keystones of bacterial networks under the whole development stage of pea. In our result, the bacteria in *Rhizobium* were found in the root of each developmental stage,but *Mesorhizobium* were only found in FR and MR. *Rhizobium* were correlated (positive and negative) with 6 and 5 other different genera in the SR, respectively (Supplement Data Table 5), which may affect the nodulation of rhizobia. Interestingly, *Rhizobium* showed almost all negative correlation with most other bacteria at the FR and FN (Fig. 4 and Supplement Data Tables 6 and 7). By contrast, we found that *Mesorhizobium* was positive correlation with most other bacteria at FR. Furthermore, *Mesorhizobium* was positive correlation with most bacteria at MR except *Bacteroides*.

Analysis of linear discriminant analysis (LDA).

To determine the microbial taxa with a significant difference in the abundance of endophytic bacteria from pea roots at different developmental stage, we performed biomarker analysis using the linear discriminant analysis (LDA) effect size (LEfSe) method. The linear discriminant analysis (LDA) effect size (LEfSe) method was used to detect potential biomarkers at OTU level with an LDA score threshold of > 4.0 and a P-value for the factorial Kruskal-Wallis test of 0.05 (Chen et al. 2020). As shown in LEfSe Bar (Fig. 5), 6, 2, 1, 0, 2, and 4 bacterial groups were enriched as biomarkers in the NS, S, SR, FR, FN, and MR, respectively. In NS, OTU 106 (*Helicobacter*), 229 (*Coriobacteriaceae_UCG_002*), 90 (*Cetobacterium*), 85 (*Bacteroides*), 143 (*Pantoea*) and 321 (uncultured) were mainly enriched. In S, OTU 416 (*Rhodoligotrophos*) and 394 (*Bacillus*) were mainly enriched. In SR, OTU 594 (*Bacteroides*) was mainly enriched. OTU 15703 and 68 were mainly enriched in FN, which all belonged to *Rhizobium*. In MR, OTU 17477 (*Rhizobium*), 5631 (*Pseudomonas*), 28978 (*Pseudomonas*) and 345 (uncultured) were detected as mainly enriched.

Discussion

Endophytic bacterial community dynamics during different developmental stages.

Plants normally associate with diverse microorganisms. The roots influence the rhizosphere by changing soil pH, oxygen utilization, soil structure, and quorum sensing mimicry, thus selecting specific bacteria to flourish in the rhizosphere from the reservoir of soil microbial diversity (Berendsen et al. 2012; Liu et al. 2019). The influence of soil context and plant species on rhizosphere bacteria composition were reported by several previous studies (Pérez-Jaramillo et al. 2016). For example, Edwards et al. (Edwards et al. 2015) found that composition of the rice root microbiome was significantly influenced by soil source and rice genotype when grown under controlled greenhouse conditions. Turner et al. (Turner et al. 2013) found

es when compared with the cereals rhizosphere

microbiomes. Although more and more researches revealed the composition and changes of rhizosphere bacteria, there are still few researches on root endophyte community dynamics.

We performed longitudinal dense sampling across the entire life cycle of different pea cultivars. The endophytic bacterial community was less diverse than those of soil (Fig. 1A), suggesting that the part of endophytic community was derived from soil bacteria. Furthermore, we found that the developmental stage significantly affected the structure of endophytic bacterial community. However, the influence of different pea cultivars was not significant and relatively weak. Several studies found that the endophyte was influenced by the host plant developmental stage, species, health and environmental conditions. Yu et al. (Yu et al. 2015) illustrated the effect of plant growth stage on the structure of endophytic bacterial communities present in the leaves of *Stevia rebaudiana*. Leo and Drik (Leo et al. 2008) concluded that plant growth stage overwhelmed any effect of plant genotype on the bacterial communities associated with potato using PCR denaturing gradient gel electrophoresis fingerprints. Our experimental results were further supported the influence of developmental stage on the composition of endophytic bacteria. Furthermore, our study detailed indicated that the pea root endophytic bacterial community shifted across pea developmental stages based on two background datasets from S and NS, suggesting that different root microbes may be recruited during different developmental stages. Importantly, *Rhizobium* began to accumulate in SR, continuously increased at flowering stage and remained at high levels during the mature stage (Fig. 3C). Interestingly, the relative abundance of *Pseudomonas* was increased in the flowering and mature stage. This indicates that the bacteria in *Pseudomonas* are powerful group to promote the colonization of *Rhizobium*.

Previous studies revealed that *Rhizobium* was diffusely distributed in plant root and acted as nitrogen fixers to host plants. For example, the *Rhizobium* in *Phaseolus vulgaris* Linn. and *Zea mays* L. (Gao et al. 2017; Yan et al.2017) acted as nitrogen fixers in their host plants. Abundant *Rhizobium* was found in tumorous stem mustard healthy roots and was speculated to fix nitrogen for tumorous stem mustard (Wang et al. 2020). Gupta et al. (Gupta et al. 2013) reported that endophytic nitrogen-fixing bacteria probably enhance the rate of nitrogen fixation and accumulation in plants residing in nitrogen limited soils. Hoflich et al. (Hoflich et al. 1995) reported inoculation of *Pseudomonas* and *Rhizobium* bacteria can promote the nodulation and root length of pea in pot experiments. Similarly, the native *Rhizobium* - *Pseudomonas* co-inoculation as compared to single *Rhizobium* inoculation increased the nodulation, growth parameters and yield of the different *Phaseolus vulgaris* L. genotypes under Cuban soil conditions (Sánchez et al. 2014). Some studies also have highlighted that *Pseudomonas* was identified as frequently occurring in agricultural crops such as legumes, potato and maize, and shown to contribute to the disease suppressive traits of soils (Bai et al. 2002; Miliute et al. 2015). In addition, *Pseudomonas* could alleviate drought, heat and salt stress of different crop plant, thus significantly increasing plant biomass and plant height (Rojas-Tapias et al. 2012). Our endophytic bacteria study indicated that *Pseudomonas* and *Rhizobium* was recruited higher amounts at the pea important flowering stage as compared to these two bacterial group in the pea seedling stage. Furthermore, this study gives a detailed report about the dynamic structure of the native endophytic bacteria in *Pseudomonas* and *Rhizobium*

 Therefore, different endophytes were indeed

accumulated in pea roots and nodules of different developmental stages, and they showed regular dynamic changes. Thus, these observations supported our first hypothesis that the pea accumulates different endophytic bacteria at different stage and these endophytic bacteria in *Pseudomonas* and *Rhizobium* play important roles during pea growth and development.

Co - network in roots and nodules specific preference for endophyte - endophyte interactions.

In natural habitats, microorganisms live together within complicated networks through various types of interactions, which could be either positive or negative (Wang et al. 2017). In recent years, people have paid more attention to two kinds of relationships. One is the relationship between microbes and microbes, the other is the relationship between microbes and their hosts. However, little is known about microbial-to-microbe dynamic relationships over the whole developmental stage. Our experimental results demonstrated that the pea root endophyte - endophyte correlation shifted across pea developmental stages, the complexity of microbial community decreased from SR to MR. As compared to the growth and differentiation in pea flowering stage, faster growth and differentiation was experienced at pea seedling stage. In the flowering and mature stages, the pea was mainly focused on reproductive development, then the complexity of root microbial communities may be reduced and focused on reproductive nutrition needs. In addition, compared to the seedling roots, the negative correlation decreased significantly in flowering and mature roots. This may be due to the fact that bacteria have just invaded the plant from the outside at seedling stage. Potential endophytes from seeds and soil cooperate and compete for invasion sites on the root (Gaiero et al. 2013), which leads to complicated correlation between positive and negative at seedling stage. Previous studies on rice root microbiota found that the rice root microbiota stabilizes after 8–10 weeks of growth in the field (Zhang et al. 2018). In our study, most of the endophyte-endophyte correlations tend to be stable and positive at flowering and maturity stage. Specially, the negative / positive rations at different developmental stages were given in our work. This is helpful to improve the understanding of the dynamic correlations of endophytic bacteria at different stages of pea root development.

Root-associated bacteria play important roles in crop growth, yield and product quality. This is particularly true for legume crops forming symbiotic relationships with rhizobia, for fixation of atmospheric N_2 (Barraza et al. 2020). Rhizobia are a key group of root-associated bacteria that can fix atmospheric nitrogen in nodules. In our experiments, the bacteria in *Mesorhizobium* were found in FR and MR, and *Rhizobium* bacteria were found in the root of each stage. At the root of seedling stage, the *Rhizobium* were positively correlated with the six bacteria (Fig. 4B), i.e. *Devosia*, *Dyella*, *Methylophilus*, *Rhodanobacter*, *Sphingomonas*, *Rhizomicrobium*. Among them, *Devosia* and *Dyella* have been shown to contribute to host nodule formation (Rivas et al. 2002; Wang et al. 2020). *Rhodanobacter* bacteria have been proved to be highly positively correlated with available nitrogen in soil (Wang et al. 2020). It has been suggested that *Methylophilu*, *Sphingomonas*, and *Rhizomicrobium* could promote plant growth (Agafonova et al. 2016; Asaf et al. 2018; Wei et al. 2019). We speculated that these bacteria might promote the colonization of *Rhizobium* and affect the nodule formation of pea roots. At the root and minant flora and showed negative correlation

with other bacteria (Fig. 4C). Interestingly, *Rhizobium* and *Mesorhizobium* began to decay at the mature stage and showed negative correlation with *Bacteroidetes* (Fig. 4E). This indicates *Bacteroidetes* may regulate the decay of *Rhizobium* and *Mesorhizobium* and influence the development of pea root nodules. These observations supported the second hypothesis that pea root endophyte - endophyte correlation shifted across pea developmental stages and the complexity of microbial community changed during the whole development stage of pea.

In our work, we have described a detailed characterization of pea root and nodule endophyte composition, and provide endophyte interactions across all stages. The endophyte community composition and endophytic bacteria interactions in pea roots and nodules advanced our understanding of the modulating role of these factors in the pea endophyte diversity. These basic knowledge provided a basis for further study on the formation, exuberance and decay of legume endophytes. The endophytic bacteria of peas should be considered as an important component of root system growth and nodule establishment and in synthetic microbial community to increase the efficiency of symbiotic nitrogen fixation.

Declarations

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Conflict of Interest The authors declare no competing interests.

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Consent to participate The authors declare consent to participate

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Figures

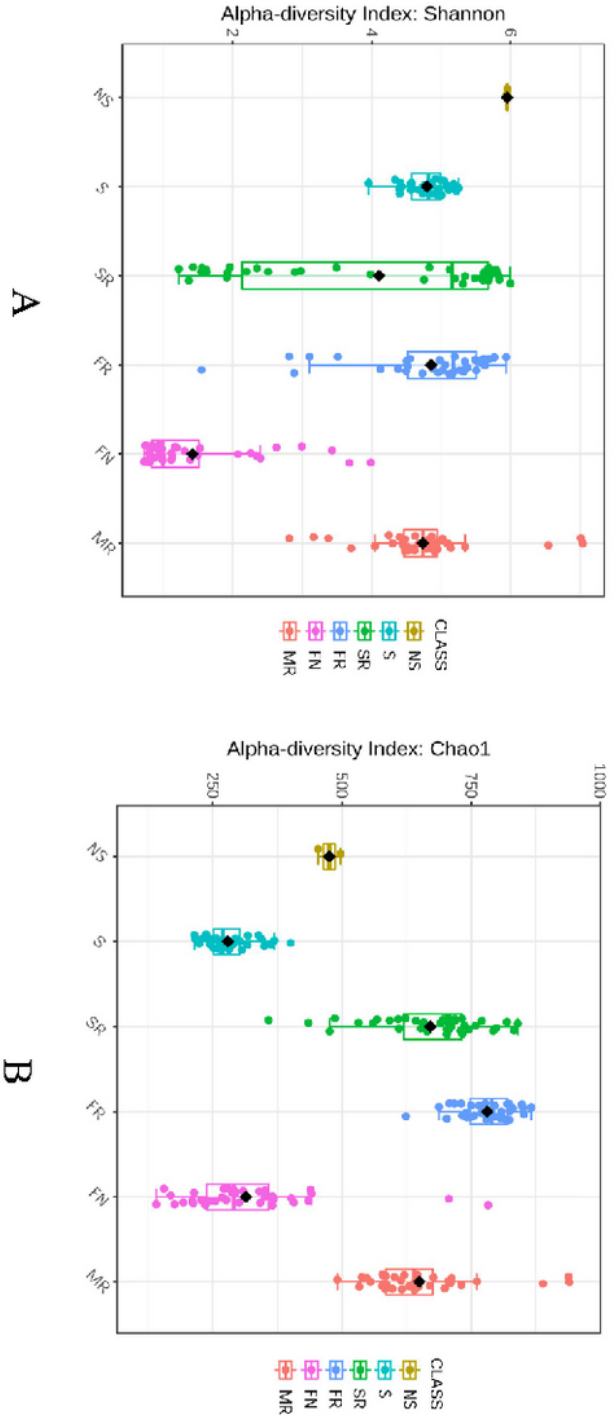

Figure 1

Alpha diversity boxplot. The horizontal bars within boxes represent medians. The tops and bottoms of boxes represent the 75th and 25th percentiles, respectively. A, The significant testing of Shannon diversity index was conducted by ANOVA among species, and Welch's t-test between different group (P < 0.05). B, The significant testing of Chao1 diversity index was conducted by ANOVA among species, and Welch's t-test between different group (P < 0.05).

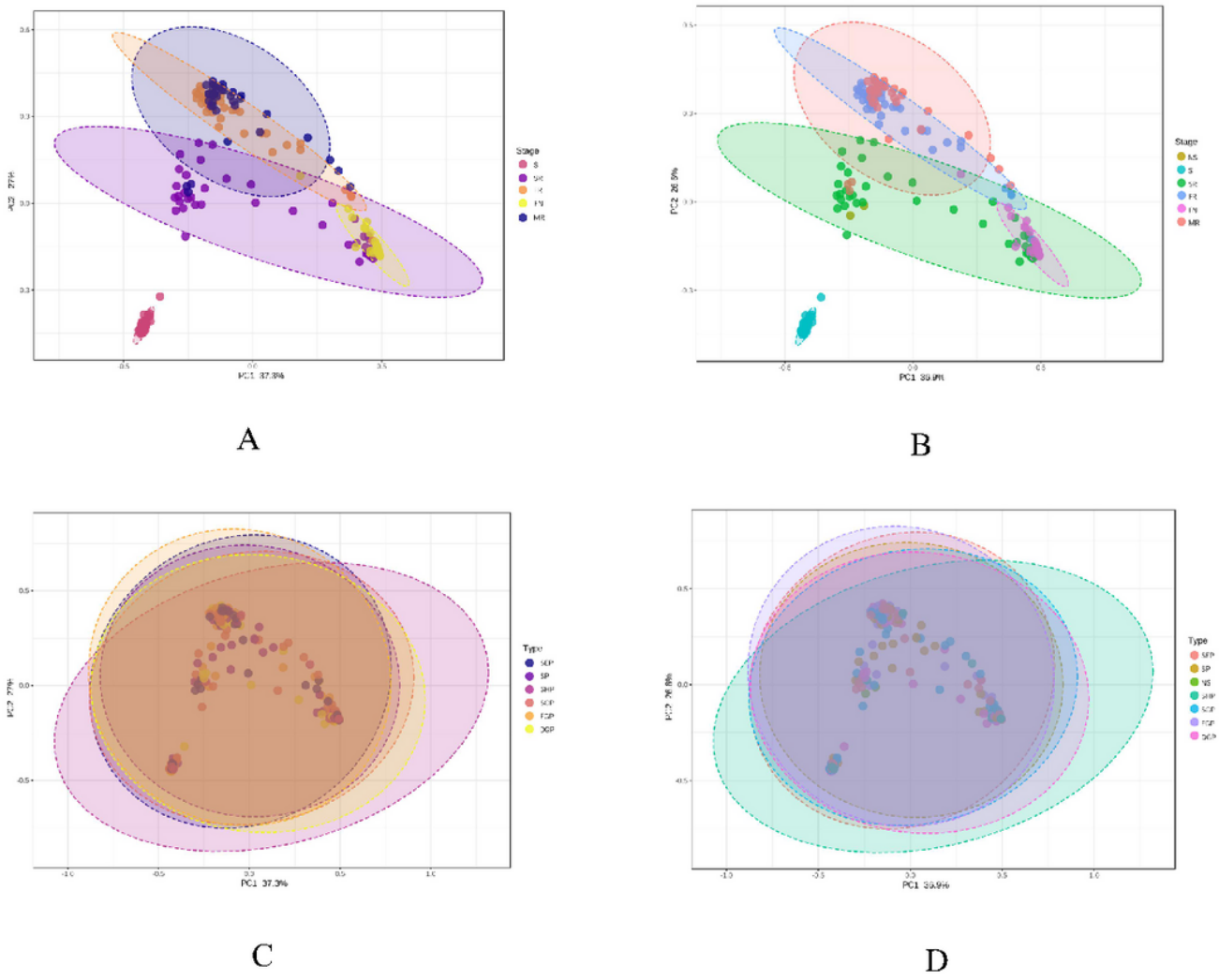

Figure 2

Alpha diversity analysis. A, B, Principle coordinate analysis (PCoA) of Bray-Curtis distances reveals that development stage is a major source of bacterial community variation in the roots(P ≤ 0.001, PERMANOVA by Adonis). C, D, Principle coordinate analysis (PCoA) of Bray-Curtis distances reveals that edible pea types is not source of bacterial community variation in the roots(P ≤ 0.05, PERMANOVA by Adonis).

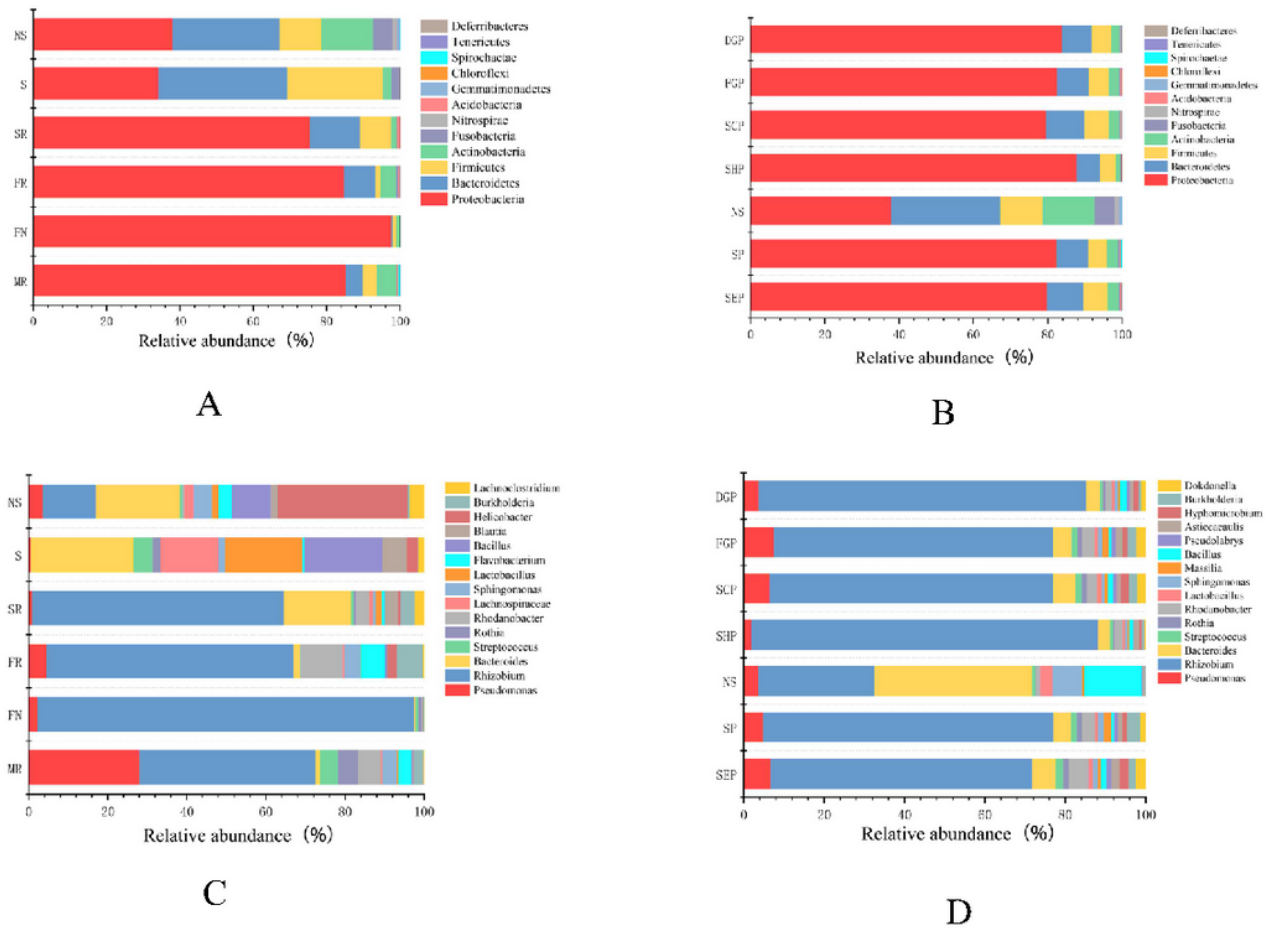

Figure 3

The community structure of pea root endophytic bacteria. A, B, Changes in the relative abundances of bacterial phyla. C, D, Changes in the relative abundances of bacterial genera.

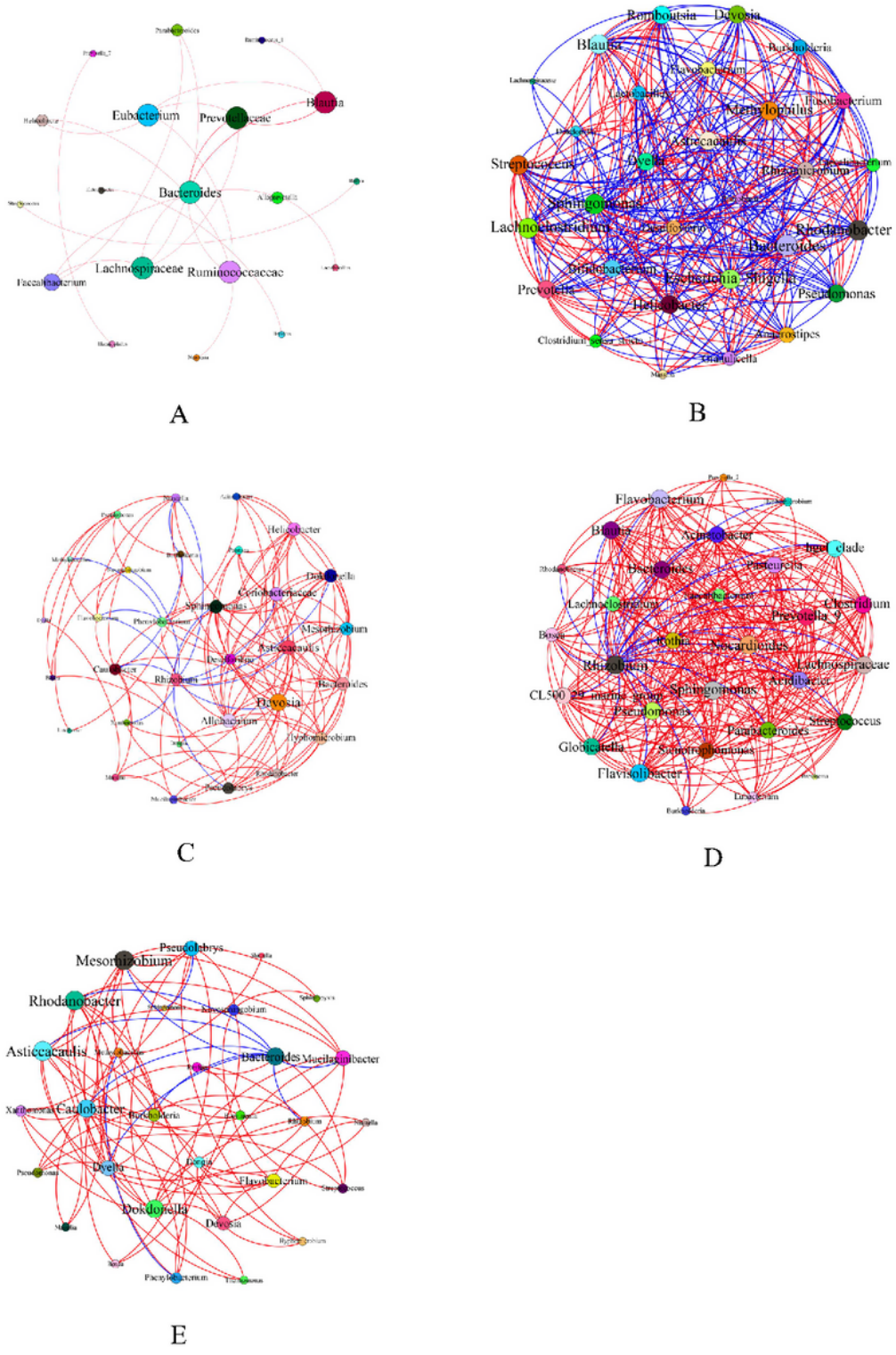

Figure 4

Co-occurring network of bacterial communities intensities based on correlation analysis. The interaction network of dominant microbiota at the genus level (top 30) in the seeds (A), seedling roots (B), flower roots (C), flower nodules(D) and mature roots (E).The nodes in network are colored by genus. A connection stands for a moderate (Spearman's ρ > 0.4) and significant (P < 0.01) correlation. The size of

each node is proportional to the number of node connections. A blue line means a negative relationship between two individual nodes and a red line indicates a positive relationship.

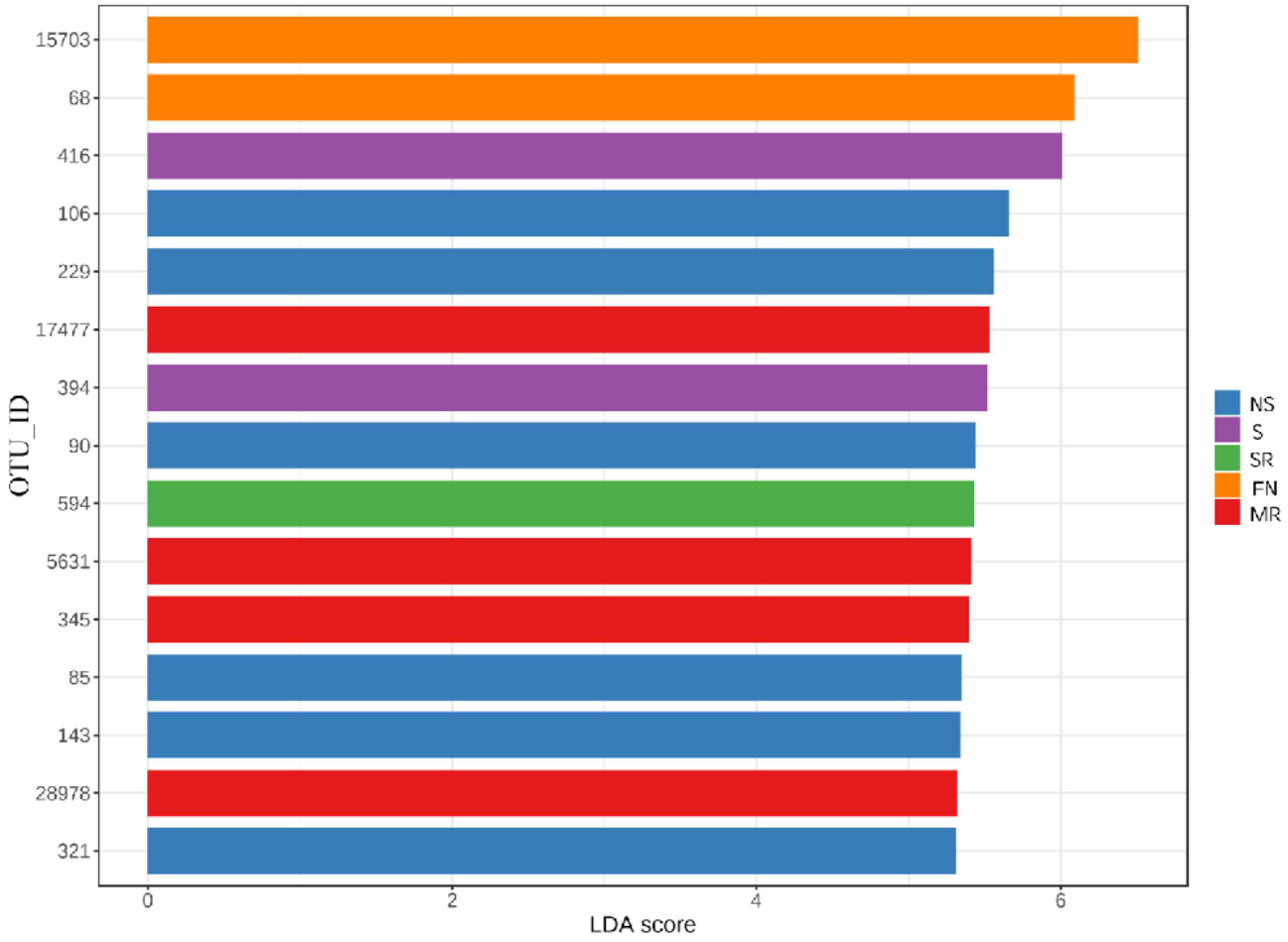

Figure 5

Linear discriminant analysis (LDA). The LDA score represents the taxa enriched in the roots at different development stages. Only taxa with LDA values greater than 4 ($P < 0.05$) are shown.

Supplementary Files

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