

Responses of Rhizosphere and Root Surface Bacterial Communities and biomass of *Cunninghamia lanceolata* and *Schima superba* Seedlings to Nitrogen Forms

Haiyan Liang

Lidong Wang

Yanru Wang

Xiaoqiang Quan

Xiaoyu Li

Yaning Xiao

Xiaoli Yan (✉ ccyanxiaoli@163.com)

Fujian Agriculture and Forestry University <https://orcid.org/0000-0002-6059-1063>

Research Article

Keywords: *Cunninghamia lanceolata*, *Schima superba*, Rhizosphere, Root surface, Nitrogen forms, bacterial community

Posted Date: October 3rd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3345365/v1>

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Abstract

Background and aims

Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) that can be absorbed and utilized by plants are heterogeneously distributed in nature soil, which will affect the plant growth and bacterial communities. This study aims to investigate the effects of single and mixed nitrogen (N) on bacterial communities in rhizosphere and root surface of *Cunninghamia lanceolata* (CR, CRS) and *Schima superba* (SR, SRS) seedlings.

Methods

Three N ratios $\text{NH}_4^+\text{-N}:\text{NO}_3^-\text{-N} = 10:0, 5:5$ (control, CK), $0:10$ were set to grow seedlings in pot culture with sand. The bacteria in the rhizosphere and root surface of the tree species were determined by High-throughput sequencing (16S rDNA), and bacterial numbers and growth indicators were fitted linearly.

Results

Compared with the CK, the $0:10$ increased the bacterial numbers, richness and diversity in CR, while the $10:0$ and $0:10$ were both decreased them in CRS, SR and SRS, which was more similar between the $0:10$ and CK. Overall, the bacterial numbers and richness were: $\text{CR} > \text{SR} > \text{CRS} > \text{SRS}$, but no obvious rule in diversity. The dominant bacterial phyla in CR and CRS were mainly *Proteobacteria* and *Bacteroidota*, while in SR and SRS were mainly *Proteobacteria* and *Acidobacteriota*. Except for the root biomass of *S. superba*, the linear relationship of other indicators in the rhizosphere were greater than root surface.

Conclusion

Mixed N was more conducive to bacterial community proliferation, and there were significant differences between the two tree species and the bacterial communities at different rhizosphere sites.

Introduction

As one of the main coniferous afforestation and timber species in subtropical China, *Cunninghamia lanceolata* has attracted much attention for its single stand planting mode, simple community structure and reduced ecological function services (Suo et al. 2019; Tian et al. 2011). Broad-leaved tree species can produce a large amount of litter, decomposition speed, provide nutrients, but also play a role in water storage and fertilizer, which now is used as soil conversion tree species with conifer species for mixed planting to create a mixed forest (Yan et al. 2019). As one of the soil modification species, *Schima superba* is widely distributed in subtropical areas, and is an important precious timber, biological fire prevention and ecological control species in China. In the existing mixed planting forest of *C. lanceolata* and *S. superba*, the quality of *C. lanceolata* plantation has been greatly improved (Wan et al. 2013). In

order to balance the economic and ecological benefits of afforestation, the mixed afforestation of needle and broad has been widely concerned, and ecological forest management is advocated (Yu 1990).

Nitrogen (N) is the most important mineral nutrient element in plants, and has become the main factor limiting plant growth in terrestrial ecosystems. The ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) that can be absorbed and utilized by plants have great spatial heterogeneity in natural soil. Due to the widespread existence of N deficiency and heterogeneous distribution environment in nature, plants have formed regulatory strategies for utilization and absorption of different forms of N in the long-term evolution process so that different plants have different N absorption preferences (Tao et al. 2017). The heterogeneous distribution results in unequal distribution of the two N forms in soil, and most studies have shown that the plant growth is higher when the mixed nitrogen content is equal (Sun et al. 2015; Zhu et al. 2021).

Plant functional characteristics, symbiotic microorganisms and soil free microorganisms were the main biological factors affecting nitrogen uptake preference. Most plant roots have mycorrhizal symbiosis, and bacterial community is also one of the hot spots in root and rhizosphere research. Numerous studies have shown that soil pH value, nutrient content, temperature and humidity, and root exudates all affect soil bacteria levels (Marschner et al. 2004; Yu et al. 2021). Soil nitrogen availability and plant tissue nitrogen levels are closely related to changes in rhizosphere bacterial community structure, and plants can change their rhizosphere microbiome by affecting nutrient availability (Bell et al. 2015). The way in which plants establish rhizosphere bacterial communities can now be seen as a selective trait related to the intrinsic nutritional needs of plant species. Compared with the non-rhizosphere soil community, the taxonomic structure and functional profile of rhizosphere soil community changed less, that is, the degree of homogeneity was higher, and the interaction between rhizosphere bacteria operating taxonomic units was stronger than that of non-rhizosphere soil (Yan et al. 2017). Most studies have shown that the main bacterial groups in rhizosphere soils are *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, etc. (Ren et al. 2016; Wang et al. 2018). The addition of $\text{NH}_4^+\text{-N}$ increased the number and biomass of bacteria, while the addition of $\text{NO}_3^-\text{-N}$ decreased these values. The N deposition of two N forms inhibited the growth of bacteria, and the change trend was related to the ratio of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in soil (Liu et al. 2016).

At present, numerous studies tend to study the differences between rhizosphere and non-rhizosphere soil microbial communities. As one of the attachment locations of microorganisms, root surface is directly connected with plant roots, and it is also a microbial site worthy of attention. In the dynamic natural soil, the ideal state such as the ratio of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ does not exist, and single $\text{NH}_4^+\text{-N}$ and single $\text{NO}_3^-\text{-N}$ rarely exist. How the single N source and mixed N affect the rhizosphere and root surface bacterial communities of *C. lanceolata* and *S. superba* seedlings remains unclear. In view of this, the effects of single N source and mixed N on rhizosphere and root surface bacterial communities of *C. lanceolata* and *S. superba* seedlings were studied by the method of pot culture with sand.

Materials and methods

Plant materials

One year of *C. lanceolata* and *S. superba* seedlings with uniform growth that were free from disease and insect pests, and had a complete root system were selected as the test seedlings. The average seedling height and ground diameter of *C. lanceolata* were (25.8 ± 3.1) cm and (4.51 ± 0.50) mm, and the average seedling height and ground diameter of *S. superba* were (16.1 ± 2.1) cm and (3.06 ± 0.46) mm. Plastic pots (26 cm diameter $\times$ 28 cm height) were used for cultivating the seedlings. The potting substrate was selected clean sand, which was repeatedly washed with distilled water until the N content in the sand was close to zero (Hao et al. 2017; Wu et al. 2011). When planting the test seedlings, the root soil was cleaned with distilled water and then transplanted into the test sand, keeping the root system intact.

Field experiment and experimental setup

In April 2021, the sand culture experiments were conducted in a rainproof greenhouse with good ventilation and light transmission at Fujian Agriculture and Forestry University, China. The average temperature was 20.1°C and the average relative humidity was about 77%. Each pot was filled with equal amount of clean sand.

According to the research results of Zhang *et al.* (Zhang et al. 2006) on the influence of fertilization on the soil nutrient content of *C. lanceolata* and the previous research results of Meng *et al.* (Meng 2016) and Liu *et al.* (Liu et al. 2017), the total N concentration was set to 2 mmol/L. In this study, three ratios were set as $\text{NH}_4^+\text{-N}:\text{NO}_3^-\text{-N} = 10:0, 5:5$ (control CK) and $0:10$. $\text{NH}_4^+\text{-N}$ supplied as $(\text{NH}_4)_2\text{SO}_4$, while $\text{NO}_3^-\text{-N}$ was supplied as NaNO_3 , and NaCl was used to regulate the Na^+ concentration among each treatment. In all treatments, except for the concentration of the two N forms were different, the concentration of the macronutrients (Hoagland formula) and micronutrients (Amon formula) remained the same. To prevent the conversion of ammonium to nitrate, the 7 $\mu\text{mol/L}$ nitrification inhibitor dicyandiamide was added to the nutrient solution. The pH of the nutrient solution was adjusted to 5.8 with 2 mol/L NaOH or HCl. Each treatment had six replicates, and the total number of pots was 36. Each pot was added with nutrient solution at a rate of 50 mL every 5 days and supplied with 150–300 mL of distilled water every 2–3 days. We did not water the pots for 24 h before and after pouring the nutrient solution.

Sample collection

After 160 days of cultivation, the seedlings were harvested and gently shaken to remove the loosely adhered sand before which rhizosphere sand and fresh roots samples were collected. Samples were stored at -80°C for further analysis. In each treatment, there were four sites: *C. lanceolata* rhizosphere (CR), *C. lanceolata* root surface (CRS), *S. superba* rhizosphere (SR), *S. superba* root surface (SRS).

Determination of rhizosphere sand and root surface bacteria

After the genomic DNA extraction, the extracted genomic DNA was detected by 1% Agarose Gel Electrophoresis. The V3-V4 region of bacterial 16S rDNA were amplified using primer 338F (5'-

ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') combined with cohesive subsequences and bar code sequences (Cao et al. 2021; Ren et al. 2020). PCR amplification system consisted of 30 ng DNA samples, 5 μ M Forward Primer and 1 μ L Reverse Primer, 3 μ L bovine serum albumin (BSA), 12.5 μ L 2 $\times$ Taq Plus Master Mix and 7.5 μ L double distilled water (ddH₂O). The PCR amplification procedure was 94°C for 5 min, 94°C for 30 s, 50°C for 30 s, 72°C for 60 s (30 cycles), and then reacted at 72°C for 7 min and finally placed at 4°C. The amplified PCR products were tested for band size using 1% Agarose Gel Electrophoresis for 30 min and purified with Agencourt AMPure XP nucleic acid purification kit. The Miseq library was used to construct the product, and the "Y" shaped joint was connected, and the self-connected segment of the joint was removed. The library template was enriched by PCR amplification, and the single stranded DNA fragment was produced by the deformation of sodium hydroxide (NaOH). The Pair-end (PE) double-ended sequence data was obtained by Miseq sequencing. Quality control was carried out on the measured Fastq data and high-quality Fasta data was finally obtained. The relative abundance of bacteria at each classification level was obtained after species annotation of genes. The sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97% (Edgar 2013) to calculate the Chao richness estimator and Shannon diversity index values. High-throughput sequencing was performed in Allwegene Gene Technology Co., LTD., Beijing, China.

Data analysis

The bacteria in the rhizosphere and root surface of the two tree species, Trimmomatic (version 0.36) and Pear (version 0.9.6) were used for quality control of Fastq data, and Flash (version 1.20) and Pear were used for concatenation of sequences at both ends to obtain Fasta sequences. The OTU cluster analysis of 97% similarity level valid series was carried out by Uparse (Edgar 2013). Qiime (version 1.8.0 <http://qiime.org/>) was used for Alpha and Beta diversity analysis, and R programming language was used for statistics and plotting. SPSS 25.0 was used to conduct T-test for the comparison of the bacterial numbers, richness and diversity between rhizosphere and root surface of the two tree species, as well as the two tree species in the rhizosphere and root surface. The linear fitting analysis was carried out to analyze the relationship between bacterial numbers and growth indicators, and Origin 2018 was used to plot.

Results

The number of the bacterial community in rhizosphere and root surface

The bacterial number of rhizosphere and root surface of two tree species under different N forms

According to the Operational Taxonomic Units (OTUs) clustering analysis (Table 1), the analysis produced a total of 11,256 OTUs, with 10,541 remaining after the leveling process, of which 7,015, 3,219,

3,635 and 3,454 were respectively contained in CR, CRS, SR, SRS. The bacterial numbers in four sites under each treatment were: CR > SR > CRS > SRS.

In the ratios of 10:0, 5:5, and 0:10 (Table 1), the mean number of OTUs in CR was 2238, 2805, 2873, and in CRS was 1384, 1849, and 1712, respectively, with no significant difference among treatments ($P > 0.05$). The mean number of OTUs in RS was 1384, 1986, 1777, among which the 5:5 treatment was significantly larger than the 10:0 ($P < 0.05$), while the difference between the ratios of 5:5 and 0:10, 10:0 and 0:10 were not significant ($P > 0.05$). The mean number of OTUs in SRS was 1148, 1738, and 1488, respectively, which were significantly different among the three treatments ($P < 0.05$). Overall, except for the CR, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ treatments reduced bacterial OTUs in other sites.

Table 1
The number of OTU in rhizosphere and root surface of the two tree species under different N forms

Treatment	OTU			
	CR	CRS	SR	SRS
10:0	2238 ± 274.90 a	1384 ± 176.36 a	1384 ± 63.28 b	1148 ± 67.67 c
5:5	2805 ± 277.11 a	1849 ± 114.05 a	1986 ± 21.26 a	1738 ± 48.01 a
0:10	2873 ± 407.56 a	1712 ± 130.35 a	1777 ± 240.68 ab	1488 ± 67.95 b
Note: Different lowercase letters in the table indicate significant differences among treatments.				

At the ratio of 5:5 (Fig. 1a, b), the OTU between CR and CRS, SR and SRS showed significant differences ($P < 0.05$), but not at the ratios of 10:0 and 0:10 ($P > 0.05$), and the rhizosphere was larger than the root surface in all cases. Under the rhizosphere and root surface sites (Fig. 1c, d), there were no significant differences in OTU between *C. lanceolata* and *S. superba* ($P > 0.05$), but the OTU of *C. lanceolata* was greater than that of *S. superba*.

The number of OTUs common and unique in rhizosphere and root surface of the two tree species under different N forms

The number of rhizosphere and root surface bacteria of the two tree species under different N forms were generated by Venn diagram (Fig. 2). The Venn diagram can count the number of OTUs common and unique among the three treatments, which can show the overlap between the groups of OTUs and their groupings among treatments in a more visual way.

The number of common OUTs under the three treatments in the four sites were 2386, 1753, 1649, 1307, respectively (Fig. 2a, b, c, d). Under the ratios of 10:0, 5:5, 0:10, the unique OTUs were 850, 854, 763 in CR (Fig. 2a), 87, 233, 255 in CRS (Fig. 2b), 226, 394, 241 in SR (Fig. 2c), and 225, 570, 303 in SRS (Fig. 2d). The results showed that the CR, SR, SRS had the most unique bacterial groups under the ratio of 5:5, and

the CRS was the most unique in the ratio of 0:10. Meanwhile, the number of common bacteria between the two treatments were both the highest at ratios of 5:5 and 0:10 under the four sites.

Analysis of Alpha diversity of rhizosphere and root surface bacteria of two species under different N forms

The diversity analysis of single sample (Alpha diversity) can reflect the richness and diversity of bacterial communities. Chao1, Shannon and Simpson indexes represent the species richness and diversity. Coverage represents the coverage of the sample library.

Under the three treatments (Table 2a), there were no significant differences in Chao1 index, Coverage, Shannon and Simpson indexes of bacteria in the CR, and the bacterial richness and diversity were: 0:10 > 5:5 > 10:0. There were no significant differences in Chao1 index, Coverage and Shannon index of bacteria on the CRS ($P > 0.05$) (Table 2b), while Simpson index reached its maximum at the ratio of 0:10, significantly greater than 10:0 ($P < 0.05$). The bacterial richness showed as 5:5 > 0:10 > 10:0, and the bacterial diversity was relatively higher at the ratios of 0:10 and 5:5.

The Chao1 index in the SR showed as: 5:5 > 0:10 > 10:0 (Table 2c), in which the ratio of 5:5 was significantly greater than 10:0 ($P < 0.05$). The bacterial Coverage, Shannon and Simpson index were not significantly different, in which the Shannon and Simpson index were both the largest under the ratio of 5:5. The differences in Chao1 index, Coverage and Shannon index under the SRS were significant ($P < 0.05$) (Table 2d), while there was no significant difference in Simpson index ($P > 0.05$). And its richness and diversity of bacteria were as follows: 5:5 > 0:10 > 10:0.

Table 2
Rhizosphere and root surface bacterial richness and diversity indexes of the two tree species under different N forms

	Treatment	Chao1	Coverage	Shannon	Simpson
(a) CR	10:0	3210.21 ± 363.36 a	0.97 ± 0.01 a	8.66 ± 0.45 a	0.9900 ± 0.0058 a
	5:5	3923.04 ± 285.33 a	0.96 ± 0.00 a	9.28 ± 0.50 a	0.9933 ± 0.0067 a
	0:10	3967.79 ± 543.98 a	0.96 ± 0.01 a	9.30 ± 0.36 a	0.9933 ± 0.0033 a
(b) CRS	10:0	1919.37 ± 216.31 a	0.98 ± 0.00 a	7.15 ± 0.27 a	0.9700 ± 0.0058 b
	5:5	2375.77 ± 86.23 a	0.98 ± 0.00 a	8.34 ± 0.26 a	0.9833 ± 0.0033 ab
	0:10	2158.26 ± 87.86 a	0.98 ± 0.00 a	8.28 ± 0.50 a	0.9900 ± 0.0058 a
(c) SR	10:0	1834.57 ± 139.76 b	0.98 ± 0.00 a	7.73 ± 0.12 a	0.9833 ± 0.0033 a
	5:5	2440.54 ± 35.06 a	0.98 ± 0.00 a	9.32 ± 0.07 a	1.0000 ± 0.0000 a
	0:10	2270.00 ± 229.55 ab	0.98 ± 0.00 a	8.08 ± 1.12 a	0.9733 ± 0.0267 a
(d) SRS	10:0	1628.26 ± 99.65 c	0.98 ± 0.00 a	7.23 ± 0.03 c	0.9767 ± 0.0033 a
	5:5	2285.61 ± 58.51 a	0.97 ± 0.00 b	8.77 ± 0.02 a	0.9900 ± 0.0000 a
	0:10	2035.83 ± 46.85 b	0.97 ± 0.00 b	8.07 ± 0.31 b	0.9800 ± 0.0100 a

There were significant differences in Chao1 index between CR and CRS under the ratios of 10:0 and 5:5 ($P < 0.05$) (Fig. 3a), and between CR and SR under the three treatments ($P < 0.05$) (Fig. 3c), but no significant differences between SR and SRS, CRS and SRS ($P > 0.05$) (Fig. 3b, d). The bacterial richness in four sites were: CR > SR > CRS > SRS. Except for the Coverage between CR and CRS, CR and SR were significantly different under the ratio of 5:5 ($P < 0.05$) (Fig. 3e, g), there were no significant differences in other treatments ($P > 0.05$) (Fig. 3e, f, g, h). It can be seen that the probability of sequence detection in each sample was high.

The bacterial Shannon indexes in CR and CRS under the ratio of 10:0 and in SR and SRS under the ratios of 10:0 and 5:5 had significant differences ($P < 0.05$) (Fig. 3i, j). Under the three treatments (Fig. 3k, l), there were no significant differences in Shannon index between CR and SR, CRS and SRS ($P > 0.05$), and in Simpson index between all the two groups under the three ratios ($P > 0.05$) (Fig. 3m, n, o, p). In conclusion, bacterial diversity in *C. lanceolata* and *S. superba* characterized by Shannon index was main as followed: rhizosphere > root surface, while bacterial diversity in the rhizosphere and root surface environments and the bacterial diversity represented by Simpson index showed no significant rule.

Analysis of Beta diversity of rhizosphere and root surface bacteria of two species under different N forms

The PCoA axis 1 and axis 2 respectively explain 29.26% and 22.23% in CR (Fig. 4a), 35.37% and 17.57% in the CRS (Fig. 4b), 34.66% and 25.92% in SR (Fig. 4c), 35.63% and 15.78% in SRS (Fig. 4d). The

composition and diversity of bacterial community were similar between the groups of 5:5 and 0:10, but were both far away from 10:0, that there were great differences. Among them, the differences of bacterial community composition and diversity in the intra-group were larger than the inter-group in CRS and SRS under the ratios of 5:5 and 0:10. That means the bacterial community composition between intra-group and inter-group was more similar in rhizosphere than root surface.

Composition of rhizosphere and root surface bacterial communities of two tree species under different N forms

Effects of single and mixed form N on bacterial community composition of rhizosphere and root surface of *C. lanceolata*

Under the three treatments, 62 and 47 bacterial phyla were detected in the CR and CRS, respectively. Under the ratio of 10:0 (Fig. 5a), the mainly dominant bacteria phyla in the CR were *Proteobacteria* (42.14%), *Bacteroidota* (11.29%), *Acidobacteriota* (9.89%), *Actinobacteria* (9.52%), *Chloroflexi* (6.31%), *Verrucomicrobiota* (5.84%). In the ratio of 5:5, the dominant rhizosphere bacteria were *Proteobacteria* (49.18%), *Bacteroidota* (10.90%), *Verrucomicrobiota* (7.06%), *Acidobacteriota* (6.56%), *Actinobacteriota* (6.21%), *Patescibacteria* (4.29%). Under the ratio of 0:10, the rhizosphere dominant bacteria were *Proteobacteria* (45.45%), *Bacteroidota* (12.19%), *Verrucomicrobiota* (6.96%), *Actinobacteriota* (6.81%), *Acidobacteriota* (6.17%), *Patescibacteria* (5.47%).

As shown in Fig. 5b, the dominant bacteria in the CRS under the ratio of 10:0 were *Proteobacteria* (58.01%), *Bacteroidota* (14.48%), *Acidobacteriota* (7.91%), *Actinobacteriota* (6.93%), *Verrucomicrobiota* (4.74%), *Cyanobacteria* (2.51%). In the ratio of 5:5, the dominant bacteria were *Proteobacteria* (53.47%), *Bacteroidota* (14.91%), *Actinobacteriota* (7.58%), *Verrucomicrobiota* (5.10%), *Acidobacteriota* (3.93%). At the ratio of 0:10, the dominant bacteria were *Proteobacteria* (56.30%), *Bacteroidota* (13.33%), *Actinobacteriota* (6.32%), *Acidobacteriota* (5.54%), *Verrucomicrobiota* (4.05%), *Chloroflexi* (2.66%).

In conclusion, the dominant bacteria phyla in CR and CRS under the three treatments were both *Proteobacteria* and *Bacteroidota*, and the dominant bacteria phyla between the ratios of 5:5 and 0:10 were more similar.

Effects of single and mixed form N on bacterial community composition of rhizosphere and root surface of *S. superba*

Under the three treatments, 45 and 44 bacterial phyla were detected in the SR and SRS, respectively. Under the ratio of 10:0 (Fig. 6a), the dominant bacteria phyla in SR were *Proteobacteria* (44.78%), *Acidobacteria* (14.70%), *Actinobacteria* (9.74%), *Verrucomicrobiota* (7.48%), *Bacteroidota* (6.05%), *Cyanobacteria* (4.89%). In the ratio of 5:5, the dominant rhizosphere bacteria were *Proteobacteria* (40.68%), *Bacteroidota* (10.11%), *Acidobacteriota* (9.35%), *Verrucomicrobiota* (6.93%), *Actinobacteriota* (6.37%), *Cyanobacteria* (4.61%). Under the ratio of 0:10, the rhizosphere dominant bacteria were

Proteobacteria (39.27%), *Cyanobacteria* (10.60%), *Bacteroidota* (10.55%), *Acidobacteriota* (8.13%), *Verrucomicrobiota* (7.40%), *Actinobacteriota* (6.24%).

Under the ratio of 10:0 (Fig. 6b), the dominant bacteria phyla in SRS were *Proteobacteria* (51.20%), *Acidobacteriota* (10.87%), *Verrucomicrobiota* (8.56%), *Cyanobacteria* (7.78%), *Actinobacteria* (6.93%), *Bacteroidota* (5.42%). In the ratio of 5:5, the dominant root surface bacteria were *Proteobacteria* (43.60%), *Acidobacteriota* (13.37%), *Bacteroidota* (8.68%), *Cyanobacteria* (6.90%), *Verrucomicrobiota* (6.72%), *Actinobacteriota* (5.70%). Under the ratio of 0:10, the dominant root surface bacteria were *Proteobacteria* (49.58%), *Acidobacteriota* (13.00%), *Bacteroidota* (8.94%), *Verrucomicrobiota* (8.70%), *Actinobacteriota* (4.70%), *Cyanobacteria* (3.08%).

In conclusion, the dominant bacteria phyla in SR under the three treatments were *Proteobacteria*, while in SRS under the three treatments were *Proteobacteria* and *Acidobacteriota*.

Linear fitting of the OTUs and the growth indicators in the rhizosphere and root surface of the two tree species

The OTUs in CR and CRS had significantly negative correlation with the increment of plant height (Fig. 7a) and ground diameter (Fig. 7b). The negative correlation between the OTUs and total biomass in the CR was significant but not significant in the CRS (Fig. 7c). The OTUs in CR and CRS had non-significantly positive correlation with the root biomass (Fig. 7d). The OTUs in the SR and SRS had significantly positive correlation with the increment of plant height (Fig. 7e). The positive correlation between the OTUs and the increment of ground diameter in the SR was significant but not significant in the SRS (Fig. 7f). The OTUs in SR and SRS had non-significantly positive correlation with total biomass (Fig. 7g). The OTUs in SR and SRS had significantly negative correlation with the root biomass (Fig. 7h).

As shown as Fig. 7, the relationships between the OTUs and the increment of height, the increment of ground diameter, and total biomass were negatively correlated with the relationship between the OTUs and root biomass in the rhizosphere and root surface of *C. lanceolata* and *S. superba*. Among them, the relationship of *C. lanceolata* were negatively correlated with *S. superba*. Meanwhile, except for the relationship between the OTUs and root biomass of *S. superba*, the other relationships were all the rhizosphere greater than root surface.

Discussion

The rhizosphere refers to the area of soil adjacent to and affected by plant roots, usually with high microbial activity (Yan et al. 2017). The abundance, diversity and composition of soil bacterial community are mainly affected by soil type and fertilization, such as sand, loam, clay, nutrient (such as N, P) concentration, pH value, temperature and humidity and other environmental factors (Chen et al. 2016; Liu et al. 2020). For example, in soils with different pH values, bacterial diversity was highest in neutral soils and low in acidic soils (Fierer and Jackso 2006). Soil N availability and plant N levels are closely related to changes in rhizosphere bacterial community structure. Plants can change their rhizosphere

microbiome by influencing nutrient availability (Bell et al. 2015), and different N form and ratio will affect the bacterial communities in soil (Liu et al. 2016).

Ammonium leads to larger in reducing the bacterial number, richness and diversity compared with nitrate

The results of this study showed that the ratio of 0:10 contributed to increase the OTU number of bacteria in the CR, while the control N level of 5:5 contributed to increase the OTU number of bacteria in the CRS. In the SR and SRS, the control N supply level contributed to increase the number of bacterial OTU, while under the ratio of 10:0, the number of bacterial OTU in the rhizosphere and root surface of *C. lanceolata* and *S. superba* was the least. In the study of N uptake preference of main tree species in subtropical forestation, it was found that the NO_3^- -N uptake rate of tree species was significantly positively correlated with soil bacterial biomass (Chen 2018; Zhang and Han 2012), and NH_4^+ -N addition would reduce its biomass (Soderberg and Bååth 2004). This is similar to this result that the overall number of bacterial OTU was as follow: CK (5:5) > 0:10 > 10:0. Contrary studies have shown that soil bacteria are more likely to absorb NH_4^+ -N compared with NO_3^- -N (Barlett and Leff 2010; Zi et al. 2020). This may be because even though NH_4^+ -N is easier to absorb than NO_3^- -N, there is a toxic effect of NH_4^+ (Li et al. 2014), which makes the number of bacteria under the 10:0 ratio treatment relatively small. Due to acidification, NH_4^+ -N may inhibit microbial content more strongly than NO_3^- -N (Zhang et al. 2017). At the same time, it may also be that bacteria in these environments prefer the coexistence environment of NH_4^+ -N and NO_3^- -N. Many bacterial groups can reduce and absorb NO_3^- -N, and use NO_3^- -N as the sole N source to survive (Allen et al. 2001), showing a greater bias than NH_4^+ -N. When the plant is at its peak growth, its N uptake rate has an inverse relationship with the N level of microbial biomass (Bell et al. 2015).

In N-applied soil, NH_4^+ -N availability can directly affect bacterial diversity (Zeng et al. 2016), or bacterial community can be enhanced with the increase of soil NO_3^- -N (Wang et al. 2020). Different tree species also showed differential responses to rhizosphere and root surface bacterial biomass with different N forms (Marschner et al. 2004). As an adherent growth site of microorganisms in the rhizosphere region, the root surface is also different from the rhizosphere soil in terms of microbial abundance, diversity and composition (Guo et al. 2020; Ryan et al. 2019). The results showed that the number of OTU in the rhizosphere matrix of *C. lanceolata* and *S. superba* was greater than that on the root surface. Pang (Pang 2019) also found that under the combined effect of natural conditions, warming and nitrogen increase, the abundance of bacteria in the root system of a Canadian yellow flower was the highest in the rhizosphere. It can be seen that the rhizosphere environment is the most abundant area of bacteria (Yan et al. 2017). In terms of bacterial taxa, the CR had the most unique bacterial taxa under the control environment of 5:5, while the CRS had the most unique bacterial taxa under the ratio of 0:10. Both rhizosphere and root surface of *S. superba* showed the most unique bacterial taxa under the control 5:5. The composition and diversity of bacterial communities in the four sites were similar in the ratios of 5:5

and 0:10, but significantly different from that of 10:0, which was consistent with the results of bacterial OTU number. Except for the CR, single NH_4^+ -N and NO_3^- -N treatments reduced bacterial richness and diversity in other sites, which was mainly caused by the change of soil pH value during the absorption of NH_4^+ and NO_3^- by roots (Zhang et al. 2021). The same results showed that the application of ammonium and nitrate was 1:1 could improve the relative abundance and diversity of soil microbial community (Liu 2022).

Nitrogen forms affect bacterial community structure in the rhizosphere and root surface of the two tree species

NH_4^+ -N and NO_3^- -N are the main environmental factors that affect the composition and distribution of bacterial communities (Li et al. 2016; Shen et al. 2016). Some studies also suggest that increasing soil NH_4^+ -N availability by N application directly affects bacterial diversity, and then indirectly affects bacterial community composition through soil acidification and plant community changes (Zeng et al. 2016). Most studies have shown that the main dominant groups of bacteria in rhizosphere soil are *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota* and *Bacteroidota* (Ren et al. 2016; Wang et al. 2018). The results of this experiment showed that bacterial community structure in the rhizosphere and root surface of *C. lanceolata* and *S. superba* were different to varying degrees under different N forms and ratios. Under the three treatments, the dominant phyla of bacteria in the CR and CRS were *Proteobacteria* and *Bacteroidota*, and the dominant phyla with the ratios of 5:5 and 0:10 at both positions were more similar, which was consistent with the results of bacterial abundance and diversity. Under the three treatments, *Proteobacteria* was the dominant phyla of *S. superba* rhizosphere bacteria. Among them, the second dominant phyla were *Acidobacteriota*, *Bacteroidota*, and *Cyanobacteria* corresponding to the ratios of 10:0, 5:5, and 0:10, respectively, and there were certain differences in the dominant phyla under each treatment. *Proteobacteria* and *Acidobacteriota* were the dominant phyla of *S. superba* root surface bacteria, and the dominant phyla of the ratios of 5:5 and 0:10 were more similar. Obviously, under the three treatments, the dominant bacteria in rhizosphere and root surface bacteria of the two tree species are both *Proteobacteria*. In all treatments, the main dominant bacterial phyla are *Proteobacteria*, *Bacteroidota*, *Actinobacteriota*, *Acidobacteriota*, *Verrucomicrobiota*, *Patescibacteria*, *Chloroflexi* and *Cyanobacteria*, etc., which is consistent with the results of most studies (Chai et al. 2019; Coolon et al. 2013; Wang et al. 2018; Zhao et al. 2020). The proportion of *Acidobacteriota* (oligotrophs) in all sites under the ratio of 10:0 and in the SRS under all treatments were relatively higher than that under the other treatments and sites, but the abundance of copiotrophs (such as *Proteobacteria*, *Bacteroidota* and *Actinobacteriota*) were higher in all treatments and sites, indicated that copiotrophs were the main bacterial communities in rhizosphere and root surface of the two tree species.

NH_4^+ -N and NO_3^- -N have different effects on soil bacterial community structure. Compared with organic N, inorganic N can reduce the number of *Acidobacteriota*, and adding high levels of inorganic N can help increase the number of *Bacteroidota* (Kavamura et al. 2018; Sun et al. 2020). The influence of NH_4^+ -N on soil bacteria groups was greater than that of NO_3^- -N, in which NH_4^+ -N concentration was negatively

correlated with the relative abundance of *Acidobacteriota*, but positively correlated with *Proteobacteria*, *Bacteroidota* and *Actinobacteriota* (Fan et al. 2021; Wang et al. 2018; Zi et al. 2020). The results showed that the first two dominant bacteria phyla in CR and CRS were *Proteobacteria* and *Bacteroidota*, which was similar to the results of previous studies. Secondly, the third dominant bacteria phyla in CR and CRS under the ratio of 10:0 were *Acidobacteriota*, and the proportion were both larger than that of the other two treatments, which was contrary to previous studies, that is, $\text{NH}_4^+\text{-N}$ was not negatively correlated with *Acidobacteriota*. This may be because in the ratio of 10:0, $\text{NH}_4^+\text{-N}$ is prone to nitrification and conversion to $\text{NO}_3^-\text{-N}$, which will produce a large amount of H^+ and reduce the soil pH value (Niu et al. 2020), thus contributing to the proliferation of *Acidobacteriota* (Ezeokoli et al. 2020). Especially the continuous nitrogen fertilizer application can significantly affect most dominant soil bacteria by change soil pH (Ren et al. 2020). The dominant bacteria phyla in SR were *Proteobacteria*, followed by *Acidobacteriota*, *Bacteroidota* and *Actinobacteria*, and the dominant bacteria phyla in SRS were *Proteobacteria* and *Acidobacteria*, followed by *Verrucomicrobiota* and *Bacteroidota*. It can be seen that the negative correlation between $\text{NH}_4^+\text{-N}$ and *Acidobacteriota* is not strong, especially in the SRS, but is positively correlated, and the ratios of 5:5 and 0:10 are also positively correlated. The rhizosphere of *S. superba* is more strongly related to the root surface and *Acidobacteriota*, which may be related to the N forms in the treatment, tree species and the environment of sand culture substrate (Liu et al. 2022; Marschner et al. 2004). In summary, under different N forms environment, there are some differences in the main dominant bacteria phyla of *C. lanceolata* and *S. superba*, which also shows the differences in rhizosphere soil bacterial communities between coniferous and broad-leaved trees (Hu et al. 2021).

Relationship between bacterial numbers and growth indicators

In this study, the linear relationship between the growth indicators and bacterial numbers showed that the bacterial numbers in rhizosphere and root surface of *C. lanceolata* decreased with the increases of seedling height, ground diameter and total biomass, but increased with the increase of root biomass. The result of *S. superba* were opposite to those of *C. lanceolata*. That was, the parts of aboveground was negatively correlated with underground in two tree species. Except for the root biomass of *S. superba*, the linear relationship of other indicators in the rhizosphere were greater than root surface. The bacterial numbers in the rhizosphere and root surface of *C. lanceolata* were the least under the ratio of 10:0, and the corresponding root growth was also less, resulting the environment for bacteria to attach was reduced. On the contrary, the root system grew well under the CK and 0:10 treatments, which could provide more attachment points for bacterial growth and facilitate the increment of its richness and diversity (Li et al. 2021; Xia et al. 2022). While the bacterial numbers in the rhizosphere and root surface of *S. superba* were negatively correlated with the root growth, that was the bacterial numbers were the lowest under the ratio of 10:0 treatment, but the root biomass was higher. It can be seen that larger roots do not always promote the increase of rhizosphere microbial biomass (Iannucci et al. 2021). At the same time, the rhizosphere secretion provides nutrient environment for the growth of bacteria, and the amount of secretion also affects the bacterial numbers (Chen et al. 2019).

It has been suggested that root biomass, rather than total biomass and aboveground biomass, is the most important factor driving soil bacterial communities and nitrogen cycling functions (Liu et al. 2022). Our results demonstrated that the increment of seedling height and increment of ground diameter were the inverse driving factors for bacterial community in rhizosphere and root surface of *C. lanceolata*, while root biomass had no significant effect on them. In contrast, *S. superba* seedling height increment significantly drove bacterial communities in rhizosphere and root surface, while root biomass was an inverse driving factor. As an important catalyst for soil nutrient conversion, rhizosphere soil microorganisms can effectively promote fertilizer decomposition and ammonium nitrification, so as to promote the uptake of nutrients by plant roots (Potthast et al. 2012). For *S. superba*, bacterial activity may promote more nutrients absorbed by roots for aboveground growth, whereas the opposite is true for *C. lanceolata*. Meanwhile, the composition of bacterial community is mainly driven by the soil (substrate) type (Schlemper et al. 2017). Sand was used as the substrate in this experiment, and its relatively loose and poor substrate environment also had a certain impact on the bacterial community in the rhizosphere and root surface (Marschner et al. 2004). It seems that the single and mixed N source of two species of tree growth and the influence of rhizosphere and root surface bacterial community is different (Ding et al. 2022).

Conclusion

In conclusion, compared with the CK, the single NO_3^- -N treatment increased the bacterial numbers, richness and diversity in CR, while the single NH_4^+ -N and NO_3^- -N treatments reduced them in CRS, SR and SRS. The bacterial numbers and richness were shown as: $\text{CR} > \text{SR} > \text{CRS} > \text{SRS}$, but there was no obvious rule in the diversity. There were similarities and differences in the dominant phyla in the rhizosphere and root surface of the two species, among which *Proteobacteria* was all the most dominant phylum. The aboveground and underground parts of the two tree species were negatively correlated, and the two tree species were also negatively correlated under each indicator. Therefore, the ratio of 5:5 was more conducive to increase the bacterial numbers, richness and diversity in the rhizosphere and root surface of the two species, and there were significant differences between the two tree species and the bacterial communities at different rhizosphere sites.

Declarations

Acknowledgements

We would like to thank the support of the project funding, the teachers and students of the College of Forestry of Fujian Agriculture and Forestry University for their advice and help this time. We thank the staff of the laboratory for providing us with a good experimental environment.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Haiyan Liang, Lidong Wang, Yanru Wang, Xiaoqiang Quan and Xiaoyu Li. Data management was performed by Haiyan Liang, Lidong Wang and Yaning Xiao. The first draft of the manuscript was written by Haiyan Liang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This research was funded by the National Natural Science Foundation of China (32171773) and the Distinguished Young Scientific Research Talent Program of Fujian Agriculture and Forestry University (KXJQ20012).

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

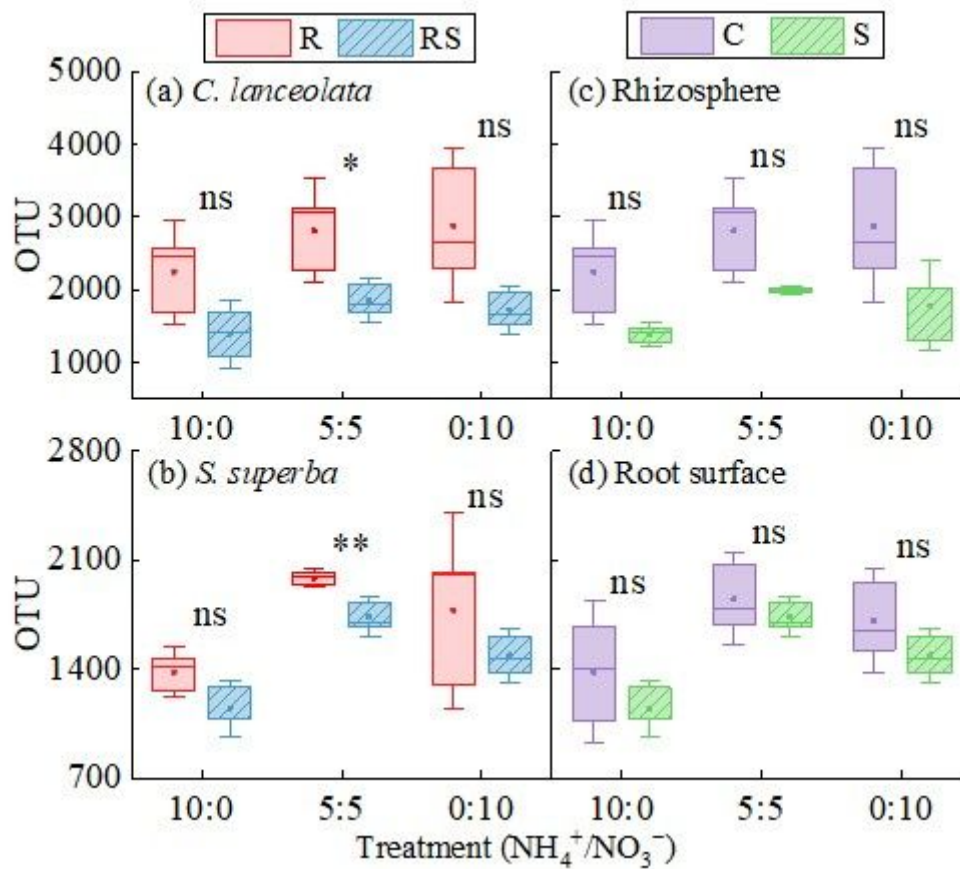


Figure 1

Comparison of the bacterial number in rhizosphere and root surface of *C. lanceolata*(a) and *S. superba* (b) and two tree species in rhizosphere (c) and root surface (d) under different N forms. * $P < 0.05$ ** $P < 0.01$ ns $P > 0.05$

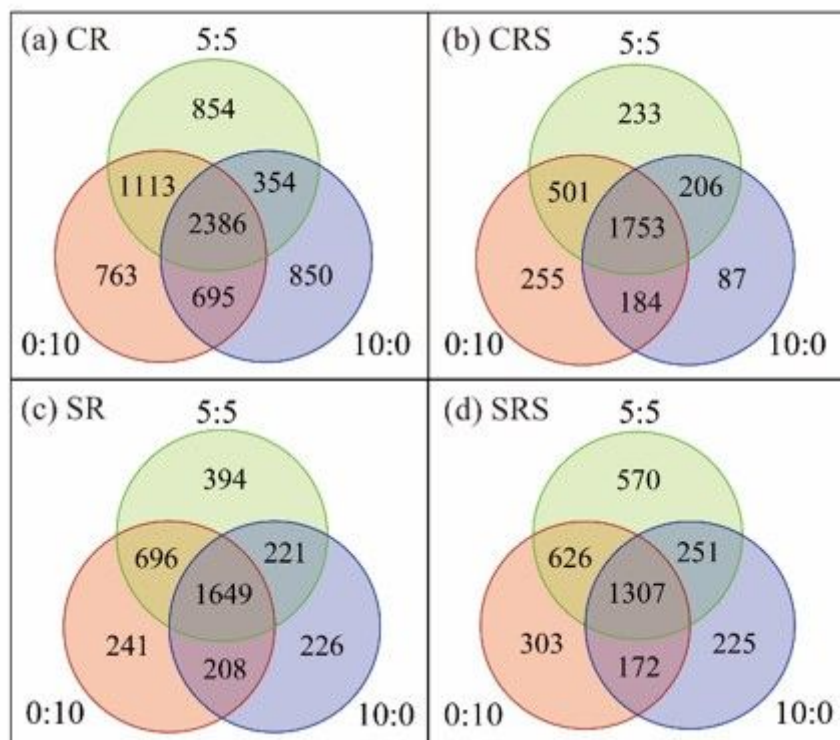


Figure 2

Bacterial OTUs distribution in rhizosphere and root surface of *C. lanceolata* and *S. superba* under different N forms

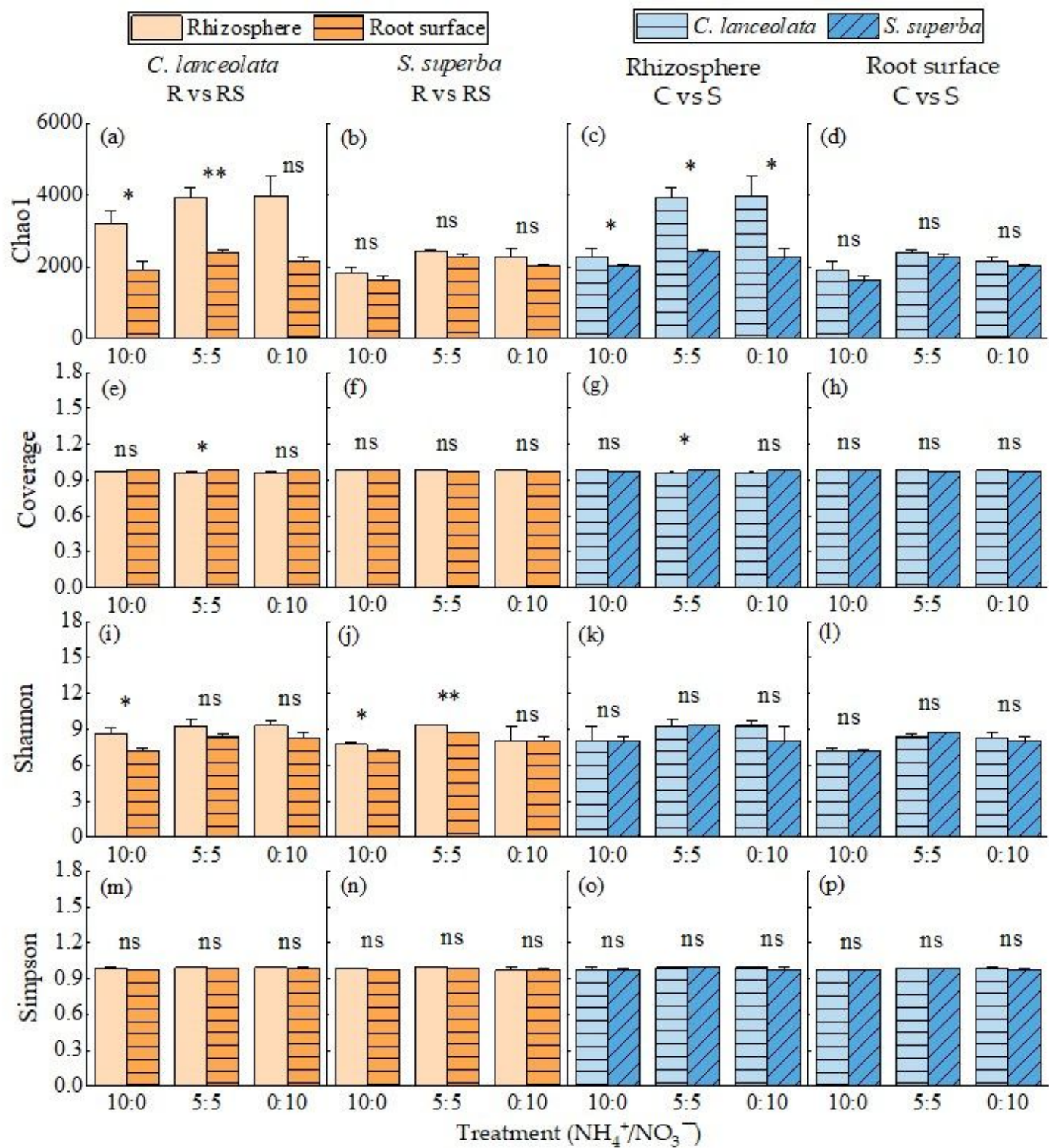


Figure 3

Comparison of rhizosphere and root surface in two tree species and two tree species in rhizosphere and root surface bacterial richness and diversity indexes under the different N forms

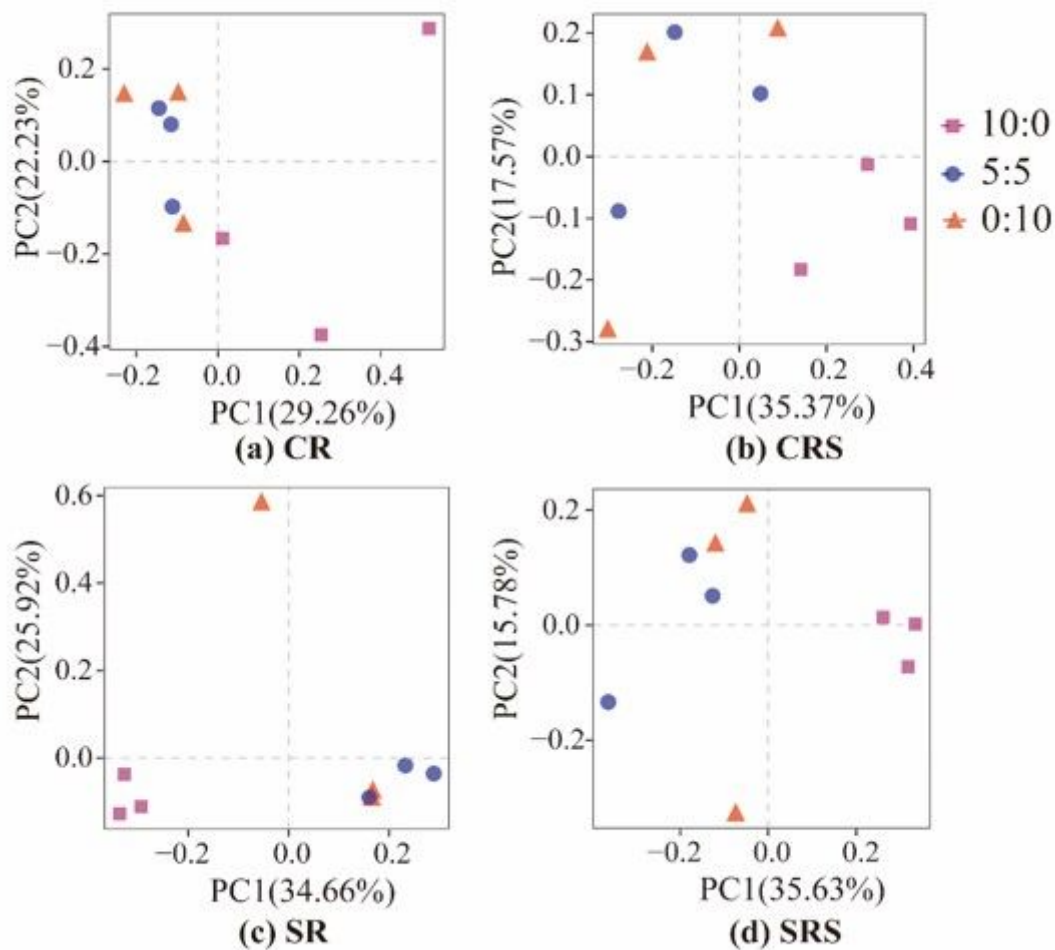


Figure 4

Principal Coordinate Analysis based on bray-curtis

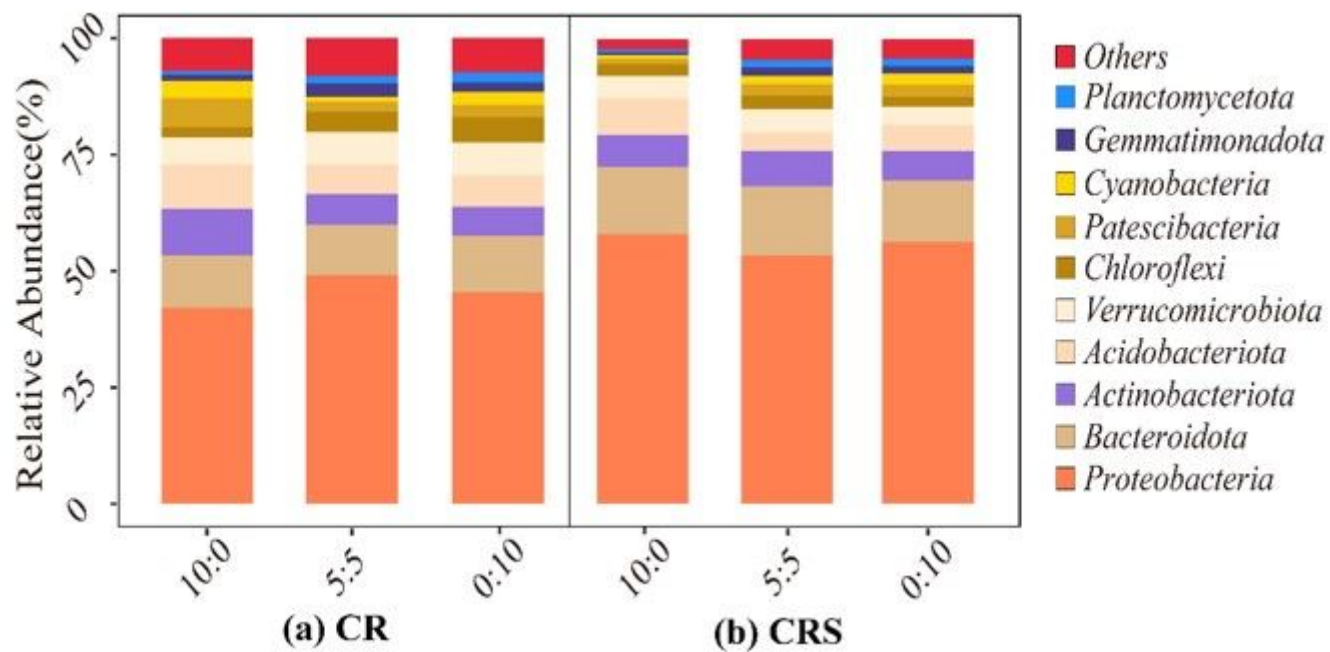


Figure 5

Composition of rhizosphere and root surface bacteria of *C. lanceolata* at phylum level under different N forms

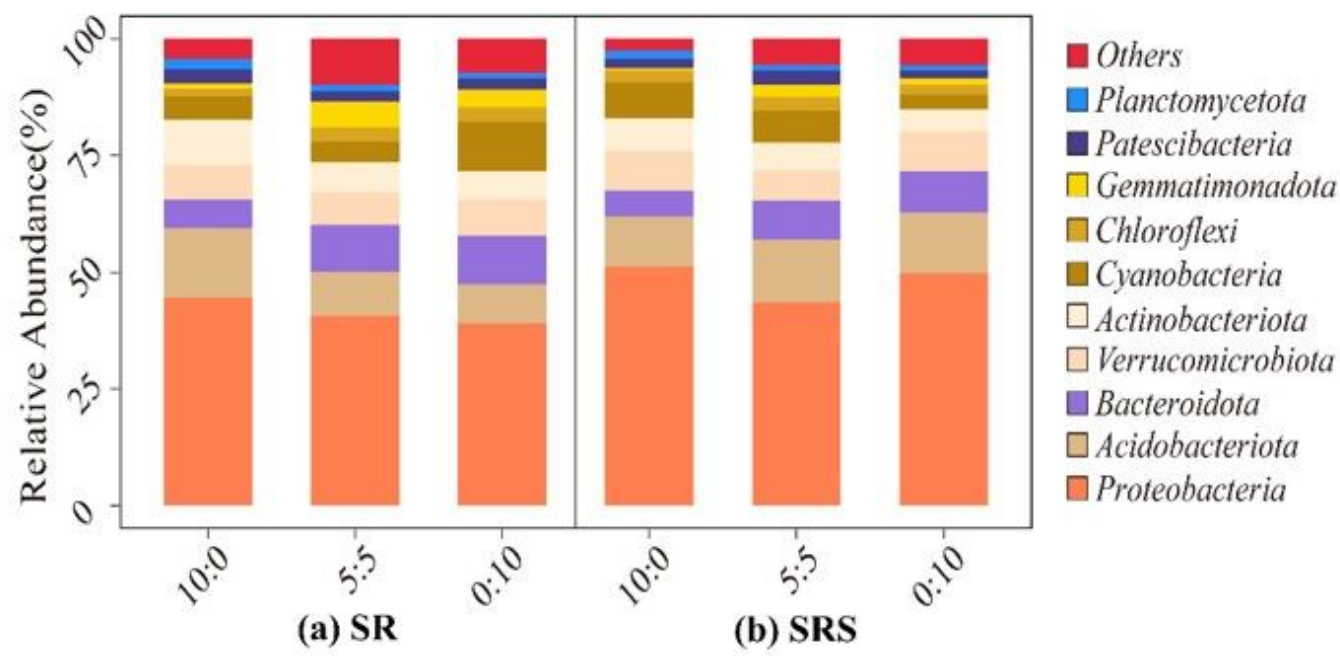


Figure 6

Composition of rhizosphere and root surface bacteria of *S. superba* at phylum level under different N forms

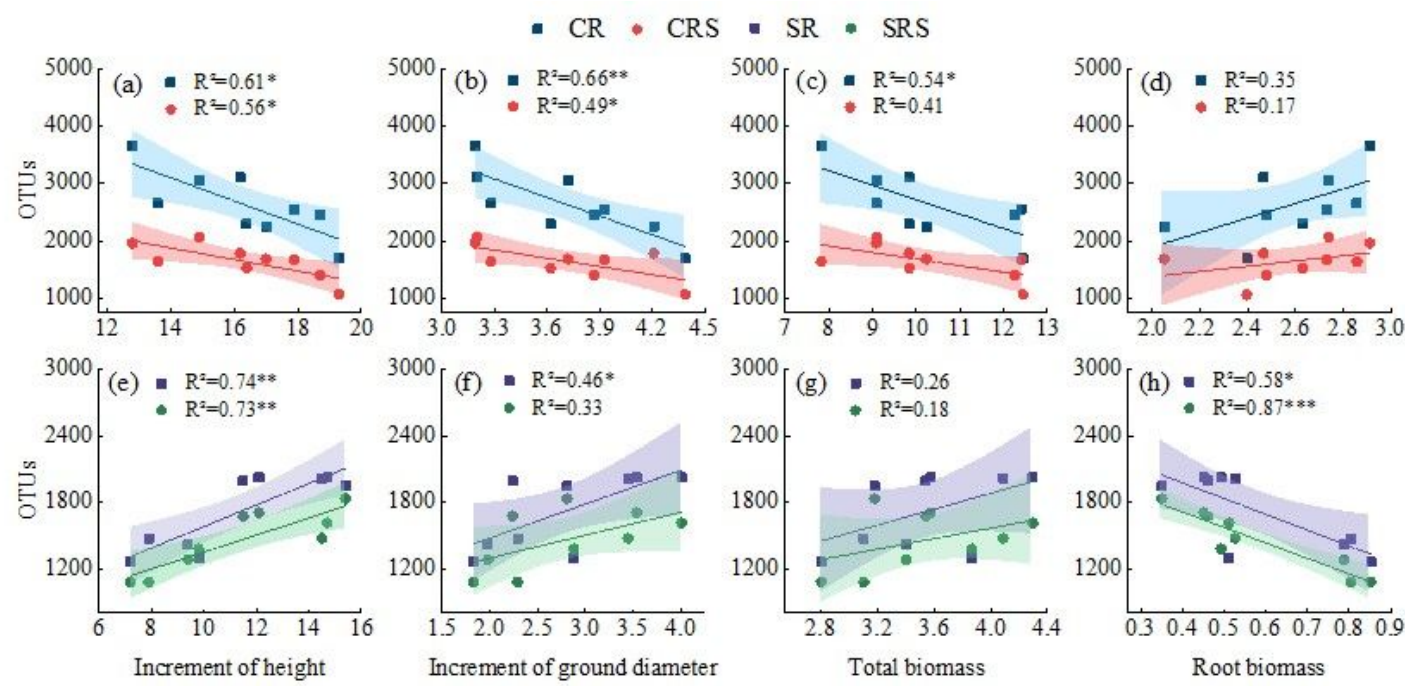


Figure 7

Linear relationships of the OTUs and growth indicators in the rhizosphere and root surface of the two tree species. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, none: $P > 0.05$.