Difference in rhizosphere soil bacterial community was among six *Cinnamomum camphora* chemotypes

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Abstract: Plant types and soil bacterial communities had a close relationship, understanding the profound association between them contributes to better learn bacterial ecological function for plant growth. In this study, rhizosphere soil of six different chemotype *Cinnamomum camphora* trees were collected, including *C. bodinieri var. citralifera*, [*C. camphora* (Linn.) Presl], camphora-type, cineole-type, linalool-type and isoborneol-type. Soil properties content and bacterial communities were analyzed. Two chemotype *C. camphora*, including [*C. camphora* (Linn.) Presl] and linalool-type, shaped similar bacterial community structure, decreased *Firmcutes* relative abundance. richness estimators (Chao1 index and Ace index) of [*C. camphora* (Linn.) Presl] were decreased compared with the others. Furthermore, soil bacterial community structure was also similar among *bodinieri var. citralifera*, camphora-type, cineole-type and isoborneol-type. Hence, different chemotype *C. camphora* altered soil nutrient and shaped rhizosphere bacterial communities.

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Keywords: *Cinnamomum camphora*; chemotype; soil nutrient; soil bacterial community diversity and structure

1. Introduction

Cinnamomum camphora (L.) Presl is an evergreen tree originating from the south of the Yangtze River in China, has been introduced to other countries [1]. At present, it mainly habitat was tropical and subtropical of Asia, particularly in China, Japan, Korea, and Vietnam [2]. *C. camphora* is not only used for wood processing and interior decoration of buildings, but has extremely medicinal value and high economic effects [3]. For instance, its wood is usually for furniture making, and it also has been commonly regarded as traditional Chinese herb medicines for the treatment of inflammation-related diseases such as rheumatism, bronchitis and indigestion [4, 5]. Furthermore, highly qualified essential oil is richness in roots, stems, leaves and wood of *C. camphora*, such as mint, camphor and patchouli [6, 7]. Therefore, *C. camphora* is classified different chemotypes according to chemical composition extracted from it, such as camphora-type and cineole-type [1, 8]. Chemotypes are organisms categorized under the same species, subspecies or varieties, with differences in the composition of secondary metabolites [9]. Although, many literatures demonstrate that no obviously difference is in morphology among different *C. camphora* chemotypes, their physiological structure and root secretions are obviously difference [1, 10]. This result drives potentially changes of surrounding environment growing of *C. camphora*.

Soil bacteria play a vital role in the terrestrial forest ecosystem, they always gather together on plant root surface to mineralize soil nutrient, decompose litters and

accelerate nutrient cycle for plant absorption and utilization [11]. However, soil bacterial community structure and diversity are significantly affected by climate, soil texture, vegetation and land use patterns, because they are very sensitive to the changes in surrounding environment [12]. Therefore, soil bacteria are considered as sensitive indicators of soil ecosystem changes. Previous studies suggest that soil bacteria community structure in rhizosphere soil of different plant types also is differences, even under the same site condition [13]. Literatures found that endophytic bacteria in leaves and stems of are differences due to various chemical composition in leaves and stems of different chemotypes *C. camphora* [3, 14]. However, many studies only focus on the endophytic bacteria and chemical composition of different chemotypes *C. camphora*, but usually pay little attention on the distribution of bacterial community diversity and structure in its growth.

Based on above, we investigated and analyzed rhizosphere soil properties and bacterial community diversity and structure of six different chemotypes *C. camphora*, and the deep connection between them. This paper links soil properties and rhizosphere bacterial community among all six chemotypes *C. camphora* to analyze their habitat. The objects of this study were to compare rhizosphere soil bacteria community diversity and structure of six chemotypes *C. camphora*. This result will not only provide some insights on rhizosphere microecology of different chemotypes *C. camphora*, also offers some new ideas for the sustainable development of *C. camphora*.

2. Materials and methods

2.1 Experimental site

The experimental site is situated in the biotechnology experimental base (115°59′38.40″ E, 28°45′29.20″ N) of Nanchang Institute of Technology, Nanchang City, China. It belongs to subtropical humid monsoon climate, with mild and humid climate, abundant sunshine, abundant rainfall, south wind in summer, north wind in winter, long frost-free period and short freezing period. The mean annual temperature and annual precipitation are 20.5 °C and 1567 mm, respectively.

2.2 Experimental treatment and samplings

Cuttings were from new branches of 3-years-old and different chemotype *camphora* trees, which include *C. bodinieri var. citralifera* (NHZ), [*C. camphora* (Linn.) Presl] (NMZ), camphora-type (NZ), cineole-type (AZ), linalool-type (FZ), isoborneol-type (ZZ). These cuttings were cut into 30 cm segments with the average ground diameter at 2.0 cm and soaked in sterile water for 15 min and then for cutting culture. The trial area was about 198 m² (36 m × 6 m), and it was divided into 6 plots with the same size, each plot was 2 m apart. All cutting were cut into the soil with Row spacing and plant spacing 30 cm, respectively. Approximately 100 g compound fertilizers were applied into surface soil as base fertilizer in that winter, and 150 g urea (≥ 30% N) were also average added to each plot in May next year. After cultivating, soil samples (depth of 0-20 cm) of different chemotype cuttings rhizosphere soil were collected after removing surface litters in July 2019. The collected test soil was passed a 2 mm sieve to remove gravel and roots before divided into two parts. One part was to analysis soil basic physical and chemical properties, and the other soil was storage at -20 °C for determining bacterial community diversity and structure.

2.3 Determinations of soil physicochemical properties

All soil properties were determined according to Kong [15]. Briefly, dried soil and 25 mL deionized water were mixed into 50 mL plastic pipe with ration of 1: 2.5 (soil: water) and shaken for 30 min, the pH value of the sample was measured using a pH meter (Sanxin, Shanghai, China). Soil samples were digested with chloric acid and sulfuric acid and then soil total phosphorus (TP) was quantified by the Discrete Auto Analyzer (SmartChem, Brookfield, USA). Soil available phosphorus (AP) content was determined at 700 nm absorbance with a spectrophotometer by the molybdenum resistance colorimetry method. Soil total N contents were analyzed with the Discrete Auto Analyzer (SmartChem, Brookfield, USA). Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents were extracted with 2 mol L^{-1} KCl solution, and they were quantified by the Discrete Auto Analyzer (SmartChem, Brookfield, USA). Soil total potassium (TK) and available K (AP) contents were measured using the Flame Photometer (F-100, Shanghai, China). Soil organic matter (SOM) content was analyzed using the titrating method and then converted into SOM content. All treatments were repeat in ten times.

2. 4 Soil DNA extraction

Soil total DNA was extracted with a Fast DNA SPIN Kit for Soil (MP Biomedicals, USA) according to the manufacturer protocol. The quantity and quality of extracted DNA were measured using the NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and 1.8% agarose gel electrophoresis, respectively. The extracted DNA was stored at -20 °C before analyzing bacterial components.

2.4 Illumina-MiSeq sequencing and data analysis

The V3-V4 regions of soil bacterial 16sRNA gene were amplified with common primers 4338F/806R [16], and PCR products were sequenced on the Illumina Miseq platform (Nonogene, China). Raw reads were filtered to obtain high-quality reads with the QIIME software according to the default value. The high-quality reads were classified into operational taxonomic units (OTUs) at 97% sequence similarity level, and which also was used to calculate soil bacterial Shannon and Simpson indices and Ace and Chao1 richness estimators. Meanwhile, bacterial taxonomies were confirmed using the OTUs by the SILVA database (http://www.arb-silva.de).

2.5 Statistic analysis

One-way ANOVA was used to analyzed soil basic properties (including soil pH, TP, TN, TK, AP, AK, NH_4^+-N, NO_3^--N and SOM content) by the Duncan test ($P < 0.05$) with SPSS 24.0 software (IBM SPSS Inc., USA). Soil bacterial diversity indices (Shannon and Simpson indices) and richness estimators (Ace and Chao1 richness estimators) were also performed by One-way ANOVA. The principal co-ordinates analysis was used to reveal soil bacterial community structure. The relationship between soil properties and bacterial relative abundance were performed in RDA. Bacterial biomarkers of different chemotype *C. camphora* trees rhizosphere soil samples were analyzed using the linear discriminant analysis effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/galaxy/root). The principal co-ordinates analysis was used to reveal soil bacterial community structure. The relationship between soil properties and bacterial relative abundance were performed in RDA. The LEfSe, PCoA, RDA and LDA were performed with the R Project (https://www.r-

project.org/).

3. Results

3.1 soil properties

There was no not statistical difference in pH value among all Camphor trees of different chemotypes (Table 1). At end of this experiment, average SOM content was significant higher at FZ, NZ ZZ and AZ than that of NMZ and NHZ. The highest TP content was 1.22 ± 0.11 at FZ among all soil samples, and AP content was significantly decreased at FZ and NHZ than NMZ, NZ and AZ. Soil K content was the highest at NHZ compared other *C. camphor* of chemotypes, and AK content was higher at NZ, ZZ and AZ than FZ, NMZ and NHZ. Soil TN content was lower at NMZ and NHZ than that of other Camphor types. For NH_4^+-N content, which was significantly enhanced at ZZ and FZ, and the highest content was ZZ at 41.16 ± 4.59, the rank of soil sample NH_4^+-N content from high to bottom was ZZ > FZ > NMZ > AX > NHZ > NZ. Soil NO_3^--N content was higher at ZZ, AZ, FZ and NZ than NMZ and NHZ.

3.2 Soil bacterial community diversity and sequence data

The similarities and differences of OTUs among the rhizosphere soil samples of different chemotype *C. camphor* trees were demonstrated in a six-set Venn diagram (Figure 1). The six different chemotype *C. camphor* trees shared 2287 OTUs, and the unique OTUs were 379, 371, 212, 238, 446 and 237 for the AZ, FZ, NZ, NHZ, NMZ and ZZ rhizosphere soils, respectively.

Soil bacterial Shannon index was lower at NMZ than that of NHZ. Shannon index at NMZ was lower than NHZ (Figure 1a). There was no significantly difference in

Simpson index among all chemotype *C. camphor* trees (Figure 2b). Surprisingly, bacterial richness estimators (Chao1 index and Ace index) were decreased at NMZ, and they were highest at AZ (3423.81±361.33, 3445±333.56) among all Camphor trees of different chemotype (Figure 2c and 2c).

3.3 Soil bacterial community structure

Based on the SILVA database, soil bacterial OTUs were assigned into predominant phyla as following: *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Gemmationadetes*, *Latescibacteria* and *Rokubacteria*. At phylum taxa level (Figure 3), *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Gemmationadetes* and *Latescibacteria* relative abundances were significantly altered at rhizosphere soil of different chemotype *C. camphor* trees, suggesting that soil bacteria were reassembled at different chemotype *C. camphor* trees. The relative abundance of *Proteobacteria* was lower at NMZ than their counterparties at ZN and AZ (Figure 4a). Soil *Actinobacteria* relative abundance significantly increased at FZ, NMZ and NHZ relative to NZ and AZ (Figure 4b). For *Bacteroidetes*, its relative abundance was higher at NMZ and NHZ than that at NZ and ZZ (Figure 4c), and there was a significant difference in *Chloroflexi* relative abundance between NMZ and AZ (Figure 4d). While, *Firmicutes* relative abundance at FZ and NMZ decreased compared with other counterparties (Figure 4e). The relative abundance of *Gemmationadetes* enhanced among ZZ, AZ and ZN relative to NMZ and NHZ (Figure 4f). Compared with both NZ and AZ, *Latescibacteria* relative abundance was higher at FZ (Figure 4g).

3.3 bacterial community structure

The LefSe analysis indicated that there were statistically significant associations among predominant bacterial taxa in rhizosphere soil of different chemotype *C. camphora* trees. The phylum *Acidobacteria* was stimulated by the root exudations of FZ, MHZ and NMZ, suggesting that these species may potentially be recruited by them and as their biomarkers for rhizosphere soil (Figure 5).

The two-dimensional PCoA plot explained 36.33% of the total variation, and the PCoA1 accounted for 23.77% of the total variance (Figure 6). The samples both of NMZ and FZ was highly similar and they mainly distributed negatively along with PCoA1 axis. Furthermore, the others samples were also extreme similar with each other, and even 95% confidence ellipse of ZZ included NHZ and AZ, and this result confirmed that soil bacterial community structure was distinct divided two parts.

3.4 Linking soil properties and bacterial community

The RDA analysis revealed the comprehensive relationship between soil properties and dominate bacterial taxa (Figure 7). A positive associate was shown between pH, the relative abundance among *Actinobacteria*, *Acidobacteria*, *Bacteroidetes* and *Latescibacteria*, but which had negative with SOM, TN and NO_3^--N content. In addition to, *Proteobacteria* relative abundance was negatively with TP, TK and NH_4^+-N content.

4. Discussion

4.1 Comparison bacterial community diversity

At the end of this study, Chao1 and Ace richness estimators of NMZ were

significantly decreased compared with other five chemotypes *C. camphora.* This result suggested that bacteria community diversity was lower than other chemotypes *C. camphora*, this was also confirmed in fig that the relative abundance of *Firmicutes* and *Gemmationadetes* was the lowest. We predicted that the life activity of bacteria may be impacted by soil SOM concentration. SOM coming from litters and microbial residuals is commonly considered as carbon by bacteria, and which can provide substantial and persistent energy for bacterial life activity [17, 18]. In this study, high relative abundance of *Acidobacteria* had negative associate with SOM content, this also can account for the decreasing of SOM content of NMZ, due to the *Acidobacteria* could degrade organic matters from litters, maintaining soil available nutrient supply [19, 20].

Apart from the effects of SOM content on soil bacterial community diversity, mineral nutrient also plays an important role on composition of bacteria [21]. At our recent study, soil bacterial relative abundance was significantly altered in rhizosphere soil of different chemotypes *C. camphora* and which shaped different bacterial communities. We predicted that root exudation may be the main driver factor. Root secretions coming from different chemotypes *C. camphora*, might be difference, even had one more distinct difference [1, 22, 23]. Some bacteria preferencing this distinct substance are recruited and appealed to rich on plant rhizosphere, but soil bacteria may also be inhibited indirectly by root secretions due to fiercely competition for nutrients and spaces was among bacteria [24, 25]. Therefore, nutrient supply was also another major influence factor. For example, *Proteobacteria* phylum participates in N transformation processes and accelerates N cycle, they also can efficiently convert and

utilize root exudation, in particular in transforming the low molecular weight substance [26]. In this paper, *Proteobacteria* phylum was richness on rhizosphere soil, this may be caused that 1) *Proteobacteria* was attracted and recruited to transform N for development and growth of *C. camphora* under N addition condition [27]; 2) *Proteobacteria* phylum may also be recruited by root exudation due to they were the major phylum convert and utilize root exudation [28].

4.2 Comparison bacterial community structure

There is a close relationship between vegetation composition and soil physical and chemical properties. Plant affects soil physical and chemical properties through litterfall and root exudates, thus affecting the composition and structure of soil bacterial communities [11]. Previous studies indicated that changes in plant species had a significant effect on bacterial community structure [11]. Therefore, it can be better understood the impact of vegetation types on soil microbial ecosystems and function by studying the relationship between soil microorganisms and vegetation.

In this study, the PCoA analysis confirmed that soil bacterial community structure was divided obviously two parts among six different chemotypes *C. camphora*, one part was FZ and NMZ, and another was AZ, ZZ, NZ and NHZ. This result suggested that rhizosphere of six different chemotypes *C. camphora* trees were mainly divided two forms. It can be explained that there was something similar between root exudations of FZ and NMZ, but which was different form root exudations that secreting by other four different chemotype *C. camphora* trees. In addition, these similar root exudations recruited countless bacteria to gather in the rhizosphere and shaped the

bacterial community structure. Previous study also found that plant genotype was one of the dominate factors affecting rhizosphere soil bacterial community structure. Such as the microbiome community of rhizosphere of wheat and chickpea was significant difference due to their root drive the composition and function of the rhizosphere microorganisms [29]. In return, changes in composition and function of the rhizosphere microorganisms can alter soil nutrients then leading to redistribution of soil microbe [30]. Besides, rhizosphere bacterial community structure was also difference at the period of plant growth due to changing plant root architecture and nutrient supply [31]. Hence, difference in bacterial community structure was among six different chemotypes *C. camphora*.

5. Conclusion

The Illumina-MiSeq sequencing analysis suggested that rhizosphere soil bacterial community diversity and structure of six different chemotype *C. camphora* trees were significant difference, the relative abundance of phylum *Firmicutes* at FZ and NMZ decreased compared the others, but *Acidobacteria* relative abundance of FZ and NMZ was higher than NZ, ZZ and AZ. Moreover, soil bacterial community structure was highly similar between FZ and NMZ, as well as among ZZ, NZ, AZ and NHZ. SOM content had reduced trend at NHZ and NMZ, and soil pH was no significant difference among all different chemotype *C. camphora* trees. In conclusion, different chemotype *C. camphora* trees altered soil properties and shaped different rhizosphere soil bacterial communities, and NMZ and FZ had highly similar aspect on bacterial community structure. Therefore, different chemotype *C. camphora* trees had different rhizosphere

bacterial communities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Table 1. Rhizosphere soil properties of six chemotype *Cinnamomum camphora* trees. Different lowercase letters represent significant differences,

$p < 0.05$. NHZ, *C. bodinieri var. citralifera*, NMZ, [*C. camphora* (Linn.) Presl], NZ, camphora-type, AZ, cineole-type, FZ, linalool-type, ZZ,

isoborneol-type.

| Treatments	FZ	NMZ	NHZ	NZ	ZZ	AZ
pH	5.85 ± 0.38 a	5.70 ± 0.14 a	5.65 ± 0.26 a	5.51 ± 0.23 a	5.70 ± 0.30 a	5.61 ±0.16 a
SOM	9.76 ± 1.46 a	6.89 ± 0.46 c	7.65 ± 0.41 b	9.83 ± 1.80 a	9.33 ± 0.71 a	10.09 ± 1.23 a
TP	0.96 ± 0.11 a	0.88 ± 0.10 ab	0.78 ± 0.07 bc	0.92 ± 0.16ab	0.84 ± 0.10 abc	0.71 ± 0.12 c
TN	1.22 ± 0.11 a	0.88 ± 0.07 c	0.81 ± 0.14 c	1.16 ± 0.16 ab	1.02 ± 0.04 b	1.12 ± 0.05 ab
TK	33.35 ± 1.19 bc	33.79 ± 2.48 b	37.87 ± 1.32 a	34.79 ± 0.99 b	31.45 ± 1.06 cd	29.35 ± 4.34 d
AP	6.12 ± 1.45 c	10.01 ± 1.54 a	5.76 ± 1.36 c	9.03 ± 1.86 ab	7.54 ± 1.70 bc	10.58 ± 0.57 a
NH_4^+-N	32.90 ± 3.89 b	23.9 6± 3.79 c	24.67 ± 6.31 c	20.51 ± 2.81 c	41.16 ± 4.59 a	23.06 ± 4.96 c
NO_3^--N	3.08 ± 0.24 a	1.95 ± 0.23 b	2.05 ± 0.19 b	2.78 ± 0.67 a	3.16 ± 0.32 a	2.77 ± 0.81 a
AK	63.08 ± 6.63 b	73.45 ± 8.98 b	61.24 ± 8.78 b	105.57 ±18.25 a	94.17 ± 10.37 a	102.12 ± 7.76 a
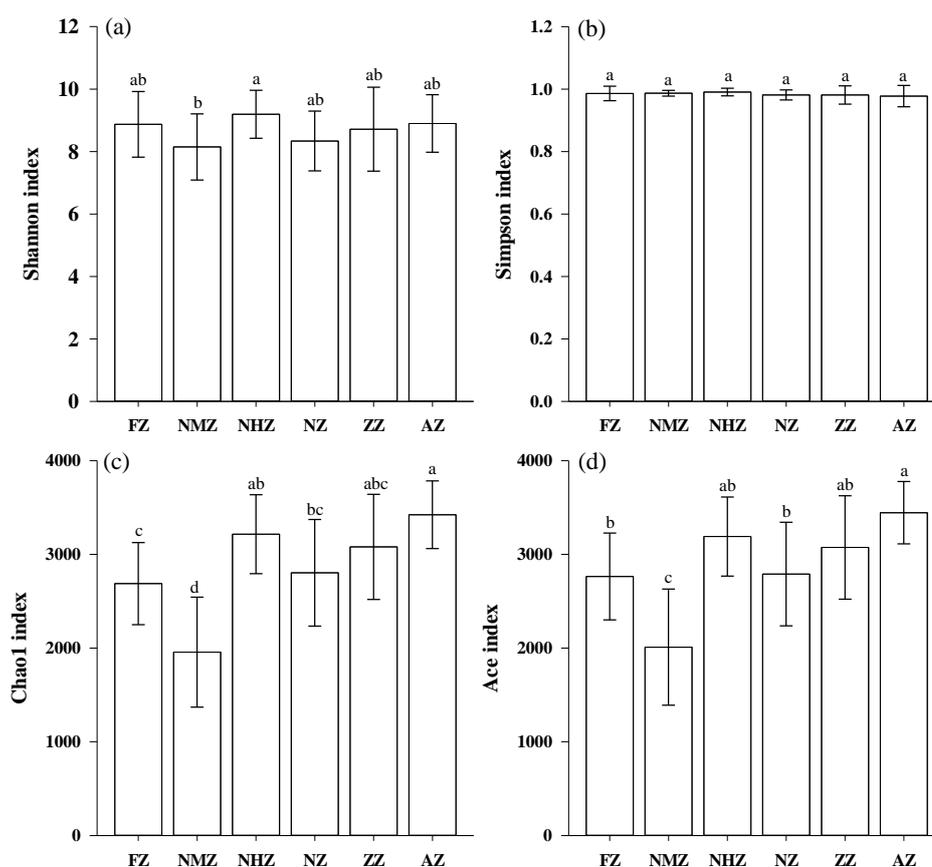
Figure 1. Soil bacterial community diversity of different chemotype *C. camphora* trees.

(a) bacterial Shannon index, (b) Simpson index, (c) Chao1index and (d) Ace index.

Different lowercase letters represent significant differences, $p < 0.05$. NHZ, *C. bodinieri var. citralifera*, NMZ, [*C. camphora* (Linn.) Presl], NZ, camphora-type, AZ, cineole-type, FZ, linalool-type, ZZ, isoborneol-type.
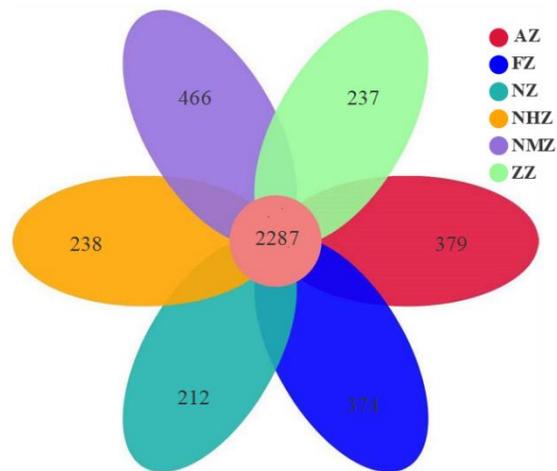
Figure 2. The Venn diagram showing the numbers of unique OTUs of the five treatments. Details are described in Figure 1.
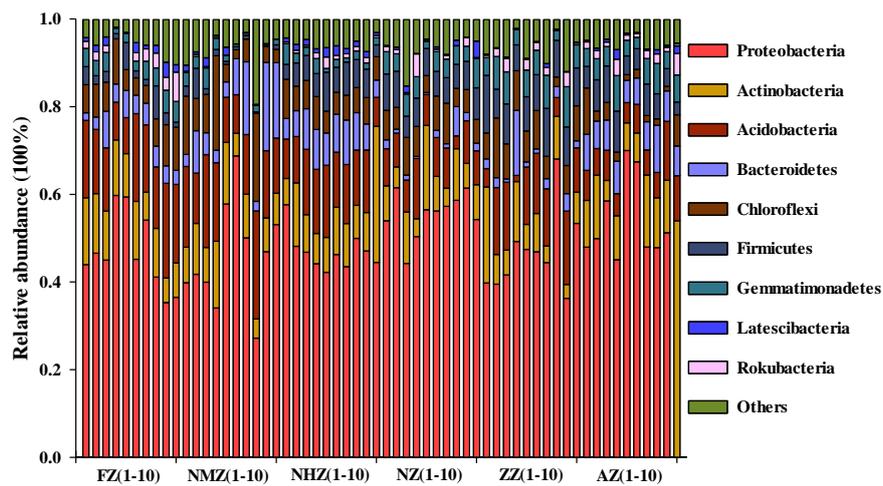
Figure 3. Soil bacterial taxonomic distribution at the phylum level. Details are described in Figure 1.
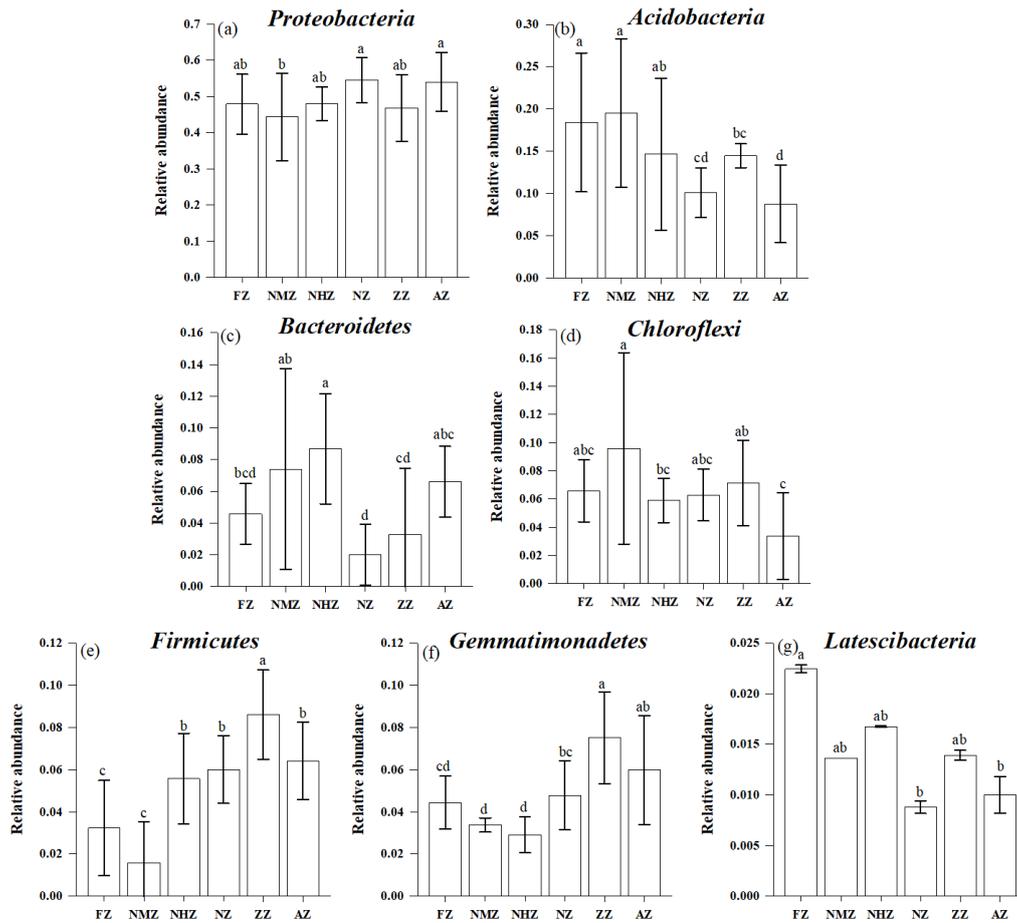
Figure 4. Soil bacterial taxonomic distribution at the phylum level. Details are described in Figure 1.
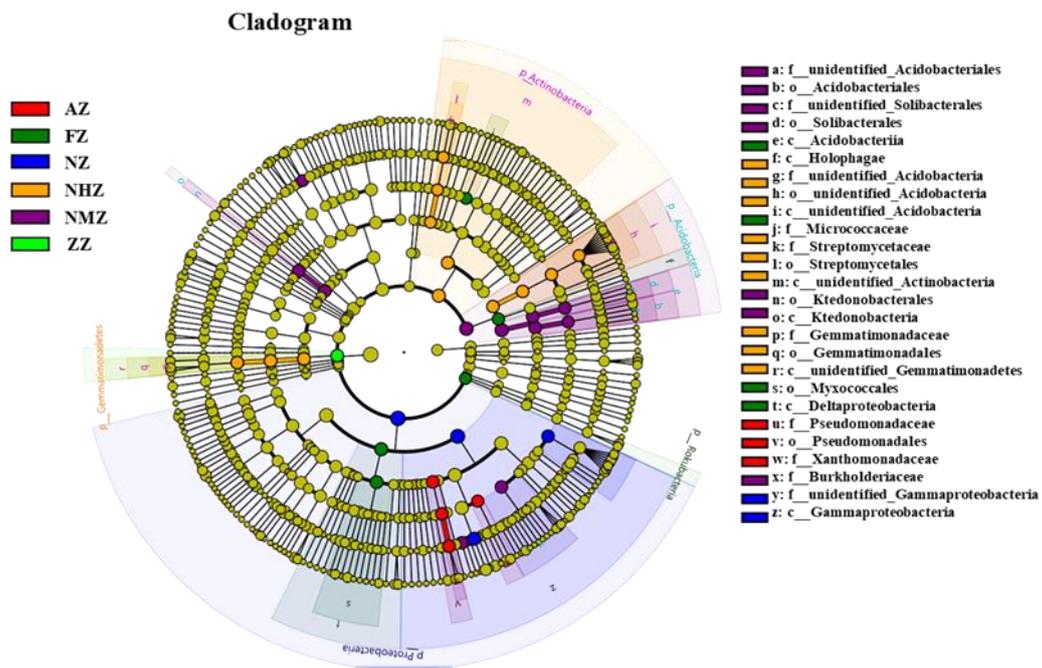
Figure 5. The significantly different abundant taxa of bacteria in the five treatments.
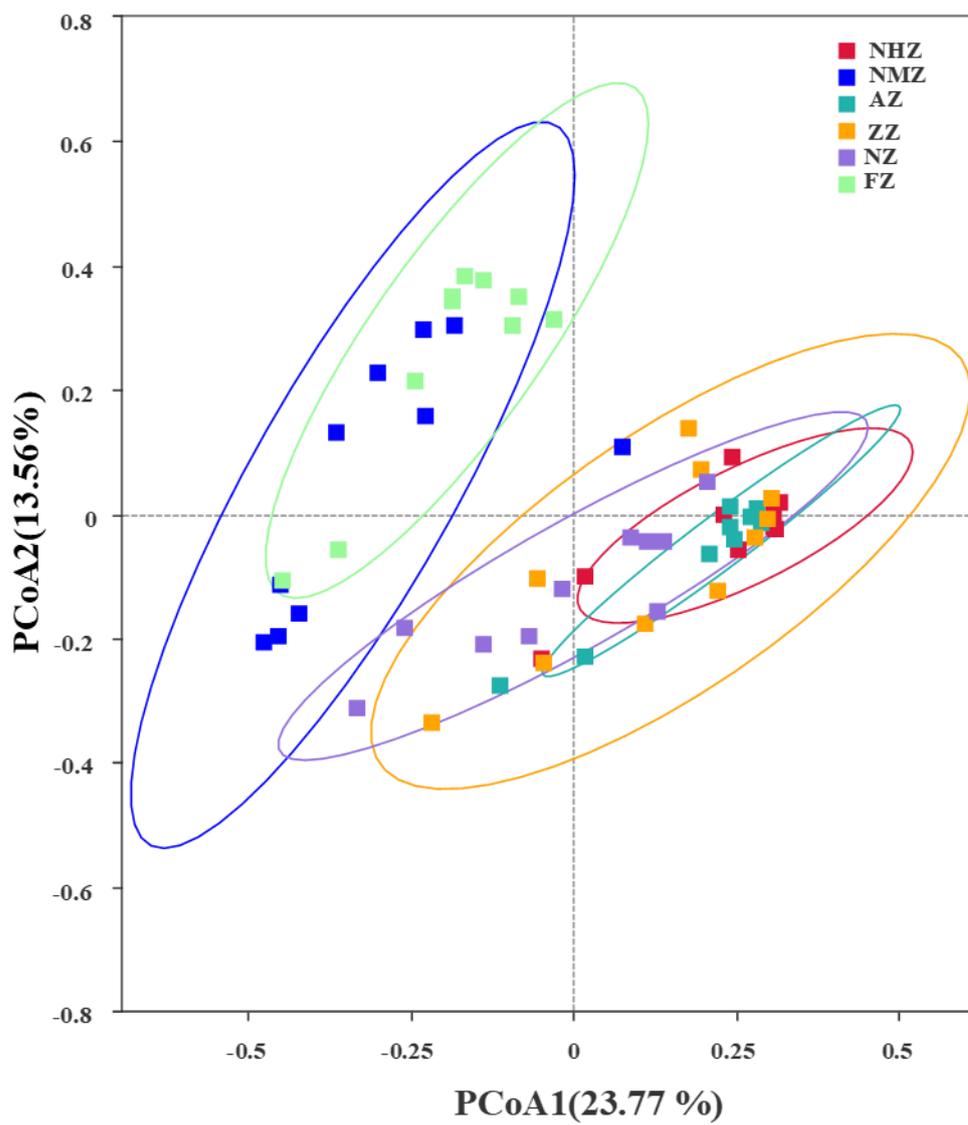
Details are described in Figure 1.

Figure 3. Principal coordinate analysis revealing the entire soil bacterial community structure. Details are described in Figure 1.
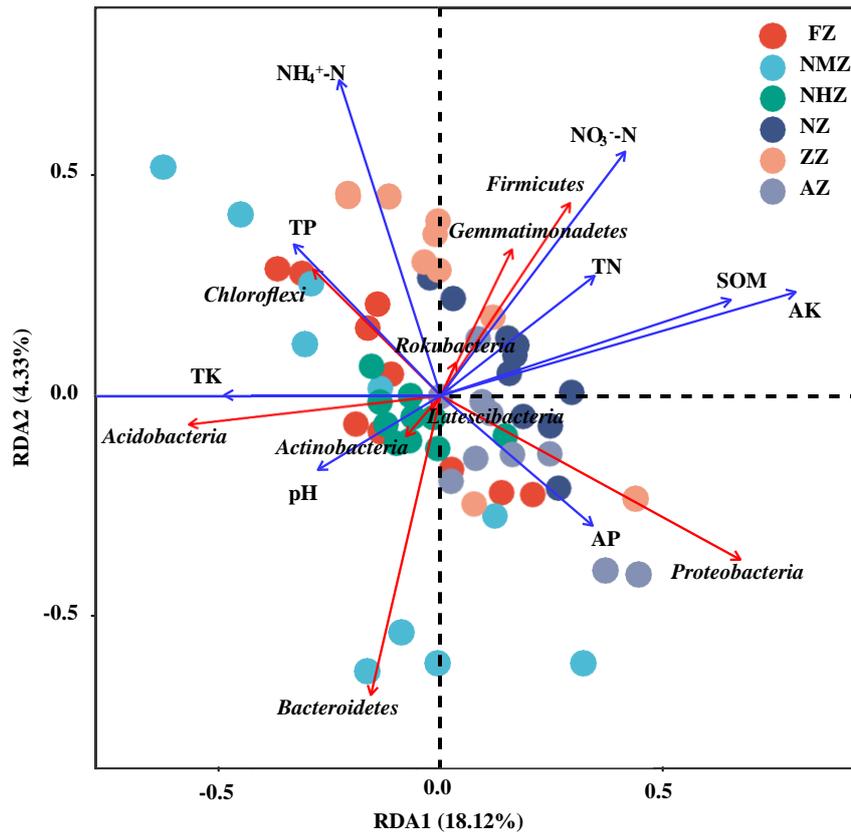
Figure 4. Principal redundance analysis revealing the comprehensive linkages between soil properties and bacterial community. Details are described in Figure 1.