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Study on the Changes in the Microbial Community in Rhizosphere Soil of Blueberry Plants at Different Growth Stages

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Abstract: In order to clarify the relationship between mineral nutrients and rhizosphere microorganisms at different growth and development stages of blueberry (*Vaccinium* spp.), this work studied the dynamic changes in element content and microbial quantity in different parts of blueberry plants. The test material was a 12-year-old half-highbush blueberry variety ('Beilu'). The changes in the mineral nutrient elements in leaves, branches and the soil of blueberry plants were studied at the full bloom stage (T1), green fruit stage (T2), mature stage (T3) and late mature stage (T4), and the correlations of the average contents of mineral elements in the four periods were studied. The bacterial community in the rhizosphere soil was determined and analyzed using 16S rRNA high-throughput sequencing technology. The results showed that the changes in other mineral elements in various parts of blueberry plants varied in different periods. Nitrogen (N) showed a downward trend in branches, leaves and soil, especially in leaves ($p < 0.05$). The N contents in T2, T3 and T4 decreased by 9.9%, 26.4% and 29.9%, respectively. The N contents in the leaves and branches showed a downward trend at different growth stages, especially in leaves. The phosphorus (P) content in leaves showed a trend of increasing first and then decreasing, while it continued to increase in branches. The content of potassium (K) in leaves changed significantly, where it increased first and then decreased. The content of calcium (Ca) in leaves decreased first and then increased, while the content of magnesium (Mg) in branches and leaves decreased first and then increased, and the relative change was significant. The contents of iron (Fe) and zinc (Zn) in leaves decreased first and then increased, while the contents of manganese (Mn) and copper (Cu) were relatively stable. Cu decreased first and then increased in leaves and soil, and it increased first and then decreased in branches. The mineral nutrients in different growth stages of blueberry showed significant correlation in leaves, branches and soil. Mn in leaves was significantly positively correlated with P, Ca, Mg, Mn, Cu and Zn in soil ($p < 0.01$). Nitrogen and calcium in leaves were significantly correlated with manganese and phosphorus in soil, respectively. Ca in branches was significantly positively correlated with N and K in soil and was significantly positively correlated with Zn in soil ($p < 0.01$). Magnesium was significantly negatively correlated with iron in soil. The bacterial community structure of the blueberry rhizosphere soil changed significantly over time ($p < 0.05$), and the relative abundance showed the following trend: T4 > T2 > T3 > T1. At the phylum level, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Verrucomicrobia were the dominant bacteria in different periods. *Candidatus solibacter* and *Bryobacter* were significantly higher in T1 and T3 than in T1 and T4. *Bradyrhizobium flora* increased significantly at T3. *Sphingomonas* increased significantly at T1 ($p < 0.05$).

Keywords: blueberry; growth stage; mineral element; high-throughput sequencing; rhizosphere soil bacterial community



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1. Introduction

Blueberry is an evergreen or deciduous shrub [1]. Its fruit has a unique flavor and is rich in nutrients, such as vitamins, anthocyanins and antioxidant enzymes. It is known

as the ‘king of berries’. It has been listed as the world’s third-generation fruit, loved by consumers at home and abroad, and it is listed as one of the five major human health foods by the Food and Agriculture Organization of the United Nations [2–6].

With improving living standards, the demand for fresh blueberries is increasing. At present, there are still some deficiencies in the cultivation and management of blueberry plants. For most growers, orchard management is relatively extensive, but there is no clear standard for fertilization, which leads to a decline in fruit quality and yield [7,8]. Blueberry roots are shallowly distributed in the soil, without main roots, and have a poor ability to compete with other plants for nutrients. It can be seen that soil and fertilization management are very important in blueberry cultivation and production. Blueberry planting faces various challenges due to its strict requirements in terms of soil conditions [9]. The growth of blueberry plants is closely related to mineral elements and soil fertilizer and water management. Blueberries need a variety of mineral elements to promote their growth, flowering and fruiting. It has been reported that there is a significant correlation between soil nutrients, crop nutrition, fruit tree mineral element contents and the crop and fruit tree yield [10,11].

Mineral nutrient elements are the material basis for the growth and development, fruit yield and quality of blueberries. When compared with other fruit tree species, blueberry plants have unique mineral nutrition characteristics. Lacking or excessive mineral nutrition will adversely affect tree growth [12,13]. Previous studies have found that mineral elements are important for plant growth and development. They not only improve plant disease resistance and stress resistance but also improve fruit quality and increase yield. However, there are few studies on the correlations between mineral elements relating to blueberry and soil bacteria. Li Yadong preliminarily discussed the suitable range of mineral nutrient contents in *Vaccinium uliginosum* leaves [14]. Bai et al. found that soil nutrients in the root distribution layer significantly affected the contents of N, P, K, Mg, Fe, Mn, Zn and other elements in the leaves of *Vaccinium uliginosum*, but there was no significant correlation between most mineral elements in the leaves and the corresponding mineral elements in the root layer soil [15]. This result is the same as the results of previous studies on fruit trees such as sweet oranges [16]. The mineral compositions of wild and cultivated blueberry plants were analyzed by Paulina Drd et al. The results showed that the contents of Ca, Na, Mg, Mn and Zn in wild blueberry fruit were higher, while similar levels of Cu, Cr, Fe and Ni were detected in wild and cultivated plants [17].

Soil is an important ecosystem that provides good growth conditions. Moreover, it is an important environment for microorganisms, which is called the ‘natural medium’ of microorganisms [18]. It has been found that rhizosphere soil microorganisms play an important role in the growth of blueberry plants; they can promote the growth of roots, affect the decomposition and transformation of soil nutrients by roots and then affect the absorption and utilization of nutrients in soil by plants [19]. Zheng Tianjiao et al. studied the rhizosphere microorganisms of different varieties of blueberry at a certain development stage, and they found that Acidobacteria, Actinobacteria and Proteobacteria were the common dominant bacteria in the rhizospheres of three kinds of blueberries [20]. The relative abundance and diversity of bacterial and fungal communities in rhizosphere soil at different plant developmental stages have changed significantly [21].

Rhizosphere soil microorganisms are an important part of soil microecology. They can form nodules with plants and produce secondary metabolites that promote plant growth. At the same time, they can also promote the participation of mineral elements in soil in the material cycle of plants, assisting plant nutrient absorption and improving plant disease resistance [22–25].

The above research provides a reference for the factors that affect the characteristics of mineral elements, growth adaptability and the fruit yield of blueberry plants. Currently, there are few reports on the relationship between soil nutrients, leaf mineral elements and the soil community structure of blueberry plants. However, this field of research is the key

factor guiding the management of blueberry soil fertilizer technology and improving the quality of blueberry fruit.

In summary, to fill a research gap, this study focused on the different growth stages of blueberry plants, and it explored the relationship between the diversity of soil microorganisms at different growth stages and the mineral elements present in blueberry trees. By analyzing the changes in the mineral element content of blueberry soil, leaves and branches and the diversity and relative abundance of the rhizosphere microbial bacterial community, the absorption and utilization characteristics of mineral nutrient elements in blueberry plants were clarified, and the changes in the mineral element content in the soil, tree body and rhizosphere microbial bacterial community at different development stages were elucidated. This work provides a theoretical basis for regulating the nutritional needs of blueberry plants, improving cultivation techniques, improving fruit quality and performing scientific fertilization.

2. Results

2.1. Blueberry Leaf, Branch and Soil Mineral Nutrient Contents and Their Correlation Analysis

2.1.1. Mineral Element Contents in Leaves, Branches and Rhizosphere Soil of Blueberry Plants at Different Growth Stages

As illustrated in Figure 1, the N contents in the leaves, branches and rhizosphere soil of blueberry plants at different growth stages showed a decreasing trend, especially in leaves ($p < 0.05$). The content of N in the leaves decreased by 9.9%, 26.4% and 29.9%, respectively, at the green fruit stage, mature stage and late mature stage compared to that at the full flowering stage. The P content showed a trend of increasing first and then decreasing, and the changes in leaves and branches were significant. The P content of leaves decreased from 0.23% to 0.14% and 0.06% in the mature period and the end of the mature period, respectively, which were 39.1% and 73.9% lower than that in the green fruit period. The content of P in the branches increased gradually with plant growth. The content of P in the green fruit stage, mature stage and late mature stage increased from 0.03% to 0.07% and 0.09% and 0.1%, respectively, which increased by 133.3%, 200% and 233.3%, respectively, compared to that in the full flowering stage. The change in K content also increased first and then decreased, and the change in the leaves was significant. The content of K in mature and late mature leaves decreased from 0.51% in the green fruit stage to 0.41% and 0.31%, respectively, which were 19.6% and 39.2% lower than that in the green fruit stage. The content of Ca showed a trend of decreasing first and then increasing, but the changes in leaves, branches and rhizosphere soil were not significant. The content of Ca in leaves increased by 17.1% and 24.4% at the middle and late maturity stages compared to that at the green fruit stage. The Mg content showed a trend of decreasing first and then increasing, in which the leaves and branches changed significantly while the rhizosphere soil did not change significantly. The content of Mg in the leaves decreased by 20.7%, 13.8% and 27.6%, respectively, at the green fruit stage, mature stage and late mature stage compared to that at full flowering stage. The content of Mg in the green fruit period was 8.3% lower than that in the flowering period, and the content of Mg in the mature period was 54.5% higher than that in the green fruit period. The content of Mg in the late mature stage decreased by 58.8% compared to that in the mature stage. Among the trace elements, the Fe content showed a trend of decreasing first and then increasing, with significant changes in leaves but not significant in branches and rhizosphere soil. However, the content of Fe in rhizosphere soil was much higher than that in plants, while the content of Mn was stable and there was no significant difference. The Cu content in leaves and rhizosphere soil showed a trend of decreasing first and then increasing, while that in branches showed a trend of increasing first and then decreasing. The change in Zn content showed a trend of decreasing first and then increasing.

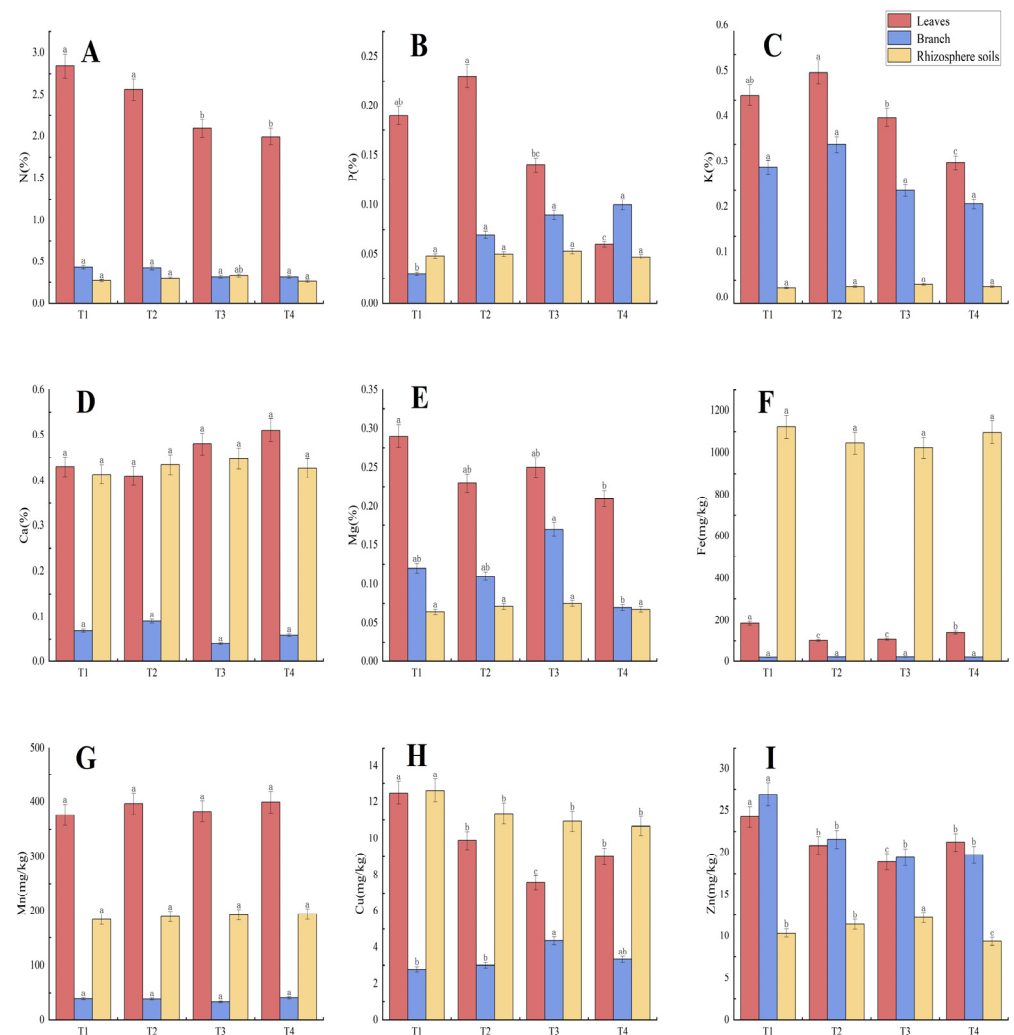


Figure 1. Mineral element contents in different parts of blueberry plants at different growth stages. T1. full bloom stage; T2. green fruit stage; T3. mature stage; T4. late mature stage. (A): The total N contents of leaves, branches and rhizosphere soil; (B): total P contents in leaves, branches and rhizosphere soil; (C): total K contents of leaves, branches and rhizosphere soil; (D): total Ca contents in leaves and branches and available Ca content in rhizosphere soil; (E): total Mg contents in leaves and branches and available Mg content in rhizosphere soil; (F): total Fe contents in leaves and branches and available Fe content in rhizosphere soil; (G): total Mn contents in leaves and branches and available Mn content in rhizosphere soil; (H): total Cu contents in leaves and branches and available Cu content in rhizosphere soil; (I) total Zn contents in leaves and branches and available Zn content in rhizosphere soil. The lower-case letters indicate a significant difference ($p < 0.05$) among different samples for each treatment.

2.1.2. Correlation Analysis of Mineral Nutrient Elements in Leaves, Branches and Soil of Blueberry Plants

The average contents of mineral elements in the rhizosphere soil, branches and leaves of blueberry plants at four growth stages were analyzed by Pearson's simple correlation coefficient method. Table 1 shows that there was a significant negative correlation between N in leaves and the Mn content in the soil, and there was a significant positive correlation between Ca in leaves and the P content in the soil. There was a very significant positive correlation between Mn in leaves and P, Ca, Mg, Mn, Cu and Zn in soil, and there was a very significant positive correlation with the N, K and Fe contents. There was no significant correlation between P, K, Mg, Fe, Cu and Zn in the leaves and the contents of various nutrient elements in the soil.

Table 1. Correlations among various mineral nutrient elements in leaves and soil.

		Leaves								
		N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
Soil	N	−0.324	−0.170	0.003	0.539	−0.227	0.122	0.669 *	0.279	0.218
	P	−0.360	−0.217	−0.185	0.627 *	−0.186	0.069	0.900 **	0.119	0.196
	K	−0.151	−0.127	−0.027	0.463	−0.108	0.280	0.698 *	0.493	0.316
	Ca	−0.406	−0.320	−0.317	0.536	−0.384	−0.079	0.848 **	−0.041	0.043
	Mg	−0.490	−0.306	−0.255	0.370	−0.574	−0.332	0.759 **	−0.069	−0.064
	Fe	−0.194	−0.141	−0.186	0.167	−0.404	−0.106	0.657 *	−0.002	0.085
	Mn	−0.601 *	−0.431	−0.490	0.566	−0.395	−0.148	0.826 **	−0.107	0.008
	Cu	−0.490	−0.199	−0.286	0.511	−0.288	−0.096	0.715 **	−0.210	0.029
	Zn	−0.108	0.069	0.023	0.483	0.051	0.205	0.837 **	0.379	0.463

Note: ‘**’ indicates $p < 0.05$; ‘***’ indicates $p < 0.01$.

Table 2 shows the correlation analysis of the nutrient elements in the soil and branches of blueberry plants. The content of N in the branches was significantly positively correlated with the contents of K and Zn in soil; the content of Mn and Cu in soil and P in branches were significantly positively correlated; and the content of Ca in branches was significantly positively correlated with the contents of N and K in soil, as well as significantly positively correlated with the content of Zn. The contents of Fe in the soil and Mg in the branches were significantly negatively correlated, and the contents of N, P, Ca and Zn in the soil and Mn in branches were significantly positively correlated. There was no significant correlation between the contents of K, Fe, Cu and Zn in branches and the nutrient contents of the soil.

Table 2. Correlations among various mineral nutrient elements in branches and soil.

		Branch								
		N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
Soil	N	0.502	0.162	0.088	0.651 *	−0.036	0.201	0.615 *	0.066	0.391
	P	0.386	0.275	−0.054	0.553	0.081	0.114	0.588 *	0.292	0.254
	K	0.669 *	−0.060	0.062	0.668 *	0.002	−0.111	0.568	0.027	0.536
	Ca	0.082	0.483	−0.018	0.441	−0.254	0.259	0.612 *	0.125	−0.037
	Mg	0.042	0.546	0.051	0.400	−0.345	0.155	0.507	0.115	−0.205
	Fe	0.060	0.405	0.081	0.473	−0.595 *	0.080	0.551	−0.282	−0.121
	Mn	−0.011	0.636 *	−0.156	0.314	−0.287	0.115	0.472	0.242	−0.209
	Cu	−0.035	0.645 *	0.019	0.425	−0.243	0.381	0.544	0.143	−0.136
	Zn	0.586 *	0.119	0.088	0.771 **	−0.091	−0.025	0.645 *	0.011	0.369

Note: ‘**’ indicates $p < 0.05$; ‘***’ indicates $p < 0.01$.

According to Table 3, the correlation analysis of nutrient element contents in the branches and leaves of blueberry plants showed that N in leaves was significantly negatively correlated with P in branches ($p < 0.01$). The correlation coefficient was 0.832, which was significantly negatively correlated with Cu. P in the leaves was significantly positively correlated with K and Ca in the branches. K in the leaves was significantly positively correlated with N and K in the branches. Ca in the leaves was significantly positively correlated with Cu in the branches. Mg and Zn in the leaves were not significantly correlated with each element in the branches. Fe in the leaves was significantly negatively correlated with P in the branches. There was a significant positive correlation between Mn and Zn, a significant positive correlation between Mn in the leaves and Ca in the branches, a very significant positive correlation between Cu in the leaves and N and Zn in the branches, and a significant negative correlation with P.

Table 3. Correlations among various mineral nutrient elements in leaves and branches.

		Leaves								
		N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
Branches	N	0.336	0.537	0.638 *	0.173	0.436	0.307	0.393	0.749 **	0.543
	P	−0.832 **	−0.376	−0.517	0.410	−0.339	−0.578 *	0.396	−0.673 *	−0.275
	K	0.506	0.643 *	0.672 *	−0.031	0.236	−0.040	0.158	0.566	0.031
	Ca	0.228	0.594 *	0.572	0.371	0.296	0.150	0.631 *	0.570	0.573
	Mg	0.005	0.412	0.421	0.388	0.529	−0.033	0.130	0.108	0.116
	Fe	−0.095	0.130	0.159	0.525	0.080	−0.052	0.180	−0.040	−0.042
	Mn	0.118	0.130	0.170	0.532	0.100	0.434	0.518	0.536	0.571
	Cu	−0.590 *	−0.016	−0.066	0.731 **	0.250	−0.297	0.379	−0.145	0.050
	Zn	0.548	0.374	0.494	0.139	0.428	0.677 *	0.120	0.747 **	0.437

Note: “*” indicates $p < 0.05$; “**” indicates $p < 0.01$.

2.2. Analysis of Bacterial Community Structure and Relative Abundance in Rhizosphere Soil of Blueberry Plants at Different Developmental Stages

High-throughput sequencing of bacterial 16S rRNA genes was performed on 20 soil samples across four growing periods. After the further removal of chimeras and short sequences, a total of 1,036,394 high-quality sequences were obtained, with a length of 351–450 bp (see Figure A1). The dilution curve of each sample showed a relatively stable trend, indicating that the sequencing data were reasonable (see Figure A2). This indicated that the sequencing depth was sufficient in terms of covering a wide range of bacterial types; therefore, subsequent bacterial community analysis continued.

Operational Taxonomic Unit (OTU) cluster analysis was carried out on rhizosphere soil bacteria. The results are shown in Figure 2. The total number of bacterial OTUs in the four periods was 16,166, the number of OTUs in the full-bloom stage (T1) was 10,110, the number of OTUs in the green fruit stage (T2) was 10,110, the number of OTUs in the mature stage (T3) was 10,439 and the number of OTUs in the late mature stage (T4) was 11,205, indicating that the number of bacterial OTUs in the rhizosphere soil of blueberry was positively correlated with the growth and development of blueberry.

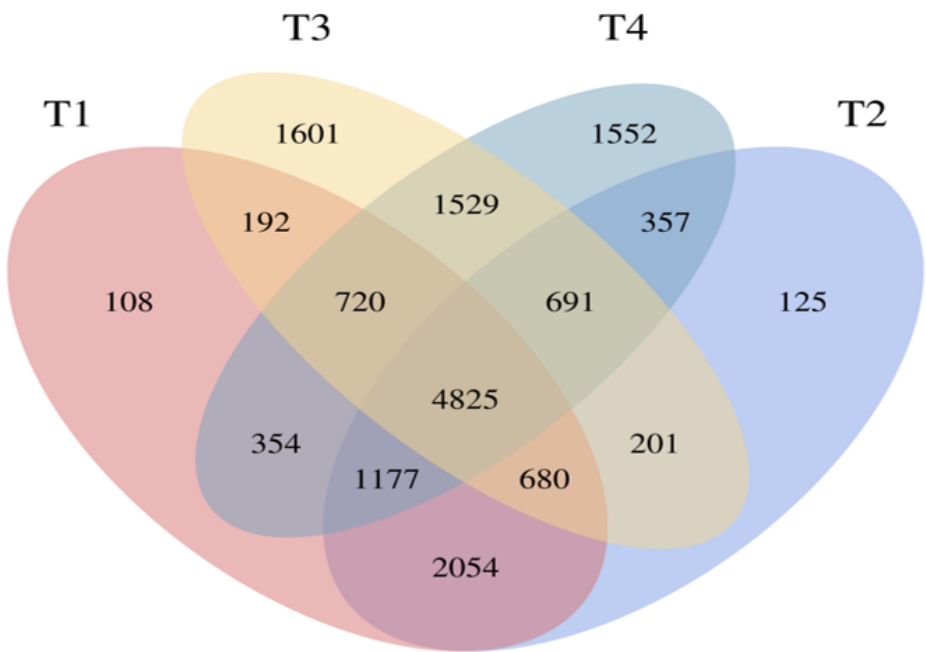


Figure 2. Venn distribution of rhizosphere soil bacteria in blueberry plants at different developmental periods. T1. full bloom stage; T2. green fruit stage; T3. mature stage; T4. late mature stage.

The total number of soil bacteria in the four periods was 4825, and the number of unique OTUs in each period were 108, 125, 1601 and 1552, respectively. The OTU number of specific bacteria in T2 soil increased by 15.7% compared to T1, that in T3 soil increased by 1180.8% compared to T2, and that in T4 soil decreased by 3.1% compared to T3. It can be seen that the number of unique bacterial OTUs in the rhizosphere soil of blueberry plants in different periods increased first and then decreased, and the number of unique bacterial OTUs in the rhizosphere soil increased significantly at the maturity stage.

2.3. Analysis of Soil Bacterial Community Diversity Index at Different Developmental Stages of Blueberry Plants (Alpha-Diversity)

Bacterial α diversity analysis can effectively reflect the abundance of species and the species diversity of a single sample. According to the statistical results of bacterial α diversity, the changes in bacterial community diversity in blueberry soil at different developmental stages are shown in Table 4. The Chao 1 index showed an upward trend, and the total Chao1 indexes in the rhizosphere of the four periods were 4651.76, 5391.47, 5270.74 and 6813.6, respectively, indicating that the total number of bacteria in the rhizosphere soil of blueberry plants gradually increased with the gradual maturity of blueberry fruit. The total Shannon indexes/Simpson indexes of blueberry rhizosphere soil samples in the four periods were 6.95/1.00 in the flowering period, 7.12/1.00 in the green fruit period, 7.01/1.00 in the mature period and 7.12/1.00 in the late mature period. Table 4 shows that there was not much difference in soil bacterial diversity in each period.

Table 4. Changes in diversity indices of inter-root soil bacterial communities at different growth stages of blueberry plants.

Period	chao1	ACE	Shannon	Simpson	Pielou	Coverage
T1	4651.76 $\pm$ 1049.78 b	4770.1 $\pm$ 1075.08 b	6.95 $\pm$ 0.12 a	1 $\pm$ 0 a	0.85 $\pm$ 0.01 a	0.95 $\pm$ 0.01
T2	5391.47 $\pm$ 924.82 b	5520.96 $\pm$ 1009.92 b	7.12 $\pm$ 0.11 a	1 $\pm$ 0 a	0.86 $\pm$ 0.01 a	0.94 $\pm$ 0.01
T3	5270.74 $\pm$ 1016.55 b	5452.9 $\pm$ 1049.14 b	7.01 $\pm$ 0.1 a	1 $\pm$ 0 a	0.85 $\pm$ 0.01 a	0.94 $\pm$ 0.01
T4	6813.6 $\pm$ 539.92 a	7040.47 $\pm$ 543.29 a	7.12 $\pm$ 0.17 a	1 $\pm$ 0 a	0.85 $\pm$ 0.01 a	0.92 $\pm$ 0.01

Note: Different lowercase letters in the same column indicate significant differences between different samples ($p < 0.05$).

2.4. Principal Coordinate Analysis of Bacterial Community in Rhizosphere Soil of Blueberry Plants at Different Growth Stages

The data in the samples were simplified for PCoA (Figure 3). After removing noise and redundancy, the similarities and differences in rhizosphere bacterial community composition in the four periods were analyzed. PC1 explained 40.86% and 25.79% of the variance variables, and PC2 explained 22.71% and 14.33% of the variance variables. This suggested that these two principal components played a key role in explaining the variability between them. The results showed that the cumulative contribution rates of the two principal components were 63.57% and 40.12%, respectively, and the degree of difference could be seen, which reflected the complex characteristics of the rhizosphere bacterial community. Combined with principal component analysis and principal coordinate analysis, it can be seen that the similarity of the samples in the four periods was generally low. There were significant differences in the community structure of rhizosphere soil microorganisms in the four growth stages ($p < 0.05$).

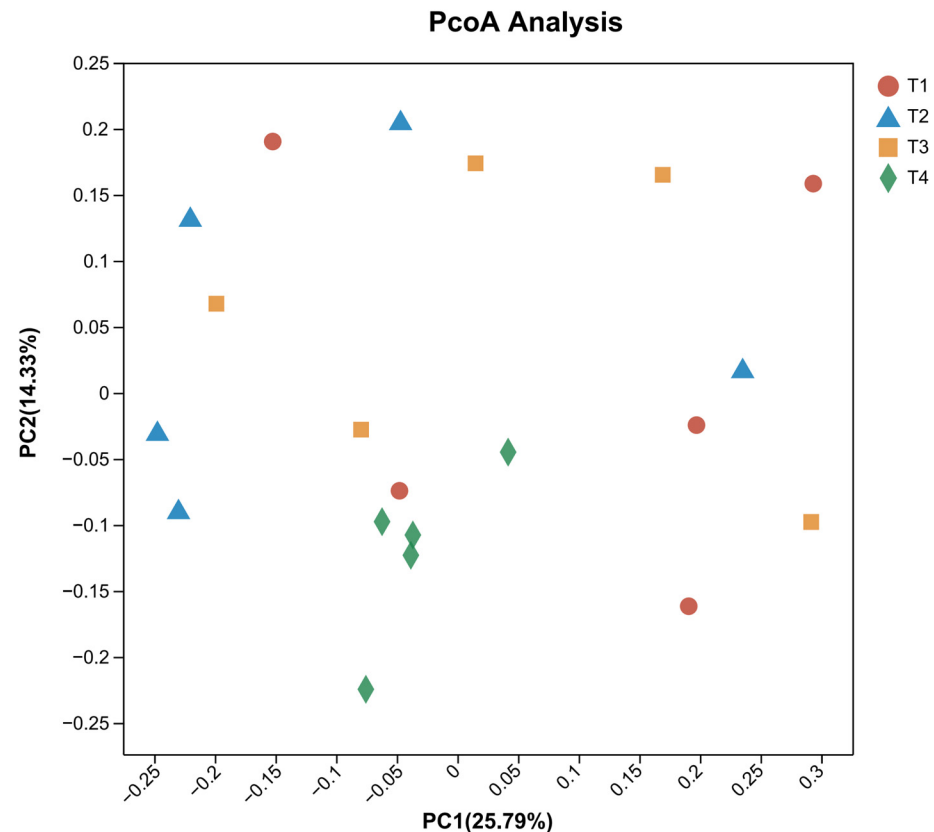


Figure 3. Analysis of rhizosphere soil bacterial communities PCoA in blueberry plants at different developmental periods. T1. full bloom stage; T2. green fruit stage; T3. mature stage; T4. late mature stage.

2.5. Analysis of Soil Bacterial Community Composition at Different Developmental Stages of Blueberry Plants

2.5.1. Phylum-Level Analysis of Bacterial Community Composition

The high-throughput sequencing results showed that a total of 52 phyla, 110 classes, 174 orders, 346 families, 705 genera and 1197 species of bacteria were detected in the rhizosphere soil of blueberry plants at different growth stages. The distribution of bacteria at the phylum level in different periods is shown in Figure 4. Among them, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Verrucomicrobia are the dominant phyla, with average relative abundance levels of 34.6%, 21.8%, 11.5%, 11.0% and 5.2%, respectively, and these five phyla account for nearly 85% of the total abundance.

The bacteria in the soil samples of the four periods were essentially the same, but there were some differences in the levels of bacterial community members in the rhizosphere soil of blueberry plants at different growth stages. Among them, Proteobacteria showed a decreasing–increasing–decreasing distribution trend with the growth of plants, and its level was relatively high at maturity. Acidobacteria showed a gradually increasing trend with the growth of plants, and its level was relatively high at the end of maturity. In addition, Gemmatimonadetes were the most abundant in the mature period. Bacteroidetes and Firmicutes were the most abundant in the green fruit period, and Saccharibacteria and Cyanobacteria were relatively stable.

2.5.2. Composition of Bacterial Community at the Genus Level at Different Developmental Stages of Blueberry Plants

The bacterial community at the genus level at different fruit development stages contains a total of 705 genera. The top 15 genus-level colonies were selected for comparative analysis (Figure 5). *Candidatus Solibacter*, *Bradyrhizobium*, *Bryobacter* and *Sphingomonas* were the dominant genera, accounting for 4.1%, 2.7%, 2.4% and 1.9% of the total abundance,

respectively. *Candidatus Solibacter* accounted for a relatively large proportion in the full-bloom stage and mature stage, and they showed a decreasing–increasing–decreasing trend with the growth of plants. *Bradyrhizobium* accounted for a relatively large proportion in the mature period and showed a trend of increasing first and decreasing with plant growth.

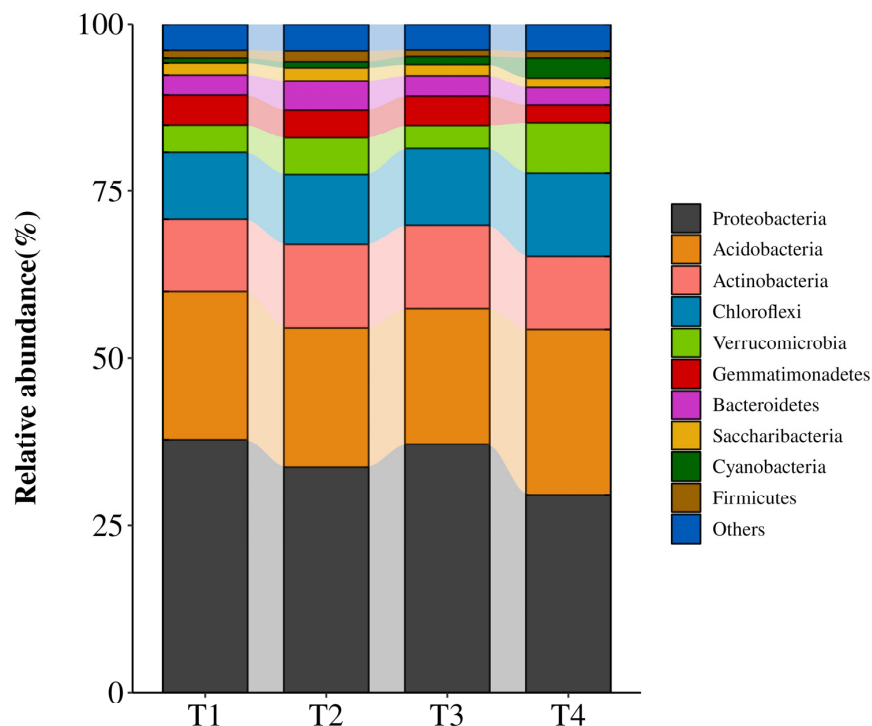


Figure 4. Relative abundance of bacteria (phylum level) at different developmental periods of blueberry plants. T1. full bloom stage; T2. green fruit stage; T3. mature stage; T4. late mature stage.

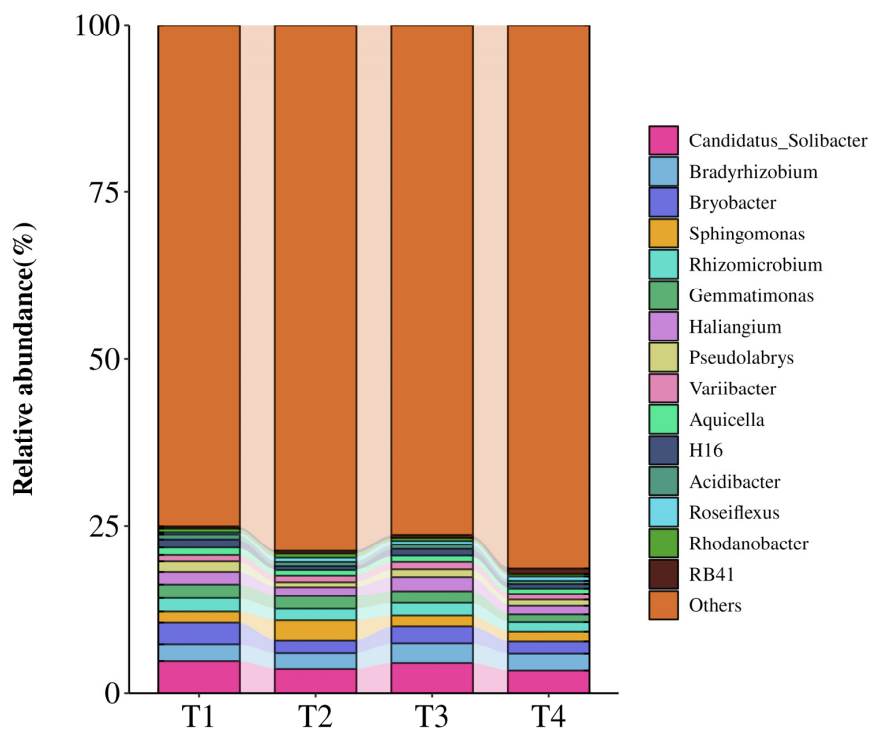


Figure 5. Relative abundance of bacteria (genus level) at different developmental periods of blueberry plants. T1. full bloom stage; T2. green fruit stage; T3. mature stage; T4. late mature stage.

2.6. Correlation Analysis between Rhizosphere Soil Bacterial Community and Soil Mineral Elements in Different Growth Stages of Blueberry Plants

In order to explore the correlation between the bacterial microbial community and soil environmental factors, 10 dominant bacterial genera and 9 soil mineral elements were selected for RDA (Figure 6). The results showed that the effects of different mineral elements on the soil bacterial community were significant. The explanatory power of the first and second axes of RDA were 65.92% and 3.68%, respectively, and the total interpretation rate was 69.6%. This result better reflects the relationship between different mineral elements and bacterial microbial communities. Among them, pH, Ca, Mn, K and Mg were negatively correlated with the dominant bacteria. *Candidatus solibacter* was significantly negatively correlated with available magnesium and available potassium. *Sphingomonas* was significantly positively correlated with total nitrogen and available phosphorus.

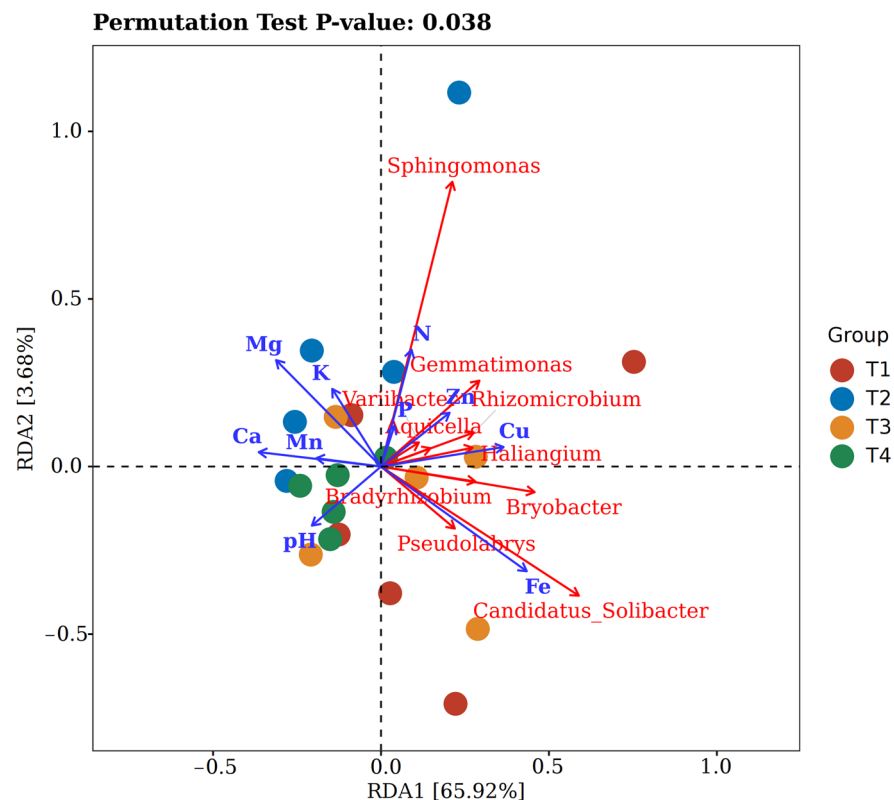


Figure 6. RDA of the top ten dominant bacterial genera and soil environmental factors. (Note: Each point represents a sample; the blue arrows represent the environmental factors, the red arrows represent the dominant bacteria, and the length of the arrows represents the degree of association with the bacteria, where a long length of the arrows indicates a high degree of association. The angle between the environmental factors and the dominant bacteria represents the size of the correlation between them. An acute angle indicates that the two factors are positively correlated, a right angle means they are not correlated, and an obtuse angle means they are negatively correlated. The p -value represents the value obtained by the random permutation nonparametric test. The smaller the p -value, the more significant the effect of the influencing factors on the composition of the flora. The percentage in the coordinate axis brackets represents the proportion of the differences in the data that the corresponding coordinate axis can explain.).

3. Discussion

3.1. Changes in Mineral Element Contents in Branches, Leaves and Rhizosphere Soil of Blueberry Plants at Different Growth Stages

During plant growth and development, the fruit quality and yield are closely related to mineral elements. Plants can transport mineral elements in the soil to the aboveground

parts through the root system for the growth and development of the whole plant. Plant nutrient levels are largely dependent on soil nutrient levels. Yang Xiaoxu et al. [26] studied the annual dynamic changes in mineral element contents in orange leaves. The contents of N and P showed a downward trend, and the contents of K, Ca and Fe showed an overall upward trend. The results of this experiment showed that the change trend in the N and P contents in different periods was essentially the same. Eaton et al. proposed that nitrogen could increase the anthocyanin content of cranberry fruit and affect fruit color [27], which may be one of the reasons for the decrease in N content. The dynamic changes in each element's content in different growth stages of blueberry plants and its relationship with soil reflect the different needs of plants for nutrient elements in the growth process. For example, in this study, the Mn content of blueberry leaves was the highest during the T1 stage, indicating that the Mn demand was highest during this period.

Li Yadong et al. researched *Vaccinium uliginosum* and showed that there was a less significant positive correlation between the annual cycle variation in mineral nutrients in the soil and the annual cycle variation in mineral nutrients in leaves of bog bilberry [28]. The results of this study showed that the content of Ca in leaves was significantly positively correlated with the content of P in soil, which may indicate that P in soil is beneficial to the absorption of Ca or the synergistic effect of the two in the metabolic process during blueberry growth. There was no significant correlation between the contents of P, K, Mg, Fe, Cu and Zn in blueberry leaves and the contents of nutrient elements in soil. The reason could be that blueberry is a shallow-root plant and its nutrient absorption capacity is weak, resulting in no significant correlation between the nutrient levels in leaves and nutrient levels in the soil.

It has been reported that Mn and Zn are higher in naturally growing plants. Manganese is an essential element that binds to many essential enzymes. For example, the activity of superoxide dismutase is inhibited by low manganese [29]. Zn is involved in the composition of many functional proteins in plants, which has a certain effect on the activity of various enzymes, subsequently affecting photosynthesis [30]. As an essential trace element for plants, Mn is present in various enzymes, activators and important redox agents. At the same time, it participates in the process of photosynthesis and plays an important role in the formation of chloroplasts [31].

In this experiment, it was found that Ca in branches was significantly positively correlated with N, K and Zn in the soil, especially Zn ($p < 0.01$). This shows that Zn plays a special role in regulating the operation of Ca in branches. The content of Mn in blueberry leaves is much higher than that found in branches, which may be caused by the large demand in the leaves and directional transportation. At the same time, the positive correlation between Mn in leaves and branches and various soil elements suggests that Mn plays a central role in the mineral nutrient balance of blueberry plants and may be involved in the transportation and absorption regulation of various elements. It has a wide range of synergistic effects and involves multiple physiological processes. The Mg content in the branches was significantly negatively correlated with Fe in the soil, reflecting that the absorption of Mg may be greatly inhibited by Fe. In addition, it was found that the content of Fe in the rhizosphere soil of blueberry plants was much higher than that in the blueberry plants themselves. The reason for this result may be that the blueberry roots are easily infected by fungi, and the formed mycorrhizal fungi will promote Fe enrichment in the soil [32–34].

3.2. Bacterial Diversity and Composition in the Rhizosphere Soil of Blueberry Plants at Different Growth Stages

The diversity and relative abundance of rhizosphere microorganisms are closely related to plant health. Rhizosphere microorganisms are indicators of soil fertility. Previous studies have confirmed that some plants at different growth stages will significantly affect the diversity of the composition and size of rhizosphere microbial communities [35–37]. Microorganisms undergo seasonal dynamic changes, and the types and quantities of bacte-

ria will be different during different seasons, greatly impacting the community distribution and life activities of microorganisms. This is also one of the reasons for the different relative abundance levels of rhizosphere microorganisms at different growth stages [38,39]. This study found that the relative abundance of bacteria in the rhizosphere soil of blueberry plants at the end of fruit ripening was the highest. The analysis also showed that the metabolism of blueberry plants was very strong during the whole fruit growth period. With a large amount of fruit ripening, the growth of blueberry roots was active and a large number of secondary metabolites were produced to promote the growth and reproduction of more microorganisms.

Tang et al. [40] pointed out that the bacterial communities of different plants were different. However, there was no significant change in bacterial diversity and evenness index in each growth period, indicating that these were relatively stable and less affected by environmental factors. The results of this study showed that, at the phylum level, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Verrucomicrobia were the dominant phyla in different blueberry developmental stages, which was consistent with the main trends relating to dominant bacteria in a forest ecosystem and the results obtained by Zheng et al. [20,41,42].

Kämpfer et al. [43] showed that bacteria can produce plant growth hormone and cytokinin and promote plant growth and development via nitrogen fixation. Li et al. [44] proposed that Proteobacteria had a positive correlation with soil nitrogen content. Studies have shown that *Acidobacteria* can increase soil organic matter by decomposing animals and plants, indicating that Proteobacteria may be related to plant nitrogen fixation. *Acidobacteria* can increase soil nutrients [45]. According to the changes in the levels of bacterial genera, we found that the dominant genera in the rhizosphere of blueberry were *Candidatus solibacter*, *Bradyrhizobium*, *Bryobacter* and *Sphingomonas*. Among them, *Candidatus solibacter* was the most abundant, and it accounted for a particularly large proportion in the flowering and maturity stages. *Candidatus solibacter* is a Gram-negative bacterium that can decompose organic matter and use carbon sources. It is positively correlated with soil organic matter. It also directly affects nitrogen transformation in soil [46,47]. *Bryobacter* is acid-resistant and strictly aerobic; it mainly lives in acidic wetlands and soils [48]. Blueberry plants are suited to slightly acidic soil environments, indicating that these blueberry root environments attract some beneficial bacteria that promote growth and development; in that regard, the bacteria identified in this study can provide a reference for actual blueberry production conditions.

3.3. Correlation Analysis Between Rhizosphere Bacterial Community and Soil Environmental Factors of Blueberry at Different Growth Stages

Studies have revealed that the structure and function of soil microbial communities are highly correlated with metal trace elements, mainly iron. The addition of iron and zinc increases the relative abundance of Proteobacteria in most soils [49].

This study found that in addition to the extremely significant correlation between N element and *Sphingomonas*, the iron in blueberry soil showed a significant correlation with its bacterial community, and an extremely significant correlation with *Candidatus solibacter* and *Pseudolabrys*. This is essentially consistent with relevant research results. The correlation analysis between the bacterial community composition of blueberry roots and soil environmental factors showed that P was significantly correlated with *Sphingomonas*, which could increase the relative abundance of *Sphingomonas*, indicating that the bacterial community composition of the blueberry rhizosphere was affected by soil environmental factors. *Sphingomonas* is a rhizosphere microorganism that is beneficial to plants. It can degrade organic pollutants, promote plant growth and inhibit plant pathogens. From another perspective, this shows that P in the soil can promote the growth of blueberry plants. In actual production, by adjusting the contents of mineral elements in soil, the microenvironment of blueberry rhizosphere soil can be changed, so as to improve soil quality and promote the yield and quality of blueberry fruit [50].

4. Materials and Methods

4.1. Overview of the Test Site

The test site is located in the base of the Jilin Jingyu Blueberry Science and Technology Institute in Jingyu County, Jilin Province (127°01' E, 42°32' N). The soil is slightly acidic black loam (pH 4.33~4.80). The average annual precipitation is 776.4~923.1 mm, the average annual temperature is 3.7~5.9 °C and the frost-free period is about 104 days.

4.2. Sample Collection

Leaf and branch sample collection: The 12-year-old half-high bush blueberries were selected as the experimental material. Their planting separation was 1 × 2 m. The collection of samples began in May 2021. Sampling transpired over four distinct developmental stages of the fruit (T1: full bloom; T2: green fruit; T3: mature fruit; T4: end of mature). Their leaves and branches were promptly transported to the laboratory in iceboxes after collection.

Five healthy and insect-free blueberry branches with relatively consistent growth were selected for research. Leaf samples were collected utilizing an S-shaped (serpentine) sampling methodology. From each bush, 8 leaves were gathered from branches oriented towards the north, south, east and west, achieving a total of 32 leaves per bush, amounting to 160 leaves collectively. Branches were sampled in four periods. Two 1-year-old branches with essentially the same growth were selected in the four directions of east, south, west and north of the five trunks. The collected branches were transported back to the laboratory for washing and dried on filter paper. Then, we cut the branches into small sections with stainless steel scissors. Finally, these branches were placed in paper bags and numbered.

Leaf and branch sample pretreatment: Firstly, sample blanching was performed at 105 °C for 20 min in a blast oven (Shanghai Boxun Industry Co. Ltd., Shanghai, China), and then the samples were dried to constant weight at 65 °C. After drying, the samples were crushed by a micro-pulverizer (Shanghai Boxun Industry Co. Ltd., Shanghai, China) and passed through a 100-mesh sieve. Finally, each sample was placed in a sample bag, numbered, sealed and placed in a dryer for testing.

Soil sample collection: Soil samples were collected under 5 blueberry trees from which we had collected leaves and branches, and the period was the same as that of leaves and branches. Surface vegetation and debris were removed, and soil around the fine roots was carefully excavated to facilitate sample collection. Sampling was conducted from a depth range of 10–20 cm beneath the surface layer. Roots were gently unearthed, and soil adhering to the roots was collected using the shaking method. The collected soil was immediately placed in sterile sealed bags and transported to the laboratory. A portion of each soil sample was allocated for nutrient analysis of the rhizosphere, while the remaining soil was reserved for sequencing of bacterial communities within the rhizosphere.

4.3. Determination of Mineral Elements

Determination of the mineral element content in leaves and branches: Total N was measured using the Kjeldahl method. The molybdenum antimony anti-colorimetric method was used to determine the total P content in the spectrophotometer. After H₂SO₄-H₂O₂ (Beijing Chemical Works., Beijing, China) digestion, the total K content was determined by a flame photometer. The effective contents of total Ca, total Mg, Fe, Cu, Zn, K and Mn were determined by atomic absorption spectrometry (AAS) [51].

Determination of mineral elements in soil: The total N content was determined by the semi-micro Kjeldahl method. The total P content was determined by the HClO₄-H₂SO₄ (Beijing Chemical Works., Beijing, China) digestion method, and the total K content was determined by a flame photometer. The Ca and Mg contents were determined by EDTA (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) titration method. The Fe content was determined by atomic absorption spectrometry (AAS). The Mn content was determined by NH₄OAc extraction-KMnO₄ (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) colorimetry. The Cu and Zn contents were determined by HCL (Beijing Chemical Works., Beijing, China) extraction-atomic absorption spectrometry (AAS) [51].

4.4. Total DNA Extraction, PCR Amplification and Sequencing from Soil

Approximately 0.5 g of soil was weighed for each sample, and total DNA was extracted using the Omega Stool DNA Kit (MoBio Laboratories, Carlsbad, CA, USA). PCR amplification targeting the V3-V4 region of the bacterial 16S rRNA gene was performed using primers 338 (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') (see Table A1 in Appendix A for details). Amplification success was confirmed via 2% agarose gel electrophoresis. Subsequently, the extracted DNA was forwarded to Shandong Kaiyuan Gene Technology Co., Ltd. (Jinan, China) for comprehensive sequencing analysis.

4.5. Data Processing

MMiseq sequencing obtains double-ended sequence data. Firstly, according to the overlap relationship between PE reads, the paired reads were spliced (merged) into a sequence. At the same time, the quality of the reads and the effect of the merge were filtered. The barcode and primer sequences at the ends of the sequence distinguish the samples to obtain an effective sequence and correct the sequence direction. The RDP classifier Bayesian algorithm was used to analyze the 97% similar OTU representative sequences, and the community composition of each sample was counted at each classification level: domain, kingdom, phylum, class, order, family, genus and species.

Single-factor analysis of variance (ANOVA) was performed using SPSS 21.0 software to analyze the significance of the difference ($p < 0.05$). Pearson's correlation coefficient was used to analyze the relationship between nutrient contents in different parts of blueberry plants. OTU clustering was performed on 97.0% high-quality sequences of reads using Usearch (v.11) software. Alpha diversity analysis (including Shannon, Simpson and Chao1 indexes) was performed using QIIME1 (v1.8.0) software. R (v3.6.0) software was used to analyze the species composition histogram. QIIME1 (v1.8.0) was used to calculate the beta diversity distance matrix and the weighted UniFrac distance. Redundancy analysis (RDA) was performed using R (v3.6.0) software.

5. Conclusions

In this study, the changes in the mineral element contents in different parts of blueberry branches, leaves and rhizosphere soil at different growth stages were not the same, which is related to the fact that the growth and development peaks of each part were not within the same period. The contents of N and P in the aboveground part gradually decreased with the growth of the plant. Among them, P in the branches gradually decreased, while the trend for N was the opposite; the contents of N, P, Zn, Cu and Mg in leaves decreased gradually. The contents of N, Zn and K in the rhizosphere soil increased first and then decreased. The mineral nutrients in different growth stages of blueberry plants showed significant correlation in the leaves, branches and soil.

The bacterial community structure in the rhizosphere soil of blueberry plants was also significantly different, and the relative abundance of the bacterial community in the rhizosphere soil showed the following trend: T4 > T2 > T3 > T1. At the phylum level, Actinobacteria, Proteobacteria, Acidobacteria, Verrucomicrobia and Chloroflexi were the main dominant bacteria in different periods. *Candidatus solibacter* and *Bryobacter* were significantly higher during the full-bloom stage and the maturity stage than at the green fruit stage and late maturity stage. *Bradyrhizobium* increased significantly at the maturity stage, and *Sphingomonas* increased significantly at the green fruit stage. The contents of N, P, Zn, Cu and Fe in the soil were significantly positively correlated with the relative abundance of dominant bacteria.

In summary, it is suggested that the key points of cultivation and management in each period should be determined according to the different changes in mineral elements in different growth stages of blueberry plants. The fertilization formula and cultivation management should be finely adjusted according to the local soil conditions and climatic characteristics to improve the yield and quality of fruit. However, this study only analyzed the rhizosphere microorganisms of blueberry plants from the perspective of bacteria. It is

suggested that researchers should further analyze the changes in rhizosphere soil fungal communities in different developmental stages of blueberry plants, and seek to fully understand the effects of rhizosphere microbial communities on the growth of blueberry plants, to provide a more comprehensive scientific basis for the cultivation and management of blueberry crops.

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Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

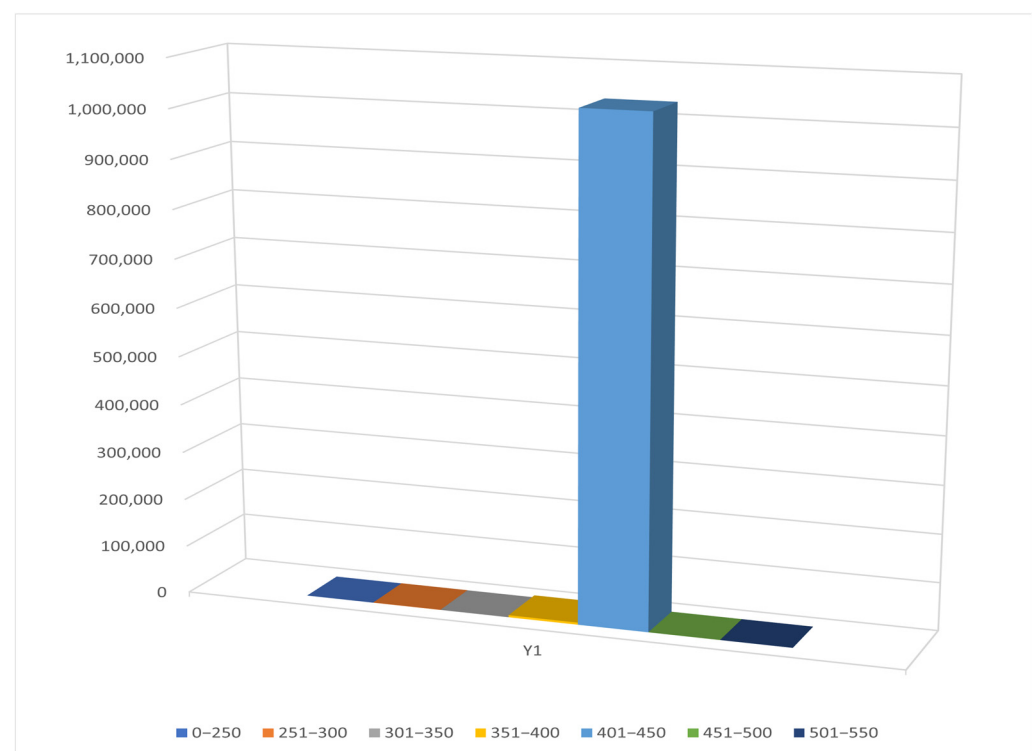


Figure A1. High-quality sample sequence distribution map.

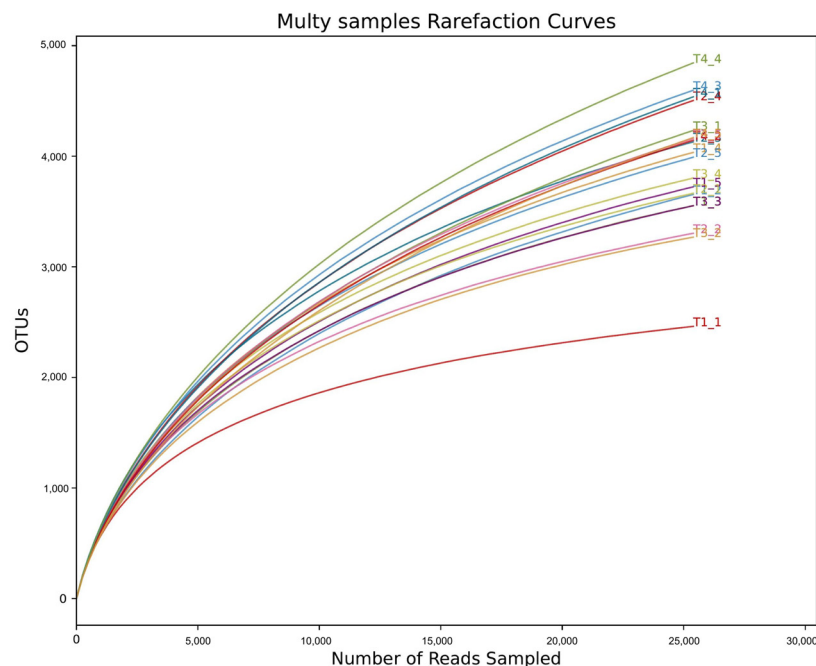


Figure A2. Rhizosphere soil bacterial dilution curves of blueberry fruits at different developmental periods. T1. full bloom period; T2. green fruit period; T3. maturation period; T4. late maturation stage.

Table A1. Reaction system of PCR.

Reaction Component	Volume (μ L)
5 $\times$ FastPfu buffer	4
dNTPs (2.5 mM)	2
Forward primer (5 μ M)	0.8
Reverse primer (5 μ M)	0.8
FastPfu polymerase	0.4
ddH ₂ O	20

References

- Wu, L. Thirty-five Years of Research and Industry Development of Blueberry in China. *J. Jilin Agric. Univ.* **2016**, *38*, 1–11.
- Zou, X.; Yang, Z.; Shi, J.; Huang, X.; Zhang, W.; Haroon, E. Preservation Effect of Gum Arabic Edible Coating Incorporated with White Roselle Extract (*Hibiscus sabdariffa* L.) on Cold-Stored Blueberries. *Food Sci.* **2019**, *7*, 204–211.
- Wu, L.; Zhang, Q.; Zang, H.; Xu, Z.; Xu, D. Determination of sugar and acid components in blueberries from Lijiang, Yunnan and Jingyu, Jilin. *China Fruits* **2019**, *6*, 54–58, 64.
- Wu, Z.; Zhang, L.; Xie, L.; Zhang, X.; Zheng, S.; Li, T. Analysis on Content and Composition of Amino Acids in Fruits of Twelve Blueberry Varieties. *South China Fruits* **2023**, *52*, 149–153.
- Basu, A.; Feng, D.; Planinic, P.; Ebersole, J.L.; Lyons, T.J.; Alexander, J.M. Dietary blueberry and soluble fiber supplementation reduces risk of gestational diabetes in women with obesity in a randomized controlled trial. *J. Nutr.* **2021**, *151*, 1128–1138. [\[CrossRef\]](#)
- Jiang, J.; Wu, L.; Tang, X.; Zhao, S.; Li, Y.; Zhang, Z.; Liu, H. Determination and Analysis of Content of Heavy Metals in Orchard Soil and Fruit of Blueberry. *J. Jilin Agric. Univ.* **2009**, *31*, 656–660.
- Li, Y.; Liu, S.; Wang, D.; Li, Q.; Wang, C.; Wu, L. Comparative Study on the Effects of Different Soil Improvement Methods in Blueberry Soil. *Agronomy* **2024**, *14*, 125. [\[CrossRef\]](#)
- Wang, J.; Tang, X.; Wang, M.; Yao, L.; Liu, X. Effects of storage nutrition on next year growth and nitrogen absorption and utilization in blueberry. *J. Northeast Agric. Univ.* **2022**, *53*, 29–36.
- Chen, S.; Zhu, Y.; Shao, T.; Long, X.; Gao, X.; Zhou, Z. Relationship between rhizosphere soil properties and disease severity in highbush blueberry (*Vaccinium corymbosum*). *Appl. Soil. Ecol.* **2019**, *137*, 187–194. [\[CrossRef\]](#)
- Meerts, P. Foliar macronutrient concentrations of forest understorey species in relation to Ellenberg's indices and potential relative growth rate. *Plant Soil* **1997**, *189*, 257–265. [\[CrossRef\]](#)
- Dris, R.; Niskanen, R.; Fallahi, E. Relationships between leaf and fruit minerals and fruit quality attributes of apples grown under northern conditions. *J. Plant Nutr.* **1999**, *12*, 1839–1851. [\[CrossRef\]](#)

12. Liu, H.; Song, Y.; Zhang, H. Research development of mineral nutrient deficiency symptoms and rectification measures of cowberry. *China Fruits* **2018**, *5*, 57–62.
13. Liu, H.; Zhang, H. Accumulation and Distribution Characteristics of Mineral Nutrients in Duke Blueberry during Maturing. *South China Fruits* **2022**, *51*, 193–197, 202.
14. Li, Y.; Hao, R.; Qu, L.; Zhng, Z. A Study of The Mineral Nutrient Status of Bog Bilberry. *J. Jilin Agric. Univ.* **1990**, *12*, 24–28.
15. Bai, Y.; Wei, X.; Chen, L.; Wan, R.; Hou, Z. Multivariate Analysis of Fruit Leaf Mineral Elements, Soil Fertility Factors and Fruit Quality of *Vaccinium uliginosum* L. *Sci. Agric. Sin.* **2018**, *51*, 170–181.
16. Khokhar, Y. Soil fertility and nutritional status of Kinnow orchards grown in aridisol of Punjab, India. *Afr. J. Agric. Res.* **2012**, *33*, 4692–4697. [[CrossRef](#)]
17. Paulina, D.; Vaida, Š.; Krystyna, P. Mineral Composition of Wild and Cultivated Blueberries. *Biol. Trace Elem. Res.* **2018**, *181*, 173–177.
18. Li, Q.; Wang, M.; Liu, J. Diversity and Differences in Soil Microbial Community Structure in the Rhizosphere of Rapeseed at Different Times. *North. Hortic.* **2023**, *24*, 72–80.
19. Dong, D.; Liu, Y.; Lu, C.; Wang, L.; Liu, A. Diversity of Microbial Community between Rhizosphere Soils of Blueberries Cultivated for Different Years. *Acta Agric. Boreali-Occident. Sin.* **2018**, *7*, 1041–1048.
20. Zheng, T.; Wang, M. Diversity and Differences of Rhizosphere Microbial Community Structure in Different Blueberries. *North. Hortic.* **2022**, *14*, 76–86.
21. Tong, L.; Gao, J.; Jin, Y. Dynamic changes of microorganisms in the rhizosphere of strawberry during different growth stages. *J. Beijing Univ. Agric.* **2019**, *4*, 10–15.
22. Liu, X.; Tang, X.; Liu, H.; Wu, L.; Zhang, Z.; Li, Y. The influence of soil amendments on cytokinin contents and their annual variations in the root zone of *Vaccinium vitisidaea*. *J. South China Agric. Univ.* **2014**, *35*, 52–57.
23. Yuan, J.; Wen, T.; Zhang, H.; Zhao, M.; Penton, C.R.; Thomashow, L.S.; Shen, Q. Predicting disease occurrence with high accuracy based on soil macroecological patterns of *Fusarium wilt*. *ISME J.* **2020**, *14*, 2936–2950. [[CrossRef](#)]
24. Zhou, Y.; Bai, Y.; Yue, T.; Li, Q.; Huang, Y.; Jiang, W.; He, C.; Wang, J. Research progress on the growth-promoting characteristics of plant growth-promoting rhizobacteria. *Microbiol. China* **2023**, *50*, 644–666.
25. Guo, L.; Zhang, C.; Wang, F.; Shen, J.; Zhang, K.; He, L.; Guo, Q.; Hou, X. Analysis of bacterial community characteristics in the rhizosphere soil of wild tree peony. *J. Nanjing For. Univ. (Nat. Sci. Ed.)* **2023**, *47*, 45–55.
26. Yang, X. Dynamics Studies on the Mineral Characteristics of Three Blueberry Cultivars in Jilin Province. Master's Thesis, Jilin Agricultural University, Changchun, China, 2013.
27. Eaton, G.W. Effects of NPK Fertilizers on the Growth and Composition of Vines in a Young Cranberry Bog. *J. Am. Soc. Hortic. Sci.* **1971**, *96*, 426–429. [[CrossRef](#)]
28. Li, Y.; Qu, L.; Hao, R. Research on the annual variation of mineral nutrient element content in the leaves of bog bilberry. *Acta Hortic. Sin.* **1990**, *3*, 185–190.
29. Li, C.; Zhou, H. The role of manganese superoxide dismutase in inflammation defence. *Enzym. Res.* **2011**, *2011*, 387176. [[CrossRef](#)]
30. Wang, H.; Jin, J. The physiological and molecular mechanisms of zinc uptake, transport, and hyperaccumulation in plants: A review. *Plant Nutr. Fertil. Sci.* **2009**, *15*, 225–235.
31. Moshe, S.; Ora, E.; Elisha, T. Manganese nutrition effects on tomato growth, chlorophyll concentration, and superoxide dismutase activity. *J. Plant Physiol.* **2004**, *161*, 197–202.
32. Wang, M. Effects and Mechanism of Arbuscular Mycorrhizal Fungi on Iron Uptake in Citrus. Ph.D. Thesis, Huazhong Agricultural University, Wuhan, China, 2008.
33. Liu, F.; Cheng, X.; Gu, L.; Feng, P.; Shao, X.; Zhao, Q. Survey on Root Endophytic Fungi Resources of *Vaccinium uliginosum* Linn. *Shandong Agric. Sci.* **2015**, *47*, 41–46.
34. Zhu, J. The Separation, Screening and Application of Mycorrhizal Fungi from *Vaccinium Bracteatum* Thunb. Master's Thesis, South Central Forestry S&T University, Changsha, China, 2016.
35. Wu, W.; Han, X.; Wang, J.; Sun, N.; Li, Y. Variation of bacterial community structure in rhizosphere soil of *Fructus aurantii* at different growth stages. *Southwest China J. Agric. Sci.* **2023**, *36*, 1694–1701.
36. Li, Q.; Xiao, Z.; Ren, M. Variation of Bacterial Community Structure in *Gardenia jasminoides* Rhizosphere at Different Growth Stages. *Chin. J. Soil Sci.* **2021**, *52*, 346–354.
37. Mei, L.; Huang, Z.; Song, Z.; Jia, Y.; Su, D.; Lin, H.; Lin, Z. Effect of *Pennisetum* sp. and its different growth periods on physical-chemical properties and bacterial diversity in sandstone areas. *Acta Microbiol. Sin.* **2019**, *59*, 2038–2050.
38. Colin, R.J.; William, C.D. Annual and Seasonal Variation in the Phyllosphere Bacterial Community Associated with Leaves of the Southern Magnolia (*Magnolia grandiflora*). *Microb. Ecol.* **2010**, *61*, 113–122.
39. Jumpponen, A.; Jones, K. Seasonally dynamic fungal communities in the *Quercus macrocarpa* phyllosphere differ between urban and nonurban environments. *New Phytol.* **2010**, *186*, 496–513. [[CrossRef](#)]
40. Tang, J.; Chen, Z.; Guo, A.; Qiu, Q. Characteristics of Microbial Community Structure in the Rhizosphere Soil of Different Crops. *J. Nucl. Agric. Sci.* **2021**, *35*, 2830–2840.
41. Ma, S.; Verheyen, K.; Props, R.; Wasof, S.; Vanhellefont, M.; Boeckx, P.; Boon, N.; De Frenne, P. Plant and soil microbe responses to light, warming and nitrogen addition in a temperate forest. *Funct. Ecol.* **2018**, *32*, 1293–1303. [[CrossRef](#)]

42. Zhao, R.; Zheng, S.; Hu, Y.; Li, H.; Chen, Y.; Chun, Z. Endophytic bacterial diversity of the medicinal orchid *Dendrobium nobile*. *S. Afr. J. Bot.* **2023**, *158*, 90–97. [[CrossRef](#)]
43. Kämpfer, P.; Ruppel, S.; Remus, R. *Enterobacter radicincitans* sp. nov., a plant growth promoting species of the family Enterobacteriaceae. *Syst. Appl. Microbiol.* **2005**, *28*, 213–221. [[CrossRef](#)]
44. Li, Y.; Lin, Z.; Lu, Z.; Liu, M. Microbial Diversity and Community Structure in Soil under Tea Bushes-*Ganoderma lucidum* Intercropping. *Fujian J. Agric. Sci.* **2019**, *34*, 690–696.
45. Yang, J.; Zhang, J.; Yu, H.; Chen, J. Community composition and cellulase activity of cellulolytic bacteria from forest soils planted with broad-leaved deciduous and evergreen trees. *Appl. Microbiol. Biotechnol.* **2013**, *98*, 1449–1458. [[CrossRef](#)] [[PubMed](#)]
46. Chen, L.; Zhao, Z.; Li, J.; Wang, J.; Guo, G.; Wu, W. Effects of muddy water irrigation with different sediment particle sizes and sediment concentrations on soil microbial communities in the Yellow River Basin of China. *Agric. Water Manag.* **2022**, *270*, 107750. [[CrossRef](#)]
47. André, M.; Julierme, Z.; Arnold, B.; Marco, A.; Ediwan, J.; Marianfela, H. Benefits of soybean co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: Large-scale validation with farmers in Brazil. *Eur. J. Agron.* **2024**, *155*, 127112.
48. Wang, R.; Zhang, Z.; Lu, F.; Wei, L.; Xiao, Y. Optimizing the bacterial community structure and function in rhizosphere soil of sesame continuous cropping by the appropriate nitrate ammonium ratio. *Rhizosphere* **2022**, *23*, 100550. [[CrossRef](#)]
49. Dai, Z.; Guo, X.; Lin, J.; Wang, X.; He, D.; Zeng, R.; Meng, J.; Luo, J.; Delgado-Baquerizo, M.; Moreno-Jiménez, E.; et al. Metallic micronutrients are associated with the structure and function of the soil microbiome. *Nat. Commun.* **2023**, *14*, 845. [[CrossRef](#)]
50. Leng, F.; Cui, X.; Zhu, N.; Zhu, X.; Wang, X.; Wang, Y. Characterization of root microbial communities associated with *Astragalus membranaceus* and their correlation with soil environmental factors. *Rhizosphere* **2023**, *25*, 100656. [[CrossRef](#)]
51. Bao, S. *Agrochemical Analysis of Soil*; China Agriculture Press: Beijing, China, 2005.

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