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Comparison between the rhizosphere microbiome of transgenic sugar beet resistant to rhizomania and non-transgenic parent, Revealed by Illumina Miseq

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Abstract

Due to the development and increasing cultivation of genetically engineered plants as an integral part of modern agriculture, the biosafety of soil microorganisms, essential elements of soil fertility, quality, and stability, has been discussed. To reveal the effect of transgenic plants on soil bio-diversity, it is necessary to compare the microflora of transgenic and parental plants. In this study, second-generation GM sugar beet seeds rendered resistant to the propagation of *Necrotic yellow vein virus* of beets (*BNYVV*) via gene silencing in a field trial were compared to their parental plant by analyzing 16S rDNA metagenomes with the use of the Illumina MiSeq platform. Analysis of the alpha and beta diversity found some influence on bacterial communication of rhizosphere between non-transgenic and transgenic (including 211S3, 219S3, 228S3, and 231S6 cultivars) sugar beets. Based on the results of the research on alpha diversity, the transgenic 211S3 and 219 S3 cultivars showed a lower average than the control sample in the Cho, phylogenetic diversity, Cho1 bias correction, and the number of OTUs indexes; The transgenic 231S6 cultivar showed a significantly higher mean than the control sample in the Simpsons index. Also, in the study of beta diversity based on the Bray-Curtis distance algorithm, all 211S3, 219S3, 228S3, and 231S6 cultivars and control samples were positioned in one group. According to the Unweighted UniFrac distance algorithm, 219S3 and 231S6 cultivars were put together in one group, and 211S3 and 228S3 cultivars were classified into one group. A comparison of the bacterial genera showed a noteworthy reduction in relative abundance. While a few genera showed a significant decrease in terms of overall abundance, other genera that stabilize molecular nitrogen and motivate plant growth, such as *Agrobacterium*, *Devosia*, *Mesorhizobium*, *Burkholderia*, and *Bradyrhizobium*, showed a significant decrease compared to the control cultivar in all transgenic beets.

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1. Introduction

Worldwide production of transgenic crops has increased to millions of hectares [11], and this trend is anticipated to continue in the future. These new transgenic crops provide numerous economic advantages. However, they have also generated concerns about their environmental impact, particularly on the bacterial societies of rhizosphere soil, which plays an essential role in plant health.

Many countries are wary of releasing transgenic plants due to concerns about the possible negative efficacies of GM plants on human health and the climate. However, many investigations have shown that plant species strongly influence the rhizosphere bacterial diversity [10-3]. Another study indicated that the composition of the soil bacterial community is determined by its host plants [22]. According to previous research, transgenic plant diffusion has little to no substantial transitory effect on the soil bacteria community [16-7-28]. In other studies, the diffusion of transgenic plants had a major influence on microbial populations of non-target bacteria, the activity of soil enzymes, or the microbial community's structure [7-29]. Another research showed the influence of the diffusion of soil microbial society on transgenic plants [18].

These serious concerns necessitate a thorough examination of the environmental impact of transgenic plants. As new GM plants are expanded, soil microbiologists must also expand the current standards, including studying their effects on soil organisms to assess the security of GM plants. Environmental agents, i.e., climate, humidity, temperature, and light, influence the quantity and composition of Root exudate. Therefore, annual repetition is urgent when analyzing the impression of GM plants on bacteria that exist in the soil. Furthermore, due to the limited research on the connection between the soil microbial society and plants [18] and the high convolution of the soil, which contains one of the most diverse communities of microbia [9], more research is necessary to evaluate the influence of the diffusion of GM plants on the soil.

However, many soil bacteria cannot be cultured in laboratory media. Therefore, this study used the new generation technology of sequencing and metagenomics to study the changes in bacterial diversity of rhizosphere soils under transgenic beet cultivation compared to non-transgenic cultivars [18]. NGS technology, such as the Illumina MiSeq platform with corresponding bioinformatics instruments, appears to be robust technology that has become far more affordable since 2013 [15]. It has been used to study the high convolution of microbial communities by many researchers [26-8]. NGS technology is important for systematic contrast research that overcomes some heterogeneity of the soil and is able to discern the considerable differences in the relative abundance of rhizosphere bacteria [20].

Like most members of the *Chenopodiaceae* family, the sugar beet is stable and resistant to salinity stress. While sugar beet cultivation has spread from Europe to different parts of the world, developed areas have suffered from many unknown diseases. Rhizomonia is one of the most critical sugar beet diseases. Rhizomania, the most harmful sugar beet illness, is caused by the beet necrotic yellow vein infection (BNYVV), which forms spores that can keep the virus in the soil for more than 15 years. The resulting considerable fall in root output and sugar content could result in considerable economic losses for sugar beet harvests. Rhizomania was first recorded in Italy more than a half-century ago. It has since then spread to nearly all sugar beet-growing regions throughout the world. The utilization of resistant varieties is instrumental in guaranteeing beneficial yield in soils infested with *BNYVV*. Recently, RNA silencing has been utilized to improve rhizomania resistance [31]. This study aims to determine whether the release of GM sugar beet affects the soil rhizosphere bacterial community. To do so, we used a 16S rDNA-based Illumina MiSeq platform to compare bacterial societies that live in the soils of GM sugar beet versus a non-GM sugar beet control.

2. Materials and methods

2.1. Plant Materials

The sugar beet line (including 211S3, 219S3, 228S3, and 231S6 cultivars) in this study prevents

the production of CRP2 virus transcription protein at the transcriptional level (mRNA) using the gene silencing method. This mRNA is degraded by the plant's defense mechanism as soon as the gene encoding the virus coat protein is transcribed, thus inhibiting the activity of the virus. Using the plant's own defense mechanism, this type of transgenic does not lead to the production of protein or any new compound, resulting in minimal interference with the plant genome and the environment (Zare et al., 2015).

2.2. Field Design and Sampling

The sugar beet plants were planted in a randomized block design in an experimental field in Shiraz, Iran, in May 2015. Three replicate plots were used for each sugar beet cultivar or line, and they were randomly scattered around the field. The soil type in the area was meadow soil, which has roughly 15% water content, 26.7 g/kg organic matter, 0.12 percent total nitrogen, NH4+-N 3.52 mg/kg, NO3-N 2.33 mg/kg, available P 56.4 mg/kg, available K 128.8 mg/kg, and pH 6.20–6.30. Plant and rhizosphere soil samples were gathered throughout a year of cultivation. Plant samples and soil from the rhizosphere were collected at the end of the beet growing season. Briefly, each cultivar had three sample points, and two sugar beet plants with adhering soil were dug out from each site and transported to the lab as quickly as feasible. Then, rhizosphere soil samples from all six sites were collected by brushing the soil adhering to the plant's root surface and combining it in one biological replicate. Finally, after repeating this three times, the biological duplicates of rhizosphere soils were frozen at −80 degrees Celsius.

2.3. DNA Extraction from Rhizosphere Soil Samples

DNA was extricated in duplicate from roughly 20.60 g of the soil from each biological replicate in this investigation, using the Power Soil DNA Isolation Kit (MoBio Laboratories Inc., USA). Electrophoresis on a 1% agarose gel was used to evaluate the DNA integrity. The DNA samples were kept frozen at −20°C.

2.4. PCR Amplification of 16S rDNA and Illumina MiSeq Sequencing

Gene-specific primers for amplifying the V3 region of 16S rDNA were 515F (5'GMGCCAGCMGCCGCGGMAA-3') and 806R (5'-GGACTACHV GGGMWTCTAAT-3') [10, 20]. High throughput sequencing of the qualified libraries was conducted by Microgen Tech Solutions Co., Ltd (Korea) using the Illumina MiSeq platform (Illumina, USA) and MiSeq Reagent Kit. A total of 7,015,054 single reads (16S rDNAs (V3 Region)-Based Illumina MiSeq) were reported by the Microgen company. The reads were deposited and are available in the NCBI with Bio Project ID: PRJNA825276 and Bio Sample accessions: SAMN27507525, SAMN27507526, SAMN27507527, SAMN27507528, SAMN27507529, SAMN27507530, SAMN27507531, SAMN27507532, SAMN27507533, SAMN27507534. SAMN27507535, SAMN27507536, SAMN27507537, SAMN27507538, SAMN27507539.Operational Taxonomic Units (OTU) Selection and Analysis of Species Composition

The raw data was filtered to remove reads with sequencing adapters, ambiguous N bases, and average base quality scores less than 0.05 to obtain clean reads. Afterward, paired-end clean reads with overlap were merged to tags utilizing Fast Length Adjustment of Short reads. The read length was set at 400 pb. The Taxonomically identified OTU representative sequences classified by the Green genes database (green genes v13 5 97 percent) with a 97 percent confidence value were considered a cutoff. Finally, a profiling histogram was created using CLC software to summarize The OTU number of each taxonomic rank in the different samples.

2.5. Alpha and Beta Diversity Analysis

The diversity of alpha indicates the abundance of a species within a population (α). Among the alpha diversity indices, the Cho1 and ACE indices show the species richness, considering the number of species present in a level or a specific sample, regardless of the number of individuals studied in each species. The Shannon and Simpson indices account for the incidence of species and express the relative frequency of taxis. Beta diversity indicates the degree of similarity or difference of ecosystems in terms of species. There are several methods for determining beta diversity, the most

important of which are similarity indices (between regions). Distance matrices, Jacquard, Euclidean Unweighted UniFrac, Weighted UniFrac, Weighted UniFrac not normalized, D-0 UniFrac, and D 0.5 UniFrac are types of computational methods for checking the similarities or differences between samples. This study used CLC to calculate the alpha diversity indices, including the observed OTU number, Chao 1, abundance coverage-based estimator (ACE), Shannon, and Simpson. In addition, the associated rarefaction curve and PCA (principal component analysis) of the OUT were drawn. Variance analysis was executed, and the value of VIF (Variance Inflation Factor) from the Analysis of Variance Tables was reported for all alpha diversity models using CLC software. CLC calculated beta diversity distances, including Bray-Curtis, Euclidean, and unweighted UniFrac, were based on the "OTU table". The Differential abundance was a by CLC, and the taxonomic composition of the rhizosphere soil was determined at the phylum level.

2.6. Statistical Analysis of Similarities

Statistical analysis of similarities according to the Bray-Curtis, Euclidean, and Unweighted UniFrac distance was executed by CLC. In addition, analysis of molecular variance (PERMANVA) and ANOVA were calculated using CLC. The Benjamini-Hochberg false discovery rate correction was applied to the obtained p-value to determine the differences in microbial community abundance between samples.

3. Results and Discussion

3.1. Analysis of 16S rDNA (V3 Region)-Based Illumina MiSeq Data

The Microgen Company reported 7,015,054 single reads (16S rDNA (V3 Region)-Based Illumina MiSeq). Of these, 3,053,331 paired reads remained after trimming, merging, and fixing length to 400 bp (CLC software). The average number of paired reads per sample was 202,555 ± 59,228 bp (Table 1). A total of 3,668 OTUs were identified based on the input database size.

Table 1. Average of paired reads per sample (n=3) Standard Deviation

sample	Number of read± SD	Number of OTU± SD
211S3	166904.3±11556.19	403.6667±114.5484
219S3	167277.3±7943.135	435±164.4353976
control	261153±83618.25	1086.667±454.6541
28S3	218839.3±82732.86	617±
S6	203603±	449.3333±

3.2. Alpha Diversity of Bacterial Community in Rhizosphere Soils

It is critical to sequence at an appropriate depth to recover all of a sample's microbial diversity. Therefore, a rarefaction study was conducted to see if the sequencing coverage was adequate (Fig. 1). The rarefaction curves for the OTUs showed that as the sequencing profundity expanded, the number of species observed increased (Fig. 1). In this analysis, the database coverage rate of each sample was close to 97 percent, meaning that of the bacteria within the samples were identified. The rarefaction curves in Fig. 1 suggest that the sequencing was sufficient to achieve the bacterial societies diversity in the samples and covered identifiable species within the bacterial community. The alpha diversity (or diversity within a sample) was computed to determine the GM sugar beet's impact on the soil.

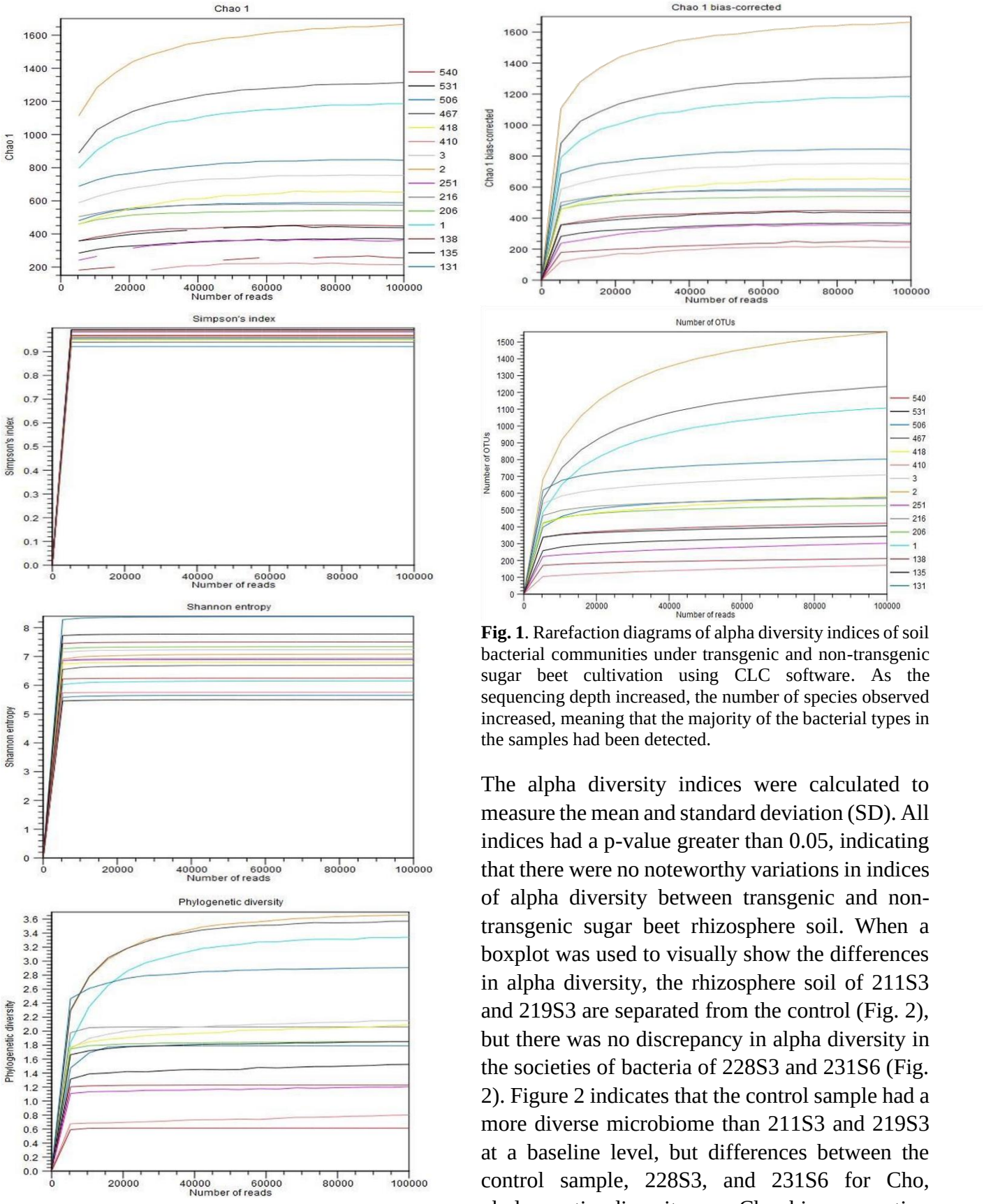
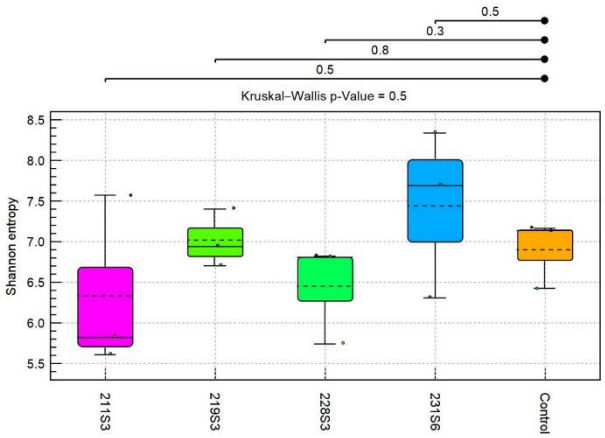
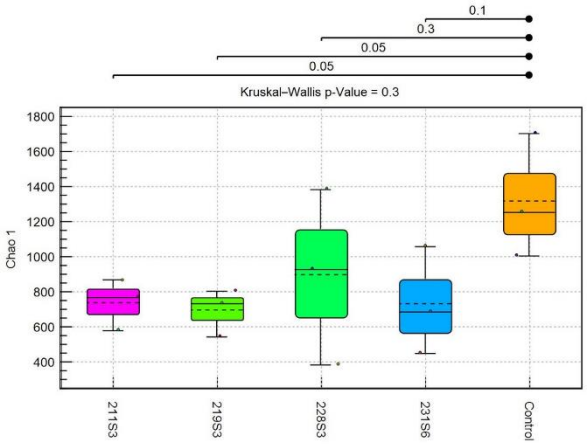


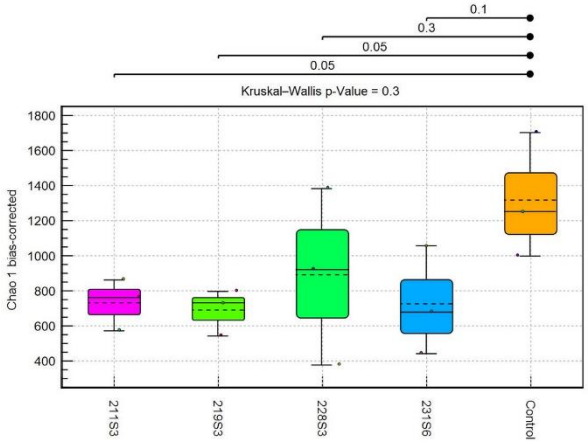
Fig. 1. Rarefaction diagrams of alpha diversity indices of soil bacterial communities under transgenic and non-transgenic sugar beet cultivation using CLC software. As the sequencing depth increased, the number of species observed increased, meaning that the majority of the bacterial types in the samples had been detected.

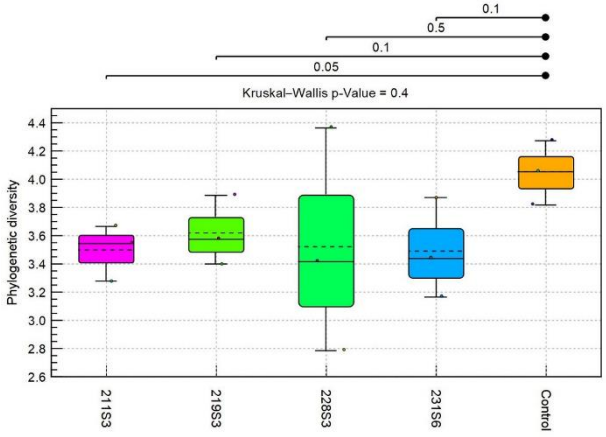
The alpha diversity indices were calculated to measure the mean and standard deviation (SD). All indices had a p-value greater than 0.05, indicating that there were no noteworthy variations in indices of alpha diversity between transgenic and non-transgenic sugar beet rhizosphere soil. When a boxplot was used to visually show the differences in alpha diversity, the rhizosphere soil of 211S3 and 219S3 are separated from the control (Fig. 2), but there was no discrepancy in alpha diversity in the societies of bacteria of 228S3 and 231S6 (Fig. 2). Figure 2 indicates that the control sample had a more diverse microbiome than 211S3 and 219S3 at a baseline level, but differences between the control sample, 228S3, and 231S6 for Cho, phylogenetic diversity, or Cho bias correction groups were insignificant. These findings revealed

that the bacterial community's species richness and evenness had decreased in the two GM sugar beets rhizospheres. Shannon and Simson's diversity indices showed no major differences across all samples, but in Simson's diversity indices, the rhizosphere soil of 231S6 was separated from the control. The alpha diversity results indicated growing transgenic sugar beet did not alter the taxonomic diversity of the soil bacterial microbiome. Analysis of variance was performed, and the value of VIF (Variance Inflation Factor) from the analysis of variance tables for all alpha diversity models was equal to 1.60 in all cultivars. This VIF value indicates the absence of alignment. As a result, we determined that this experiment (transgenic sugar beet cultivation) had no significant impact on alpha diversity indices of rhizosphere soil bacterial population according to ANOVA tables, and the experimental hypothesis in this regard is rejected.









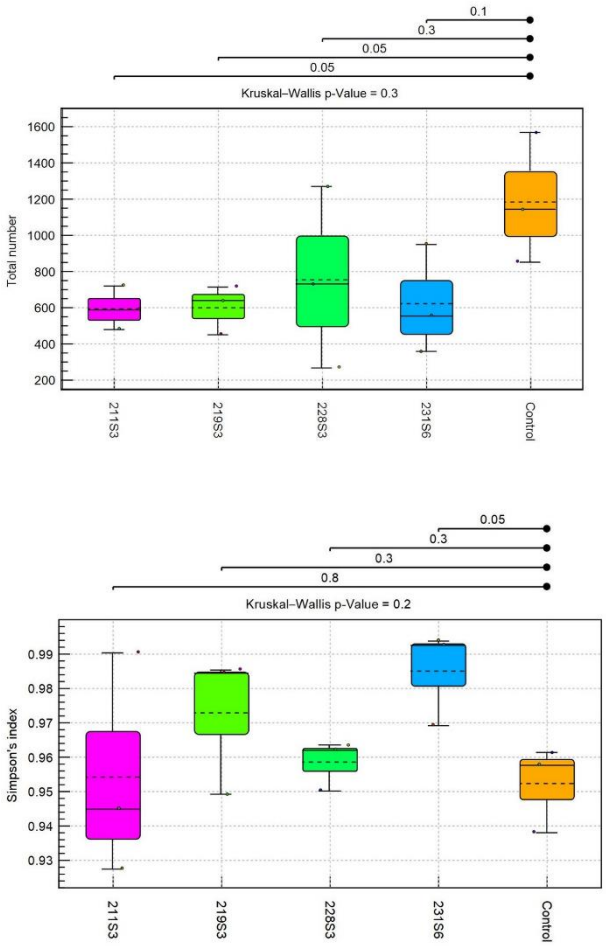


Fig. 2. Box plot diagram of alpha diversity indices, including Cho1, phylogenetic diversity, Cho1 bias correction, Shannon, Simpson, and Number of OUT indexes, under cultivation of transgenic and non-transgenic sugar beet. The transgenic cultivars 211S3 and 219 S3 showed a lower average than the control sample in the Cho, phylogenetic diversity, Cho1 bias correction, and the number of OTUs indexes. However, in the Simpsons index, the transgenic cultivar 231S6 showed a significantly higher mean than the control sample.

3.3. Beta Diversity of Bacterial Community in Rhizosphere Soils

Beta diversity compares the microbiological community composition between samples and groups based on their compositional similarity. Beta diversity analysis was performed to determine the change in the microbiome composition in different samples. In this study, the beta diversity analysis was performed using the Bray-Curtis, Euclidean, and Unweighted UniFrac distance Matrices. The PCoA results revealed that the bacterial population composition was identical in all cultivars. PCo1 and PCo2 explained 13% and 18% of the variation in the society of bacteria based on the Bray-Curtis distance, and 21% and 32% based on the Unweighted UniFrac distance. The PCoA diagram based on the Bray-Curtis distance shows that all 211S3, 219S3, 228S3, and 231S6 cultivars were placed in one group. Beta phylogenetic diversity analysis on the soil samples based on the Euclidean distance was not significant, and the P-value of the PERMANOVA table was about 0.09. In the Unweighted UniFrac algorithm, the OTU composition in the rhizosphere soil of transgenic and non-transgenic sugar beet was 32% (Fig. 3). Analysis of beta variability diagrams of the soil samples based on Unweighted UniFrac distance showed that the 219S3 and 231S6 cultivars were classified in one group, and the 211S3 and 228S3 cultivars were classified in one group.

The bacterial populations of the rhizosphere soil were used to construct a phylogenetic tree at the genus level. Phylogenetic beta diversity analyses were conducted using the unweighted UniFrac. The variance coordinate analysis (PCoA) did not separate the transgenic rhizosphere soil from the control (Fig. 3). The PERMANOVA, based on the Bray-Curtis, Euclidean, and Unweighted distances, showed that while the transgenic sugar beet rhizosphere soil was different from that of the control in PCoA, the distinction was not statistically significant. So, PERMANOVA research revealed that the samples' bacterial compositions were similar. We then used PCoA analysis based on OTU relative abundance profiles to demonstrate the similarity of different samples. The results showed no statistically significant variations in societies of bacteria between transgenic and non-transgenic sugar beets.

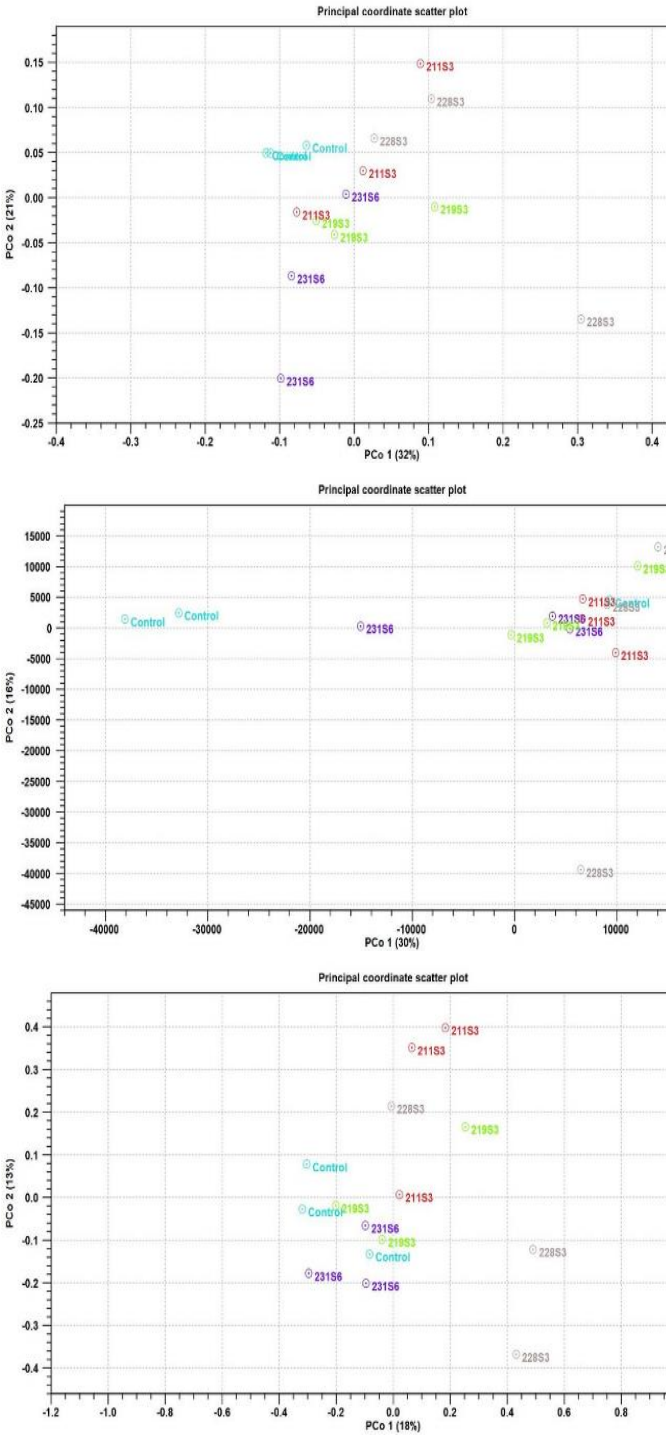


Fig 3. Beta biodiversity in the form of a PCoA diagram based on the Bray-Curtis, Euclidean, and Unweighted UniFrac distance Matrices.

3.4. DIFFERENTIAL ABUNDANCE ANALYSIS

3.4.1. Comparison of the Major Bacterial Phyla in the Rhizosphere Soil

Figure 4 demonstrates the taxonomic compound of the soil at the phylum level. The most abundant phylum in the rhizosphere of GM sugar beets and wild parents was *Proteobacteria*. *Proteobacteria* and *Actinobacteria* were also essential components of the rhizosphere's core microbiome. Following *Proteobacteria*, *Actinobacteria* was the most common bacteria in the control group. *Firmicutes*, *Bacteroidetes*, *Gemmatimonadetes*, *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *TM7*, and *Verrucomicrobia* were the next largest phyla,

followed by *Bacteroidetes*, *Gemmatimonadetes*, *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *TM7*, and *Verrucomicrobia* (Fig. 4).

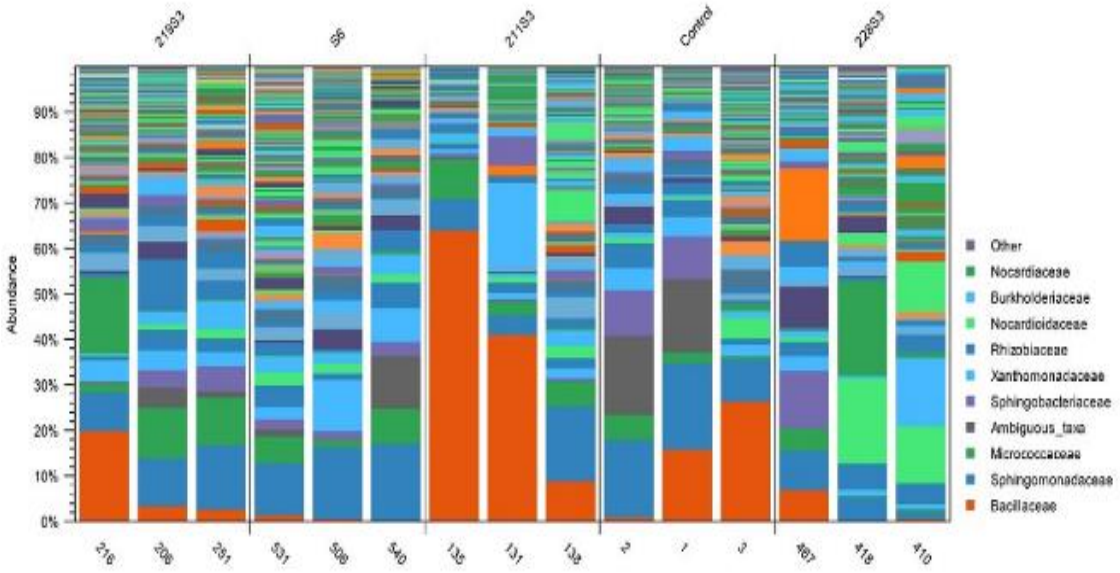


Fig 4. Bar chart (A) and Area chart (B) of the relative abundance of taxis at the genus level.

3.4.2. Comparison of the Main Bacterial Genera in the Rhizosphere Soil

In the rhizosphere soil of the GM sugar beet and the control, 3670 genera were found. The relative abundances of 954 genera in the GM and control rhizospheres were significantly different (Table 2). *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Gemmatimonadetes*, *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *TM7*, and *Verrucomicrobia* were some of the bacterial phyla related to the GM sugar beet in the current findings. *Cellvibrio*, *Janthinobacterium*, *Arthrobacter*, *Sphingomonas*, *Rhodoplanes*, *Stenotrophomonas*, *Nitrospira*, *Mesorhizobium*, *Bradyrhizobium*, and *Burkholderia*, *Pseudomonas*, and *Lysobacter* decreased in all GM rhizosphere soils (Table 2). Additionally, the relative amounts of *Cellvibrio*, *Luteibacter*, *Dyadobacter*, and *Pseudoxanthomonas* in the rhizosphere of resistance GM decreased by more than 10-fold compared to the control (Table 2).

3.4.3. Comparison of the Composition of the Main Nitrogen-Fixing Bacteria in the Rhizosphere Soil

Rhizobium-Legume symbioses in soil ecosystems are important in terms of the ammonium supply for nitrogen fixation. Among the fifteen families of bacteria that fix nitrogen and coexist with legumes, five genera (*Azorhizobium*, *Methylobacterium*, *Rhizobium*, *Cupriavidus*, and *Microvirga)* were not observed in this study. Among ten other genera reported in this research, the relative abundance of six genera (*Agrobacterium*, *Devosia*, *Mesorhizobium*, *Burkholderia*, and *Bradyrhizobium*) showed a noteworthy reduction in all transgenic beets compared to the control cultivar, and The FDR p-value of these six genera was less than 0.05. Although the abundance of these bacterial genera is low in terms of the significance of the nitrogen cycle, the population of symbiotic bacteria and nitrogen-fixing bacteria were analyzed and fall into the category of soil functional groups. Because the sugar beet is a sucrose-producing plant, molecular nitrogen-fixing bacteria that need more carbon can be present around the roots of this plant. According to Tsurumaru et al. [28], *Mesorhizobium*,

Bradyrhisobium, and *Streptomyces* are the predominant nitrogen-fixing species associated with sugar beets. The relative abundance of some *Agrobacterium* strains was, on average, 158–951 times lower than the control, and the abundance of the *Bradyrhizobiaceae* family was 50 times lower than the control on average (Table 2). Among growth-promoting bacteria, transgenic sugar beet cultivation showed no observable statistically significant effect on the abundance of *Pseudomonas*. However, the abundance of some genera of *Bacillus* and the relative abundance of one genus belonging to the order *4391287 Burkholderiales* showed a 88–502 and 145-fold decrease, respectively. The relative abundance of other nitrogen-fixing bacteria, such as *Stenotrophomonas 3330580* (as a plant growth stimulant), was 1200 times lower in the transgenic rhizosphere soil compared to its control (Table 2). Also, the relative abundance of 139278 *Achromobacter* in the rhizosphere of transgenic beet decreased 347 times in all samples of transgenic sugar beet. *Devosia* relative abundance decreased in GM resistance sugar beet compared to the wild parent, and *Mesorhizobium 227344*'s relative abundance decreased in the GM against the control. In GM, the number of *Burkholderiales 4391287* was significantly lower than in the control group. The relative amount of the *Bradyrhizobiaceae* family within the control rhizosphere soil was remarkably higher than the GM rhizosphere soil (Table 2). Among the nitrogen-fixing bacteria, *Stenotrophomonas 3330580* was significantly less abundant in the GM rhizosphere soil, and *Pseudomonas'* relative abundance did not shift significantly (Table 2). The relative abundance of *Achromobacter 139278* significantly decreased in GM beets.

Table 2. Relative abundance of 954 genera that significantly differed between transgenic and non-transgenic sugar beet rhizosphere soils.

Genus	Control vs							
	219S3	211S3	228S3	231S6	219S3	211S3	228S3	231S6
	P-value				Change fold			
Devosia, 959590	0.02	0.03	0.02	0.01	158.53	92.54	220.51	119.48
Legionella, 881104	0.03	0.04	0.04	0.01	105.58	61.92	146.94	79.77
Thermus, 867135	0.01	0.02	0.01	0.00	214.05	33.19	298.36	162.78
Nesterenkonia, 845354	0.03	0.04	0.04	0.01	101.76	59.84	141.66	76.98
Salinimicrobium, 836539	0.02	0.03	0.03	0.01	131.13	76.85	182.48	99.03
Devosia, 818484	0.01	0.02	0.02	0.00	263.38	153.19	366.21	198.14
Hymenobacter, 782955	0.02	0.03	0.02	0.01	158.53	92.54	220.51	119.48
Gemmata, 591358	0.00	0.00	0.00	0.00	1286.52	754.12	1790.36	971.69
Devosia, 573013	0.01	0.01	0.01	0.00	322.54	46.40	449.28	244.59
Devosia, 570988	0.01	0.01	0.01	0.00	432.52	250.16	600.99	324.42
Bacillus, 548055	0.03	0.04	0.04	0.01	94.42	55.58	131.46	71.47
Amycolatopsis, 544457	0.00	0.00	0.00	0.01	706.33	417.62	30.27	535.76
Azohydromonas, 536678	0.01	0.02	0.02	0.01	197.25	115.06	274.34	148.61
Bdellovibrio, 525171	0.00	0.00	0.00	0.00	1246.91	717.46	1731.58	932.63
Luteolibacter, 512867	0.01	0.01	0.01	0.00	307.48	178.58	427.45	231.14
Pedobacter, 465948	0.01	0.01	0.01	0.00	329.53	191.27	458.07	247.63
Kaistobacter, 367995	0.00	0.00	0.00	0.00	1217.18	713.05	1693.75	919.05
Lactobacillus, 333178	0.01	0.01	0.01	0.00	371.25	215.53	516.08	279.02
Planctomyces, 328826	0.03	0.04	0.04	0.01	94.96	55.77	132.18	71.79

Rhodoplanes, 319907	0.01	0.02	0.02	0.00	197.69	117.26	275.49	150.18
Agrobacterium, 306515	0.00	0.01	0.00	0.00	543.78	77.09	757.17	411.70
Mycetocola, 288552	0.01	0.01	0.01	0.00	345.17	204.45	480.93	262.04
Novosphingobium, 281742	0.00	0.00	0.02	0.00	855.60	31.73	330.80	87.51
Bacillus, 278426	0.00	0.01	0.00	0.00	809.14	466.91	1124.01	606.15
Bacillus, 256974	0.02	0.03	0.03	0.01	112.01	66.56	156.13	21.80
Devosia, 255206	0.00	0.01	0.03	0.00	590.22	21.84	820.92	444.70
Rhodoplanes, 253167	0.01	0.02	0.02	0.01	204.59	119.30	284.55	154.12
Caulobacter, 248395	0.01	0.02	0.02	0.01	175.25	104.14	244.27	133.25
Kribbella, 247758	0.01	0.02	0.02	0.01	204.59	119.30	284.55	154.12
Paenibacillus, 241963	0.00	0.00	0.00	0.00	918.59	529.61	1275.93	687.82
Sporichthya, 237887	0.02	0.02	0.02	0.01	175.20	102.33	243.72	132.09
HTCC, 235311	0.03	0.04	0.04	0.01	116.44	68.35	162.07	88.01
Chryseobacterium, 229949	0.01	0.01	0.01	0.00	356.05	209.39	495.68	269.36
Mesorhizobium, 227344	0.01	0.01	0.01	0.00	495.64	286.37	688.62	371.55
Cellvibrio, 225453	0.00	0.00	0.00	0.00	933.19	537.97	1296.19	698.70
Leptospirillum, 221094	0.00	0.01	0.01	0.00	632.34	364.71	878.37	473.58
Flavobacterium, 219727	0.03	0.04	0.04	0.01	105.58	61.92	146.94	79.77
Lutibacterium, 219175	0.02	0.02	0.02	0.01	182.55	106.58	253.93	137.60
Paenibacillus, 172955	0.01	0.02	0.02	0.00	239.88	139.83	333.62	180.67
Enterobacter, 164915	0.00	0.00	0.00	0.00	703.79	414.14	979.86	532.59
Pontibacter, 153137	0.00	0.00	0.00	0.00	1486.19	887.96	2072.83	1132.87
Lysobacter, 142320	0.01	0.02	0.01	0.00	213.28	126.22	297.14	161.85
Achromobacter, 139278	0.01	0.01	0.01	0.00	373.62	216.62	519.30	280.61
Clostridium, 137816	0.00	0.00	0.00	0.00	1543.46	891.77	2144.40	1157.04
Limnohabitans, 90543	0.01	0.02	0.02	0.01	211.33	122.99	293.86	159.03
Marinococcus, 14475	0.01	0.00	0.01	0.00	525.49	296.52	728.14	377.15
Pimelobacter, 12798	0.00	0.00	0.00	0.00	2005.49	1150.65	2784.11	1497.62
Opitutus, 1559	0.00	0.00	0.00	0.00	2343.10	110.63	3250.72	1744.11
Pseudoxanthomonas, 1115104	0.01	0.01	0.01	0.00	398.09	235.02	554.45	301.73
Gemmata, 1719556	0.01	0.02	0.02	0.01	204.59	119.30	284.55	154.12
Sphingobacterium, 1977254	0.00	0.00	0.00	0.00	1101.05	633.29	1528.95	823.35
Streptomyces, 2115777	0.03	0.04	0.04	0.01	88.44	53.12	123.43	67.58
Pontibacter, 2187047	0.03	0.04	0.04	0.01	109.10	64.10	151.87	82.50
Dyadobacter, 915265	0.01	0.02	0.02	0.00	248.69	144.72	345.79	187.14
Flavisolibacter, 888623	0.01	0.02	0.02	0.01	210.69	122.98	293.07	158.79
Gemmata, 833489	0.01	0.02	0.01	0.00	285.43	165.89	396.83	214.64
Agrobacterium, 1105689	0.00	0.00	0.00	0.00	1015.92	601.70	1415.48	771.22
Sphingopyxis, 722895	0.01	0.02	0.02	0.01	211.33	122.99	293.86	159.03
Niabella, 2880504	0.02	0.03	0.03	0.01	145.82	85.35	202.89	110.06
Bacillus, 898949	0.00	0.01	0.00	0.00	658.83	386.41	916.91	47.25

Planctomyces, 2951283	0.02	0.02	0.02	0.01	169.10	98.64	235.19	127.40
Gemmata, 3163659	0.00	0.00	0.00	0.00	1159.36	667.40	1610.09	867.37
Rhodoplanes, 1140993	0.02	0.02	0.02	0.01	152.48	90.92	212.61	116.12
Gemmata, 3205243	0.01	0.02	0.02	0.00	233.99	136.25	325.38	176.13
Amycolatopsis, 1130641	0.01	0.01	0.01	0.00	495.33	286.88	688.37	371.80
Sphingobium, 1125832	0.02	0.03	0.03	0.01	104.33	62.48	145.55	79.61
Ammoniphilus, 686219	0.01	0.01	0.01	0.00	410.37	237.74	570.32	308.07
Opitutus, 813557	0.03	0.04	0.04	0.01	94.42	55.58	131.46	71.47
Devosia, 3926111	0.00	0.00	0.00	0.00	1850.74	1060.28	2568.86	1380.91
Agrobacterium, 645742	0.01	0.02	0.02	0.00	192.01	27.69	267.47	145.64
Fluviicola, 4043609	0.01	0.01	0.01	0.00	314.83	182.81	437.65	236.64
Microlunatus, 4110006	0.03	0.04	0.04	0.01	105.58	61.92	146.94	79.77
Arenimonas, 4222822	0.00	0.00	0.00	0.00	1010.73	599.42	1408.46	767.76
Sphingomonas, 1024743	0.00	0.01	0.01	0.00	543.88	320.18	226.32	411.67
Actinomyces, 4300121	0.02	0.03	0.03	0.01	147.96	86.43	205.82	111.55
Pseudonocardia, 4303162	0.00	0.00	0.00	0.00	1282.46	97.46	1785.73	971.01
Flavobacterium, 4308303	0.00	0.03	0.00	0.01	3242.90	152.67	71.88	42.22
Sphingobacterium, 4316539	0.01	0.01	0.01	0.00	516.68	298.43	717.82	387.26
Segetibacter, 4333673	0.01	0.02	0.02	0.00	239.88	139.83	333.62	180.67
Sphingobium, 4336568	0.01	0.01	0.01	0.00	390.43	226.00	542.55	292.97
Flavisolibacter, 4373079	0.01	0.01	0.01	0.00	422.34	244.92	587.03	317.23
Planctomyces, 4378677	0.02	0.03	0.03	0.01	131.13	76.85	182.48	99.03
Aquicella, 4381254	0.01	0.02	0.02	0.00	177.10	104.83	246.74	134.40
Bdellovibrio, 4398570	0.02	0.03	0.03	0.01	123.79	72.60	172.28	93.52
Rhodoplanes, 4402900	0.03	0.04	0.04	0.01	116.44	68.35	162.07	88.01
Flavisolibacter, 4417921	0.01	0.02	0.02	0.01	204.59	119.30	284.55	154.12
Asticcacaulis, 4437030	0.02	0.03	0.03	0.01	126.79	74.19	176.41	95.68
Mycoplana, 4457554	0.01	0.02	0.02	0.01	200.78	116.91	279.20	151.13
Pirellula, 4460790	0.00	0.00	0.00	0.00	1166.07	684.70	1623.06	881.48
Pontibacter, 4468617	0.00	0.00	0.00	0.00	2662.11	1587.12	3711.97	2027.20
Asteroleplasma, 4473685	0.00	0.01	0.01	0.00	692.38	399.98	961.94	518.99
Planctomyces, HWI-M04844	0.00	0.00	0.00	0.00	1245.22	730.16	1732.96	940.66
Aquicella, HWI-M04844	0.00	0.00	0.00	0.00	904.00	521.25	1255.68	676.93
Ardenscatena, HWI-M04844	0.00	0.01	0.00	0.00	801.84	462.73	1113.89	600.70
Flavisolibacter, HWI-M04844	0.00	0.01	0.00	0.00	779.95	450.19	1083.50	584.37
Aquicella, HWI-M04844	0.01	0.01	0.01	0.00	393.15	228.13	546.49	295.40
Sporolactobacillus, HWI-M04844	0.01	0.01	0.01	0.00	444.24	257.51	617.43	333.61
Aquicella, HWI-M04844	0.01	0.01	0.01	0.00	483.86	279.94	672.36	362.99
Adhaeribacter, HWI-M04844:	0.01	0.01	0.01	0.00	568.32	328.79	789.71	426.34
Aquicella, HWI-M04844	0.01	0.01	0.01	0.00	331.09	194.99	461.00	250.65

Nocardioides, HWI-M04844	0.00	0.01	0.01	0.00	506.45	299.14	705.41	383.96
Glaciecola, HWI-M04844	0.01	0.01	0.02	0.00	535.03	37.95	743.21	400.72
Rubricoccus, HWI-M04844	0.01	0.01	0.01	0.00	264.10	156.43	367.97	200.49
Tatlockia, HWI-M04844	0.01	0.02	0.02	0.01	211.94	123.54	294.76	159.62
Pirellula, HWI-M04844	0.02	0.03	0.03	0.01	145.82	85.35	202.89	110.06
Aquicella, HWI-M04844:12	0.01	0.01	0.01	0.00	295.67	171.55	410.98	222.14
Fimbriimonas, HWI-M04844	0.02	0.03	0.03	0.01	131.13	76.85	182.48	99.03
Planctomyces, HWI-M048441	0.02	0.03	0.02	0.01	158.53	92.54	220.51	119.48
Gemmata, HWI-M04844	0.02	0.03	0.02	0.01	167.86	98.09	233.51	126.58
Aquicella, HWI-M04844	0.01	0.01	0.01	0.00	256.82	152.16	357.84	29.87
Chthonomonas, HWI-M04844	0.02	0.03	0.03	0.01	137.38	80.31	191.12	103.62
Opitutus, HWI-M04844	0.01	0.01	0.01	0.00	360.35	211.96	501.67	272.64
Sediminibacterium, HWI-M04844	0.01	0.02	0.01	0.00	242.97	141.22	337.80	182.72
Planctomyces, HWI-M04844	0.03	0.04	0.04	0.01	109.10	64.10	151.87	82.50
Parachlamydia, HWI-M04844	0.03	0.04	0.04	0.01	101.30	59.66	141.05	76.69
Sporichthya, HWI-M04844	0.03	0.04	0.04	0.01	109.10	64.10	151.87	82.50
Pirellula, HWI-M04844	0.03	0.04	0.04	0.01	105.58	61.92	146.94	79.77
Flavobacterium, HWI-M04844	0.02	0.03	0.03	0.01	126.79	74.19	176.41	95.68
Flavobacterium, HWI-M04844	0.02	0.03	0.03	0.01	126.79	74.19	176.41	95.68
Glycomyces, HWI-M04844	0.01	0.01	0.01	0.04	424.77	249.38	15.89	321.08
Flavobacterium, HWI-M04844	0.02	0.03	0.03	0.01	126.79	74.19	176.41	95.68

4. Conclusions

The impact of transgenic sugar beets on the community of the rhizosphere bacteria has seldomly been studied with the Illumina MiSeq technique, although the Illumina MiSeq platform has been used to determine the impact of other cultivars within the rice species or glyphosate on the society of rhizosphere bacteria [8-22]. According to prior studies, the bacterial society of Quest canola, the root-endophytic of EPSPS-GM plants, was noticeably distinct from Excel canola at the mid-flowering growth stage. [27-6]. In this study, reads were deposited and are available in the NCBI with Bio Project ID: PRJNA825276 and Bio Sample accessions: SAMN27507525, SAMN27507526, SAMN27507527, SAMN27507528, SAMN27507529, SAMN27507530, SAMN27507531, SAMN27507532, SAMN27507533, SAMN27507534. SAMN27507535, SAMN27507536, SAMN27507537, SAMN27507538, SAMN27507539. According to the results of this investigation, the cultivation of all transgenic sugar beet did not substantially impact the biodiversity of rhizosphere bacteria. This means that the relative abundance of dominant and rare species under transgenic sugar beet cultivation did not significantly change. Based on the findings of Tsurumaru et al. [28], the most significant branch of sugar beet bacteria was *Proteobacteria*, which agrees with the results in this study on transgenic beets. In another study by Zuo et al. [33], the effect of EPSPS transgenic soybean culture on the ZUTS1 line of alpha diversity of soybean root bacteria was not found to be significant in the three stages of plant development, vegetative growth, flowering, and grain filling. In another study, Zuo et al. [33] found the effect of 5-year cultivation of transgenic poplar Bt line 741 on the Chao1 and Ace richness index and Simpson and Shannon diversity index of soil microbial communities were not significant.

In this study, the β-diversity was analyzed by PCA. Based on beta diversity indices (Bray-Curtis algorithm), the 211S3, 219S3, 228S3, and 231S6

cultivars and the control sample were placed in one cluster, and based on the unweighted unifrac algorithm, the 219S3 and 231S6 cultivars were placed in a group, and 211S3 and 228S3 were classified in a group. Beta diversity analysis on soil samples based on the Unweighted UniFrac distance matrix did not separate transgenic sugar beet rhizosphere soil from the control, showing that other distance matrices were unsuccessful in grouping the samples. Among these methods, the unweighted UniFrac distance pays attention to the rare categories, while the weighted UniFrac distance pays attention to the larger categories. The variance of the Bray-Curtis and Unweighted UniFrac statistical algorithms was 18% and 32%, respectively. Based on the matrix of similarity or beta diversity, the amount of variance in the sample classification was less than the acceptable value [4]. Therefore, it is concluded that the composition of bacterial societies of GM and non-GM sugar beet had high similarity and overlap and the differences were not significant. Furthermore, when an efficient comparison test of transgenic sugar beet and its control soil was made, the relative abundance of specific rhizosphere bacterial phyla and genera were measurably different between the GM transgenic sugar beet and its wild parents. According to the results, some definitive bacterial phyla in the GM sugar beet were *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Gemmatimonadetes*, *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *TM7*, and *Verrucomicrobia.* Other definitive bacterial genera included *Cellvibrio*, *Janthinobacterium*, *Arthrobacter*, *Sphingomonas*, *Rhodoplanes*, *Stenotrophomonas*, *Nitrospira*, *Mesorhizobium*, *Bradyrhizobium*, and *Burkholderia*. According to the results of Tsurumaru et al. [28], *Proteobacteria* was the most significant branch of sugar beet bacteria, which agreed with the results obtained in this study on transgenic beets. In other studies of different bacterial genera in rhizosphere soils, 3670 genera of bacterial strains were identified in the non-GM and GM sugar beet soils of the rhizosphere. The relative abundance of 954 strains with a P value less than 0.05 varied between the rhizosphere soils of transgenic and non-transgenic sugar beets. However, the FDR-P value or FDR-adjusted p-value of many of these taxonomic units became insignificant after calculating the false discovery rate. Different soil sample positions and the effect of environmental conditions are factors that can lead to these differences. Bacteria that fix nitrogen and bacteria that promote plant growth in the rhizosphere soil were among the bacteria that showed considerable abundance differences (Table 2). Legumes are important in rhizobium ecosystem symbioses because they supply ammonia for plant growth by nitrogen fixation [14]. Among the 15 original bacterial genera that fix nitrogen [21], five genera (*Azorhizobium*, *Ensifer*, *Shinella*, *Cupriavidus*, and *Microvirga*) were not found in this study.

In another genera, only *Methylobacterium* and *Rhizobium* were not influenced. Also, GM exerted essential influence on six genera in comparison with with its control cultivar; such as, *Agrobacterium*, *Devosia*, *Mesorhizobium*, *Burkholderia*, and *Bradyrhizobium*. Furthermore, in GM plants the growth of *Ochrobactrum* and *Achromobacter* repressed. Moreover, *Phyllobacterium* vanished in the soils of rhizosphere. *Pseudomonas* with *Bacillus* have been utilized as a model for bacteria related to plant-, and it is involved in plant growth and health [2].

Some differences observed in the abundance of some species could be linked to the plant growth status and the effect of environmental conditions, especially since this experiment was not performed in a greenhouse and the effect of environmental conditions on the changes cannot be ignored. Therefore, it will be necessary to increase the number of repetitions and samples in future studies to reduce the environmental effects. The findings demonstrated that transgenic beet cultivation during the first year of growth will not have a substantial impact on the diversity of the soil microbial community. However, achieving definitive results will require the analysis of more than a thousand samples. Other researchers have indicated significant changes at different stages of development, such as flowering time. Therefore, in addition to preparing many samples, future studies should include different stages of plant development and continue for at least another year to ensure the long-term effects of these plants. The

measurement of soil properties in the rhizosphere, especially soil chemical properties, can help interpret the effects of transgenic plants on soil microbial diversity, so it is recommended. Hence, the comparative investigation of bacterial communities within the rhizosphere of GM sugar beet versus its control cultivar or wild parent at the flowering level needs more study.

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Conflict of Interest

Others have no conflict of interest.

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