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Diversity of microbial communities in the rhizosphere soil of the transgenic (*AtAREB1*) and conventional (BR 16) soybean plants

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ABSTRACT. Rhizosphere soil is one of the most diverse microbial environments worldwide and is recognized as an ecosystem undergoing continuous transformation. Microorganisms inhabiting the rhizosphere soil play diverse roles in maintaining and stabilizing the environment. Consequently, plant-associated habitats serve as a dynamic environment influenced by various microbial composition factors. Genetically modified plants have introduced numerous traits into agriculture to address productivity-limiting factors, such as drought. This study aimed to assess the microbial diversity in the rhizosphere soils of drought-tolerant transgenic (*AtAREB1*) and conventional (cultivar BR 16) soybean plants. Bacterial communities were investigated using a cultivation-independent approach, utilizing pooled samples from the respective soils, followed by pyrosequencing of the 16S rRNA gene. In addition, physicochemical parameters of the soils were evaluated. The results indicated that the microbial diversity in the rhizosphere soil of both transgenic and conventional plants remained unchanged, with similar taxonomic distributions observed in both bacterial communities. Analysis of physicochemical parameters in both soils suggested a potential direct relationship between these properties and the microbial community profile, with implications for soil nutrient content and physical composition. These results are positive for biosafety when using the transgenic cultivar compared with the conventional crop.

Keywords: Soil microorganisms; *Glycine max* L. Merrill; pyrosequencing; water deficit.

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Introduction

The rhizosphere is considered to be one of the most complex ecosystems on Earth, consisting of a narrow zone of nutrient-rich soil that is influenced by plant roots. Diverse microorganisms, including fungi, bacteria, protists, nematodes, and invertebrates, constitute the rhizosphere microbiome (Kumar & Dubey, 2020).

Soil microbial communities play a crucial role in nutrient cycling by mineralizing and decomposing organic materials and releasing nutrients essential for plant growth into the soil. Various processes can influence the availability of the nutrients supplied by these communities. Additionally, soil microorganisms may affect nutrient uptake and plant growth through the release of growth-stimulating or inhibiting substances that affect root physiology and architecture (Faria et al., 2021).

Thus, during coevolution, soil microorganisms have developed strategies to interact with plants, forming important associations for plant development (Faria et al., 2021). The interaction between plants and their environment is a dynamic process in which the root system not only provides anchorage and facilitates nutrient and water uptake but also serves as a crucial interface for plant interaction with its surroundings. Evidence suggests that plants can influence the structure of the soil microbiome by secreting root exudates. Chemical signals emitted by soil microorganisms are received and recognized by plants, prompting the release of chemical compounds in the form of root exudates, which constitute a complex mixture of compounds (Sun et al., 2021). Given the sensitivity of the soil microbiome to small environmental changes or stresses resulting from natural disturbances, it can serve as an indicator of soil quality (Hartmann and Six, 2023). The capacity of root exudates to mediate and sustain the soil microbiome presents opportunities to exploit this mechanism. It is conceivable that engineered or selectively bred plants could cultivate specific soil microbes that are necessary for or beneficial to plant health (Sun et al., 2021).

The soybean crop (*Glycine max* L. Merrill), native to Asia, holds significant global importance, playing a crucial role in the production of various products and by-products in the agribusiness, chemical, and food industries, with considerable potential to address nutritional needs. Soybean seeds are valuable for both protein meal and vegetable oil production (Hartman et al., 2011). Brazil is the world's largest soybean producer, with the 2023/2024 crop estimated at 146 million tons of grains (Companhia Nacional de Abastecimento [CONAB], 2024; United States Department of Agriculture [USDA], 2024). However, this crop faces challenges due to water stress, which severely hampers plant growth and yield, and is likely to be exacerbated by climate change (Shin et al., 2015).

The adoption of genetically modified (GM) crops and their associated benefits has introduced new and promising features to agriculture, with the aim of mitigating the detrimental effects of biotic and abiotic factors, including water deficits. Barbosa et al. (2013) demonstrated that the introduction of the foreign AREB1 (abscisic acid responsive element binding) gene into soybean plants enhanced water deficit tolerance, with GM plants showing more efficient survival than wild-type plants after 5 days of water deprivation. Soybean plants overexpressing the transcription factor *AtAREB1*, identified in *Arabidopsis thaliana* and involved in the abscisic acid (ABA)-dependent stress response pathway, were generated from embryos of the conventional cultivar BR 16, which is known for its sensitivity to drought. The AREB1 gene, a basic domain/leucine zipper transcription factor, binds to the abscisic acid (ABA)-responsive element motif in the promoter region of ABA-inducible genes (Leite et al., 2014), thereby activating expression. GM plants containing the AREB1 gene exhibit normal growth, high survival rates, no leaf damage after water stress, and superior physiological performance under water deficit compared to their genetic background (Barbosa et al., 2013; Leite et al., 2014). Moreover, they also displayed a greater total number of pods and seeds and an increased dry matter content of seeds, indicating an improved capacity to withstand drought with no loss in yield (Marinho et al., 2016).

Genetically modified plants can directly or indirectly influence the structure, function, and diversity of soil and rhizosphere microbial communities, potentially driven by plant genotypes and changes in agricultural management associated with transgenic plant cultivation. The rhizospheric microbiome comprises organisms of interest in studies assessing the environmental impact of transgenic crops (Montanari-Coelho et al., 2018; Turrini et al., 2015). Consequently, the construction and sequencing of 16S rRNA gene clone libraries via Next-Generation Sequencing (NGS) provides an avenue to explore microbial diversity, serving as a method to assess bacterial communities across various habitats, including rhizosphere soil (Garcia et al., 2016).

Therefore, studying the diversity of bacterial species in soils cultivated with transgenic and conventional plants is crucial, given the essential role that these microorganisms play in sustaining this environment. Moreover, this investigation is of significant importance for GMO biosafety. Hence, this study aimed to examine the existing microbiome in rhizosphere soils cultivated with transgenic and conventional soybean plants and to analyze the chemical composition of the soil in this region.

Material and methods

Experimental design

The soybean [*Glycine max* (L.) Merrill] conventional cultivar BR 16 (non-GM plants) and the transgenic event 1Ea2939 (GM plants) (BR 16 cultivar modified with *Areb1* gene from *Arabidopsis thaliana* - 35S: AREB1 construction) were grown in an irrigated area belonging to the experimental field of Embrapa Soybean (23°11' S, 51°11' W), in Londrina, PR, Brazil, during the 2014/15 crop season.

Collection of rhizosphere soil material and bacterial DNA extraction

The rhizosphere soil was collected at the Embrapa Soybean cultivation field on February 2, 2015, on a day with an average temperature of 23.7°C and a relative humidity percentage of 82.4%. During the soybean crop season, these plots were irrigated and not subjected to any type of treatment in addition to natural field conditions. For soil collection, a Dutch auger was used at a depth of 0–15 centimeters from four non-GM plants, and four GM plants randomly chosen, totalizing 8 samples. The samples were packed separately in sterile plastic bags and stored in an insulated box with ice for transport. In the Laboratory of Microbial Biotechnology João Alencar Pamphile (LBIOMIC*), Universidade Estadual de Maringá* (UEM), samples were mixed and sieved to obtain a pool of rhizosphere soil samples corresponding to each soybean cultivar/GM event. Total rhizosphere soil DNA was extracted using the Power Soil DNA Isolation Kit (MO BIO Laboratories,

Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. After extraction, DNAs were sent to the company DSMA (Sustainable Development and Environmental Monitoring), located in Mogi das Cruzes, SP, for pyrosequencing.

Analysis of rhizosphere microbiome via PCR-Pyrosequencing of 16S ribosomal genes

The pool of DNA extracted from the rhizosphere soil was assessed for quantity and quality on agarose gel (1%). Bacterial diversity was evaluated using the 16S rRNA gene, which was amplified using the primers 968F and 1378R. PCR was performed in a final volume of 50 µL containing 1x enzyme buffer, 5 mM Mg Cl_2, 10 mM dNTPs, 0.1 µM of each primer, and 2U Taq DNA Polymerase. The amplification reaction was programmed as follows: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 40 s, and a final extension at 72°C for 10 min. After amplification, the PCR reaction was assessed using agarose gel electrophoresis (1%).

The PCR products were purified using a purification kit (GFX PCR DNA and Gel Band Purification Kit, GE Healthcare), according to the manufacturer's instructions. After purification, the purity and quantity of the amplicons were checked on agarose gel (1%) and spectrophotometry (NanoDrop®, Wilmington, USA). The products of each PCR reaction resulting from each treatment were mixed proportionally in agreement with the quantification and sequenced in high yield in GS-FLX Titanium (Roche Applied Science®) by pyrosequencing.

Pyrosequencing analysis and data processing

First, DNA sequences were selected based on the quality and size of the bases using the Pipeline Initial Process tool available on the Ribosomal Database Project (RDP) pipeline. The selection criteria were quality bases, fragment size, identified primer sequences, and unidentified bases. In order to obtain a set of sequences with satisfactory quality for analysis, only base sequences with scores greater than 20 and bases with a maximum probability of 1% error were used. Sequences with unidentified bases and those smaller than 380 bp were excluded. There was a 20% tolerance range for primer similarity (without adapter sequences). Thus, the bases of adapters were cut off in the region flanked by the primers, eliminating the risk of truncated sequences reducing the quality and interfering with the classification of the sequences.

Sequences were classified using the Mothur software (v. 1.34.4), based on the RDP database, where there are gene sequences 16S rRNA of bacteria tested and evaluated for quality and the identification of microbial isolates. The search parameters were based on similarity, where the higher the similarity, the lower the taxonomic level identified.

Estimate of richness and diversity index

The level of alpha diversity (diversity estimator Chao-1, diversity index Shannon-Wiener, and Simpson) in the samples was calculated using the PAST software.

Analysis of the physicochemical composition of the rhizosphere associated with the plant

Analyses of the physicochemical composition of the rhizosphere soil sampled from cultivated plots with GM and non-GM plants (pH, organic matter, macro and micronutrients) were outsourced and were performed by the company Laborfort Chemical Analysis, located in Cambira, PR.

Results

Analysis of the diversity of bacterial communities of rhizosphere soil

Pyrosequencing yielded 27,510 bacterial 16S rRNA sequences from the rhizosphere soil of GM plants and 15,432 sequences from the rhizosphere soil of non-GM plants. After screening using the RDP Pipeline, 8,829 sequences were obtained from the GM plant rhizosphere soil sample, and 4,082 sequences were obtained from the non-GM plants rhizosphere soil sample.

Based on the total number of sequences obtained, approximately 496 and 377 operational taxonomic units (OTUs) were obtained for the GM and non-GM plants-rhizosphere soil samples, respectively (Figure 1). A rarefaction curve was generated to assess OTU richness at the 95% identity level, corresponding to the gene's taxonomic level.

Observing the midpoint of the rarefaction curve, as from about 4,000 sequences, there was a subtle difference in richness of phylotypes between the two bacterial communities found in rhizosphere soil samples from GM soybean plants (Figure 1). Both samples showed ascending curves but did not reach a plateau (Figure 1).

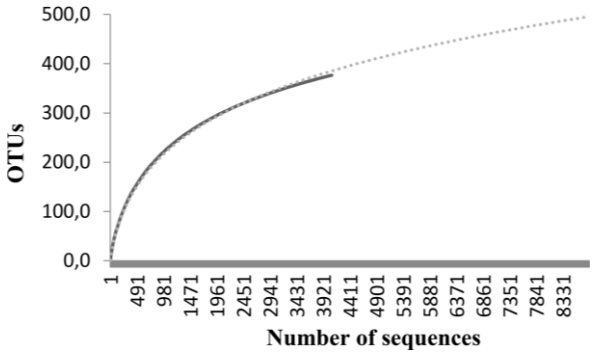

Figure 1. Rarefaction curve indicating the sampling effort at the taxonomic genus level (95%). The continuous line corresponds to soil samples from the conventional soybean cultivar (BR 16), and the dashed line corresponds to rhizosphere soil samples from the transgenic soybean (1Ea2939 GM AREB1).

The values obtained with the Simpson diversity index indicated a diversity of OTUs in the rhizosphere soil samples of GM and non-GM plants (Table 1). Similarly, the Shannon-Wiener index also demonstrated a pronounced similarity between samples (Table 1), reflecting a consistent pattern of OTU diversity across the two groups.

Table 1. Simpson diversity index, Shannon-Wiener diversity, and Chao-1 richness calculated for rhizosphere soil samples of the different soybean cultivars.

Index	GM plants (1Ea2939 AREB1)	Non-GM plants (BR 16)
Simpson	0.9748	0.9747
Shannon-Wiener	4.647	4.659
Chao-1	653.4	486.1

Regarding the Chao-1 richness index, the values suggested a higher richness of bacteria in the rhizosphere soil of GM plants compared to the rhizosphere soil of non-GM plants, as presented in Table 1.

Analysis of rhizosphere soil from GM soybean plants revealed that Acidobacteria (26.47%), Actinobacteria (24.10%), and Proteobacteria (21.42%) were the predominant phyla. Similarly, analysis of the rhizosphere soil from non-GM plants revealed a similar pattern, with the predominant phyla being Acidobacteria (24.77%), Actinobacteria (28.29%), and Proteobacteria (20.90%) (Table 2). Other phyla accounted for less than 1% of the total sequences obtained from both samples (Table 2).

Table 2. Taxonomic distribution of the microbiota associated with rhizosphere soil samples from soybean transgenic plants (1Ea2939 AREB1) and conventional soybean plants (BR16) at the phylum level.

Phyla	GM plants (1Ea2939 AREB1)		Non-GM plants (BR 16)	
	Number of sequences	(%)	Number of sequences	(%)
Actinobacteria	2128	24.1	1155	28.29
Acidobacteria	2337	26.47	1011	24.77
Proteobacteria	1891	21.42	853	20.9
Gemmatimonadetes	556	6.3	279	6.83
Chloroflexi	685	7.76	267	6.54
Verrucomicrobia	602	6.82	230	5.63
Nitrospira	229	2.59	119	2.92
Firmicutes	127	1.44	61	1.49
Not classified	77	0.87	23	0.56
Bacteroidetes	31	0.35	19	0.47
Chlamydiae	13	0.15	15	0.37
Planctomycetes	35	0.4	11	0.27
Synergistetes	38	0.43	10	0.24
Spirochaetes	18	0.2	8	0.2
Deinococcus-Thermus	14	0.16	5	0.12
Thermodesulfobacteria	7	0.08	5	0.12
Aquificae	14	0.16	4	0.1
Chlorobi	10	0.11	3	0.07
Thermotogae	3	0.03	2	0.05
Deferribacteres	8	0.09	1	0.02
Elusimicrobia	2	0.02	1	0.02
Chloroplasts	3	0.03	0	0
Armatimonadetes	1	0.01	0	0
TOTAL	8829	100	4082	100

The microbiota of the rhizosphere soil of GM plants exhibited the following orders of Actinobacteria: Actinomycetales (16.04%), Solirubrobacterales (5.26%), and Acidimicrobiales (1.99%). Acidobacteria accounted for 21.64% of the sequences classified at the phylum level, with 2.21% classified under the order Bryobacter. Within the phylum Proteobacteria, the orders Rhizobiales (5.05%), Myxococcales (2.54%), and Xanthomonadales (1.88%) were prevalent (Table 3). Similarly, Actinobacteria from the non-GM cultivar were of the orders Actinomycetales (20.14%), Solirubrobacterales (5.12%), and Acidimicrobiales (2.06%). Acidobacteria accounted for 19.60% of the sequences classified only at the phylum level, with 3.33% classified under the order Bryobacter. Proteobacteria belonged to the orders Rhizobiales (4.41%), Myxococcales (2.84%), and Xanthomonadales (2.60%). Other orders had proportions of less than 1% of the total sequences obtained in both samples (Table 3).

Table 3. Taxonomic distribution of the microbiota associated with rhizosphere soil samples from soybean transgenic plants (1Ea2939 AREB1) and conventional soybean plants (BR16) at the order level.

Orders	Transgenic event 1Ea2939 (AREB1)		Conventional soybean cultivar (BR 16)	
	Number of sequences	%	Number of sequences	%
Acidimicrobiales	176	1.99%	84	2.06%
Actinomycetales	1416	16.04%	822	20.14%
Anaerolineales	260	2.94%	104	2.55%
Bryobacter	195	2.21%	136	3.33%
Chromatiales	117	1.33%	51	1.25%
Desulfuromonadales	80	0.91%	49	1.20%
Edaphobacter	161	1.82%	50	1.22%
Gemmatimonadales	556	6.30%	279	6.83%
Acidobacteria *	1911	21.64%	800	19.60%
Ktedonobacterales	116	1.31%	33	0.81%
Myxococcales	224	2.54%	116	2.84%
Nitrospirales	229	2.59%	119	2.92%
Rhizobiales	446	5.05%	180	4.41%
Rhodospirillales	273	3.09%	78	1.91%
Solirubrobacterales	464	5.26%	209	5.12%
Spartobacteria	342	3.87%	119	2.92%
Sphaerobacterales	95	1.08%	29	0.71%
Verrucomicrobia*	141	1.60%	56	1.37%
Syntrophobacterales	129	1.46%	56	1.37%
Thermomicrobiales	113	1.28%	53	1.30%
Xanthomonadales	166	1.88%	106	2.60%
Other orders with frequency <1%	1219	13.81%	553	13.55%
TOTAL	8829	100.00%	4082	100.00%

Physicochemical composition of the rhizosphere

Analysis of the physicochemical properties of rhizosphere soils sampled from plots cultivated with GM and non-GM plants revealed pH values ranging from 6.27 to 6.32, respectively (Table 4). Both soils were classified as Type 3, which is characterized as clay soil. This classification is supported by the lower proportion of sand particles and higher proportions of clay, which enhance the soil water-holding capacity (Table 4). Table 4 presents the presence and quantity of organic matter, macro- and micronutrients in these soils. Notable differences in macronutrients were observed, particularly in phosphorus; the soil of non-GM plants exhibited higher levels of phosphorus. Regarding micronutrients, the soil of non-GM plants had higher levels of zinc, manganese, boron, and organic matter content compared to the soil of GM. The manganese content in the soil of non-GM plants was 20 mg dm^3 higher than in the soil of GM.

Table 4. Values of pH, macro and micronutrients in the rhizosphere soils of the GM plants (1Ea2939 (AREB1)) and the non-GM plants (BR 16).

Parameter	GM plants	non-GM plants
pH SMP*	6.27	6.32
Macronutrients (mg dm^3)		
Phosphorus (P)	18.35	23.14
Potassium (K)	0.54	0.51
Calcium (Ca)	5.69	5.95
Magnesium (Mg)	2.19	2.06

Micronutrients (mg dm^3)		
Zinc (Zn)	3.33	4.42
Iron (Fe)	25.85	22.36
Manganese (Mn)	174.17	194.39
Boron (B)	0.56	0.58
Copper (Cu)	19.88	19.62
Organic matter (%)	2.44	2.64

* Method Shoemaker, Mac Lean and Pratt.

Discussion

The soil microbial community is considered to be fundamental to all terrestrial ecosystems because of its ecological functions and interactions with vegetation. Understanding this community is essential for determining the plant carrying capacity. In addition, introducing microorganisms that supply essential nutrients to plants can improve the growth and development of plant communities.

Through analysis of 16S rRNA clone libraries using pyrosequencing, the bacterial diversity present in rhizosphere soil samples from GM and non-GM plants was identified. Major phylogenetic groups, including Acidobacteria, Actinobacteria, and Proteobacteria, were found in both samples. These phyla are commonly found in soils across various terrestrial ecosystems and exhibit similar bacterial communities, although group levels vary largely due to soil pH values (Chaparro et al., 2012).

Montanari-Coelho et al. (2018) described the endophytic microbiome associated with the leaves of the same plant cultivars (GM soybean 1Ea2939 *AREB1* event and BR 16 conventional cultivar). They noted minimal differences in the microbial communities. In this niche, Proteobacteria, Bacteroidetes, and Firmicutes were identified as the dominant endophytic bacterial groups, in contrast to rhizospheric soil, where Acidobacteria and Actinobacteria were the predominant microbial groups. This variation reflects the capacity of the host plant to selectively modulate its microbial architecture, resulting in distinct interactions between the plant and its microbiome (Verma et al., 2021). The microbial diversity of endophytic microbiota is expected to be lower and more selective than that in other niches, such as epiphytic and rhizospheric habitats, because the level of control within plant tissues is higher than that observed in soil (Trivedi et al., 2020; Bashir et al., 2022; Sohrabi et al., 2023). However, it is inappropriate to compare the diversity index obtained in this study with the data of Montanari-Coelho et al. (2018) because of differences in NGS techniques. For example, this study employed pyrosequencing, resulting in 4,000–8,000 reads, whereas another study used the Illumina platform, yielding 80,000–90,000 reads. Therefore, comparisons between the two studies may lead to misinterpretation of the results.

An analysis method utilizing rarefaction curves is considered excellent for comparing microbial community types, where a community with a higher curve is deemed the most diverse (Hughes et al., 2001). In this analysis, the diversity and richness indices of the microbial communities in the samples (GM and non-GM soybean) showed no significant changes. Using the Shannon-Wiener diversity index to determine the most diverse bacterial community and the Simpson index, which assigns values from 0 to 1 to each community (with values closer to 0 indicating greater diversity), we demonstrated that rhizosphere soil samples from GM and non-GM soybean cultivar exhibited similar bacterial diversity.

The nonparametric Chao-1 method was used to assess microbial diversity. According to this evaluation, the values indicated greater bacterial richness in the rhizosphere soil of GM soybean plants than in the rhizosphere soil of non-GM plants. However, nonparametric methods such as Chao-1 can be influenced by sample size.

Furthermore, genetic alterations may affect soil microbiota because communities associated with genetically modified plants may differ from those present in wild plants (Stuart et al., 2010). However, given the close similarity of the diversity index values obtained in this study, the increase in microbial richness found in the rhizosphere soil of GM soybean plants is unlikely to be solely attributed to genetic modifications in these plants.

The phylum Actinobacteria was predominant in the rhizosphere soil samples of GM and non-GM soybean plants. Actinobacteria are among the largest taxonomic units (Stackebrandt & Schumann, 2006) and are widely distributed in aquatic and terrestrial environments. Actinobacteria are Gram-positive bacteria with a wide variety of forms. While the characteristics of this phylum are still being studied, it is known that these bacteria require iron for development and are often found in arid and acidic soils (Yan et al., 2021).

The phylum Acidobacteria was the second most prevalent phylum in the analyzed rhizosphere soil samples. Acidobacteria are widely distributed in various environments; however, their cultivation and

maintenance in the laboratory are challenging (Eichorst et al., 2011). Acidobacteria are acid-tolerant microorganisms that are an important component of soil microbes. They play crucial roles in soil ecology by regulating biogeochemical cycles, decomposing complex polymers, secreting polysaccharides, and promoting plant growth by producing phytohormones, antibiotics, siderophores, and other metabolites that can control plant pests and diseases (Kalam et al., 2020).

The phylum Proteobacteria was abundant in the rhizosphere soil samples of both GM and non-GM plants. This phylum is known among prokaryotes because of its diverse phenotypic characteristics, encompassing a wide range of Gram-negative bacteria, including autotrophic microorganisms, and aerobic and anaerobic heterotrophs. Proteobacteria thrive in systems with varying environmental conditions, owing to their physiological diversity (Andreote et al., 2009).

The phyla Gemmatimonadetes, Chloroflexi, Verrucomicrobia, Nitrospira, and Firmicutes were found in both rhizosphere soil samples, with values of less than 7%. Gemmatimonadetes are widely distributed in soils with neutral pH, but are difficult to cultivate in laboratory settings (DeBruyn et al., 2011). Verrucomicrobia are present in the microbial communities of soil, marine, and freshwater environments (Lee et al., 2009). Nitrospira participates in the nitrogen cycle in aquatic environments through nitrate oxidation, and is also found in soil samples (Dunbar et al., 1999). This genus belongs to a group of microorganisms called complete ammonia oxidizers (Comammox), which can directly oxidize ammonia to nitrate. This makes them important bacteria for the nitrogen biogeochemical cycle (Meng et al., 2023). Firmicutes are microorganisms that have adapted to rapid development in soils with limited moisture (O'Neill et al., 2009).

Understanding how environmental factors influence the microbial community structure and the relationship between microbial taxonomic and functional diversity remains unclear (Prosser, 2007). Dini-Andreote et al. (2010) analyzed soil microbiomes in sugarcane cultivation by comparing soils with and without herbicide application and examining the effects of transgenics on the microbiome. The authors observed the presence of the phyla Proteobacteria (34.7%), Actinobacteria (28.1%), Firmicutes (16.2%), and Acidobacteria (9.7%). These findings are consistent with those obtained in the present study and with the review conducted by Bulgarelli et al. (2013) on the structure and function of the bacterial microbiota in plants. The review emphasized that the taxonomic structure of the phyllosphere, including the rhizosphere and endosphere of roots and leaves, is primarily composed of Actinobacteria (in the rhizosphere and endosphere of roots and leaves), Bacteroidetes and Firmicutes (in the endosphere of roots), and Proteobacteria (in the rhizosphere).

The primary determinants of the soil microbial community structure include organic matter content, pH, nutrients, and various root exudates (Garbeva et al., 2004). These factors can alter the chemical composition of the rhizosphere and consequently affect the soil microbial diversity. Studies have suggested that species richness and diversity are strongly influenced by pH (Lopes et al., 2021). Phyla such as Proteobacteria, Chloroflexi, and Bacteroidetes respond positively to alkaline soil conditions, whereas Actinobacteria, Verrucomicrobia, and Firmicutes tend to thrive in neutral to acidic environments (Lopes et al., 2021).

According to Lauber et al. (2009), soil harbors various bacterial populations, and the composition of soil bacterial communities can vary widely across space. However, our understanding of the specific changes in soil bacterial community structure that occur on larger spatial scales is limited. This is because most studies either surveyed a small number of soils in detail or analyzed a large number of soils using techniques that provided limited information about the phylogenetic structure of bacterial communities. Similar to the approach used in this study, Lauber et al. (2009) used independent cultivation techniques based on pyrosequencing to characterize soil bacterial communities. These authors characterized the microbiome of 88 soils from North and South America and obtained an average of 1,501 sequences per soil. They found that the overall bacterial community composition was significantly correlated with the differences in soil pH ($R^2 = 0.79$). This correlation was primarily driven by changes in the relative abundance of Acidobacteria, Actinobacteria, and Bacteroidetes across a range of soil pH values. Furthermore, soil pH explained a significant portion of the variability associated with the observed changes in phylogenetic structure within each dominant lineage. The overall phylogenetic diversity of the bacterial communities was also related to soil pH ($R^2 = 0.50$), with peak diversity observed in soils with near-neutral pH levels. Taken together, these results suggest that the structure of soil bacterial communities is predictable at higher spatial scales and that the influence of soil pH on bacterial community composition is evident at small taxonomic resolutions.

Phosphorus is the second most important essential inorganic nutrient in the environment after nitrogen due to its structural, functional, and energy transfer roles. It is a limiting factor for plant development and a component of biomolecules such as nucleic acids and ATP (Fernandez et al., 2005).

The phosphorus values observed here were 18.35 mg dm^3 for GM soybean soil samples and 23.14 mg dm^3 for non-GM soil samples. These values are significant and contribute to the stability of the bacteria. This difference in phosphorus concentration could account for the observed differences in certain groups, such as *Nitrospora*, which respond positively to this nutrient in the soil (Zhalnina et al., 2015).

Regarding the organic matter (OM) content, the observed values were 2.44% for the GM rhizosphere soil samples and 2.64% for the non-GM rhizosphere soil samples. Soils with higher OM content tend to have more stable microbial populations because the type and amount of OM influence functional diversity and microbial abundance in soil ecosystems (Grayston et al., 2001).

Micronutrients, such as B, Cu, Fe, Mn, and Zn, are essential for plant growth, although they are required in smaller quantities than macronutrients, such as N, P, K, Ca, Mg, and S. High concentrations of these micronutrients in the soil can reach toxic levels for microorganisms (Lopes et al., 2006). The amounts of these elements found in the analyzed rhizosphere soils were similar for both samples, indicating no significant impact on the existing microbial communities.

Therefore, when comparing soils from the GM and non-GM cultivar, no significant differences in micro- and macronutrients or pH (Table 4) were observed. This confirms our hypothesis that the homogeneity of the bacterial communities in the rhizosphere soil of GM and non-GM plants was similar and that the transgenic event had no adverse impact on microbial environment richness.

Conclusion

Based on our data, we can conclude that transgenic event 1Ea2939 does not affect the rhizospheric microbiota of soybean plants. This finding highlights a positive aspect of GMO biosafety compared to conventional farming, indicating that genome modification does not affect plant-microbiome interactions. The rhizosphere is primarily composed of Acidobacteria, Actinobacteria, and Proteobacteria, with minimal differences observed between them. Furthermore, the diversity index demonstrated minimal variation, supporting the hypothesis that this genetic modification did not affect the microbiomes of the plants.

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References

Andreote, F. D., Carneiro, R. T., Salles, J. F., Marcon, J., Labate, C. A., Azevedo, J. L., & Araújo, W. L. (2009). Culture-independent assessment of *Rhizobiales*-related Alphaproteobacteria and the diversity of *Methylobacterium* in the rhizoplane of transgenic eucalyptus. *Microbial Ecology, 57*, 82-93. https://doi.org/10.1007/s00248-008-9405-8

Bashir, I., War, A. F., Eafiq, I., Reshi, Z. A., Rashid, I., & Shouche, Y. S. (2022). Phyllosphere microbiome: Diversity and functions. *Microbiological Research, 254*, 126888. https://doi.org/10.1016/j.micres.2021.126888

Barbosa, E. G. G., Leite, J. P., Marin, S. R. R., Marinho, J. P., Carvalho, J. F. C., Fuganti-Pagliarini, R., Farias, J. R. B., Neumaier, N., Marcelino-Guimarães, F. C., Oliveira, M. C. N., Yamaguchi-Shinozaki, K., Nakashima, K., Maruyama, K., Kanamori, N., Fujita, Y., Yoshida, T., & Nepomuceno, A. L. (2013). Overexpression of the ABA-dependent AREB1 transcription factor from *Arabidopsis thaliana* improves soybean tolerance to water deficit. *Plant Molecular Biology Reporter, 31*, 719–730. https://doi.org/10.1007/s11105-012-0541-4

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology, 64*, 807-838. https://doi.org/10.1146/annurev-arplant-050312-120106

Companhia Nacional de Abastecimento. (2024). *Acompanhamento da safra brasileira de grãos - v. 11 - SAFRA 2023/23 - n. 6 - Sexto levantamento | Março, 2024*. https://www.conab.gov.br/info-agro/safras/graos/monitoramento-agricola

Debruyn, J. M., Nixon, L. T., Fawaz, M. N., Johnson, A. M., & Radosevich, M. (2011). Global biogeography and quantitative seasonal dynamics of *Gemmatimonadetes* in soil. *Applied and Environmental Microbiology, 77*, 6295-6300. https://doi.org/10.1128/aem.05005-11

Dini-Andreote, F., Andreote, F. D., Costa, R., Taketani, R. G., van Elsas, J. D., & Araujo, W. L. (2010). Bacterial soil community in a Brazilian Sugarcane field. *Plant and Soil, 336*, 337–349. https://doi.org/10.1007/s11104-010-0486-z

Dunbar, J., Takala, S., Barns, S. M., Davis, J. A., & Kuske, C. R. (1999). Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Applied and Environmental Microbiology, 65*, 1662-1669. https://doi.org/10.1128/aem.65.4.1662-1669.1999

Eichorst, S. A., Kuske, C. R., & Schmid, T. M. (2011). Influence of plant polymers on the distribution and cultivation of bacteria in the phylum Acidobacteria. *Applied and Environmental Microbiology, 77*, 586-596. https://doi.org/10.1128/aem.01080-10

Faria, M. R., Costa, L. S. A. S., Chiaramonte, J. B., Bettiol, W., & Mendes, R. (2021). The rhizosphere microbiome: Functions, dynamics, and role in plant protection. *Tropical Plant Pathology, 46*, 13-25. https://doi.org/10.1007/s40858-020-00390-5

Fernández, V., Tapia, G., Varela, P., & Videla, L. A. (2005). Redox regulation of thyroid hormone-induced Kupffer cell-dependent IkB-a phosphorylation in relation to inducible nitric oxide synthase expression. *Free Radical Research, 39*, 411-418. https://doi.org/10.1080/10715760400029637

Garbeva, P., van Veen, J. A., & van Elsas, J. D. (2004). Microbial Diversity in soil: selection of microbial populations by plant soil type and implications for disease suppressiveness. *Annual Review of Phytopathology, 42*, 243-270. https://doi.org/10.1146/annurev.phyto.42.012604.135455

Garcia, A., Polonio, J. C., Polli, A. D., Santos, C. M., Rhoden, S. A., Quecine, M. C., Azevedo, J. L., & Pamphile, J. A. (2016). Rhizosphere bacteriome of the medicinal plant *Sapindus saponaria L.* revealed by pyrosequencing. *Genetics and Molecular Research, 15*(4), gmr15049020. https://doi.org/10.4238/gmr15049020

Grayston, S. J., Griffith, G. S., Mawdsley, J. L., Campbell, C. D., & Bardgett, R. D. (2001). Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biology & Biochemistry, 33*, 533-551. https://doi.org/10.1016/S0038-0717(00)00194-2

Hartman, G. L., West, E. D., & Herman, T. K. (2011). Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. *Food Security, 3*, 5–17. https://doi.org/10.1007/s12571-010-0108-x

Hartmann, M., & Six, J. (2023). Soil structure and microbiome functions in agroecosystems. *Nature Reviews - Earth & Environment, 4*, 4-18. https://doi.org/10.1038/s43017-022-00366-w

Hughes, J. B., Hellmann, J. J., Ricketts, T. H., & Bohannan, B. J. (2001). Counting the uncountable statistical approaches to estimating microbial diversity. *Applied and Environmental Microbiology, 67*, 4399-4406. https://doi.org/10.1128/AEM.67.10.4399-4406.2001

Kalam, S., Basu, A., Ahmad, I., Sayyed, R. Z., El-Enshasy, H. A., Dailin, D. J., & Suriani, N. L. (2020). Recent understanding of soil acidobacteria and their ecological significance: a critical review. *Frontiers in Microbiology, 11*, 580024. https://doi.org/10.3389/fmicb.2020.580024

Kumar, A., & Dubey, A. (2020). Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production. *Journal of Advanced Research, 24*, 337-352. https://doi.org/10.1016/j.jare.2020.04.014

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied Environmental Microbiology, 75*, 5111–5120. https://doi.org/10.1128/aem.00335-09

Lee, K. C., Webb, R. I., Janssen, P. H., Sangwan, P., Romeo, T., Staley, J. T., & Fuerst, J. A. (2009). Phylum Verrucomicrobia representatives share a compartmentalized cell plan with members of bacterial phylum Planctomycetes. *BMC Microbiology, 9*, 5. https://doi.org/10.1186/1471-2180-9-5

Leite, J. P., Barbosa, E. G. G., Marin, S. R. R., Marinho, J. P., Carvalho, J. F. C., Pagliarini, R. F., Cruz, A. S., Oliveira, M. C. N., Farias, J. R. B., Neumaier, N., Guimarães, F. C. M., Yoshida, T., Kanamori, N., Fujita, Y., Nakashima, K., Shinozaki, K. Y., Desidério, J. A., & Nepomuceno, A. L. (2014). Overexpression of the

activated form of the *AtAREB1* gene (AtAREB1ΔQT) improves soybean responses to water deficit. *Genetic and Molecular Research, 13*, 6272-6286. https://doi.org/10.4238/2014.august.15.10

Lopes, A. S., Abreu, C. A., & Santos, G. C. G. (2006). Micronutrientes. In *Tópicos em Ciências do Solo*. Sociedade Brasileira de Ciência do Solo.

Lopes, L. D., Hao, J., & Schachtman, D. P. (2021). Alkaline soil pH affects bulk soil, rhizosphere and root endosphere microbiomes of plants growing in a Sandhills ecosystem. *FEMS Microbiology Ecology, 97*, fiab028. https://doi.org/10.1093/femsec/fiab028

Marinho, J. P., Kanamorio, N., Ferreira, L. C., Fuganti-Pagliarini, R., Carvalho, J. F. C., Freitas, R. A., Marin, S. R. R., Rodrigues, F. A., Mertz-Henning, L. M., Farias, J. R. B., Neumaier, N., Oliveira, M. C. N., Marcelino-Guimarães, F. C., Yoshida, T., Fujita, Y., Yamaguchi-Shinozaki, K., Nakashima, K., & Nepomuceno, A. L. (2016). Characterization of molecular and physiological responses under water deficit of genetically modified soybean plants overexpressing the AtAREB1 transcription factor. *Plant Molecular Biology Reporter, 34*, 410–426. https://doi.org/10.1007/s11105-015-0928-0

Meng, S., Liang, X., Peng, T., Liu, Y., Wang, H., Huang, T., Gu, J-D., & Hu, Z. (2023). Ecological distribution and function of comammox *Nitrospira* in the environment. *Applied Microbiology and Biotechnology, 107*, 3877-3886. https://doi.org/10.1007/s00253-023-12557-6

Montanari-Coelho, K. K., Costa, A. T., Polonio, J. C., Azevedo, J. L., Marin, S. R. R., Fuganti-Pagliarini, R., Fujita, Y., Yamaguchi-Shinozaki, K., Nakashima, K., Pamphile, J. A., & Nepomuceno, A. L. (2018). Endophytic bacterial microbiome associated with leaves of genetically modified (AtAREB1) and conventional (BR 16) soybean plants. *World Journal of Microbiology and Biotechnology, 34*, 56. https://doi.org/10.1007/s11274-018-2439-2

O'Neill, B., Grossman, J., Tsai, S. M., Gomes, J. E., Lehmann, J., Peterson, J., Neves, E., & Thies, J. E. (2009). Bacterial community composition in Brazilian anthrosols and adjacent soils characterized using culturing and molecular identification. *Microbial Ecology, 58*, 23-35. https://doi.org/10.1007/s00248-009-9515-y

Prosser, J. L. (2007). Microorganisms cycling soil nutrients and their diversity. In J. D. van Elsas, J. K. Jansson, & J. T. Trevors (Eds.), *Modern soil microbiology* (pp. 237-261). CRC Press.

Sohrabi, R., Paasch, B. C., Liber, J. A., & He, S. Y. (2023). Phyllosphere microbiome. *Annual Reviews in Plant Biology, 74*, 539-568. https://doi.org/10.1146/annurev-arplant-102820-032704

Shin, J. H., Vaughn, J. N., Abdel-Haleem, H., Chavarro, C., Abernathy, B., Kim, K. D., Jackson, S. A., & Li, Z. (2015). Transcriptomic changes due to water deficit define a general soybean response and accession-specific pathways for drought avoidance. *BMC Plant Biology, 15*, 26. https://doi.org/10.1186/s12870-015-0422-8

Stackebrandt, E., & Schumann, P. (2006). Introduction to the taxonomy of Axtinobacteria. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, KH., Stackebrandt, E. (Eds) *The Prokaryotes*. Springer.

Stuart, R. M., Romão, A. S., Pizzirani-Kleiner, A. A., Azevedo, J. A., & Araújo, W. L. (2010). Culturable endophytic filamentous fungi from leaves of transgenic imidazolinone-tolerant sugarcane and its non-transgenic isolines. *Archives in Microbiology, 192*, 307-313. https://doi.org/10.1007/s00203-010-0557-9

Sun, H., Jiang, S., Jiang, C., Wu, C., Gao, M., & Wang, Q. (2021). A review of root exudates and rhizosphere microbiome for crop production. *Environmental Sciences and Pollution Research, 28*, 54497-54510. https://doi.org/10.1007/s11356-021-15838-7

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., & Singh, B. K. (2020). Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews - Microbiology, 18*, 607-621. https://doi.org/10.1038/s41579-020-0412-1

Turrini, A., Sbrana, C., & Giovannetti, M. (2015). Belowground environmental effects of transgenic crops: a soil microbial perspective. *Research in Microbiology, 166*, 121-131. https://doi.org/10.1016/j.resmic.2015.02.006

United States Department of Agriculture [USDA]. (2024, March). *World Agricultural Production. Circular Series, WAP 3-24*. Foreign Agricultural Service/USDA. https://fas.usda.gov/data/world-agricultural-production

Verma, S. K., Sahu, P. K., Kumar, K., Pal, G., Gond, S. K., Kharwar, R. N., & White, J. F. (2021). Endophyte roles in nutrient acquisition, root system architecture development and oxidative stress tolerance. *Journal of Applied Microbiology, 131*, 2161-2177. https://doi.org/10.1111/jam.15111

Yan, B., Liu, N., Liu, M., Du, X., Shang, F., & Huang, Y. (2021). Soil actinobacteria tend to have neutral interactions with other co-occurring microorganisms, especially under oligotrophic conditions. *Environmental Microbiology, 23*, 4126-4140. https://doi.org/10.1111/1462-2920.15483

Zhalnina, K., Dias, R., de Quadros, P. D., Davis-Richardson, A., Camargo, F. A., Clark, I. M., McGrath, S. P., Hirsch, P. R., & Triplett, E. W. (2015). Soil pH determines microbial diversity and composition in the park grass experiment. *Microbial Ecology, 69*, 395-406. https://doi.org/10.1007/s00248-014-0530-2