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Impact of Kiwifruit Waste Compost on Soil Bacteriome and Lettuce Growth

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Abstract: Composts produced with kiwifruit waste from the calibration process (KW), mixed with 5%, 10%, and 20% wheat straw (WS), were evaluated as crop fertilizers through a pot experiment with lettuce, arranged as a randomized block design. Highest lettuce yields were achieved with 20 and 40 t·ha^{-1} 5%WS compost and 40 t·ha^{-1} 10%WS compost, suggesting that the physical characteristics of the composts increased soil water holding capacity and root growth, whereas chemical characteristics such as pH, organic matter, and nutrient contents contributed to improving soil reaction and nutrient availability. The type of soil amendment used influenced the development of different bacterial consortia in the bulk soil and rhizosphere, leading to increased levels of potentially beneficial bacteria and enhanced levels of relevant functions for plant growth, such as nitrogen fixation. Composted KW as an organic amendment can be used to improve soil quality and the circular economy.

Keywords: bacteria; composting; nitrogen; soil organic amendment; wheat straw

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1. Introduction

The use of mineral N fertilizers has contributed to increased crop production over the last decades. However, these fertilizers increase the emission of greenhouse gases and pollutants to the environment and promote the loss of biodiversity [1,2]. Mineral fertilizers are directly available for plant uptake, while organic fertilizers consist of complex molecules that contribute to feeding plants over time and soil microorganisms, increasing soil microbial biomass and activity, and promoting soil structural stability, soil porosity, water holding capacity, and carbon sequestration [3–5], which contribute to reducing greenhouse gases emission [6].

Agricultural production involves the generation of a large amount of organic waste derived from farming cultural practices and calibration processes. The kiwi industry in 2021 produced in Portugal approximately 55,460 tons of kiwifruits [7], which implies a great amount of waste. The environmental impact of kiwi waste is particularly high for landfill deposition due to methane and greenhouse gas emissions [8]. One way for recycling this waste for agronomic use is through composting in accordance with the principles of the circular economy [9], as the aerobic decomposition of organic matter (OM), under conditions that allow thermophilic temperatures, results in a stabilized and sanitized product, free of seeds and pathogenic microorganisms with agronomic and environmental advantages [10].

The advantages of compost application for lettuce production have been reported by several authors [11–13]. Lettuce grown with organic fertilizers showed a reduced risk of lettuce contamination by nitrates [6,12], and the risk of nitrate leaching to the deep layers of soil also decreased [14,15]. Moreover, similar or higher lettuce yield and nutrient uptake with the application of organic compared with mineral fertilizers has also been widely documented [16,17]. For example, solid sewage sludge compost was able to supply nutrients to achieve similar or higher lettuce yields compared with inorganic fertilizers [12,16]. The same was found for solid urban waste at the rate of 30 t·ha^{-1} [6] and with cattle manure in sandy soils [18].

The soil-associated microbiota naturally influences soil health and its ability to improve crop growth. In this regard, the use of mineral or organic-based fertilizers, such as compost, does indeed matter significantly. There are several studies demonstrating that the use of mineral fertilizers negatively impacts the diversity and richness of microbial species in the soil. Conversely, the use of organic-based fertilizers appears to have a beneficial effect on increasing microbial richness and diversity, as well as enhancing the robustness of the network of microbial interactions established in the soil [19–21].

OM decomposition during the composting process depends on the composition of the feedstock material and on environmental conditions, such as moisture content and aeration [8]. The degradation of the kiwi fruit waste through composting requires a bulking agent in order to increase aeration, and the wheat straw from bundles used to protect kiwi stems from frost offers adequate properties to promote aeration [22]. Therefore, the characteristics of the mixture of the kiwi waste with wheat straw indicate the possibility of achieving a good-quality compost to be used as a soil organic amendment to improve soil quality and increase crop yields. The composts used in this study were analyzed after a maturation period of 176 days, as detailed in a previously published study [23]. The bacterial composition of all three composts was dominated by aerobic species, with varying levels of beneficial microorganisms among them. These composts were also evaluated for stability, phytotoxicity, and organic matter content. Despite their potential as effective soil organic amendments, there is limited information on the composting process of kiwi waste with other materials and on the effects of this type of compost on vegetable growth. Thus, the aim of this study was to assess the changes in the physicochemical properties and bacterial composition of soil with the application of compost from kiwi waste mixed with wheat straw, compared with mineral N fertilizers, and their effects on lettuce growth.

2. Materials and Methods

2.1. Pot Experiment

A pot experiment with lettuce (*Lactuca sativa* L. cv. Madie) was set up inside a greenhouse in NW Portugal (41°47′ N e 8°32′ W), with composts produced with kiwi waste mixed with 5%, 10%, and 20% (fresh weight) wheat straw (5S, 10S, and 20S, respectively) for a period of 208 days. The wheat straw was obtained from bundles used to protect kiwi stems from frost. The kiwifruit waste was obtained from the kiwi calibration process. Three composting piles were built outdoors with alternate layers of kiwi and straw to approximately 1.6 m wide, 1.3 m high, and 8 m long. The two materials were mixed with a backhoe loader and covered with TenCate Toptex ® (TenCate Geosynthetics GmbH, Linz, Austria) to avoid rainfall and simultaneously allow gas exchange. The piles were turned after 28, 56, 90, and 130 days from the beginning of the composting process with the backhoe loader. The characteristics of the composts are shown in Table 1.

The composts were matured as the temperatures decreased to stable temperatures close to ambient levels, and the amount of NH_4^+-N (25–149 mg·kg^{-1}) was below 400 mg·kg^{-1} [24]. The electrical conductivity (EC) was below the maximum value (3 dS·m^{-1}) recommended for soil application [25]. The trial was established as a randomized block design with 4 blocks. Each block included 12 treatments (as in Table 2): (i) the composts 5S, 10S, and 20S with the levels of 10, 20, and 40 t·ha^{-1} (5S10, 5S20, 5S40, 10S10, 10S20, 10S40, 20S10, 20S20, and 20S40); (ii) mineral N fertilizer (20.5% N) at the rates of 30 and 70 kg N·ha^{-1} (N30 and N70); and

(iii) a control treatment without soil fertilizers (C). The compost application was based on 100,000 plants·ha^{-1}. The transplanting occurred on 6 January 2023 to pots with 8 kg of soil collected from 0 to 20 cm of depth (Table 3).

Table 1. Chemical characteristics of composts 5S, 10S, and 20S produced with kiwi waste mixed with 5%, 10%, and 20% of wheat straw, respectively.

		5S	**10S**	**20S**
pH		7.8 ± 0.1	7.7 ± 0.1	7.4 ± 0.2
EC	(dS·m^{-1})	0.8 ± 0.1	0.4 ± 0.2	0.3 ± 0.0
OM	(g·kg^{-1})	762 ± 12	834 ± 4	920 ± 7
N	(g·kg^{-1})	28.7 ± 1.0	27.7 ± 0.8	12.9 ± 1.4
C/N		15 ± 1	17 ± 1	40 ± 4
N-NH_4^+	(mg·kg^{-1})	149 ± 62	25 ± 13	39 ± 10
N-NO_3^-	(mg·kg^{-1})	26 ± 3	157 ± 69	48 ± 20
P	(g·kg^{-1})	4.3 ± 0.6	3.7 ± 0.4	1.3 ± 0.2
K	(g·kg^{-1})	12.5 ± 0.5	12.5 ± 1.1	7.7 ± 1.0

Note: Nutrient contents are expressed on a dry matter basis.

Table 2. The fertilizing products.

Fertilizer	**Code**	**Dose**
Control	C	
Mineral N fertilizer (20.5% N)	AA30	146 kg·ha^{-1}
Mineral N fertilizer (20.5% N)	AA70	341 kg·ha^{-1}
Compost 5% straw	5S10	10 t·ha^{-1}
Compost 5% straw	5S20	20 t·ha^{-1}
Compost 5% straw	5S40	40 t·ha^{-1}
Compost 10% straw	10S10	10 t·ha^{-1}
Compost 10% straw	10S20	20 t·ha^{-1}
Compost 10% straw	10S40	40 t·ha^{-1}
Compost 20% straw	20S10	10 t·ha^{-1}
Compost 20% straw	20S20	20 t·ha^{-1}
Compost 20% straw	20S40	40 t·ha^{-1}

Table 3. Soil chemical characteristics at the beginning of the pot experiment.

pH	**EC (dS m^{-1})**	**OM * (%)**	**Extractable Phosphorus ** (mg kg^{-1})**	**Extractable Potassium ** (mg kg^{-1})**	**Extractable Calcium *** (mg kg^{-1})**	**Extractable Magnesium *** (mg kg^{-1})**
5.9 ± 0.1	0.02 ± 0.00	4.3 ± 0.5	121 ± 13	263 ± 18	263 ± 24	81 ± 14

* Tinsley method ** Egner-Riehm method. *** Extraction with ammonium acetate. Organic matter and nutrient contents are expressed on a dry matter basis.

Lettuces were harvested 62 days after planting. During this period, the plants were irrigated to prevent water stress, and manual weeding was carried out to prevent weed competition.

2.2. Analytical Methods

Lettuce shoots were dried at 65 °C until reaching a constant weight to determine dry matter content. Dry samples were milled to determine total N content using the modified Kjeldahl method based on sulfuric acid digestion, with a Kjeldahl digestion unit (DK 20, VELP Scientifica Srl, Usmate Velate, Italy) and a compact distillation unit (UDK 139, VELP Scientifica). Mineral N was extracted from 100 g of fresh compost with 1 M KCl 1:5 solution and determined by molecular absorption spectrophotometry using a continuous flow auto-analyzer (SanPlus System, Skalar, Breda, The Netherlands). The organic nitrogen mineralization rate of the compost was calculated by the difference between the accumulated N in the lettuce produced with the compost and the control lettuce, minus the compost mineral N, divided by the compost organic N.

2.3. Sampling and Preparation of Soil Samples for DNA Extraction

Bulk soil, from a total of 48 pots prepared according to 2.1, was sampled at up to 10 cm depth using a sterile 15 mL polypropylene tube. For maximal representativity, each sample was composed of sub-samples taken from different positions of each pot. The refrigerated soil samples were weighted (200 mg) and divided into three DNAse-free 2.0 mL polypropylene tubes that were kept at −80 °C until used for DNA extraction. Rhizosphere soil was prepared essentially according to a previously published method [26], with some adaptations. The roots of lettuce were placed in a 50 mL polypropylene tube containing 10 mL of phosphate buffer solution at pH 7.0. These 50 mL tubes were then positioned horizontally on an orbital shaker (Certomat B. Braun, Melsungen, Germany) for 20 min at 80 rotations per minute, followed by centrifugation for 10 min at 10,000× *g*. The resulting pellet was regarded as the soil of the lettuce rhizosphere.

2.4. Extraction, Purification, and Sequencing of Microbial DNA

Each individual sample underwent total DNA extraction, with three replicate tubes per sample, utilizing the DNeasy® PowerSoil® Pro-Kit (QIAGEN, Hilden, Germany). The DNA extraction, purification, quality control, PCR, and library generation were performed as previously described [23]. Sequencing of amplicons generated by PCR of 16S rRNA gene V3-V4 fragments was conducted on an Illumina platform at Novogene Company Limited (Cambridge, UK) to generate paired-end reads.

2.5. Bioinformatics and Sequencing Results Analysis

The sequencing reads were merged, quality-filtered, aligned to SILVA138, and clustered into OTUs as described previously [23]. Functional annotation was conducted using the FAPROTAX database [27], and corresponding abundance maps were used to generate heatmaps [28].

2.6. Nucleotide Sequences Accession Number

Raw reads were deposited in the SRA database under BioProject RJNA1012906.

2.7. Statistical Analyses

Analysis of variance (ANOVA) was performed using the SPSS general linear model procedure. Statistical significance was indicated at a probability level of $p = 0.05$ to compare means between treatments. All statistical calculations were performed using SPSS v. 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Effect of Compost and Mineral N Fertilizer on Lettuce Growth

Lettuce fresh weight increased with kiwi waste composts with 5% and 10% wheat straw applied at the rates of 20 and 40 t·ha^{-1} compared with the unfertilized treatment and the treatments with mineral N fertilizer (Figure 1).

However, the same was not always true for the compost with 20% wheat straw, which showed a very high C/N ratio (Table 1).

The increase in lettuce yield with composts 5S and 10S compared with the control treatment can be attributed to the organic matter (OM) mineralization that increased the availability of nutrients, as previously reported by other authors [10,29,30]. However, compared with mineral N treatments, the increase in lettuce yields with composts will have to be explained by other beneficial factors as a result of compost application, such as increased soil water holding capacity and porosity, that improved root growth, as well as increased salinity caused by the mineral fertilizer.

Unlike conventional mineral fertilizers, which can lead to soil degradation, changes in soil osmolarity, pH imbalance, and a decline in microbial diversity [31], the use of organic amendments such as compost offers a sustainable alternative. The improvement in chemical and physical soil properties, including pH, nutrient availability, bulk density, porosity, and aggregation stability, has been widely reported [32,33]. For example, after two successive crops of lettuce fertilized with manure compost or sewage sludge compost, soil showed a higher percentage of stable aggregates and increased water holding capacity compared with soil fertilized with mineral fertilization [12], whereas in a laboratory experiment with maize, OM addition has been reported [34] to contribute to decreasing bulk density of the soil, allowing the development of root length. Thus, the volume of the rhizosphere increases and, consequently, the absorption of water and nutrients is more efficient with compost application [35]. Moreover, the application of inorganic N forms may decrease microbial activity [36], whereas the application of compost (pH between 7.4 and 7.8) may increase soil pH and, consequently, may precipitate aluminum and iron, decreasing soil toxicity and may increase the release of nutrients such as phosphorus and calcium [33] and N mineralization rates [37]. Composting, as highlighted by Sayara et al. [38], not only produces a stable product that can be used as an organic amendment but also enhances soil fertility and may even help suppress soil-borne diseases.

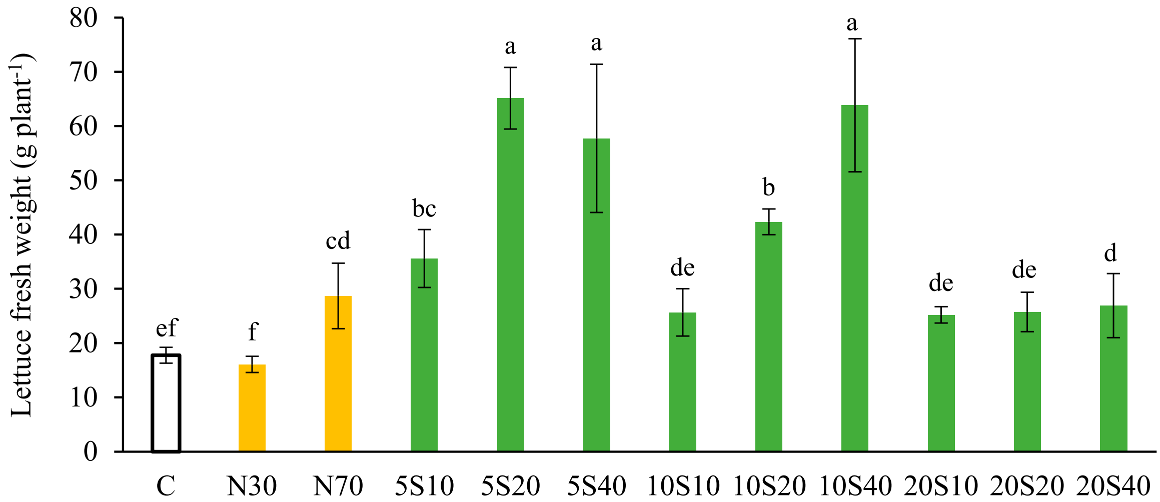

Figure 1. Fresh weight of lettuce shoots in response to the control treatment without fertilization (C), mineral N fertilizer at the rates of 30 and 70 kg·ha^{-1} (N30 and N70), and composts 5S, 10S, and 20S with levels of 10, 20 and 40 t·ha^{-1} (5S10, 5S20, 5S40, 10S10, 10S20, 10S40, 20S10, 20S20, and 20S40). Uncolored bars: control samples; yellow bars: samples with mineral N fertilizer; green bars: samples with compost fertilizer. Bars with different letters are significantly different ($p < 0.05$).

For the overall rates of compost application (Figure 2a), lettuce yield was enhanced by 48% with compost 5S, with a lower proportion of straw, compared with compost 20S, with a very high C/N ratio, which may have contributed to the immobilization of N by soil microorganisms in the beginning of the experiment, and a small rate of organic N mineralization (0.7%) over the period of lettuce growth, compared with composts 10S (1.6%) and 5S (2.7%). Several studies have documented that the C/N ratio is a good indicator of N availability [39–41], and there are reports in the literature in which organic materials with a C/N ratio of >15 promoted N immobilization [39,42]. These results are in agreement with Ribeiro et al. [43], who reported that lettuce yield increased with hen manure (C/N = 10 and total N = 39 g·kg^{-1}) in comparison to on-farm compost (C/N = 17 and total N = 11 g·kg^{-1}). Comparing the effects of the application rate for the average of the three composts, there is a clear tendency for the lettuce yield to increase as the compost application rate increases, although this is not the case for compost 20S (Figure 2b).

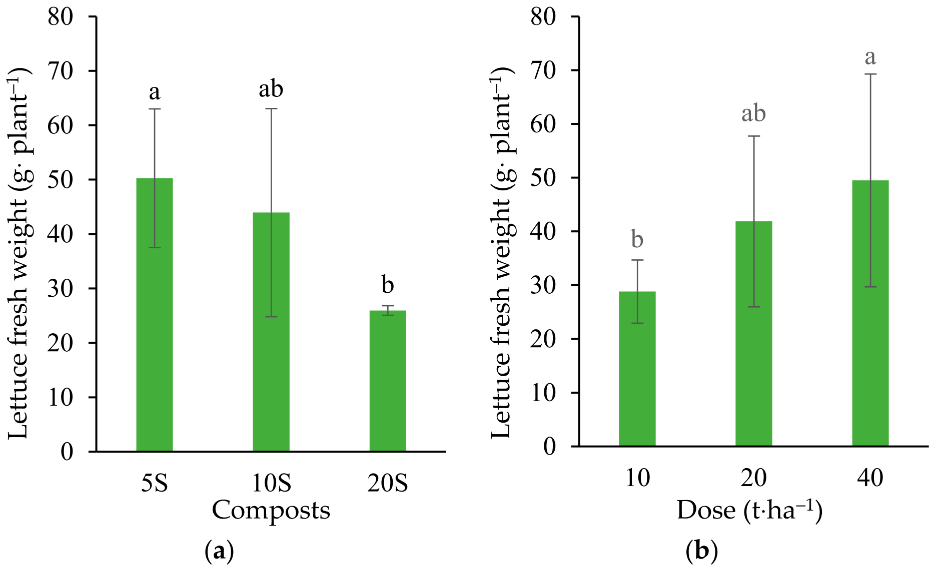

Figure 2. Lettuce fresh weight in response to (**a**) the application of composts 5S, 10S, and 20S for the average compost rates and (**b**) the application of doses 10, 20, and 40 t·ha^{-1} for the average of the composts. Bars with different letters are significantly different ($p < 0.05$).

As expected, the lettuce shoot dry matter content decreased for lettuces with increased fresh weight (Table 4).

Table 4. Dry matter content (DM), N content, and N accumulation in lettuce shoots, mineral N available at the beginning of the experiment, and organic N mineralized from composts during the period of lettuce growth.

	DM (%)	N Content (g kg^{-1})	N Accumulation (mg plant^{-1})	Mineral N (mg pot^{-1})	Mineralized N (mg pot^{-1})
C	10.7 ± 0.6	15.8 ± 0.2	30.0 ± 3.4	-	-
N30	12.4 ± 1.5	18.8 ± 1.0	37.5 ± 6.2	300	-
N70	10.0 ± 0.8	24.1 ± 1.6	69.0 ± 12.2	700	-
5S10	8.0 ± 1.7	15.1 ± 1.6	43.2 ± 4.9	3.1	10.1
5S20	8.1 ± 1.0	16.5 ± 2.1	86.9 ± 8.4	6.3	50.5
5S40	6.9 ± 0.9	16.5 ± 2.2	65.8 ± 16.3	12.5	23.2
10S10	8.8 ± 0.9	16.0 ± 1.9	35.9 ± 6.2	2.8	3.0
10S20	7.7 ± 1.1	15.9 ± 1.3	51.9 ± 4.8	5.6	16.2
10S40	7.1 ± 0.1	17.8 ± 1.2	80.4 ± 17.3	11.2	39.2
20S10	8.8 ± 0.8	17.0 ± 2.0	36.5 ± 8.6	1.6	4.8
20S20	8.5 ± 0.5	14.9 ± 2.4	32.5 ± 16.2	3.2	0.0
20S40	8.0 ± 0.5	14.1 ± 0.7	30.6 ± 5.4	6.4	0.0
LSD	1.4	2.5	14.9		

LSD: least significant difference ($p < 0.05$); Treatments: control treatment without fertilization (C), mineral N fertilizer at the rates of 30 and 70 kg·ha^{-1} (N30 and N70), and composts 5S, 10S, and 20S with levels of 10, 20 and 40 t·ha^{-1} (5S10, 5S20, 5S40, 10S10, 10S20, 10S40, 20S10, 20S20, and 20S40).

The maximum N content of lettuce shoots was registered with the application of 70 kg·ha^{-1} of mineral N fertilizer (24.1 g·kg^{-1}). The same happened in other experiments where the nitrate content of lettuces fertilized with compost from different materials such as solid urban waste, sewage sludge, sheep manure, and poultry litter was below the nitrate content of lettuces fertilized with mineral fertilizers [6,12–14]. This may be explained by the slow release of mineral N from compost, leading to a more gradual uptake of N by plants [12] or by mineral N immobilization by soil microorganisms as a result of the increased amount of available C from compost [6].

For the compost treatments, mineral N available in the composts at the start of the experiment was generally below the difference between N accumulation in the treated lettuces and the control lettuces (Table 3). Therefore, the plant absorbed the mineral N and the organic N mineralized, reducing the risk of N losses. On the contrary, the mineral N available in the mineral N treatments N30 and N70 (300 mg and 700 mg, respectively) was above the increase in accumulated N (7.5 mg and 39 mg plant^{-1}, respectively), revealing risks of N losses by leaching, as reported by several authors [6,12,15,44].

3.2. Effect of Compost and Mineral N Fertilizer on Soil Bacteriological Composition

The analysis of the 16S sequencing data of all samples unveiled the presence of 74 distinct phyla, with only 6 of them exhibiting abundances exceeding 1%. The prevailing phylum was *Pseudomonadota* (ex-*Proteobacteria*), constituting an average abundance of 19.5% (ranging from 8.9% to 42.1%), followed by *Firmicutes*, with an average abundance of 14.2% (ranging from 3.8% to 28.4%). In Figures 3 and 4, the most abundant genera identified are represented for all the soil samples analyzed.

Figure 3. Relative abundances of dominant sequences assigned to genus level identified in all bulk soil samples based on partial sequence analysis of the V3–V4 region of the bacterial 16S rRNA.

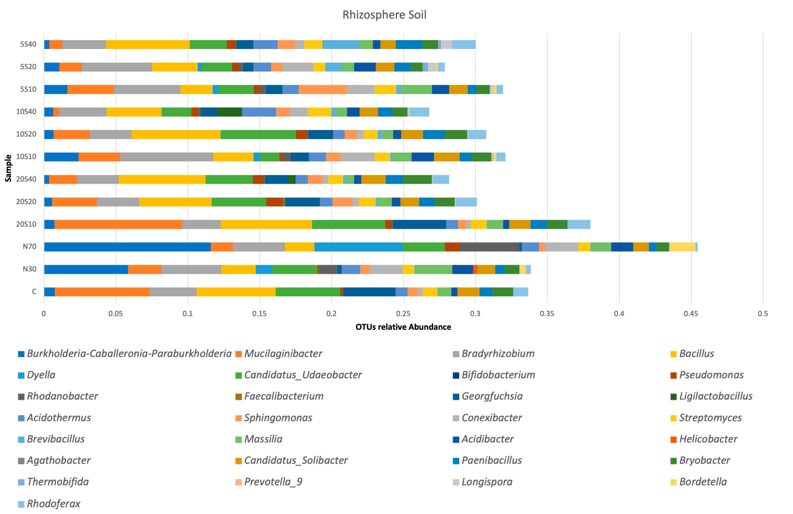

Figure 4. Relative abundances of dominant sequences assigned to genus level identified in all rhizosphere samples based on partial sequence analysis of the V3–V4 region of the bacterial 16S rRNA.

A total of 975 genera were identified, *Bradyrhizobium* being the most abundant genus, with an average abundance of 3.43% (ranging from 1.8% to 6.5%), followed by *Bacillus*, which averaged 3.39% (ranging from 1.8% to 6.4%). This bacterial genus is comprised of several rhizobia species with nitrogen-fixing capabilities and can be found free living in soil or in symbiosis mainly associated with leguminous roots [45,46]. The introduction of mineral N led to a decline in the relative abundance of *Bradyrhizobium* over time, which seems to be a logical outcome given the presence of a readily available nitrogen source, eliminating the need for diazotrophic activity in atmospheric nitrogen fixation. *Bradyrhizobium* species has been shown to enhance N uptake, dry biomass, and grain yield in some commercially relevant crops, such as soybeans [47–49]. In a field trial in Mozambique using soybeans, these strains doubled the number of nodules and increased dry weight by up to 5.8 times [50]. A study in Northeast Germany evaluated three indigenous *Bradyrhizobium* isolates with various soybean cultivars. Results indicated significant strain and cultivar interactions affecting nodulation, with inoculated plants showing higher nodulation and increased N uptake compared with field conditions [51].

Another effect of the addition of mineral N, as revealed by the α-diversity analysis (Table 5), namely observed OTUs and Shannon and Simpson indices, is a slight reduction in the diversity and richness of the bacterial populations present in the bulk soil at the end of the experiment, which is in contrast with what happens when most of the organic composts are added to the soil. In fact, this influence of soil mineral N supplementation in the bacterial community structure has been already described for the wheat, bulk, and rhizosphere soil microbiome [52].

Table 5. Summary of α-diversity indices.

Sample	Observed Species	Shannon	Simpson	Chao1	ACE
C (e)	3465	9.125	0.991	4588.199	4529.034
N30 (e)	2756	8.581	0.986	3126.095	3336.335
N70 (e)	2781	8.929	0.993	3080.517	3233.258
20S10 (e)	3390	9.567	0.996	3792.223	4040.761
20S20 (e)	3441	9.371	0.994	4391.518	4364.381
20S40 (e)	4017	9.953	0.996	4757.697	4853.611
10S10 (e)	3875	9.909	0.996	4629.201	4715.741
10S20 (e)	4129	10.016	0.996	4869.104	4961.429
10S40 (e)	4301	9.949	0.997	5692.096	5733.125
5S10 (e)	3648	9.648	0.995	4323.995	4402.814
5S20 (e)	3321	9.396	0.994	4106.534	4073.586
5S40 (e)	3513	9.492	0.994	4150.914	4268.635
C (r)	2607	8.621	0.990	2914.944	3076.161
N30 (r)	2763	8.804	0.993	3360.813	3489.190
N70 (r)	2877	8.512	0.988	3549.633	3637.165
20S10 (r)	2774	8.643	0.991	3125.921	3335.398
20S20 (r)	3216	9.288	0.995	3655.835	3770.027
20S40 (r)	3422	9.318	0.994	3872.488	3989.121
10S10 (r)	3327	9.241	0.992	4076.302	4175.466
10S20 (r)	3211	9.248	0.995	3664.377	3785.656
10S40 (r)	3641	9.616	0.996	4111.319	4312.322
5S10 (r)	3540	9.530	0.995	4282.607	4387.217
5S20 (r)	3696	9.661	0.995	4435.924	4535.316
5S40 (r)	3265	9.520	0.996	3652.223	3757.082

Note: (e)—bulk soil samples at the end of the experiment and (r)—lettuce rhizosphere soil.

In relation to lettuce rhizospheres grown in mineral N-supplemented soils, the analysis of the most prevalent bacterial genera shows a noticeable prevalence of *Burkholderia*, *Caballeronia*, and *Paraburkholderia*. Species from these genera are recognized for their metabolic adaptability [53–55] and may thrive in environments rich in accessible nitrogen sources, incorporating nitrogen into their biomass as they grow and reproduce. This, in turn, can potentially lead to nitrogen immobilization and a decrease in nitrogen and other relevant nutrient availability for the plants.

When analyzing the effect of the addition of organic composts in different doses to the soil, some significant and highly significant differences can be observed (Figure 5).

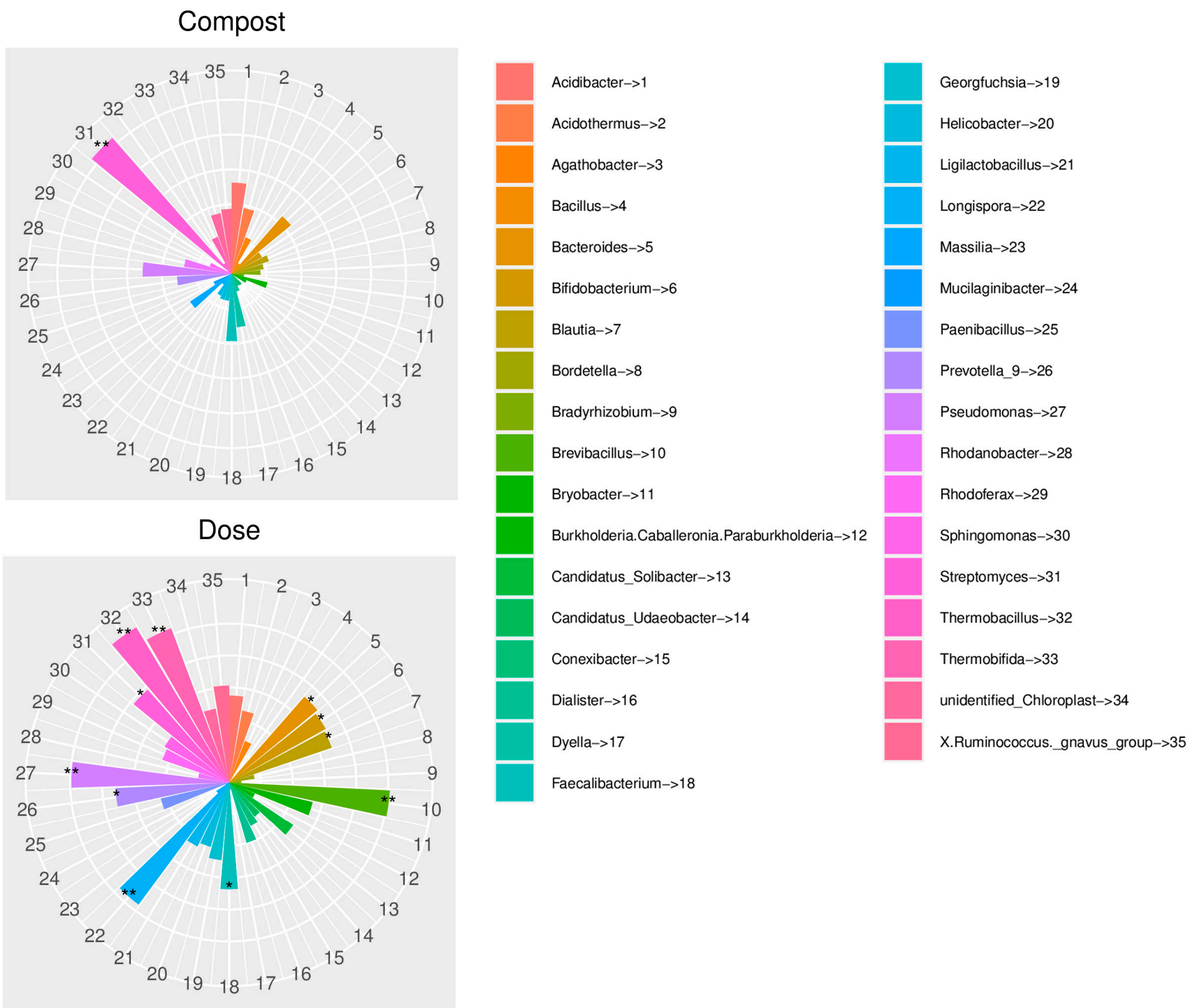

Figure 5. Influence of the addition of compost (5S, 10S, and 20S) in different doses (10, 20, and 40 t·ha^{-1}) on the bacterial composition of the soil samples. * Significant differences; ** highly significant differences.

Especially considering the taxa with highly significant differences (according to Figure 5), we can observe their relative abundances in the bulk soil of the pots and in the lettuce rhizosphere soil in Figures 6 and 7, respectively.

The presence of *Brevibacillus* and *Thermobacillus* at higher levels in the bulk soil of pots, where lettuce growth was significantly higher, can be observed. Similarly, in the rhizosphere soil of lettuces where growth was more pronounced (namely 5S20 and 5S40), the levels of *Brevibacillus*, *Thermobacillus*, and *Longispora* appear to be increased. Some species of *Brevibacillus* have been reported as plant growth-promoting rhizobacteria [56] and also as a biocontrol agent [57,58].

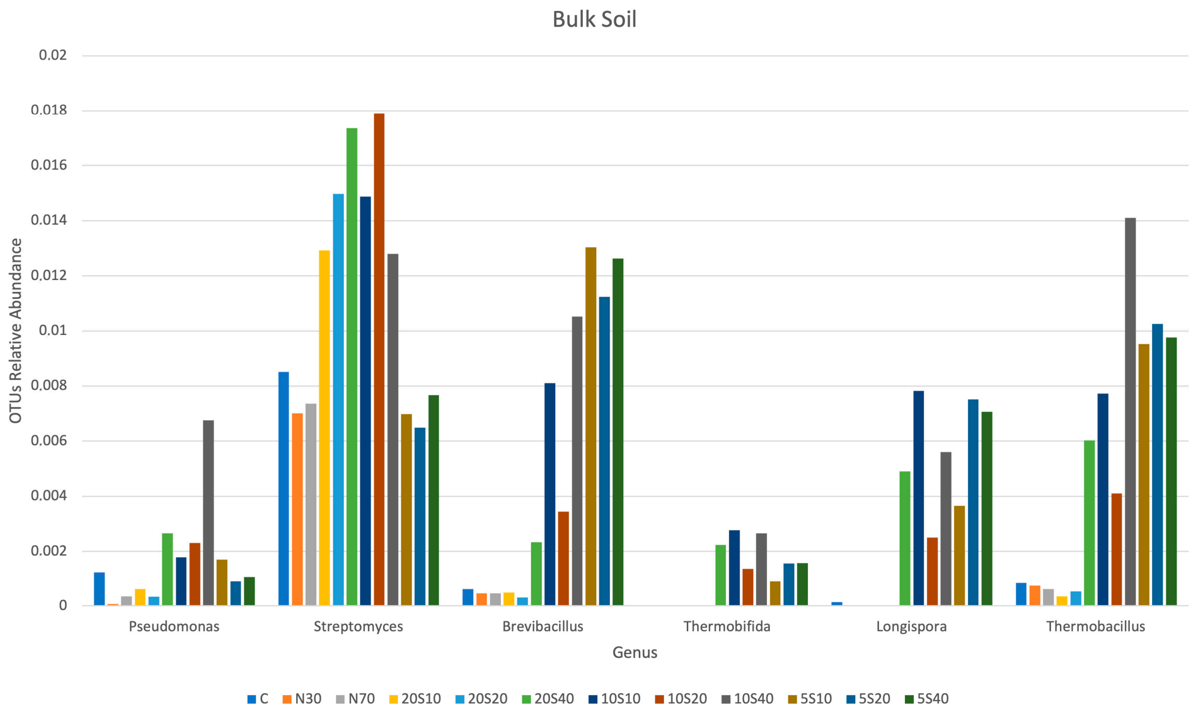

Figure 6. Relative abundances of *Pseudomonas*, *Streptomyces*, *Brevibacillus*, *Thermobifida*, *Longispora*, and *Thermobacillus* identified in the several samples of bulk soil at the end of the experiment.

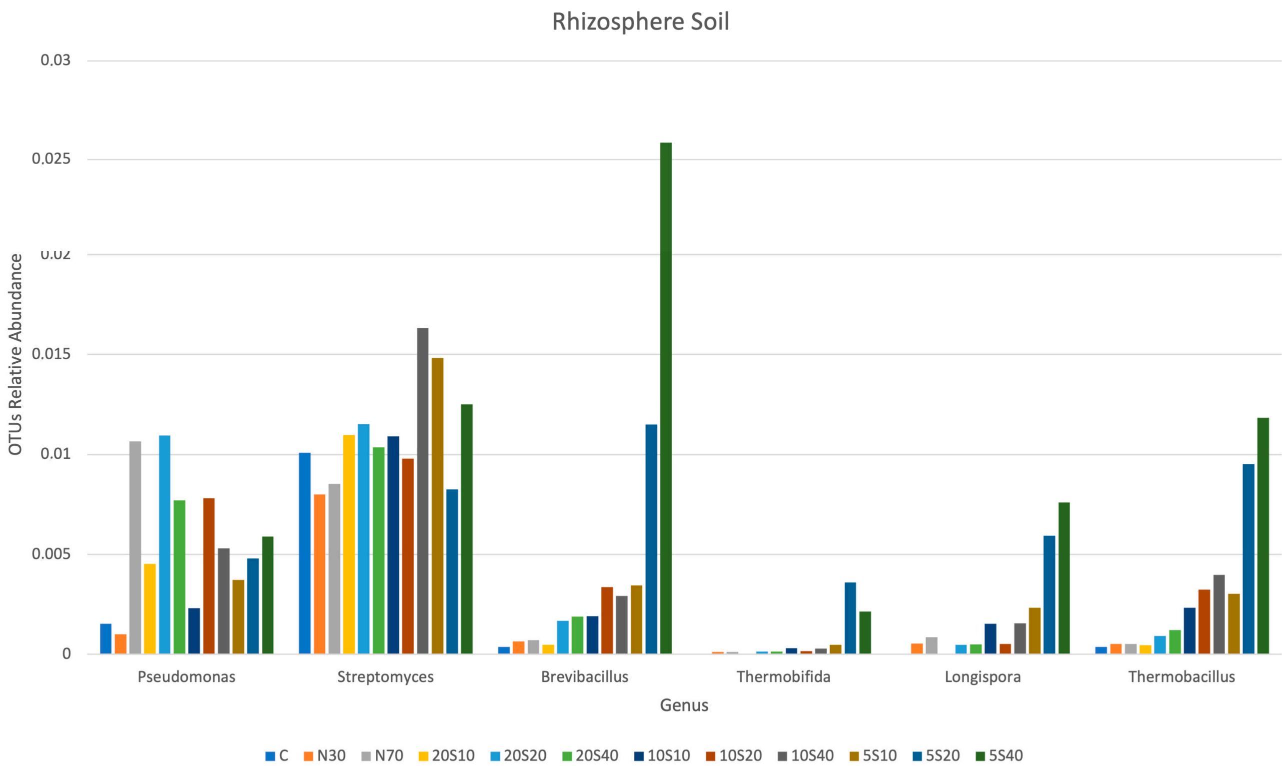

Figure 7. Relative abundances of *Pseudomonas*, *Streptomyces*, *Brevibacillus*, *Thermobifida*, *Longispora*, and *Thermobacillus* identified in the several samples of rhizosphere soil of the lettuces.

3.3. Influence of Soil Bacteriological Composition on Lettuce Growth

3.3.1. Bacteriological Composition

The analysis of Figure 1 clearly demonstrates that the use of organic compost results in increased lettuce growth. However, this growth depends on both the type of compost and the applied dosage. Based on the results from Figure 1, conditions with the highest and lowest growth were selected, and the samples were grouped into two groups (High for higher growth—5S20, 5S40, 10S40; and Low for lower growth—10S10, 20S10, 20S20). Biomarker research aimed at identifying differences in bacterial abundances between the low-growth and high-growth group samples was performed using a LEfSe analysis, with the results filtered for high significance and a minimum LDA effect of three. The summary of the most significant outcomes of the LEfSe analysis can be observed in Figure 8.

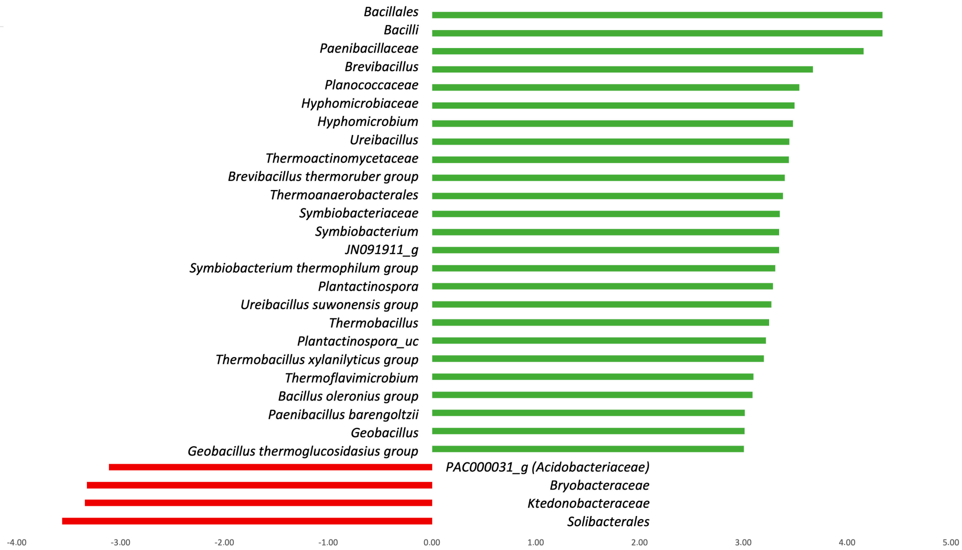

Figure 8. Linear discriminant analysis (LDA) scores computed for bacterial features differentially abundant in soil from the "Low" group (red) and "High" group (green) samples.

Soil microorganisms display vast diversity, forming intricate interrelations, making it challenging and inaccurate to attribute a specific soil effect to a single microorganism. Nevertheless, when observing two sample groups with significantly disparate growth-promoting capacity due to distinct organic fertilizer additions, substantial differences in the relative abundance of certain bacteria can be relevant. These bacteria, functionally, may influence soil qualities critical for adequate lettuce growth. The majority of taxa showing substantial differences between the "Low" and "High" groups are more prevalent in the "High" group. This is particularly evident for bacteria of the *Bacilli* class, such as the *Paenibacillaceae* family, containing species capable of enhancing crop growth via biological nitrogen fixation, production of indole-3-acetic acid (IAA), phosphate solubilization, siderophore release, and protection against some phytopathogens [59]. Moreover, the genus *Brevibacillus* is frequently associated with plant growth, possessing nitrogen fixation capacity and biocontrol activity against certain pathogens [56,60]. Conversely, members of *Solibacterales* order in soils, already described as indicators of soil degradation [61], are heightened in the "Low" group samples. Characterization of the bacterial community in the composts after a maturation period of 176 days [23] revealed that each compost harbored a distinct microbial community. The 5S and 10S composts were particularly rich in potentially beneficial bacteria that promote plant growth and protect against pathogens, such as *Brevibacillus*, *Bradyrhizobium*, *Bacillus*, and *Paenibacillus*. This may have positively influenced the development of the microbial flora in the bulk soil and rhizosphere during lettuce growth, thereby contributing to the efficacy of the 5S and 10S composts in enhancing lettuce yield.

3.3.2. Functional Analysis

The functional roles of the bacterial populations in the various samples were determined using the FAPROTAX database, which places a strong emphasis on the ecological and metabolic functions of bacteria [27]. This database-driven approach links taxonomic classification with current knowledge of microbial metabolism and behavior to infer metabolic pathways and enzymatic functions, and it excels in its ability to predict functions based on the literature of cultured taxa. The use of this method in soil samples has already been evaluated [62], and like all functional inference methods, it has both advantages and limitations. One of the most notable limitations is the fact that certain species are not yet considered, particularly in some functions important for soil microbiology, such as nitrification [62]. The application of this method across all studied samples, with an awareness of the mentioned limitations, clearly demonstrates (Figure 9) a distinct functional profile of the samples over time, as expected.

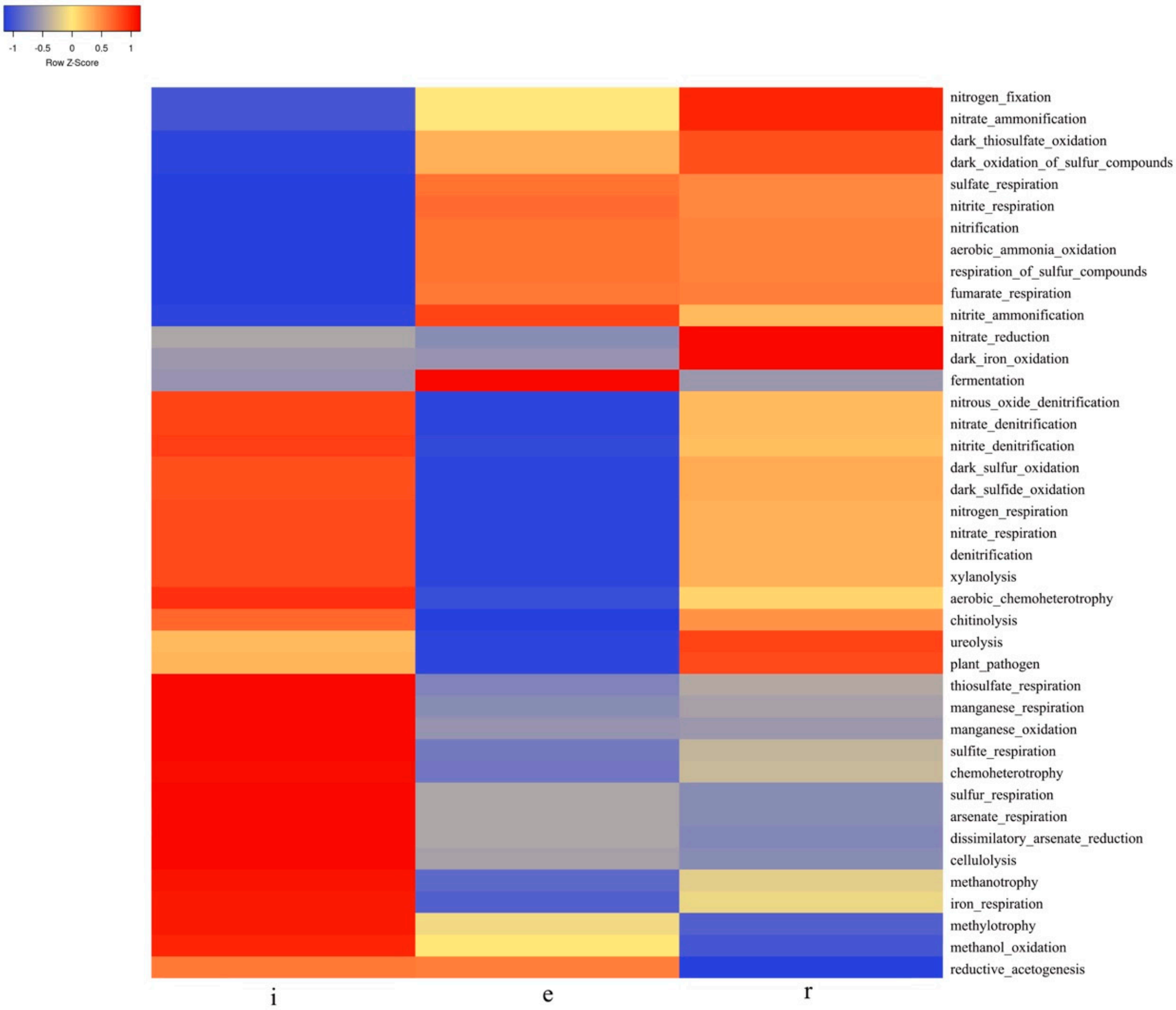

Figure 9. Features abundance cluster heatmap based on dominant functions that were identified in samples based on FAPROTAX database; i—bulk soil at the beginning of the experiment; e—bulk soil at the end of the experiment; r—rhizosphere soil.

The initial samples (group "i") were analyzed immediately after mixing the soil with different compost products (each with its own bacterial population) in varying proportions. In this case, the functional inference may not accurately reflect the bacterial functions in that specific soil, as there was insufficient time for a true microbial community and network of microbial relationships to be established. As a result, the inferred functions represent a somewhat meaningless sum of the functions that could be inferred from the taxonomic composition of the soil and the compost products. Conversely, the samples analyzed at the end (group "e"), when the lettuces were harvested, naturally reflect the functional profile of the bacterial population present in that soil, as there was time for a true microbial consortium to develop in each pot. These samples, as can be observed from Figure 9, present a functional profile clearly distinct from that determined for the bacterial population of the rhizosphere, as expected, particularly due to the complex interrelationships between bacteria and plants observed in this site.

A more detailed comparative analysis of the functions associated with soils of the pots where organic compost was added and the lettuce growth was the highest or the lowest (according to Figure 1) can be observed in the heatmap in Figure 10.

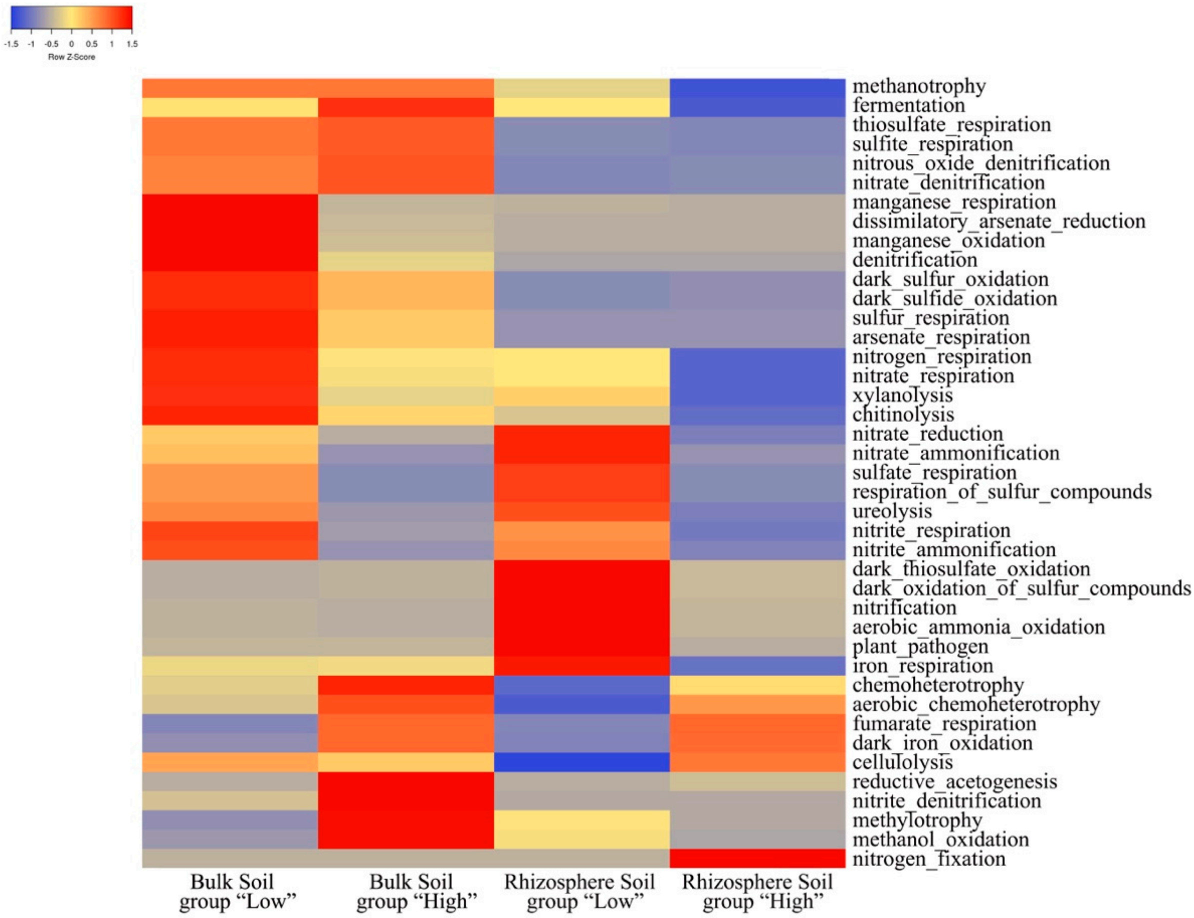

Figure 10. Features abundance cluster heatmap based on dominant functions that were identified in samples based on FAPROTAX database.

In fact, a comparison of the functional profile of the two sample groups already mentioned in Section 3.3 (high and low lettuce shoots fresh weight) reveals some relevant differences that may help to explain the observed difference in lettuce shoot fresh weight. Indeed, when compared, the soil of the pots where growth was most reduced ("Low" group) exhibits a functional profile with some elevated functions, such as denitrification and dissimilatory arsenate reduction, which could have a negative effect on the availability of essential nutrients for the plant, such as nitrate, leading to a negative impact on its growth. The potential conversion of arsenate (AsO_4^-) into arsenite (AsO_3^-) under anaerobic conditions can also have negative consequences for nutrient uptake and root development, particularly due to the presence of arsenite. In fact, although poorly understood, the presence of arsenite may cause root growth inhibition [63]. In contrast, the soil of the pots of the "High" group plants shows a functional profile with a higher incidence of functions that can contribute to nutrient cycling, improve soil structure and water retention, remove potentially toxic compounds from the soil, and produce plant growth-promoting substances. All of these functions can, in fact, directly or indirectly benefit plant growth and productivity. In fact, these soils are more abundant in functions related to chemoheterotrophy, which is a fundamental microbial process in which organic matter is broken down, playing a crucial role in nutrient cycling by releasing essential nutrients, such as nitrogen, phosphorus, and sulfur, from organic matter back into the soil, allowing these nutrients to become available for plant uptake, supporting their growth and development. Additionally, some methylotrophic bacteria can produce plant growth-promoting substances, such as phytohormones, which can stimulate root elongation, shoot growth, and nutrient uptake [64,65].

The rhizosphere soil also presents some highly relevant differences between the two groups ("High" and "Low") as the nitrogen fixation function is much increased in the rhizosphere of the "High" group. This is a crucial process in the nitrogen cycle, converting atmospheric nitrogen into ammonia, which, after conversion to ammonium, can then be assimilated by plants for their growth and development. An increase in nitrogen fixation in the lettuce rhizosphere would provide plants with a readily available source of nitrogen, which could lead to enhanced plant growth, resulting in increased lettuce shoot fresh weight. Soil from the rhizosphere of lettuce shoots from the "Low" group exhibits some activities, potentially influencing essential nutrient balance and availability, such as increased rates of aerobic ammonia oxidation, nitrate ammonification, and nitrate reduction, which could impact plant growth. In addition, plant pathogens are increased in this soil, mainly due to the presence of *Rathayibacter tritici*, *Rhodococcus fascians*, and *Stenotrophomonas maltophilia*, classified as plant pathogens in the FAPROTAX database. Interestingly, the presence of bacterial genera, usually recognized as having biocontrol activities, is significantly increased in the "High" group, as described previously. There is evidence that organic amendment, biostimulation, and biodegradation using organic substrates can be useful for soil health [66]. The use of biochar, for instance, has been shown to increase the levels of beneficial microbes as biocontrol agents, thereby enhancing disease resistance in crops like strawberries [67]. The use of bio-based materials (BBMFs) in fertilizers has also shown promising results. Barquero et al. [68] demonstrated that these materials modulate the soil's bacterial community, increasing the abundance of plant growth-promoting rhizobacteria (PGPR). The predominance of PGPR in soils treated with BBMFs may explain why these fertilizers perform similarly or even better than conventional ones, as PGPR can enhance nutrient availability, improve plant growth, leading to higher productivity, and provide protection against pathogens [69], making them a critical component in sustainable agriculture.

4. Conclusions

Composts combining kiwifruit waste and wheat straw exhibit potential for enhancing lettuce yield. However, excessive straw in compost hampers short-term lettuce growth and nutrient absorption. To optimize crop yields, it is advisable to apply compost at rates between 20 and 40 t·ha^{-1}, with 5% or 10% straw. Compost containing 20% straw may increase soil organic matter, but it does not immediately enhance crop yield. Soil amendment choices significantly influence bacterial communities in both soil and rhizosphere, with mineral N negatively impacting diversity. Kiwi waste compost, serving as an alternative to mineral N fertilizer, improves soil conditions, contributing to enhanced lettuce yield and promoting the circular economy.

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