

## ARTICLE

# The Impact of Bio Compost Use on the Agricultural Soil Microbiome: Evidence from a Laboratory-Scale Study

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## ABSTRACT

Composted organic amendments are increasingly recognized as viable alternatives to synthetic fertilizers, driven by the rising interest in organic waste recycling and sustainable soil management. This study aims to evaluate the long-term effects of bio compost, derived from organic waste, on the chemical and microbiological properties of agricultural soils. A one-year laboratory-scale experiment was conducted using nine microcosm replicates with increasing doses of compost (ratios 1:1, 1:2, 1:4), applied in both powder and granular forms, with and without the addition of water. Microbiological analysis based on 16S rRNA gene sequencing revealed significant shifts in microbial composition. The results demonstrated a consistent increase in microbiological DNA concentration in samples amended with bio-compost, showing a 50% increase from the initial concentration. Furthermore, regarding species composition, bio-compost altered the bacterial population in favor of the predominant species introduced into the soil, indicating a selective enhancement of the bacterial community. Additionally, the addition of water did not affect either the quantity or quality of the bacterial composition. Microbial biomass significantly improved following compost application, with powdered formulations proving more effective than granular ones, and drought conditions often eliciting more pronounced responses. These findings demonstrate that the amendment can improve soil quality by minimizing disruption to microbial communities and promoting long-term soil fertility. Adopting this approach appears beneficial for circular, bio-based agricultural systems.

**Keywords:** Microbial Consortium; Bio Compost Impact; Metagenomics Analysis; Bacterial Community; Microbiome Evolution

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# 1. Introduction

Compost is a nutrient-rich organic fertilizer; therefore, composting represents a sustainable method for reusing organic waste and minimizing the use of synthetic fertilizers in agriculture<sup>[1,2]</sup>. The excessive use of chemical fertilizers leads to environmental pollution, underscoring the need for alternative fertilization strategies<sup>[3,4]</sup>. A promising alternative to chemical fertilizers is compost derived from organic waste, which enhances soil quality and health and may help mitigate greenhouse gas emissions<sup>[5]</sup>.

Compost produced from the organic fraction of municipal solid waste (OFMSW) has demonstrated potential benefits for soil properties and plant growth<sup>[6–8]</sup>.

Using compost derived from waste organic matter is one of the most effective approaches to increasing soil organic matter (SOM). However, its effectiveness depends on several factors, including the quality and quantity of the compost, its moisture content, soil texture, clay content, and the management practices employed. Mature compost, which contains higher levels of stabilized organic carbon, tends to be more effective than immature compost in boosting organic matter content<sup>[9,10]</sup>. Higher organic matter levels are closely associated with higher soil organic carbon<sup>[11]</sup>. The application of compost has demonstrated benefits for soil physical structure, particularly by reducing bulk density. This reduction occurs as low-density organic material is incorporated into the mineral matrix, leading to structural improvements. Lower bulk density typically enhances porosity, especially through increased meso- and macropores, which result from improved soil aggregation driven by biological activity<sup>[12]</sup>. Reduced bulk density corresponds to greater pore space and improved soil workability<sup>[13]</sup>. Clay and sandy soils, when combined with well-moistened organic matter and readily decomposable organic compounds, promote the formation of micro- and macro-aggregates, thereby improving stabilization and aggregation. Additionally, fungal hyphae, fine roots, and polysaccharide-rich microbial exudates contribute to the stabilization of macro-aggregates<sup>[12]</sup>. Multiple factors influence the effects of compost on soil properties, including its maturity, application frequency, application rate, and the characteristics of the receiving soil. The literature suggests that compost produced from domestic waste or manure can significantly improve soil structure. It also indicates

that the application of mature compost improves soil physical, chemical, and biological properties, primarily due to its high organic matter content and active microbial populations<sup>[12,13]</sup>.

Compost microbial communities are influenced by the type of raw material and the composting process employed<sup>[14,15]</sup>. In these composts, the dominant bacterial taxa belong to the phyla *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*<sup>[16,17]</sup>. These taxa have been detected in varying proportions across different organic waste composts. However, the total microbiota, including both beneficial and pathogenic microorganisms, in composts derived from domestic waste have not been investigated<sup>[18]</sup>.

Recent advances in metagenomics have revolutionized the study of microbial communities across diverse environments, including composts derived from organic waste. Among the methodologies, 16S rRNA sequencing has demonstrated significant potential for analyzing microbial populations in environmental samples such as compost. This approach enables the identification of bacterial and archaeal taxa and the quantification of their relative abundance under various composting conditions. Furthermore, analyzing microbial dynamics in organic waste compost provides valuable tools for distinguishing between beneficial and pathogenic microorganisms across different compost types. Understanding these microbial dynamics helps harness the advantages of composts and evaluate their suitability as bio-organic fertilizers, thereby enhancing soil health and promoting plant growth<sup>[19]</sup>.

Despite the growing body of research on compost amendments, several knowledge gaps remain. There is limited information on how different physical formulations of compost (e.g., powder versus granular) influence soil microbial community dynamics. Furthermore, most studies have focused on plant–soil systems, while the direct interactions between compost and soil microbiota in the absence of plants remain poorly understood. Additionally, the selective effects of commercial bio-composts on native soil microbial communities under varying moisture conditions have not been thoroughly investigated.

Considering the above, this study aimed to investigate the interactions between commercial bio compost and microbiologically characterized soil. Specifically, the research focused on the influence of the soil microbiome over time.

Using a laboratory-scale microcosm experiment, this study enabled detailed observation of soil changes induced by compost application and provided insights into the broader environmental and agronomic impacts of using this amendment. This study contributes to clarifying the roles of compost formulation, dosage, and moisture conditions in shaping soil microbial communities, thereby addressing key gaps in current knowledge.

## 2. Materials and Methods

### 2.1. Laboratory Test Setup

The research activity involves the use of bio compost in its two commercial forms: powder and granular. It was added to soil from uncultivated agricultural land. Nine treatments were set up with increasing concentrations of bio compost, as indicated in the treatment scheme. The treatments were divided into two groups: (a) the dry line, where no water was added to the soil throughout the experiment, and (b) the

water line, in which 5 mL of water per day was added. The treatments (**Figure 1**) are listed below:

- Treatment 1 control soil (dry and water lines);
- Treatment 2 control granular bio compost (dry and water lines);
- Treatment 3 control powder bio compost (dry and water lines);
- Treatment 4 soil: granular bio compost = 1:1 (dry and water lines);
- Treatment 5 soil: powder bio compost = 1:1 (dry and water lines);
- Treatment 6 soil: granular bio compost = 1:2 (dry and water lines);
- Treatment 7 soil: the amendment powder ratio = 1:2 (dry and water lines);
- Treatment 8 soil: the amendment granular ratio = 1:4 (dry and water lines);
- Treatment 9 soil: the amendment powder ratio = 1:4 (dry and water lines).

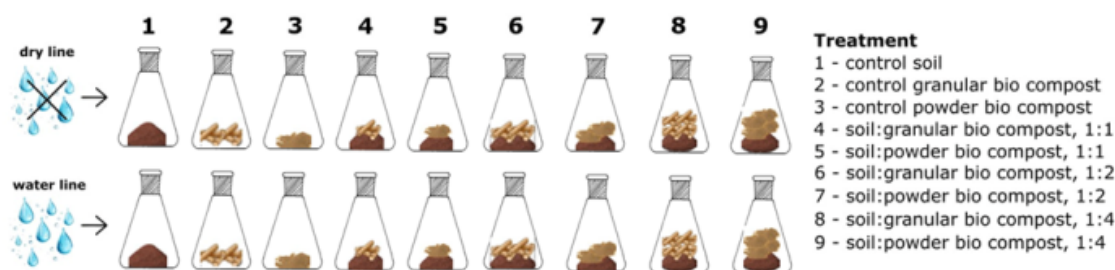


**Figure 1.** Treatment scheme of research activity.

The quantities of soil and bio compost were calculated based on the guidelines provided for the bio compost used, calculated considering the soil's specific weight of  $1,500 \text{ kg}\cdot\text{m}^{-3}$ .

For each test, 5.0 kg of pelletized amendment compost was premixed with the excavated soil and then returned to the excavation site. Additionally, 2.5 kg of powdered amendment compost was premixed with the excavated soil and similarly reintegrated into the excavation. Based on these quantities, the individual test amounts were calculated by simulating a soil sample of 187.5 g. The resulting values for the tests are as follows:

- a) Treatment soil: granular bio compost = 1:1 = 187.5 g of soil + 5 g of granular bio compost;
- b) Treatment soil: granular bio compost = 1:2 = 187.5 g of soil + 10 g of granular bio compost;
- c) Treatment 8 soil: the amendment granular ratio = 1:4 = 187.5 g of soil + 20 g of granular bio compost;
- d) Treatment soil: powder bio compost = 1:1 = 187.5 g of soil + 2.5 g of powder bio compost;
- e) Treatment soil: the amendment powder ratio = 1:2 = 187.5 g of soil + 5 g of powder bio compost;
- f) Treatment soil: the amendment powder ratio = 1:4 = 187.5 g of soil + 10 g of powder bio compost.

For each series of nine replicas of the dry line, and the water line, monitoring was carried out at 1 month, 2 months, 3 months and one year after the start of the trial.

## 2.2. DNA Extraction

The protocol used for DNA extraction from soil is the standard bead-beating protocol (NucleoSpin Soil, Macherey-Nagel, Germany). DNA was quantified with Qubit™4 Fluorometer (Invitrogen, Thermo Fisher Scientific).

## 2.3. Sequencing

Sequencing pipeline was performed using the MinION device from Oxford Nanopore Technologies (ONT). The library was prepared through a one-step PCR process using the Oxford Nanopore Technologies (ONT) 16S library protocol (Oxford Nanopore Technologies, n.d.). The first step was to amplify and barcode the ~1,500 bp 16S V1V9 region using the 16S Barcoding Kit (SQK-RAB204; Oxford Nanopore Technologies, Oxford, UK). The primers used were: 27F: 5'-TTTCTGTTGGTGCTGATATTGC AGRGTTYGATYMTGGCTCAG-3' and 1492R: 5'-ACTT GCCTGTCGCTCTATCTCCGGYTACCTTGTTACGACTT-3'. PCR conditions were as follows: initial denaturation at 95 °C for 1 min, 35 cycles of 95 °C for 20 s, 55 °C for 30 s, and 65 °C for 2 min, followed by a final extension at 65 °C for 5 min. Barcoded fragments were purified using the AMPure XP system (Beckman Coulter Inc., California, USA). Quantification of the eluted samples was performed using a Qubit fluorimeter (Thermo Fisher Scientific, Massachusetts, USA). Barcoded libraries were pooled to enable sequencing of all samples in one experiment, achieving a final ratio of 50–100 femtomoles in 10 µL of 10 mM Tris + HCl buffer (pH 8.0) with 50 mM NaCl. Library preparation was performed with Rapid Sequencing Adapter (RAP), which contains both adapter sequences and motor proteins, attached to the tagged DNA ends. The prepared library was loaded into the MinION flow cell (Oxford Nanopore Technologies, Oxford, UK), and sequencing was performed.

## 2.4. Bioinformatics Analysis

For each sample, Read1 (forward) and Read2 (reverse) reads from the MiSeq were downloaded from Illumina Basespace in fastq.gz format. The DADA2 pipeline was then used in R (version 4.2.1) to recover Amplicon Sequence Variants (ASVs) from the amplicon data. Considering the quality scores of the reverse reads, the dual reads were truncated at

300 bp, at 275 bp, and reverse reads at 250 bp. Only reads with quality scores higher than 25 were selected for further analysis. Reads were dereplicated, merged, and chimeras were removed. The Phyloseq package in R was used to construct Operational Taxonomic Unit (OTU) tables. Taxonomy was assigned using the SILVA 132 training set, with a sequence assignment threshold of 97%, which is the default setting in DADA2. The OTU table was filtered to remove OTUs with an abundance below 0.25% to remove spurious OTUs. The “transform\_sample\_counts” function from the Phyloseq package was used to normalize the data. The “estimate richness” function from phyloseq was used to compute the alpha diversity metrics (effective number of species, calculated as the exponential of the Shannon entropy index). MinKNOW was the operating software for Oxford Nanopore Technologies (ONT), which was used for data acquisition, real-time analysis, feedback runs, local base-calling, and data streaming. MinKNOW generated FAST5 (HDF5) and FASTQ files. Raw signal data were stored in FAST5 format and were converted to FASTQ files by Guppy. Guppy, the data processing toolkit for ONT devices, was responsible for barcoding/demultiplexing, adapter cutting, and alignment<sup>[20]</sup>. The software filtered out FASTQ files with quality scores below 7. Passed FASTQ files were uploaded to the cloud-based data analysis platform EPI2ME for further analysis. Reads were then filtered based on quality, and taxonomic classification was performed using the Basic Local Alignment Search Tool (BLAST) against the NCBI 16S bacterial database (v2020. 04. 06) with default parameters of minimum horizontal coverage of 30% and minimum accuracy of 77%<sup>[21]</sup>. Quality control filtering, adapter trimming, and alignment parameters (including identity and coverage thresholds) were applied using the software’s default configuration. ONT MinION reads were aligned to the SILVA 132 database using the GALAXY platform for supplementary taxonomic classification beyond the default MinION database. Sequencing adapters were trimmed from the filtered FASTQ files using the Porechop tool. Single-end reads were pre-processed using the fast tool. Qualified quality phred was selected as 9. Reads shorter than 1,000 and longer than 2,000 were filtered. Taxonomic assignment was performed using Kraken2 (V2.0.8-beta). The confidence threshold was set to 0.1, meaning that at least 10% of the k-mers must match the database records. Kraken2 reports

were visualized in R using the Pavian package.

### 3. Results

Microbiological analysis was performed on both the concentrations of microbial DNA extracted from the different replicates at different sampling times. Subsequently, the microbiological composition of the extracted DNA was analysed.

#### 3.1. Microbial DNA Analysis

Microbiological analysis was performed on the concentrations of microbial DNA extracted from different replicates at various sampling times. Subsequently, the microbiological composition of the extracted DNA was analyzed.

The analysis carried out on the different replicates (**Figure 2**) showed an increase in DNA concentration over time. In all replicates, both with and without the addition of water, compared to the replicate containing only soil, an initial increase in DNA amount was observed up to T1. The maximum values reached were  $753.75 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil for the replicate with water and  $668.75 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil for the replicate without water.

Regarding the replicates with only the amendment, it was observed that for the granular amendment, both in the presence and absence of water, there was a consistent increase over time. The DNA quantity in the replicate without water reached  $1,087.59 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil, while in the replicate with water, it reached  $978.25 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil. For the powder amendment, a constant increase over time was also noted both without water and with water addition, but the patterns differed. In the absence of water, a steady growth was observed, reaching a maximum concentration of  $1,215.86 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil at T4. In the replicate with water, continuous growth occurred up to T2, followed by a greater increase, reaching  $1,079.56 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil. It was noted that the DNA concentration in the powder amendment replicate without water exceeded that of the granular amendment replicate as early as T1. In contrast, for the replicates with water, the DNA concentration in the powder amendment only matched that of the granular amendment

at T3. Furthermore, for both types of amendments, greater growth was observed in the replicates without water compared to those with water addition.

In the soil replicates, a similar trend was observed under both wet and dry conditions. However, diversity was noted in the different concentrations of the amendment. Although the DNA concentration in all replicates consistently increased, the trends of the curves varied. Specifically, for the replicates with a 1:1 ratio, the DNA concentration of the amendment in powder form exceeded that of the granular amendment at T1. The DNA concentration in the replicate with added water was lower than in the replicates without water addition. For the powder product, the DNA concentration was  $1,238.55 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil with water added and  $1,495.82 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil without water. For the granular amendment, the concentrations were  $1,078.53 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil with water and  $1,275.26 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil without water.

Regarding the replicas with a 1:2 ratio, the replicate containing the DNA concentration of the amendment in powder form exceeded the DNA concentration of the granular amendment at T1. Additionally, the DNA concentration in the replicate where water was added was lower than in the replicas without water addition.

Specifically, for the powdered amendment with water added, the concentrations reached were  $1,487.60 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil, and without the addition of water,  $1,684.76 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil. For the granular amendment, the concentrations were  $1,394.59 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil with water added, and  $10,521.52 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil without water.

Regarding the replicas with a 1:4 ratio, the replicate containing the powdered amendment exhibited a higher DNA concentration than the replicate with the granular amendment at T2. The DNA concentration in the replicate where water was added was lower than in the replicas without water addition. For the powdered product, DNA concentrations reached  $2,354.85 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil with water addition and  $2,515.84 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil without water addition. For the granular amendment, concentrations were  $2,258.20 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil with water addition and  $2,318.53 \mu\text{g DNA}\cdot\text{kg}^{-1}$  of soil without water addition.

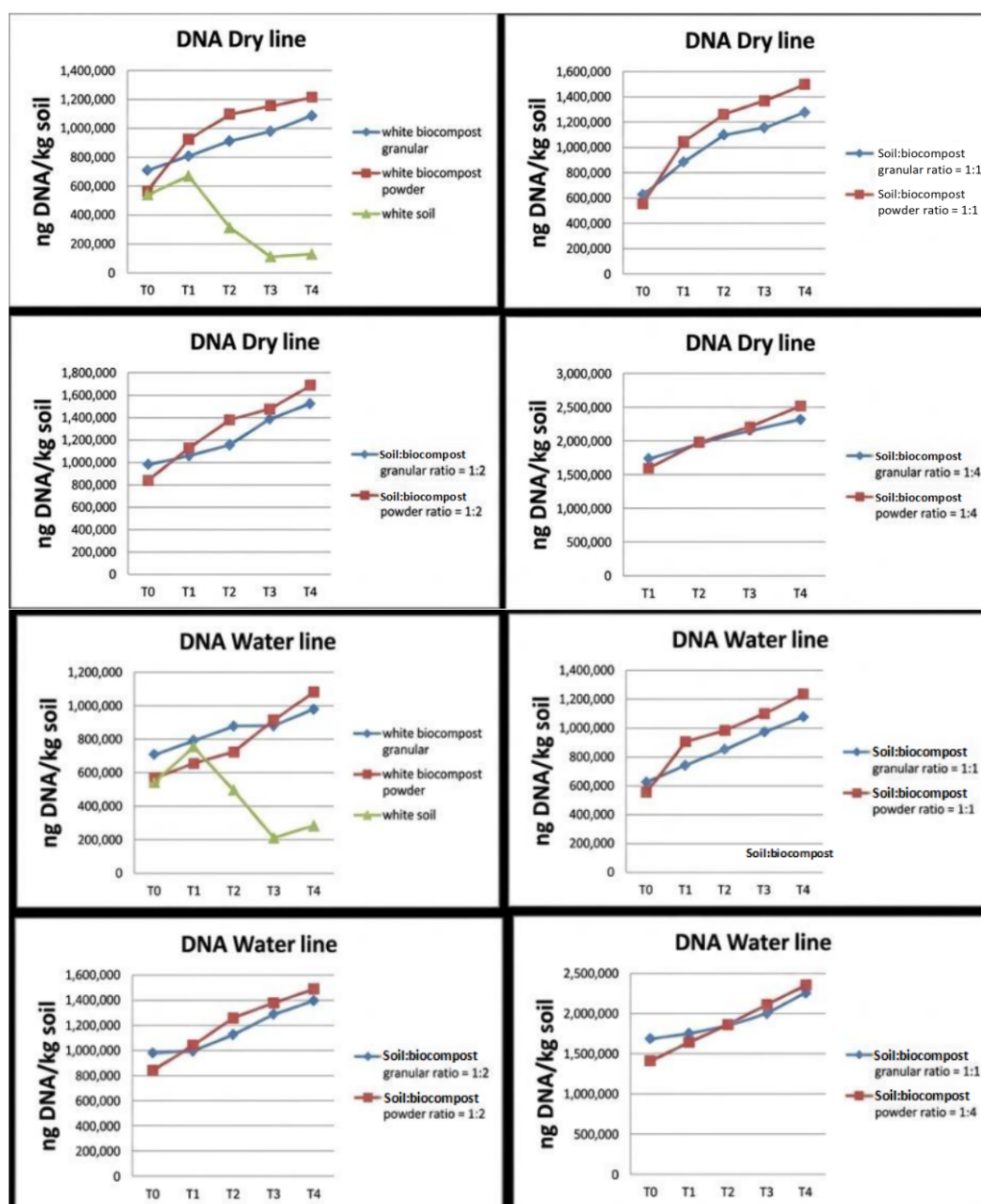


Figure 2. Trend of laboratory tests replicates with and without the addition of water in relation to the DNA concentration.

### 3.2. Sequencing

The sequencing of the three matrices used for the experimentation allowed the identification of the starting bacterial pool. The soil presented a microbiological pool composed of 228 species with a percentage of presence that varies between 9% (*Streptomyces* sp.) and 0.01% (*Thermomonas* sp.). The Granular, the amendment presented a bacterial pool mainly composed of 25 species with a high percentage of presence of *Brevibacillus* sp. 15.78% and *Paenibacillus* sp., 34.01%. Powder amendment presented a bacterial

pool mainly composed of 25 species with a high percentage of presence of *Bacillus* sp. (29.14%), *Saccharopolyspora* sp. (19.94%), *Corynebacterium* sp. 22.63%, *Saccharomonospora* sp. 7.34%, *Enteractinococcus* sp. (2.47%), *Atopococcus* sp. (2.26%). As regards the composition of the bio compost pellets and the bio compost powder, they have only a few species in common and in particular *Actinomadura* sp. (0.05% and 0.40%), *Bacillus* sp. (0.85% and 29.14%), *Mesorhizobium* sp. (0.50% and 0.08%), *Paenibacillus* sp. (34.01% and 0.91%), *Planifilum* sp. (2.87% and 1.09%), *Streptomyces* sp. (4.50% and 0.24%) and *Thermoactinomyces* sp. (0.01% and 0.91%)

(Figure 3a). The tests on the individual matrices show that the species in the soil do not undergo changes over time, with a constant presence of all the previously absent species. The

only peculiarity is the difference in the presence of water, where all species show a greater presence, with an increase between 0.2% and 0.3% (Figure 3b).

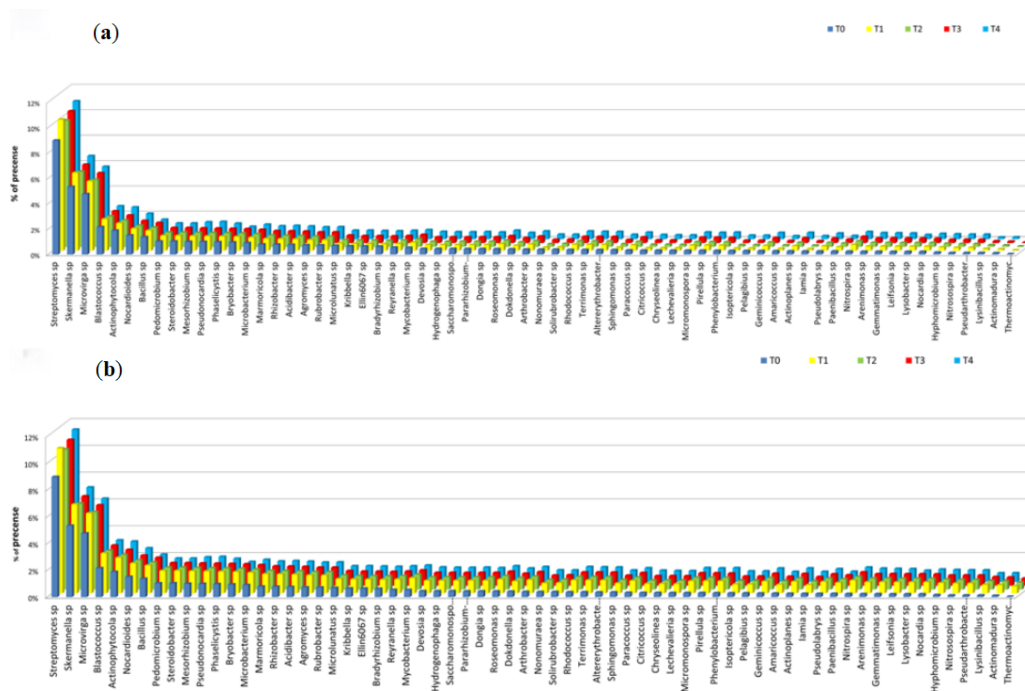


Figure 3. Trend of species in the soil matrix in (a) absence of water, and (b) with the addition of water.

Granular bio compost, at the end of the experiment, 8 species were no longer present (*Agromyces sp.*, *Mesorhizobium sp.*, *Microbacter sp.*, *Phaselicystis sp.*, *Skermanella sp.*, *Steroidobacter sp.*, *Streptomyces sp.* and *Streptosporangiaceae sp.*). Furthermore, a significant decrease was noted for *Brevibacillus sp.*, which went from a presence at T0 of 15.78% to T4 of 6.24% and *Paenibacillus sp.*, which went from a presence at T0 of 34.01% to T4 of 9.54, while an increase was noted for *Planifilum sp.*, which went from a

presence at T0 of 2.87% to T4 with a presence of 31.59% and *Pseudonocardia sp.*, which went from a presence at T0 of 0.50% to T4 with a presence of 28.56%. All other species show a constant trend over time (Figure 4a). The same situation is observed in the sample in the presence of water, with an increase of 0.2% in all species (Figure 4b).

It is noted in powder compost that all the species remain at a stable concentration over time, both in the absence and presence of water (Figure 5).

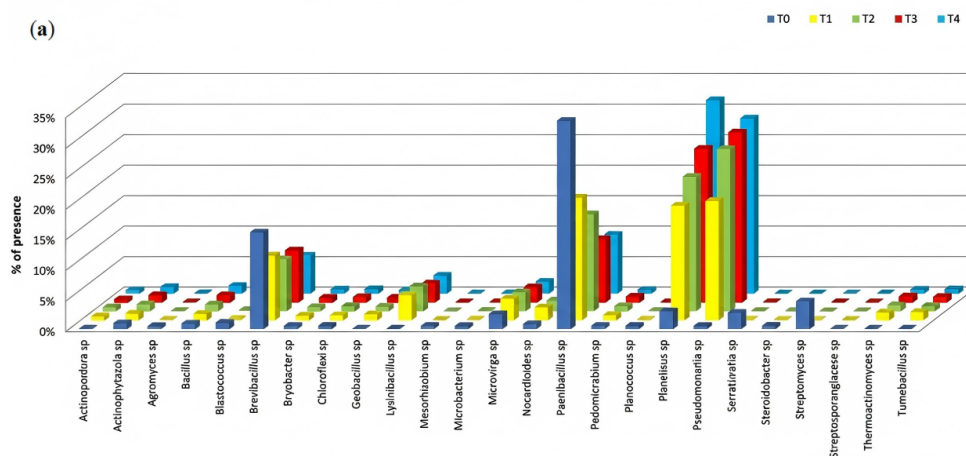


Figure 4. Cont.

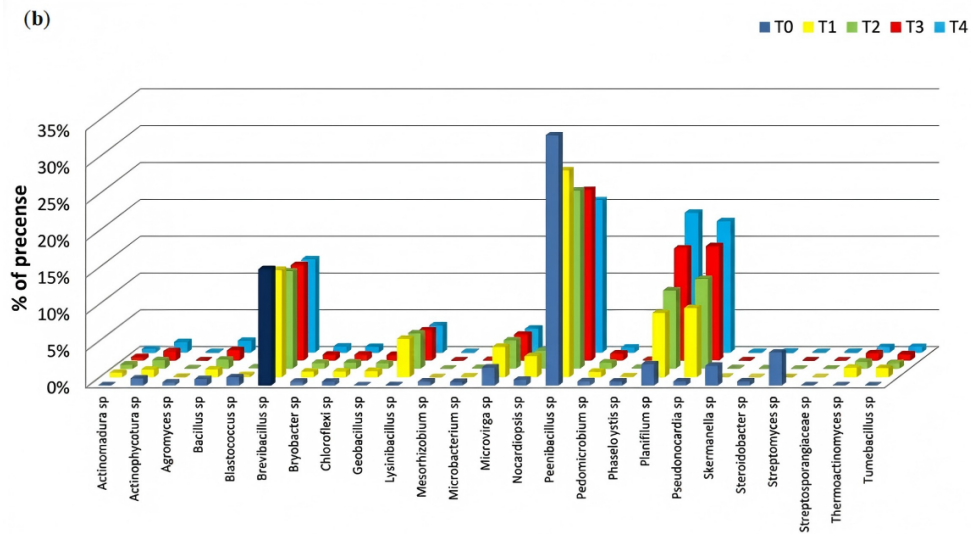


**Figure 4.** Trend of species in the granular bio compost in (a) absence of water, and (b) with the addition of water.

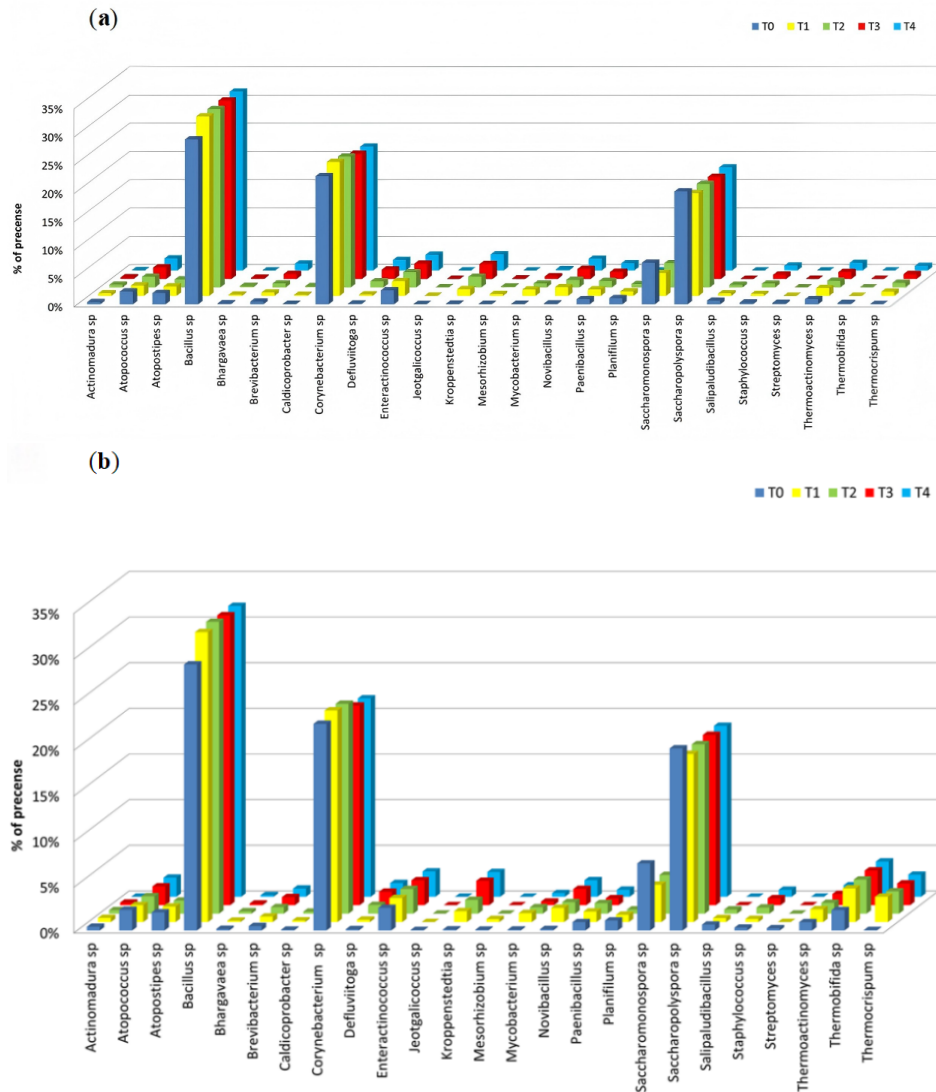


**Figure 5.** Trend of species in the powder bio compost in (a) absence of water, and (b) with the addition of water.

The samples with soil and added bio compost, it is noted that in all the dry tests in which granular bio compost was added, i.e., with concentrations of 1:1, 1:2 and 1:4, the addition of compost caused the disappearance of 30% of the species present in the initial soil sample, a 1% increase in the presence of 36% of the species present in the initial soil and the remaining 34% remained constant over time. Regarding the native species in the bio compost, it is noted that the species that increased their presence over time are *Penibacillus* sp., which increased its presence by 5% in both the sample with a

1:1 and 1:2 soil:bio compost ratio and by 3% in the sample with the 1:4 ratio, and *Pseudonocardia* sp., which showed a 2% increase in all samples. Two species, however, show a decrease in presence over time: in particular, *Brevibacillus* sp. shows a decrease in all samples with the different concentrations of bio compost equal to 5.5% and *Streptomyces* sp. shows a decrease in all samples of 6%. The disappearance of *Streptosporangiaceae* sp. is noted in all the final samples, while all the remaining species (19 species) show a constant concentration over time for all samples (Figure 6).

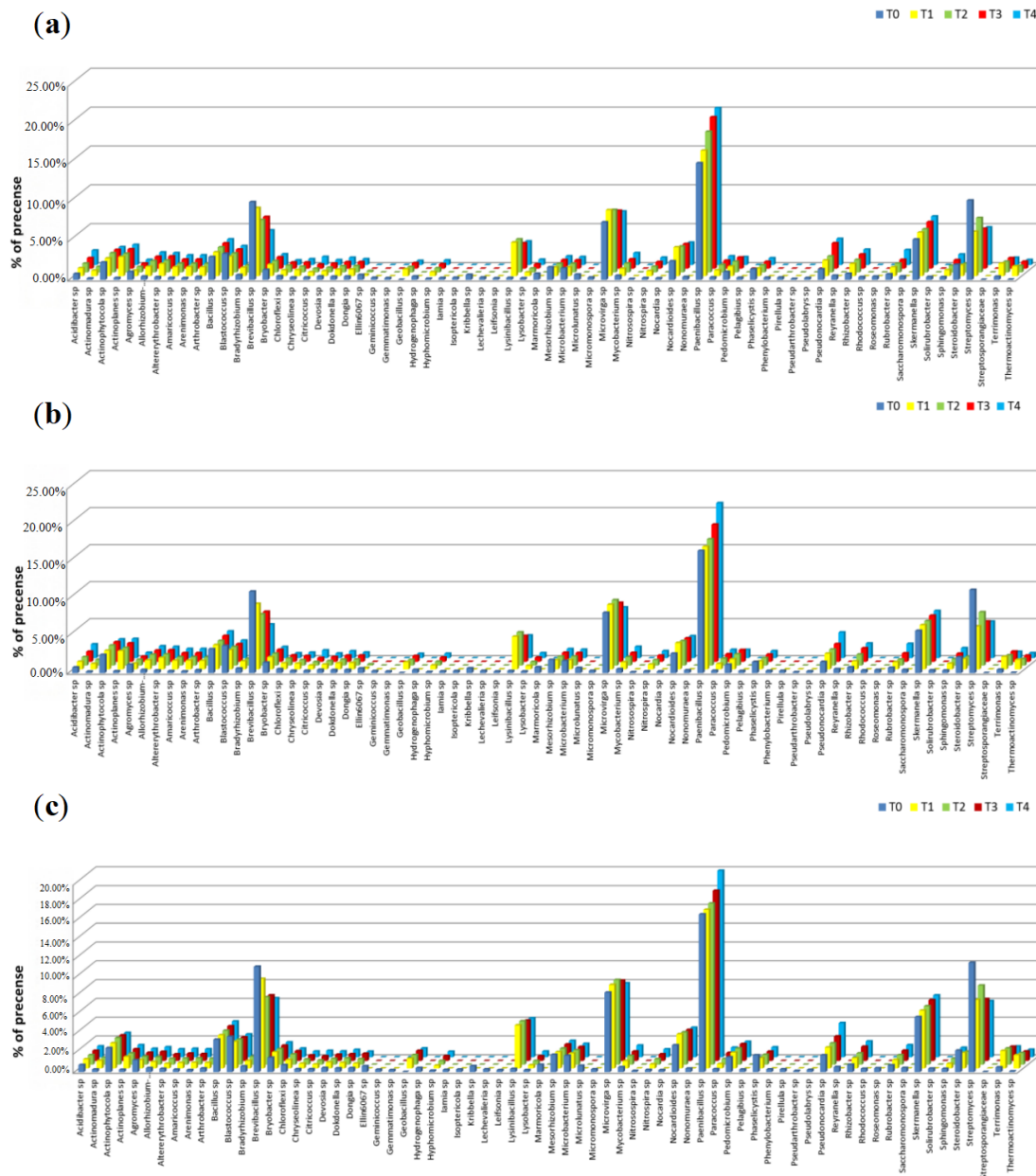


**Figure 6.** Trend of species in: (a) The soil + granular bio compost with 1:1 ratio without water; (b) The soil + granular bio compost with 1:2 ratio without water; (c) The soil + granular bio compost with 1:4 ratio without water.

The series of samples with the addition of water in different proportions shows the same situation as the dry samples with a slight increase in all percentages of presence of 0.2% (**Figure 7**).

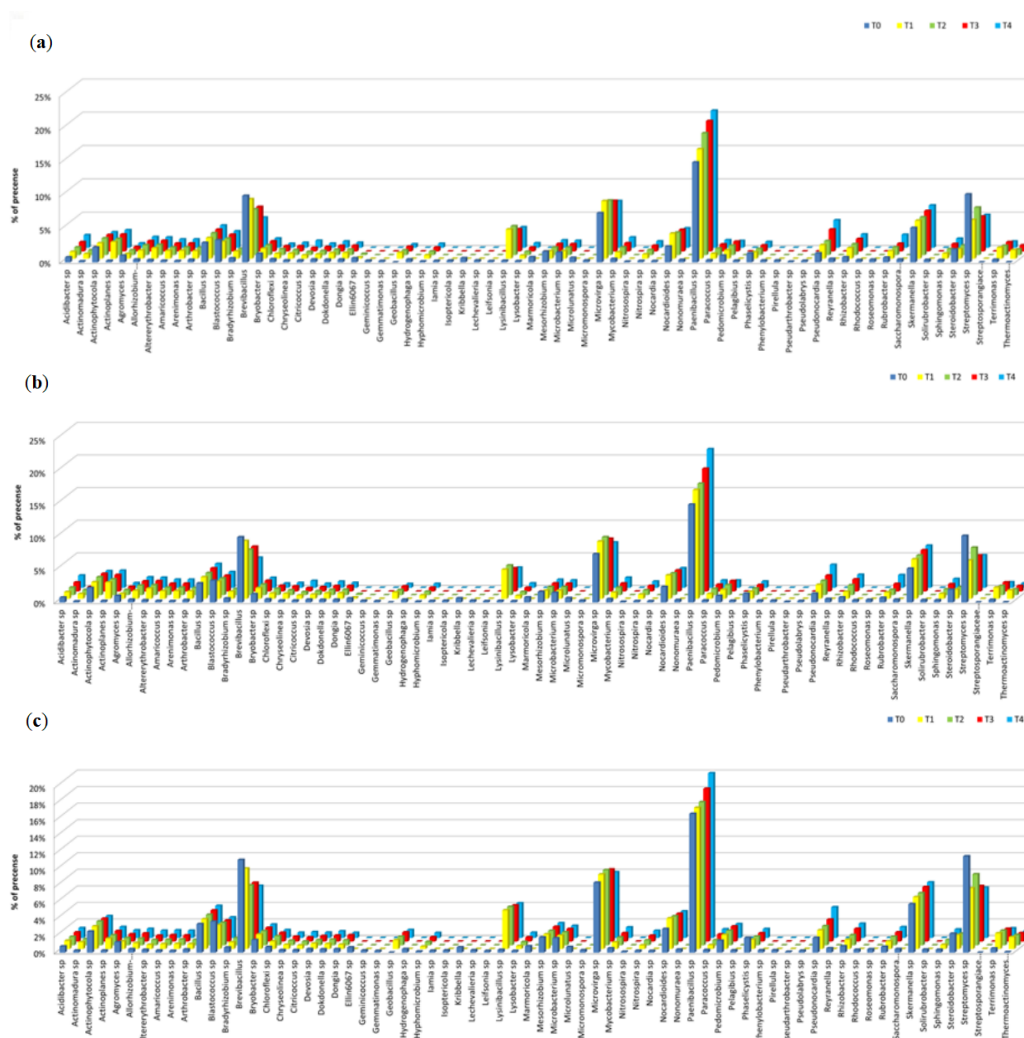


**Figure 7.** Trend of species in: (a) The soil + granular bio compost with 1:1 ratio with water; (b) The soil + granular bio compost with 1:2 ratio with water; (c) The soil + granular bio compost with 1:4 ratio with water.

Samples to which powdered biocompost was added in all dry tests, i.e., with the concentrations 1:1, 1:2 and 1:4, caused the disappearance of 30% of the species present in the starting soil sample, an increase in the presence of 1% for 23% of the species present in the starting soil and the remaining 47% remained constant over time. With regards to the native species of the bio compost, it is noted that the species that increase their presence over time are with an increase of 3% *Penibacillus* sp., an increase of 1% *Thermoactinomyces* sp. and of 0.5% *Brevibacterium* sp., *Caldicoprobacter* sp., *Kroppenstedtia* sp., *Mycobacterium*

sp., *Novibacillus* sp. and *Staphylococcus* sp. Only one species shows a decrease of 0.5% compared to the initial presence, *Saccharopolyspora* sp., while there are 8 species that disappear at the end of the experiment (*Atoposhas* sp., *Bhargavala* sp., *Jeogalicoccus* sp., *Mesoribhozium* sp., *Planifilium* sp., *Salipaludibacillus* sp., *Streptomyces* sp. and *Thermobifida* sp.) (**Figure 8**).

Regarding the series of samples with the addition of water in different proportions, the same situation is shown as the dry samples, with a slight increase in all percentages of presence of 0.2% (**Figure 9**).



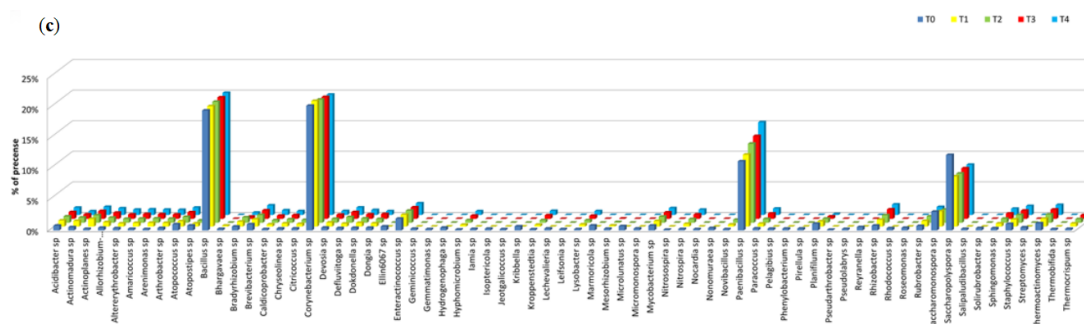


**Figure 9.** Trend of species in: (a) The soil + powder bio compost with 1:1 ratio with water; (b) The soil + powder bio compost with 1:2 ratio with water; (c) The soil + powder bio compost with 1:4 ratio with water.

## 4. Discussion

### 4.1. Microbial Community Shifts and Functional Implications

The results of this study provide strong evidence of the multiple benefits of an organic waste-derived amendment in enhancing biological properties. Based on the experiments conducted, it was possible to define the microbiological interactions between the soil improver and the soil matrix in the absence of plants, across all replicates, both with and without water.

The microbiological data obtained from the study further validate the improvement in soil quality. The microbiome appears more oriented toward nutrient acquisition and symbiotic interactions, as evidenced by the increased concentration of microbial DNA in the soil and the shift in community composition, including a proliferation of species such as *Mesorhizobium* sp.

This shift toward functionally beneficial taxa suggests a transition from a relatively neutral microbial assemblage to a more active, nutrient-driven community. Such changes are typically associated with increased availability of organic substrates introduced by compost, which favor copiotrophic microorganisms capable of rapid growth and nutrient transformation.

Phosphate solubilization and plant growth promotion are known functions of *Bacillus* sp.<sup>[22]</sup>, while biological nitrogen fixation, particularly in the rhizospheres associated with legumes, is attributed to *Mesorhizobium* sp.<sup>[23]</sup>. Furthermore, the suppression of genera such as *Pedomicrobium* sp. and *Pseudonocardia* sp., which are less competitive, has been observed, indicating microbial succession driven by nutrient enrichment<sup>[24]</sup>.

This competitive exclusion can be explained by the increased availability of labile carbon sources, which promote fast-growing taxa over slower, oligotrophic microorganisms. Similar patterns have been observed in other studies where organic amendments induce a shift toward metabolically versatile bacterial groups.

### 4.2. Effect of Compost Formulation (Powder vs. Granular)

Microbiological analyses have shown that, despite a constant concentration of microbial biomass and a stable composition of species present in the soil, the application of powder or granular amendments with or without water increases microbial biomass. Both types of amendments influence the composition of the soil's microbial community, promoting the proliferation of certain species, such as *Bacillus* sp., *Microvirga* sp., and *Thermoactinomyces* sp., while leading to the disappearance of others.

The distinction between powder and granular formulations provides valuable agronomic insights. Powder formulations of the amendment consistently outperformed granular variants, especially under drought conditions.

This difference can be attributed to the higher specific surface area and faster solubilization rate of powdered compost, which likely enhance microbial accessibility to organic substrates. Increased surface contact between compost particles and soil microorganisms may accelerate colonization and enzymatic activity.

The higher solubility and larger surface area of powder particles likely promote faster microbial colonization, as observed by Reeve et al.<sup>[25]</sup> in a comparative study on particle size and the bioavailability of organic fertilizer particles.

### 4.3. Effect of Moisture Conditions

This differential response between irrigation and drought regimes underscores the critical role of soil moisture in regulating microbial activity. Drought-adapted microbial consortia, such as *Bacillus* spp. and *Actinobacteria* spp., tend to dominate under conditions of limited water availability.

The persistence and proliferation of these taxa under both irrigated and non-irrigated conditions suggest that the amendment may mitigate environmental stress by providing protective niches and accessible nutrients, thereby enhancing microbial resilience.

Their proliferation in our study suggests that the amendment may improve drought resilience<sup>[26]</sup>.

### 4.4. Effect of Compost Dose

From a microbiological perspective, a 1:1 ratio is insufficient for improving soil quality, whereas ratios of 1:2 and 1:4 are significantly more effective both quantitatively and qualitatively.

This finding indicates the existence of a threshold level of organic input necessary to significantly alter microbial dynamics. At lower doses, the amount of available carbon and nutrients may be insufficient to sustain microbial growth and community restructuring.

From a systemic standpoint, the results support incorporating the amendment into circular agricultural models, where waste flows are recycled into high-value inputs.

### 4.5. Agronomic and Environmental Implications

The observed improvements in nutrient retention and microbial diversity align with the key objectives of regenerative agriculture and the strategies outlined in the European Green Deal.

The aim of this study is to assess and confirm the critical role of microbial diversity in regulating key biochemical processes and maintaining soil health. A diverse and functionally active microbial community is fundamental for the long-term sustainability and resilience of agroecosystems.

In this context, the selective stimulation of beneficial microbial taxa through compost amendments may contribute not only to improved soil fertility but also to a reduced de-

pendence on chemical fertilizers, yielding positive environmental outcomes.

As highlighted by Schlöter et al.<sup>[27]</sup>, the amendment appears to benefit such a community by supplying nutrients and creating selective ecological niches.

### 4.6. Limitations of the Study

Despite these promising results, several limitations must be acknowledged. The experiment was conducted under controlled laboratory conditions using a microcosm approach, which may not fully represent field-scale dynamics. The absence of plants excludes rhizosphere interactions, which play a crucial role in shaping microbial communities. Additionally, the relatively short experimental duration does not permit assessment of the long-term effects of compost application. Therefore, future studies should include field trials, extended monitoring periods, and plant–soil systems to validate and expand upon these findings.

## 5. Conclusions

This article investigates the effects of two commercial forms of bio compost, granular and powdered, on the microbiome of agricultural soil under controlled conditions. The results indicate that both forms positively affect the microbiome, increasing microbial biomass by up to 50%. Experiments conducted with and without the addition of water demonstrated that water did not influence microbial growth, as similar results were achieved in both cases. The findings are significant for characterizing the microbial species present in both the soil and the bio compost. The study demonstrates that compost amendments act as selective agents, reshaping the native soil microbiome by promoting functionally beneficial taxa involved in nutrient cycling and plant growth promotion. This characterization revealed that the species introduced with the bio compost selectively increased only the non-competitive native species. Notably, an increase was observed in species crucial for the bioavailability of nutrients beneficial to agricultural plants, including *Bacillus* sp., *Mesorhizobium* sp., *Microvirga* sp., *Thermoactinomyces* sp., *Pedomicrobium* sp., and *Pseudonocardia* sp. Finally, the experiment provided essential insights regarding the appropriate doses of biocompost to apply, highlighting that a 1:1 ratio was insufficient and ineffective for proper fer-

tilization, while 1:2 and 1:4 ratios yielded good results with no significant differences between them, indicating similar impacts on agricultural soil. Overall, this study highlights the importance of compost formulation, dosage, and environmental conditions in shaping soil microbial communities and provides useful insights for optimizing the use of bio-composts in sustainable agricultural systems.

## Author Contributions

Conceptualization, A.C. and M.C.; methodology, A.C. and M.C.; validation, A.C. and M.C.; formal analysis, A.C. and M.C.; investigation, A.C. and M.C.; resources, A.C.; data curation, A.C., F.T.L. and M.C.; writing—original draft preparation, A.C., F.T.L. and M.C.; writing—review and editing, A.C., F.T.L. and M.C.; visualization, A.C., F.T.L. and M.C.; supervision, A.C.; project administration, A.C. and M.C.; funding acquisition, A.C. All authors have read and agreed to the published version of the manuscript.

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## Data Availability Statement

All data are available upon request, and through forthcoming publications.

## Conflicts of Interest

The authors declare no conflict of interest.

## AI Use Statement

The authors declare that no artificial intelligence (AI) tools were used in the preparation of this manuscript.

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