Chapter

Advances in Microbial Biotechnology: Lessons from Intensive Agriculture Compatible with Organic Farming

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Abstract

The use of plant-growth-promoting microbes in agriculture is expected to double over the next decade due to several advantages. For example, they have a positive impact on soil health, and product yields and can increase traditional fertilizer's bioavailability, reducing their use. This is based on the diverse metabolic capabilities conferred by microbes which are required by plants for a healthy development. Their application can be based either on microbial isolates or communities. The former comprises a reductionist approach that maximizes microbial load and few metabolic traits. Inversely, the latter focuses on metabolic diversity. Since fertile soils have diverse microbial communities or microbiomes, methods that replicate this habitat at an industrial scale can unlock a new class of bioadditives for organic and traditional farming. Moreover, since microbes can reduce traditional fertilizer use, which is the main contributor to greenhouse gas (GHG) emissions from agriculture, these biotechnologies can help decarbonize this industry. Therefore, in view of the role of microbes in soil health and nutrient management, efforts in fundamental and translational research on this topic are further needed. Thus, this chapter will explore the use of microbial biotechnology in agriculture, with a focus on a case study of a microbiome-based bioadditive.

Keywords: microbial biotechnology, microbiome, bacteria, soil, fertilizers, organic farming, plant-growth promoting bacteria, bioinoculants, bioactivators

1. Introduction

The health of soil depends largely on the microorganisms that colonize this habitat. This microbial community is known as the soil microbiome [1–7]. Similarly, the optimal development of plants is determined by the quality of the soil, in terms of its physicochemical characteristics as well as its microbial load and diversity. Members of the soil microbiome communicate with plants through phytohormones and other small molecules, establishing a mutually beneficial symbiotic relationship [8]. For example, some bacteria help plants obtain nitrogen from the atmosphere and

nutrients from the soil, including metals, phosphates, and others. They can also help plants in situations of physical stress such as drought, or in the fight against pathogens [2–7, 9]. Taken together, the metabolic versatility conferred by microbes can increase crop yields with relatively less fertilizer input. In view of these characteristics, the use of soil microbes as inoculants has gained traction over the past years, especially in traditional and extensive agriculture. However, in this context, organic farming can take advantage of the knowledge gained about microbes and their benefits.

There are two main approaches for formulating microbial inoculants: (i) a reductive approach that focuses on isolating microbes from the complexity of the soil or rhizospheric microbiome, and (ii) the formulation of microbial consortia, or even complex microbial communities. The former focuses on specific metabolic traits [10], whereas the latter takes advantage of the synergies among microbes and their corresponding diverse metabolic capabilities [11]. While there are numerous examples of products based on reductive technologies, microbiome-based solutions are still emerging. However, considering the advantages of the synergistic interplay between the different microorganisms, i.e., the presence of a beneficial bacterium promotes the growth of others alike, there is a need for further research and development on this type of technology.

Using molecular techniques, such as the sequencing of microbial genes present in the soil, it is possible to study and design microbiomes with the desired metabolic attributes [5]. Likewise, it is feasible to compare these engineered microbial consortia with natural microbiomes of rich and unperturbed soils, such as those from a primary forest, or soil associated with the rhizosphere of healthy and vigorous plants. This can potentially serve as a baseline to define how an optimal soil microbiome is constituted. However, plant-associated microbiomes are dynamic in terms of space (plant anatomy) and time (development phase) [11], meaning that plants will be associated with microbes needed, however, only based on those already present in the soil. This underscores the importance of soil microbiome.

Therefore, acknowledging the importance of soil microbial diversity in plant health and crop yields, this chapter will focus on microbiome-based approaches to lower the reliance on traditional fertilizers, while lowering the carbon footprint of agriculture.

2. Microbial biotechnology in agriculture

2.1 Fundamentals in microbial ecology

The layer of our planet that contains life, which is known as the biosphere, contains trillions of microbes. Aside from this large number, what really impacts our life is the genetic, and therefore, metabolic diversity conferred by these microorganisms. They are responsible for the biogeochemical homeostasis in our planet, which involves nutrient cycling including carbon, sulfur, nitrogen, oxygen, phosphorous, and other elements. Since microbes, including bacteria and archaea, have been around for hundreds of years prior to plants and animals, they have set the biochemical stage where multicellular organisms have developed. This points towards the coevolution of microbes with every other organism that evolved afterwards, from fungi to plants, to animals [12, 13].

Due to our intertwined dependency, several metabolic functions that are required by larger organisms, including plants, are strictly conferred by microbes, such as

nitrogen fixation, nutrient acquisition, vitamin synthesis, and others [14]. Therefore, the soil microbiome, or the pool of microbes present in this habitat, determines how rich and dynamic it is, and thus, the extent of metabolic activities that plants can rely upon. The higher the soil microbial diversity, the higher the chances plants will develop optimally. Unfortunately, current farming practices that do not protect the soil, such as tilling, intensive use of agrochemicals, and others, have reduced soil microbiome diversity, which in turn, makes plants require higher fertilizer input, potentially creating a vicious cycle [15, 16].

Microbial communities are composed of individuals, of the same or different species, or even strains. These individuals, in turn, are part of populations that interact with others, forming communities. Every level has an impact on each other. Therefore, considering genetic diversity, each individual contributes to the function of the ecosystem. Moreover, the interactions can take different forms, depending on the benefit each member acquires. For instance, there is mutualism when both partners benefit. Commensalism, when one of them benefits. Amensalism when one of them inhibits the growth of the other, for instance, by generating antibiotics. It can be a competing relationship when both need the same nutrients. One organism can consume the other, in a predator-prey-type relationship. And finally, synergism, when there is greater benefit for both partners.

The study of microbial communities, or microbiomes, greatly benefited from advances in high-throughput DNA sequencing techniques. Microbiome members can be identified by sequencing the prokaryotic 16S rRNA gene, which functions as a bar code. Other technologies include shotgun metagenomic sequencing, which, in addition to answering the question of "who", also identifies other genes present in the sample. This allows for the functional determination of the microbiome. The community can also be studied in terms of its diversity, via the calculation of alpha and beta diversity indices [17]. The former describes how diverse a sample is (or community), whereas the latter compares diversity between samples. Typically, the soil and rhizosphere are colonized by bacteria belonging to the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. The biological and physicochemical conditions determine their relative abundance [18].

A previous study on different soil types in terms of their microbial cellular abundance and genome complexity revealed that pasture and arable soil contain one order of magnitude higher cell count than forest and even marine soils [19]. However, when looking at the genome complexity, forest and marine soils can be 17 and 32 times more complex, respectively, than arable soil. Remarkably, pasture soil was equivalent to forest. Since genome complexity is required for metabolic diversity, these observations highlight that microbial cell quantity does not necessarily confer quality. Moreover, the fact that pasture soil is more diverse than arable soil might be due to cow dung. Interestingly, manure is a key element in organic fertilization. In that sense, a recent study demonstrated an impact on the soil microbiome by compost application, increasing its diversity, and thus its quality [20]. Overall, these observations suggest that using complex microbial communities on the soil can impact its microbiome, improving its qualities. In other words, they work as soil amendments. Such practices have been traditionally used in organic farming, successfully. This emphasizes the role of microbes impacting the soil microbiome and its properties, which leads to the success of this type of fertilization.

As previously mentioned, microbes interacting with the rhizosphere have a fundamental role during the development of the plant [8, 21, 22]. The structure of the

rhizospheric microbiome depends on the developmental stage of the plant. Thus, as the plant grows, it generates compounds, such as phytohormones, that can be recognized by microbes, promoting their assembly around the root. This dynamic is the foundation for the synergetic interrelation between plants and their root microbiome. Thus, a diverse soil microbiome is needed to supply plants with the key microbes that will sustain their optimal growth.

Considering that microbes are metabolic powerhouses, advances in microbial biotechnology have resulted in technologies that take advantage of their metabolic capabilities in agriculture, and beyond.

2.2 Bioactivators, biofertilizers, bioinoculants, *etc.* what are they?

Biofertilizers are products whose active ingredients are not "chemical" based, but rather of biological origin. However, there are many terms that are applied to this general description, depending on the geographical location and alternatives in the formulations. Some of those terms are bioactivators, biostimulants, phytostimulants, biologics, bioinoculants, bioformulations, bioadditives, etc. However, to consolidate its definition, the European Biostimulants Industry Council (EBIC), defines biostimulants as: "substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality." Similarly, the 2018 United States Farm Bill defines bioinoculants as "a substance or microorganism that, when applied to seeds, plants, or on the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, or crop quality and yield". Thus, both agree that it must be of biological origin and enhance plant growth by any means. In addition to the characteristics previously mentioned, others include (i) the increase of beneficial microbes associated with the plants; (ii) their capacity to provide non-traditional plant nutrients; and (iii) their ability to improve soil quality and health. In the context of organic farming, the fact that biostimulants can increase nutrient uptake indicates that they can act as "bioactivators" of traditional fertilizers. This can help transition large-scale agriculture to a more sustainable path. Similarly, organic farming can get a boost from the use of microorganisms.

When developing biostimulants, there are several characteristics that must be followed [14]. For instance: (i) they should not be toxic, and must be safe for the environment and animals; (ii) they should be of natural origin (i.e., isolated from the natural environment); (iii) they should be bioactive (i.e., being able to interact with plants or intended target)'; (iv) ideally, robust in terms of their compatibility with formulation ingredients; (v) they must be cost-competitive with the established market; (vi) they must have a positive effect either on the plant (biomass or product yields), and finally, in order to protect producers from technologies of dubious bioactivity, (vii) the biostimulants must be evaluated in the field, ideally over multiple seasons.

The active ingredients of biostimulants can consist of numerous biological agents. Some of those can include beneficial bacteria, which are also known as plant-growth-promoting bacteria. It can also include beneficial fungi, such as *Trichoderma spp*. In addition to these whole cells, it can also include microbial byproducts, including humic acids and fulvic acid, seaweed extract, protein hydrolysates, amino acids and peptides, and biopolymers, including chitosan. Some formulations also include inorganic compounds, which are basically trace elements and other nutrients.

In most cases, microbes are isolated from the environment, such as soil, rhizosphere, lakes, etc. They can also be isolated from environments exposed to extreme

conditions, such as drought, with the purpose of conferring those attributes to plants [23, 24]. As previously mentioned, the approach can be based on using microbial isolates with their limited metabolic capabilities (i.e., reductive), or communities, to take advantage of their diversity and synergetic interactions (i.e., microbiomes). This chapter will focus on the latter.

While advances in microbiome-based technologies are still in their infancy, organic farming has successfully relied on this approach for fertilization technologies, such as manure and compost, as previously mentioned. However, its underlying microbiology has been largely overlooked. Therefore, the next logical stage in advancing organic farming is understanding and, potentially, replicating complex natural microbiomes. This would allow for their optimization, development of new varieties with desired metabolic traits, and more importantly, their scaling for large-scale application.

3. Case study: MicroBios S.A. technology

3.1 Overview: a complex microbial community produced at large-scale that mimics the rhizospheric microbiome

In view of the advantages of microbiome-based approaches, this chapter will present data about a technology developed that promotes plant growth while increasing the efficiency of traditional fertilizers. Since each kg of synthetic fertilizer is "bioactivated" by this microbial community, less input is needed to achieve the same or higher yields. This leads to a more sustainable agricultural practice, reducing its carbon footprint. While the data presented below focuses on NPK, organic fertilizers can also be bioactivated by microbes. As mentioned before, this is due to the synergistic interplay among bacteria that helps in nutrient uptake and efficiency.

In this sense, a microbiome-based bioadditive was kindly provided but its developer, MicroBios (Montevideo, Uruguay). This was previously developed inspired by natural soil microbiomes, focused on desired and optimal microbial capabilities for plants. For example, nitrogen fixation, reduction of nitrification, and the presence of microorganisms that promote plant growth and increase the bioavailability of nutrients, among other traits. To achieve this, state-of-the-art fermentation techniques were employed: a defined microbial consortium cultured in bioreactors and subsequent solid-state fermentation, employing organic materials in each stage. The microbial consortia contained species, and therefore genes, required for the desired metabolic functions. The microbes included bacteria belonging to the phyla Actinobacteria, Firmicutes, and others.

The fermentative process, along with the optimal consortia members, modifies the structure, diversity, and function of the solid substrate microbiome. Once the fermentation is complete, the bioadditive is granulated for easy field implementation or formulated for liquid application.

3.2 Comparison with other microbiome-based technologies

Existing microbiome-based organic fertilizers include manure, compost, and "fermented" products, or those that were developed by a hypothesis-driven approach, such as the bioadditive described herein. Manure represents the least developed technologically, as it depends on the animal's dung microbiome, and the biotransformation process is carried out in the field under uncontrolled conditions (**Figure 1**).

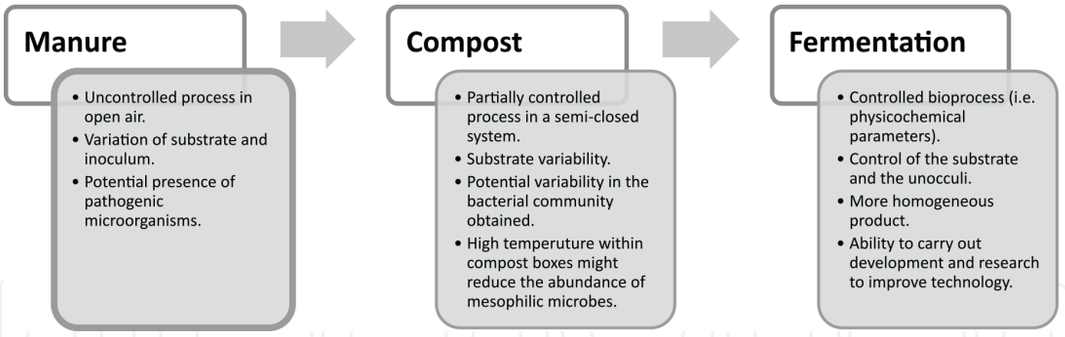

Figure 1.
Evolution of microbiome-based organic fertilizers.

Compost is a more controlled process as it is performed in a semi-closed system, where a new substrate is added over time. Here, however, the inocula and the substrate represent the biggest variables. Finally, there are the fermented products. These are obtained through controlled bioprocesses in bioreactors with pre-established inoculums and substrates. This controlled environment also allows for optimization. Therefore, fermented products are the most advanced among microbiome-based organic fertilizers.

The second generation of this technology is the one developed through continuous R&D. For example, the biotechnology described herein is based on years of research and development. The optimization (not shown here) involved the selection of microbes with synergetic interactions, the optimum fermentation conditions, and the selection of the best and most sustainable substrates, aiming towards a circular economy. Thus, this bioadditive can be regarded as a next-generation microbiome-based bioadditive.

3.3 Deciphering the bioadditive' s microbial structure and diversity

3.3.1 Relative abundance of microbiome members of substrates and products

The bioadditive is formulated on a microbiome-based approach where microbial and metabolic diversity is key for its function. Understanding its composition can only be achieved via non-culture techniques such as 16S rRNA sequencing [25]. Thus, we performed a metagenomic analysis of the solid substrate, the rhizosphere of healthy and robust plants, the bioadditive as currently used and with variations on its fermentation parameters, and worm humus (**Figure 2**).

Figure 2 demonstrates the microbial relative abundance at the phylum level of the solid substrate, the bioadditive generated under two conditions, A: aerobically, and B: microaerobically, and worm humus. The latter is colloquially known as high quality and was used as an external comparison. Additionally, we analyzed the rhizosphere of healthy and vigorous adult plants. As can be seen, the substrate microbiome can be modified by fermentation conditions. The substrate contained Proteobacteria as the most abundant phylum. In terms of the bioadditive, the anaerobic method promoted Proteobacteria, whereas in the microaerophilic condition, currently used in the field, Firmicutes were the most abundant. This phylum is comprised of many microaerophilic, facultative, as well as strict anaerobic microorganisms, such as *Clostridium* sp. Known probiotics such as lactic acid bacteria and bacillus are also members of

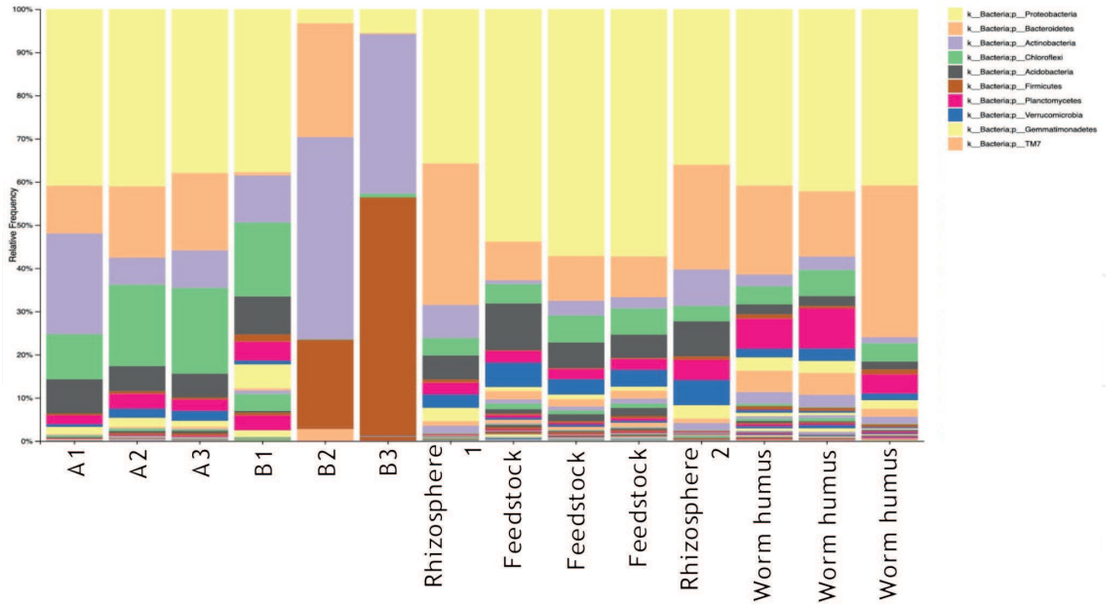

Figure 2.
Relative abundance at the phylum level of the bioadditive generated under two conditions, A and B. The rhizosphere of two vigorous plants, the solid substrate, and worm humus. Data generated via 16S rRNA sequencing at the Core microbiome of the University of North Carolina at Chapel Hill, NC, USA. The 10 most abundant phylum are shown.

this phylum [4, 26–28]. The three anaerobic samples contain different abundance levels of Firmicutes, due to the sampling depth within the solid-state fermentation reactors. For instance, sample B1 looks more like A1, A2, and A3, because it was closer to the surface. On the other hand, worm humus and the rhizosphere contained Bacteroidetes as the most abundant phylum. Overall, this data reveals that the substrate's microbial community can be modified, and the fermentation conditions have a profound effect on the resulting microbiome. As previously mentioned, the bioadditive was submitted to iterative rounds for process development until the current composition was achieved (not shown).

3.3.2 Beta diversity analysis of the bioadditive reveals that its composition resembles a rhizospheric microbiome

Another microbiome analysis we performed was beta diversity, which is a comparison of diversities between samples. Similarly, data visualization is carried out via principal coordinate analysis (PCoA). This allows us to visually cluster samples according to their similarity. **Figure 3** shows the presence of four clusters among the samples analyzed: aerobic bioadditive (Cluster 1), microaerobic additive with rhizospheres (Cluster 2), the substrate (Cluster 3), and earthworm humus (Cluster 4). This result demonstrates two key points: (a) the substrate microbiome composition is modified during the fermentation process, and (b) the anaerobic bioadditive has similarities to rhizospheric microbiomes. In other words, the optimized fermentative process and inocula achieve the generation of a naturalized synthetic microbial community.

Therefore, the anaerobic bioadditive provides seeds or seedlings, at the beginning of their growth cycle, the microbiological environment that they would naturally

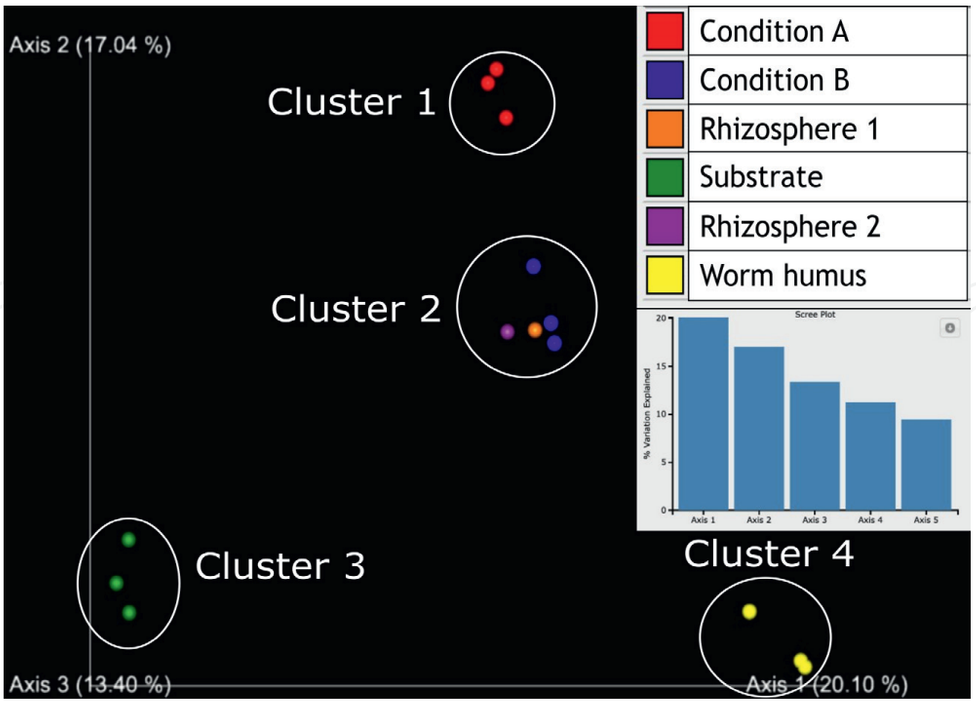

Figure 3.
Beta diversity analysis (PCoA, bray-Curtis) based on 16S rRNA gene sequencing data. Data were generated by 16S rRNA gene sequencing at the Core microbiome of the University of North Carolina at Chapel Hill, NC, USA.

develop under optimal conditions. This makes the plant "save" metabolic resources and time, allowing it to develop its biomass. In addition, this growth is enhanced due to the high relative abundance of microorganisms that promote plant growth. Another notable point is that the bioadditives are closer to worm humus, compared to the substrate, when analyzed from axis 1.

3.4 Microbial beta-diversity analysis of soils from forests, and fields treated or not with the bioadditive

In addition to comparing the bioadditive with healthy rhizospheres and other samples, we compared the soil of forests and production fields treated or not with this microbiome-based fertilizer. The treated fields were exposed to the bioadditive for nine consecutive years. The forest and field soils analyzed were in the district of Carlos Antonio Lopez, department of Itapúa, Paraguay. The samples were randomly selected, within the first 20 cm from the top, and were not associated with the rhizosphere of any vegetation.

Figure 4 shows three clusters: soil samples (Cluster 1), the bioadditive inoculated with different inocula (Cluster 2), including control (i.e., the bioadditive as commercialized), and the substrate (Cluster 3). Interestingly, while every soil sample is clustered together, the treated soil appears closer to the forest, suggesting a modification of the soil microbiome due to the bioadditive multi-year treatment. While this data represents a single time point, it agrees with previous observations of soil amendment practices with compost [20]. Longitudinal experiments would reveal the soil microbiome dynamics upon treatment with this microbiome-based additive. However, these results reveal a key point: the bioadditive, in addition to providing plants with a rhizospheric-like microbiome, can potentially modify the soil microbial community

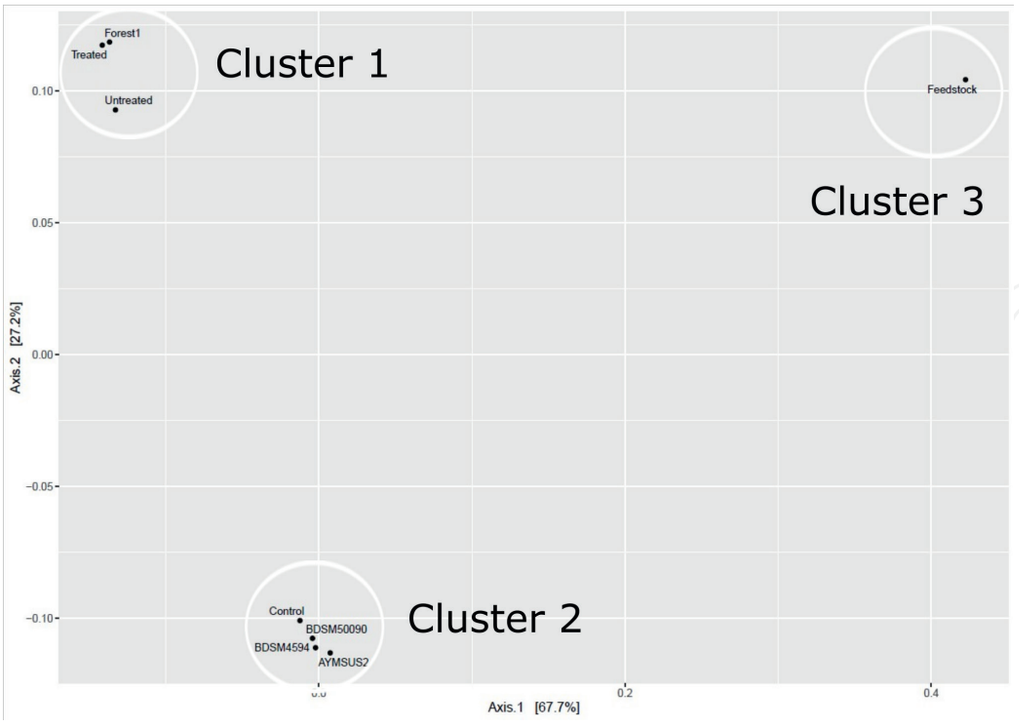

Figure 4.
Beta diversity analysis (PCoA, bray-Curtis) based on 16S rRNA gene sequencing. Data was generated by 16S rRNA gene sequencing at the Core microbiome of the University of North Carolina at Chapel Hill, NC, USA. Cluster 2 corresponds to different samples of the bioadditive inoculated with different sets of microbes. Control refers to the currently commercialized product.

to resemble that of the forest. Thus, it suggests the potential of this technology for soil restoration activities and regenerative agriculture.

3.5 The multiyear large-scale bioadditive application demonstrates a significant yield increase with lower fertilizer input

Despite the microbial characteristics of a biostimulant, its effectiveness in increasing yields is what finally matters. Therefore, its evaluation is critical. While greenhouse- or lab-scale data are relevant, field applications are fundamental for obtaining robust and reproducible data. This will assure consumers and regulators of the effectiveness of each developed technology. For this purpose, the microbiome-based bioadditive was evaluated in the field by replacing approximately 30% of nitrogen, phosphorous, and potassium (NPK) fertilizer, and comparing it with 100% NPK, under identical conditions.

As can be seen in **Figure 5**, per hectare, 168 kg of bioactivated NPK (4–30-10, proportion) with 72 kg of the bioadditive, increases the yield significantly (ANOVA, $p < 0.05$), compared to negative non-fertilized control (2812 vs. 2299 kg) and to 200 kg of NPK alone (2812 vs. 2364 kg). Furthermore, while not significant ($p > 0.05$), this condition produced more than 240 kg of NPK alone (2812 vs. 2557 kg). This field assay was performed in collaboration with the Paraguayan Institute of Agricultural Technology (IPTA, in Spanish).

In addition to this field test, we analyzed production data from producers who have used this microbial biotechnology over the years. **Figure 6** shows the percentage yield variation among different crops from 2015 to 2021, across an applied area of over 2.000.000 acres. These values were calculated considering the treatment (30%

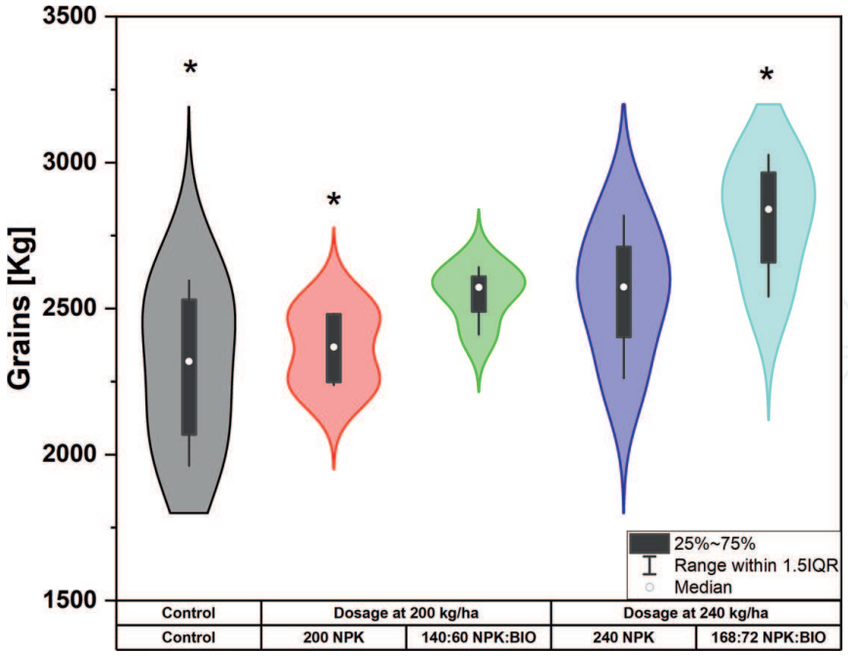

Figure 5.
*Evaluation of the microbiome-based additive in the field by the Paraguayan Institute of Agricultural Technology using soybean (var. SOJAPAR R 19). Assay was performed in Tomas Romero Pereira, Department of Itapúa, Paraguay (−26,453,196. -55,264,015), altitude of 330 meters above sea level (masl), and soil type Rodic Kandiudox, in November 2022 to march 2023. Each treatment was performed with 4 repetitions, each consisting of 9 m long lines. Separated by 0.45 m, each. * p < 0.05.*

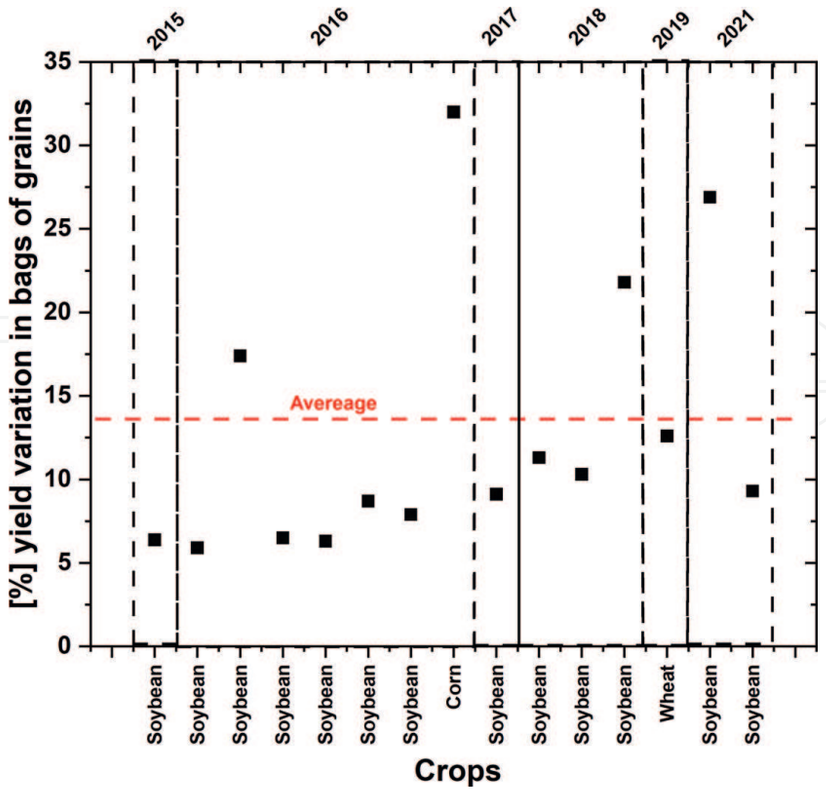

Figure 6.
Percentage yield variations of crops treated with the bioadditive by replacing 30% of NPK, compared to 100%. Average increase of 12.8%. Data represents field applications from 2015 to 2021, in a total area of approximately 2.000.000 acres.

bioadditive: 70% NPK) *versus* control (100% NPK), under the same conditions, side by side. As can be seen, the average percentage yield increase was 12.8%, across multiple years and phylogenetically distinct crops, i.e., legumes and grasses. This confirms that the use of this microbiome-based bioadditive, in tandem with NPK, has a positive effect on crop yield, at a large scale.

Considering the microbial diversity within this bioadditive, potential mechanisms of action might involve increased nutrient bioavailability, production of growth-promoting phytohormones, and stress tolerance, among others. In this way, production is maximized, while lowering the requirements for chemical fertilizers.

4. Potential for reducing greenhouse gases in agriculture through the implementation of bioactivators

4.1 The carbon-footprint of agriculture in Paraguay

The agricultural industry is one of the pillars of the Paraguayan economy. Like most industrial processes, this activity contributes to the country's carbon footprint. Among the most important greenhouse gases (GHG) is nitrous oxide (N_2O), where each molecule represents approximately 310 CO_2 equivalents (CO_{2eq}), in terms of its greenhouse effect capacity (IPCC Guide 1996). This gas originates mainly from biogeochemical processes linked to nitrogen, where soil microorganisms are key players. Due to this, agriculture plays an important role in GHG emissions, specifically, because of the use of synthetic fertilizers, such as NPK. However, not only nitrogen but also phosphorus and potassium, have a significant carbon footprint according to their life cycle analysis (LCA).

With adequate land management and agro-industrial processes focused on the use of biotechnologies, Paraguay and other countries can continue producing more food, while reducing GHG generation. For instance, by using biostimulants that increase agro-industrial performance, while reducing the use of synthetic fertilizers.

Considering that Paraguay is one of the top agricultural producers worldwide, this would set a positive precedent positioning it as a leader in the fight against climate change, without harming production, and potentially opening new markets.

4.2 NPK carbon footprint

The NPK fertilizer has a significant carbon footprint related to direct and indirect processes. For instance, these include the planting and harvesting of crops, and emission factors, or the production and transport of fertilizers, respectively [29–31]. Analyzing every active ingredient of NPK, i.e., nitrogen, phosphorus, and potassium, for each Kg of N in the soil, it releases approximately 5.9 kg of CO_2eq in the form of N_2O (IPCC Guide 2006). According to data from the 2015 National Inventory of Greenhouse Gas Emissions of Paraguay (INGEI), N_2O represents almost 20% of the total GHG emissions in this country. Adding to this value the carbon footprint of its life cycle (LCA), which is on average 8.8 Kg CO_2eq per Kg of N, we have a total of 14.7 kg of CO_2eq per kg of nitrogen used (**Table 1**) [32–34]. This value agrees with a recent study arguing that cutting the use of nitrogen fertilizers is needed for a significant reduction in GHG emissions [35].

	Kg CO_2eq/Kg of N, P, o K.			Ref.
	N	**P**	**K**	
LCA	9.51	40.02	12.31	[33, 34]
	9.55	38.14	47.73	[33, 34]
	7.97	55.82	16.74	[33, 34]
	8.66	45.47	25.98	[33, 34]
	8.6	51.61	41.29	[33, 34]
Average LCA	8.84	45.73	25.39	[33, 34]
Emissions (soil N_2O)	5.9	NA	NA	IPCC 2006
Total CO_2eq/component [kg]	14.74	45.73	25.39	This work

Table 1.
Carbon footprint in terms of kg CO2eq per kg of each active ingredient of NPK.

On the other hand, according to LCA data and emission factors, the carbon footprint of P and K is approximately, per kg, 45.7 and 25.3 Kg CO_2eq, respectively (**Table 1**) [32, 33]. As was suggested by Gao and coworkers [35], not only emission factors are important when considering the potential for carbon-savings, but also LCA. Therefore, in this work, we considered these values for each component of NPK to calculate how much can be saved when a portion of it is bioactivated.

4.3 CO_2eq saving potential in Paraguay by replacing 30% of NPK with the microbiome-based bioadditive

According to the Paraguayan Ministry of the Environment and Sustainable Development (MADES), in its latest 2021 Nationally Determined Contribution (NDC) report, this country generated 82,399 Mt. CO_2eq in 2021. Of these, 26,499 Mt. CO_2eq corresponded to agriculture, equivalent to 32% of the national total.

Among the Climate Change Mitigation Plans of Paraguay by 2030 is the decrease in the use of nitrogenous fertilizers such as NPK (Point AG.2.). Considering the carbon footprint of each NPK active ingredient and the amount of fertilizer imported into the Republic of Paraguay, it is possible to calculate the potential CO_2eq savings for each percentage of NPK that is no longer used. Assuming a 30% reduction in NPK, and its replacement with bioadditives, a total saving of approximately 2 Mt. CO2eq per year would be obtained (**Table 2**). Part of these values could be accounted for by Paraguay.

Paraguay is a signatory to the Paris Agreement and committed to reduce GHGs by 20% with respect to a Business-as-usual (BAU) baseline by 2030 (Law No. 5681/16). Of these, 10% are unconditional, while the remainder are conditional on the international provision of means for implementation. Therefore, the present strategy to replace NPK with bioactivators, such as the microbiome-based bioadditive described herein, can represent an important tool to achieve the objectives and international commitments of the Republic of Paraguay.

Along with GHG reduction by this strategy, the bioactivation of NPK by microbes achieves an average of 12.8% higher yields compared to the non-bioactivated version, as previously shown. Therefore, the present strategy of reducing GHGs using microbial biotechnologies also achieves a positive impact on producers.

Total CO_2eq (Kg) saved by reducing the use of NPK by 30% in Paraguay			
Year	**2019**	**2020**	**Total (2019 + 2020)**
N	45,30,48,350.56	36,42,48,105.22	81,72,96,455.79
P	1,16,90,59,938.36	1,05,50,84,632.42	2,22,41,44,570.78
k	99,77,60,815.72	79,81,78,463.12	1,79,59,39,278.85
Total (Kg)	2,61,98,69,104.65	2,21,75,11,200.77	4,83,73,80,305.42
Mt (Megaton)	2.62	2.22	4.84

Table 2.
Potential for CO_2eq savings by reducing the use of NPK by 30%. The quantity of each active component was calculated based on the percentage to which it corresponded in each fertilizer. Values calculated according to annual import quantity for 2019 and 2020.

4.4 Increase in plant biomass as an atmospheric carbon-sink tool

In addition to decarbonizing agriculture by replacing chemical fertilizers with microbes, another potential saving is due to the increase in atmospheric CO_2 fixation within plant biomass. It was previously shown that using fertilizers increases atmospheric CO_2 fixation up to 5 times, due to increased photosynthesis [31]. Therefore, the 12.8% increase in agricultural yield mentioned above implies a greater CO_2 fixation, concomitant with the decrease in synthetic fertilizers use.

On the other hand, each ton of plant biomass fixes 1.6 tons of atmospheric CO_2 [36, 37]. Therefore, it is feasible to quantify the net carbon footprint reduction due to the 12.8% increase in productivity achieved by this microbiome-based bioadditive. Experiments are underway.

5. Conclusion

Taken together, these data demonstrate that by studying the soil microbiome and its interaction with plants, it is possible to isolate and identify plant-growth-promoting microbes. Moreover, their development through microbial biotechnology techniques can lead to the creation of important tools for increasing agricultural yields while achieving decarbonization. Likewise, organic farming has traditionally relied on microbiome-based fertilization solutions, achieving great and sustainable results. However, with the advent of molecular and fermentation technologies, new and improved solutions can be developed inspired by nature, such as the bioadditive described herein. In this sense, this biotechnology can also be applied in organic farming as a tool to bioactivate their compatible fertilizers. Importantly, however, research and development must be accompanied by field tests over multiple seasons and crops, to safeguard producers. Many questions and challenges remain that academia, government, and industry can tackle together in the pursuit of sustainable food production. Some of those questions include the evaluation of bioactivity of these technologies across geographies or their exact mechanism of action. Deciphering the soil microbiome is another frontier to be pushed. In conclusion, the strategy to activate fertilizers with next-generation microbiome-based bioactivators represents a great opportunity to achieve GHG reduction objectives while boosting agricultural production.

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Conflict of interest

WJS-E is current principal investigator of MicroBios but was not involved in the development of the bioadditive described herein.

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