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Exploring Microbial Diversity in Forest Litter-Based Fermented Bioproducts and Their Effects on Tomato (*Solanum lycopersicum* L.) Growth in Senegal

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Abstract: Reducing the use of chemical inputs (fertilizers, pesticides) in agriculture while maintaining crop productivity is the main challenge facing sub-Saharan African family farming systems. The use of effective microorganisms (EM) is among the various innovative approaches for minimizing chemical inputs and the environmental impact of agricultural production and protecting soil health while enhancing crop yields and improving food security. This study sought to characterize the microbial biodiversity of local beneficial microorganisms (BMs) products from locally fermented forest litter and investigate their ability to enhance tomato plant growth and development. Beneficial microorganisms (BMs) were obtained by anaerobic fermentation of forest litter collected in four agroecological regions of Senegal mixed with sugarcane molasses and various types of carbon sources (groundnut shells, millet stovers, and rice bran in different proportions). The microbial community composition was analyzed using next-generation rDNA sequencing, and their effects on tomato growth traits were tested in greenhouse experiments. Results show that regardless of the litter geographical collection site, the dominant bacterial taxa in the BMs belonged to the phyla *Firmicutes* (27.75–97.06%) and *Proteobacteria* (2.93–72.24%). Within these groups, the most prevalent classes were *Bacilli* (14.41–89.82%), *α-proteobacteria* (2.83–72.09%), and *Clostridia* (0.024–13.34%). Key genera included *Lactobacillus* (13–65.83%), *Acetobacter* (8.91–72.09%), *Sporolactobacillus* (1.40–43.35%), and *Clostridium* (0.08–13.34%). Fungal taxa were dominated by the classes *Leotiomycetes* and *Sordariomycetes*, with a prevalence of the acidophilic genus *Acidea*. Although microbial diversity is relatively uniform across samples, the relative abundance of microbial taxa is influenced by the litter's origin. This is illustrated by the PCoA analysis, which clusters microbial communities based on their litter source. Greenhouse experiments revealed that five BMs (DK-M, DK-G, DK-GM, NB-R, and NB-M) significantly ($p < 0.05$) enhanced tomato growth traits, including plant height (+10.75% for DK-G and +9.44% for NB-R), root length (+56.84–62.20%), root volume (+84.32–97.35%), root surface area (+53.16–56.72%), and both fresh and dry shoot biomass when compared to untreated controls. This study revealed that forest-fermented litter products (BMs), produced using litter collected from various regions in Senegal, contain

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beneficial microorganisms known as plant growth-promoting microorganisms (PGPMs), which enhanced tomato growth. These findings highlight the potential of locally produced BMs as an agroecological alternative to inorganic inputs, particularly within Senegal's family farming systems.

Keywords: local beneficial microorganisms; plant growth; tomato; agroecology; family farming; Senegal

1. Introduction

In Senegal, family farming plays a crucial role in supplying both local and national markets with fresh and perishable agricultural products. However, the high added value of these crops, combined with their high sensitivity to pests, leads producers to use unsustainable farming methods. These methods heavily rely on imported chemical inputs (fertilizers, pesticides), exposing the environment and consumers to multiple pollutants [1,2]. Therefore, the current challenge for agriculture in sub-Saharan Africa is to design sustainable and resilient low-input farming systems and practices that improve ecosystem services such as primary productivity (yields), nutrient recycling (fertility), and soil health. Many technologies have been developed to increase agricultural production. Nevertheless, some, including the use of mineral fertilizers and agrochemical products, have high costs and harmful effects on the environment.

The use of effective microorganisms (EM) is among the various agroecological techniques that can minimize the environmental impact of agricultural production while enhancing efficiency and profitability. These bioproducts are formed by fungi and bacteria isolated from soil or litter and can coexist in a liquid fermentation medium. Effective microorganism (EM) technology, first described by Teruo Higa in [3,4], is a robust and versatile approach already tested in Latin America and Southeast Asia "https://www.emrojapan.com/what/ (accessed on 22 January 2025)". Although known by a few in sub-Saharan Africa, its use remains relatively limited. These bioproducts are based on the principle of simple fermentation by farmers using native microorganisms collected locally from forest litter areas.

The beneficial effects of EM on seed germination and the vigor of certain plant species and soil have been well-documented. Studies by [5–7] demonstrated EM's ability to stimulate wheat seed germination and enhance the vigor of maize, sorghum, and sunflower seedlings. Previous studies have shown that applications of EM to the soil and plant ecosystem can improve soil quality, soil health [8,9], and the growth yield and quality of crops [10–13]. Others studies reported that EM enhanced organic composting [14] and is used in agriculture as bio-fertilizer or bio-pesticide [12,15,16]; it was also found that EM at concentrations of 1% and 2% improved seed germination and seedling vigor in *Albizia saman* and *Acacia auriculiformis*. Additionally, Ney et al., 2020 highlighted the impact of EM inoculation on increasing nitrogen availability in soils, thereby boosting legume productivity [17,18]. EMs appeared therefore as an innovation with great potential to support the agro-ecological transition by strengthening the autonomy of producers in terms of inputs by making their practices greener and their products safer. It seems clear that developing farming system practices based on the use of EM can lead to sustainable, productive agriculture in the Sahelian conditions, especially for market gardening.

In Senegal, tomatoes (*Solanum lycopersicum*) are a widely cultivated vegetable used as fresh fruit, puree, and sauces. Tomato is ranked as the second most important horticultural crop after onions in Senegal, playing a significant socio-economic role in the country's

economy [19]. The production is among the high-gross-margin vegetable crops that could help small farmers modernize family agriculture. The production remained relatively stable over the past five years, reaching 152,950 metric tons in 2023, with an average yield of 1.7 metric tons per hectare [20].

For this study, forest-fermented litter products—referred to as local beneficial microorganisms (BMs)—were produced by anaerobically fermenting local litter collected from various agroecological regions of Senegal. Thus, this study examined the BM products for the presence of beneficial microorganisms with potential plant growth-promoting characteristics to be used as biofertilizers or biostimulants.

This study aims to perform microbial characterization of bioproduct BMs and to evaluate their ability to improve tomato production in Senegal.

2. Materials and Methods

2.1. Litter—Collection and the Local Fermented Forest Litter Preparation

Local fermented forest litters, referred to in this study as local beneficial microorganisms (BMs), are mixed cultures of different components with forest litters collected in four different agroecological regions of Senegal following a climatic gradient (Figure 1). They were collected in the North Groundnut Basin (14°57.562′ N; 16°27.489′ O), Saint-Louis region (16°12.468′ N; 16°23.145′ O), and South Groundnut Basin (14°36.125′ N; 16°26.682′ O), and an urban forest spot in Dakar (14°42.125′ N; 17°25.593′ O).

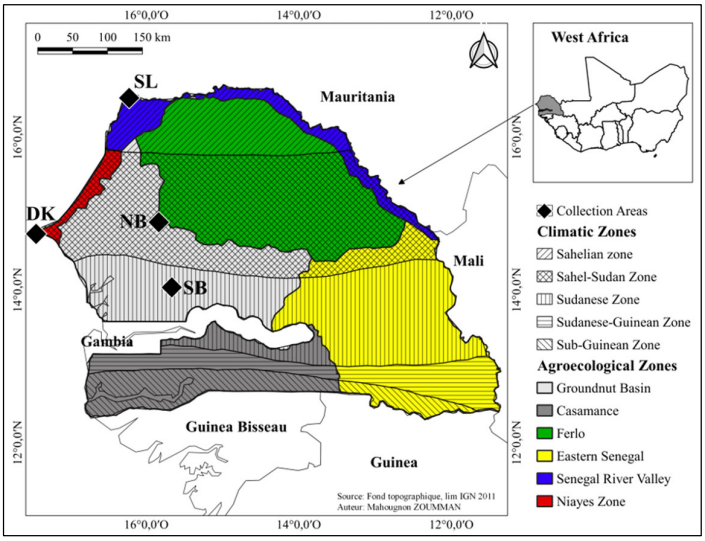


Figure 1. Map showing climatic zones and the forest litter collection sites along the climatic gradient in Senegal. DK: forest litter collection site in Dakar, located in the Sahel-Sudanese climatic zone, within the agroecological zone of the Niayes, in a peri-urban setting. SL: forest litter collection site in Saint-Louis, situated in the Sahelian climatic zone, within the agroecological zone of the Senegal River Valley. NB: Forest litter collection site in the Sahel-Sudanese climatic zone, within the agroecological zone of the Groundnut Basin. SB: Forest litter collection site located in the Sudanese climatic zone, within the agroecological zone of the Groundnut Basin.

The preparation process of the local fermented forest litters containing beneficial microorganisms (BMs) consists of two successive fermentation steps to produce liquid containing beneficial microorganisms originating from forest litter [16,21,22]. In a 42-L container, 4.8 kg of litter was mixed to 2.1 kg of sugarcane molasses and 1.05 kg of plain yogurt. Then, 9.6 kg of each of the following different residues (groundnut shells, millet stovers, rice bran, and a 50:50 mixture of peanut shells and rice bran) was added as carbon sources to compare their effect on the microbial communities. The mixture was

supplemented with water until the optimal humidity was obtained. The containers were then closed hermetically and left to ferment in an anaerobic condition at room temperature until the pH reached 5, usually in one month.

To create an active liquid product, 2.1 kg of the previously fermented material was combined with 42 L of water, 1.05 kg of yogurt, and 2.1 kg of sugarcane molasses in a second fermentation phase. After being put in airtight containers, the mixture was allowed to ferment anaerobically at room temperature for seven to fourteen days, or until the pH fell to four or below. After fermentation was finished, plant remnants were removed from the liquid BMs result by coarsely filtering it through a screen before storing it at room temperature. A total of fifteen local liquid-fermented products were produced, each made with a distinct carbon source and trash from a different origin (Table 1). These products are expected to contain a diverse and substantial number of effective microorganisms. For reference, our study also included a commercial bioproduct obtained from the Songhai Center in Benin (BJ-CCS).

Table 1. Litters and different carbon resources used for BMs preparations. The various carbon sources are agricultural residues readily available throughout Senegal. These residues are low in nutrients and rich in carbon and serve as a growth support for microorganisms.

Number of BMs Products	Litter Collection Zones	Carbon Source Used	BMs Products Code *
1	Dakar	Groundnut shell	DK-G
2		Groundnut shell and Millet stover	DK-GM
3		Millet stover	DK-M
4		Rice bran	DK-R
5	North Groundnut Basin	Groundnut shell	NB-G
6		Millet stover	NB-M
7		Rice bran	NB-R
8	South Groundnut Basin	Groundnut shell	SB-G
9		Groundnut shell and Millet stover	SB-GM
10		Millet stover	SB-M
11		Rice bran	SB-R
12	Saint-Louis	Groundnut shell	SL-G
13		Groundnut shell and millet stover	SL-GM
14		Millet stover	SL-M
15		Rice bran	SL-R
16	Commercial product Songhai Center Benin	Unknown	BJ-CCS

* The first two letters of the code correspond to the litter collection area and the letters after the dash correspond to the type of agricultural residue used in the production.

2.2. DNA Extraction and Sequencing

The total genomic DNA was extracted from 50 mL of each BMs product using the FastDNA™ SPIN kit for Soil (MP Biomedicals, 29525 Fountain Parkway, Solon, OH-44139 USA), with modification of the manufacturer's instructions [23]. Agarose gel (1.5% w/v)

electrophoresis was used to confirm adequate genomic DNA, which was then quantified using a Thermo Scientific spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific 3411 Silverside Road, Bancroft Building, Suite 100, Wilmington, DE 19810, USA). High-throughput sequencing was performed by the ADNID company (Montferrier, France; http://www.adnid.fr) with the Illumina MiSeq system (Illumina, San Diego, CA, USA) by targeting the 16S rRNA gene with the 515F/806R primers set and the ITS gene with the ITS3F-ITS4R primers. The sequences were deionized, and operational taxonomic units (OTU) were defined by clustering at 3% divergence (97% similarity) followed by the removal of singletons and chimeras. Final OTUs were taxonomically classified using BLASTn https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 25 January 2021) against a curated database derived from GreenGenes and SYLVA. We then produced the final OTU tables containing the number of sequences per sample per OTU matching the designated taxonomic classification. The whole process was conducted by ADNID (Montferrier, France; http://www.adnid.fr).

2.3. Effect of BMs Product on Tomato

The agronomic efficacy of BMs on the tomato plants was conducted for 28 days under natural light in a greenhouse at the ISRA/IRD Bel Air Campus in Dakar (latitude: 14.701778, longitude: −17.426229, altitude: 9 m). The experimentation took place from January to February 2021, with the monthly average midday temperature ranging between 23 °C and 29 °C. The variety AMIRAL F1 hybrid was used in the greenhouse test. This trial was conducted in 600 mL gardening pots with horticultural potting soil as substrate (NFU42001 1 kg/m^3; NPK 10/10/20; pH 5.8). Tomato seeds were soaked in 2% of BMs for 1 h before pre-sprouting on agarose water (0.8% agarose) in Petri dishes. After pre-germination, the seedlings were transplanted into pots. Ten days after potting, the seedlings were treated with 10 mL of BMs at 2% concentration (0.2 mL of BMs per pot). Each treatment was replicated 12 times, including the untreated control, which received 10 mL of water. Growth parameters such as plant height, leaf chlorophyll content, and fresh and dry biomass aerial parts were evaluated. Chlorophyll content of leaves was measured with a chlorophyll meter (SPAD-502 Plus) in the middle of the day, between 10 and 11 AM, under maximum light capacity. Plant height (cm) was measured from the ground to the tip of the stem with a tape measure. The plants were uprooted at 28 days post-inoculation (DPI). The roots were carefully washed and scanned, and root traits (root area, root length, root volume, and root diameter) were evaluated using WinRhizo version 2012b.

3. Results

3.1. Composition of Bacterial and Fungal Communities of the Liquid BMs Samples

The taxonomic inventory of the bacterial sequences across all the BM samples identified, respectively, three bacterial phyla and five classes. The most dominant bacterial phyla (>1% of total relative bacterial abundance) in terms of relative abundance were *Firmicutes* (27.75–97.06%) and *Proteobacteria* (2.93–72.24%), which together accounted for more than 99.99% of the bacterial sequences (Figure 2a). *Actinobacteriota* was a rare class (<1% of total relative bacterial abundance) found across the BM samples. The most predominant bacteria classes within the BM samples were *Bacilli* (14.41–89.82%), *Alphaproteobacteria* (2.83–72.09%), and *Clostridia* (0.024–13.34%), followed by the rare classes *Gammaproteobacteria* and *Actinobacteria*. At the genus level, the BM samples compositions were predominated by *Lactobacillus* (13–65.83%), Acetobacter (8.91–72.09%), *Sporolactobacillus* (1.40–43.35%), *Clostridium* (0.08–13.34%), and *Bacillus* (0.17–1.79%), followed by the rare genus *Brevibacillus*, *Rummeliibacillus*, *Brevibacterium*, and *Anaerocolumna* (Figure 2b). The fungal community composition of the BM samples was also subject to variation in the relative abundance of

classes dominated by *Leotiomycetes* (88.72–100%) followed by *Sordariomycetes* (0.00–11.28%) and *Cystobasidiomycetes* (0.00–4.27%) (Figure 3a). The most dominant fungal genus was *Acidea* accounting for 82.72–100% of the fungal abundance in each sample, followed by *Gaeumannomyces* (0.00–9.77%), *Bannoa* (0.00–4.27), and *Knoxdavieasia* (0.00–3.26%) (Figure 3b).

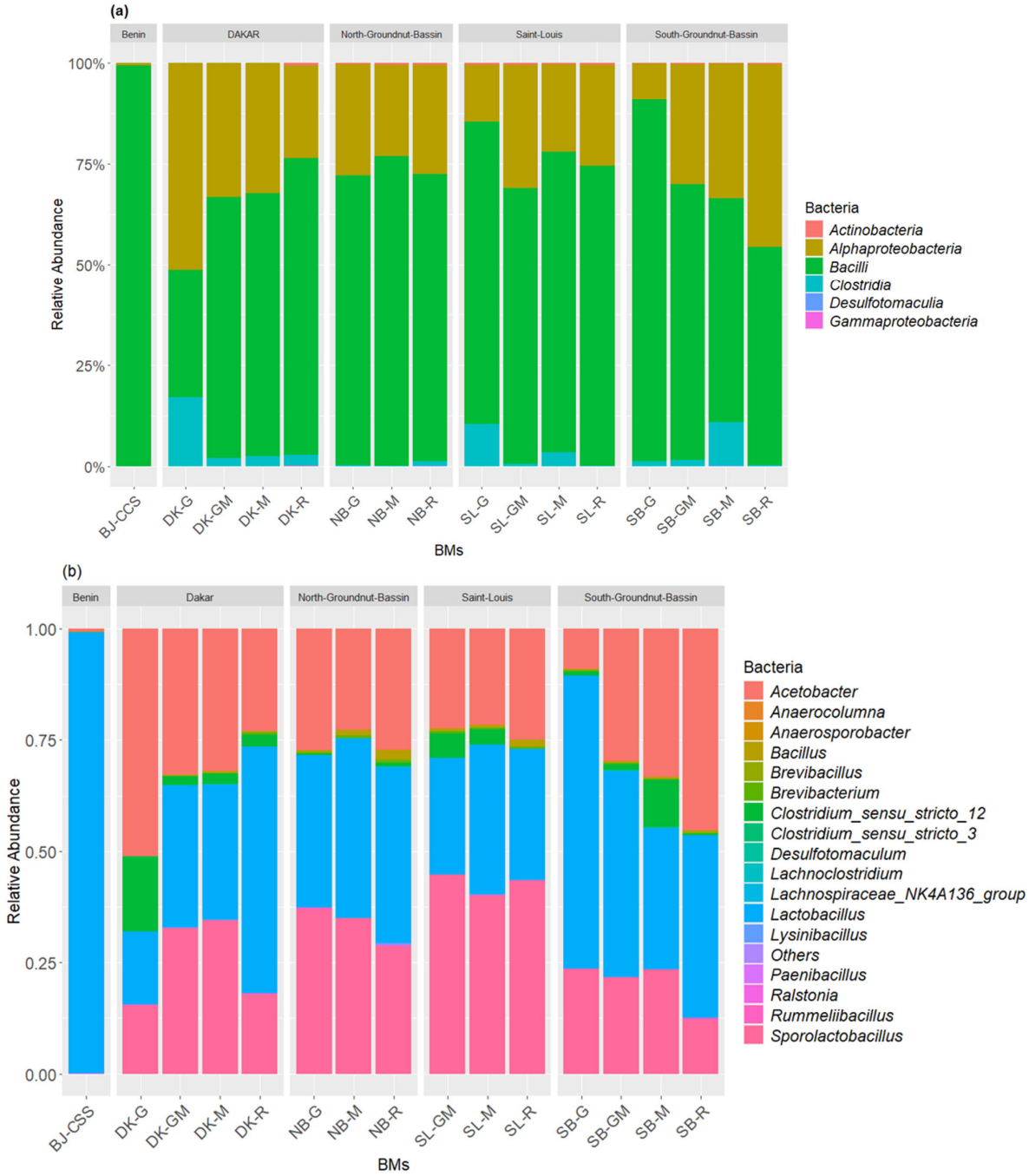


Figure 2. Taxonomic composition of the bacterial communities at the class (**a**) and genus (**b**) levels in the BM samples. The graph illustrates the different taxonomic groups (at the class and genus levels) present in the BMs, with each group represented by a distinct color. The size of each taxonomic group corresponds to its relative abundance in the BMs.

A notable diversity of fungi was observed, particularly in the composition of BMs made with ingredients from St-Louis. These compositions showed a higher dominance of the genus *Acidea* (77% to 82% in SL-G, SL-GM, and SL-R). Additionally, they contained the genera *Gaeumannomyces* (100% in SL-M, and 3% to 13% in SL-GM, SL-G, and SL-R)

and *Knoxdavieasia* (1% and 3% in SL-GM and SL-R, respectively). Additionally, the genus *Bannoa* was identified in BMs from the Dakar region, with respective proportions of 0.9%, 0.6%, and 4.2% in DK-M, DK-R, and DK-GM. Similarly, BMs derived from litter collected in the Saint-Louis region (SL-R, SL-G, and SL-GM) showed proportions of 0.5%, 11.8%, and 6.2%, respectively.

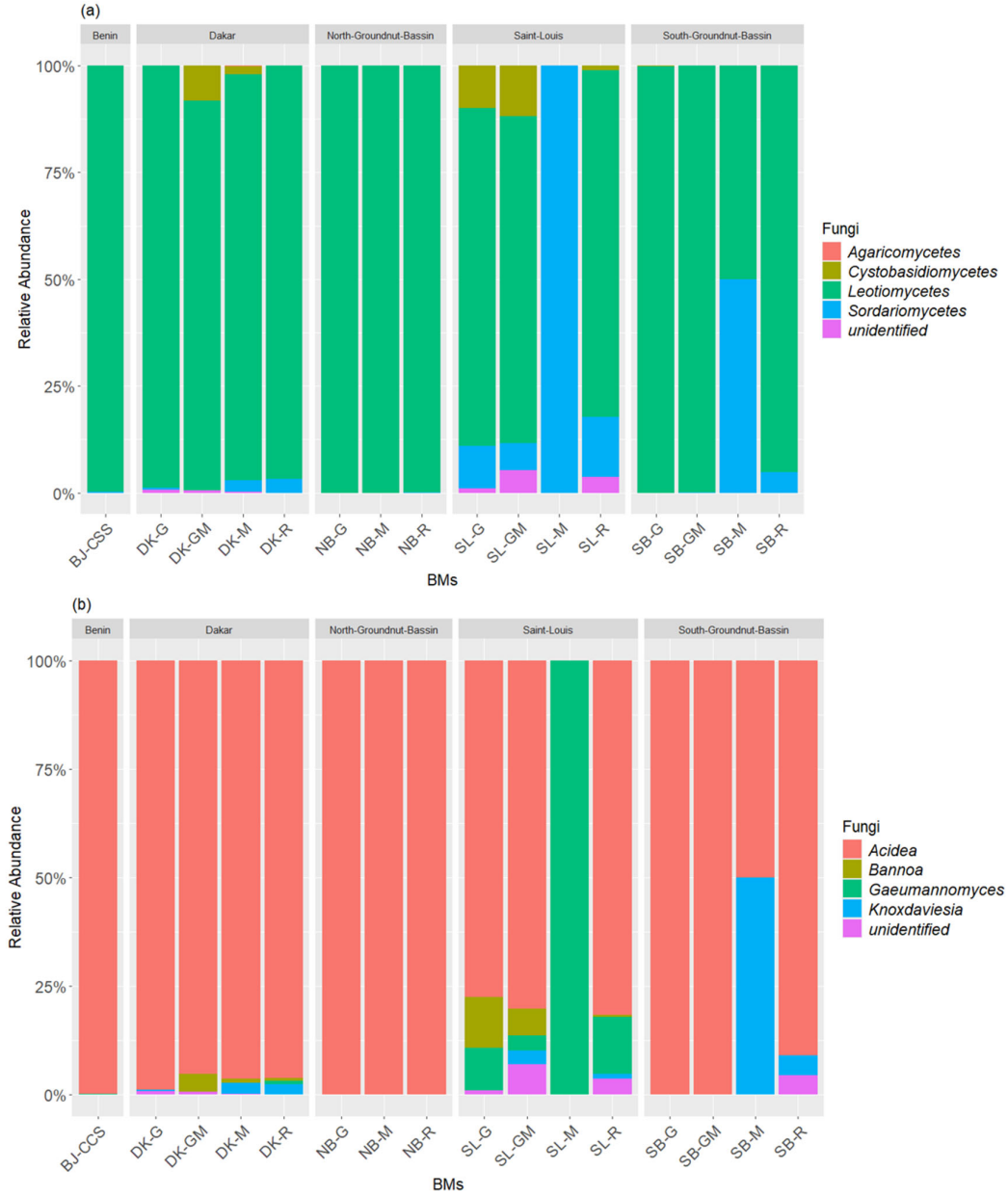


Figure 3. Taxonomic composition of the fungal communities at the class (**a**) and genus (**b**) levels in the BM samples. The graph illustrates the different taxonomic groups (at the class and genus levels) present in the BMs, with each group represented by a distinct color. The size of each taxonomic group corresponds to its relative abundance in the BMs.

The commercial BM (BJ-CCS) studied was predominantly composed of Firmicutes (97.06%) and Proteobacteria (2.93%). Among bacterial classes, *Bacilli* dominated at 97.04%, followed by *Alphaproteobacteria* at 2.83%. Regarding fungi, the *Leotiomycetes* class overwhelmingly dominated, accounting for 99.99% of the fungal composition. At the genus level, BJ-CCS was primarily characterized by *Lactobacillus* (96.92%), *Acetobacter* (2.84%), and the fungal genus *Acidea* (99.99%).

3.2. Bacterial α and β-Diversity

Alpha diversity (OTU richness and Shannon indices) of the bacterial community was higher than that of the fungal community in all samples (Table 2). OTU richness and Shannon indices did not differ significantly between litter collection sites.

Table 2. Bacterial and fungal community richness and diversity of the BM samples.

	Bacterial α-Diversity			Fungal α-Diversity		
	OTU Observed	Chao1	Shannon Indices	OTU Observed	Chao1	Shannon Indices
BJ-CCS	105	191.13	1.48	19	19.17	1.31
SL-R	69	104.10	1.92	14	15.00	1.53
SL-M	74	83.10	2.07	1	1.00	0.00
SL-G	87	108.08	2.17	8	8.25	0.86
SL-GM	100	117.10	1.86	13	16.00	1.54
NB-R	106	144.15	2.29	6	6.50	0.00
NB-M	87	94.77	2.03	2	2.00	0.01
NB-G	96	114.07	2.16	2	3.00	0.69
SB-R	88	121.46	2.02	4	7.00	0.55
SB-M	121	146.83	2.38	2	3.00	0.69
SB-G	80	101.08	2.11	3	3.00	0.00
SL-GM	96	153.00	2.30	3	4.00	0.01
DK-R	94	117.21	2.29	7	7.00	0.22
DK-M	126	138.50	2.12	17	17.00	0.69
DK-G	49	57.75	1.81	7	8.00	0.23
DK-GM	92	132.07	2.16	4	4.00	0.28

The β-diversity analysis of the bacterial community among the different BM samples, assessed using principal coordinates analysis (PCoA) (Figure 4), indicated that the samples clustered according to their litter collection origin. This clustering suggests that spatial factors significantly influenced the β-diversity of the bacterial communities.

The PCoA clustering was statistically supported by PERMANOVA analysis, (F-value of 4.2700, adonis R^2 of 0.59, and p-value < 0.001; Table 3). These results confirm the presence of significant dissimilarities in the bacterial community compositions among the different collection origins. However, no significant dissimilarities were observed in the fungal community, regardless of the collection zone or the carbon sources used in the BM fabrication.

Table 3. Effect of litter origin and carbon sources on BM microbial β-diversity.

	Bacteria β-Diversity		Fungi β-Diversity	
	Structure	Dispersion	Structure	Dispersion
	R^2	F	R^2	F
Origin	0.59132 ***	4.2700	0.33199	1.3518
Carbon_Sources	0.13171	1.2682	0.17683	0.9600

p value 0 < *** < 0.001.

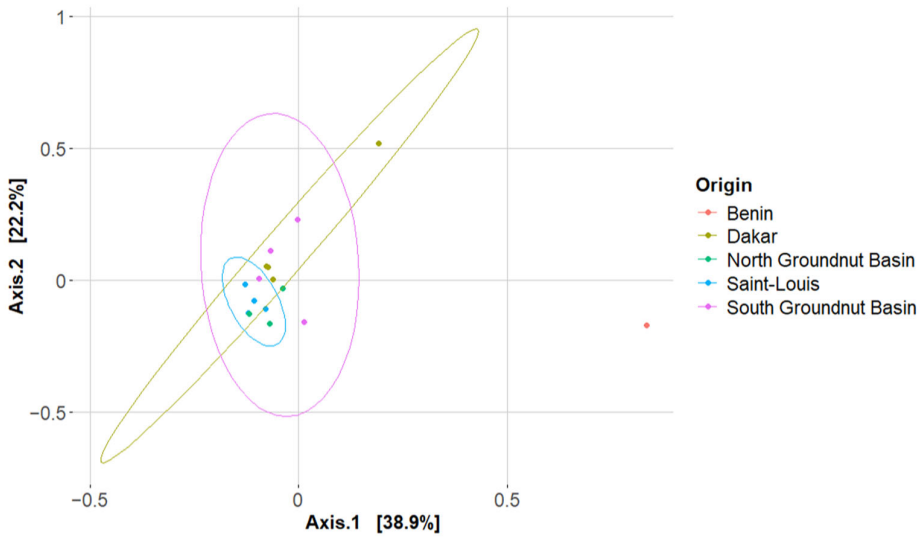


Figure 4. β-diversity of microbial communities in BMs according to litter origin. The first two axes explain nearly 61% of the total variance. Each point represents the observed OTU count of BMs from a specific litter origin, with grouping patterns reflecting geographic variation in microbial composition.

3.3. Effects of BMs on Aerial Growth of Tomato

Data on the effects of BM treatment on tomato plants after 28 days indicate that 5 of 15 products had a significantly positive effect on the chlorophyll content of leaves and plant growth (Figure 5 and Table 4). Results indicate that the products DK-G, DK-GM, and DK-M significantly enhanced ($p < 0.001$) the chlorophyll content in tomato leaves compared to control plants. The leaves' chlorophyll content increased by 17.94% for DK-G, 18.21% for DK-GM, and by 15.37% for DK-M compared to the controls and the reference BJ-CCS. Moreover, plant height significantly increased by 10.75%, 10.98%, 13.10%, and 9.44% for DK-G, DK-GM, DK-M, and NB-R, respectively, compared to the control.

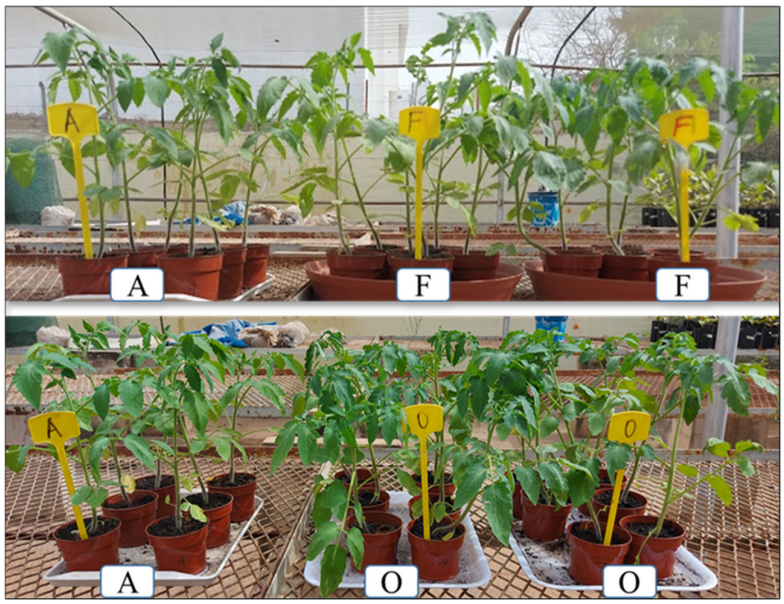


Figure 5. Effect of BMs on the growth of tomato at 28 days after treatment. A: Untreated control plants; F: plants treated with NB-R; and O: plants treated with DK-G. An increase in the height of the treated plants compared with the untreated control showed that some bioproducts have a better effect on chlorophyll content than others (fewer green plants).

Table 4. Effect of BMs on tomato growth properties, BMs that induced a significant increase in chlorophyll content and plant height are highlighted with asterisks indicating the level of statistical significance. The letters indicate the bioproducts grouped in the same statistical group by the comparison test.

BMs	Chlorophyll Content in Tomato Leaves	Plant Height (cm)
Untreated control	38.182 cde	44.318 def
BJ-CCS	38.183 cde	43.500 efg
DK-G	45.033 a ***	49.083 ab ***
DK-GM	45.136 a ***	49.182 ab ***
DK-M	44.050 a ***	50.125 a ***
DK-R	37.600 de	43.818 defg
NB-G	39.864 bcd	42.909 efg
NB-M	41.318 b *	42.909 efg
NB-R	40.467 bc	48.500 abc **
SB-G	36.492 e	41.625 fg
SB-GM	37.942 de	44.333 def
SB-M	36.658 e	41.083 g
SUB-R	39.845 bcd	43.818 defg
SL-G	38.000 de	46.583 bcd
SL-GM	36.045 e	44.636 def
SL-M	38.067 de	45.667 cde
SL-R	36.845 e	44.955 de

Tukey test, p-value 0 < *** < 0.001 < ** < 0.01 < * < 0.05.

3.4. Effect of BMs on Tomato Root Traits

At 28 DAT, treatments with NB-R, NB-M, DK-M, DK-G, and DK-GM significantly ($p < 0.001$) increased root length, root volume, and root surface area in tomato plants compared to the control. Notably, root length increased by 56.84%, 75.81%, and 62.20% for DK-G, NB-M, and NB-R, respectively. Interestingly, root volume increased by 59.21% for DK-M, 84.32% for DK-G, 85.44% for DK-GM, and 97.35% for NB-R. Root surface area also saw significant increases: 53.16% for DK-G, 51.61% for DK-GM, and 56.72% for NB-R. However, there was no statistical difference in root diameter (Table 5). Tomato plants treated with BJ-CCS showed no significant difference compared to the control.

Table 5. Effect of BMs on tomato root traits. BMs with significant variation for each root trait.

BMs	Length (cm)	Surface (cm^2)	Diameter (mm)	Volume (cm^3)
C-NI	11 921.47	99.05	0.57	2.68
BJ-CCS	12 743.54	98.28	0.54	2.54
DK-G	18 697.76 *	151.71 **	0.67	4.94 ***
DK-GM	19 208.93	150.17 *	0.67	4.97 ***
DK-M	16 479.96	133.92	0.64	4.267 *
DK-R	17 740.28	122.10	0.54	3.21
NB-G	92 92.78	76.02	0.50	1.67
NB-M	20 959.97 **	141.52	0.58	3.971 *

Table 5. *Cont.*

BMs	Length (cm)	Surface (cm^2)	Diameter (mm)	Volume (cm^3)
NB-R	19 336.82 *	155.24 **	0.69	5.289 ***
SB-G	15 106.81	108.89	0.54	2.73
SB-GM	18 328.67	118.15	0.52	2.87
SB-M	10 632.62	91.97	0.56	2.39
SB-R	14 974.29	97.89	0.48	2.15
SL-G	18 078.49	113.17	0.49	2.70
SL-GM	14 198.09	96.86	0.49	2.18
SL-M	14 782.35	114.65	0.62	3.60
SL-R	14 901.43	118.29	0.58	3.09

Tukey test, *p*-value 0 < *** < 0.001 < ** < 0.01 < * < 0.05.

3.5. Effects of BMs on the Aerial and Root Biomass of Tomato Plants

Figure 6 illustrates the effects of BM products on tomato plant aerial and root biomass, focusing on fresh shoot biomass, dry shoot biomass, fresh root biomass, and dry root biomass. Analysis of biomass data indicates that the fresh and dry shoot biomass weight of plants treated with DK-G, DK-M, and NB-R significantly increased ($p < 0.0001$), ranging from 8% to 52% compared with the non-inoculated control (C-NI) for dry above-ground biomass (Figure 6a,c). The best performances are obtained with beneficial microorganisms (BMs) from the Dakar region. For root fresh biomass, plants treated with NB-R and SL-GM showed a significant increase ($p < 0.0001$) compared to the control. In contrast, no significant difference was obtained in root dry biomass, although there was an increase in the mean of about 29% in NB-R compared with the control (Figure 6b,d).

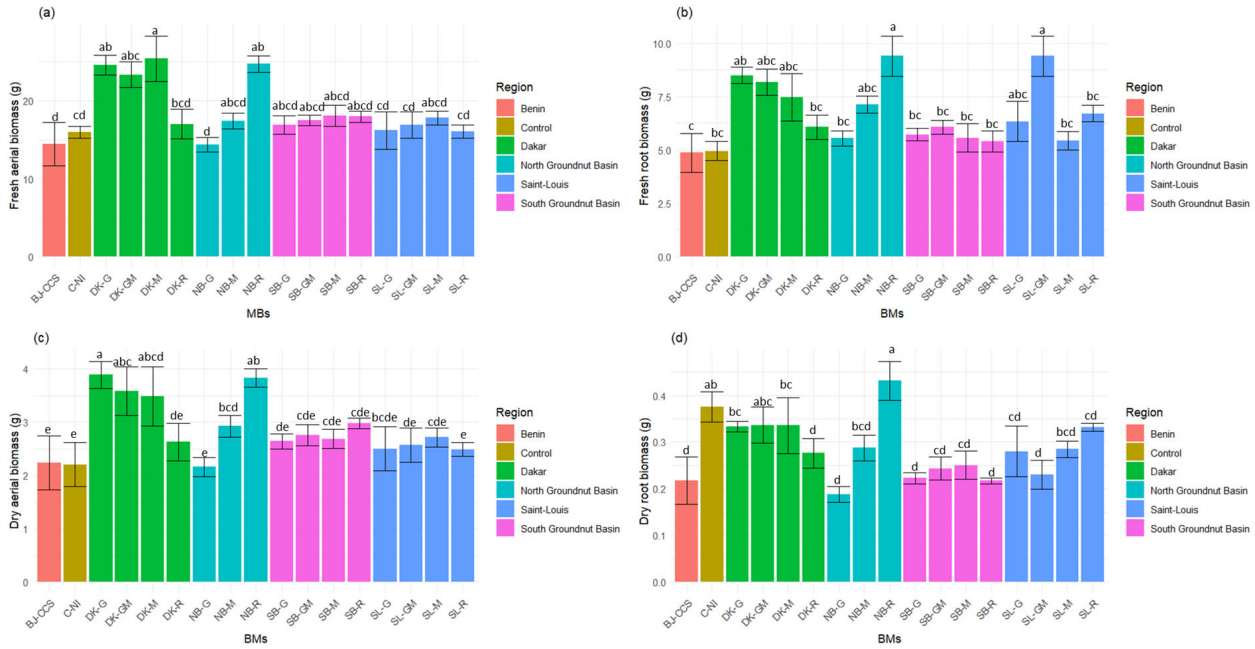


Figure 6. Effect of BMs on tomato plant shoot and root biomass; (**a**) fresh aerial biomass; (**b**) fresh root biomass; (**c**) dry aerial biomass; and (**d**) dry root biomass. Different colors represent forest litter collection areas. Overall, BMs DK-G, DK-GM, DK-M, NB-M, and SL-GM positively impacted tomato plant biomass compared to non-inoculated plants. The letters indicate the bioproducts grouped in the same statistical group by the comparison test.

4. Discussion

Effective microorganisms are a robust technology for improving soil quality, plant growth, and yield. The main microorganisms found in EMs are photosynthetic bacteria (*Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*), lactic acid bacteria (*Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*), yeasts (*Saccharomyces* spp.), actinomycetes (*Streptococcus* spp.), and fermenting fungi such as yeasts and actinomyces (*Streptomyces* spp.) [24–26]. While several studies highlight their abundance and potential benefits, the existing research remains incomplete, leaving their effectiveness insufficiently demonstrated. This gap often leads to legitimate questions regarding their real usefulness [27].

The metabarcoding analysis conducted in our study confirms the presence of these microorganisms in BMs produced with local ingredients collected from four different agroecological regions in Senegal. The microbial content revealed the predominance of the bacterial genera *Lactobacillus*, *Acetobacter*, and *Sporolactobacillus*, as well as an extremely acidophilic fungal genus, *Acidea*. This finding is consistent with the few studies that describe the composition of microbial community groups that constitute effective microorganisms [12,28–30]. *Lactobacillus* are widely recognized for their holistic and ecological contributions to both agriculture and human health. Their remarkable capabilities include preserving and enhancing nutritional quality, serving as biocontrol agents, improving soil conditions, and stimulating plant growth [31–33].

Our results show some variations in the structure of the BMs' microbial communities due to the litter's origin. These results align with those of Marois et al. [16], who observed the relative importance of the impact of the origin of litter from Mediterranean and temperate climates in fermented forest litter (LFF) on the structure of microbial communities. Previous studies revealed that most of the microbes belonging to those genera are well known as plant growth and health-promoting microorganisms [34–37]. The presence of those microbial communities is linked to the decrease in the pH of the medium, which can reach 4 during the fermentation process [25]. The locally derived Senegal BMs demonstrated greater microbial community diversity compared to commercial BMs. Among their shared microbial communities are genera such as *Lactobacillus* and *Acetobacter*. These effective microorganisms play diverse roles in crop enhancement, including boosting seed germination, fostering seedling growth, increasing chlorophyll content in plant leaves, and promoting overall plant development [25,38–40]. In the present work, the application of 15 indigenous beneficial microorganisms (BMs) to tomato plants revealed that 3 of them significantly contributed to the improvement of plant growth and chlorophyll content in tomato leaves 28 days after treatment. Indeed, the BMs DK-M, DK-G, and DK-GM made with forest litter collected in the Dakar region, mixed with millet stover stalks, groundnut shells, or a mixture of both, were shown to be very effective in optimizing growth and chlorophyll synthesis in tomato plants. These results are in line with those of Kalaji et al. [41], who showed that the inoculation of 10 mL of EM to *Arabidopsis* plants significantly improves the leaves' chlorophyll content. A similar study carried out on *Kalanchoe daigremontiana* [42] showed that the microorganisms effectively contribute to increased root development and germination of *K. daigremontiana*.

All tomato plants treated with the three most effective BMs in this study showed a significant increase in plant height, number of leaves, root weight, and chlorophyll content. This improvement can be attributed to their ability to degrade organic matter in the substrate, making essential nutrients such as iron and magnesium available, which are necessary for plant growth [43,44]. Furthermore, these microorganisms have been identified to enhance the absorption capacity of minerals, resulting in increased chlorophyll content and enhanced photosynthetic abilities [37,45]. The production of biologically active substances, such as phytohormones and growth factors, by these microorganisms, can also

contribute to the observed improvements in phenotypic traits [46,47]. Indeed, the effect of several phytohormones, such as AIA, allows root growth, thus increasing the plant's ability to explore the rhizosphere and mobilize more nutrients [48–50].

The studies of Youssef et al. [51] show an improvement in the size and fresh and dry aboveground biomass of *Stevia rebaudiana* inoculated with BMs compared to non-inoculated plants. Our results corroborate their conclusions, showing a significant increase in plant height and fresh and dry aerial biomass for plants inoculated with BMs NB-R, NB-M, DK-G, DK-M, and DK-GM. The performance of these BMs observed in 28 days could be maintained by a double or triple inoculation during the plant cycle. Indeed, studies conducted by Javaid and Bajwa [24] showed that multiple inoculations of EM at 0.2% at 15-day intervals to wheat increased aboveground dry biomass by nearly 272%.

The Dakar region of Senegal has been recognized as the source of the most effective locally beneficial microorganisms (BMs). Molecular studies revealed a spatial effect on the beta diversity of these microorganisms, suggesting that the highly diverse BM compositions for DK-G, DK-GM, and DK-M may contribute to their beneficial properties through complementary functional interactions [52]. This hypothesis is supported by observations that microorganisms with different ecological and functional characteristics can collaborate to enhance both root and aerial plant traits [53,54].

A similar study on *Kalanchoe daigremontiana* by Domenico [42] showed that the use of EMs led to a significant increase in plant height, number of leaves, vegetative and radical weight, number and weight of new shoots, and leaf area. Limanska et al. [55] reported that inoculation of tomato seeds and seedlings with *Lactobacillus plantarum* strains resulted in stimulated plant growth, with an increase in shoot, main root, and lateral root length, as well as the development of root hairs. Additionally, their studies showed that applying a mixture of effective microorganisms resulted in an acceleration of the decomposition of organic materials in soil, which in its turn led to the quicker and better release of nutrients for plant growth. A recent study showed a yield increase of 0.23 t/ha following the inoculation of EM-based fertilizers [56]. These findings agree with our results that show an increase in root length, root area, and root diameter for plants inoculated with DK-M, DK-G, and DK-GM at 28 DAT.

The significant positive effects of BMs from the Dakar region might be due to the limited microbial diversity in this area, which consists of only three or four microbial genera, all belonging to the Bacilli class. This suggests that they share similar functions, such as promoting growth, protecting against pathogens, and enhancing plant health. In other regions, in addition to *Bacilli*, there are bacteria from the *Alphaproteobacteria* class, primarily involved in nitrogen fixation. These bacteria also aid in promoting plant growth. However, due to their different mechanisms of action, they may disrupt the synergy with beneficial bacteria from other classes. Moreover, as the diversity of microbial groups increases, competition for resources can arise, potentially hindering their ability to benefit the plant.

The uniqueness of this study lies in the use of local resources (forest litters, rice bran, millet stover, and peanut shells available throughout Senegal) to produce local BM. It allowed us to confirm the presence of beneficial microbial communities, regardless of the type of carbon source used or the region where forest litter was collected. These results will enable farmers to produce BM using any available resources.

5. Conclusions

This study aimed to determine the composition and abundance of effective microorganisms present in forest litter in Senegal and to evaluate their agronomic efficacy on tomatoes. A total of 18 bacterial genera were identified in forest litter. Among these, Lacto-

bacillus was prominent, along with *Acetobacter*, *Sporolactobacillus*, *Clostridium*, and *Bacillus*. The composition of beneficial microorganisms (BMs) in fungi was very low, with the dominant fungal genus being *Acidea*, a non-pathogenic genus. Inoculating tomato plants with the 15 Senegalese BMs from our study revealed that three of them were highly effective compared to uninoculated plants. They significantly improved plant growth, chlorophyll content, and both above-ground and root biomass, among other parameters. These results highlight the value of local beneficial microorganisms as a low-cost, agroecological solution to boost crop resilience and productivity in Senegal. To consolidate these findings, further studies are needed. The metabarcoding analysis will be complemented by total genomic DNA sequencing to enable precise species-level identification of beneficial microbes within the BMs. Additionally, field trials under real farming conditions will be conducted using a dual inoculation strategy in order to validate the effects of the best-performing BMs on tomato yield. This next phase is crucial for assessing the scalability of the approach and its relevance for widespread adoption by smallholder farmers.

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