



## Population and Diversity of Arbuscular Mycorrhizal Fungi and Bacteria in Soil Amended With Compost

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### ABSTRACT

This study investigated the impact of soil amendment with compost on arbuscular mycorrhizal fungi (AMF) population/spore numbers and diversity, and also on soil bacterial population and diversity. Six (6) levels of compost (0g –Control soil, 200g, 400g, 600g, 800g, and 1000g), in a Randomized Complete Block Design (RBCD) was adopted to determine soil biota diversity. AMF spore were extracted from the soil and identified, and AMF colonization of the roots of Okra (*Abelmoschus esculentus*) was also determined. Cultured bacteria from soil samples were enumerated, isolated, characterized. Analysis of variance (ANOVA) at  $P < 0.05$  was performed on the data obtained and Duncan multiple range test was used to separate the mean values. Results of the study shows that microbial counts were higher in soil, with total bacterial and fungal counts of  $9.2 \times 10^6$  CFU/g and  $2.67 \times 10^4$  CFU/g respectively, compared to that of compost  $3.63 \times 10^6$  CFU/g and  $2.30 \times 10^4$  CFU/g respectively; the highest spore count was observed at the 1000g compost level (225 spores), followed by the 800g level (166.67 spores), while the lowest count was in 0g compost rate –Control soil (72.67 spores). On the other hand, the total bacteria population ranged from  $5.10 \times 10^6$  CFU/g to  $12.51 \times 10^6$  CFU/g, with significant differences indicating higher microbial presence in soils with increased compost application. A total of four AMF morphological types were identified belonging to four different genera, these include *Acaulospora*, *Rhizophagus*, *Gigaspora*, *Funneliformis*. The study concluded that adding compost to soil brings many benefits for soil health and diversity of soil microorganisms.

**Keywords:** Arbuscular Mycorrhizal Fungi (AMF), Compost, Diversity, Soil Microbes, Cropping System, *Abelmoschus esculentus*.

### Introduction

A thriving soil harbors a diverse community of microorganisms, including bacteria, fungi, and archaea (Chen *et al.*, 2018), which contribute significantly to nutrient cycling, decomposition processes, and the promotion of plant growth (Harrier & Watson, 2016). Also, soil microbes like Arbuscular mycorrhizal fungi (AMF) are responsible for the biological fertility of the soil (Chen *et al.*, 2018), and are great determinants for soil performance through the decomposition of plant materials and residues to increase the organic matter content of the soil, thereby improving soil quality (Rillig *et al.* 2019). AMF contribute to soil aggregation and structure through the production of glomalin, a glycoprotein that helps bind soil particles together (Rillig *et al.*, 2019).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous root symbionts with low host specificity and wide geographic distribution, and belong to the phylum Glomeromycota (Bücking & Kafle, 2015). AMF play a vital role in nutrient uptake and plant growth (particularly in low nutrient soil) by forming symbiotic associations with plant roots (Gupta & Sharma, 2017). Notably, Compost enriches the soil with organic matter and nutrients, providing food and habitat for soil microbes, while soil microbes, break down compost materials, releasing nutrients and enhancing soil fertility (van der Heijden *et al.* 2015). Additionally, soil amended with compost has been reported to increase the abundance and diversity of soil microbes including AMF (Gupta & Sharma, 2017).

Compost is a soil amendment that is rich in organic matter and nutrients and is commonly used to improve soil fertility and structure (Gul *et al.*, 2015), plant health as well as suppression of diseases caused by soil borne pathogens (Bonanomi *et al.*, 2015b). Compost is a valuable organic material produced through the controlled decomposition of organic waste (Bernal *et al.*, 2018). Compost is typically composed of 30-60% organic carbon, which is essential for soil carbon sequestration and serves as a food source for soil microbes (Bernal *et al.*, 2017). It is a haven for diverse beneficial soil microbes like bacteria, fungi, and actinomycetes that break down organic matter, a process that not only recycles nutrients but also improves soil aeration (Bünemann, 2018). This high organic matter content enhances soil structure, water retention, creating a favorable environment for microbial activity and root penetration (Bonanomi *et al.*, 2015a; Gul *et al.*, 2015). The organic matter in compost also improves soil cation exchange capacity (CEC), enabling it to retain and release nutrients more effectively (Berruti *et al.*, 2016).

Additionally, compost amendments generally lead to an increase in soil microbial biomass, as the organic matter in compost serves as a substrate for microbial growth, providing energy and nutrients that support microbial populations (Chaudhary, 2021).

Compost application often results in shifts in the relative abundance of microbial taxa within the soil community, because certain groups of bacteria, such as Actinobacteria and Proteobacteria, and fungi, such as Ascomycota and Basidiomycota, may proliferate in response to compost inputs (Chen *et al.*, 2018). Different compost types influence the composition of functional microbial groups in soil; for example, compost rich in plant residues may favor cellulolytic bacteria and fungi, whereas compost with animal manure may support nitrogen-fixing bacteria (Kranz *et al.*, 2020).

Understanding how soil amendment with compost influences the soil microbial community composition, particularly interactions with AMF, can provide valuable insights for optimizing soil health (Javot, 2017).

This study investigated the impacts of soil amendment with compost on arbuscular mycorrhizal fungi (AMF) population/spore numbers and diversity, and also on soil bacteria population and diversity.

## Materials and Methods

### Study Location

The research was conducted at the Faculty of Agriculture, Department of Crop and soil Science green house at Abuja campus, in University of Port Harcourt. The site is located between latitude 4°54'27.80"N and longitude 6°55'1.73"E, with an average temperature of 27°C, relative humidity of 78% and average rainfall that ranges from 2500-4000mm. The soil of Port Harcourt is of recent alluvial soil, dominated by low-lying coastal plains which structurally belong to the sedimentary formation of Niger delta.

### Experimental Design and Planting

Randomized Complete Block Design (RBCD) (Kranz *et al.*, 2020) was adopted for this study. Six (6) experimental set-ups consisting of 15kg each of soil were amended with different concentrations of the compost (0g, 200g, 400g, 600g, 800g, and 1000g). The 0g represents the control which had no compost. The compost was formulated using organic wastes (plant leaves, yam peels, plantain peels, saw dust, rice left overs, paper, and egg shells. This was allowed to mature for 6 months. Okra (*Abelmoschus esculentus*) seeds were purchased from the Community Market Choba, Port Harcourt Rivers State, Nigeria. Seeds were planted in the various bags bearing the different concentrations of compost stated above in 15kg of soil.

### Extraction of AMF Spores

The AM fungal spores were separated from the soil by wet sieving and decanting technique. Then 50g of rhizosphere soil sample was mixed in 200ml of distilled water in a large beaker. After 1hr, the contents of the beaker were decanted through sieves which were arranged in a descending order from 200 µm to 25 µm size. The process was repeated thrice, until the upper layer of the soil suspension was transparent. The retained material on the sieve was decanted into a beaker with a stream of water and estimation of spores was carried out.

### Identification of Mycorrhiza Fungi

Identification of AMF spore was performed by morphological observation of colour, shape, size, hyphal attachment, spore ornamentation and spore reaction towards Meizer's solution. Spore enumeration was conducted under a stereo microscope and spore identification was conducted under a microscope with 200x magnification (Berruti *et al.*, 2016).

### Arbuscular Mycorrhizal Fungi (AMF) Root Colonization

For the analysis of mycorrhizal colonization in the plants, the root samples were washed free of soil and cut into 1cm long bits, cleared in 2.5% KOH at 90°C for 20-30 minutes, rinsed in water, acidified with 5N HCL and stained in lactophenol containing 0.05% trypan blue 50 segments approx. stained root samples were mounted on slides and examined for AM colonization under a compound microscope at 10x10 magnification. Percent root colonization was calculated using the following formula; %Root colonization = (No of positive segments - No of segments observed) × 100 (Berruti *et al.*, 2016).

### Cultivation and Enumeration of Bacteria from Soil Samples

The standard plate count method as reported by Chouhan (2015) was used in cultivating the soil samples so as to enumerate the bacteria loads including isolating the bacteria types in the samples. A 10-fold serial dilution was carried out. In this method, stock solution of the soil sample was first prepared by transferring 1g of soil sample into test tube containing 10ml sterile normal saline. After which, 1ml of the sample was withdrawn from the stock with the aid of a sterile pipette to a test tube containing 9ml sterile normal saline. This was done serially until a dilution of  $10^{-6}$  was obtained. Aliquot (0.1ml) of  $10^{-2}$  was inoculated on pre-dried Eosin methylene blue agar and cetrimide agar plates while aliquot of  $10^{-3}$  was inoculated on nutrient agar plates. Plates were inoculated in duplicates and were spread using sterile bent glass rod before they were incubated at 37°C for 24-48 hours. After the incubation, plates were observed for growth and the colonies were counted for enumeration of bacterial populations in the soil samples. The counts/ population was reported as colony forming unit per gram (CFU/g).

### Isolation of Bacteria

Pure cultures of bacteria were obtained by aseptically streaking representative discrete colonies of different morphological types which appeared on the cultured plates onto freshly prepared pre-dried Nutrient agar plates and were later incubated at 37°C for 24hours. After pure cultures were obtained, each isolates was preserved frozen in 10% glycerol in bijou bottles for later use.

The 10% glycerol was prepared by adding 90ml of water into a 10ml glycerol solution in a conical flask and thereafter 5 ml was dispensed into bijou bottles, which were sterilized and allowed to cool before the isolates were transferred using sterile wire loop.

### Characterization and Identification of Bacteria Isolates

The bacterial isolates were characterized by observing them microscopically and subjecting them to series of biochemical and physiological tests such as Gram stain, catalase, citrate, oxidase, coagulase, Methyl Red, Motility, indole, starch hydrolysis, Voges Proskauer and sugar fermentation tests. Further confirmation was done by comparing their characteristics with those of known taxa as outlined in Bergey's Manual of Systematic Bacteriology and advanced bacterial identification system (ABIS) reported by Garrity (2007).

### Statistical Analysis

Analysis of variance (ANOVA) at  $P < 0.05$  was performed on the data obtained in this study and Duncan multiple range test was used to separate the mean.

### Results

The results of the initial physiochemical and biological properties of soil and compost are presented in Table 1. The results indicated a moderately acidic pH of 5.37, moderate moisture content of 18.00%, a porosity of 40.67%, while the compost had a neutral pH of 7.29, a higher moisture content of 27.5% and a porosity of 60.65%. Compost contained significantly higher levels of organic carbon (4.25%), organic matter (7.37%), and available phosphorus (94.75 mg/kg) compared to the soil, which had levels of 1.40%, 2.43%, and 28.23 mg/kg, respectively. Microbial counts were higher in soil, with total bacterial and fungal counts of  $9.2 \times 10^6$  CFU/g and  $2.67 \times 10^4$  CFU/g, compared to that of compost  $3.63 \times 10^6$  CFU/g and  $2.30 \times 10^4$  CFU/g respectively. All bacteria species that were isolated from the initial soil sample were also present in compost except *Micrococcus* sp. and *Proteus* sp. which were present in soil only, fungi species identified in the soil were also present in the compost except *Rhodotorula* sp.

**Table 1: Initial Physiochemical and Biological Properties of Soil and Compost**

Properties	Soil	Compost
Texture	Sandy loam	loamy
Moisture Content (%)	18.00	27.5
Porosity (%)	40.67	60.65
Bulk Density (g/cm <sup>3</sup> )	1.46	0.42
pH	5.37	7.29
Organic Carbon (%)	1.40	4.25
Organic Matter (%)	2.43	7.37
Total Nitrogen (%)	0.21	0.17
Available Phosphorus (mg/kg)	28.23	94.75
Potassium (K, cmol/kg)	0.00	0.17
Sodium (Na, cmol/kg)	0.03	0.19
Calcium (Ca, cmol/kg)	3.53	19.73
Magnesium (Mg, cmol/kg)	1.11	6.82
Exchangeable Acidity	1.85	2.68
Cation Exchange Capacity (cmol/kg)	4.72	27.07
Total Bacterial Count (CFU/g)	9.2 x 10 <sup>6</sup>	3.63 x 10 <sup>6</sup>
Total Fungal Count (CFU/g)	2.67 x 10 <sup>4</sup>	2.30 x 10 <sup>4</sup>

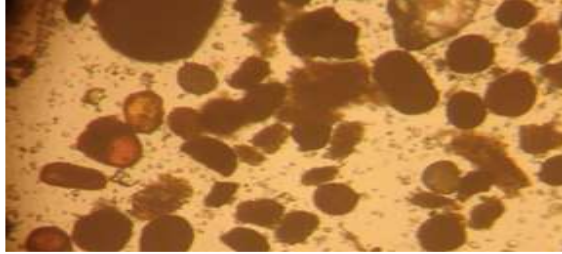
The results of Arbuscular Mycorrhizal Fungi (AMF) population and number of spores in the rhizosphere soil of *Abelmoschus esculentus* (okra) is presented in Table 2. The AMF spore population increased across compost levels, with a range of 72.67 to 225 spores per 50g dry weight. The highest spore count was observed at the 1000g compost level (225 spores), followed by the 800g level (166.67 spores), while the lowest count was in 0g compost rate (72.67 spores). Significant differences ( $p < 0.05$ ) indicated that increasing compost levels positively influenced AMF spore production. AMF population/ number of spores followed the order 1000 g > 800 g > 200 g > 600 g > 400 g > 0 g.

The results on individual Arbuscular mycorrhizal fungi (AMF) species diversity identified from the rhizosphere soil of Okra under the different compost levels are also presented in Table 2. A total of four AMF morphological types were identified belonging to four different genera, these include *Acaulospora*, *Rhizophagus*, *Gigaspora*, *Funneliformis* (Plate 1). *Acaulospora* spp. had a range 41.5 – 114 g/dwt, *Rhizophagus* spp. ranged from 16 – 57 g/dwt. *Gigaspora* spp. ranged from 0 – 5.67 g/dwt, *Funneliformis* spp. ranged from 15.5 – 45 g/dwt. The Mycorrhiza genera followed the order *Acaulospora* > *Rhizophagus* > *Funneliformis* > *Gigaspora* for spore abundance at 50g soil.

**Table 2: Individual Arbuscular Mycorrhizal Fungi (AMF) Species Population and Diversity**

Compost level	<i>Acaulospora</i> spp.	<i>Rhizophagus</i> spp.	<i>Gigaspora</i> spp.	<i>Funneliformis</i> spp.	Spore / 50gdwt
0 g	41.5 <sup>a</sup>	16 <sup>a</sup>	0 <sup>a</sup>	15.5 <sup>a</sup>	72.67 <sup>a</sup>
200 g	88.1 <sup>b</sup>	28 <sup>c</sup>	4 <sup>b</sup>	16 <sup>a</sup>	137.5 <sup>b</sup>
400 g	74.67 <sup>c</sup>	16.5 <sup>a</sup>	0 <sup>a</sup>	17.33 <sup>a</sup>	109 <sup>c</sup>
600 g	80.5 <sup>d</sup>	23.17 <sup>b</sup>	0 <sup>a</sup>	21 <sup>b</sup>	126 <sup>d</sup>
800 g	98 <sup>e</sup>	40.33 <sup>d</sup>	5.67 <sup>c</sup>	22.67 <sup>b</sup>	166.67 <sup>e</sup>
1000 g	114 <sup>f</sup>	57 <sup>e</sup>	5.33 <sup>c</sup>	49 <sup>c</sup>	225 <sup>f</sup>

The letters represent mean differences (Means with similar letters showed no significant difference,  $P > 0.05$ )



Arbuscular mycorrhizal fungi (AMF) Spores



*Acaulospora* spp.



*Gigaspora* spp.



*Rhizospora* spp.

Plate 1: AMF Species Identified Under Different Compost Levels

The results of the arbuscular mycorrhizal fungi (AMF) root colonization status and total bacteria and total fungi populations (counts) are presented in Table 3. Photos of root colonization are presented in Plate 2. The roots of all okra plants under different compost levels were all infected by mycorrhiza hyphae during the study (plate 2). Total number of roots ranged from 100.97-115.5, total root infected ranged 9.17 - 62.67 under the various compost levels respectively (Table 3), % AMF Colonization ranged from 7.94% - 55.13% with the highest colonization observed under 1000 g compost level, followed by 600 g compost level while 0 g recorded the lowest % AMF colonization. The % AMF colonization followed the order 1000 g > 600 g > 800 g > 400 g > 200 g > 0 g.

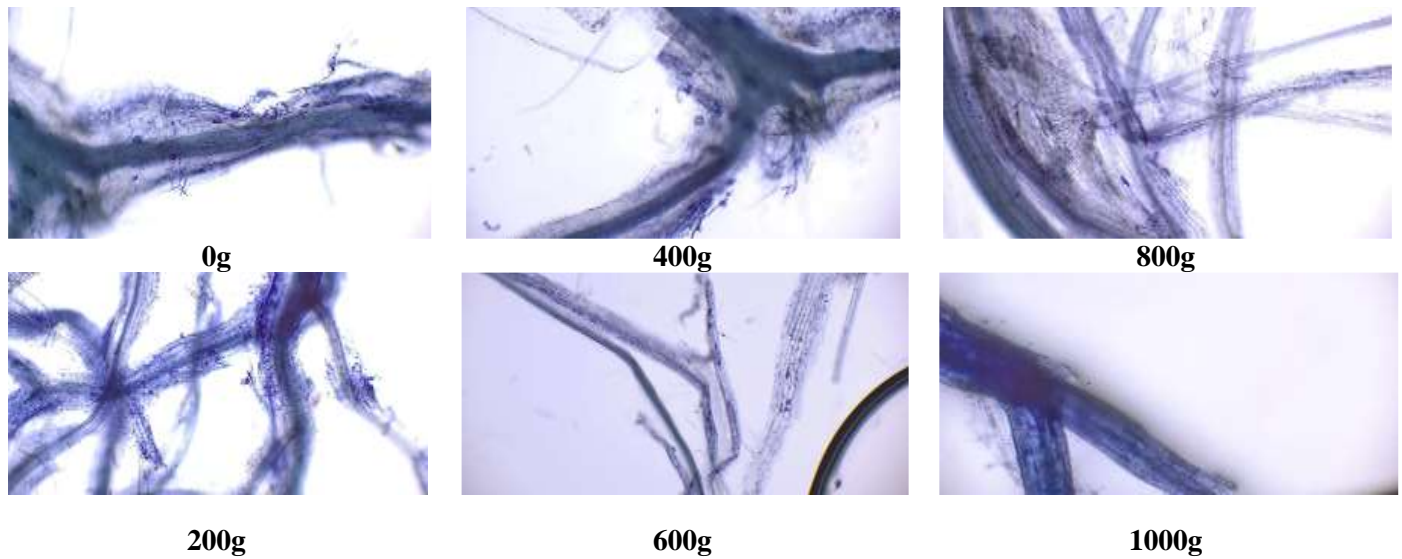
The total bacteria population ranged from 5.10 x 10<sup>6</sup> CFU/g to 12.51 x 10<sup>6</sup> CFU/g, with significant

differences indicating higher microbial presence in soils with increased compost application. The highest total bacteria population (12.51 x 10<sup>6</sup> CFU/g) was observed at 800g, followed by 200g with a value of 11.22 x 10<sup>6</sup> CFU/g, the lowest bacteria population (5.10 x 10<sup>6</sup>) was recorded at 400g. Total bacteria population followed the order 800g > 200g > 1000g > 600g > 0g > 400g. Total fungi count ranged from 0.6x10<sup>4</sup> CFU/g to 3.37x10<sup>4</sup> CFU/g. The highest total fungi population (3.37x10<sup>4</sup> CFU/g) was observed at 200g, followed by 0g with a value of 1.67x10<sup>4</sup> CFU/g, the lowest total fungi population (0.6x10<sup>4</sup> CFU/g) was recorded at 400g. Total Fungi population followed the order 200g > 0g > 800g > 1000g > 600g > 400g. Bacteria and fungi population showed significant differences (p = 0.05) under different levels of compost.

Table 3: Arbuscular Mycorrhizal Fungi (AMF) Root Colonization and microbial counts under Compost Levels

Compost Level	Arbuscular Mycorrhizal Fungi Root Colonization			Microbial Counts	
	Total root count	Total root infected	% AMF	Total Bacteria Count (x10 <sup>6</sup> )	Total Fungi Count (x10 <sup>4</sup> )
0 g	115.51 <sup>d</sup>	9.17 <sup>a</sup>	7.94 <sup>a</sup>	6.51 <sup>b</sup>	1.67 <sup>c</sup>
200 g	109.03 <sup>c</sup>	26.79 <sup>b</sup>	24.57 <sup>b</sup>	11.22 <sup>e</sup>	3.37 <sup>d</sup>
400 g	100.97 <sup>a</sup>	33.00 <sup>c</sup>	32.68 <sup>c</sup>	5.10 <sup>a</sup>	0.6 <sup>a</sup>
600 g	105.17 <sup>b</sup>	46.14 <sup>d</sup>	43.87 <sup>d</sup>	7.21 <sup>c</sup>	0.75 <sup>a</sup>
800 g	102.15 <sup>a</sup>	33.60 <sup>c</sup>	32.89 <sup>c</sup>	12.51 <sup>f</sup>	1.10 <sup>b</sup>
1000 g	113.67 <sup>d</sup>	62.67 <sup>e</sup>	55.13 <sup>e</sup>	9.92 <sup>d</sup>	0.75 <sup>a</sup>

(P = 0.05) Means with the same letters in each section were not significantly different at p<0.05



**Plate 2: Arbuscular Mycorrhizal Fungi (AMF) Root Colonization under Different Levels of Compost**

The diversity and distribution of bacterial isolates under different compost level is presented in Table 4. *Bacillus* sp., *Staphylococcus* sp. and *Pseudomonas* sp. were present in across compost levels, *Alcaligenes* sp., were present in all except 1000g compost level, *Micrococcus* sp. were present in only 400g, 600g, 800g compost levels, *Cronobacter* sp. were identified in 200g, 600g, 1000g compost levels, *Serratia* sp. was not present in all compost levels except 200g.

The result of the phenotypic characterization of bacterial isolates is presented in Table 5. A total of nine bacteria species were identified, these include *Staphylococcus* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Cronobacter* sp., *Proteus* sp., *Serratia* sp., *Alcaligenes* sp., *Tatumella* sp., *Staphylococcus* sp., and *Pseudomonas* sp.

**Table 4: Distribution of Bacteria across Various Compost Levels**

Bacterial Isolate	Initial Soil	Initial Compost	Compost treated 15kg soil					
			0g	200g	400g	600g	800g	1000g
<i>Staphylococcus</i> sp.	+	+	+	+	+	+	+	+
<i>Serratia</i> sp.	-	-	-	+	-	-	-	-
<i>Pseudomonas</i> sp.	+	+	+	+	+	+	+	+
<i>Bacillus</i> sp.	+	+	+	+	+	+	+	+
<i>Micrococcus</i> sp.	+	-	-	-	+	-	+	+
<i>Alcaligenes</i> sp.	+	+	+	+	+	+	+	+
<i>Cronobacter</i> sp.	+	+	-	+	-	+	-	+
<i>Tatumella</i> sp.	-	-	-	-	+	-	+	-
<i>Proteus</i> sp.	+	-	-	-	-	-	-	+

**Key:** + = isolated; - = not isolated

The diversity and distribution of fungi across different compost level; and macroscopic and microscopic characteristics of isolates is presented in Table 6 and 7 respectively. A total of nine Fungi species were identified, these include *Rhizopus* sp., *Candida* sp., *Aspergillus niger*, *Geotrichum* sp., *Rhodotorula* sp., *Aspergillus flavus*, *Penicillium* sp., *Mucor* sp., and *Scopulariopsis* sp.

*Rhizopus* sp. and *Mucor* sp. were present in all compost levels, while *Candida* sp. were present in all except 1000g compost level, *Geotrichum* sp. were identified in all compost level except 0g compost level, *Penicillium* sp. were present in all compost level except 0g and 800g compost levels, *Scopulariopsis* sp. were absent in all compost level except 1000g.

**Table 5: Phenotypic Characterization of Bacterial Isolates**

Isolate Code	Macroscopy	Microscopy	Motility	Catalase	Glucose	Mannitol	Lactose	Sucrose	Oxidase	Methyl red	Voges Proskamer	Indole	Citrate	Probable Identity
1.	Green small round moist	-ve rods	+	+	-	+	-	-	+	-	-	-	+	<i>Pseudomonas</i> sp
2.	Green small round moist	-ve rods	+	+	-	+	-	-	+	-	-	-	+	<i>Pseudomonas</i> sp
3.	Green small round moist	-ve rods	+	+	-	-	-	-	+	-	-	-	+	<i>Alcaligenes</i> sp
4.	Green-brown, round flat	-ve short rods	+	+	-	+	-	-	+	-	-	-	+	<i>Pseudomonas aeruginosa</i>
5.	Red round smooth	-ve short rods	+	+	+	+	-	+	-	-	+	-	+	<i>Serratia</i> sp
6.	Cream, raised slimy	+ve cocci	-	+	+	+	+	+	-	+	+	+	+	<i>Staphylococcus</i> sp
7.	Cream flat dry	+ve chained rods	+	+	+	+	-	+	-	-	+	-	+	<i>Bacillus</i> sp
8.	Cream flat dry	+ve chained rods	+	+	+	+	-	+	-	-	+	-	+	<i>Bacillus</i> sp
9.	Yellow small round	+ve small cocci	-	+	-	+	+	+	-	-	-	-	+	<i>Micrococcus</i> sp
10.	Green small round moist	-ve rods	+	+	-	-	-	-	+	-	-	-	+	<i>Alcaligenes</i> sp
11.	Pink large round smooth	-ve short rod	+	+	+	+	+	+	-	-	+	-	+	<i>Cronobacter</i> sp
12.	Brown round raised	-ve short rods	-	+	+	-	-	+	-	-	-	-	-	<i>Tatumella</i> sp
13.	Cream round flat dry	-ve rods	+	+	+	+	-	+	-	+	+	-	+	<i>Cedecea</i> sp
14.	Pale small round	-ve rods	+	+	+	-	-	+	-	-	+	-	+	<i>Proteus</i> sp
15.	Pale round smooth	-ve short rod	+	+	+	-	-	-	-	+	-	+	+	<i>Providencia</i> sp

**Table 6: Distribution of fungi across various compost levels**

Fungal Isolate	Initial Soil	Initial Compost	Compost treated 15kg soil					
			0g	200g	400g	600g	800g	1000g
<i>Rhizopus</i> sp.	+	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	-	-	-	+	-	-	-	-
<i>Rhodotorula</i> sp.	+	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	-	+	+	-	-	-	-	-
<i>Candida</i> sp.	+	+	+	+	+	+	+	+
<i>Geotrichum</i> sp.	-	-	-	+	+	+	+	+
<i>Penicillium</i> sp.	+	+	-	+	+	-	+	+
<i>Scopulariopsis</i> sp.	-	-	-	-	-	-	-	+
<i>Mucor</i> sp.	+	+	+	+	+	+	+	+

**Table 6: Macroscopic and microscopic characteristics of fungal isolates**

Isolate code	Macroscopy	Microscopy	Probable identity
F10	White cottony, brownish grey to black-grey coloration, brown reverse	Smooth walled and non-septate branched sporangiophores, presence of rhizoids	<i>Rhizopus</i> sp.
F1	White periphery, dense black spores, dark brown reverse	Hyaline conidiophore phialides borne on vesicles, chains of conidia with septate hyphae	<i>Aspergillus niger</i>
F3	Pink-red smooth colonies	Ovoid, elongate budding cells	<i>Rhodotorula</i> sp.
F6	Dark green slimy suede-like conidia	Erect conidiophores with phialides bearing one-celled hyaline conidia	<i>Gliocladium</i> sp.
F4	White periphery, dense green spores, dark brown reverse	Hyaline conidiophore phialides borne on vesicles, chains of conidia with septate hyphae	<i>Aspergillus flavus</i>
F11	Cream to shiny round colonies	Large oval cells with budding cells	<i>Candida</i> sp.
F5	Flat white dry sued-like colonies, white reverse	Cylindrical arthroconidia	<i>Geotrichum</i> sp.
F2	Green powdery surface surrounded by white lawn, brown reverse	Septate hyphae with septate conidiophores bearing conidia	<i>Penicillium</i> sp.
F7	Buff to brown like growth	chains of single-celled conidia produced in basipetal succession	<i>Scopulariopsis</i> sp.
F8	Flat white dry sued-like colonies, white reverse	Cylindrical arthroconidia	<i>Geotrichum</i> sp.
F9	Fluffy white cottony, white reverse	Aseptate hyphae bearing round sporangiospores	<i>Mucor</i> sp.

## Discussion

The findings show the initial physiochemical and biological characteristics of both soil and compost, providing essential insights into their baseline status before the application of amendments. These results play a crucial role in understanding how different levels of compost influence soil health, microbial activity, and plant responses. Microbial analysis revealed higher total bacterial counts in soil than in compost, suggesting that the natural soil supports a broader bacterial community. However, the fungal count in compost ( $2.30 \times 10^4$  CFU/g) was similar to that in soil ( $2.67 \times 10^4$  CFU/g). These results indicate that while the compost may have fewer total bacteria initially, it still supports a robust microbial community essential for nutrient cycling and soil fertility (Smith & Taylor, 2020).

It was observed from the results that AMF spore population increased with increasing compost levels.

Compost amendments are known to boost AMF populations significantly, as organic matter enhances soil structure, moisture retention, and nutrient availability, creating favourable conditions for AMF proliferation as stated by Smith & Taylor (2020). Previous studies show that higher compost levels generally correlate with increased spore numbers, a reflection of AMF's positive response to organic substrates. Enhanced spore populations in amended soils contribute to the mycorrhizal network's stability, increasing its ability to support plant nutrient acquisition (Bender *et al.*, 2015). This relationship supports the hypothesis that compost acts as a stimulant for AMF reproduction, especially in species like *Acaulospora* and *Rhizophagus*, which have been observed to dominate in compost-treated environments due to their adaptability and efficient symbiosis with plant roots (Singh *et al.*, 2018). The diversity of AMF species within a soil ecosystem is crucial for the functional resilience of plant-microbe interactions, as varied AMF communities can provide a broader range of benefits to the host plant.



However, studies reveal that compost amendments often favour certain dominant species, sometimes limiting the establishment of a broader AMF community (Lekberg & Helgason, 2018). From the results in this study, it was observed that species from genera like *Acaulospora* and *Rhizophagus* frequently show higher increases in abundance under compost levels, whereas other genera may not establish as readily due to environmental and competitive dynamics in the soil microbiome this is in agreement with reports by Lekberg & Helgason (2018). This selective enrichment of specific AMF species suggests that compost amendments may create conditions that preferentially support species well-adapted to organic substrates, potentially affecting overall AMF community structure and reducing diversity. Limited diversity may constrain the functional benefits that a broader range of AMF species could otherwise provide, such as enhanced stress resilience and varied nutrient acquisition pathways (Singh *et al.*, 2018).

The extent of AMF root colonization often mirrors the nutrient quality and biological activity of the soil, with compost-treated soils consistently showing higher colonization rates (Zhang *et al.*, 2015). The results in this study shows that AMF colonization in plant roots generally increases with moderate compost levels, Compost provides a sustained nutrient source, which not only supports AMF development but also facilitates deeper root penetration by AMF hyphae, thereby increasing the plant's access to nutrients such as phosphorus, nitrogen, and trace minerals as observed in studies by Smith & Taylor (2020). Optimal compost levels enhance colonization without risking nutrient excess, providing a balanced environment that enables AMF to effectively contribute to soil nutrient cycles and plant health. AMF colonization and spore density were positively influenced by compost levels, with higher compost levels showing significant increases in both spore density and colonization rates, supporting findings by Holland *et al.* (2018), which demonstrated that organic amendments enhance AMF growth by providing necessary nutrients.

The percentage of root colonization increased with higher compost levels, suggesting a favourable environment for AMF symbiosis. This symbiosis is essential for improved nutrient uptake, especially phosphorus, as AMF facilitate nutrient acquisition in host plants (Zhang *et al.*, 2018).

The study's results show the potential of compost in promoting AMF, which are beneficial for plant health and nutrient efficiency.

Studies have shown that compost amendments significantly increase the total bacteria population in soil by providing a rich source of organic carbon and nutrients, which support bacterial growth (Singh *et al.*, 2018). Higher bacterial populations in compost-treated soils contribute to faster organic matter decomposition and enhanced nutrient cycling, making nutrients more available to plants. As observed in the results, increased bacterial counts particularly at compost levels between 200 g and 800 g, align with previous research indicating that moderate compost additions create a balanced environment that optimizes bacterial growth without causing nutrient saturation or competition among microbes. The microbial population may peak at optimal compost levels before stabilizing or declining if nutrient saturation or other inhibitory conditions occur (Zhang *et al.*, 2015).

At high compost levels, TBC tends to increase further, showing the ability of bacteria to utilize the additional nutrients and adapt to organic-rich conditions, this in agreement with studies by Holland *et al.* (2018). This trend shows the role of bacteria in enhancing soil fertility, as they help decompose organic compounds into simpler forms accessible to plants, such as nitrogen and phosphorus.

It was observed from the results that compost amendments also impact the total fungal population, although the response of fungi to compost can differ from that of bacteria due to their different ecological roles and nutrient requirements. Fungal populations generally prefer environments with stable organic content and lower pH, which compost can help maintain in certain concentrations (Zhang *et al.*, 2018). However, studies suggest that fungi may be less responsive to very high compost levels due to competition with bacteria, especially in highly organic conditions where bacterial activity is dominant (Sharma, 2021) as observed from the results.

Fungal populations often thrive at moderate compost levels, as these conditions provide sufficient organic matter without the excessive nutrient loads that can favour bacterial dominance (Singh *et al.* 2018). Fungi play a crucial role in decomposing complex organic matter, such as lignin and cellulose, which contribute to soil structure and the slow release of nutrients.

Compost-treated soils with moderate fungal activity can therefore support a balanced microbial ecosystem, facilitating nutrient availability and promoting long-term soil health (Holland *et al.*, 2018). Fungal diversity also reflects the influence of compost amendments on soil microbial structure. Fungi play a key role in decomposing complex organic materials and supporting long-term soil health, and their diversity in compost-amended soils can greatly benefit plant growth by improving nutrient cycling. It was observed that *Rhizopus* sp., *Candida* sp., and *Mucor* sp. were present across all compost levels, indicating their robustness in different compost conditions. These fungi are known to adapt well to organic-rich environments, playing crucial roles in organic matter breakdown (Zhang *et al.*, 2018). *Aspergillus niger* and *Aspergillus flavus* appeared selectively at lower compost levels, suggesting that certain compost levels may create niche environments suited to specific fungal taxa. This selective presence may be influenced by changes in soil moisture, pH, and organic matter composition, factors that typically fluctuate with compost addition (Rillig *et al.*, 2019). *Geotrichum* sp. and *Penicillium* sp. were more consistently isolated in various compost levels, highlighting that intermediate compost levels might optimize fungal diversity by balancing nutrient richness without overwhelming the soil with organic matter (Altieri *et al.*, 2016).

The distribution of fungi across compost levels supports findings that moderate compost applications can sustain diverse fungal communities, whereas very high or low compost levels may limit the diversity of fungal species in soil (Zhang *et al.*, 2018). This variation in fungal diversity emphasizes the importance of balanced compost use to maintain fungal populations that support soil fertility and plant health. The results indicate a range of bacteria genera present across different compost levels. Compost levels generally enhanced bacteria diversity by supporting beneficial bacteria, although some bacterial isolates were less prevalent at certain levels. *Staphylococcus* sp., *Pseudomonas* sp., *Bacillus* sp., and *Alcaligenes* sp. were consistently present across all compost levels, reflecting their resilience and adaptability to a wide range of soil environments. These genera are well-known for their beneficial roles, including pathogen suppression, organic matter decomposition, and nutrient solubilization which is in agreement with findings by Bhatnagar & Reddy (2017).

*Serratia* sp. was observed only at the 200g compost level, while other species like *Proteus* sp. and *Tatumella* sp. appeared sporadically across compost levels, suggesting that certain compost levels provide niche environments for these microbes. Previous research suggests that moderate compost levels may temporarily foster conditions that support species typically absent in soils with no compost or high compost concentrations (Adekiya *et al.*, 2019). The appearance and disappearance of some bacterial species, such as *Cronobacter* sp. and *Micrococcus* sp., across compost levels, suggest that compost levels modulate microbial diversity by altering soil pH, nutrient availability, and organic matter content (Chen *et al.*, 2018). This pattern of bacterial diversity aligns with findings that organic amendments can create favourable conditions for diverse microbial populations, enhancing functional benefits for plant systems (Bhatnagar & Reddy, 2017). However, the selective occurrence of certain bacteria highlights that compost effects on diversity may vary, as specific conditions may favour the establishment of certain taxa over others.

In conclusion, this study has shown that adding compost to soil brings many benefits for soil health and diversity of soil microorganisms, higher compost levels increased beneficial fungi and microbes, which help cycle nutrients and improve soil quality, and the diversity of bacteria and fungi in the soil increased with more compost, likely due to the added organic matter that supports a variety of microorganisms.

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