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In vitro Evaluation of the Effect of Inorganic Fertilizer on Rhizosphere Soil Microbial Populations during Early Growth of *Zea mays* and *Phaseolus vulgaris*

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Authors' contributions

This work was carried out in collaboration between all authors. Author ECC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EOE, MCO, OO, COJ, MCE, NUN and POC managed the analyses of the study. Author EOE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study assessed the effects of different regimen of inorganic fertilizers on the microbial community structure in the rhizosphere soil of *Zea mays* and *Phaseolus vulgaris* during early growth.
Study Design: Seeds of *Zea mays* and *Phaseolus vulgaris* were planted and Inorganic fertilizers were added to the soil after two weeks of planting to determine their effects on the microbial structure as well as the microbial succession pattern in the rhizosphere soils of *Zea mays* and

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Phaseolus vulgaris during early growth. Statistical analysis were carried out using a two-way ANOVA.

Place and Duration of Study: This study was carried out at the farmland of School of Agriculture and Agricultural Technology of the Federal University of Technology, Owerri Nigeria for a period of five weeks.

Methodology: Seeds of *Zea mays* and *Phaseoulus vulgaris* were planted in a soil under laboratory condition and different regimen of inorganic fertilizers added after two weeks using the placement method of fertilizer application, and a control experiment maintained without addition of fertilizers. Rhizosphere soil from *Zea mays* and *Phaseolus vulgaris* were collected every week for the remaining weeks for microbiological analysis to determine the microbial community and microbial populations present in the soil.

Results: In this study the presence of *Bacillus* sp*, Staphylococcus aureus, Micrococcus luteus, Enterococcus faecalis,* and *Rhizobium* sp, were evident in the rhizosphere soil of both *Zea mays* and *Phaseolus vulgaris* all through the five week period of study. The fungal community structure revealed the presence of *Saccharomyces* sp, *Fusarium* and *Penicilium notatum* for *Zea mays* rhizosphere soil, while *Phaseolus vulgaris* rhizosphere soil had *Saccharomyces* sp, *Streptomyces* sp, *Fusarium* and *Penicilium notatum.* The microbial succession pattern revealed that *Bacillus* sp, *Staphylococcus aureus, Rhizobium* sp and *Saccharomyces* sp were mostly predominant in the rhizosphere soil of *Zea mays* and *Phaseolus vulgaris* all through the period of study.

Conclusion: The results from this study revealed that inorganic fertilizers had significant effect on the microbial community structure present in the rhizosphere soil of *Zea mays* and *Phaseolus vulgaris* during early growth when compared to the control without inorganic fertilizer. This result suggests that the increased microbial community in rhizosphere soil of *Zea mays* and *Phaseolus vulgaris* resulted from increased biological interaction in the soils between the roots of plants, microorganisms and the inorganic fertilizer.

Keywords: Microbial succession; microbial community structure; inorganic fertilizers; Zea mays; Phaseolus vulgaris.

1. INTRODUCTION

Soil is a mixture of minerals, organic matter, gases, liquids, and countless organisms that together support life on earth [1]. It plays a major role in determining how fast or well a crop would grow. The soil microorganisms are also known to exert profound influences on the status of soil fertility, in particular, on the availability of plant nutrients [2]. Critical factors such as soil organic matter decomposition, nutrient cycling, soil degradation and bioremediation of polluted soils is chiefly determined by these soil microorganisms [3]. The presence of these microbes in a soil play important role in shaping the community structure and function of the plant [4] and also the distribution of nutrients to the plants. One other factor known to influence community structure as well as availability of nutrients is the presence or absence of amendments in the form of fertilizers.

The application of fertilizers to soils can cause changes to the physical, chemical as well as the biological properties in the soil [5]. It is known that inputs of agrochemicals and fertilizers into soil can increase the sustainability of the cropping systems that result from biological interactions in the soil [6]. Clegg et al. [7] had reported that fertilizers have significant impact on the total microbial community structure and also increased soil microbial densities. The application of these soil amendments in the form of fertilizers has always been a pivotal principle of sustainable agriculture [8]. These amendments cause biological changes in the rhizosphere. The rhizosphere is the region which includes plant roots and its surrounding soil [9] and it is extremely important for active root metabolism. The rhizosphere that surrounds the plant root surface is affected by plant root activities. Plant roots secrete mucilage which supplies carbohydrate sources to soil micro-organisms [10].

The community structures of soil microorganisms in the rhizosphere are expected to differ greatly from that in bulk soil (non-rhizosphere soil). This is basically due to the difference in the amount of nutrient being supplied to the soil through the biological interactions that takes place between the roots and the soil microbial community. Rhizosphere microorganisms, bacteria in particular, strongly influence plant growth.

This study was aimed at understanding of the microbial community present in the rhizosphere of maize (*Zea mays*) and bean (*Phaseolus vulgaris*) plants and their response to different regimens of fertilizer treatments at early growth of the seedlings.

2. MATERIALS AND METHODS

2.1 Study Area

The farmland of the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo state, Nigeria at coordinate of 5.3905℃N, 6.9907℃E.

2.2 Collection of Samples

2.2.1 Soil sample

Soil samples were randomly collected from an uncultivated portion of the farmland using a shovel to a depth of 20 cm. The collected soils were bulked to form a composite sample and 5 kg each was measured and stored in polythene bags where the pot planting experiment was carried out.

2.2.2 Maize and bean seed sample

Maize and Bean seeds were obtained from Imo Agricultural Development Program [ADP] Centre, Owerri, Imo state, Nigeria

2.2.3 Chemical fertilizers

The two regimens of chemical fertilizers used, namely NPK 10-10-10 and NPK 15-15-15 which were also obtained from Imo Agricultural Development Program [ADP] Centre, Owerri, Imo state, Nigeria were used for this trial.

2.2 Planting of Seed and Treatment Application

In total, *Zea mays* and *Phaseolus vulgaris* seeds (five per bag) were planted in duplicate in two different set ups. In one set up, the seeds were planted without the application of chemical fertilizer and in the second experimental set up, the seeds were planted and Chemical fertilizers applied three weeks after seed germination using the placement method as applied [11]. It refers to the application of fertilizers into the soil close to the seed or plant in order to supply the nutrients in adequate amounts to the roots of growing plants. The seeds were left to grow for five weeks and the effect of the fertilizers on the rhizosphere microbial community during the early growth of maize and beans was determined. Bare soils without these plants were used as control to compare changes in microbial population of Bare soil with and without inorganic amendments.

2.3 Enumeration of Microbial Community

Soil samples were analyzed during the period of growing weeks; at the beginning and at the end of planting to determine the microbial populations present in the soil. The microbiological analysis carried out using the method of dilution on specific media as described by [12]. Total heterotrophic bacterial count were determined using Nutrient Agar, total fungal counts were determined using Potato Dextrose Agar (PDA), while total rhizobium count was determined using Congo red yeast extract mannitol Agar (CREYEMA). Upon incubation and development of colonies, colonies formed on the respective media were counted and calculated as colony forming units per 1 g of dry soil.

2.4 Statistical Analysis

The data were subjected to two-way analysis of variance (two-way ANOVA) using SPSS 16.0 statistical program followed by post hoc testing. Mean values were separated using the Duncan and Student-Newman Keuls method at P=0.05 respectively.

3. RESULTS AND DISCUSSION

3.1 Microbial Analysis

Microbial analysis of maize and beans rhizosphere soil revealed the presence of bacteria, fungi and rhizobium species as shown in Table 1. Bacterial populations isolated from the rhizosphere soils revealed the presence of *Bacillus* sp, *Staphylococcus aureus, Micrococcus luteus, Enterococcus faecalis* and *Rhizobium* sp for both beans and maize. Fungi isolated from the rhizosphere of bean plants included *Saccharomyces* sp, *Streptomycetes* sp, *Fusarium* sp, and *Penicillium notatum,* while the maize rhizosphere soils revealed the presence of *Saccharomyces* sp, *Fusarium* sp and *Penicillium* sp.

Table 2 shows the total heterotrophic bacterial count (THBC), total fungal count (TFC), total Rhizobium count (TRC) is as shown in Table 2, Table 3 and Table 4 respectively. The results revealed a difference in count at different week of microbial analysis during the five weeks of maize and Beans planting.

3.2 Microbial Succession Pattern during the Five Weeks of Maize and Beans Plant Growth

Tables 5 and 6 shows the results for the microbial succession pattern for beans and maize rhizosphere soils. *Bacillus* sp was present all through the period of study while *Micrococcus luteus* was only present in the bulk soil and absent went the maize and beans plant were planted. Rhizobium was present in all through the study period for maize plant. In the beans plant, Rhizobium was present in the bulk soil and was absent when the beans seed was planted but later reappeared when the fertilizers regime were added to the soil. *Saccharomyces* sp was presented all through the study period for beans and maize plant. All other fungi species of *Streptomyces, Fusarium* and *Penicillium* showed varying degree of fluctuation all through the period of study.

4. DISCUSSION

This study reveals the presence of *Bacillus* sp, *Staphylococcus aureus, Micrococcus luteus, Enterococcus luteus, Rhizobium sp, Fusarium sp, Penicillium sp* and *Streptomyces sp* in the

Table 1. Bacteria and fungi species isolated from the rhizosphere of Beans and Maize

Plant	Bacteria	Fungi
Beans	*Bacillus sp*	*Saccharomyces sp*
	Staphylococcus aureus	*Streptomycetes sp*
	Micrococcus luteus	*Fusarium sp*
	Enterococcus faecalis	*Penicillium notatum*
	Rhizobium sp	
Maize	*Bacillus sp*	*Saccharomyces sp*
	Staphylococcus aureus	*Fusarium sp*
	Micrococcus luteus	*Penicillium notatum*
	Enterococcus faecalis	
	Rhizobium sp	

Table 2. Total heterotrophic bacterial count (THBC) during the five weeks of maize and beans plant growth

Sample	Week 1 (cfu/g)	Week 2 (cfu/g)	Week 3 (cfu/g)	Week 4 (cfu/g)	Week 5 (cfu/g)
Soil only	3.0×10^8	3.5×10^8	2.8×10^8	2.2×10^8	2.0×10^8
Soil + maize	ND	3.8×10^8	5.0×10^8	5.1×10^8	5.7×10^8
Soil + Bean	ND	4.1×10^7	5.5×10^7	6.8×10^7	1.6×10^7
Soil + maize + NPK 10-10-10	ND	ND	4.3×10^8	6.2×10^8	7.1×10^8
Soil + Beans + NPK 10-10-10	ND	ND	6.6×10^8	9.7×10^8	4.2×10^8
Soil + maize + NPK 15-15-15	ND	ND	5.2×10^8	7.0×10^8	8.7×10^8
Soil + Beans + NPK 15-15-15	ND	ND	1.2×10^7	2.2×10^7	7.6×10^7

Key: cfu/g – Colony forming unit / gram; ND – Not determined

Table 3. Total fungal count (TFC) during the five weeks of maize and beans plant growth

Sample	Week 1 (cfu/g)	Week 2 (cfu/g)	Week 3 (cfu/g)	Week 4 (cfu/g)	Week 5 (cfu/g)
Soil only	1.6×10^7	1.0×10^7	1.3×10^7	1.2×10^7	1.1×10^7
Soil + maize	ND	5.0×10^6	1.5×10^7	1.7×10^7	1.9×10^7
Soil + Bean	ND	1.0×10^6	2.1×10^6	1.4×10^6	4.8×10^6
Soil + maize + NPK 10-10-10	ND	ND	1.3×10^7	1.9×10^7	2.2×10^7
Soil + Bean + NPK 10-10-10	ND	ND	1.7×10^6	2.3×10^6	5.1×10^6
Soil + maize + NPK 15-15-15	ND	ND	1.3×10^7	2.5×10^7	2.9×10^7
Soil + Bean + NPK 15-15-15	ND	ND	2.7×10^6	3.1×10^6	1.2×10^7

Key: cfu/g – colony forming unit / gram; ND – Not determined

Table 4. Total rhizobacterial count (TRC) during the five weeks of maize plant growth

Sample	Week 1 (cfu/g)	Week 2 (cfu/g)	Week 3 (cfu/g)	Week 4 (cfu/g)	Week 5 (cfu/g)
Soil only	1.6×10^7	1.0×10^7	1.3×10^7	1.2×10^7	1.1×10^7
Soil + maize	ND	6.0×10^6	2.0×10^7	3.1×10^7	3.3×10^7
Soil + Bean	ND	5.0×10^6	1.4×10^6	1.1×10^6	1.3×10^7
Soil + maize + NPK 10-10-10	ND	ND	3.7×10^7	3.7×10^7	3.9×10^7
Soil + Bean + NPK 10-10-10	ND	ND	2.1×10^6	2.1×10^6	6.2×10^6
Soil + maize + NPK 15-15-15	ND	ND	4.0×10^7	4.0×10^7	4.4×10^7
Soil + Bean + NPK 15-15-15	ND	ND	8.0×10^5	8.0×10^5	6.5×10^6

Key: cfu/g – Colony forming unit / gram; ND – Not determined

Table 5. Microbial succession pattern of bacteria and rhizobium

Samples	*Bacillus sp*	*Staphylococcus aureus*	*Micrococcus luteus*	*Enterococcus faecalis*	*Rhizobium sp*
Week 1					
Soil only (Beans)	+	+	+	+	+
Soil only (Maize)	+	+	+	+	-
Week 2					
Soil only	+	-	-	-	-
Soil+Beans	+	+	-	+	-
Soil + Maize	+	+	-	-	+
Week 3					
Soil only	+	+	-	-	+
Soil + Beans	+	-	-	+	+
Soil+Beans+NPK10:10:10	+	+	-	+	+
Soil + Beans + NPK15:15:15	+	+	-	+	+
Soil + Maize	+	+	-	- -	+
Soil + Maize + NPK10:10:10	+	+	-	+	+
Soil + Maize + NPK15:15:15	+	+	-	+	+
Week 4					
Soil only	+	+	-	-	-
Soil + Beans	+	-	-	+	+
Soil + Beans + NPK10:10:10	+	-	-	-	+
Soil + Beans + NPK15:15:15	+	+	-	+	+
Soil + Maize	+	+	-	+	+
Soil + Maize + NPK10:10:10	+	-	-	-	+
Soil + Maize + NPK15:15:15	+	+	-	+	+
Week 5					
Soil only	+	+	-	-	+
Soil + Beans	+	-	-	-	+
Soil + Beans + NPK10:10:10	+	+	-	+	+
Soil + Beans + NPK15:15:15	+	-	-	+	+
Soil + Maize	+	+	-	+	+
Soil + Maize + NPK10:10:10	+	+	-	+	+
Soil + Maize + NPK15:15:15	+	+	-	+	+

Key: - = absent, + = present,

maize rhizosphere soil during the different weeks of planting. This is in agreement with previous studies carried out by Cavaglieria et al. [13] who reported the presence of *Bacillus, Micrococcus, Fusarium* and *Penicillium* spp as part of the bacterial and fungal community structure of maize rhizosphere soil and Chen et al. [14] who also reported the presence of Actinomycetes, Bacillaes and Rhizobiales in a study to determine the bacterial community structure of maize plant. Bokati et al. [15] further reported the presence of *Fusarium* sp in the rhizosphere of maize plant an endophytic fungi associated with most plant. During the early growth of maize, the presence or colonization of the roots of maize by *Fusarium* sp is thought to provide competitive advantage to the maize plant [16]. It is also postulated by Bokati et al. [15] that the early colonization of the roots of maize by *Fusarium* sp may play vital roles in enhancing

the adsorption of nutrients and water thus conferring accelerated development to maize. *Enterobacter* species isolated in the roots of maize from this study can also be collaborated with studies conducted by Da Silva et al. [16] and Taghavi et al. [17] who described *Enterobacter* species as plant growth promoting rhizobacteria.

The addition of different regimen of fertilizers resulted in increase in the total heterotrophic bacterial count, total rhizobial count and total fungal count. This can be correlated with studies conducted by Fengping et al. [18] who reported an increase in the overall biomass of the microbial community in the rhizosphere of maize following the addition of inorganic amendments. This increase in overall microbial biomass could have resulted from the mineralized nitrogen from the NPK regimen which increased with increase in the regimen of NPK. It can be deduced that an increase in the NPK regimen resulted in an increase in the total microbial biomass. A comparison to the bulk soil and the soil with maize shows an obvious shift in the overall microbial biomass. This increase can be said to be as a result of the development of maize plant roots which released exudates and nutrients beneficial to the microbes present which in turn helps the plant assimilate certain nutrients during nutrient cycling [19]. The observed increase in the microbial population over the five weeks of maize growth is an indication that there is a higher microbial activity in a rhizosphere soil than the bare soil. This assertion was also suggested by Geisseler and Scow, [20] and this could also have resulted from the presence of exudates in the maize plant rhizosphere.

The microbial succession pattern of the maize shows that *Bacillus* sp. *Enterococcus* sp, *Rhizobium* sp and *Saccharomyces* sp were mostly predominant throughout the five weeks

Table 6. Microbial succession pattern of fungi during five weeks of maize and beans plant growth

Samples	*Saccharomyces sp*	*Streptomycetes sp*	*Penicillium notatum*	*Fusarium sp*
Week 1				
Soil only (Beans)	+	+	-	+
Soil only (Maize)	+	-	-	-
Week 2				
Soil only	+	-	+	-
Soil+Beans	+	-	-	+
Soil + Maize	+	-	-	-
Week 3				
Soil only	-	-	+	+
Soil + Beans	+	-	-	+
Soil + Beans + NPK10:10:10	+	-	-	+
Soil + Beans + NPK15:15:15	+	-	-	-
Soil + Maize	+	-	-	-
Soil + Maize + NPK10:10:10	+	-	-	-
Soil + Maize + NPK15:15:15	+	-	-	-
Week 4				
Soil only	+	-	+	+
Soil + Beans	+	-	+	-
Soil + Beans + NPK10:10:10	+	-	+	-
Soil + Beans + NPK15:15:15	+	-	-	-
Soil + Maize	+	-	-	+
Soil + Maize + NPK10:10:10	+	-	+	+
Soil + Maize + NPK15:15:15	+	-	+	-
Week 5				
Soil only	+	-	-	-
Soil + Beans	+	-	+	-
Soil + Beans + NPK10:10:10	+	-	+	-
Soil + Beans + NPK15:15:15	+	-	-	-
Soil + Maize	+	-	-	+
Soil + Maize + NPK10:10:10	+	-	-	+
Soil + Maize + NPK15:15:15	+	-	+	+

Key: - = absent, + = present,

planting period of maize. The addition of different regime of NPK had no effect on the microbial community structure but had effect on the overall microbial biomass.

For the beans seed plant, the microbial community comprised of *Bacillus* sp, *Staphylococcus aureus*, *Enterococcus* sp, *Micrococcus* sp, *Rhizobium* sp, *Saccharomyces* sp, *Streptomyces* sp, *Fusarium* sp and *Penicillium* sp. Some of this isolates have been reported in previous studies. Patkowska [21] reported the presence of *Bacillus* sp, *Fusarium* sp and *Penicillium* sp The results from this present study are in agreement with [22] who isolated *Staphylococcus aureus*, *Enterococcus* sp, *Bacillus* sp, *Rhizobium* sp and *Streptomyces* as part of the microbial community present in a study to determine novel bacteria and endophytic community in the roots of *Phaseoulus vulgaris*. *Fusarium* sp was also isolated in this present study. The presence of *Fusarium* sp in the roots of Beans plant is in agreement with studies carried out by Askar and Rashad, [23] who reported the presence of *Fusarium* in the roots of *Phaseoulus vulgaris*. It is thought that the presence of *Fusarium* in the roots of *Phaseoulus vulgaris* is due to the fact that *Fusarium* sp is a common root rot disease at the roots of *Phaseoulus vulgaris* which mostly results in loss of yield of the bean plant [23].

The succession pattern of microorganisms in the bean rhizosphere showed that *Bacillus* sp, *Staphylococcus aureus* and *Rhizobium* sp were predominant, while *Enterococcus* sp fluctuated during the five week of planting. The total bacteria in the rhizosphere of beans increased after application of the inorganic fertilizer compared to the bare soil (control). This may be due to some exudates secreted in the rhizosphere of beans plant that stimulates microbial activities thus resulting in increase in microbial populations in the rhizosphere before addition of inorganic fertilizers. The addition of inorganic fertilizers resulted in slight increase in the total microbial populations in the rhizosphere soil of the bean plant when compared to the bare soil that had no fertilizer. This increase in total microbial populations in both the rhizosphere of *Zea mays* and *Phaseoulus vulgaris* could be due to the interactions between the roots of the plants, the soil and the microbes which in turn influenced the microbial populations in the soil, releasing exudates in forms of soluble amino acids and sugars [24-26].

5. CONCLUSION

The use of inorganic fertilizers is a dynamic process which exerts beneficial effects on plant growth and also plays a major role in the replenishment of soil nutrients. This study demonstrates the microbial population changes and the dominant groups that occur in the soil of both maize and bean plant. It further demonstrate the potential effects of fertilizers on the microbial population in the rhizosphere soil of both maize and bean, which inadvertently will result in greater biological interactions between the roots and the microorganisms presents in the rhizosphere. However, the addition of inorganic fertilizers had effect on the total microbial populations when compared but did not show any affect against the microbial community structure as the succession pattern was intact. The results from this study further justifies the possible positive effects inorganic fertilizers on the total microbial populations in the rhizosphere of maize and bean plant which if harnessed can support sustainable agriculture and food production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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