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Microbial Community of Soil Cultivated with Corn Intercropped with Cowpea under different Phosphorus Fertilizer Treatments

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Abstract: A field experiment was carried out in Al-Jazirah Althanea region , Shatt Al-Arab district , Basra Province on silty clay soil to study the effect of the intercropping system on microbial community in soil under different treatments of phosphorus . The experiment was designed as a factorial experiment under complete randomized block design. The treatments included phosphate fertilizer type (concentrated superphosphate and diammonium phosphate), the level of phosphorus fertilizer (0, 25 and 50 kg P ha^{-1}) and the cropping system (sole corn and intercropping of corn and cowpea). Phosphorus fertilizers were added as side dressing at the time of sowing. Corn and cowpea seeds were sowing on August 5, 2019. Soil samples were taken from the rhizosphere zone and the zone away from the rhizosphere (bulk soil) of corn plant, at the flowering stage of the cowpea, the number of bacteria, fungi, and actinomycetes were estimated. The number of bacteria, fungi and actinomycetes were increased with increasing level of phosphorus at both the rhizosphere soil and bulk soil. Diammonium phosphate recorded the higher number of bacteria and actinomycetes, while the type of fertilizer did not affect the number of fungi in both the rhizosphere soil and bulk soil. Higher values of bacteria and actinomycetes were obtained in intercropping system as compared of sole corn system, while the results were reversed in terms for fungi number. The results of the study also indicated an increase in the number of all organisms in the rhizosphere soil compared to their numbers in the bulk soil.

Keywords: Diammonium phosphate, Bacteria, Fungi, Corn -cowpea, Intercropping

Phosphate fertilizers are of several types, some of which consist of phosphorus only, and some of them consist of two nutrients. Most of the compound fertilizers contain. Many studies indicated that diammonium phosphate is more efficient than the concentrated superphosphate in processing with available phosphorous in the soil due to its physical characteristics such as the increase in the diffusion coefficient of the fertilizer and its low ability to aggregate in addition to the high solubility which reaches 100% (Khan et al 2010). Measuring the numbers and activity of microorganisms in the soil is an indicator for nutrients availability including phosphorus, which is one of the important nutrient for formation of nucleic acids, phospholipids and protoplasmic compounds, which are very important for the organisms to analyze the organic matter in the soil. Phosphorus content reaches 0.5-1% of the fungi mycelium dry weight and 1-3% of the dry weight of actinomycetes (Liu et al 2012). Likewise, microbial communities and their activity are affected by the cropping system used. Presence of two different crops in such place produces different types of root exudates, which changes the composition of the biological community in the rhizosphere and changes the activity and competition of the neighborhoods (Xu et al 2018). Qin et al (2017) stated that the intercropping system leads to an increase in root secretions and root residues, which provides greater energy sources that encourage the reproduction of organisms and increase their numbers.

MATERIAL AND METHODS

Location and treatments: A field experiment was carried out during 2019 in the AL-Jazeera Althanea region (30°, 36 '15 "N ; 47° 47' 26" E 3m above sea level) at Shatt Al-Arab district, which is 7.5 km from the center of Basra province, in silty clay soil classified as Typic torrifluvents (Al-Atabb 2008) to test the effect of the intercropping system of corn and cowpea and phosphorus fertilization on the numbers of microorganisms in the soil . Before planting, soil samples were taken from 0-30 cm depth and initial properties were estimated according to standard methods mentioned (Black 1965, Page et al 1982).

Field preparation and implementation of treatments: The field was plowed twice, smoothing, and leveling, then divided into three equal blocks. Each block represents a replicate and each replicate contains 18 experimental units that represent the overlap of the three study factors. The size of the experimental unit for sole corn was 4 m length x 2.7 m width contains 3 rows at 90 cm leaving a distance of 45 cm side borders. As for the experimental unit of corn and cowpea, it was 4 m long x 3.6 m wide contains 3 rows of corn at 90 cm and 4 rows cowpea of interspersed corn rows leaving a distance of 45 cm as side borders. Plant to plant distance within row for corn and cowpea were kept as 40 and 25 cm, respectively. Corn (variety Furat) and cowpea (variety Ramshorn) were used. Sowing was done on 5 August 2019 at 3-4 seeds in each hole for both plants and after 20 days of planting the plants were thinned out and one plant was left farm operations were carried out for the crop whenever required all over the growing season . River water was used for irrigation for all experimental units. The Table 2 represents the number of lines and plant density for each treatment.

Table 1. Soil characteristics of soil before planting

Property		Quantity	Unit
pH		8.2	----------
EC		12.30	dS m^{-1}
CEC		13.10	Cmol (+) kg^{-1} soil
Total carbonate minerals		81.60	g kg^{-1}
Organic matter		8.03	g kg^{-1}
Ca^{++}	Dissolved cations	37.50	mmol L^{-1}
Mg^{++}		18.03	mmol L^{-1}
K^{+}		3.12	mmol L^{-1}
Na^{+}		1.50	mmol L^{-1}
$CO_3^{=}$	Dissolved anions	0.00	mmol L^{-1}
HCO_3^{-}		4.33	mmol L^{-1}
$SO_4^{=}$		20.08	mmol L^{-1}
Cl^{-}		90.60	mmol L^{-1}
N available		57.30	mg kg^{-1}
P available		30.80	mg kg^{-1}
K available		171.65	mg kg^{-1}
The total number of bacteria		2.2×10^{5}	CFU g^{-1} soil
The total number of fungi		4.00×10^{3}	CFU g^{-1} soil
The total number of actinomycetes		7.43×10^{5}	CFU g^{-1} soil
Sand		42.70	g kg^{-1}
Silt		550.50	g kg^{-1}
Clay		406.80	g kg^{-1}
Texture		Silty clay	

Three factors were used in the experiment were cropping system includes sole crop of corn and intercropping of corn with cowpea, type of phosphorus fertilizer includes concentrated superphosphate fertilizer (20.21% P) and diammonium phosphate fertilizer (21% P) and phosphorus fertilizer levels includes 0, 25 and 50 kg P ha^{-1}

Phosphorus fertilizer as concentrated superphosphate or diammonium phosphate were added close to corn rows at one dose at the time of sowing. Potassium sulfate fertilizer (43% K) was added at level of 83 kg K ha^{-1} in two doses, the first equivalent to 15% of the level was added immediately before planting and the rest was added at the stage of flowering near to corn rows. Nitrogen was applied at a level of 180 kg N ha^{-1} through urea (46% N) by two equal doses, the first at planting time and the other at the stage of flowering near to corn rows. The nitrogen was added through diammonium phosphate was subtracted from the nitrogen dose and remaining nitrogen was added through urea.

Estimating the number of microorganisms in soil: Soil microbial populations (bacteria, fungi, and actinomycetes) were counted separately in the rhizosphere and the zone far from the rhizosphere (bulk soil) of the maize plant when the cowpea entered the flowering stage after storing the samples in the refrigerator until the analysis was carried out . Two plants were taken from each experimental unit and their roots were extracted and cleaned from the surrounding soil, shake the plant by hand several times and collect the soil around the root with a small brush and combined to one composite sample. The (dilution method) was used in which 1 g of soil was added to 9 ml sterile distilled water and shaken well in attest tube and dilution series to10^{-6} were prepared . One ml of the 10^{-5} and 10^{-6} dilutions were spread on (yeast beef – extract peptone media) to determine the bacteria, 1 ml of the 10^{-3} and 10^{-4} dilutions were spread on (potato Dextrose Agar) to determine fungi population, and 1 ml of 10^{-5} and 10^{-6} dilutions were spread on starch casein agar to determine the actinomycetes population (Njeage et al 2017). The number of colonies was calculated after incubation for 7 days at 28°..

Statistical analysis: The separate data of rhizosphere soil or bulk soil were subjected to analysis of variance using GenStat Procedure Library Replacement PL 18.2 program to obtain the means. The data of rhizosphere soil and bulk soil were compared using t-test analysis (Al-Rawi and Khalaf Allah 2000).

RESULTS AND DISCUSSION

Number of bacteria: There was an significant increase in the number of bacteria at rhizosphere soil comparted bulk soil with increasing the level of phosphorus respectively

Table 2. Number of lines and plant density according to the cropping system

Cropping system	No. of lines		Planting distances (m)		Plant density (Plant ha-^{1})		Total plant density (Plant ha^{-1})
	Corn	Cowpea	Corn	Cowpea	Corn	Cowpea	
Sole corn	3	0	0.90	-------	27777	0	27777
Corn+Cow pea	3	4	0.90	0.90	20833	44444	65277

(Table 3). These results were similar to those of AL- Bahrani (2015). Phosphorus enhances the biological activity in the soil and increases the number of microorganisms. Liu et al (2012) indicated that soils in which carbon and nitrogen availability are high, are more sensitive to the addition of phosphorus with respect to number and activity of microbes. They also stated that phosphorus is an important factor for plant growth and this growth increases the amount of carbon secreted to microorganisms in the soil , which leads to an increase in their numbers.

Diammonium phosphate fertilizer was superior to the concentrated superphosphate fertilizer. The values were increasing at the rhizosphere soil comparison for bulk soil. The is due to the high solubility of diammonium phosphate and thus the increase in the source of nutrients (P and N), which is reflected in the increase of the number of microorganisms, especially bacteria. In addition to the neutral effect of this fertilizer, which reduces the phosphorus degradation and increases its availability for microorganisms and that the addition of nitrogen with phosphorus is synchronized by the same fertilizer which can have a complementary effect in increasing the number of bacteria or increasing plant growth, which improves the growth of soil microorganisms. The diammonium phosphate is superior to the concentrated superphosphate at all phosphorous levels, and this superiority was significant only in the rhizosphere soil. The intercropping was superior to the single cultivation with at the rhizosphere soil comparison at bulk soil. Xu et al (2018) also found that presence of two crops together produce different types of root exudates than they are in a single crop, which changes the composition of the biological community in the rhizosphere and changes the effectiveness and numbers of neighborhoods and the competition between them. The superiority of intercropping over single cultivation at most levels and types of phosphate fertilizers was observed and the differences were significant in the rhizosphere soil .This indicates the superiority of intercropping under different conditions of phosphate treatments including the treatment of non-addition of fertilizer. The highest value at rhizosphere soil was values by 20 - 96%. The highest value at bulk soil was surpassing the rest values by 8-10%. The highest differences between the two cropping system was at 50 kg P ha^{-1}which indicate efficiency of intercropping is more at the high level of phosphorus, and perhaps this is due to the improvement of plant growth at this level.

There was a significant increase in the number of bacteria in the rhizosphere soil of the maize plant compared to the bulk soil (Fig. 1). This may be explained by the fact that the root exudates in the rhizosphere lead to develop a good environment for the growth of organisms and increasing their numbers, including bacteria (Qin et al 2017). Tang et al (2015) also observed obtained bacteria in the rhizosphere soil compared to the bulk soil. The large differences may arise in the number of neighborhoods between one site and another in the soil a few centimeters apart, which is the result of variation in terms of moisture content, organic matter or pH.

Al- Hammadi (2014) confirmed that the number of bacteria in the rhizosphere of beans and barley increased by 108 times and 200 times, respectively, compared to their numbers in the bulk soil indicating that the concentration of microorganisms is on the newly growing root hairs that represent the root secretions of sugars, amino acids and salts , and these root hairs rot easily, resulting in addition of organic matter to the soil, a source of many materials that the organisms feed . In addition both roots and microorganisms consume O_2 and produce CO_2 by respiration and decomposition of organic matter, which produces carbonic acid, which controls the soil pH and availability of an important elements to the reproduction of organisms. When calculating the R/S reached 14 indicating that the number of

Table 3. Soil bacteria number (x10^5 CFU g^{-1} soil) of different P fertilizer treatments under sole and intercropping system of corn

(kg P ha^{-1})	P source	Rhizosphere soil			Bulk soil		
		Sole corn	Corn+cowpea	Average	Sole+corn	Corn+cowpea	Average
0	CSP	77.44a	82.27a	79.85a	5.98a	6.32a	6.15a
	DAP	77.44a	82.27a	79.85a	5.98a	6.32a	6.15a
25	CSP	78.60a	102.66b	90.63b	6.45a	6.52a	6.48a
	DAP	125.88c	107.66bc	121.77cd	7.41a	7.46a	7.44a
50	CSP	106.21b	126.33c	116.27c	8.73a	9.66a	9.19a
	DAP	106.60b	151.77d	129.18d	10.79a	9.84a	10.32a
Average		95.36A	110.49B		7.56A	7.69A	

CSP: Concentrated superphosphate; DAP: Diammonium phosphate. Values followed by different letters are significantly different among treatments with P< 0.05

bacteria has been clearly affected by the rhizosphere, and numbers have doubled compared to the bulk soil, and this percentage may be appropriate in the root zone of a non-leguminous plant.

Number of fungi: The number of fungi increased by increasing the levels of phosphorus at the rhizosphere soil comparison at bulk soil for levels of 0, 25 and 50 kg P ha^{-1} (Table 4). These results are similar to Liu et al(2012) and Tang et al (2015). This may be attributed to the ability of fungi to compete and grow in acidic media after addition of phosphate fertilizers with an acidic effect and phosphorous represents 0.5 - 1% of the dry weight for fungi mycelium.

No significant differences between the two types fertilizers were observed in the rhizosphere soil and the bulk soil. Sole cropping of corn significantly increased the number of fungi as compared to intercropping of corn with cowpea , and this result was contrary to the results of the bacteria (Table 3). Increasing the numbers of bacteria and actinomycetes at intercropping system leads to a decrease in the number of fungi and improved of the ratio of bacteria: fungi. Increasing the number of bacteria is important to increase the microbial activity and improve the chance of eliminating pathogens Yang et al (2016). This result is may be in favor of the intercropping system, because fungi are among the organisms that can cause many diseases for plants (Brussaard et al 2007).

The number of fungi of concentrated superphosphate treatments was higher than the numbers of fungi of diammonium phosphate treatments at the level of 50 kg P ha^{-1} (Table 4) while the fungi number of diammonium phosphate treatments was higher than the number of fungi of concentrated superphosphate treatments at the level 25 kg P ha^{-1}. It is observed that the treatment included the addition of superphosphate fertilizer at a level of 50 kg P ha^{-1} surpassed the rest treatments , with an increase ranging between 5 - 47% at the rhizosphere soil and ranged between 14-209% at bulk soil . The lowest values were in control treatment (no phosphate fertilizer was added). The superiority of sole cropping over intercropping in most phosphate fertilizer treatments, but without significant effect on both the rhizosphere soil and the bulk soil.

It was showed that there is a significant increase in the number of fungi in the rhizosphere soil compared to the bulk soil respectively this is due to the diversity of root exudates and their interaction with each other leads to the creation of a rich environment for biodiversity and an increase in the number of microbes in the rhizosphere. These results are in agreement with Yan et al (2008), Qin et al (2017) and Xu et al (2018). Many studies indicate that the effect of the rhizosphere on fungi and actinomycetes is very small compared to the numbers of bacteria, as the roots do not cause the quantitative change of fungi, but only encourage the growth of certain species at the expense of others. In addition to that the dominant species in the rhizosphere differ according to the type of plant, plant age and the soil type. It is clear when calculating the R/S ratio as it reached within 2, while the bacteria had about 14 (Fig. 1). Al-Hammadi (2014) obtained similar results as the number of fungi increased in the rhizosphere of barley or beans by about 10 times as compared with the bulk soil. It concluded that the root exudates may help germinate the dormant stages of many fungi that remain dormant due to their inability to compete with other microorganisms.

Number of actinomycetes: Number of actinomycetes in rhizosphere soil and bulk soil were increased significantly with increasing phosphorus levels (Table 5). for the rhizosphere soil for the bulk soil at levels of 0, 25 and 50 kg P ha^{-1}. These results are in agreement with Tang et al (2015) However, Mander et al (2012) found that increasing the level of phosphorous reduces the numbers of actinobacteria while

Table 4. Soil fungi number (x 10^3 CFU soil) of different P fertilizer treatments under sole and intercropped system of corn

(kg P ha^{-1})	P source	Rhizosphere soil			Bulk soil		
		Sole corn	Corn+cowpea	Average	Sole+corn	Corn+cowpea	Average
0	CSP	13.66a	10.33a	11.99a	4.77a	4.11a	4.44a
	DAP	13.66a	10.33a	11.99a	4.77a	4.11a	4.44a
25	CSP	14.22a	13.33a	13.77ab	8.77a	8.99a	8.88b
	DAP	15.66a	15.66a	15.66b	10.55a	9.33a	9.94b
50	CSP	18.44a	16.38a	17.66c	13.66a	13.77a	13.71d
	DAP	17.10a	16.55a	16.82bc	12.66a	11.33a	11.99c
Average		15.45B	13.84A		9.19A	8.60A	

See Table 3 for details

increases their numbers at phosphorus deficiency. Liu et al (2012) indicated that the exploitation of microorganisms of available soil carbon as a source of energy depends on the availability of nutrients, and phosphorus is one of the most specific elements in that and thus in the development of microbes numbers and activity Phosphorus constitutes 1-3% of the dry weight of actinomycetes cells form of nucleic acids, phospholipids, monoester phosphate or multiple phosphates. The fertilizer type had significant effect on the number of actinomycetes, as the diammonium phosphate was superior to the concentrated superphosphate at the rhizosphere soil comparison at bulk soil, respectively. These results were similar with the results of the bacteria (Table 3), which were attributed to the high solubility of the diammonium phosphate and the abundance of nutrients (N and P) together, and this is reflected in the increase in the number of organisms in the soil, including actinomycetes.

The significant higher value of actinomycetes was recorded when corn cultivated with cowpea as compared with sole corn (Table 5). These results were similar to the results for bacteria (Table 3), but it differed from the results for fungi (Table 4). These results are in agreement with Tang et al (2015) and Qin et al (2017) and this to the fact that the presence of two different crops in the same place leads to increased exudates of roots and residues of roots, which provide greater energy sources and encourages the reproduction and increase of their numbers . The number of actinomycetes increased by increasing the levels of addition of phosphate fertilizer for both sources and in the rhizosphere soil and bulk soil (Table 5). This result shows the superiority of the diammonium phosphate at all levels due to the presence of two elements in the fertilizer component added at the same time in addition to the neutral reaction for this fertilizer, which does not allow to lose a large percentage of phosphorus. Intercropping system recorded higher values of actinomycetes number at all fertilization treatments in the

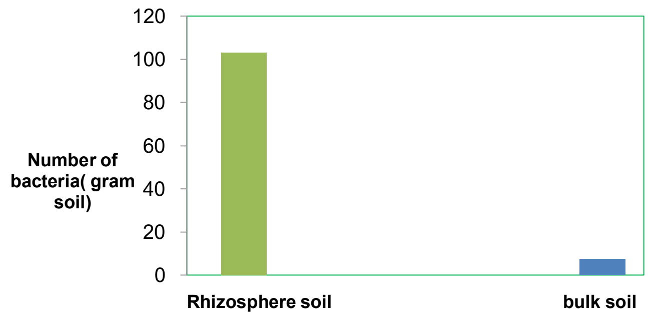

Fig. 1. Number of bacteria (10^5 CFU g^{-1}soil) in the rhizosphere and bulk soils of maize plant

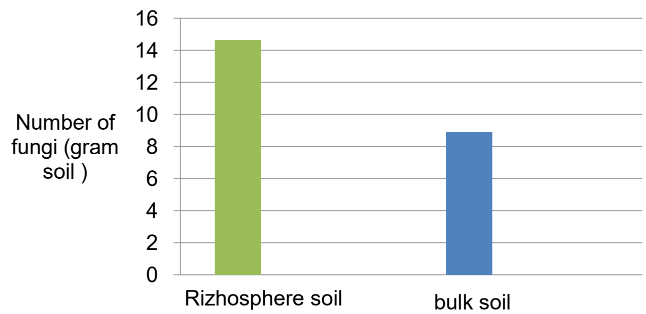

Fig. 2. Number of fungi (x 10^3 CFU g^{-1} soil) in the rhizosphere soil and bulk soil of the maize plant

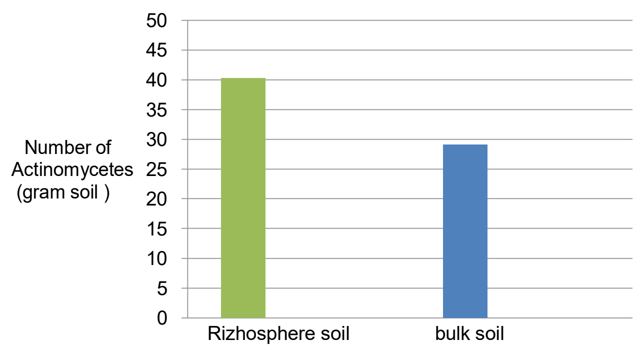

Fig. 3. Number of actinomycetes (10^5 CFU g^{-1} soil) in the rhizosphere soil and bulk soil of the maize plant

Table 5. Soil actinomycetes r (x 10^3 CFU g^{-1} soil) of different P fertilizer treatments under sole and intercropped system of corn

(kg P ha^{-1})	P source	Rhizosphere soil			Bulk soil		
		Sole corn	Corn+cowpea	Average	Sole+corn	Corn+cowpea	Average
0	CSP	24.00a	31.99a	27.99a	20.99a	20.99a	20.99a
	DAP	24.00a	31.99a	27.99a	20.99a	20.99a	20.99a
25	CSP	35.88a	46.33a	41.10b	28.00a	28.55a	28.27b
	DAP	44.60a	51.33a	47.96c	32.66a	32.55a	32.60bc
50	CSP	40.11a	48.22a	44.16bc	35.00a	32.99a	33.00cd
	DAP	47.33a	58.33a	52.83d	38.99a	36.33a	37.66d
Average		35.98A	44.70B		29.44A	28.73A	

See Table 3 for details

rhizosphere soil, while there were no clear differences between the two cropping systems at bulk soil, and this confirms the effective role of root exudates in controlling living numbers and improving this role by the presence of two different plants in the quantity and type of secretions. There were greater numbers of actinomycetes in the rhizosphere soil compared with bulk soil with significant differences (Fig. 3). This is due to the root exudates in the rhizosphere encourage the growth of organisms and increase theirnumbers . Al Hammadi (2014) and Tang et al (2015) observed an increase in the number of actinomycetes in the root zone compared to the bulk soil. The roots is little compared to bacteria, as the R/S value ranges between 1/2 - 1/3, but under certain conditions, such as those available around the roots of old plants this percentage may increase. Al-Hammadi (2014) also obtained an increase in the number of actinomycetes in the rhizosphere of beans and barley at the last stages of growth due to the increase of difficult decomposed substances such as peptides, organic acids, amino acids, biotin, inositol and choline, which are needed in their growth after the end of the easy decomposed substances. Calculating the R/S value for the present study, it was 1.4, which is less than that of bacteria and fungi.

CONCLUSIONS

The addition of diammonium phosphate fertilizer is more suitable for the soil under study compared to concentrated superphosphate fertilizer concerning of increasing the biological activity of the soil. To confirm this, experiments can be conducted on other soils from the region. The use of cowpeas as a support crop for corn led to the improvement of the micro-phylogenetic condition of the soil and increased the number of microbes, especially in the rhizosphere compared to the cultivation of sole corn, which resulted in increasing the availability of nutrients in the root zone and improving plant growth.

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