



## Effectiveness of Gypsum Application and Arbuscular Mycorrhizal Fungi Inoculation on Ameliorating Saline-Sodic Soil Characteristics and their Productivity



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**T**HE DEGRADATION of soil characteristics caused by salinity and sodicity gives rise to severe limitations on crop production. Therefore, the current study was conducted in a split block design with three replicates during the winter (2019/2020) and summer (2020) seasons at Sakha Agriculture Research Station Farm, Kafr El-Sheikh Gov., Egypt to study the integrated effect of gypsum with mycorrhizae fungi inoculation to improve saline-sodic soil properties and bacterial communities, physiological and productivity of wheat and maize plants. The treatments included different soil gypsum levels ( $G_0$ : none,  $G_1$ : 7.5 Mg ha<sup>-1</sup>, and  $G_2$ : 10 Mg ha<sup>-1</sup>) of the requirements and mycorrhizal fungi inoculation (without inoculation AMF<sub>0</sub> and inoculated AMF<sub>1</sub>). The results showed that the combination of  $G_2 + AMF_1$  caused significant changes in the photosynthetic activity, antioxidant enzyme activities (catalase, peroxidase and superoxide dismutase), mycorrhizal root colonization, total bacterial counts, P solubilizer bacteria, and respiration of microbial soil in the Rhizosphere of wheat and maize. In addition, after two growing seasons, electrical conductivity (EC) decreased from 7.09 to 4.57 dS m<sup>-1</sup>, exchangeable sodium percentage (ESP) from 19.35 to 15.47 and soil bulk density (BD) from 1.38 to 1.31 Mg m<sup>-3</sup>. While the exchangeable calcium percentage (ECaP) increased from 11.51 to 15.47, available phosphorus (Av. P) from 8.19 to 11.17 mg kg<sup>-1</sup>, infiltration rate (IR) from 0.7 to 0.97 cm hr<sup>-1</sup>, and total porosity (TP) from 47.92 to 50.44 % and  $G_2 + AMF_1$  treatment gave the highest grain yield of wheat and maize were 7.72 and 8.52 Mg ha<sup>-1</sup>, respectively. Current findings concluded that the incorporation of gypsum application with AMF inoculation could be applied as an effective way of ameliorating saline-sodic soil characteristics and alleviating the hazardous effects of soil salinity and sodicity on wheat and maize plants.

**Keywords:** Saline-sodic soil; Gypsum; Arbuscular mycorrhizal fungi; Bacterial communities; photosynthetic activity; Wheat-maize productivity.

### 1. Introduction

Globally, salt-affected soils were estimated an area of 11.74 million km<sup>2</sup>, and the continents which contained the highest areas were Asia, Africa, and Australia (Hassani, et al., 2020; FAO, 2021). In Egypt, it occupied approximately 33% of the Nile Delta Region; the remaining areas are threatened due to the promotion of soil degradation (Mohamed, 2016), while in Kafr El-Sheikh Gov. (Northern Nile Delta, Egypt) more than 50% are salt-affected soil (Ab-

delRahman et al., 2019; Aboelsoud et al., 2022). The salt-affected soil is formed by the simultaneous effect of high salinity and Na concentrations in the soil solution or at the exchange sites which caused the degradation of soil characteristics (Alcívar et al., 2018; Kheir et al., 2019). The salt-affected soil characteristics induce morphological and physiological changes, increased ion toxicity, decreases leaf water potential, alter the biochemical processes in plants; and the photosynthesis process. This reflects subsequently on

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crop production (Shrivastava and Kumar, 2015; Evelin et al., 2019; Doklega et al., 2021; El-Nahrawy et al., 2022)

Recommendations approach for amelioration and management of salt-affected soils. One, to reclaim salt-affected soils; two, to manage salt-affected soils, i.e., without reclamation, using alternate suitable agricultural options such as increasing the salt-tolerant of plants, saline aquaculture, etc. The choice depends on the feasibility of reclamation and its cost-effectiveness (Mandal et al., 2018; Yonggan et al., 2020; El-Ramady et al., 2022). using gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) technology for the reclamation of sodic soil, which is the oldest soil amendment (Kost et al., 2018; Presley et al., 2018). Gypsum application alleviated the hazardous impacts (Aboelsoud et al., 2020) by facilitating efficient removal of Na on the soil exchange sites through increasing exchangeable Ca which promotes clay aggregation, thereby increasing water infiltration and movement through the soil physiochemical and enzymatic properties of the soil (Fontoura et al., 2019; Aiad et al., 2019 and Zhang et al., 2020).

Arbuscular mycorrhiza fungi (AMF) can form a mutualistic symbiosis with most terrestrial plants, where, the arbuscules are the site of nutrients exchange between the plant and the fungi. Also, AMF plays a dynamic role in plant growth under various conditions through composing useful symbiotic associations with most plants, modulating the root system and enhancing the mobilization of elements (Wu et al., 2014), and increasing the plant tolerance by up-regulating enzymatic as well as non-enzymatic antioxidant defense systems (Ahmad et al., 2015); lipid peroxidation (Abd\_Allah et al., 2015) and phytohormone synthesis (Navarro et al., 2014). Also, endophytic bacteria colonize the internal tissues of host plants to enhance their growth, stress tolerance and protect plants from soil pathogens (Malfanova et al., 2011; and Sessitsch et al., 2012). Also, AMF could be improving some soil characteristics such as pH, nutrient availability, aeration, soil aggregates, and water relationships, that by producing glycol sugar protein glomalin (Schreiner et al., 1997; Sheng et al., 2011).

Overall, the major target of this current research is to study the integration effect of gypsum and AMF inoculation on ameliorating soil characteristics and productivity of wheat and maize under saline-sodic soil conditions.

## 2. Materials and methods

### 2.1. Materials

2.1.1. Gypsum: It was obtained from the Executive Authority for Land Improvement Projects (EALIP) in Kafr El-Sheikh Gov., Egypt. Gypsum treatments were  $G_0$ : none,  $G_1$ :  $7.5 \text{ Mg ha}^{-1}$ , and  $G_2$ :  $10 \text{ Mg ha}^{-1}$  (as 0, 75, and 100% gypsum requirement, respectively) mixed by plowing in the upper layer (0–30 cm) before cultivation. The soil gypsum requirement was determined according to Richards (1954) as follows:

$$GR = \frac{ESP_i - ESP_f}{100} \times CEC \times 4.1$$

Where GR: gypsum requirement for 30 cm soil depth ( $\text{Mg fed}^{-1}$ ),  $ESP_i$ : initial soil ESP,  $ESP_f$ : the required soil ESP decreased to 13 and CEC: cation exchange capacity ( $\text{cmol}_c \text{ kg}^{-1}$ ).

2.1.2. The arbuscular mycorrhizal fungi (AMF): it was obtained from the Microbiology Dept., Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. The 200 g of soil inoculum containing a starting AMF concentration of  $10 \text{ spores g}^{-1}$  soil was mixed with pure sand and then inoculated to each hill. The inoculum was placed 5 cm below the surface of the soil before planting

### 2.2. Experimental field setup and treatments

In a split block design, the experimental field was carried out in the winter (2019–2020) and summer (2020) seasons at Sakha Agriculture Research Station Farm, Kafr El-Sheikh Gov., Northern Nile Delta, Egypt ( $31^\circ 05' 19.8'' \text{ N}$  and  $30^\circ 56' 13.2'' \text{ E}$ ) with an elevation of about 6 meters above sea level. The soil was heavy clay saline-sodic (Table 1). The experimental field consists of 18 plots (6 treatments x 3 replications), in which each plot area was  $10 \text{ m}^2$  ( $4 \text{ m} \times 2.5 \text{ m}$ ). Gypsum formed the main plots and the subplots were occupied by AMF treatments. The meteorological data obtained from NASA's MERRA-2 assimilation model <https://power.larc.nasa.gov/> are illustrated in Table 2.

Ca-superphosphate ( $15.5\% \text{ P}_2\text{O}_5$ ) was mixed by plowing in the upper layer (0–30 cm) before cultivation. The seeds of wheat (*Triticum aestivum* L., CV. Sakha 94) and maize (*Zea mays* L., CV. Hybrid 368) were obtained from the Field Crops Research Institute, Sakha Agricultural Research Station, Kafr El-Sheikh Gov., Egypt. Wheat seeds were sown on 11 Nov. 2019 at a rate of  $143 \text{ kg ha}^{-1}$  and maize was sown on 15 May, 15<sup>th</sup>, 2020 at a rate of  $24 \text{ kg ha}^{-1}$  (2 grains per hole with 20 cm spacing). The agricultural practices for both crops were follow-up through the Ministry of Agriculture recommendations in the North Delta.

## 2.3. Measurements and Analyses

### 2.3.1. Physiological Characteristics:

After 40 days from sowing, the antioxidant enzymes were determined in plant roots samples. In spectrophotometer T70, Catalase activity  $\mu\text{M}$  ( $\text{H}_2\text{O}_2$   $\text{mg}^{-1} \text{min}^{-1}$  FW) was determined at 240 nm for 1 min following the decomposition of  $\text{H}_2\text{O}_2$ , while peroxidase activity ( $\text{U mg}^{-1} \text{h}^{-1}$ FW) was determined at 240 nm for 1 min following the decomposition of  $\text{H}_2\text{O}_2$

(Kato and Shimizu, 1987). Superoxide dismutase ( $\text{U g}^{-1}$  FW) was assayed based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (Beauchamp and Fridovich, 1971). Photosynthetic activity [ $\mu\text{m}$  ( $\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )] was measured in leaves, as  $\text{CO}_2$  was consumed using a closed gas-analytical system LI 6000 (Li-Cor, USA), a portable measuring device.

**Table 1: Main soil characteristics investigated before cultivated.**

Character		Physical characteristics				
		Particle size distribution ( $\text{g kg}^{-1}$ )				
Season	Sand	Silt	Clay	Texture		
2019	179.3	296.8	523.9	Heavy clay		
2020	180.6	297.4	522.0	Heavy clay		
	$\text{CaCO}_3$ ( $\text{g kg}^{-1}$ )	Bulk density ( $\text{Mg m}^{-3}$ )	Total porosity (%)	Infiltration rate $\text{cm hr}^{-1}$	CEC $\text{cmol}_c \text{ kg}^{-1}$	
2019	24.6	1.38	47.92	0.70	38.54	
2020	24.7	1.38	47.92	0.70	38.68	
		Chemical characteristics				
	pH (1:2.5)	EC $\text{dS m}^{-1}$	SAR	ESP	ECaP	
2019	8.32	7.09	17.12	19.35	11.51	
2020	8.29	6.84	16.92	19.16	11.58	
	O.M ( $\text{g kg}^{-1}$ )	O.C ( $\text{g kg}^{-1}$ )	Available macronutrients ( $\text{mg kg}^{-1}$ )			
			N	P	K	
2019	11.6	6.73	21.47	8.19	345.6	
2020	11.2	6.50	23.18	8.21	358.3	
		Soluble ions $\text{mmol L}^{-1}$				
	$\text{Na}^+$	$\text{Ca}^{2+}$	$\text{Mg}^{2+}$	$\text{K}^+$		
2019	51.97	10.35	8.07	0.441		
2020	50.27	9.92	7.74	0.443		
	$\text{CO}_3^{2-}$	$\text{HCO}_3^-$	$\text{Cl}^-$	$\text{SO}_4^{2-}$		
2019	-	3.5	39.5	27.8		
2020	-	4.5	43.2	20.6		
		Biological characteristics $\text{Log CFU g}^{-1}$ dry soil				
		Total bacterial counts			P-solubilizers	
2019		6.18			4.12	
2020		6.34			4.32	

\*  $\text{CaCO}_3$ : Total  $\text{Ca}^{+2}$  carbonate, O.M: Soil organic matter, O.C: Soil organic carbon and ECaP: exchangeable calcium percentage were analyzed as described by Nelson and Sommers, 1983; CEC: Cation exchange capacity determined as the Rengasamy and Churchman, 1999 method; available N was determined according to Jackson 1973; Available P and K were determined according to Soltanpour 1985. EC and soluble ions were determined in soil paste extract.

**Table 2. Meteorological data for winter and summer seasons (2019/2020).**

Seasons	month	Temperature, $\text{C}^\circ$			Relative humidity %	Wind Speed Mph	Pressure Hg	Precipitation ( $\text{mm month}^{-1}$ )
		Max.	Min.	Mean				
Winter 2019/2020	11-Nov.	22.85	10.95	16.90	55.45	6.96	29.97	0.60
	Dec.	17.35	8.65	13.00	64.00	8.40	30.03	18.60
	Jan.	14.03	7.35	10.69	71.00	8.66	30.11	33.90
	Feb.	15.52	7.79	11.66	70.31	7.67	30.09	17.50
	Mar.	18.77	8.29	13.53	63.77	9.20	29.91	13.70
	18- Apri.	20.94	9.72	15.33	62.33	8.63	29.97	1.90
Summer 2020	15- May.	29.18	12.12	20.65	49.94	9.65	29.92	0.00
	Jun.	29.53	15.07	22.30	58.17	8.72	29.84	0.00
	Jul.	31.35	19.42	25.39	65.39	8.60	29.72	0.00
	Aug.	31.84	20.03	25.94	65.10	8.56	29.72	0.00
	11- Sep.	32.00	20.73	26.36	67.27	7.91	29.78	0.00

\* Mph: miles per hour; mmHg: millimeters of mercury (Manometric units)

### 2.3.2. Bacterial communities:

#### 2.3.2.1. Mycorrhizal colonization:

At the flowering stage, the fine roots were collected from the lateral root system of each treatment and fixed in formalin/acetic acid/alcohol Ratio (v/v/v) (FAA) solution until further processing. Root mycorrhizal infection was estimated after being stained with trypan blue in lactophenol (Phillips and Hayman, 1970). Roots were pigmented by 0.5% NH<sub>4</sub>OH and 0.5% H<sub>2</sub>O<sub>2</sub> v/v in water to clear some phenolic compounds (Kormanik and McGraw, 1982) before acidification with (0.05 M HCl). One cm stained root on glass slides with lactophenol was surveyed by a digital computerized microscope at 40–10X magnification. The infection and development of mycelium, vesicles and arbuscules in the roots were calculated by the formula:

$$\% \text{Colonization} = \frac{\text{Total number of AM positive segments}}{\text{The total number of segments studied}} \times 100$$

#### 2.3.2.2. Total bacterial counts:

Total bacteria and phosphate solubilizing bacterial counts were determined in the rhizosphere of wheat and maize plants for each treatments by using a soil dilution plate technique (Olsen and Bakken, 1987).

Tryptic-soya agar medium was used for total bacterial counts after three days of incubation at 30 C°, and Pikovskaya medium (Goenadi and Sugiarto 2000) was used for phosphate-solubilizing bacterial counts; clear zones around the colonies were recorded after five days of incubation at 30 C°.

#### 2.3.2.3. Measurement of Respiration microbial soil:

Respiration microbial soil (CO<sub>2</sub> (mg) 10g<sup>-1</sup> 72h<sup>-1</sup>) was estimated according to (Jaggi, 1976). This method is based on the measurement of CO<sub>2</sub> released during the microbial activity in the soil. 10 g of soil was placed in 50 ml plastic tube, and then plastic tubes were fitted into a Duran bottle containing 25 ml 0.05 NaOH (prepared with CO<sub>2</sub> free distilled water) where the tubes were kept at the neck of the bottle. The same system but without soil was used as a blank and system with uninoculated carrier as control. Bottles were incubated for 72 h at room temperature. After incubation, plastic tubes were removed and 5 ml of (0.5 M) barium chloride was add to NaOH solution followed by a few drops of phenol phthalin, Mixture was titrated with (0.05 M) HCl with continuous stirring until red color turned to colorless. The rate of respiration was calculated according to the following relationship:

$$CO_2(\text{mg})CW/t = \frac{(V_0 - V) \times 1.1}{d. wt}$$

CW: the amount of soil dry weight in gram

t: incubation time (h)

V<sub>0</sub>: volume of HCl titration of blank

V: volume of HCl titration of sample

d.wt: dry weight of 1 g soil

Conversion factor (1 ml 0.05 M NaOH= 1.1 mg CO<sub>2</sub>).

### 2.4. Crop production

At the maturity stage, grain and straw yields of wheat and maize were recorded in 1 m<sup>2</sup> for each plot (kg m<sup>-2</sup>) and transformed as Mg ha<sup>-1</sup>.

### 2.5. Soil analysis

Soil samples at the start of the experiment soil, and after crop harvest, were collected from 0–30 cm depth from each plot. Soil samples were air-dried, gently crushed, and sieved (2 mm). Some physical characteristics i.e. particle size distribution, bulk density, Total porosity and infiltration rate were analyzed according to standard methods as described by (Piper, 1950, Campbell, 1994 and Tricker, 1978, respectively). While the chemical characteristics were measured according to (Page et al., 1982). Microbial estimations in the rhizosphere of soil samples were also carried out. The total count of bacteria was estimated by soil extract agar media (Allen, 1959), while the most probable number of nitrogen fixing bacteria was estimated using semisolid N free LGI and JNfb medium with 0.3% agar-agar (Cavalcante and Dobereiner, 1988) and calculated using tables of (Cochran, 1950).

### 2.6. Statistical analysis

All data were subjected to the variance (ANOVA) according to the methods described by Snedecor and Cochran (1980) using the Costat package program, version 6.311 (Cohort Software, USA) at a P < 0.05.

## 3. Results

In this study, the soil's properties and bacterial communities, physiological and productivity of wheat and maize plants showed significant responses due to the applications of gypsum and AMF inoculation treatments as well as their combinations.

### 3.1. Physiological Characteristics

#### 3.1.1. Photosynthetic activity

Data presented in Table (3) illustrate the photosynthetic activity of wheat and maize plants as affected by gypsum or/and AMF inoculation treatments. The photosynthetic activity was increased from 38.17 and 30.53 μm (CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) with AMF<sub>0</sub> to 42.80 and 34.50 μm (CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) with AMF<sub>1</sub> for wheat and maize plants, respectively. In addition, the photosynthetic activity increased with increasing gypsum application rate, since its values for wheat and maize plants were increased with G<sub>2</sub> by 27.55% and 32.36%, respectively, compared to G<sub>0</sub>. Also that photosynthetic activity was increased with the different

combinations of G and AMF (Figure 1A). This increase could be arranged as follows:  $G_2+AMF_1 > G_1+AMF_1 > G_2+AMF_0 > G_1+AMF_0 > G_0+AMF_1 > G_0+AMF_0$ , whereas  $G_2+AMF_1$  treatment increased of

photosynthetic activity by 41.87% and 49.03% in wheat and maize, respectively compared to the control ( $G_0+AMF_0$ ).

**Table 3. Photosynthetic and antioxidant enzyme activities in wheat and maize plants as affected by gypsum and AMF treatments as well as their combinations after 40 day from plants sowing.**

Crops	Treatments	Photosynthetic [ $\mu\text{m}(\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1})$ ]	Superoxide dismutase ( $\text{u g}^{-1} \text{FW}$ )	Peroxidase ( $\text{u mg}^{-1} \text{h}^{-1}\text{FW}$ )	Catalase $\mu\text{M}(\text{H}_2\text{O}_2 \text{ mg}^{-1} \text{min}^{-1}\text{FW})$
After wheat harvesting	AMF <sub>0</sub>	38.17	33.43	11.90	3.22
	AMF <sub>1</sub>	42.80	16.10	16.70	1.91
	F-test	**	ns	**	*
	LSD <sub>0.05</sub>	<b>2.87</b>	<b>23.01</b>	<b>2.21</b>	<b>1.27</b>
	G <sub>0</sub>	34.85	46.50	9.75	4.35
	G <sub>1</sub>	42.15	16.60	15.40	1.79
	G <sub>2</sub>	44.45	11.20	17.75	1.56
	F-test	*	*	*	*
	LSD <sub>0.05</sub>	<b>3.51</b>	<b>28.18</b>	<b>2.70</b>	<b>1.56</b>
	G <sub>0</sub> AMF <sub>0</sub>	33.2	60.4	7.2	5.3
	G <sub>0</sub> AMF <sub>1</sub>	36.5	32.6	12.3	3.4
	G <sub>1</sub> AMF <sub>0</sub>	39.5	21.7	13.5	2.3
	G <sub>1</sub> AMF <sub>1</sub>	44.8	11.5	17.3	1.27
	G <sub>2</sub> AMF <sub>0</sub>	41.8	18.2	15	2.06
G <sub>2</sub> AMF <sub>1</sub>	47.1	4.2	20.5	1.06	
F-test	*	*	*	*	
LSD <sub>0.05</sub>	<b>5.43</b>	<b>20.9</b>	<b>4.76</b>	<b>1.66</b>	
After maize harvesting	AMF <sub>0</sub>	30.53	70.33	10.27	1.83
	AMF <sub>1</sub>	34.50	103.23	14.53	0.81
	F-test	**	ns	**	ns
	LSD <sub>0.05</sub>	<b>1.46</b>	<b>40.03</b>	<b>1.8</b>	<b>1.68</b>
	G <sub>0</sub>	27.35	61.85	8.75	2.70
	G <sub>1</sub>	34.00	88.50	13.10	0.70
	G <sub>2</sub>	36.20	110.00	15.35	0.57
	F-test	**	ns	**	ns
	LSD <sub>0.05</sub>	<b>1.78</b>	<b>49.02</b>	<b>2.2</b>	<b>2.06</b>
	G <sub>0</sub> AMF <sub>0</sub>	25.7	46.5	6.8	3.6
	G <sub>0</sub> AMF <sub>1</sub>	29	77.2	10.7	1.8
	G <sub>1</sub> AMF <sub>0</sub>	31.8	79.5	11.2	1.04
	G <sub>1</sub> AMF <sub>1</sub>	36.2	97.5	15	0.36
	G <sub>2</sub> AMF <sub>0</sub>	34.1	85	12.8	0.86
G <sub>2</sub> AMF <sub>1</sub>	38.3	135	17.9	0.28	
F-test	*	*	*	*	
LSD <sub>0.05</sub>	<b>4.52</b>	<b>29.54</b>	<b>3.69</b>	<b>0.67</b>	

\*G<sub>0</sub>: without gypsum application; G<sub>1</sub>: 75% gypsum requirement; G<sub>2</sub>: 100% gypsum requirement; AMF<sub>0</sub>: without mycorrhizal fungus inoculation ; AMF<sub>1</sub>: Mycorrhizal fungus inoculation treatment.

### 3.1.2. Antioxidant enzymes activities:

Data presented in Table (3) showed that the change in antioxidant enzyme activities in wheat and maize plants were affected by the combination of gypsum with AMF. The data in Figure (1D) noted that the soil supplemented with  $G_2+AMF_1$  achieved the highest decrease in catalase enzyme activity in wheat and maize plants (80.0 and 92.2 %, respectively). While, peroxidase enzyme activities in wheat and

maize plants were increased with  $G_2+AMF_1$  by 184.72 and 163.24%, respectively Figure (1C).

On the other hand, the superoxide dismutase activity gave un-similar trends according to the plant type Figure (1B). The combination treatment of  $G_2+AMF_1$  decreased the superoxide dismutase activity of wheat plants by 93.05%, while it was increased in maize plants by 190.32% with this treatment compared to the control treatment ( $G_0+AMF_0$ ).

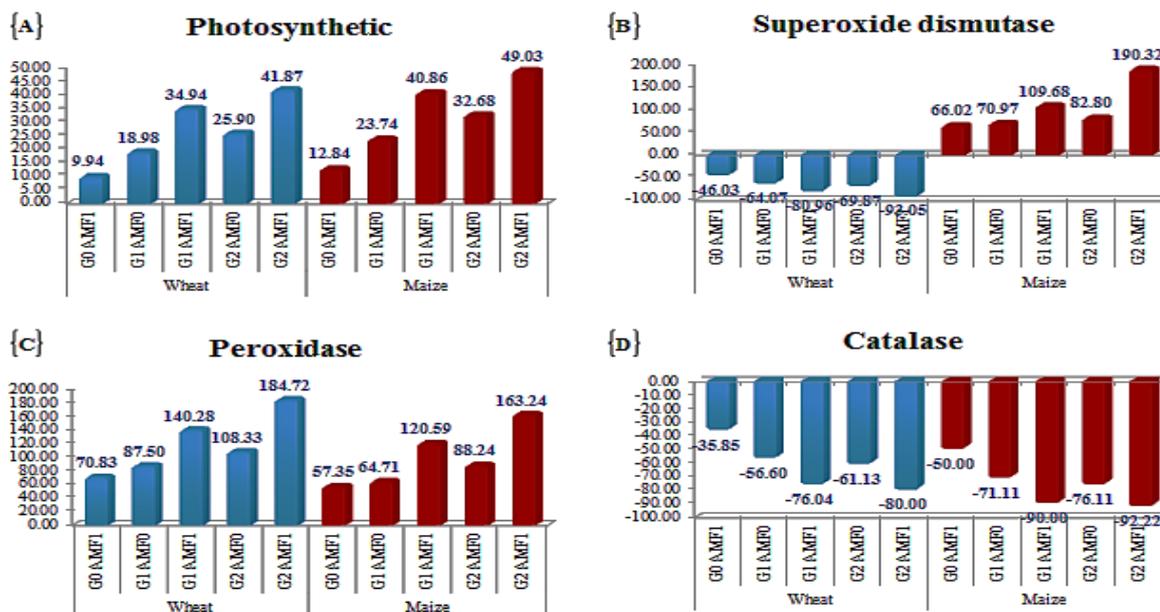


Fig. 1. The changes of photosynthetic activity, superoxide dismutase, peroxidase and catalase as affected by the combined effect of gypsum applications and AMF inoculation treatment on G<sub>0</sub>: without gypsum application; G<sub>1</sub>: 75% gypsum requirement; G<sub>2</sub>: 100% gypsum requirement; AMF<sub>0</sub>: without mycorrhizal fungus inoculation; AMF<sub>1</sub>: mycorrhizal fungus inoculation treatment.

3.2. Bacterial communities

Results shown in Figure (2) revealed that the combined application of gypsum with AMF enhanced the mycorrhizal root colonization, total bacte-

rial counts, P solubilizer bacteria, and respiration of microbial soil in the Rhizosphere of wheat and maize at the flowering stage compared with the control (G<sub>0</sub> AMF<sub>0</sub>).

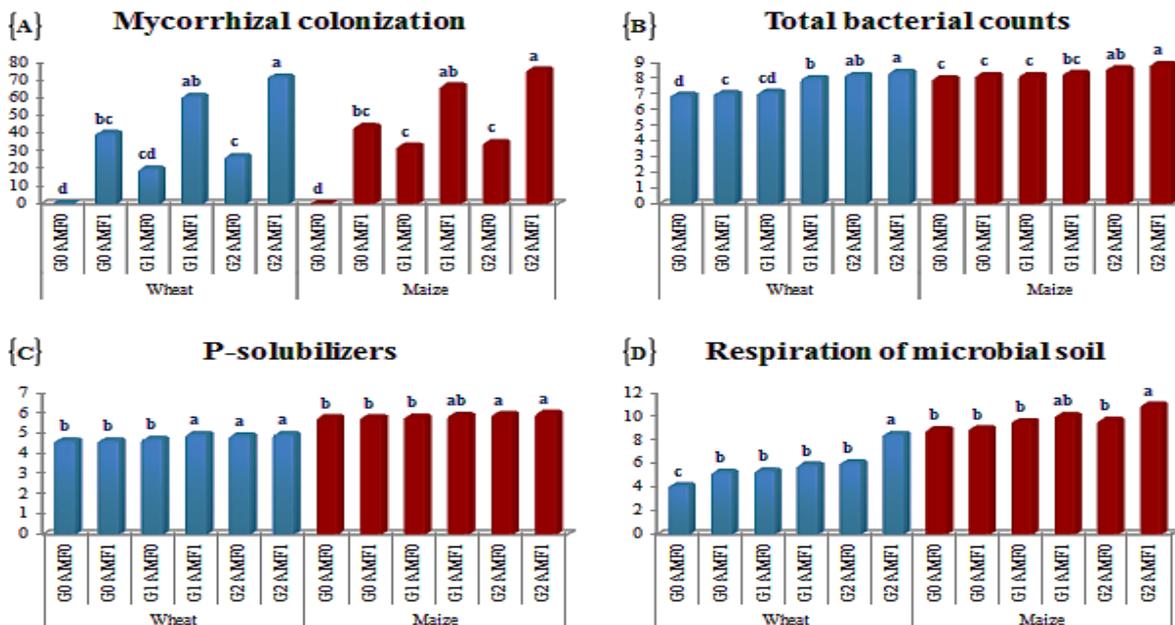


Fig. 2. The combined effect of different rates of gypsum and AMF inoculation on the mycorrhizal root colonization, total bacterial counts, P solubilizer bacteria, and respiration of microbial soil in the Rhizosphere of wheat and maize at the flowering stage. G<sub>0</sub>: without gypsum application; G<sub>1</sub>: 75% gypsum requirement; G<sub>2</sub>: 100% gypsum requirement; AMF<sub>0</sub>: without mycorrhizal fungus inoculation; AMF<sub>1</sub>: mycorrhizal fungus inoculation treatment.

The  $G_2$  AMF<sub>1</sub> treatment increased significantly mycorrhizal root colonization in roots of wheat and maize (Figure 2A). The total bacterial load varied from 6.94 to 8.40 log CFU g<sup>-1</sup> in dry soil with wheat plants, while it varied from 8.01 to 8.90 log CFU g<sup>-1</sup> in dry soil with maize plants (Figure 2B). P-solubilizers were increased by 6.91 and 3.62% in wheat and maize, respectively compared with the  $G_0$  AMF<sub>0</sub> treatment (Figure 2C). In addition, the respiration of microbial soil varied from 4.15 to 8.5 CO<sub>2</sub> mg 10g dry soil 72 h<sup>-1</sup> in wheat and from 8.91 to 11.00 CO<sub>2</sub> mg 10g dry soil 72 h<sup>-1</sup> in maize (Figure 2D).

### 3.3. Wheat and Maize Productivity:

The results in Table (4) showed that the yields of wheat and maize were significantly influenced by the applications of gypsum and/or inoculation by AMF.

An increase in the grain and straw yields of wheat and maize in the plots inoculated by AMF (1.46, 2.47 and 22.10, 11.72%, respectively) compared to the control.

The addition of 100% gypsum requirement ( $G_2$ ) achieved the highest significant increases in grain and straw yields of wheat (23.51 and 18.10%, respectively) and maize (16.43 and 18.14%, respectively) over that without gypsum application. The yields of both crops were affected by the interaction between gypsum and AMF. Subsequently, the maximum grain, and straw yields of wheat (7.72 and 11.42 Mg ha<sup>-1</sup>, respectively) and maize (8.52 and 11.52 Mg ha<sup>-1</sup>, respectively) were obtained with the combination treatment of  $G_2$  AMF<sub>1</sub>.

**Table 4. The yield as affected by different gypsum rates and AMF inoculation treatments, as well as their combination.**

Treatments	Wheat		Maize	
	Grain yield	Straw yield	Grain yield	Straw yield
AMF <sub>0</sub>	6.83	10.12	6.20	9.13
AMF <sub>1</sub>	6.93	10.37	7.57	10.20
F-test	**	**	**	*
LSD <sub>0.05</sub>	<b>0.029</b>	<b>0.125</b>	<b>0.277</b>	<b>0.503</b>
G <sub>0</sub>	6.21	9.56	6.39	8.82
G <sub>1</sub>	6.77	9.89	6.84	9.74
G <sub>2</sub>	7.67	11.29	7.44	10.42
F-test	**	*	**	**
LSD <sub>0.05</sub>	<b>0.074</b>	<b>0.945</b>	<b>0.274</b>	<b>1.042</b>
G <sub>0</sub> AMF <sub>0</sub>	6.16	9.43	5.91	8.80
G <sub>0</sub> AMF <sub>1</sub>	6.26	9.69	6.86	8.84
G <sub>1</sub> AMF <sub>0</sub>	6.72	9.76	6.34	9.26
G <sub>1</sub> AMF <sub>1</sub>	6.82	10.02	7.33	10.23
G <sub>2</sub> AMF <sub>0</sub>	7.62	11.16	6.36	9.32
G <sub>2</sub> AMF <sub>1</sub>	7.72	11.42	8.52	11.52
F-test	**	*	**	*
LSD <sub>0.05</sub>	<b>0.050</b>	<b>0.216</b>	<b>0.480</b>	<b>0.872</b>

\*G<sub>0</sub>: without gypsum application; G<sub>1</sub>: 75% gypsum requirement; G<sub>2</sub>: 100% gypsum requirement; AMF<sub>0</sub>: without Mycorrhizal fungus inoculation; AMF<sub>1</sub>: Mycorrhizal fungus inoculation treatment.

### 3.4. Soil characteristics

#### 3.4.1. Some soil's physicochemical:

The data illustrated in Table (5) showed the clear effects of the individual applications of gypsum and AMF individually or in combined together on electrical conductivity (EC), exchangeable sodium percentage (ESP), exchangeable calcium percentage (ECaP), available phosphorus (Av. P), infiltration rate (IR), soil bulk density (BD) and total porosity (TP) after wheat and maize harvesting. The data revealed that the inoculation of plant seeds by AMF decreased the values of soil EC, ESP and BD by 18.96, 10.13, and 2.22%, respectively, after wheat harvesting, and 33.86, 15.21 and 3.76%, respectively, after maize harvesting. On the other hand, AMF increased the ECaP, Av. P, IR and TP after wheat harvesting by

16.47, 22.07, 14.63 and 2.32%, respectively and by 19.08, 22.55, 19.54 and 3.79%, respectively, after maize harvesting in comparison to the initial values. Also, the addition of 100% of gypsum requirement ( $G_2$ ) decreased the values of EC, ESP and BD after harvesting wheat by 28.91, 15.04, and 2.99%, respectively, while after maize these parameters were decreased by 47.41, 22.98, and 4.55%, respectively. Also,  $G_2$  increased the values of ECaP, Av. P, IR and TP after harvesting wheat by 21.06, 22.74, 23.08 and 3.19%, respectively and by 24.17, 22.33, 27.08 and 4.64%, respectively after maize harvesting comparing to the initial values. In addition, the change of soil physicochemical characteristic as affected by the integration of gypsum with AMF could be arranged as follows:  $G_2$ AMF<sub>1</sub>>  $G_1$ AMF<sub>1</sub>>  $G_2$ AMF<sub>0</sub>>

$G_1AMF_0 > G_0AMF_1 > G_0AMF_0$  (Figure 3). So, the application of 100% gypsum requirement with AMF inoculation ( $G_2AMF_1$ ) caused the highest change in

the soil characteristic values over that before planting, while the lowest change was observed in the control ( $G_0AMF_0$ ).

**Table 5.** Some soils physicochemical properties as affected by gypsum and AMF treatments as well as their combinations.

Crops	Treatments	EC	ESP	ECaP	Av. P	IR	BD	TB
After wheat harvesting	AMF <sub>0</sub>	6.10	17.77	13.28	9.29	0.80	1.36	48.68
	AMF <sub>1</sub>	5.96	17.57	13.78	10.51	0.82	1.35	49.06
	F-test	**	**	**	**	**	**	**
	LSD <sub>0.05</sub>	<b>0.04</b>	<b>0.13</b>	<b>0.22</b>	<b>0.31</b>	<b>2.13E-09</b>	<b>0.004</b>	<b>0.003</b>
	G <sub>0</sub>	6.81	19.03	12.03	9.07	0.71	1.37	48.24
	G <sub>1</sub>	5.77	17.18	13.99	10.03	0.81	1.36	48.87
	G <sub>2</sub>	5.50	16.82	14.58	10.60	0.91	1.34	49.50
	F-test	**	**	**	**	**	**	**
	LSD <sub>0.05</sub>	<b>0.31</b>	<b>0.27</b>	<b>0.16</b>	<b>0.51</b>	<b>1.14E-16</b>	<b>0.009</b>	<b>0.35</b>
	G <sub>0</sub> AMF <sub>0</sub>	6.83	19.08	11.59	8.23	0.70	1.38	48.05
	G <sub>0</sub> AMF <sub>1</sub>	6.79	18.97	12.47	9.90	0.72	1.37	48.43
	G <sub>1</sub> AMF <sub>0</sub>	5.89	17.33	13.87	9.47	0.80	1.36	48.68
	G <sub>1</sub> AMF <sub>1</sub>	5.65	17.02	14.11	10.60	0.82	1.35	49.06
	G <sub>2</sub> AMF <sub>0</sub>	5.57	16.91	14.38	10.17	0.90	1.34	49.31
G <sub>2</sub> AMF <sub>1</sub>	5.43	16.72	14.77	11.03	0.92	1.33	49.69	
F-test	*	*	**	*	**	*	*	
LSD <sub>0.05</sub>	<b>0.15</b>	<b>0.23</b>	<b>0.38</b>	<b>0.54</b>	<b>1.34E-12</b>	<b>0.008</b>	<b>0.37</b>	
After maize harvesting	AMF <sub>0</sub>	5.27	16.85	13.73	9.27	0.85	1.34	49.31
	AMF <sub>1</sub>	5.11	16.63	14.31	10.60	0.87	1.33	49.81
	F-test	**	**	**	**	**	**	**
	LSD <sub>0.05</sub>	<b>0.08</b>	<b>0.11</b>	<b>0.22</b>	<b>0.15</b>	<b>1.22E-09</b>	<b>0.002</b>	<b>0.003</b>
	G <sub>0</sub>	5.89	18.46	12.19	9.27	0.76	1.36	48.81
	G <sub>1</sub>	5.04	16.18	14.60	9.97	0.86	1.34	49.62
	G <sub>2</sub>	4.64	15.58	15.27	10.57	0.96	1.32	50.25
	F-test	**	**	**	**	**	**	**
	LSD <sub>0.05</sub>	<b>0.24</b>	<b>0.36</b>	<b>0.23</b>	<b>0.36</b>	<b>1.14E-16</b>	<b>0.009</b>	<b>0.35</b>
	G <sub>0</sub> AMF <sub>0</sub>	5.94	18.53	11.66	8.40	0.75	1.37	48.43
	G <sub>0</sub> AMF <sub>1</sub>	5.84	18.39	12.72	10.13	0.77	1.35	49.18
	G <sub>1</sub> AMF <sub>0</sub>	5.15	16.34	14.46	9.43	0.85	1.34	49.43
	G <sub>1</sub> AMF <sub>1</sub>	4.93	16.01	14.74	10.50	0.87	1.33	49.81
	G <sub>2</sub> AMF <sub>0</sub>	4.70	15.68	15.06	9.97	0.95	1.32	50.06
G <sub>2</sub> AMF <sub>1</sub>	4.57	15.47	15.47	11.17	0.97	1.31	50.44	
F-test	*	*	*	**	*	**	**	
LSD <sub>0.05</sub>	<b>0.10</b>	<b>0.26</b>	<b>0.38</b>	<b>0.27</b>	<b>1.07E-10</b>	<b>0.006</b>	<b>0.31</b>	

\*G<sub>0</sub>: without gypsum application; G<sub>1</sub>: 75% gypsum requirement; G<sub>2</sub>: 100% gypsum requirement; AMF<sub>0</sub>: without Mycorrhizal fungus inoculation; AMF<sub>1</sub>: Mycorrhizal fungus inoculation treatment.

### 3.4.2. Bacterial Communities:

The bacterial counts that are the total bacterial counts (log CFU g<sup>-1</sup> soil), P solubilizer bacteria (log CFU g<sup>-1</sup> soil), and respiration of microbial (Log CO<sub>2</sub> mg 10g dry soil 72 h<sup>-1</sup>) in the root rhizosphere after wheat and maize harvesting were affected significantly by gypsum applications combined with AMF (Fig-

ure 4). The highest total bacterial counts (7.20 and 8.40 Log CFU g<sup>-1</sup> soil), P solubilizer bacteria (4.89 and 5.89 Log CFU g<sup>-1</sup> soil), and respiration of microbial (6.70 and 9.69 Log CO<sub>2</sub> mg 10g dry soil 72 h<sup>-1</sup>) with wheat and maize, respectively were recorded in soil treated by G<sub>2</sub>AMF<sub>1</sub>.

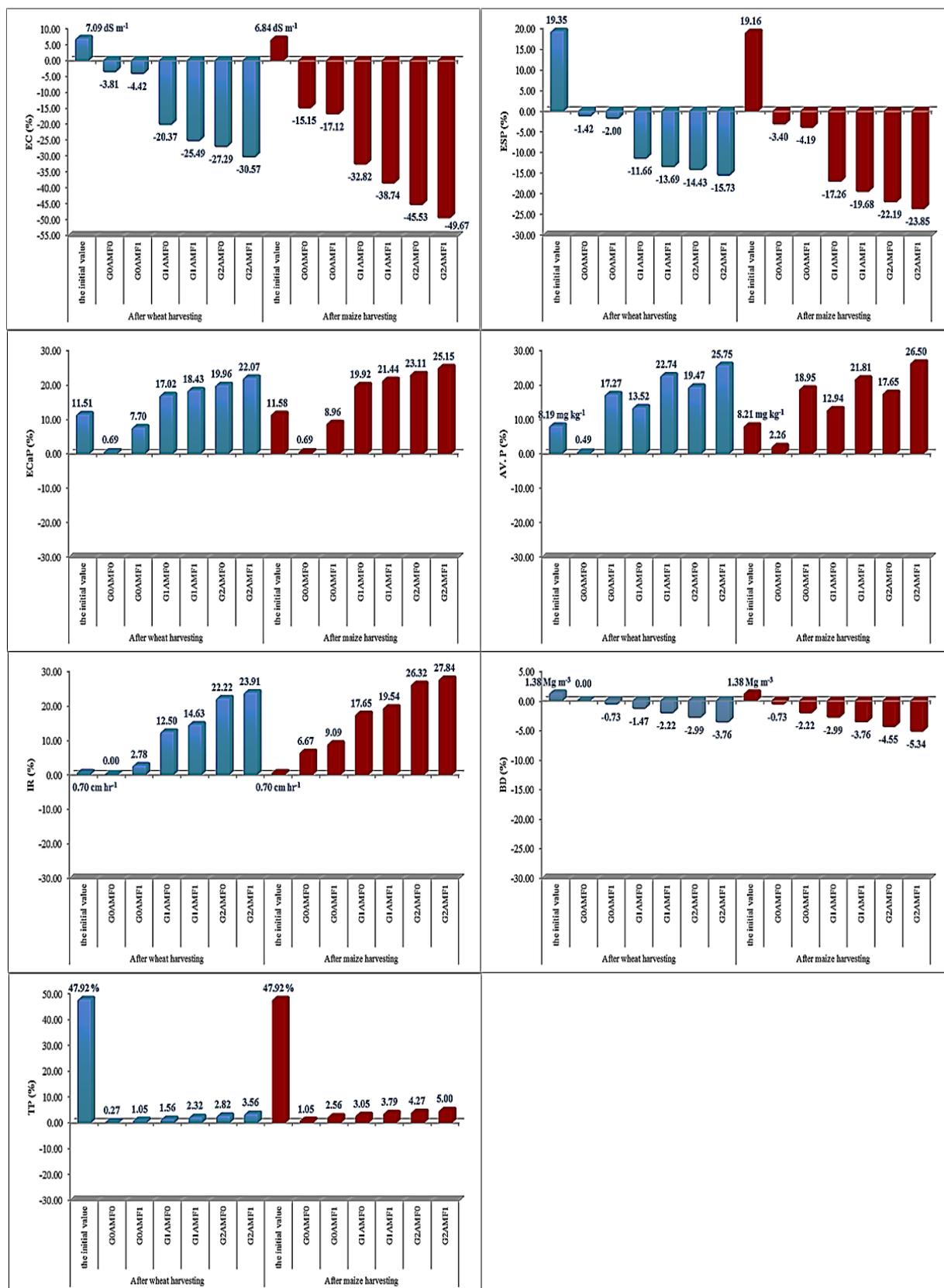
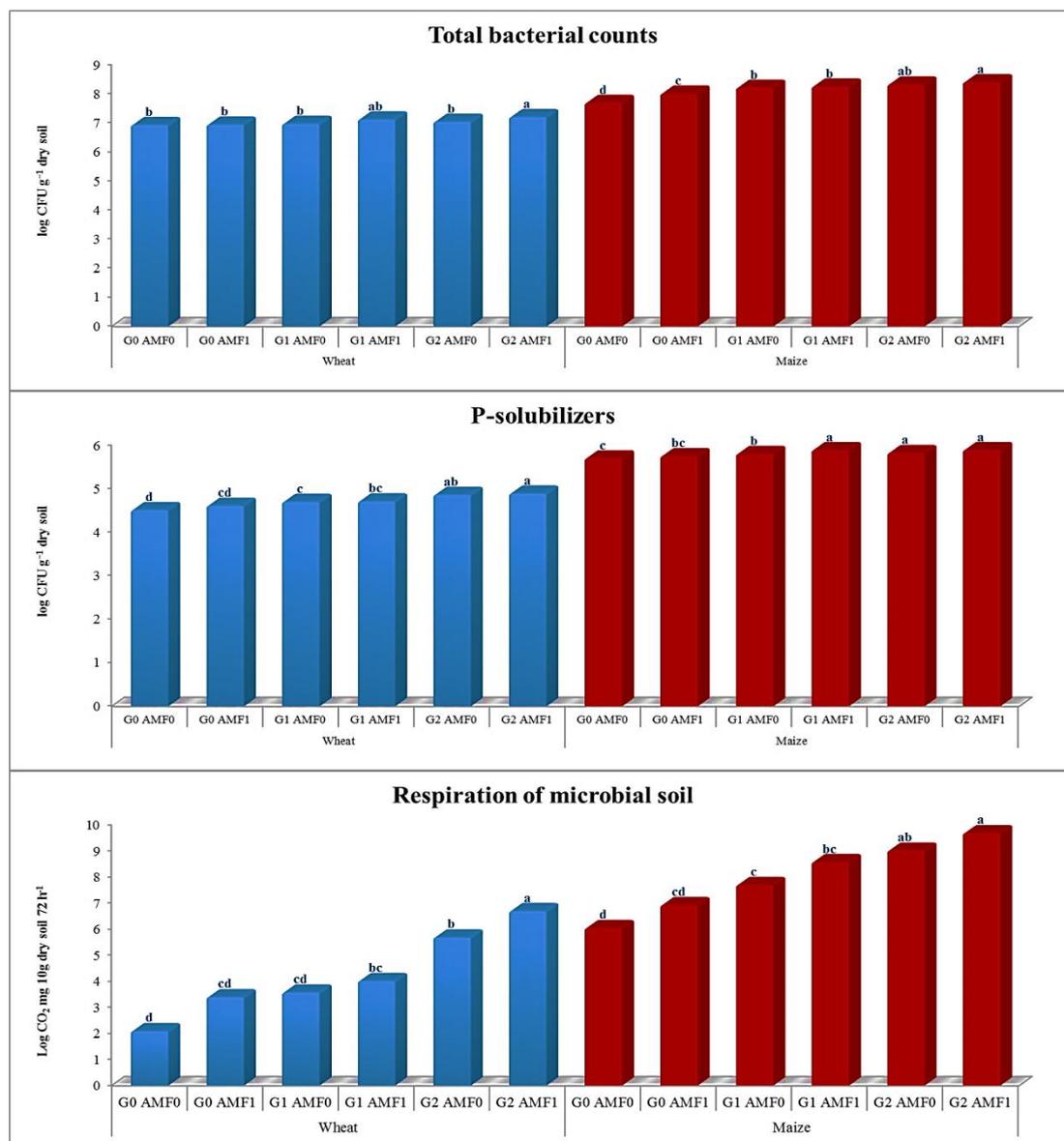


Fig. 3. The changes of soils physicochemical properties as affected by the combined effect of gypsum applications and AMF inoculation treatment on G<sub>0</sub>: without gypsum application; G<sub>1</sub>: 75% gypsum requirement; G<sub>2</sub>: 100% gypsum requirement; AMF<sub>0</sub>: without mycorrhizal fungus inoculation; AMF<sub>1</sub>: mycorrhizal fungus inoculation treatment.



**Fig. 4.** The combined effect of different rates of gypsum and AMF inoculation on the total bacterial counts, P solubilizer bacteria, and respiration of microbial in the root rhizosphere after wheat and maize harvesting. G<sub>0</sub>: without gypsum application; G<sub>1</sub>: 75% gypsum requirement; G<sub>2</sub>: 100% gypsum requirement; AMF<sub>0</sub>: without mycorrhizal fungus inoculation; AMF<sub>1</sub>: mycorrhizal fungus inoculation treatment.

### 3.5. The relationship between all studied traits as affected by the interaction among gypsum and AMF inoculation

The integration effect of gypsum applications with AMF inoculants on some physiological and biological activities and the productivity of wheat-maize plants resulted in a correlation coefficients heat map between soil properties and all traits of wheat and maize are shown in Figure (5 A and B). The results showed high positive correlation ( $p < 0.05$ ) after wheat harvesting between the soil EC, ESP, BD, superoxide

dismutase activity, catalase activity as well as between ECaP, AV. P, IR, TB, photosynthetic, peroxidase, total bacterial counts, P solubilizer bacteria, and respiration of microbial, grain and straw yields (Figure 5A). While after maize harvesting, the high positive correlations ( $p < 0.05$ ) were found between the soil EC, ESP, BD, catalase activity, also between soil ECaP, AV. P, IR, TB, photosynthetic activity, superoxide dismutase activity, peroxidase activity, total bacterial counts, P solubilizer bacteria, respiration of microbial grain and straw yield (Figure 5B).

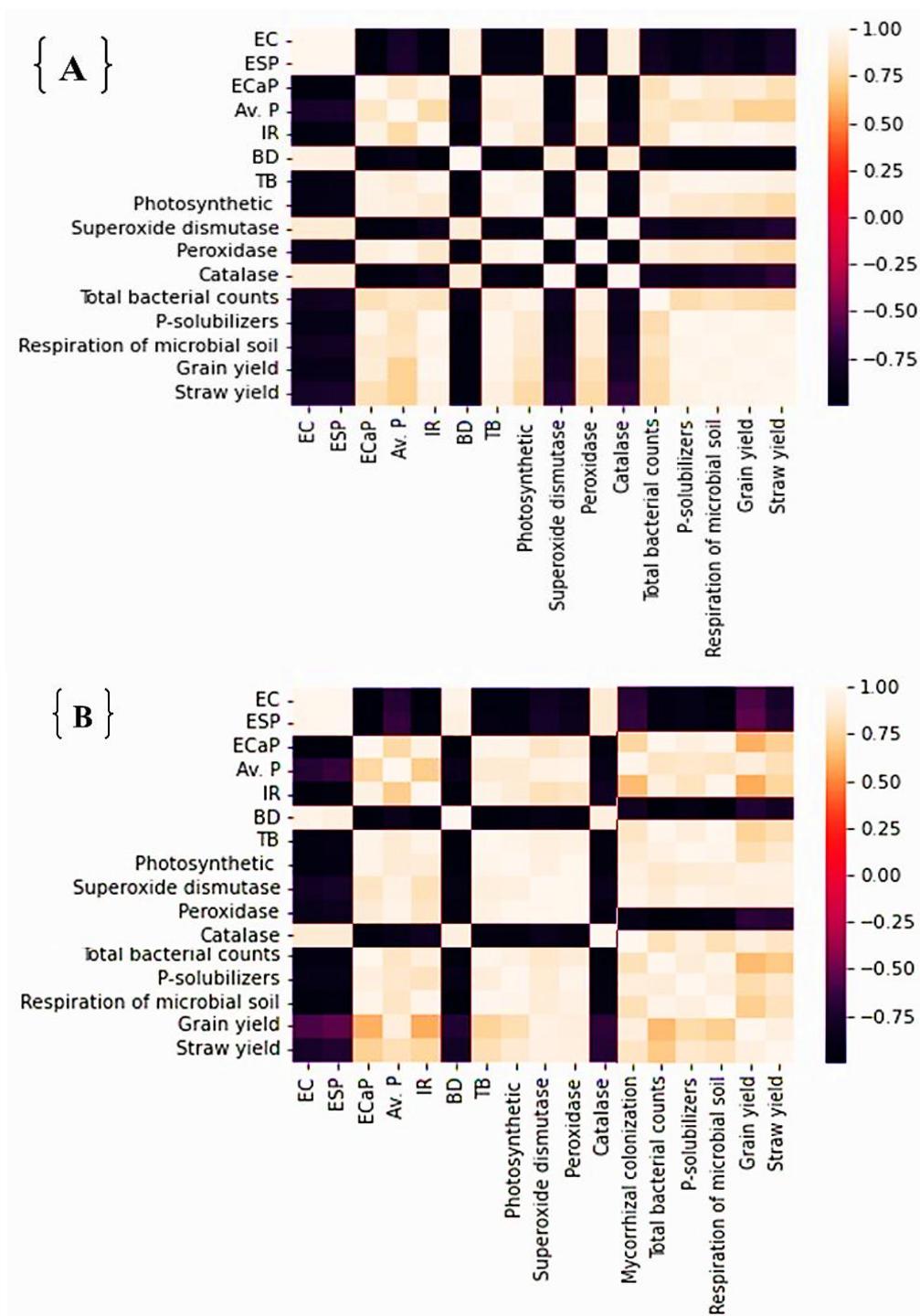


Fig. 5. Correlation coefficients heat map, displaying the interaction of the gypsum with AMF on saline-sodic soil properties and bacterial communities, physiological and productivity of wheat and maize plants. The light yellow color represents high values and the black color represents low values.

## 5. Discussions

The saline-sodic soil with poor properties adversely affects the growth and yield of most crops, so the amelioration processes are very important for increasing crop productivity (Zaka et al., 2018). For this purpose, the application of gypsum (as chemical amendments) with arbuscular mycorrhizal fungi in-

oculants (as bio-amelioration) were used to improve saline-sodic soil characteristics (Table 5). These may be probably attributed to the release of more  $\text{Ca}^{2+}$  replacing  $\text{Na}^+$  in the sorption complex, improving soil aggregate stability and infiltration rate in the soil which lead to clear decreases in soil salinity, sodicity and bulk density. These results were in agreement

with those of (Chaganti et al. 2015; Anikwe et al., 2016; Gupta et al., 2016; Tirado-Corbalá et al., 2019; Fontoura et al., 2019). they indicated that the gypsum application provided high volumes of  $\text{Ca}^{2+}$ , thus facilitating its release into the soil solution and exchanged by  $\text{Na}^+$ , which reduced the soil dispersion that improves its physical properties e.g. bulk density, total porosity, and increasing water infiltration rates by promoting clay aggregation through the soil profile. Gypsum is proved to be the best soil amendment for improving the chemical propertie (Bayoumy et al., 2019; Santos et al., 2019; Zhang et al., 2020; El-Sharkawy et al., 2021). The addition of gypsum to the soil had positive effects on mycorrhiza colonization; it may be due to the improvement of the soil properties as well as decreasing the  $\text{Na}^+$  in soil. The reason behind the increase of colonization at low concentrations of salt and decrease at high concentrations of salt may be that AMF species have varying tolerance to salinity. AMF improves some soil characteristics such as pH, nutrient availability and soil aggregates (Schreiner et al., 1997), while the glycol sugar protein glomalin produced by AMF improves soil aggregation, structure and aeration, retention, percolation and water relationships. AMF colonization also influences the concentration of organic acids which play important role in lowering pH and increasing the availability of N, P and K in soil (Sheng et al., 2011). Our results indicated that the decreasing root colonization corroborates with salinity-sodicity stress. These findings were supported by Juniper and Abbott, (2006), which reported that salt stress can affect AMF root colonization through slowing down colonization, spore germination, and hyphal growth. The results also showed that soil inoculation with AMF activated the microorganisms in the rhizosphere of plants. Soil microbes are one indicator of soil fertility. The high population of microbes in the soil, the high fertility of the soil. Total bacterial counts and phosphate solubilizing bacteria were increased in the rhizosphere of plants treated with AMF compared to the untreated control treatment. Increased bacterial counts may be due to nutrient availability in the rhizosphere of AMF-treated plants that provide the needed energy for soil microorganisms to decompose organic matters. These results agree with those obtained by El-Sawah *et al.* (2021) who found that the AFM inoculation individually or in combinations with nitrogen fixing bacteria, increased bacterial counts in the rhizosphere of guar plants. The current study showed that the application of mycorrhizal resulted in an increase of P and Ca content in wheat and maize plants. This increase can be attributed to acid and alkaline phosphatase enzymes in the rhizosphere soil produced by AM fungal hyphae. These enzymes help phosphorus availability to plants,

which can be easily absorbed by extra radical hyphae and transferring them to the root tissues (Etesami et al., 2021). Various microbial activities in the soil can be measured one of them is by measuring the amount of oxygen consumed or the amount of carbon dioxide produced by microbial activities in the soil or measuring the process of respiration (Haney et al., 2018). AM fungi can increase microbial activity in saline soil. However, in our study, AMF inoculation increased soil respiration compared to the control treatment which suggested an improvement in microbial activity after the inoculation of AMF in the soil. Soil respiration can be affected by AMF fungi due to a presence of AMF hyphal exudates like glomalin, amino acid, and sugar (Toljander et al., 2008) which is considered as a vital stimulator for microbial activity (Abiven et al., 2007)

Salinity induces oxidative stress in plants resulting in reactive oxygen species (ROS) which attack several biomolecules such as nucleic acid, protein, and membrane lipid, and in sequence diminishes crop yield (Gill and Tuteja, 2010). Plants employ an enzymatic system to counteract the adverse effects of ROS such as catalase, peroxidase, and superoxide dismutase enzymes. The results are shown in Table (3) and Figure (1A, B, C, D) indicated that the application of gypsum with AMF inoculants enhanced plant tolerance against salinity, decreased the stress on the plant, consequently increased the photosynthetic activity, and decreased the formation of ROS. This led to the regulation of some antioxidant enzymes and photosynthetic activities of wheat and maize plants. The mechanisms by which gypsum enhances plant tolerance against salinity may be due to the improvement of the soil properties as well as the harmful effect of salinity and sodium in wheat and maize plants which is reflected in an increase in yield. These findings are supported by a previous study (Bello et al., 2021), which revealed that gypsum provided soluble  $\text{Ca}^+$ , this makes more  $\text{Ca}^+$  available for plant uptake. supports crop tolerance to salinity stress by decreasing  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation in the cell membrane (Grattan, 1999), increasing the  $\text{K}^+/\text{Na}^+$  ratio in salinity-stressed plants (Al-shareef and Tester, 2019), and increasing P uptake by plants (Cuevas et al., 2019). In addition, gypsum is provided sulfur element. which enhances the antioxidant defense by decreasing cellular-redox conditions and scavenging excessive ROS (Hasanuzzaman et al., 2018) and improves the chlorophyll content, C/N metabolism, photosynthetic enzyme activity, protein synthesis, and electron transport in the plant cells (Shaban et al., 2019). Also, gypsum increases yield production by reducing the salinity stress on the plant (Zaka et al., 2018). Finally, gypsum increased the yield of wheat and maize crop (Bayoumy et al., 2019;

Iqbal et al., 2020; Aboelsoud et al., 2020; and El-Sharkawy et al., 2022). Regarding the effect of AMF on photosynthetic activity and antioxidant enzyme activities. AMF is considered to promote plant growth and salinity tolerance (Evelin et al., 2019). The AMF improved antioxidant systems in stress plants have been reported in Wheat (Abdel Latef, 2010) and Maize (Wang et al., 2020). Additionally, it can alleviate salt stress by decreasing oxidative stress in plants and improved concentrations of osmolytes such as proline, polyamines, and glycine betaine (Frosi et al., 2018); enhancing lipids and proteins, quenching free radicals, and buffering cellular redox potential under salinity stress (Yang et al., 2008); improving in photosynthetic activity (Wang et al., 2022) due to mycorrhizal colonization in plants are limited the  $\text{Na}^+$  transport (Zhu et al., 2010), energizing carbohydrate transport and metabolism (Kaschuk et al., 2009), increasing the leaf area and higher stomatal conductance and consequently better assimilation  $\text{CO}_2$  due to improving water situation in plants (Chen et al., 2017). Finally, using AMF can be increased the yield of wheat and maize crop (Cozzolino et al., 2013; Pérez et al., 2016; Hussain et al., 2021; Elliott et al., 2021)

## 5. Conclusion

Salinity and sodicity in soils induced stress in wheat and maize and reduced antioxidant enzyme and photosynthetic activities, which decreased both crop production. Therefore, gypsum application and AMF treatments were effective in reducing the harmful effect of salinity and sodium on wheat and maize plants and it reflected in an increase in their yields and improved soil characteristics. Thereby, according to the results of the current study, the application of gypsum with AMF inoculants could be recommended to improve saline-sodic soil characteristics and crop productivity.

## Conflicts of interest

There are no conflicts to declare.

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