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Actinobacterial and fungal strain-enriched wheat straw as an effective strategy for alleviating the effect of salinity stress on soil chemical and biochemical properties

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Abstract

Background: Soil salinization influences the physical and chemical properties of soil and disturbs soil biodiversity. Application of wheat straw in saline soils with enhance soil fertility could mitigate the effects of salinity on soil microbial properties under laboratory conditions. However, knowledge is inadequate regarding the effects of adding enriching plant residues with beneficial organisms on soil quality in saline soil. To enhance this knowledge, an incubation experiment was performed to evaluate the effect of wheat straw (0 and 1%, w/w) enriched with microbial strains (control, *Streptomyces chartreusis*, *Pleurotus ostreatus* and a mixture of *P. ostreatus* and *S. chartreusis*.) on some soil chemical and biochemical properties under salinity stress (0, 8 and 15 dS m⁻¹).

Results: Salinity stress led to reducing soil available phosphorus (13–23%), available potassium (5–7%), total nitrogen (3–18%). Wheat straw inoculated with *S. chartreusis* and *P. ostreatus* improved microbial respiration rate (108–305%), soil microbial biomass carbon (80–110%), microbial biomass phosphorus (50–115%), catalase activity (20–140%), urease activity (25–45%), soil organic carbon (70–100%) and dissolved organic carbon (15–20%) under all salinity levels. The effect of *S. chartreusis* enriched wheat straw on enzymatic and microbial properties was higher than that of wheat straw inoculated with *P. ostreatus* under salinity stress conditions.

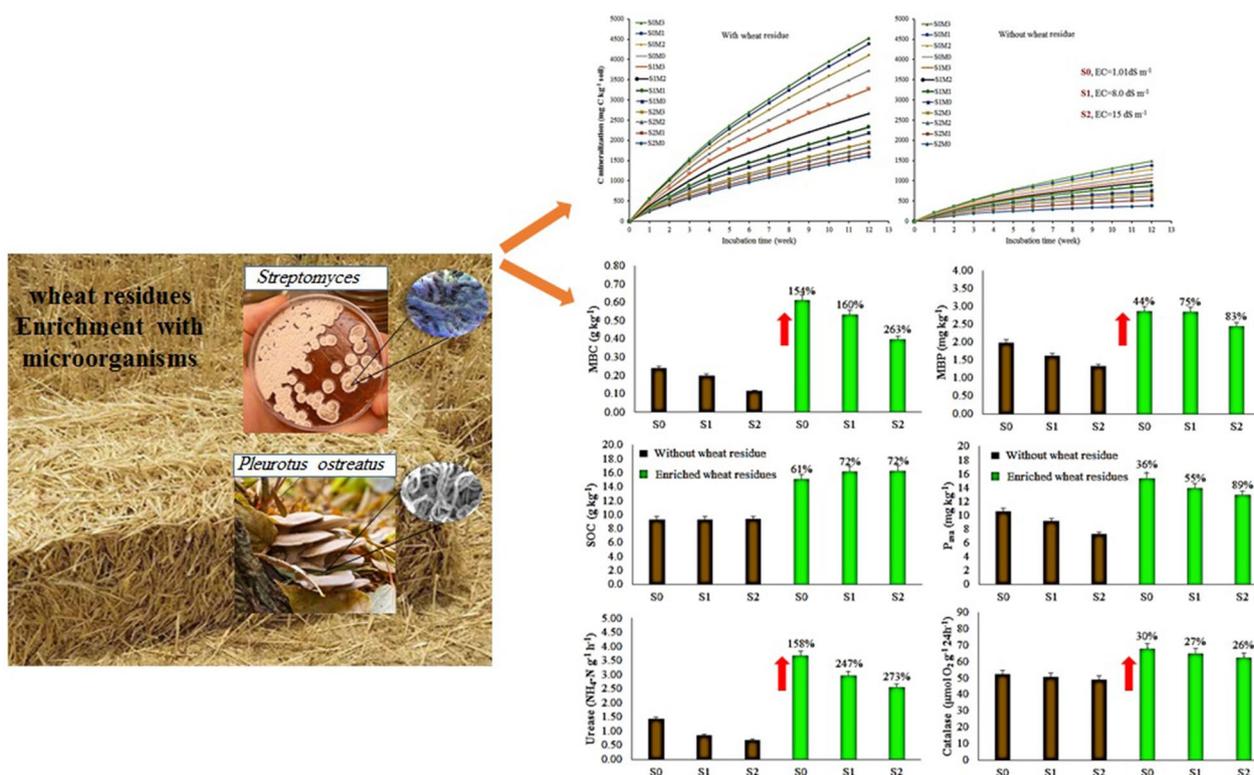
Conclusions: The results of this study demonstrated that the enrichment of wheat straw with *S. chartreusis* and *P. ostreatus* act synergistically and improve soil fertility and microbial properties. It can be concluded that the combined application of wheat straw and actinobacterial and fungal strain can be an effective strategy to ameliorate soil salinity stress in agriculture.

Keywords: Microbial respiration, Microbial biomass C, *Streptomyces chartreusis*, Microbial biomass P, Dissolve organic carbon, *Pleurotus ostreatus*

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Graphical Abstract



Introduction

Soil salinization is a widespread issue worldwide, influencing the physical and chemical properties of soil [47]. This, in turn, leads to decreasing soil biodiversity, water quality, and agricultural productivity [30, 49]. Salinity stress affects plant growth through osmotic stress and ion toxicity, thereby disturbing soil biodiversity [46]. Over the past few decades, soil salinity has reduced the production of major world crops by more than 50% [9]. Salinity reduces microbial biomass activities and the decomposition of soil organic matters [15]. It also alters microbial community structure, because microbial genotypes differ in their tolerance to low osmotic potential [18]. The microbial and biochemical properties of soil are used as sensitive bio-indicators to salinity stress in agroecosystems [15]. The sensitivity of fungi to high salinity is thought to be relatively low, but the sensitivity of bacteria is comparatively high [30].

In saline soils, the addition of plant residues increases microbial activity and biomass temporarily; as a result, microbial activity and biomass return to values similar to those in the unamended soils [5]. Similarly, previous

studies have reported that application of plant residues could mitigate the effects of salinity on soil microbial properties under laboratory conditions [13] and also could improve soil fertility [48]. In fact, the addition of plant residues is a widely used practice to enhance soil fertility [39]. Therefore, plant residues (e.g., wheat straw or rice stalks) can be effective in improving the degradation rate of soil organic matter by microorganisms [14]. There is increasing evidence showing that bacteria and fungi act together to decompose plant residues [11].

Soil microbial community plays a crucial role in some key biochemical processes including soil organic matter transformation and carbon (C) and nutrient cycling through complex enzymatic reactions [34, 40]. Soil microorganisms are key regulators of C and N dynamics [24], changing nutrient availability and influencing the release of C and N [20, 44]. In addition to the beneficial effects of wheat residue on improving soil fertility [16], enriching plant residues with beneficial organisms may further increase the agricultural and environmental benefits of wheat residues [36]. It has been reported that beneficial organisms such as *Streptomyces* and *Pleurotus*

promote phosphate solubilization [19], nutrient cycling [1] and enzymic activity [12, 21].

It seems that the interactive effects of microorganisms with different capability would be, probably, more effective on plant residue decomposition. To illustrate how two filamentous microorganisms and wheat straw influence soil fertility and microbial activities, a pot experiment was conducted to investigate the effect of wheat straw residues enriched with *Streptomyces chartreusis* and *Pleurotus ostreatus* on mobilization of nutrients and microbial and enzymatic activities of a soil under various salinity levels. To our knowledge, this study for the first time demonstrated that the inoculation of wheat straw residue with *S. chartreusis* and *P. ostreatus* acted synergistically and improved soil fertility and microbial properties.

Materials and methods

Soil sampling

A silty clay loam soil was sampled from an uncultivated field located at Gorgan University of agricultural sciences and natural resources (mean annual rainfall, 469 mm and mean annual temperature, 27 °C). The soil was then air-dried and passed through a 2-mm mesh sieve before analysis. The soil is classified as Typic Calcixerolls (USDA 2014) or Haplic Kastanozems (WRB 2014). Samples were analyzed for their chemical and physical properties. The soil studied had the following physical and chemical properties: clay, 32.6%; silt, 50.9%; sand, 16.5%; CaCO₃, 58 g kg⁻¹; pH, 7.4 (in a 1:5 soil:water solution), electrical conductivity (EC) of saturated extract, 1.01 dS m⁻¹; soil organic carbon (SOC), 9.1 g kg⁻¹; soil field capacity (w/w), 26.0%; total nitrogen (TN), 90 mg kg⁻¹; available P, 10 mg kg⁻¹; and available K, 423 mg kg⁻¹.

Preparation of *S. chartreusis* and *P. ostreatus* inocula

Streptomyces chartreusis strain (Accession number KJ152149) was isolated in our previous study [12]. *S. chartreusis* strain was cultured on yeast extract-malt extract (ISP2) broth (10 g L⁻¹ malt extract, 4 g L⁻¹ yeast extract, 4 g L⁻¹ glucose, 2 g L⁻¹ CaCO₃, and pH 7.4) [26] and incubated at 28 °C for 7 days at 150 rpm. *P. ostreatus* strain was provided by Plant Pathology Department, Gorgan University of Agricultural Sciences and Natural Resources, Iran. The fungus isolate was cultured on potato dextrose agar (4 g L⁻¹ potato starch, 20 g L⁻¹ dextrose, 15 g L⁻¹ agar, and pH 5.6) and incubated at 28 °C for 7 days. In this study, the result mixture including mycelium and spores was used as fungal inoculum.

Experiment design and soil incubation

The experiment was performed as a 4 × 3 × 2 factorial test including 24 treatments with three replications

based on a completely randomized design under laboratory conditions. Experimental factors were: (1) microbial factor at four levels (control, M0; *P. ostreatus* strain, M1; *S. chartreusis* strain, M2; and the mixture of *P. ostreatus* strain and *S. chartreusis* strain, M3). The two isolates also had no antagonistic effects on each other's growth assayed in vitro; (2) salinity stress factor at three levels (0, S0, 8, S1, and 15, S2, dS m⁻¹); and (3) and wheat straw factor at two levels (0, R0 and 1%, R1, w/w). At first, the different levels of saline water were prepared by addition of NaCl, KCl and MgCl₂ salts (at a 3:2:1 ratio) to obtain EC levels in the experiment. These salinity levels were selected based on what is found in Iran's soils [26, 28]. Then, the wheat straw residues were inoculated with the strain of *S. chartreusis* and *P. ostreatus*. Wheat straw residues were chopped into 1 cm pieces. Thereafter the wheat residue was inoculated) with each microbial strain (5%, v/w) including *S. chartreusis* (10⁷ CFU mL⁻¹), *P. ostreatus* strain (10⁶ spore mL⁻¹) and mixture of both isolates. Inoculated and non-inoculated wheat straw was mixed into the soil with different salinity levels. The samples were incubated in the dark at 25 ± 2 °C for 90 days. The soil moisture content was maintained at 70% water holding capacity (WHC) by adding distilled water.

Soil analysis

Soil pH, soil EC_e, soil available potassium (K_{ava}), soil available phosphorus (P_{ava}), soil total N (TN), soil organic carbon (SOC), soil dissolved organic carbon (DOC), soil microbial respiration rate (MRR) soil microbial biomass carbon (MBC) and soil microbial biomass phosphorus (MBP) were determined for 30, 60 and 90 days after incubation. K_{ava} was determined by flame photometry, P_{ava} was analyzed by the Olsen method and TN was determined by an elemental analyzer [23]. The fumigation–incubation method, adapted from Vance et al. [42], was also used to determine MBC. Soil microbial biomass phosphorus (MBP) was measured using the method described by Ren et al. [31]. Catalase (CAT) and urease enzymes (URE) were measured with the method Trasar-Cepeda et al. [41] and Alef and Nannipieri [3], respectively. SOC and DOC in incubated soil samples were assessed under standard conditions, following the methods developed by Nelson and Sommers [23] and Corre et al. [8], respectively. To measure MRR, soil samples (100 g) were placed into 1-L plastic jars (three jars per treatment). The jars were immediately incubated at constant humidity (70% WHC) and temperature (25 ± 2 °C). Microbial respiration rate was determined as the CO₂ emitted over a 90-day period [3]. Plastic jars containing 10 mL of 0.5 M NaOH were placed in the jars to trap the respired CO₂. The amount of CO₂ was measured by titration with 0.1 M hydrochloric acid in the presence of

15% barium chloride. Then, the respiration rate of soil (mg C kg^{-1} soil per day) was calculated for 30, 60 and 90 days after incubation. The microbial metabolic quotient ($q\text{CO}_2$) was calculated from soil respiration rate and microbial biomass as an indicator of environmental stress and maintenance energy demand of actively metabolizing microbial populations [4].

Statistical analysis of data

Prior to analysis of variance (ANOVA), soil data were checked for normality and homogeneity of variance using the Anderson Darling test and Leven's test, respectively. Data were analyzed as a factorial experiment with three factors of inoculation with microorganisms, salinity levels and application of wheat residues. Soil chemical and microbiological properties data were analyzed using SPSS statistical software to identify the most important properties separating salinity, microorganisms and wheat residues. Three-way analysis of variance was used to analyze the importance of these three main factors and their

interactions during the incubation period. Intergroup effects related to microorganisms, salinity, wheat residues and their interactions were considered. In addition, intragroup effects were related to measuring incubation time and its interaction with the three mentioned factors. The Mauchelli test was performed to confirm the sphericity of the variance-covariance matrix (identical correlations). In the event that data sphericity could not be verified, Huynh-Feldt test was used to adjust all F values for intergroup factors. Mean values were separated by the Fisher's least significant difference (LSD) test at $\alpha=0.05$. All statistical tests were performed using SPSS 17 software. Values in tables and figures represent mean \pm standard error of the mean ($n=3$).

Results

Effect of microbial strains-enriched wheat straw on soil chemical properties

The results showed that microorganisms decreased soil pH (4–6%). Salinity stress caused a slightly increase in pH

Table 1 Effects of different treatments on EC, pH and soil nutrients

Treatment	Incubation time (day)			Incubation time (day)		
	30	60	90	30	60	90
	pH			EC (dS m^{-1})		
Control	7.58 \pm 0.03 ^{Ac}	7.56 \pm 0.01 ^{Ab}	7.57 \pm 0.01 ^{Ab}	1.01 \pm 0.01 ^{Ad}	1.00 \pm 0.02 ^{Ad}	1.01 \pm 0.01 ^{Ad}
M1	7.15 \pm 0.01 ^{Bf}	7.29 \pm 0.04 ^{Ac}	7.28 \pm 0.03 ^{Ac}	0.90 \pm 0.01 ^{Ae}	0.85 \pm 0.02 ^{Be}	0.84 \pm 0.03 ^{Be}
M2	7.15 \pm 0.04 ^{Bf}	7.25 \pm 0.01 ^{Ad}	7.25 \pm 0.04 ^{Ad}	0.87 \pm 0.03 ^{Ae}	0.85 \pm 0.01 ^{Ae}	0.82 \pm 0.02 ^{Be}
M3	7.10 \pm 0.01 ^{Cf}	7.21 \pm 0.04 ^{Bf}	7.25 \pm 0.03 ^{Ad}	0.88 \pm 0.03 ^{Ae}	0.80 \pm 0.03 ^{Be}	0.78 \pm 0.03 ^{Be}
R	7.45 \pm 0.01 ^{Ad}	7.28 \pm 0.02 ^{Bc}	7.21 \pm 0.01 ^{Ce}	1.12 \pm 0.04 ^{Ac}	1.12 \pm 0.01 ^{Ac}	1.09 \pm 0.02 ^{Bc}
S1	7.70 \pm 0.01 ^{Bb}	7.75 \pm 0.03 ^{Aa}	7.76 \pm 0.04 ^{Aa}	8.07 \pm 0.02 ^{Cb}	8.17 \pm 0.03 ^{Bb}	8.21 \pm 0.01 ^{Ab}
S2	7.74 \pm 0.02 ^{Ba}	7.76 \pm 0.01 ^{Aa}	7.78 \pm 0.03 ^{Aa}	15.1 \pm 0.04 ^{Ba}	15.1 \pm 0.02 ^{Ba}	15.3 \pm 0.03 ^{Aa}
	TN (g kg^{-1})			P_{ava} (mg kg^{-1})		
Control	0.60 \pm 0.06 ^{Bb}	0.66 \pm 0.02 ^{Ad}	0.65 \pm 0.04 ^{Ae}	10.6 \pm 0.18 ^{Ad}	10.6 \pm 0.21 ^{Ae}	10.4 \pm 0.11 ^{Be}
M1	0.44 \pm 0.06 ^{Bf}	0.80 \pm 0.03 ^{Ab}	0.81 \pm 0.04 ^{Ad}	10.8 \pm 0.32 ^{Bd}	11.1 \pm 0.20 ^{Ad}	10.9 \pm 0.18 ^{Bd}
M2	0.48 \pm 0.05 ^{Be}	0.85 \pm 0.03 ^{Ab}	0.86 \pm 0.03 ^{Ac}	11.3 \pm 0.15 ^{Bc}	11.7 \pm 0.16 ^{Ac}	11.4 \pm 0.21 ^{Bc}
M3	0.38 \pm 0.02 ^{Bf}	0.90 \pm 0.04 ^{Aa}	0.89 \pm 0.06 ^{Ab}	11.6 \pm 0.34 ^{Cb}	12.0 \pm 0.20 ^{Ab}	11.8 \pm 0.15 ^{Bb}
R	0.63 \pm 0.03 ^{Ca}	0.78 \pm 0.01 ^{Bc}	0.91 \pm 0.02 ^{Aa}	14.1 \pm 0.18 ^{Ca}	15.5 \pm 0.11 ^{Aa}	15.0 \pm 0.12 ^{Ba}
S1	0.52 \pm 0.05 ^{Bc}	0.61 \pm 0.02 ^{Ae}	0.63 \pm 0.01 ^{Ae}	9.18 \pm 0.28 ^{Ae}	9.12 \pm 0.22 ^{Bf}	8.90 \pm 0.17 ^{Cf}
S2	0.50 \pm 0.02 ^{Bd}	0.55 \pm 0.03 ^{Af}	0.56 \pm 0.04 ^{Af}	8.52 \pm 0.10 ^{Af}	8.28 \pm 0.19 ^{Bg}	8.00 \pm 0.11 ^{Cg}
	K_{ava} (mg kg^{-1})			DOC (mg kg^{-1})		
Control	429 \pm 0.4 ^{Ad}	429 \pm 0.6 ^{Ad}	423 \pm 0.3 ^{Bd}	77.8 \pm 0.26 ^{Ab}	63.1 \pm 0.16 ^{Bb}	59.9 \pm 0.12 ^{Cb}
M1	448 \pm 0.3 ^{Ac}	437 \pm 0.1 ^{Bc}	432 \pm 0.1 ^{Cc}	75.5 \pm 0.14 ^{Ac}	61.6 \pm 0.17 ^{Bc}	50.9 \pm 0.16 ^{Cc}
M2	450 \pm 0.3 ^{Ac}	439 \pm 0.2 ^{Bc}	434 \pm 0.3 ^{Cc}	75.9 \pm 0.18 ^{Ac}	61.9 \pm 0.10 ^{Bc}	52.0 \pm 0.15 ^{Cc}
M3	464 \pm 0.4 ^{Ab}	458 \pm 0.1 ^{Bb}	441 \pm 0.3 ^{Cb}	76.5 \pm 0.21 ^{Ac}	59.7 \pm 0.12 ^{Bd}	47.3 \pm 0.15 ^{Cd}
R	481 \pm 0.4 ^{Aa}	470 \pm 0.4 ^{Ba}	452 \pm 0.2 ^{Ca}	88.2 \pm 0.23 ^{Aa}	81.4 \pm 0.13 ^{Ba}	69.5 \pm 0.11 ^{Ca}
S1	427 \pm 0.3 ^{Ad}	408 \pm 0.4 ^{Be}	400 \pm 0.4 ^{Ce}	55.6 \pm 0.17 ^{Ad}	43.9 \pm 0.15 ^{Be}	41.6 \pm 0.09 ^{Ce}
S2	428 \pm 0.4 ^{Ad}	401 \pm 0.2 ^{Bf}	396 \pm 0.1 ^{Ce}	32.4 \pm 0.11 ^{Ae}	33.4 \pm 0.13 ^{Bf}	29.2 \pm 0.15 ^{Cf}

Control, (SOM0R0); M1, *P. ostreatus*; M2, *S. chartreusis*; M3, mixture of *P. ostreatus* and *S. chartreusis*; R, wheat residue; S1, 8 dS m^{-1} ; S2, 15 dS m^{-1} . Within each column the means sharing similar lowercase letters do not have significant differences among treatments at 5% level according to the LSD test. Within each row the means sharing similar uppercase letters do not have significant differences at 5% level between different sampling times at 5% level according to the LSD test. Values are the mean \pm standard error of the mean ($n=3$)

(2–4%) during 3 months of incubation ($p < 0.01$). Wheat residue treatments showed no significant difference in soil pH (Table 1). A significant positive correlation ($p < 0.05$, $r = 0.48$) was also found between ECe and pH (Table 3). Soil ECe values were significantly affected by salinity, wheat residue and microorganisms (Table 1). As expected, salinity caused a significant increase in soil ECe during the incubation period; these changes remained constant over time in many treatments. The addition of wheat residues also in some treatments significantly increased soil ECe (0.08–0.12 units). The inoculation of microorganisms decreased ECe (0.14–0.23 units); this decrease was higher in the soil treated with a mixture of *P. ostreatus* and *S. chartreusis* than the soil treated with each of them alone (Table 1).

The results also showed that P_{ava} , K_{ava} and TN were significantly ($p < 0.001$) affected by salinity, microorganisms and wheat residues. In the non-treated soil with microorganisms and wheat residues, salinity of 8 and 15 dS m^{-1} reduced the soil P_{ava} (13–15% and 20–23%, respectively), K_{ava} (1–5% and 1–7%, respectively) and TN (13–17% and 18–32%, respectively) during 90 days of incubation, as compared to the control soil (Table 1). In contrast, in *S. chartreusis*-inoculated soil, P_{ava} (7–10%) and k_{ava} (3–7%) increased during 3 months of incubation (Table 1). Meanwhile, in *P. ostreatus*-inoculated soils, P_{ava} (2–5%) and K_{ava} (2–4%) increased during the incubation period (Table 1). In addition, *S. chartreusis* and *P. ostreatus* decreased TN (26 and 20%, respectively) during the first month and increased TN (25–27 and 32–34%, respectively) during the second and third months, respectively. Similarly, the TN, P_{ava} and K_{ava} values tended to increase by 5–40, 33–44 and 7–12% following wheat residue application, respectively. According to the results, the simultaneous application of both microorganisms and wheat residue led to the least P_{ava} , K_{ava} and TN reduction due to salinity stress (Table 1). A significant negative correlation was also found among ECe and soil P_{ava} , K_{ava} and TN in most treatments (Table 3). Correlation analysis also showed that soil P_{ava} , K_{ava} and TN were positively correlated with DOC and SOC (Table 3).

Soil dissolved carbon was significantly ($p < 0.001$) affected by salinity, microorganisms and wheat residues, as well as their two-way and three-way interactions. Soil dissolved carbon values were decreased with incubation time in most treatments (Table 1). Salinity levels of 8 and 15 dS m^{-1} reduced the soil DOC by 28–31 and 51–58%, as compared to the control soil, respectively (Table 1). Wheat residues increased soil DOC in different treatments by 13–30%, during 3 month incubation (Table 1). This increase was greater in *S. chartreusis*-inoculated saline soils than *P. ostreatus*-inoculated ones. Wheat residues inoculated with microorganisms caused a further

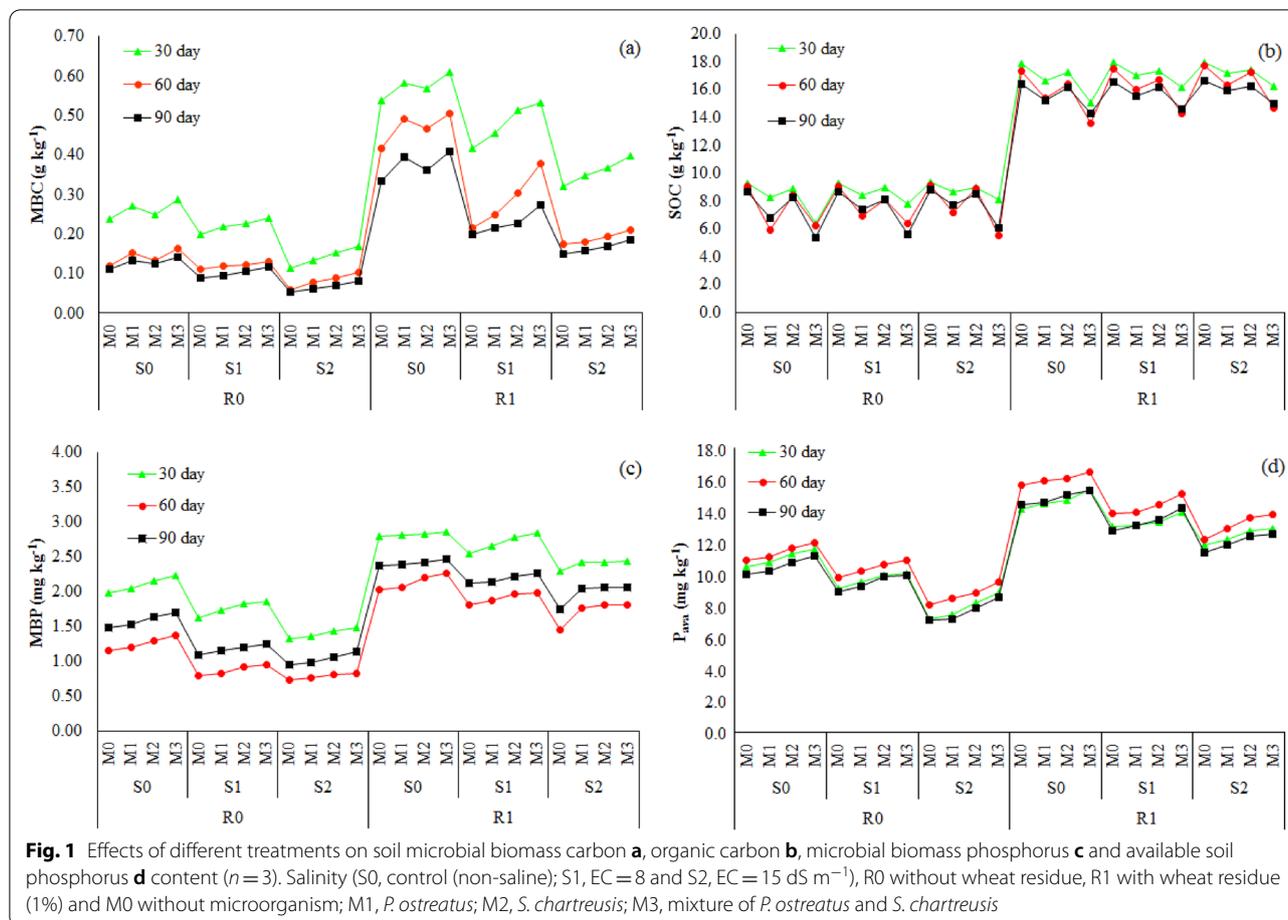
increase in the soil DOC (Table 1). SOC values were not changed significantly in most treatments during the incubation time (Fig. 1b). Wheat residues increased the SOC content by 80–110% (Fig. 1b). Increased DOC, due to providing easily degradable materials, improved soil microbial activity and biomass. Correlation analysis also showed that DOC was positively correlated with MRR and MBC ($r = 0.84$ and $r = 0.89$, $p < 0.01$; Table 3).

Effect of microbial strains-enriched wheat straw on soil biochemical properties

The results showed that all the main and interactive effects of salinity, microorganisms and wheat residues on microbial biomass were significant ($p < 0.001$). In the non-treated soil with microorganisms and wheat residues, salinity levels of 8 and 15 dS m^{-1} reduced MBC by 24–39% and 60–71%, respectively, during 3 months of incubation (Fig. 1a). In *S. chartreusis*-inoculated saline soils, MBC increased in the soils with salinity levels of 8 (22%, 23% and 31% at days 30, 60 and 90, respectively) and 15 dS m^{-1} (41%, 30% and 34% at days 30, 60 and 90, respectively) (Fig. 1a).

The inoculation with *P. ostreatus* at salinity level of 8 dS m^{-1} (16%, 18% and 14% at days 30, 60 and 90, respectively) and at salinity level of 15 dS m^{-1} (11%, 13% and 10% at days 30, 60 and 90, respectively) increased MBC compared to the control soils (non-treated saline soil) (Fig. 1a). The effect of the mixture of two microorganisms (*S. chartreusis* and *P. ostreatus*) in saline soil (8 and 15 dS m^{-1}) on MBC was greater (30–48% and 55–60%, respectively) than their effect alone (Fig. 1a). However, in the soil with EC 8 dS m^{-1} , wheat residues increased MBC by 80–90%, while at salinity level of 15 dS m^{-1} they increased it from 90% to 110% during 3 months of incubation. In addition, the interaction effects of wheat residues and microorganisms resulted in an average increase of MBC from 88% to 118% at the salinity levels of 8 and 15 dS m^{-1} , respectively (Fig. 1a).

In the control soil (soil non-treated with microorganisms and wheat residues), salinity levels of 8 and 15 dS m^{-1} reduced the soil MBP by 18–31% and 33–36%, respectively, during the 3-month incubation period (Fig. 1c). In *S. chartreusis*-inoculated soil, MBP was increased in the soil with EC 8 dS m^{-1} (13%, 15% and 10% during 3 months of incubation) and 15 dS m^{-1} (9%, 10% and 12%, respectively, during 3 months of incubation) compared with the control (*S. chartreusis*-untreated soil) (Fig. 1c). In *P. ostreatus*-inoculated soil, with EC 8 dS m^{-1} (8%, 5% and 6% at days 30, 60 and 90, respectively) and EC 15 dS m^{-1} (6%, 5% and 4% at days 30, 60 and 90, respectively) MBP was increased compared with the control (*P. ostreatus*-untreated soil) soil. However, this increase was less than that found in the soil inoculated



with *S. chartreusis* (Fig. 1c). MBP was increased at the salinity levels of 8 and 15 dS m^{-1} in the soil inoculated with *P. ostreatus* and *S. chartreusis* by 17–28% and 12–20%, respectively (Fig. 1c).

Also, the interactive effect of wheat residues with the mixture of *P. ostreatus* and *S. chartreusis* increased the amount of MBP at the salinity levels of 8 (53–108%) and 15 dS m^{-1} (63–115%), which was the highest amount of MBP measured in this study (Fig. 1c). Correlation analysis showed that the MBP was positively correlated with MRR ($r = 0.96$, $p < 0.01$), MBC ($r = 0.98$, $p < 0.01$) and P_{ava} ($r = 0.96$, $p < 0.01$) (Table 3). In this study, *S. chartreusis* and *P. ostreatus* significantly increased the amount of soil P_{ava} (Fig. 1d); however, this increase was greater in the soil treated with *S. chartreusis* than in the soil treated with *P. ostreatus*. In addition, *S. chartreusis* produced more MBP than *P. ostreatus* (Fig. 1c).

The results also showed that all the main and interactive effects on MRR were significant ($p < 0.001$). Most MRR changes were observed in the first 3 weeks of incubation. The amount of soil MRR was increased during the

first 6 weeks of incubation and then decreased; finally, it reached a constant value (Fig. 2).

As expected, the inoculation of microorganisms increased MRR of the soil. This was higher in *S. chartreusis*-treated saline soil than in the *P. ostreatus*-treated one (Fig. 2). The highest MRR was measured in non-saline soil treated with the residues inoculated with *S. chartreusis* and *P. ostreatus*, while the lowest MRR was observed in saline soil (15 dS m^{-1}) non-treated with microbial strains-enriched wheat residues (Table 2).

Wheat residues increased MRR by 90–200% as compared to the control soil (soil non-inoculated and treated with wheat residues) (Table 2). Salinity levels of 8 and 15 dS m^{-1} reduced MRR by 26–55% and 60–81% in the non-treated soil with the wheat residues and microorganisms, respectively (Table 2). In the soil treated with both microorganisms (without wheat residues), salinity levels of 8 and 15 dS m^{-1} reduced the average MRR by 20–48 and 34–51%, respectively, for 12 consecutive weeks (Table 2). In addition, the wheat residues inoculated with microorganisms increased MRR by 180–305%,

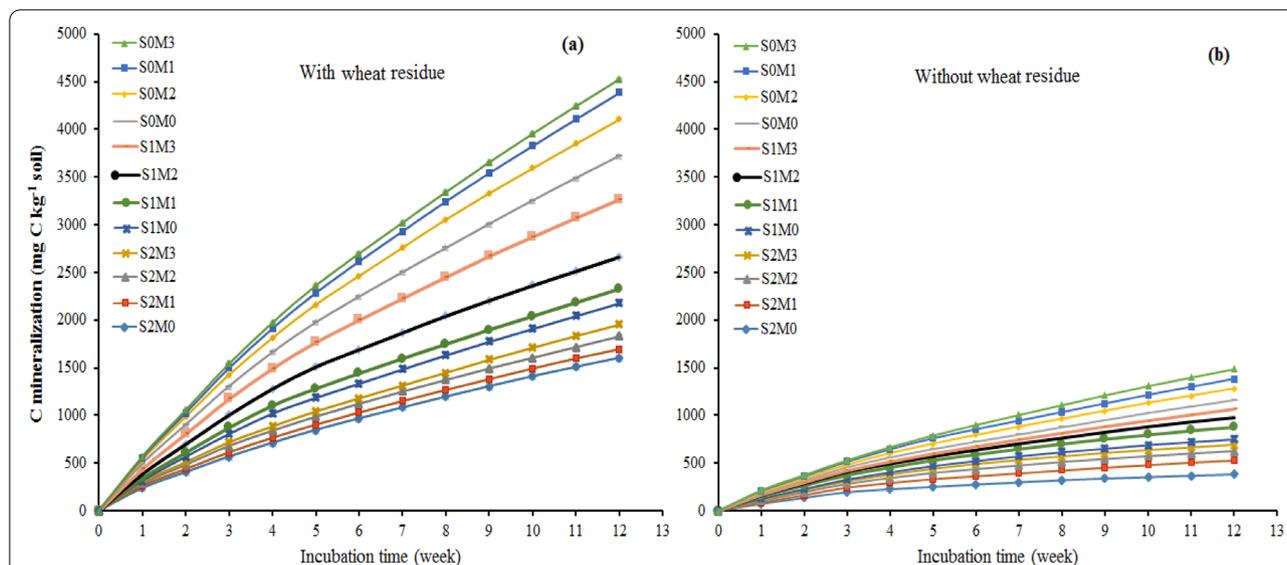


Fig. 2 Patterns of cumulative carbon mineralization at different levels of wheat residue. with wheat residue **a** and without wheat residue **b**. Salinity, S0, control (non-saline); S1, EC = 8 and S2, EC = 15 dS m⁻¹, and M0 without microorganisms; M1, *P. ostreatus*; M2, *S. chartreusis*; M3, mixture of *P. ostreatus* and *S. chartreusis* (n = 3)

Table 2 Effect of salinity, microorganisms and wheat residue on microbial respiration rate (MRR) and metabolic quotient (*q*CO₂)

Micro-Organism	Salinity (dS m ⁻¹)	Incubation time (day)			Incubation time (day)		
		30	60	90	30	60	90
Without plant residue							
Microbial respiration rate (MRR, mg kg ⁻¹ day ⁻¹)							
M-	0	24.6 ± 0.01 ^{Ah}	14.1 ± 0.15 ^{Bh}	13.2 ± 0.40 ^{Bh}	57.9 ± 0.15 ^{Ab}	37.3 ± 0.30 ^{Bb}	34.6 ± 0.34 ^{Cb}
	8	19.6 ± 0.09 ^{Aj}	10.1 ± 0.01 ^{Bj}	7.50 ± 0.24 ^{Cj}	35.0 ± 0.38 ^{Ad}	21.4 ± 0.01 ^{Bd}	19.3 ± 0.01 ^{Cd}
	15	16.3 ± 0.19 ^{Al}	8.60 ± 0.13 ^{Bl}	7.25 ± 0.13 ^{Bl}	28.0 ± 0.25 ^{Af}	16.7 ± 0.16 ^{Bf}	14.3 ± 0.14 ^{Cf}
M+	0	27.9 ± 0.28 ^{Ag}	16.5 ± 0.13 ^{Bg}	15.7 ± 0.10 ^{Cg}	69.3 ± 0.01 ^{Aa}	46.5 ± 0.13 ^{Ba}	42.1 ± 0.23 ^{Ca}
	8	23.6 ± 0.25 ^{Ai}	13.7 ± 0.10 ^{Bi}	12.1 ± 0.33 ^{Cl}	52.5 ± 0.16 ^{Ac}	33.2 ± 0.21 ^{Bc}	29.3 ± 0.38 ^{Cc}
	15	20.2 ± 0.01 ^{Ak}	11.4 ± 0.10 ^{Bk}	8.82 ± 0.14 ^{Bk}	30.3 ± 0.37 ^{Ae}	19.3 ± 0.01 ^{Be}	18.2 ± 0.01 ^{Ce}
M-	0	104 ± 0.17 ^{Ac}	87.9 ± 0.07 ^{Bd}	84.7 ± 0.32 ^{Be}	93.6 ± 0.10 ^{Ab}	83.0 ± 0.41 ^{Ba}	77.8 ± 0.23 ^{Cb}
	8	102 ± 0.44 ^{Ade}	74.6 ± 0.26 ^{Be}	54.0 ± 0.21 ^{Cg}	83.9 ± 0.04 ^{Ac}	83.4 ± 0.13 ^{Aa}	74.2 ± 0.22 ^{Bbc}
	15	106 ± 0.32 ^{Af}	76.5 ± 0.44 ^{Bf}	63.6 ± 0.15 ^{Ch}	87.8 ± 0.09 ^{Ade}	78.8 ± 0.13 ^{Bb}	68.4 ± 0.17 ^{Cd}
M+	0	96.7 ± 0.23 ^{Ad}	81.3 ± 0.22 ^{Bbc}	78.9 ± 0.22 ^{Bde}	102 ± 0.07 ^{Aa}	88.4 ± 0.25 ^{Ba}	81.0 ± 0.27 ^{Ca}
	8	107 ± 0.03 ^{Ac}	92.0 ± 0.45 ^{Bc}	80.4 ± 0.47 ^{Cf}	93.3 ± 0.12 ^{Ab}	79.7 ± 0.06 ^{Bb}	74.3 ± 0.13 ^{Cc}
	15	112 ± 0.52 ^{Ae}	89.3 ± 0.41 ^{Be}	69.2 ± 0.11 ^{Cg}	77.4 ± 0.35 ^{Ade}	77.1 ± 0.21 ^{Abc}	74.6 ± 0.08 ^{Bb}

M-, without microorganism; M+, with microorganism (*P. ostreatus* and *S. chartreusis*). Within each column the means sharing similar lowercase letters do not have significant differences among treatments at 5% level according to the LSD test. Within each row the means sharing similar uppercase letters do not have significant differences at 5% level between different sampling times at 5% level according to the LSD test. Values are mean (n = 3) with standard error (SE) of the mean

as compared to the control soil (soil non-inoculated and treated with wheat residues) (Table 2).

Microbial metabolic coefficient (*q*CO₂) was significantly (*p* < 0.001) influenced by salinity, microorganisms, wheat residues and their interactions. Microbial metabolic coefficient levels were decreased in most treatments during the incubation time. Salinity

increased soil *q*CO₂ during the first month of incubation, as compared to the control soil (Table 2). While *q*CO₂ was decreased in the second and third months with the increase of salinity levels (Table 2). Wheat residues reduced *q*CO₂ in most treatments. Correlation analysis also showed that *q*CO₂ was

Table 3 Pearson correlation coefficients (r) between soil properties across treatments averaged over incubation time ($n = 72$)

Property	EC	PH	MBC	MBP	MRR	URE	CAT	DOC	SOC	TN	K _{ava}	P _{ava}	qCO ₂
EC	1												
PH	0.48*	1											
MBC	-0.49*	-0.94**	1										
MBP	-0.44*	-0.95**	0.98**	1									
MRR	-0.52*	-0.89**	0.99**	0.96**	1								
URE	-0.4	-0.96**	0.98**	0.97**	0.94**	1							
CAT	-0.4	-0.91**	0.96**	0.99**	0.96**	0.95**	1						
DOC	-0.47*	-0.93**	0.89**	0.90**	0.84**	0.93**	0.87**	1					
SOC	0.1	-0.78**	0.76**	0.79**	0.70**	0.85**	0.80**	0.83**	1				
TN	-0.6**	-0.98**	0.95**	0.95**	0.92**	0.95**	0.90**	0.92**	0.70**	1			
K _{ava}	-0.3	-0.93**	0.95**	0.96**	0.92**	0.98**	0.95**	0.91**	0.90**	0.90**	1		
P _{ava}	-0.46*	-0.99**	0.96**	0.96**	0.90**	0.97**	0.92**	0.94**	0.78**	0.98**	0.95**	1	
qCO ₂	0.15 ^{ns}	0.62**	0.45*	0.53**	0.33 ^{ns}	0.59**	0.50*	0.67**	0.78**	0.52*	0.64**	0.62**	1

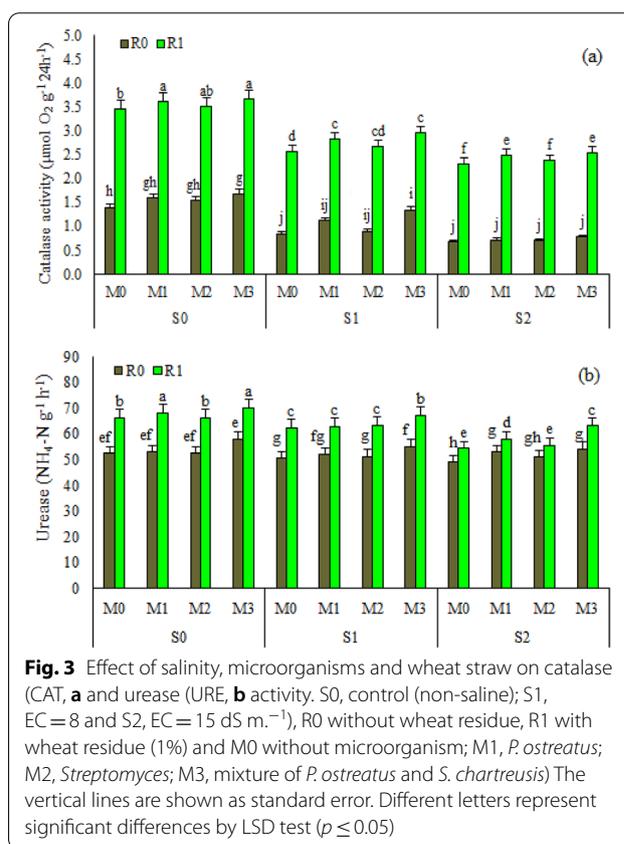
EC electrical conductivity, MBC microbial biomass carbon, MBP microbial biomass phosphorus, MRR microbial respiration rate, URE urease activity, CAT catalase activity, DOC soil dissolved carbon, SOC soil organic carbon, TN total nitrogen, K_{ava} available potassium, P_{ava} available phosphorus, qCO₂ metabolic quotient

negatively correlated with MRR and MBC ($r = -0.49$ and $r = -0.44$, respectively, $p < 0.05$; Table 3).

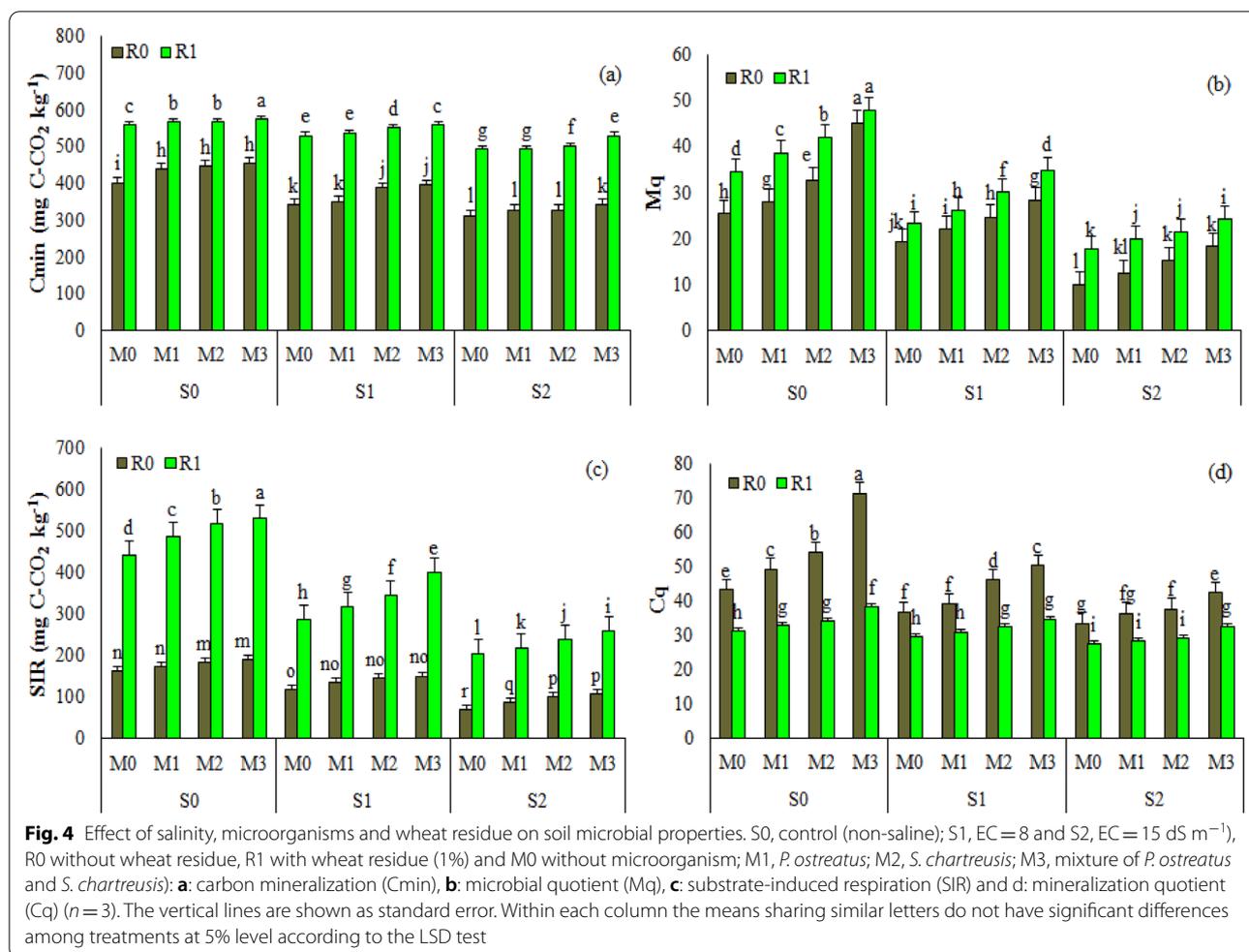
The results showed that all main and interaction effects of salinity, microorganisms and wheat residues on soil enzyme activities were significant ($p < 0.001$). Furthermore, the treatment effect with soil enzyme activities interact significantly with incubation time.

In the non-treated soil with microorganisms and wheat residues, the salinity of 8 and 15 dS m⁻¹ reduced the activity of CAT (7–9% and 10–22%, respectively) and URE (25–41% and 32–54%, respectively), during 3 months of incubation, as compared to the control soil (Fig. 3). In *S. chartreusis*-inoculated soil, salinity levels of 8 and 15 dS m⁻¹ reduced the activity of CAT (3–5% and 6–17%) and URE (22–28% and 27–50%), respectively (Fig. 3). In the soil inoculated with *P. ostreatus*, salinity levels of 8 and 15 dS m⁻¹ reduced the activity of CAT (5–7% and 9–20%) and URE (23–41% and 29–52%), respectively (Fig. 3). In addition, in the soil inoculated with microorganisms (*P. ostreatus* and *S. chartreusis*) and without wheat residues, salinity levels of 8 and 15 dS m⁻¹ reduced the activity of CAT (3–4% and 5–8%) and URE (12–15% and 15–48%), respectively, during 3 months of incubation (Fig. 3).

The simultaneous inoculation of the soil with wheat residues, *S. chartreusis* and *P. ostreatus* led to the least activity of CAT and URE reduction due to salinity stress. In the soil treated with wheat residues, the inoculation of microorganisms increased activity of CAT (29–34%) and URE (161–273%), which was greater in *S. chartreusis*-treated soil than in *P. ostreatus*-treated one (Fig. 3). Correlation analysis also showed that enzyme activities were



positively correlated with microbial activity and biomass ($r = 0.94$, $p < 0.01$; Table 3). In all treatments, wheat residues increased soil activity of CAT (10–27%) and URE (114–245%) (Fig. 3).



A general view of the changes in some microbial and biochemical properties of soil is shown in Fig. 4. All measured characteristics were decreased with the increase of soil salinity levels in the range of 15–60%, as compared to the control soil (Fig. 4). In addition, these characteristics gave a positive response to the residues of wheat inoculated with microorganisms, resulting in a 7–30% increase, as compared to the control soil (Fig. 4).

Substrate-induced respiration (SIR) and carbon mineralization (Cmin) in the soils treated with wheat residues were higher than soils non-treated with wheat residues (Fig. 4a and c). Salinity decreased SIR, Cmin, Cq (mineralization coefficient) and Mq (microbial coefficient) in the soil (Fig. 4a–d). The highest and lowest values of Cq (Fig. 4d) and Mq (Fig. 4b) were in control soil and salinity level of 15 dS m⁻¹, respectively. The values of Cq in the non-treated soil with wheat residue were higher than those in the soil treated with wheat residues (Fig. 4d). In addition, Cq changes were much higher than Mq changes in the saline soil treated with wheat residues (Fig. 4b and

d). In this experiment, respiration and microbial biomass C had a significant positive correlation with SOC and DOC ($r=0.85$, $p<0.01$; Table 3), thus indicating the greater availability of substrate in wheat residues for microbial activity. In addition, the parameters measured in this experiment showed that *S. chartreusis* had a greater effect on increasing saline soil microbial biomass (Fig. 1a and c) and respiration (Table 2) than *P. ostreatus*.

Discussion

Soil biochemical properties in saline soil treated with wheat residues and microbial consortium

Microorganisms that have the ability to dissolve insoluble phosphate and mineralize organic phosphates can increase the microbial biomass phosphorus [33]. The results of this study showed that inoculation of microorganisms and increased carbon input through wheat straw enhanced soil MBP. Changes in the content of MBP in the soil depend on seasonal changes, diversity of plant species, phosphate fertilizers and increased content of

biomass C [6]. Several studies showed that crop straw provides substrate and energy for soil microorganisms. It also creates a good environment for soil microbes, promoting their growth and reproduction [27].

In this study, salinity reduced MRR (Table 2), substrate respiration (Fig. 4) and microbial biomass (MBC, MBP; Fig. 1a and c) of soil and this decrease was greater in saline soil (15 dS m^{-1}) untreated with wheat residue, which is consistent with previous studies [29]. The microbial strains inoculated wheat straw reduced negative effect of salinity on respiration (Table 2) and microbial biomass (Fig. 1a and c) in all treatments during the 3-month incubation period. This can be attributed to the production of specific compounds and metabolites as well as the improvement of the soil fertility to the less decrease of microbial biomass in bacterial and fungal treatments under stress condition [16]. It seems that increased soil microbial biomass and their activity in soil treated with wheat residues could be due to the availability of easily degradable materials that provided nutrients and a suitable substrate for microorganisms [10, 13].

Enzymatic activity in saline soils treated with wheat residues enriched with microbial strains

In general, the results of this study showed that salinity significantly reduced enzymatic activity. Microorganisms are one of the major sources of enzymes in soil [2] and enzymatic activities are correlated with microbial biomass [34]. It is assumed that the performance of enzymes activity can be attributed to reducing soil microorganisms under salinity stress. However, it should be considered that all enzyme activity is not related to microbial activity. It has been widely suggested that high osmotic pressure due to the presence of soluble salts is one of the most important factors reduced soil microorganisms in saline soil [46]. The lowest and highest levels of the activity of enzymes were in saline soil ($>15 \text{ dS m}^{-1}$) and non-saline soil treated with wheat residues, respectively (Fig. 3). In addition, the enzymatic activity of *S. chartreusis*-inoculated treatments in saline soil was higher than that of *P. ostreatus*-inoculated treatments (Fig. 3). This may be due to the greater sensitivity of fungi to salinity and reduction of their activity in saline soils [30]. Our result is similar to previous studies that demonstrated plant residue increased URE and CAT activity and salinity stress under laboratory conditions declines enzyme activity [28, 29].

Microbial strains enriched wheat residues effects on soil organic carbon and nutrient mobilization

Plant straw return to soil is a suitable management strategy to improve soil organic carbon and increases soil microbial biomass and activities in agricultural

ecosystems [7]. Based on the results of this study, wheat residues increased organic carbon (Fig. 1b) and soluble carbon (Table 1) in the soil and wheat straw inoculated with microorganisms provided a further increase in the soil DOC (Table 1). Soil microbes play an important role in the conversion of straw organic carbon to soil organic carbon [1]. Straw incorporation increases DOC and MBC as reactive pools in soil organic matter [11]. Wang et al. [44] confirmed that microbial biomass C was a dominant factor in soil amended with crop straw involved in the SOC change. This result is similar to the previous studies, showing that plant residues increase the soil DOC [35]. The results of this study showed that soil salinity had a significant positive effect on the SOC (Fig. 1b) and negative effect on the DOC (Table 1). In addition, microorganisms reduced soil SOC, which was greater in *S. chartreusis*-treated saline soils than *P. ostreatus* treated saline soils (Fig. 1b). Soil salinity greatly influences soil organic carbon stocks [45]. Previous studies have also shown that soil organic carbon mineralization is affected by many factors including soil salinity and organic carbon content [17]. In this study, the highest amount of DOC and SOC was in the soil treated with wheat residues. DOC can be produced by microbes using SOC as a carbon source during incubation [43].

A significant negative correlation was also found among ECe and soil P_{ava} , K_{ava} and TN in most treatments (Table 3). Correlation analysis also showed that soil P_{ava} , K_{ava} and TN were positively correlated with DOC and SOC (Table 3). Tan and Thanh [38] reported a decrease in P_{ava} in soils with more than 12% salt concentration. It seems that the increased salinity stress effect on P_{ava} is dependent on reducing microbial community and secretion of extracellular enzymes, such as alkaline phosphatase [32]. Our results showed that the use of wheat residues positively improved soil P_{ava} , K_{ava} and TN. This result is consistent with the results reported by Sukitprapanon et al. [37]. The highest amount of P_{ava} is related to the second incubation month, which is probably due to the decrease in MBP in the second incubation month and the increase in phosphorus mineralization in the soil (Fig. 1c and d). TN and K_{ava} content in saline soil decreased significantly compared to control soil (Table 1). However, during 3 months of incubation, the amount of TN increased significantly. This result shows that despite the salinity, due to the adaptation of microorganisms to the stress, microbiological activity in the mineralization process of nitrogen still occurs over time, thus promoting the mineralization of nitrogen [38]. The use of organic residues has environmental benefits, such as accumulation of soil organic carbon and reduction of greenhouse gases and availability of plant nutrients in soil [37].

Conclusions

Based on the results of this study, the application of wheat residue with or without microorganisms promoted soil nutrients and soil microbial properties. Wheat residues had almost the same effect on the activity of *S. chartreusis* and *P. ostreatus*. In addition, the inoculated microorganisms had a large effect on the amount of TN, K_{ava} , P_{ava} , SOC, DOC, MRR, MBC and MBP in the soil, and this effect was greater in *S. chartreusis* treatment than in *P. ostreatus*. The results also showed that increased salinity in the soil decreased nutrients, microbial biomass, microbial activity and enzymatic activity. The effects of salinity on these properties were decreased when wheat residues created a suitable substrate and provided nutrients for microorganisms. Statistical analysis showed that better microbial and biochemical performance of soil is associated with the use of residues of wheat inoculated with microorganisms. In general, the wheat residue enriched with bacterial and fungal strain could alleviate the negative effect of salinity on the activity and biomass of microbial community and enzyme activity in the saline soils. The microbial biomass was positively correlated with soil nutrients and soil enzyme activities. Therefore, it can be concluded that the combined application of wheat residues and microorganisms might be an effective and eco-friendly option to ameliorate soil salinity in agricultural soils.

Supplementary Information

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Additional file 1. Wheat straw microbial enrichment to enhance soil properties.

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Author contributions

E.L., investigation—writing original draft; S.A.M., methodology; H.E., writing, analysis; R.G.N., review, editing, interpretation, supervision. All authors read and approved the final manuscript.

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Data availability

The data sets in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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