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Research Article

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Carbon, nitrogen, phosphorus and microbial parameter changes in response to different frequencies of salinity

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ABSTRACT

In arid and semiarid regions, increasing the rate of soil salinization is unavoidable over the coming years, which impairs the functioning of soil ecosystems. The aim of this study was to evaluate the impact of different dimensions of salinity disturbance on soil chemical and microbial parameters as proxies of soil functioning. Soil used in the experiment was collected from an agricultural field at Meet Al Mokhles village, Zefta center, Al-Gharbia governorate, Egypt. Pot experiments were prepared and left for four months to permit soil stabilization. Six soil salinity levels were assessed, (0.5, 1, 3, 5, 10 and 15%) using NaCl, comparing to control (distilled water) at different frequencies of addition at days 1, 40 and 80, under the greenhouse conditions. Soil samples were collected and analyzed from each pot on days 40, 80 and 120. The obtained results revealed that increasing disturbance intensity directly increased soil electrical conductivity, but decreased pH and available phosphorus. Organic matter content decreased at high level of salinity Total N% showed significant fluctuations between different treatments in the long term under high salinity stress. The frequency of addition did not show a direct influence on pH, total N, available P and organic matter content. Soil basal respiration and microbial biomass showed significant variation in response to different concentrations and frequencies of salinity. Overall, we conclude that while soil microbial parameters are resilient to different aspects of salinity disturbance, soil chemical environment may change at high intensities in the long term, which effectively makes soil an unfit medium for plant growth.

Introduction

Climate change strikes ecosystems through deviations from average conditions, together with supplementary changes such as increased carbon dioxide concentrations in the atmosphere, and consequently elevated temperatures **(Malhi *et al*., 2020).** Global climate change accelerates soil salinization through different ways. Rising mean temperature will indirectly increase soil salinity **(Szabolcs, 1990; Várallyay, 1994)**. Melting of glaciers and ice caps would be enhanced, resulting in sea-level rise **(Pereira *et al*., 2015)**, that increases the possibility of saltwater intrusion into agricultural soils **(Szabolcs, 1990; Várallyay, 1994; Dasgupta *et al*., 2015; El-Marsafawy *et al*., 2019**). Soil salinization is one of the most ecological hazards facing agricultural productivity, particularly in arid and semi-arid regions **(FAO, 2011)**, and a main cause of soil degradation **(Pereira *et al*., 2019)**. It is a major challenge that obstructs global food production and poses the danger of expansion to areas that are unaffected now as a consequence of climate change **(Chele *et al*., 2021; Mukhopadhyay *et al*., 2021)**.

Disturbances driven by climate changes have showed unprecedented increase, which cause a "chronic state of disequilibrium" in ecological systems **(McDowell *et al*., 2018).** Understanding how ecosystems respond to such disturbances is critical in appreciating and predicting present-day and future ecosystem dynamics **(Hillebrand *et al*., 2018)**. However, disturbances are multidimensional and have four key properties; magnitude or intensity, duration, frequency and variation **(Battisti *et al*., 2016; Donohue *et al*., 2016)**. Magnitude encompasses both absolute and relative intensities **(Battisti *et al*., 2016)**. Absolute intensity denotes the quantitative measure of the disturbance event, whereas relative intensity, also referred to as severity, represents the impact of the event on the target environmental components **(Turner, 2010; Battisti *et al*., 2016)**. According to duration, disturbances are divided into pulse and press events, where pulse disturbances occur one time and press disturbances continuously occur **(Krebs, 1989)**. From another perspective, **Ives and Carpenter (2007)** illustrated that disturbances may occur in the form of frequent and stochastic or rare shocks "pulses" or may cause constant changes in the ecological system "presses". Pulse disturbances are therefore short and sharp shocks, while press disturbances represent a constant, long-term change **(Donohue *et al*., 2016; Radchuk *et al*., 2019)**. Frequency refers to the number of times a disturbance event occurs within a predefined time period **(Turner, 2010; Battisti *et al*., 2016)**. Variation represents how disturbances change over spatiotemporal scale **(Donohue *et al*., 2016)**. Multiple aspects of disturbance must be taken into consideration to precisely expect the impact of nearby changes on ecological systems **(Kéfi *et al*., 2019)**.

Quality of soil is evaluated from analysis of its physicochemical and biological components, more specifically microbial parameters, which are key factors for soil functioning **(Schoenholtz *et al*., 2000; Janvier *et al*., 2007; Muñoz-Rojas *et al*., 2016; Maurya *et al*., 2020; Gao *et al*., 2022)**. Several studies have focused on salinization impact on soil physicochemical properties; some of which have focused on a soil suffering from high salt concentrations for many years **(Allotey *et al*., 2008; Kumar *et al*., 2018; Meena *et al*., 2022)**, whereas other studies have focused on impact of saline water irrigation **(Tedeschi and Dell'Aquila, 2005; Huang *et al*., 2011; Cucci *et al*., 2013; Wei *et al*., 2019)**. In addition, few studies have focused on the impact of salinity stress on microbial parameters **(Wong *et al*., 2008; Cao *et al*., 2021)**. However, the response of soil components to multiple aspects of salinity disturbance and expectation of soil functioning under salinity stress as a factor of climate change, to our knowledge, is greatly underestimated. So, the aim of this study was to determine the impact of different levels of salinity on soil chemical and microbial parameters, in addition, to evaluating the impact of repeated salinity stress on soil in a semi-arid environment.

Materials and methods

A pot experiment was conducted for 120 days in greenhouse conditions, at the Faculty of Science, Tanta University to investigate the impact of various soil salinity levels and frequencies of addition on certain soil chemical and microbial parameters properties.

Sampling site

Soil samples used in this experiment were collected from an agricultural field, cultivated with maize, at Meet Al Mokhles village, Zefta center, Al-Gharbia Governorate, Egypt (30°47′37″N 31°09′59″E). The soil of the field could be described as silty clay loam texture (33% clay, 53% silt and 15% sand). The chemical and microbial parameters of the used soil were showed in Table (1).

Table (1): Chemical and microbial parameters of soil used in the experiment.

Chemical parameters	Mean ± SD	Microbial Parameters	Mean ± SD
pH	7.6 ± 0.2	**Soil basal respiration (mg CO_2.g^{-1}.h^{-1})**	3.58 ± 0.23
EC (mS/cm)	0.69 ± 0.08	**Microbial biomass-C (mg/Kg)**	419.37 ± 19.52
OM%	4.12 ± 0.1		
Total N%	0.12 ± 0.01		
Available phosphorus (ppm)	27.77 ± 2.36		

Soil collection and preparation

After the collection of soil samples, plant debris and large roots were discarded, large aggregates were gently crushed by hand, and the soil was mixed thoroughly in order to reduce spatial heterogeneity. Each pot was filled with about 300 g of soil and pre-incubated for 4 months, in order to mimic field conditions, reduce the initial disturbance and allow their stabilization **(Pereira *et al*., 2019 and Edwards, 2002)**. Pots were moistened day by day with distilled water in order to obtain soil moisture of nearly 50%.

The desired soil salinity levels were artificially obtained by dissolving the calculated amounts of NaCl in distilled water, according to the preliminary experiment, the chosen concentrations represent 6 different concentrations with three salinity levels; low (0.5% and 1% NaCl), intermediate (3% and 5% NaCl) and high (10% and 15% NaCl), in addition to control (distilled water).

Experimental design

Pots were divided into seven treatments (Control, 0.5, 1, 3, 5, 10 and 15% NaCl). Treatments with NaCl were further subdivided into 3 sets according to the frequency of NaCl addition. Set 1, received NaCl once at the start of the experiment (day 1) (one frequency of addition). Set 2, received NaCl at days 1 and 40 (two frequencies of addition). Set 3, received NaCl at days 1, 40 and 80 (three frequencies of addition). Each treatment with 5 replicates (with total replicates of 220 pots). All pots were moistened with distilled water in order to obtain soil moisture of nearly 50% during the whole period of the experiment. Randomized complete block design was used in this study. Soil samples were collected from experimental pots in different sets at days 40, 80 and 120.

Soil chemical analysis

Hydrogen ion concentration (pH) and soil electrical conductivity (EC) were determined in 1:5 soil/water extract according to **Jackson (1973)**. Organic matter (OM%) was determined using a muffle furnace at 550°C for 4 hours. The loss in soil weight represents the organic matter content which was expressed as percentage of the original dry weight of the soil sample **(Margesin and Schinner, 2005)**. 50% of organic matter content was considered to be organic carbon (OC) **(Pribyl, 2010)**. Total nitrogen was determined by Kjeldahl method **(Stevenson, 1982)** after digestion of soil samples with nitric acid and hydrogen peroxide mixture. C/N ratio was computed from total N% and organic C%. Soil available phosphorus was determined using the $NaHCO_3$ solution colorimetry method **(Watanabe and Olsen, 1965)**.

Microbial parameters measurement

Soil basal respiration was determined by measuring CO_2 released by titration in a static system according to **BS EN ISO 16072 (2011)**, whereas microbial biomass-C in soil was determined using substrate-induced respiration (SIR) method according to **BS EN ISO 14240-1 (2011)**.

Statistical analysis

Data was presented as mean ± SD. In set one experiment, in order to understand the effect of one frequency of salinity, (7 levels), and time (3 levels) on soil chemical and microbial parameters, a two-way mixed ANOVA was run. Two-way ANOVA was conducted to understand the effect salinity (7 levels), and frequency of addition (2 and 3 levels) on measured parameters at days 80 and 120 respectively. In all tests, if there is a significant difference between means was detected, Dunnett and Tukey's method for multiple comparisons were used to detect all pairwise differences between different treatments. Principal component analysis (PCA) was done to reveal the relationships between microbial parameters with chemical parameters in soil and implemented disturbance in an ordination plot, using PAST, V4.08 **(Hammer *et al.*, 2001)**. Pearson's correlation analysis was performed to assess the correlation between soil parameters. All data were analyzed using IBM SPSS Statistics for Windows, Version 25, Minitab software package version 19.0 and Microsoft Excel 365. For all statistical tests p-value < 0.05 is considered significant. Treatments that are statistically different from control are marked with *. In addition, means that do not share a letter are significantly different.

Results

Soil chemical analysis

Soil pH values showed significant decreases toward high salinity treatments. pH varied from slightly alkaline at control treatments (7.37 to 7.5) to neutral in case of 15% treatments (6.87 to 7.03) (Tables 2, 3 and 4) and (Fig. 1). Frequency did not have any significant impact on lowering pH values (Table 4) and (Fig. 1).

Soil electrical conductivity (EC) significantly increased across the salinity gradient; from 0.69 to 0.82 mS/cm in control to 8.1 to 8.8 mS/cm in 15% treatment (Table 2). Moreover, increasing frequencies of addition significantly increased EC at different days of analysis (Tables 3 and 4). For instance; at day 120, EC in case of 15% treatment increased from 8.42 mS/cm to 16.32 mS/cm, then exceeded 20 mS/cm with increasing frequency of addition (Fig. 1).

Organic matter content showed significant variation with time and between different treatments (Tables 2, 3 and 4). In most treatments, OM contents showed significant increases at day 80 (Table 3).

Total N content significantly increased from day 40 to day 80 in all treatments. At day 120, total N contents decreased again at all treatments except 5% and 10% treatments (Fig. 1). However, total N contents showed significant fluctuations between different treatments (Tables 2, 3 and 4). No correlation was detected between increasing EC values and organic matter % in addition to total N% (r = 0.073, p = 0.754 and r = 0.002, p = 0.994, respectively). On contrary, soil available phosphorus showed significant decreases from day 40 to day 80 in all treatments. However, at day 120 the available P contents were significantly increased except in the case of 15% treatment (Tables 2, 3 and 4) and (Fig. 1). A negative correlation was detected between EC soil available phosphorus concentrations (r = -0.503, p= 0.02).

Table (2): Averages of pH, electrical conductivity (EC), organic matter content (OM%), total nitrogen (N%), C/N ratio and available phosphorous (P) for soil different salinity levels after 40 days of exposure. Data expressed as (Mean ± SD)

Treatment	pH	EC (mS/cm)	OM (%)	Total N (%)	C/N	Available P (ppm)
Distilled H_2O	7.5 ± 0.0	0.69 ± 0.06	3.32 ± 0.72	0.07 ± 0.01	23.71	32.14 ± 1.24
0.5% NaCl	7.45 ± 0.07	1.03 ± 0.07	3.14 ± 0.09	0.09 ± 0.0*	17.44	27.19 ± 2.11*
1% NaCl	7.2 ± 0.0*	1.28 ± 0.07	2.95 ± 0.15	0.07 ± 0.0	21.07	23.75 ± 1.19*
3% NaCl	7.35 ± 0.07	2.38 ± 0.28*	3.79 ± 0.17	0.06 ± 0.0	31.58	28.23 ± 1.03*
5% NaCl	7.1 ± 0.1*	4.18 ± 0.13*	2.6 ± 0.23	0.06 ± 0.0	21.67	27.52 ± 0.99*
10% NaCl	6.9 ± 0.2*	5.86 ± 0.03*	2.79 ± 0.03	0.05 ± 0.0*	27.90	29.64 ± 1.07
15% NaCl	7.03 ± 0.21*	8.84 ± 0.53*	2.68 ± 0.11	0.06 ± 0.01	22.33	28.23± 2.14*
F-value	10.94	244.86	5.69	15.29		8.95
p-value	<0.001	<0.001	0.004	<0.001		<0.001

* Significant different from control (Dist. H_2O) $P < 0.05$

Table (3): Averages of pH, electrical conductivity (EC), organic matter content (OM%), total nitrogen (N%), C/N ratio and available phosphorous (p) for soil exposed to different salinity levels after 80 days of exposure. Data expressed as (Mean ± SD)

Treatment		pH	EC (ms/cm)	OM (%)	Total N%	C/N	Available P (ppm)
Distilled H_2O		7.37 ± 0.12	0.76 ± 0.06	3.96 ± 0.34	0.10 ± 0.01	19.80	26.86 ± 0.09
0.5% NaCl	**F1**	7.27 ± 0.06^{a}	0.96 ± 0.06^{b}	4.02 ± 0.16	0.09 ± 0.00^{a}	22.33	26.44 ± 0.51^{a}
	F2	7.25 ± 0.07^{a}	$1.71 \pm 0.16^{*a}$	3.9 ± 0.09	0.1 ± 0.02^{a}	19.50	$22.24 \pm 0.52^{*b}$
1% NaCl	**F1**	$7.03 \pm 0.07^{*b}$	$1.36 \pm 0.12^{*b}$	$2.7 \pm 0.04^{*}$	0.13 ± 0.01^{a}	10.38	$23.28 \pm 1.04^{*b}$
	F2	7.35 ± 0.21^{a}	$2.06 \pm 0.16^{*a}$	3.65 ± 0.85	0.07 ± 0.01^{b}	26.07	28.02 ± 0.27^{a}
3% NaCl	**F1**	7.02 ± 0.12^{a}	$2.3 \pm 0.17^{*b}$	$2.6 \pm 0.24^{*}$	$0.22 \pm 0.02^{*a}$	5.91	26.08 ± 0.21^{a}
	F2	7.17 ± 0.12^{a}	$5.01 \pm 0.07^{*a}$	$2.95 \pm 0.03^{*}$	$0.06 \pm 0.01^{*b}$	24.58	$24.8\ 8 \pm 1.13^{a}$
5% NaCl	**F1**	$6.97 \pm 0.06^{*a}$	$4.22 \pm 0.08^{*b}$	3.94 ± 0.64	0.11 ± 0.01^{a}	17.91	$21.39 \pm 0.09^{*a}$
	F2	$7.07 \pm 0.06^{*a}$	$5.19 \pm 0.15^{*a}$	3.91 ± 0.24	0.11 ± 0.01^{a}	17.77	$20.82 \pm 1.3^{*a}$
10% NaCl	**F1**	$6.93 \pm 0.06^{*b}$	$5.42 \pm 0.30^{*b}$	4.16 ± 0.21	0.09 ± 0.00^{a}	23.11	24.29 ± 3.12^{a}
	F2	7.2 ± 0.00^{a}	$11.24 \pm 0.17^{*a}$	4.21 ± 0.24	0.09 ± 0.02^{a}	23.39	25.12 ± 2.24^{a}
15% NaCl	**F1**	$6.93 \pm 0.06^{*a}$	$8.1 \pm 0.16^{*b}$	4.11 ± 0.29	0.13 ± 0.02^{a}	15.81	$15.85 \pm 0.83^{*a}$
	F2	$6.9 \pm 0.14^{*a}$	$15.27 \pm 0.67^{*a}$	$2.83 \pm 0.10^{*}$	0.08 ± 0.01^{b}	17.69	$13.51 \pm 2.11^{*a}$

Means within each concentration that do not share the same letter are significantly different (Tukey's test, $p < 0.05$)

Table (4): Averages of pH, electrical conductivity (EC), organic matter content (OM), total nitrogen (N), C/N ratio and available phosphorous (p) for soil exposed to different salinity levels after 120 days of exposure. Data expressed as (Mean ± SD)

Treatment		pH	EC (ms/cm)	OM (%)	Total N%	C/N	Available P (ppm)
Distilled H_2O		**7.5 ± 0.10**	**0.82 ± 0.13**	**3.19 ± 0.61**	**0.09 ± 0.01**	**17.72**	**39.79 ± 2.16**
0.5% NaCl	**F1**	7.27 ± 0.12	1.13 ± 0.12^{a}	3.92 ± 0.13^{a}	0.07 ± 0.00^{b}	28.00	$30.42 \pm 3.66^{*b}$
	F2	7.33 ± 0.12	1.46 ± 0.05^{a}	3.90 ± 0.14^{a}	$0.12 \pm 0.01^{*a}$	16.25	38.09 ± 2.24^{a}
	F3	7.33 ± 0.06	$1.62 \pm 0.24^{*a}$	3.81 ± 0.41^{a}	$0.06 \pm 0.00^{*b}$	31.75	37.90 ± 1.05^{a}
1% NaCl	**F1**	7.25 ± 0.21	$1.68 \pm 0.27^{*a}$	3.21 ± 0.66^{a}	$0.08 \pm 0.00^{*c}$	20.06	$31.00 \pm 2.28^{*a}$
	F2	$7.23 \pm 0.15^{*}$	$1.93 \pm 0.11^{*a}$	3.12 ± 0.45^{a}	0.12 ± 0.00^{b}	13.00	37.57 ± 3.09^{a}
	F3	$7.23 \pm 0.12^{*}$	$2.37 \pm 0.34^{*a}$	3.29 ± 0.86^{a}	0.20 ± 0.02^{a}	8.23	$30.87 \pm 4.14^{*a}$
3% NaCl	**F1**	$7.10 \pm 0.06^{*}$	$2.16 \pm 0.08^{*b}$	3.81 ± 0.12^{a}	0.10 ± 0.01^{a}	19.05	39.36 ± 1.27^{a}
	F2	$7.10 \pm 0.00^{*}$	$4.92 \pm 0.11^{*a}$	3.07 ± 1.17^{a}	0.11 ± 0.00^{a}	13.95	$25.63 \pm 1.42^{*b}$
	F3	$7.07 \pm 0.06^{*}$	$5.50 \pm 0.38^{*a}$	3.56 ± 0.84^{a}	0.085 ± 0.01^{a}	20.94	$21.32 \pm 0.09^{*b}$
5% NaCl	**F1**	$7.23 \pm 0.06^{*}$	$4.12 \pm 0.13^{*c}$	3.90 ± 0.28^{a}	$0.13 \pm 0.02^{*a}$	15.00	35.07 ± 1.11^{a}
	F2	$7.00 \pm 0.00^{*}$	$5.00 \pm 0.17^{*b}$	$3.59 \pm 0.76^{a,b}$	$0.12 \pm 0.00^{*a}$	14.96	$20.3 \pm 0.87^{*b}$
	F3	$6.93 \pm 0.12^{*}$	$8.83 \pm 0.38^{*a}$	2.56 ± 0.22^{b}	0.08 ± 0.00^{b}	16.00	$19.08 \pm 0.50^{*b}$
10% NaCl	**F1**	$6.93 \pm 0.12^{*}$	$5.20 \pm 0.21^{*c}$	4.12 ± 0.28^{a}	$0.13 \pm 0.03^{*a}$	15.85	$30.35 \pm 2.18^{*a}$
	F2	$6.93 \pm 0.06^{*}$	$10.90 \pm 0.32^{*b}$	$3.64 \pm 0.77^{a,b}$	$0.12 \pm 0.01^{*a}$	15.17	$27.76 \pm 1.25^{*a}$
	F3	$6.90 \pm 0.00^{*}$	$16.10 \pm 0.65^{*a}$	2.55 ± 0.11^{b}	0.10 ± 0.01^{a}	12.75	$18.54 \pm 1.13^{*b}$
15% NaCl	**F1**	$6.87 \pm 0.12^{*}$	$8.40 \pm 0.36^{*c}$	3.70 ± 0.19^{a}	0.08 ± 0.00^{a}	23.13	$13.44 \pm 1.63^{*b}$
	F2	$6.87 \pm 0.06^{*}$	$16.30 \pm 0.10^{*b}$	3.81 ± 0.01^{a}	0.09 ± 0.01^{a}	21.17	$23.61 \pm 3.49^{*a}$
	F3	$6.87 \pm 0.06^{*}$	$22.71 \pm 0.21^{*a}$	2.93 ± 0.17^{b}	0.08 ± 0.01^{a}	18.31	$18.20 \pm 1.23^{*a,b}$

Means within each concentration that do not share the same letter are significantly different (Tukey's test, $p < 0.05$)

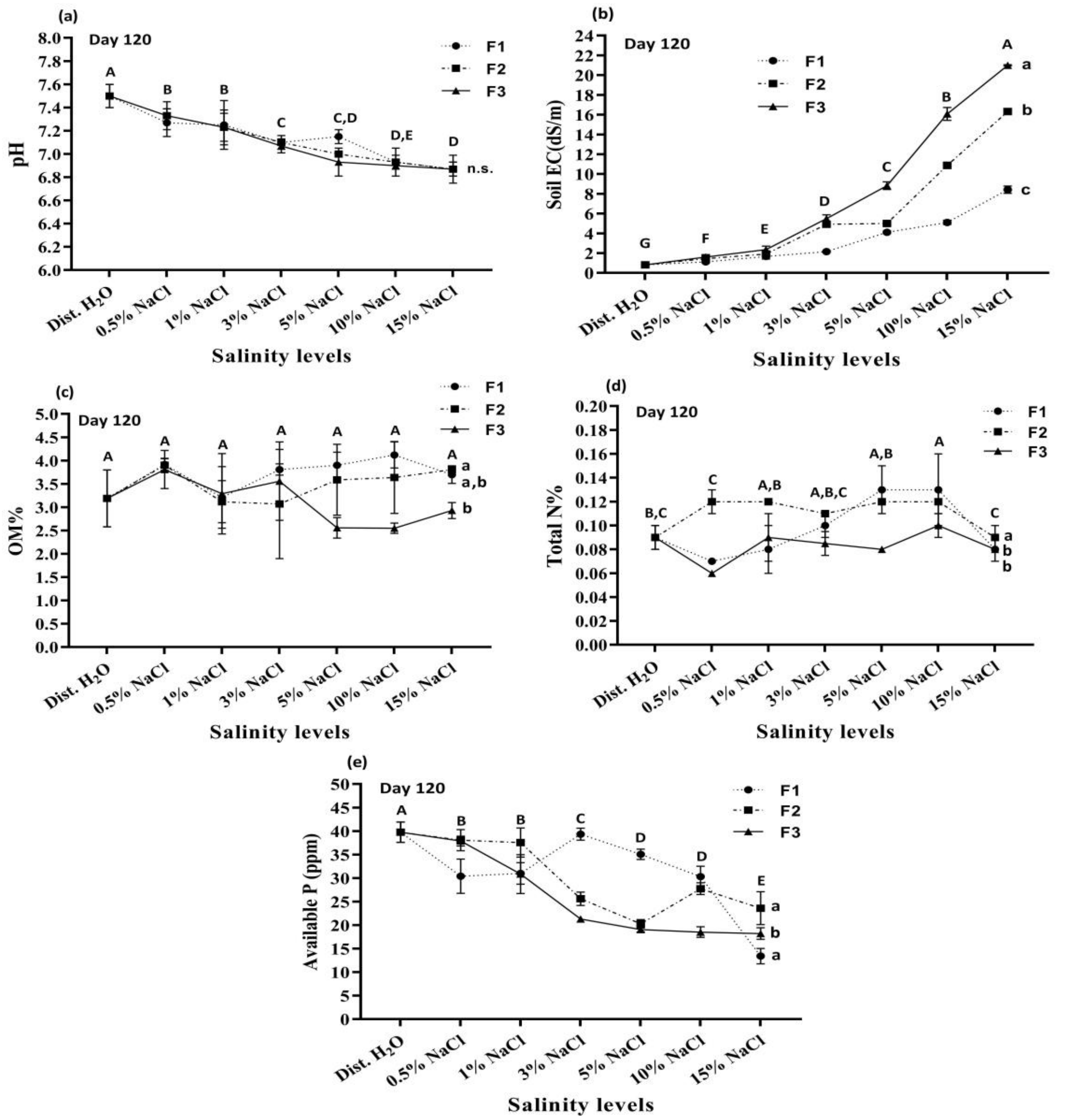

Fig. (1): Averages of pH, electrical conductivity (EC), organic matter content (OM), total nitrogen (N), C/N ratio and available phosphorous (p) for soil exposed to different salinity levels after 120 days of exposure. Data expressed as (Mean ± SD), Means that do not share the same letter are significantly different (Tukey's test, $p < 0.05$)

Microbial analysis

Soil basal respiration showed significant variation with time and across the salinity gradient (Table 5). In the majority of treatments, respiration rate significantly increased at the end of the experiment, despite decreases at day 80. The highest respiration rates were detected in the low and mid-salinity treatments at all days of analysis (Table 5). Frequency of addition significantly decreased basal respiration rates in most treatments at day 80. However, at day 120, significant increases occurred in the majority of treatments with increasing addition’s frequency (Fig. 2). Microbial biomass-C was significantly different through time and between different treatments (Table 5). The highest microbial biomasses were detected at day 40. At day 80, increasing frequency of addition significantly decreased microbial biomass in most treatments except high salinity treatments (10% and 15% treatments). At day 120, microbial biomass continued to increase in the majority of treatments in set (2), whereas significant decreases were obvious in set (3) (Table 5) and (Fig. 2). Disparities in soil microbial parameters response to different intensities at different subsets through the period of the study are illustrated in Figs. (3 and 4). Basal respirations in set (1) was positively correlated with microbial-biomass C through the period of the study (r= 0.728, p< 0.001, r= 0.720, p< 0.001 and r= 0.462, p= 0.035 at days 40, 80 and 120, respectively). However, at set (2) and set (3) no similar correlations could be detected (Figs 3 and 4).

The PCA biplot (Fig. 5) clearly showed that, the two principal components (PC1 and PC2) explained 64.14 % of the total variation in chemical and microbial parameters, and showed clear partitioning of control and low salinity level (LS) from intermediate and high salinity levels along the PC1. PC1 and PC2 strongly correlated to variables; available phosphorous, Electrical conductivity (EC), microbial biomass; respectively. Other parameters were not clearly correlated to PC1 and PC2. The data revealed a positive correlation between organic matter, pH and available P. Also, between microbial biomass and total N. A negative correlation was detected between EC, soil available phosphorus. Overall, the PCA biplot clearly indicated that salinity level variation in soil was more than frequented exposure to salinity stress.

Table (5): Averages of basal respiration and microbial biomass-C for soil exposed to different salinity levels after 40, 80 and 120 days of repeated exposure. Data expressed as (Mean ± SD)

Day	**Treatment**		**Basal respiration**	**Microbial biomass-C**
Day 40	**Distilled H_2O**		**3.20 ± 0.34**	**396.26 ± 30.14**
	0.5% NaCl		3.35 ± 0.22	406.41± 25.18
	1% NaCl		2.05 ± 0.15*	456.07 ± 27.03*
	3% NaCl		3.55 ± 0.24	453.58 ± 14.52*
	5% NaCl		1.71 ± 0.13*	253.72 ± 13.87*
	10% NaCl		2.26 ± 0.27*	210.94 ± 22.07*
	15% NaCl		0.71 ± 0.1*	195.68 ± 11.03*
ANOVA Test	F-value		64.52	86.35
	p-value		<0.001	<0.001
Day 80	**Distilled H_2O**		2.26 ± 0.11	90.92± 12.7
	0.5% NaCl	**F1**	1.17 ± 0.09^{*a}	23.85 ± 6.42^{*a}
		F2	1.38 ± 0.13^{*a}	28.02 ± 4.41^{*a}
	1% NaCl	**F1**	3.58 ± 0.21^{*a}	110.63 ± 13.25^{a}
		F2	1.53 ± 0.12^{*b}	22.85 ± 3.15^{*b}
	3% NaCl	**F1**	2.23 ± 0.14^{a}	170.57± 9.18^{*a}
		F2	0.71 ± 0.17^{*b}	34.44 ± 4.39^{*b}
	5% NaCl	**F1**	2.93 ± 0.25^{*a}	127.86 ± 16.24^{*a}
		F2	1.65 ± 0.29^{*b}	104.29 ± 11.05^{a}
	10% NaCl	**F1**	1.18 ± 0.12^{*a}	23.75 ± 2.12^{*a}
		F2	1.14 ± 0.01^{*a}	51.84 ± 7.6^{*a}
	15% NaCl	**F1**	1.65 ± 0.13^{*a}	26.73 ± 5.1^{*b}
		F2	0.08 ± 0.07^{*b}	164.18 ± 13.38^{*a}
Day 120	**Distilled H_2O**		2.15 ± 0.27	88.74 ± 5.68
	0.5 NaCl	**F1**	3.18 ± 0.15^{*a}	123.45 ± 12.25^{*b}
		F2	2.91 ± 0.14^{*a}	214 ± 15.28^{*a}
		F3	3.28 ± 0.29^{*a}	159.22 ± 17.16^{*b}

Table (5): Continue

Day	Treatment		Basal respiration	Microbial biomass-C
Day 120	1% NaCl	**F1**	3.55 ± 0.22^{*c}	114.59 ± 4.12^{*c}
		F2	6.17 ± 0.31^{*a}	220.00 ± 4.15^{*a}
		F3	5.20 ± 0.24^{*b}	138.22 ± 5.06^{*b}
	3% NaCl	**F1**	3.86 ± 0.14^{*c}	148.16 ± 5.73^{*b}
		F2	5.21 ± 0.26^{*b}	243.79 ± 6.27^{*a}
		F3	6.48 ± 0.22^{*a}	171.36 ± 7.27^{*b}
	5% NaCl	**F1**	3.13 ± 0.23^{*b}	150.0 ± 12.2^{*a,b}
		F2	4.87 ± 0.22^{*a}	178.17 ± 12.8^{*a}
		F3	5.18 ± 0.18^{*a}	122.7 ± 12.04^{*b}
	10% NaCl	**F1**	2.56 ± 0.12^{c}	157.34 ± 15.0^{*a}
		F2	4.11 ± 0.17^{*b}	153.34 ± 5.24^{*a}
		F3	4.82 ± 0.21^{*a}	94.12 ± 5.30^{b}
	15% NaCl	**F1**	2.56 ± 0.10^{c}	22.17 ± 4.17^{*b}
		F2	3.53 ± 0.15^{*b}	144.15 ± 3.61^{*a}
		F3	5.45 ± 0.13^{*a}	32.14 ± 2.12^{*b}

* Significant different from control (Dist. H_2O) $P < 0.05$. Means within each concentration that do not share the same letter are significantly different (Tukey's test, $p < 0.05$)

Table (6a): Two-way mixed ANOVA as a function of salinity , and time and two-way ANOVA as a function of salinity, and frequency for soil pH, EC and OM at days 80 and 120, respectively.

Time	Source of variation	df	pH		EC		OM%	
			F-value	P-value	F-value	P-value	F-value	P-value
Two-way mixed ANOVA	Concentration	6	39.32	<0.001	728.42	<0.001	8.18	< 0.001
	Time	2	7.57	0.002	3.83	0.34	21.37	< 0.001
	Conc.*Time	12	1.81	0.069	3.72	0.00	6.42	0.002
Day 80	Concentration	6	18.44	<0.001	1880.35	<0.001	12.83	< 0.001
	Frequency	2	10.47	0.00	1337.26	<0.001	0.01	0.91
	Conc.*Freq.	12	4.27	0.00	226.81	<0.001	5.42	< 0.001
Day 120	Concentration	6	59.83	<0.001	4100.01	<0.001	1.59	0.174
	Frequency	2	0.82	0.446	1880.53	<0.001	5.80	0.006
	Conc.*Freq.	12	0.76	0.686	357.13	<0.001	1.61	0.126

Table (6b): A Two-way mixed ANOVA as a function of salinity, and time and two-way ANOVA as a function of salinity, and frequency for soil total N%, available P, soil chemical and microbial parameters at days 80 and 120, respectively.

Time	Source of variation	df	Total nitrogen%		Available P		Basal respiration		Microbial biomass	
			F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Two-way mixed ANOVA	Concentration	6	8.33	0.001	25.17	<0.001	27.66	<0.001	60.35	<0.001
	Time	2	199.17	<0.001	377.63	<0.001	810.75	<0.001	5543.9	<0.001
	Conc.*Time	12	35.1	<0.001	72.58	<0.001	395.36	<0.001	176.47	<0.001
Day 80	Concentration	6	10.32	<0.001	59.78	<0.001	104.27	<0.001	73.51	<0.001
	Frequency	1	92.18	<0.001	0.92	0.35	334.41	<0.001	13.69	<0.001
	Conc.*Freq.	6	35.23	<0.001	6.68	<0.001	50.57	<0.001	120.48	<0.001
Day 120	Concentration	6	27.63	<0.001	104.39	<0.001	221.77	<0.001	205.96	<0.001
	Frequency	2	9.99	<0.001	30.13	<0.001	330.91	<0.001	329.93	<0.001
	Conc.*Freq.	12	25.09	<0.001	24.34	<0.001	35.49	<0.001	31.22	<0.001

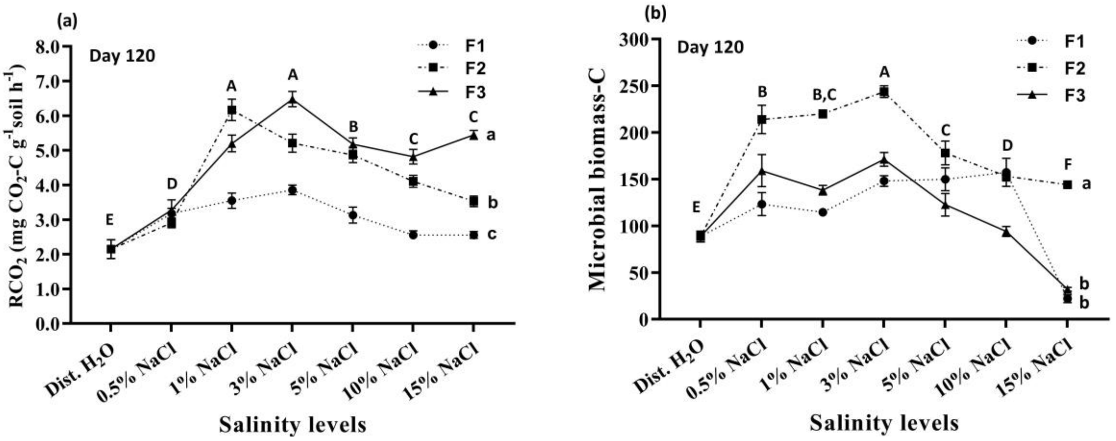

Fig. (2): Averages of soil microbial parameters (Basal respiration and Microbial biomass) for soil exposed to different salinity levels after 120 days of exposure. Data expressed as (Mean ± SD).

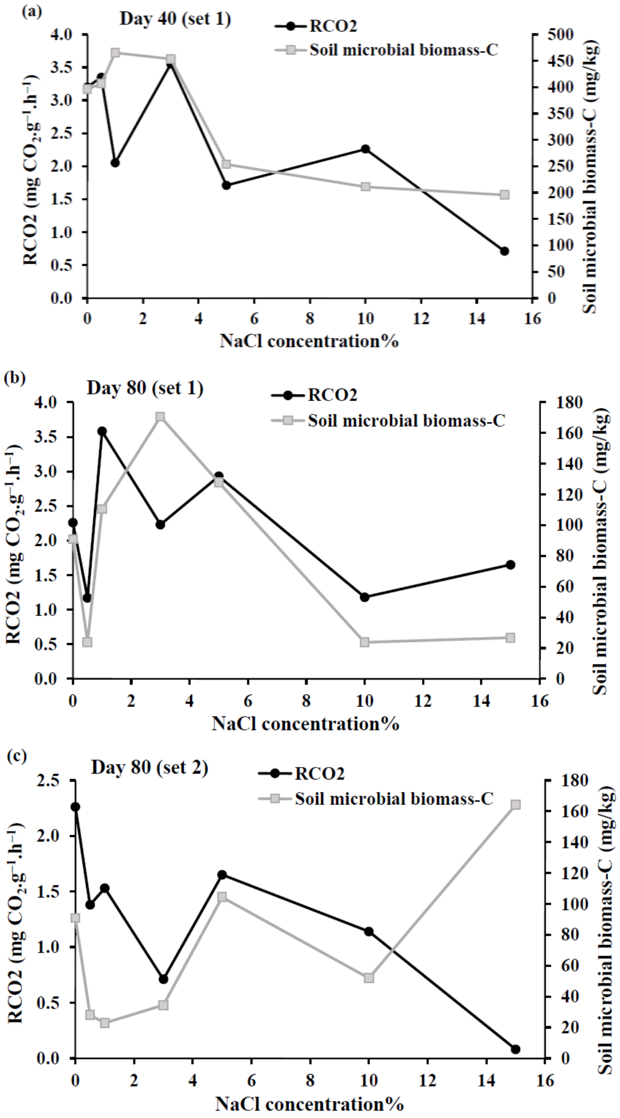

Fig. (3): Disparities in mean values of soil microbial parameters (Basal respiration and Microbial biomass) in (a) day 40 and (b, c) day 80, in response to different salinity levels in set (1) received NaCl once at the start of the experiment (day 1) (one frequency of addition) and set (2) received NaCl at days 1 and 40 (two frequencies of addition).

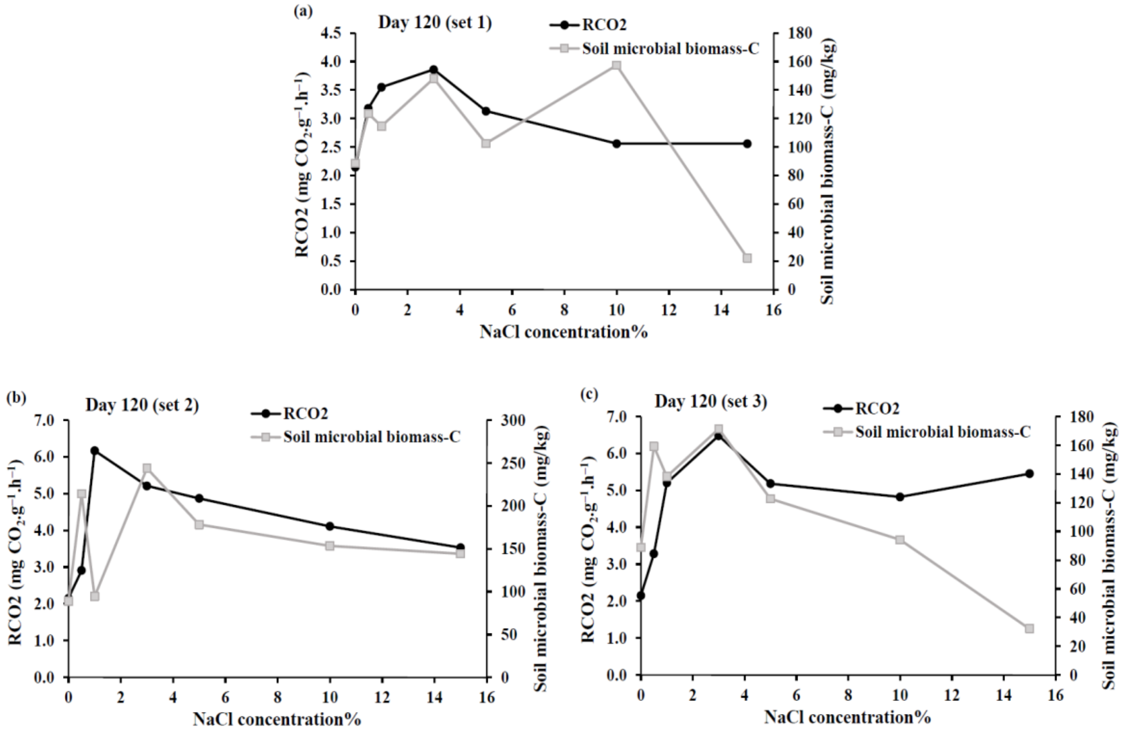

Fig. (4): Disparities in mean values of soil microbial parameters (Basal respiration and Microbial biomass) in day 120 in response to different salinity levels in (a) set 1 received NaCl once at the start of the experiment (day 1) (one frequency of addition) and (b) set 2 received NaCl at days 1 and 40 (two frequencies of addition) and (c) set 3 received NaCl at days 1, 40 and 80 (three frequencies of addition)

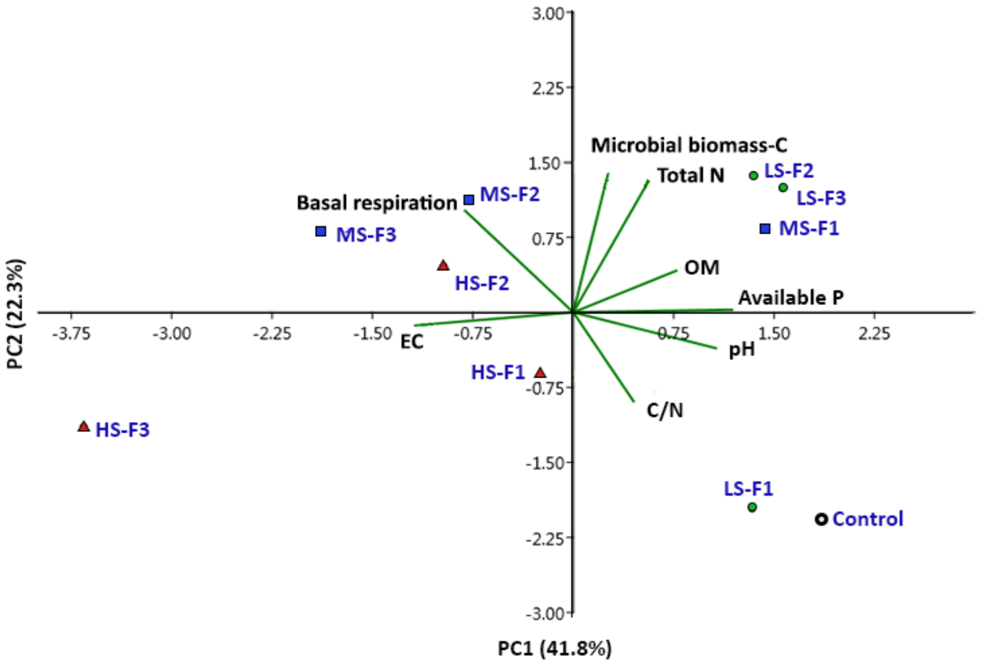

Fig. (5): Biplot of the principal component analysis (PCA) for chemical and microbial parameters of experimental soil with different levels of salinity in different sets (LS; low, SM; intermediate and HS; High salinities) and control soil with distilled H_2O. F1; one frequency of addition of NaCl, F2; two frequencies of addition of NaCl and F3; three frequencies of addition of NaCl

Discussion

Different previous studies warned from the shortage of fresh water in the nearby future and the chronic problem of soil salinization particularly in arid and semiarid areas, which may actually pose the biggest threat to human **(Letey and Feng, 2007; Dasgupta *et al.*, 2015; Zhou *et al.*, 2016).** In addition, complete understanding of how disturbance dynamics respond to climate change is lacking **(Seidl *et al.*, 2017)**. Therefore, assessment of the impact of different dimensions of salinity disturbance on the soil ecosystem in nearby future was of central priority in this experiment.

Unlike previous investigations, pH values showed a significant decline along the salinity gradient. **Mostafazadeh-Fard *et al.*, (2007)** found no correlation between increased irrigation water salinity and pH values. On contrary, **Wei *et al.*, (2019)** observed that irrigation with high-salinity brackish water was responsible for increasing soil pH because of the high availability of monovalent sodium ions in highly saline water. This discrepancy in results might arise from differences in the source from which sodium ions are generated or added to soil. Carbonate and bicarbonate are the basic hydroxyl generating anions **(Tavakkoli *et al.*, 2015; Brady and Weil, 2016)**. Consequently, the availability of high levels of salts from sources other than carbonates and bicarbonates in the soil solution, such as NaCl in the present study rather than Na_2CO_3 for example, tends to lower pH values instead of rising it because the dissolution of carbonates will be reduced **(Brady and Weil, 2016)**. The impact of salinity on soil organic matter content, total nitrogen and available phosphorus is a controversial issue. **Rietz and Haynes (2003)**, **Pan *et al.*, (2013) and Min *et al.*, (2016)** showed that increasing salinity leads to decreased plant production because of reduced water uptake under saline conditions, and consequently lower organic inputs to soil. **Pan *et al.*, (2013)** also reported significant decreases in soil total N contents with increasing salinity. On contrary, **Chen *et al.*, (2015)** indicated that soil organic matter content and total nitrogen increased due to long term irrigation with saline water. In addition, **Zhou *et al.*, (2016)** reported that increased salinity significantly increased soil total N. They assumed that soil salinization enhances plant N content and leads to increased plant litter input to soil. However, in this study, no correlation could be detected between increasing salinity, and organic matter% or total N%. Similar findings are reported by **Liang *et al.*, (2022)**. It may be suggested that, salinity disturbances with low intensity levels will not decrease organic matter and nitrogen contents. However, organic matter and nitrogen contents might increase instead because of high microbial activities at low salt concentrations. On contrary, high salt concentrations in soil decrease plant water uptake due to ionic strength. So, organic matter and nitrogen addition to soil would be declined in the long term. Regarding available phosphorus, the present results revealed that increasing salinity decreased soil available P contents. **Khoshgoftarmanesh and Nourbakhsh (2009)** found more available P in saline soil compared to non-saline soil. In contrast, **Hu and Schmidhalter (2005)** showed less available P can be found in saline soils, because of ionic-strength effects that reduce the activity of P.

Despite fluctuations observed in microbial parameters, in general, increasing disturbance intensity significantly decreased soil basal respiration, which indicates the negative impact of salinity stress on microbial activity. The present results are consistent with **Muhammad *et al*., (2006)** and **Tripathi *et al*., (2006)**, where increased salinity significantly decreased basal respiration. As salt concentration increases, Na^+ and Cl^- toxicities inhibit microbial growth **(Zahran *et al*., 1992)**. In addition, osmotic stress restricts microbial growth and activity; gradually microorganisms tend to dehydrate **(Oren, 1999; Rietz and Haynes, 2003; Wong *et al*., 2008)**. Frequent additions of saline caused significant increase in soil basal respiration at the end of the experiment even at high soil electrical conductivities. **Wong *et al*., (2008)** illustrated that high salinity disperses soil aggregates and causes hydrolysis of soil organic matter, as a consequence substrate availability increases, which counterbalances excessive stress on microbial communities exerted by high salt intensities. Regarding soil microbial biomass-C, the present results lacked a negative correlation when the analysis was performed using data obtained throughout the entire period of the study. Surprisingly, at day 40 separately, a significantly strong negative correlation between soil microbial biomass-C and disturbance intensity was detected ($r = -0.85$, $p < 0.01$). In addition, despite decreases noticed at day 80, some increases occurred at the end of the experiment. Moreover, significant decrease in most treatments in set (2) at day 80 were followed by significant increase at day 120. However, microbial biomass-C decreased in set (3). Response of microbial biomass-C may indicate some degree of recovery of microbial communities or a shift in composition to species that are more tolerant to salinity stress. **Griffiths and Philippot (2013)** suggested that soil microbial communities may tolerate and adapt to frequent disturbances, and such adaptation could be a shift in microbial community structure **(Wong *et al*., 2008)**.

In conclusion, increasing NaCl concentration, in this experiment increased soil electrical conductivity, but decreased pH and available phosphorus. Organic matter content and total N% may be decreased in the long term under high salinity stress. Frequency of addition did not show direct influence on decreasing or increasing pH, total N, available P and organic matter content. Increasing disturbance intensity from low or moderate to high levels, in the present experiment, decreased microbial activity, measured by soil basal respiration, and microbial biomass-C at day 40. However, at the end of the experiment values tend to increase again in most treatments indicating resilience of microbial communities to long term salinity stress or press disturbances even at high salt concentrations. Moreover, frequent additions increased microbial activity at the end of the experiment to values much higher than those at the beginning of the study. On contrary, microbial biomass-C increased again but was much fewer than those at the beginning of the experiment, which may indicate that the reason of microbial resilience may be a shift in microbial communities to species that consume more C. Therefore, frequent additions may change the structure of microbial communities as a whole.

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التغييرات في الكربون والنيتروجين والفسفور و المعايير الميكروبيولوجية استجابةً لتأثيرات متكررة من الملوحة

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تعد الزيادة فى نسبة الملوحة في التربة ، خاصة في المناطق الجافة وشبه الجافة من المشكلات البيئية الهامة والتى تؤدي إلى عدم قدرة النظام البيئي على أداء وظائفه بكفاءة . وتهدف هذه الدراسة إلى تقييم تأثير تعرض التربة إلى مستويات متعددة ومتكررة من الملوحة على الخواص الكيميائية للتربة إضافةً إلى المعايير الميكروبيولوجية كونها معبرة عن الأداء الوظيفي للتربة. تم تجميع التربة المستخدمة في هذه الدراسة من حقول زراعية بقرية ميت المخلص، مركز زفتى، محافظة الغربية حيث أعدت بيئات مصغرة للتربة في أوعية بلاستيكية صغيرة وتركت لمدة أربعة أشهر للسماح باستقرار التربة. وتم أعداد ستة تركيزات من كلوريد الصوديوم (٠,٥%، ١%، ٣%، ٥%، ١٠% و١٥%)، بالإضافة للماء المقطر كمعالجة ضابطة، ووضعت التركيزات المختلفة بتكرارات محددة في اليوم (١، ٤٠ ،٨٠). ثم تم تجميع العينات من الوحدات المصغرة لإجراء التحاليل المختلفة عليها في الأيام (٤٠ ،٨٠ ،١٢٠). أظهرت النتائج التي تم الحصول عليها إلى أنه بزيادة التعرض للملوحة تزداد التوصيلية الكهربية للتربة طرديًا، لكنها على النحو الآخر تقلل الأس الهيدروجيني وتركيز الفسفور المتاح. وأشارت النتائج إلى احتمالية انخفاض المحتوى العضوي للتربة والنيتروجين الكلي على المدى البعيد تحت تأثير التعرض إلى مستويات عالية من الملوحة. وأظهرت النتائج أن تكرار التعرض للملوحة لم يؤد إلى تأثيرات مباشرة على الخصائص الكيميائية للتربة. وبالنسبة للنشاط الميكروبي للتربة وكذلك الكتلة الحيوية للميكروبات فأظهرت درجة من التعافي لمظاهر الاضطراب المختلفة. وفى المجمل نستنتج أنه رغم مرونة المعايير الميكروبية للتربة للتغيرات المختلفة من الملوحة فإن الخصائص الكيميائية للتربة تتغير، خاصة تحت تأثير التركيزات العالية من الملوحة، مما يجعل التربة وسط غير مناسب لنمو النباتات.