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A novel approach toward better use of saline soils *vis-á-vis* the evaluation of microbial responses

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Abstract

The refurbishment of soil microbial properties is important for better use of saline soils. This study aimed to evaluate the efficiency of amendments *viz.* vermicompost (VC), wood ash (WA) and zeolite (ZL) on microbial properties of two different coastal saline soils *viz.* soil A (ECe 9.25 mS m^{-1}) and soil B (ECe 37.64 mS m^{-1}). Amendments were incorporated at the rates of 1% and 2% both as single and combined applications resulting in 14 different treatments. After pre-incubation, the soils were amended and incubated for 72 days at 60% of water holding capacity (WHC). Microbial activity (MA) as measured by absorption in alkali and microbial biomass carbon (MBC) by chloroform fumigation-incubation increased after amendment treatments which were lower at higher salinity. Treatment T6, combination of VC and WA (132 and 112% increase in MA and 248 and 391% increase in MBC respectively for the soil A and soil B compared to their respective control) in both soils opted as the most effective treatment while the effect of ZL addition was not significant. The increase of soil pH and ECe was proportional to the amendment type and application rate. The metabolic quotient ($q\mathrm{CO_2}$) data also supported the salt stress abatement by amendment application. Higher rates were not necessarily efficient in improving soil microbial properties as they imposed further salinity.

1. Introduction

Salt-affected soils are increasing worldwide threatening the production of crops, particularly in coastal areas. The accumulation of salts during the dry winter season in coastal areas of Bangladesh results in an increase in soil salinity high enough to hinder the soil nutrient cycle, seed germination and growth of plants (Shrivastava and Kumar, 2015). Land reclamation is inevitable in these soils for the production of a second crop in rabi season (November-March), which can be possible through the improvement of soil health. Currently, the importance of studying microbial biomass carbon (MBC) and microbial activity (MA) as well as metabolic quotient ($q\mathrm{CO_2}$) featuring soil health, increased in reclamation study as soil microorganisms are of utmost importance in regulating soil fertility (Garcia et al., 2000).

Soil salinity is a major threat to soil microorganisms, as it adversely affects soil microbial biomass (SMB) and their activity through osmotic effect, nutritional imbalance and toxic effect (Chowdhury et al., 2011). Some salt-tolerant microorganisms can adjust osmotic stress by producing osmolytes in saline soil. Producing osmolytes is a heavy nutrient-demanding system as a high amount of energy is required to synthesize osmolytes (Hagemann, 2011). The response of the microbes could be improved as well as crop production could be possible if the nutrient cycle reactivation is possible in degraded coastal saline soils through replenishment with amendment application. Studying this is also timely as climate change is likely to result in long dry periods in the coastal parts of Bangladesh. Many researchers have reported amendment complementation by the addition of organic and inorganic amendments on saline soils (Wang et al., 2014; Tripathi et al., 2007; Yazdanpanah et al., 2013). Attention has been focused on different easily available amendment materials to minimize the expensive use of chemical fertilizers to reclaim saline soils. Vermicompost (VC), wood ash (WA) and zeolite (ZL) are abundant in Bangladesh. Cow manure derived VC is a nutrient-rich organic fertilizer that is the byproduct of organic matter (OM) consumption of an earthworm and has large particulate surface areas with rich microbial populations (Atiyeh et al., 2000). The application of VC in saline soils can supply nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) (Lakhdar et al., 2009). As an acid-neutralizing amendment, WA is regularly used in soils because of its high alkalinity (Ca and Mg) as well as its abundance of P and K (Atkinson et al., 2010). As an inorganic amendment, ZL, the crystalline aluminosilicate, can be used for mitigating salt damage by adsorbing Na because of its large sorption and ion exchange capacity. Application of ZL increases soil N, P, Ca, Mg (Abdi et al., 2010), SMB (Chander and Joergensen, 2002). Soil salinity coupled with low pH is one of the problems in the southeastern coastal parts of Bangladesh (Chowdhury, 2016; Roy et al., 2021). Studies on the reclamation of acidic-salt-affected soils are scanty. Moreover, the effect of WA alone or in combination with VC as well as with inorganic amendments such as ZL in improving microbial biomass and activity in saline soils has not yet been studied. Therefore, this study aimed to determine the reclamation potential of VC, WA and ZL in acidic saline soils in relation to soil microbiological properties. If these soils could be ameliorated so that second crops can be grown, they could play an important role in increasing food production for the growing human population.

2. Materials and Methods

2.1. Collection and processing of soil samples and amendments

Three soil sub-samples from a depth of 0–15 cm (the most biologically activity layer) were collected from two different sites hereinafter referred to as soil A and soil B and formed a homogenous matrix of each of the sample sites. The sampling sites belong to Roypur Union (*a sub-unit of upazila*) of Anwara Upazila (*a sub-unit of District*) which is located in the south-eastern part of Chattogram District (22.2167°N, 91.9111°E), Bangladesh. The Union covers an area of 2,456 ha, where site A is experienced with the cultivation of 1–2 crops (paddy in kharip and chilli in rabi season) in a year and site B is experienced with no cropping practice (according to local people's perception). Based on the General Soil Type (GST) system of classification, the soils analyzed in this study were classified as the Chittagong Coastal Plain (Agroecological zone 23), which is correlated as Gleysols of the FAO-UNESCO soil unit and Inceptisols according to the USDA soil taxonomy (Huq and Shoaib, 2013). The land types range from moderately low to moderately high. The soils of the study area are characterized by clay loam texture, high consistency, low drainage capacity, and low OM content (LRUG, 1997). The area is under a tropical monsoon climate with an average maximum temperature of 32.3°C during May, and the minimum of 13.9°C during January. The annual average rainfall is 2877 mm. The monsoon starts in June and stays up to October. This period accounts for 80% of the total annual rainfall. The mean monthly evaporation varies from a minimum of 51 mm in winter to a maximum of 183 mm in summer (Misbahuzzaman and Alam, 2006). Due to low atmospheric moisture and high temperatures during the dry season, the region is vulnerable to salt accumulation on the soil surface (LRUG, 1997).

Soil sampling was done with a stainless-steel spade. All of the samples were put in polythene bags and transported to the laboratory on the day of sampling. After collection, the composite soil samples were air-dried by spreading them out to air at room temperature (26±2°C) for two days avoiding direct sunlight and sieved through a 2 mm mesh sieve to remove large detritus especially roots before pre-incubation and soil analyses. The physicochemical properties of the two soils are shown in Table 1. Soil A is classified as highly saline and soil B is extremely saline. The pH of soils was acidic in nature. Three types of amendments from both organic and inorganic origins used in this study were VC of cow dung, WA and calcium type ZL ($CaAl_2Si_4O_{12}.\ nH_2O$) (Table 2; Roy and Chowdhury, 2020). The materials used in this study are representative of typical amendments available to growers. Cow dung originated VC was collected from an organic farm while ZL was procured from a local market distributed by National Agricare, Indonesia. Wood was burned in mud stoves to produce WA. All amendments were ground and sieved to particle size 0.25–2 mm and added to the soils.

2.2. Incubation Experiment

The experiment was conducted in the Department of Soil Science, University of Chittagong. Saline soils were moistened with distilled water to 60% of WHC and pre-incubated for 10 days at ≈ 25°C before the start of the incubation experiment to stabilize the MA after the flush of respiration following rewetting of air-dried soil (Setia et al., 2011). Air-drying and rewetting of soils are common in coastal soils, therefore this pre-treatment is not unnatural. After pre-incubation, the aforementioned amendments i.e., VC, WA and ZL were applied in both soil A and B singly and in combinations at the rates of 1% or 2% (w/w) according to treatment arrangement (Table 3). Similarly, only inorganic fertilizers i.e., NPK (treatment T2 for both soil A and soil B) in forms

Table 1.
Properties of two different saline soils used in the study

Parameters	Soil A	Soil B
$EC_{1:5}$ (mS cm^{-1})	1.08	4.38
ECe (mS cm^{-1})	9.25	37.64
	Highly Saline	Extremely Saline
pH	5.01	5.22
OC (%)	1.20	1.11
Sand (%)	34	31
Silt (%)	39	42
Clay (%)	27	27
Textural class	Clay loam	Clay loam
Total N (%)	0.23	0.17

Table 2.
Properties of the three amendments used in the study

Properties	Amendments		
	ZL	VC	WA
pH	7.47	7.89	11.77
$EC_{1:10}$ (mS cm^{-1})	3.09	1.92	11.27
OC (%)	0.17	18.06	0.17
Total N (%)†	0.10	2.01	0.10
Total P (%)†	0.12	0.49	1.16
Total Na (%)†	0.47	–	0.11
Total K (%)†	1.81	2.34	4.13
Total Ca (%)†	3.04	2.56	10.40
Total Mg (%)†	0.88	0.42	1.28

†On a dry weight basis

of urea, triple superphosphate and muriate of potash was added as recommended (N-P-K: 60-18-0 Kg/ha) by BARC for common vegetable crop cultivation (FRG, 2012). All the amendments and NPK were mixed in such a way that a homogeneity was maintained. The un-amended soils (control) were incubated for the same period as for soil samples receiving amendments. There were seven separate sets of fourteen treatments (Table 3). The experiment was designed in a complete randomized way with three replications. An amount of 100 g soil (on oven dry weight basis) was placed individually into 1L airtight plastic jars. To each jar, a carbon dioxide (CO_2) trap containing 20 ml of 0.5 N sodium hydroxide (NaOH) was placed and then jars were sealed back. Sealed jars were incubated at ≈25°C and the release of CO_2 was measured over 72 days at 3, 7, 14, 28, 42, 56 and 72 days of incubation. In this way, the sets of soil samples were taken at different incubation periods for analyses of ECe, pH, OC, total N and MBC.

2.3. Soil chemical analyses

Soil pH and EC measurements were done in suspension prepared at 1:5 soil to water ratio (w/v), whereas the organic amendments were measured at 1:10 ratio of OM to water (w/v) (Yue et al., 2016) by using glass electrode pH (Seven Compact TM pH/Ion S220) meter and EC meter (Adwa AD 330). The $EC_{1:5}$ was converted to ECe according to Hardie and Doyle (2012). The particle size of the soil samples was analyzed by the hydrometer method as described in Huq and Alam (2005). Organic carbon (OC) was determined by the Walkley and Black wet oxidation method (Nelson and Sommers, 1982). For the analysis of total concentrations of elements, soils were digested by the method as described in Parkinson and Allen (1975). Total nitrogen (TN) was determined by the Kjeldahl method as recommended by Bremner and Mulnaney (1982), whereas total P was measured by vanadomolybdo yellow color method using UV-visible spectrophotometer at a wavelength of 490 nm (Huq and Alam, 2005). Total concentrations of Na and K were determined by atomic absorption spectrometer (AAS) (Agilent Technologies 200 Series AA), whereas total Ca and Mg were determined by ethylene di-amine tetra acetic acid method as described in Huq and Alam (2005).

2.4. Soil microbiological analyses

Microbial activity was determined by soil respiration, trapping the CO_2 in 0.5 N NaOH which was evolved from the soil during incubation in a closed system (Alef, 1995). The trapped CO_2 was determined by measuring the EC (Rodella and Saboya, 1999). Biomass carbon was measured by the method described in Jenkinson and Powlson (1976). The microbial cells in soil were killed by fumigation with ethanol-free chloroform. Immediately after pre-incubation, duplicate, 5 g subsamples for each were taken in falcon tubes. One set of samples was fumigated with ethanol-free chloroform for 24 hours at ≈ 25°C in a sealed desiccator. Non-fumigated set of samples in falcon tubes were capped and stored in a refrigerator. After fumigant removal, both fumigated and non-fumigated soils were extracted with freshly prepared 0.50 M potassium sulfate at 1:4 ratios and filtered. Dissolved OC in the extracts was determined after dichromate digestion by titrating with 0.03 M acidified ferrous ammonium sulfate. The amount of MBC was calculated based on the following equation:

Microbial biomass carbon = E_c / k_{ec},

Where, E_c = (OC extracted from fumigated soils) – (OC extracted from non-fumigated soils) and k_{ec}= 0.45 (Wu et al., 1990).

The qCO_2 was calculated from basal respiration (respiration of control soil without amendment application) based on the following equation (Anderson and Domsch, 1990):

$qCO_2 = (\text{mg } CO_2\text{–C·g}^{-1}\text{soil·h}^{-1}) / (\text{mg MBC· kg soil}^{-1}) = r / \text{SMB-C}$

Where r is the respiration rate (mg C·g^{-1}soil·h^{-1}) and SMB-C is the soil microbial biomass carbon (mg C· kg $soil^{-1}$).

2.5. Statistical analyses

All the results were expressed on an oven-dry weight basis which was measured with three replications. Pearson's correlations between the parameters and standard deviation were determined using the Microsoft Excel 2016 program. Regression between soil parameters and soil microbial activities was fitted to linear functions. The effects of amendment treatments were determined by one-way analysis of variance (ANOVA) using the least significant difference multiple range test at $p < 0.05$. The paired-samples T-test was measured to determine the statistical differences between pairs of means by the Statistical Package for the Social Sciences (SPSS) program. The dendrogram grouping for cluster analysis was also performed by SPSS.

3. Results

Different physicochemical and biological properties of the incubated soils were significantly influenced by the treatment applied to the saline soils. Depending on the treatments (Table 3), T12 received the highest amount of OC, TN, total P and total K and T14 received the highest amount of total Na, total Ca and total Mg. Higher content of OC and N were incorporated in the soils by the application of VC compared to WA and ZL, the amount varied depending on the application rate of the amendment.

3.1. Changes in soil chemical properties after amendment application

There was an interaction between soil chemical properties and the treatments. Soil pH and ECe were increased significantly ($p < 0.05$) in both soils compared to control after NPK fertilizer, VC, WA and ZL incorporation (Table 4, Fig. 1). The average ECe varied from 9.31 to 12.69 mS cm^{-1} in soil A and from 39.20 to 42.35 mS cm^{-1} in soil B during the incubation. The increase was proportional to the application rate in both soils (Table 3).

The treatments with VC (T3, T6, T7, T9, T12, T13) had the highest OC content (1.51 to 1.70% for soil A and 1.35 to 1.48% for soil B), whereas T1 with no amendment application had the lowest value (1.33% in soil A and 1.16% in soil B). Salinity showed inconsistent effects on changes in soil chemical properties es-

Table 3.
Treatment legend, EC_e, pH, OC and TN values (mean ±SD) after the addition of different treatments to two saline soils

Legend	Treatment description	Soil A				Soil B			
		ECe (mS cm^{-1})	pH	OC (%)	TN (%)	ECe (mS cm^{-1})	pH	OC (%)	TN (%)
T1	Control	9.31±0.06^{aA}	5.18±0.00^{aA}	1.33±0.10^{aA}	0.23±0.00^{aA}	39.20±0.48^{aB}	5.71±0.02^{aB}	1.16±0.02^{abB}	0.17±0.00^{aB}
T2	NPK (60-18-0 kg/ha)	11.82±0.02^{iA}	5.65±0.01^{cA}	1.38±0.04^{aA}	0.28±0.00^{eA}	43.77±0.37^{gB}	6.65±0.03^{dB}	1.18±0.01^{abB}	0.21±0.00^{fB}
T3	VC (1%)	9.71±0.03^{bA}	5.57±0.02^{bA}	1.54±0.01^{bA}	0.25±0.00^{cA}	39.66±0.47^{abB}	6.21±0.03^{bB}	1.35±0.03^{cB}	0.19±0.00^{cB}
T4	WA (1%)	11.15±0.09^{fA}	7.08±0.02^{gA}	1.40±0.04^{aA}	0.23±0.00^{aA}	40.96±0.28^{deB}	7.67±0.03^{gB}	1.18±0.01^{abB}	0.17±0.00^{aB}
T5	ZL (1%)	9.93±0.08^{cA}	5.57±0.03^{bA}	1.38±0.02^{aA}	0.23±0.00^{aA}	39.94±0.51^{bcB}	6.59±0.01^{cB}	1.20±0.03^{bB}	0.17±0.00^{aB}
T6	VC (1%) + WA (1%)	11.39±0.09^{gA}	7.01±0.05^{fA}	1.54±0.05^{bA}	0.25±0.00^{cA}	40.87±0.40^{deB}	7.72±0.02^{gB}	1.38±0.02^{cB}	0.19±0.00^{bB}
T7	VC (1%) + ZL (1%)	10.39±0.08^{dA}	5.89±0.01^{eA}	1.51±0.03^{bA}	0.25±0.00^{cA}	39.99±0.38^{bcB}	6.70±0.01^{dB}	1.38±0.03^{cB}	0.19±0.00^{cB}
T8	WA (1%) + ZL (1%)	11.64±0.07^{hA}	7.10±0.05^{gA}	1.40±0.02^{aA}	0.24±0.00^{bA}	41.28±0.40^{eB}	7.73±0.01^{gB}	1.18±0.04^{abB}	0.17±0.00^{aB}
T9	VC (2%)	9.77±0.13^{bA}	5.77±0.01^{dA}	1.70±0.03^{cA}	0.27±0.00^{dA}	39.67±0.41^{abB}	6.68±0.03^{dB}	1.48±0.04^{dB}	0.21±0.00^{dB}
T10	WA (2%)	12.31±0.12^{jA}	7.25±0.02^{hA}	1.40±0.02^{aA}	0.23±0.00^{aA}	41.22±0.40^{deB}	8.14±0.00^{hB}	1.15±0.04^{abB}	0.17±0.00^{aB}
T11	ZL (2%)	10.65±0.10^{eA}	5.80±0.01^{dA}	1.40±0.03^{aA}	0.23±0.00^{aA}	40.54±0.39^{cdB}	6.80±0.01^{eB}	1.16±0.03^{abB}	0.17±0.00^{aB}
T12	VC (2%) + WA (2%)	12.69±0.14^{kA}	7.27±0.01^{fA}	1.64±0.03^{cA}	0.27±0.00^{dA}	41.49±0.27^{eB}	8.14±0.01^{hB}	1.48±0.04^{dB}	0.21±0.00^{efB}
T13	VC (2%) + ZL (2%)	11.32±0.11^{gA}	5.91±0.02^{eA}	1.65±0.06^{cA}	0.27±0.00^{dA}	40.87±0.26^{deB}	6.98±0.11^{fB}	1.45±0.01^{dB}	0.21±0.00^{deB}
T14	WA (2%) + ZL (2%)	12.63±0.09^{kA}	7.32±0.01^{iA}	1.41±0.02^{aA}	0.24±0.00^{bA}	42.35±0.05^{fB}	8.14±0.03^{hB}	1.13±0.05^{aB}	0.17±0.00^{aB}

Correlation between soil A and soil B	ECe	pH	OC	TN
	0.79***	0.98***	0.89***	0.97***

†Values in the same column followed by the same small letter(s) are not significantly different at $p<0.05$ according to Analysis of variance (ANOVA)
‡Values in soil A and soil B followed by the same capital letter(s) are not significantly different at $p<0.05$ according to ANOVA
***Correlation is significant at the 0.001 level

Table 4.
MBC, MA, CR and qCO_2 values (mean ±SD) after the addition of different treatments to two saline soils

Treatment	Soil A				Soil B			
	MBC mg C·kg soil^{-1}	MA mg C·g^{-1} soil·h^{-1}	CR mg C·g^{-1} soil·h^{-1}	qCO_2 mg CO_2–C·mg^{-1} Cmic·h^{-1}	MBC mg C·kg soil^{-1}	MA mg C·g^{-1} soil·h^{-1}	CR mg C·g^{-1} soil·h^{-1}	qCO_2 mg CO_2–C·mg^{-1} Cmic·h^{-1}
T1	112.38±1.07^{aA}	0.23±0.02^{aA}	1.61±0.13^{aA}	0.36±0.01^{hA}	39.93±1.28^{aB}	0.10±0.02^{aB}	0.72±0.11^{aB}	0.32±0.02^{fA}
T2	175.23±0.12^{bA}	0.39±0.08^{deA}	2.76±0.53^{deA}	0.22±0.02^{gA}	56.67±1.16^{cB}	0.28±0.05^{cdB}	1.96±0.33^{cdB}	0.25±0.00^{deA}
T3	216.86±1.25^{hA}	0.31±0.01^{bcA}	2.19±0.10^{bcA}	0.17±0.00^{dA}	65.81±0.17^{deB}	0.23±0.05^{bcB}	1.61±0.32^{bcB}	0.20±0.01^{abcdA}
T4	197.69±0.21^{eA}	0.63±0.02^{gA}	4.41±0.11^{gA}	0.21±0.00^{fgA}	61.06±2.56^{cdB}	0.31±0.02^{dB}	2.16±0.15^{dB}	0.21±0.03^{cdA}
T5	185.59±0.26^{cA}	0.26±0.04^{abA}	1.79±0.27^{abA}	0.20±0.01^{fA}	48.25±0.61^{bB}	0.20±0.01^{bA}	1.39±0.10^{bA}	0.28±0.00^{efB}
T6	260.50±2.41^{lA}	0.80±0.03^{hA}	5.60±0.24^{hA}	0.13±0.00^{aA}	84.56±2.42^{gB}	0.51±0.03^{eB}	3.56±0.22^{eB}	0.15±0.03^{abA}
T7	221.39±1.71^{iA}	0.43±0.08^{efA}	3.00±0.56^{efA}	0.16±0.00^{cdA}	66.36±4.50^{deB}	0.27±0.05^{cdB}	1.92±0.33^{cdB}	0.18±0.00^{abcA}
T8	202.02±0.07^{fA}	0.60±0.03^{gA}	4.21±0.21^{gA}	0.20±0.00^{fA}	64.48±1.37^{deB}	0.33±0.06^{dB}	2.32±0.43^{dB}	0.18±0.00^{abcA}
T9	220.92±3.72^{iA}	0.41±0.02^{eA}	2.87±0.14^{eA}	0.15±0.00^{bA}	68.87±6.31^{eB}	0.34±0.02^{dB}	2.36±0.14^{dB}	0.19±0.00^{abcB}
T10	202.94±2.56^{fgA}	0.57±0.03^{gA}	3.97±0.18^{gA}	0.21±0.00^{fgA}	65.47±4.52^{deB}	0.31±0.03^{dB}	2.18±0.20^{dB}	0.21±0.03^{bcdB}
T11	192.78±1.82^{dA}	0.33±0.04^{cdA}	2.34±0.31^{cdA}	0.19±0.00^{eA}	49.46±5.53^{b}	0.20±0.07^{bB}	1.41±0.49^{bB}	0.31±0.09^{fA}
T12	255.49±2.45^{kA}	0.77±0.05^{hA}	5.36±0.37^{hA}	0.13±0.01^{aA}	84.74±1.91^{gB}	0.48±0.01^{eB}	3.37±0.05^{eB}	0.14±0.00^{aA}
T13	233.52±3.32^{jA}	0.50±0.02^{fA}	3.47±0.11^{fA}	0.15±0.00^{bcA}	75.05±2.12^{fB}	0.34±0.03^{dB}	2.35±0.24^{dB}	0.17±0.01^{abcA}
T14	206.00±0.58^{gA}	0.60±0.02^{gA}	4.17±0.15^{gA}	0.20±0.00^{fA}	65.12±4.54^{deB}	0.27±0.03^{cdB}	1.91±0.24^{cdB}	0.20±0.01^{bcdA}

Correlation between soil A and soil B	MBC	MA	CR	qCO_2
	0.91***	0.83***	0.83***	0.67***

†Values in the same column followed by the same small letter(s) are not significantly different at $p<0.05$ according to Analysis of variance (ANOVA)
‡Values in soil A and soil B followed by the same capital letter(s) are not significantly different at $p<0.05$ according to ANOVA
***Correlation is significant at the 0.001 level
Treatment legend description in Table 3.

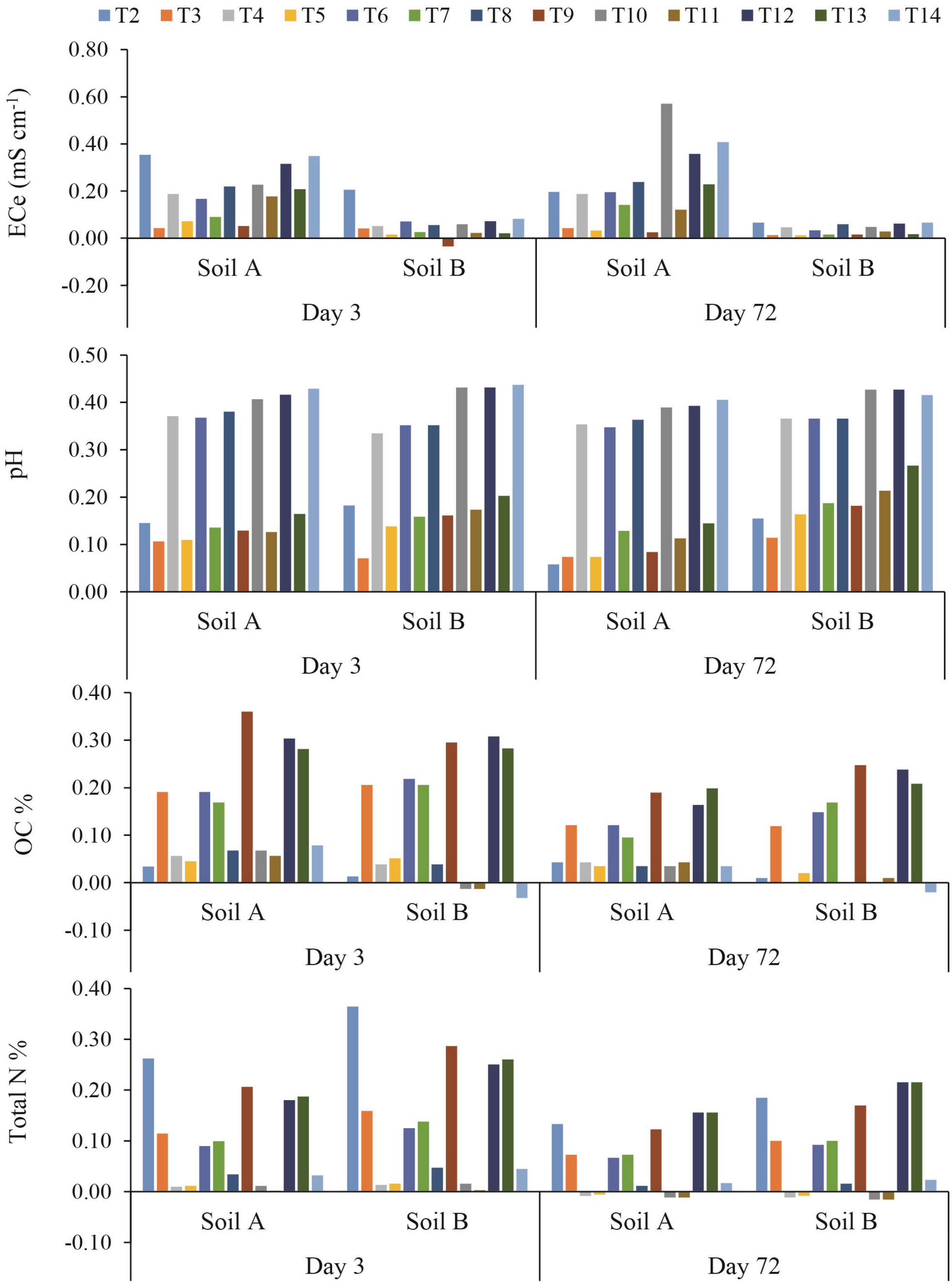

Fig 1. Relative increase (+) and decrease (-) of chemical properties on day 3 and day 72 to control after the addition of different treatments. Treatment legend description in Table 3.

pecially in the various treatments of soil B after 72 days of incubation (Fig. 1). The treatment with NPK fertilizer (T2) had the highest total N content (0.28% for soil A and 0.21% for soil B) which was significantly higher than the other treatments and it decreased in all treatments with time. Treatment with WA contained relatively high concentrations of nutrients (P, Ca, Mg, K) (Table 2) whereas, ZL contained a high concentration of Na and medium concentration of Ca and Mg but low concentrations of OC and N than VC. This imbalance resulted in a relatively low content of soil N in treatments T4, T5, T8, T10, T11 and T14.

3.2. Changes in soil microbial properties after amendment application

All the treatments showed significantly higher biomass concentrations on day 3 compared to those on following harvesting days (7, 14, 28, 42, 56 and 72 days) (Fig. 2). Microbial biomass and activity were highly variable, with increasing and decreasing trends in incubation time of 72 days. On day 3, after amendment addition, MBC ranged from 237.71 to 326.56 mg C·kg^{-1} in soil A and 52.26 to 97.32 mg C·kg^{-1} in soil B and MA ranged from 0.33 to 1.27 mg C·g^{-1}·h^{-1} in soil A and 0.20 to 0.60 mg C·g^{-1}·h^{-1} in soil B, that decreased with the range of 83.47 to 150.54 mg C·kg^{-1} in soil A and 33.50 to 68.28 mg C·kg^{-1} in soil B for MBC and 0.28 to 0.80 mg C·g^{-1}·h^{-1} in soil A and 0.15 to 0.52 mg C·g^{-1}·h^{-1} in soil B for MA on day 72.

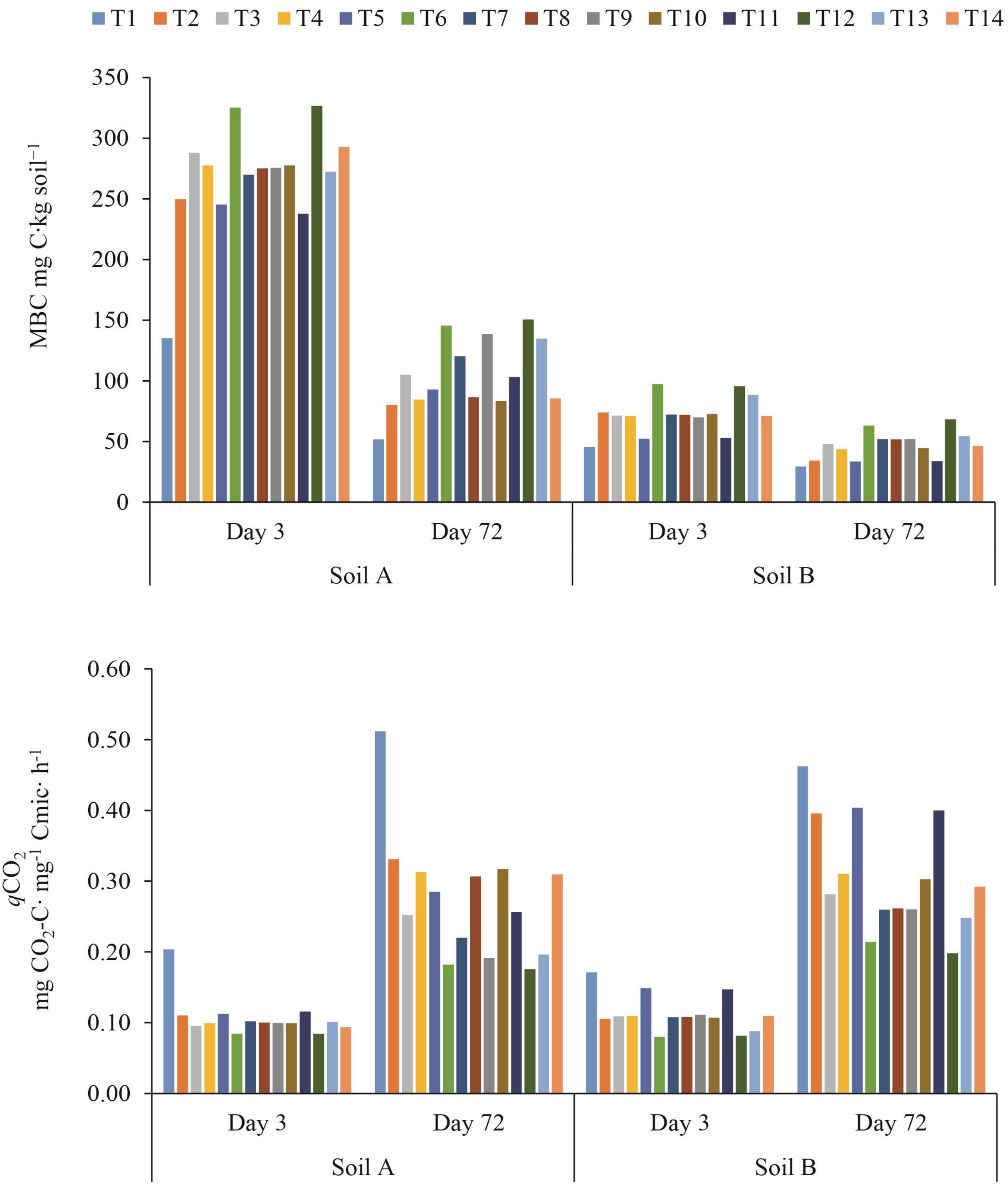

Fig 2. MBC and qCO2 on day 3 and day 72 after addition of different treatments to two saline soils. Treatment legend description in Table 3).

In both soils, the addition of amendments increased the MBC, MA and cumulative respiration (CR) compared to the unamended soil (Table 4, Fig. 2, 3, 4). The increase was more pronounced in MA than MBC after amendment addition. The highest average increase of MBC was observed in T6 (260.50 mg C·kg^{-1} in soil A and 84.56 mg C·kg^{-1} in soil B) and in T12 (255.49 mg C·kg^{-1} in soil A and 84.74 mg C·kg^{-1} in soil B) which were about 132% in soil A and 112% in soil B compared to their respective T1.

There were significant differences in MBC, MA and CR among the treatments in soil A and soil B (Table 4). Values were generally higher in soil A compared to soil B for all treatments. With both 1 and 2% dose of amendment combination, soil microbial properties after amendment application increased with the proportion of VC and WA compared to ZL (Table 4). The average soil respiration was low in soil B compared to soil A in all the amendment treatments (Table 4) but relative respiration was high in soil B during the whole incubation predominantly in the treatments with VC (Fig. 3). In both soils, CR in amended soil per unit soil OC was highest in T6 treatment (5.60 mg C·g^{-1}·h^{-1} in soil A and 3.56 mg C·g^{-1}·h^{-1} in soil B) and lowest in T5 treatment (1.79 mg C·g^{-1}·h^{-1}in soil A and 1.39 mg C·g^{-1}·h^{-1} in soil B) (Table 4, Fig. 4). The qCO$_2$, varied significantly between the treatments, with lower values in T6 and T12 (Table 4, Fig. 2).

Pearson correlation study revealed a strong significant negative effects of ECe on the basal respiration rate (0.23 mg C·g^{-1}·h^{-1} in soil A and 0.10 mg C·g^{-1}·h^{-1} in soil B). Correlation between average change after amendment addition between soil chemical and microbial properties showed ECe had strong relation with MBC, MA, CR and qCO$_2$ in soil A than in soil B. qCO$_2$ was significantly correlated with the modification of soil pH, OC and TN,

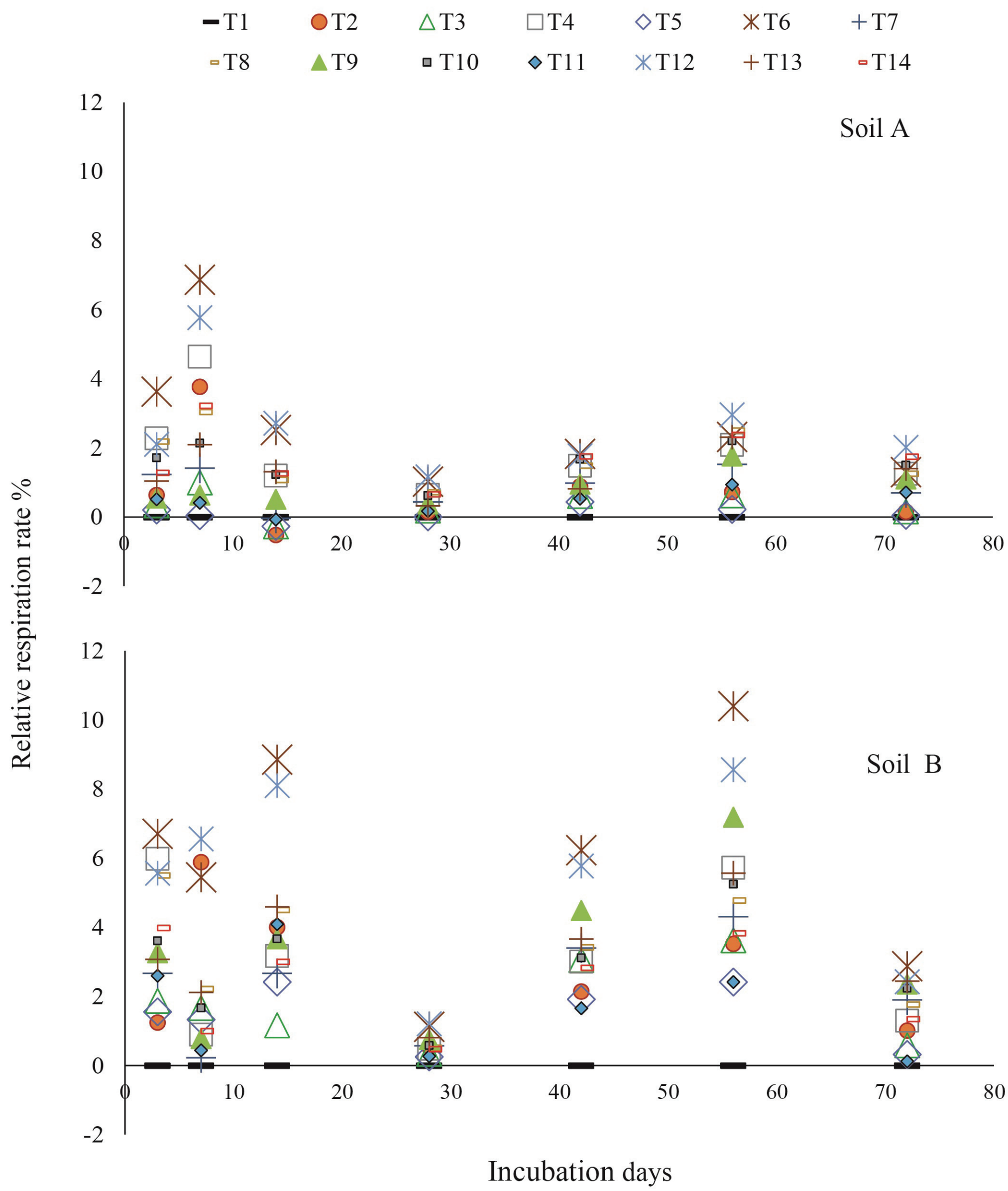

Fig 3. Relative respiration rates (%) compared to control in 72 days of incubation after the addition of different treatments to two saline soils. Treatment legend description in Table 3.

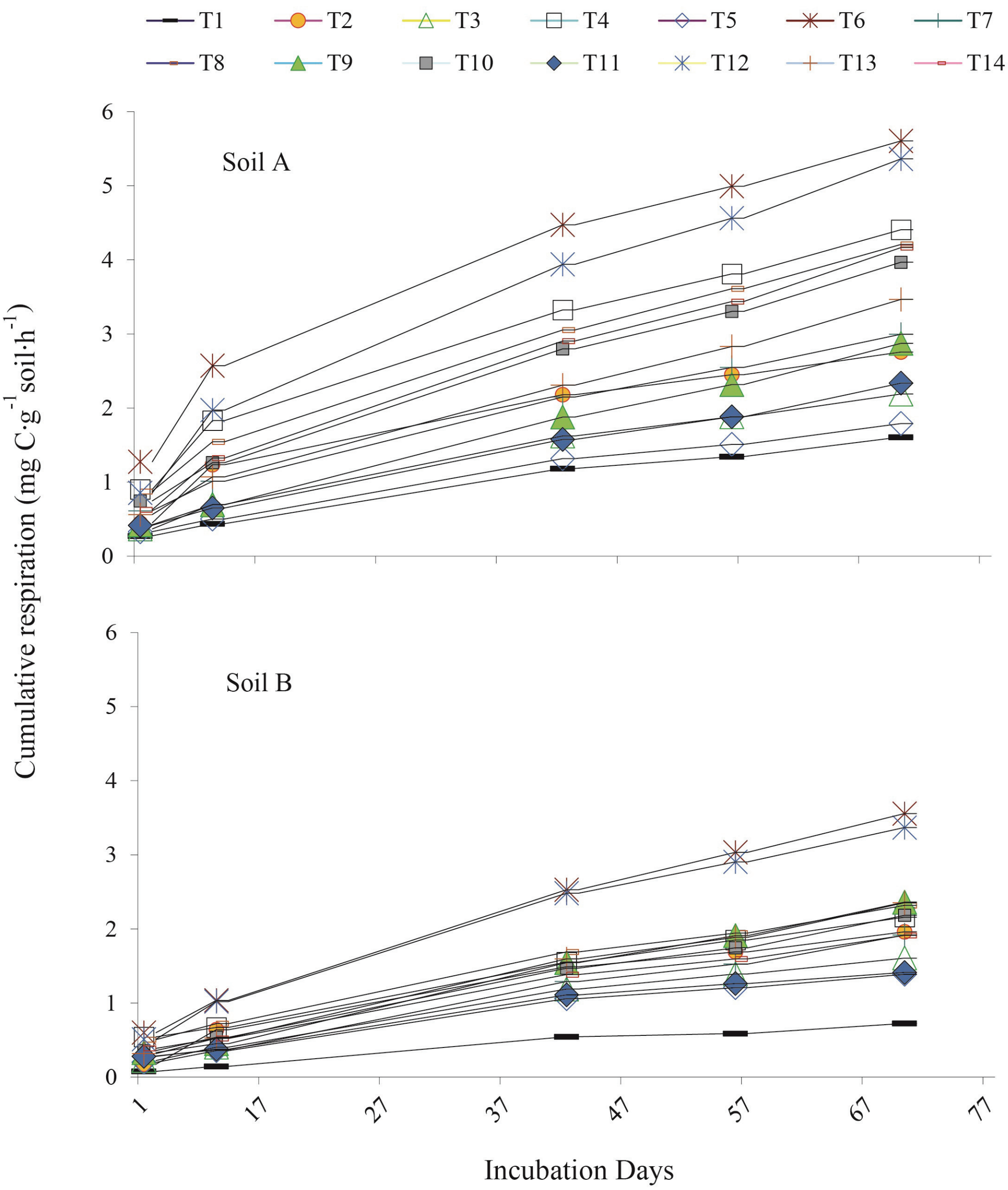

Fig 4. Cumulative respiration in 72 days of incubation after the addition of different treatments to two saline soils. Treatment legend description in Table 3.

for amendment addition in both soils which was very high with OC and TN. Results interpret no strong positive relationship between CR and increasing amendment dose both in soil A and soil B as there were no significant differences between T6 and T12, T4 and T10.

When the correlation between soil chemical and microbial properties was compared between the harvesting days, it demonstrated that the correlation of ECe with MBC and MA were positive in both soils (Table 5, $p < 0.05$). Soil pH was positively correlated with MBC and MA in amended treatments and the effects exceeded those of ECe. The MBC was significantly interrelated with the OC content of the soils; the impact was stronger as the incubation time proceeded (Table 5). Based on their overall impact, the fourteen treatments in two saline soils were clustered at a distance threshold into three groups when analyzed with Agglomerative Hierarchical Clustering (AHC) (Fig. 5). Visualizing the geometry of AHC dendrogram indicated that T6 and T12 are separately clustered (Z) and T2, T3, T4, T7, T8, T9, T10, T14 are clustered in a chunk (Y). Y and Z were different from un-amended treatment, T1 (X) in both soils.

4. Discussion

Additional nutrition can alleviate salt stress on microorganisms by improving the chemical conditions of saline soil (Wong et al., 2009). In this study, amendment addition improved the overall nutritional quality and the microbial dynamics of the soil.

Table 5.
Correlation co-efficient (r) values among soil chemical properties and soil microbial properties after the addition of different treatments to two saline soils

	Dependent	Variables	Day 3		Day 72	
		Independent	R˛	r	R˛	r
Soil A	MA	pH	0.61	0.79***	0.64	0.80***
		ECe	0.14	0.38**	0.49	0.70***
		OC	0.02	0.15	0.11	0.34*
		TN	0.00	–0.06	0.04	0.21
	MBC	pH	0.48	0.69***	0.03	0.18
		ECe	0.21	0.45**	0.00	0.02
		OC	0.23	0.48***	0.73	0.85***
		TN	0.10	0.31*	0.43	0.66***
Soil B	MA	pH	0.60	0.77***	0.32	0.57***
		ECe	0.01	0.08	0.06	0.25
		OC	0.06	0.24	0.30	0.55***
		TN	0.00	–0.05	0.23	0.47**
	MBC	pH	0.27	0.52***	0.30	0.55***
		ECe	0.09	0.29	0.07	0.27
		OC	0.33	0.58***	0.45	0.67***
		TN	0.22	0.47**	0.27	0.51***

†Legend description in table 3 and 4.
***Correlation is significant at the 0.001 level
**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

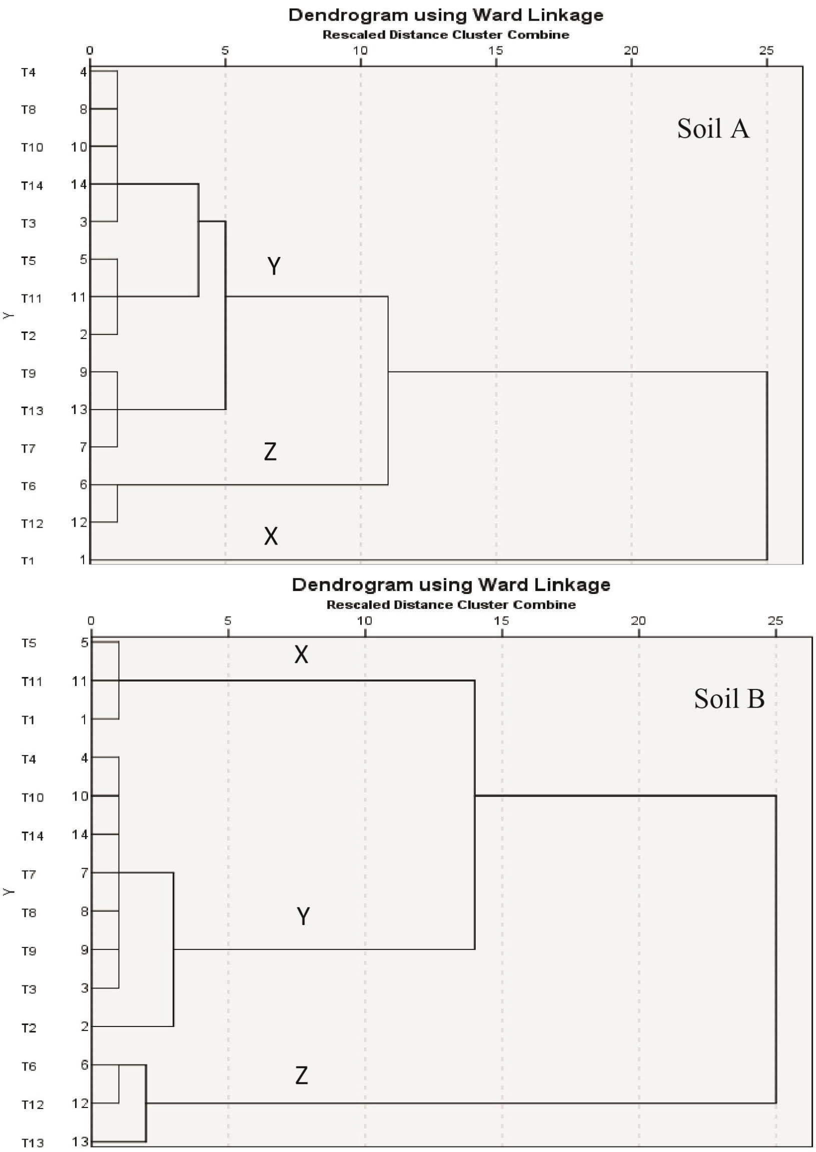

Fig 5. Dendrogram showing similarities of soil properties after addition of different treatments to two saline soils. Treatment legend description in Table 3.

Soil pH and ECe increased in saline soils following amendment application. The increase was proportional to the application rate and nature of amendments. Such a result was supported by several studies (Ouni et al., 2013; Lakhdar et al., 2008; Mkhabela and Warman, 2005). The statistically significant increase in ECe of incubated soils after the addition of VC was probably due to the high concentration of Na and K cations in VC (Trivedi et al., 2017). The WA, characterized by a higher EC and pH value than VC and ZL caused greater increases in soil ECe and pH. Hydroxyl (OH^-) ions produced by ligand exchange after carbon (C) mineralization and the increase of basic cations (K, Ca and Mg) could contribute to this modification (Mkhabela and Warman, 2005). Calcium cations have ameliorative effects in saline and acid soils (Yang et al., 2018). The treatments with increased Ca content can improve the remediation efficiency of saline soils by Na replacement from the exchange sites. The contents of OC and TN in the soil increased with the addition of VC either by a single application or combined with WA and ZL.

Following amendment incorporation, MBC and MA were stimulated in both soils, even at high salinity levels. Tejada et al. (2010) reported that SMB responds rapidly to the additions of readily available C. But the biomass and activity were significantly low at higher ECe for similar treatments. Basal respiration is an indicator of soil quality and the available OC for heterotrophic microorganisms. The lower basal respiration in soil B (higher ECe soil) compared to soil A indicated the low inherit microbiological properties especially microbial biomass content due to osmotic and or specific ion effects. Soils with high content of labile C generally have higher microbial biomass as it supplies a readily available energy source for microbial decomposition (Yan et al., 2007). Further low amendment decomposition in higher saline soil can explain the lower build-up of microbial biomass. Surprisingly, although the MBC and MA after amendment application were lower in the soil with higher ECe, the soil biochemical activity amplified better with the application of amendments in the soils with higher ECe. The relative respiration rates were higher in the extreme saline soil (soil B), indicating a stronger response of soil microorganisms to applied amendment in soil B as compared to soil A which also propounded that the microbial biomass in this soil was highly active.

An increase in soil respiration indicates efficiencies of soil microorganisms as a whole to decompose OM. The higher amount of CO_2 released per unit of soil corresponds to the higher amount of degradable substrate for the growth of microbial biomass (Martins et al., 2020). The rate of MA was more strongly increased than MBC by amendment addition. Thus, under these saline soils, C is utilized preferentially for energy (respiration) rather than growth. This may be due to the high energy demand for the synthesis of osmolytes (Mavi and Marschner, 2013). For detoxification and cell repair, soil microorganisms use C-containing compounds from the amendments to synthesize osmolytes for counteracting the osmotic stress from salinity or to devote to metabolic processes therefore MA increases (Chowdhury et al., 2011).

There were higher average MBC in single VC treatment (T3 and T9) than in single WA (T4, T10) and ZL (T5, T11) amended soils that may be due to a greater OC in the former product. Increased OC can revive soil nutrient cycles and increase the reminiscence of soil nutrients and thus support the reclamation of degraded soil. Again, supplementation can change the volume of the SMB (de Souza Silva and Fay, 2012). It seems more likely that the higher MBC was due to the higher surface microbial biomass at the VC compared to the smaller surface biomass in WA and ZL. The rising biomass of soil organisms may improve MBC through the decomposition of OM and nutrient cycling (Chaganti and Crohn, 2015). However, MA was lower in the single VC treatments compared to WA and ZL. Microbes can rapidly increase biomass in saline soil in the presence of available C (Yan and Marschner, 2012). To decompose VC- OC, microbes might use some of their energy for the synthesis of enzymes (cellulases and ligninases) (Wu et al., 1993). A small proportion of the whole microbial community possesses cellulose and lignin decomposing enzymes. Partitioning of the activity for enzyme production caused lower MA with the treatments with VC alone. Higher MBC values were found at the beginning of the incubation days at all treatments indicating greater metabolic activity in this period. This greater increase can be explained by the greater substrate availability per unit microbial biomass (Yan and Marschner, 2012). However, the significant ($p < 0.05$) decline in MBC concentration from day 3 to day 72 shows that a large proportion of the biomass dies when this labile C and nutrients were diminished. The estimation of MBC by chloroform fumigation and MA measurement by soil respiration does not allow for a comprehensive evaluation of microbial responses in the soil due to a number of potential limitations, such as selectivity, lack of specificity, incomplete recovery, and interference. However, these methods are frequently used in ecological studies because they allow for a relatively quick determination microbial biomass and activity in soils.

The addition of WA with exclusively higher nutrients (P, K, Ca, Mg) compared to VC showed substantially higher MA as that supplies labile nutrients to the microbes. The lower C mineralization from soils amended with ZL may be due to a lack of sufficient OC and N. Some active or adapted microbes in saline soils were highly active in response to the high and readily available nutrient content of WA would have a greater impact on respiration rates than by VC. However, the single ZL treatment demonstrated no significant difference with un-amended soils. Overall, the results were conditioned by the composition of amendments, the rate of application and the soil type. However, the highest concentration of MBC and MA were observed in the treatments receiving integrated use of VC with WA (T6 and T12) in both soil A and soil B coincided with CR values among all the treatments. Nutrients in WA helped microorganisms to reactivate which may further have increased the OC availability from applied VC.

Microorganisms require balanced nutrients for cellular physiology and metabolic processes. Among the treatments, the combination of VC with WA and ZL provides better remediation results than each applied singly and has substantial potential for ameliorating coastal saline soils. The rate of CR decreased (T8 and T14 in soil B) or remained the same (T4 and T10; T6 and T12) in both soils when VC, WA and ZL applied alone or in combination at a 2% rate than 1% rate, the results indicated that adequate nutrient supply was essential for decomposition of organic amendments with C and N utilization by stressed soil microbes but excessive nutrient supply showed no positive impact on microbial biomass and activity. So, it was not necessarily the fact that the increase of respiration will be higher with higher amendment addition (2%) due to increased OC and nutrient supply. A higher application of amendments can also increase ion concentration which is high enough to suppress MA (Chahal et al., 2017). A general increase in ECe was also seen in the treatments with NPK fertilizer (T2) compared with other treatments. The rate at which ECe of this study increased in the initial stage compared to control was greater for the treatments incorporated with WA and ZL.

Soil factors such as soil moisture were of utmost importance. After pre-incubation of the soils at their optimum WHC, dormant microorganisms from the soils were activated. Maintaining optimum water content throughout the incubation study kept the activated soil microorganisms functioning. Microbial biomass of dormant population of salt-tolerant microorganisms multiplied quickly when substrate became available (de Souza Silva and Fay, 2012). An increase in pH favours C mineralization over the high ECe of the soils. The present results in saline soils showed the addition of amendments significantly increased soil pH compared to control especially with the application of WA (pH 11.77). There were differences in the treatments for pH modification and soil pH governed the MA in the acid soils during the whole incubation time. Amendment addition may reduce acidity in unproductive low pH soils through liming (Zheng, 2010). Increasing the pH of acidic soil is an effective way to ameliorate soil biological activity. The increase in soil pH after the addition of biofertilizers showed a beneficial impact in degraded acid soils (Walkiewicz et al., 2020). Microbial biomass and activity inclined better at pH values between 5 and 7 (Pietri and Brookes, 2008). Soil CO_2 evolution increased as pH increased and an increase in soil pH also reduced qCO_2 (Anderson, 2003).

The qCO_2 can be used as a microbial stress indicator (Fernandes et al., 2005). Higher qCO_2 values denote soils under stress than non-stressed soils. Decreased qCO_2 can be dependent on the nutrient status of the system in question (Wong et al., 2008). Following the addition of amendments, the change of qCO_2 in amended soil was generally lower than un-amended soil (T1). Impact of amendment application masked the salt stress. The qCO_2 also varied with treatments, generally lower in the treatment with VC compared to control, synthetic fertilizer, ZL and WA, demonstrating that more C was utilized in microbial synthesis than respiration. With the addition of OC and nutrients, the qCO_2 decreased as the microbial biomass increased despite salt stress in saline soils. Interestingly, the qCO_2 was not significantly different for similar treatments between the two investigated soils, indicating that the survival of the microbes in these soils was better adapted to stress and likely having adapted energy-intensive metabolic mechanisms. Again the application of amendments could increase soil salinity (Ouni et al., 2013). The qCO_2 was generally higher in soils that were amended with WA and ZL. This may have been due to an increase in stress following the addition of WA and ZL due to the increase in ECe, particularly in soil A. A study by Usman et al. (2004) also found an increase in salts following the addition of sewage sludge that increased the qCO_2, which was imputed by increased salt stress. The importance of bona fide nutrient supply for increasing MA in salt stress situations was also found in this experiment. The dendrogram of soil A and soil B by hierarchical cluster analysis using ward linkage also supported the result of qCO_2. There was a clear pattern in the distribution of treatments in the cluster indicating that the variation in the data set was mainly related to OC and nutrient content in the two soils. In this study, the hierarchical analysis showed that cluster Z is the combination of amendments with VC and WA in both soils.

5. Conclusion

Soil salinity adversely affects soil inhabitaing organisms and growth of higher plants. Remediation of saline soils can be possible by amendment addition. Soil salinity coincides with soil acidity was a major challenge of the studied soils. The application of amendment in these soils is a very convoluted system. The effectuality depends on the nutrient status of the amendments. The addition of WA with acid-neutralizing capacity presented an interesting alternative and contributed to the improvement of the fertility of the soils when incorporated with VC. So, the comprehensive application of VC and WA in combination could be an efficient reclamation method for the amelioration of coastal saline soils. The main effect of this acidic coastal saline soils exposed to amendments was a significant increase in pH, OC concentration and TN availability, consequently an increase in MBC, MA and a decrease in qCO_2 (indicating stress relief). This study indicated that the combination of amendment with mineral sources to organic amendments may be beneficial to abide salt stress in saline soils. However, enhanced amendment application in saline soils could increase salt stress for soil microorganisms as reflected by decreased C mineralization. Moreover, the application rate of amendments should be managed for the transport cost and environmental (salinity level) peril. Therefore, VC in combination with WA at the rate of 1% could be adopted for the improvement of the coastal saline soils as it manifested to be a cost-effective and energy-efficient treatment. The positive response of microorganisms to the application of specific amendments in saline soils suggests that crops may respond better to a certain level of soil salt. Therefore, additional research is necessary to determine the impact of these amendments under field settings for better soil utilization as well as how salt-tolerant higher plant species respond to the reclaimed soils.

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