Ecological Indicators 150 (2023) 110227

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Response of soil microbial Communities, inorganic and organic soil carbon pools in arid saline soils to alternative land use practices

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A R T I C L E I N F O

Keywords:
Total soil carbon
Indicator for carbon sequestration
Saline soil microbial structure
Wastewater irrigation
Gypsum amendment

A B S T R A C T

Soil organic and inorganic carbon (SOC & SIC) and microbial community structure are key indicators of soil quality and productivity in arid-saline soils. Salinity stress and diminishing availability of freshwater (FW) for irrigation are major constraints for productivity and improving soil quality indicators. Using treated wastewater (TW) and implementing climate-smart cropping systems are promising alternatives to replace freshwater usage in intensive cropping systems, however, impacts on the microbial community and net-carbon sequestration potential are not clearly understood. This field study was conducted in an arid saline-soil to investigate soil microbial community structure and soil carbon contents under a combination of treatments comparing bioenergy sorghum (So) and switchgrass (Sg), two irrigation water sources (FW & TW), and gypsum amendment (GA), to a native-soil control. After three years of implementing these treatments, soil samples were collected and analyzed for total carbon (TC), SOC, SIC, microbial biomass-carbon (MBC) and microbial community structure. Results showed that TW increased microbial diversity and shifted the community structure towards copiotroph-dominated prokaryotes. Several predominant and responsive taxa were associated with divergent trends of SOC, SIC and salinity parameters. Both SOC and SIC pools were sensitive to treatments and demonstrated divergent trends, as contents of TC and SOC were higher in TW-treatments, but of SIC were significantly lower in several So_TW treatments. Treatment TW_So_GA assembled a distinctive microbial community structure, accumulated the highest content of SOC (7.66 g kg^{-1}) but recorded the lowest content of SIC (6.63 g kg^{-1}). The lowest content of SOC was observed in native soil (4.58 g kg^{-1}) but contained the highest SIC (8.15 g kg^{-1}). The study results revealed the agronomic systems with higher potential for increasing TC and SOC content in arid-saline soils. Surface soil SIC was responsive to agronomic management, and several treatments produced disparate impacts on SOC and SIC stocks, which warrant for considering TC as the key indicator for assessing carbon sequestration in arid lands.

1. Introduction

Approximately-one-third of irrigated agricultural lands and 20 % of total cultivated lands globally are estimated to be impacted by high salinity (Jamil et al., 2011; Shrivastava and Kumar, 2015; Wang et al., 2003). Salinization of agricultural land is rapidly increasing at approximately 0.3–1.5 million ha of additional land salinized every year (FAO, 2015; Shrivastava and Kumar, 2015), and at this rate more than 50 % of the arable land is estimated to be salinized by the year 2050 (Jamil et al., 2011). Main drivers behind increased salinization are climate change-induced aridity, increased usage of saline water for crop irrigation and failure to implement stewardship agronomic practices (Al-Karaki, 2006; Cantrell and Linderman, 2001; Parihar et al., 2015).

Irrigation with saline groundwater or wastewater sources is increasingly implemented due to increasing scarcity of freshwater resulting from prolonged drought conditions and urban growth (Dery et al., 2019). Treated wastewater (TW) from municipal sewage treatment plants is a reliable source for irrigation but could be of marginal quality due to high total dissolved salts (TDS), which may impose additional salinity/sodicity stress on soils (Toze, 2006). Studies reported aggravating soil salinity and sodicity and negative impacts on soil quality including increased osmotic potential, destruction of soil structure due to clay dispersion, reduced water infiltration, poor aeration, and reduced nutrient availability, as major concerns for continuous

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https://doi.org/10.1016/j.ecolind.2023.110227
Received 13 January 2023; Received in revised form 24 March 2023; Accepted 5 April 2023
Available online 12 April 2023

usage of TW (Chaganti et al., 2020; Gao et al., 2021; Suarez, 2013; Toze, 2006). Remediation options are available to alleviate salinity/sodicity stress to sustainably make use of the TW resources in arid regions. Application of gypsum as soil amendment can mitigate sodium effects on soil structure and reduce salinity stress by improving soil permeability and thus facilitating leaching of salts from the rooting zone (Oster and Frenkel, 1980; Shainberg et al., 1989).

Additionally, adopting salt-tolerant crops could be a strategy to utilize TW and increase biomass production, as comparable crop productivity was noted for some crops under saline irrigation (Pannell and Ewing, 2006). High biomass-producing crops such as bioenergy sorghum and switchgrass have attracted attention for their salt tolerance and higher water use efficiency (Rooney et al., 2007; Tari et al., 2013; Zhuo et al., 2015) and have proven successful in saline soils (Chaganti et al., 2020). These high biomass-producing crops could be suitable options to increase organic matter return to soil and improve soil health by utilizing TW (Jin et al., 2014; Wu et al., 2021). However, there is lack of data on soil carbon dynamics and the soil microbial community responses and it is not clear if using TW for irrigation and high biomass systems can improve carbon sequestration and soil quality in arid saline soils.

It is important to assess soil carbon dynamics to realize the carbon sequestration potential in arid lands under alternative practices, and to ensure preservation of carbon stocks and minimize further decline of soil quality and productivity. Both soil organic carbon (SOC) and inorganic carbon (SIC) stocks are equally important in arid lands (Lal, 2009), as more than half of total carbon is present in inorganic forms, which is estimated to be between 695 and 1,738 Pg in 1 m depth (Batjes, 1996; Eswaran et al., 1995). Several reports revealed that changes in both SOC and SIC pools in arid soils are closely linked (Díaz-Hernández, 2003) and may exhibit divergent trends under different land-use management practices (Fu et al., 2021; Raza et al., 2020), but their repsosnes to TW irrigation is not clearly understood (Ferdush and Paul, 2021). Thus, agronomic practices must be carefully evaluated for their impacts on both pools of soil carbon to ensure SOC sequestration represents net gains in total soil carbon stocks (An et al., 2019; Raza et al., 2021). Sequestration of SOC refers to net increase in SOC stocks, largely impacted by plant productivity and soil microbial interactions (Liang and Zhu, 2021; Sokol and Bradford, 2019). Microbial community interactions are primarily responsible for organic matter decomposition and stabilization (Bardgett and van der Putten, 2014). Beneficial soil microbial interactions are also critical for nutrient cycling and nutrient availability to plants (Chowdhury et al., 2011), and improving plant tolerance to salinity (Abdelaziz et al., 2017; Shahzad et al., 2017). However, overall microbial diversity and abundance will be lower in saline soil, as microbial communities are sensitive to salinity changes and some taxa are negatively impacted by higher salinity (Jahromi et al., 2008) and soil aridity (Štovícek and Gillor, 2022), which may significantly alter community composition and functions (Rath et al., 2019; Zhou et al., 2017). Several studies noted rapid shifts in some abundant taxa in response to irrigation water induced salinity compared to rare taxa (Ji et al., 2020; Obayomi et al., 2020), whereas some noted positive influence of wastewater irrigation on several dominant taxonomic groups in arid soils (Dang et al., 2019; Ibekwe et al., 2018). Higher crop productivity was noted under wastewater irrigation (Chaganti et al., 2021), whcih may elicit a positive feedback loop for plant–microbe interactions resulting in higher organic matter return and sequestration potential. However, long-term effects of salinity stress may increase under TW, which could interfere with the positive feedback on soil carbon pools and impact net sequestration potential (Rath and Rousk, 2015). Moreover, it is not clear how changes in microbial community structure impacts SIC stocks, as variable effects were noted from no effect of microbial activity to increasing SIC pools through carbonate precipitation (Zhao et al., 2020; Zheng et al. 2022) or driving losses through dissolution (Chang et al. 2012). Direct and indirect role of TW needs to be accounted as well as many nutrients in TW including Ca^{2+} and HCO_3^{-1} can induce pedogenic carbonate formation (Bughio et al. 2016), while nutrients such as DOC and nitrogen may stimulate microbial biomass and SOC sequestration (Hashem and Qi, 2021). However, there is lack of field data to understand these dynamics and particularly, more data is needed on microbial community changes with concurrent data on impacts on SOC and SIC pools in arid saline soils (Ferdush and Paul, 2021).

This study was conducted to understand soil carbon dynamics and soil microbial community changes in response to treated wastewater irrigation in bioenergy sorghum and perennial switchgrass cropping systems. We hypothesized that wastewater irrigation with gypsum amendment would increase microbial biomass and microbial community structure and increase SOC content. The objective of this study was to evaluate the impacts of experimental factors on SOC, SIC, microbial biomass and soil microbial community structure.

2. Material and methods

2.1. Study sites and experimental design

This study was conducted at the long-term research sites located near the Texas A&M AgriLife Research Center, El Paso, TX, USA. This region falls under arid climatic conditions as average annual precipitation is approximately 17 cm and has a potential evapotranspiration of approximately 195 cm. The soil type at the study site is Saneli Silty Clay loam (clayey over sandy or sandy-skeletal, montmorillonitic calcareous, thermic Vertic Torrifluvents). A soil analysis report for the study site is provided in Table 1. A split-plot randomized block experimental design was used in this study with two bioenergy cropping-systems of sorghum (So) and switchgrass (Sg) as the main experimental factor. Sub-experimental factors included two irrigation water sources [freshwater (FW) and treated wastewater (TW)] and two amendment treatments (gypsum amendment (GA) and no-amendment (NA) control). Gypsum amendment treatment received gypsum and sulfur application, at a rate of 10 and 1 Mg ha^{-1}, respectively. Gypsum and sulfur were incorporated into the top 15 cm of the soil before planting in only the first year. Eight treatment combinations were evaluated in this study; (1) So_FW_GA, (2) So_FW_NA, (3) So_TW_GA, (4) So_TW_NA, (5) Sg_FW_GA, (6) Sg_FW_NA, (7) Sg_TW_GA, (8) Sg_TW_NA.

All treatment combinations were replicated three times. Each experimental plot measured 5.6 m long and 2.6 m wide with a total of 24 experimental plots. Individual plots were separated from each other by a 0.60 m wide buffer strip to avoid any edge effects of treatments and lateral percolation of irrigation water into adjacent plots. For both switchgrass and sorghum an inter-row spacing of 0.90 m and intra-row spacing of 0.05 m was followed. Sorghum cultivar ES5200 (Ceres Inc., Thousand Oaks, CA, USA) and switchgrass cultivar "Alamo" were used

Table 1
Physical and chemical properties of soil sample collected from the experimental field plots at 0–15 cm soil depth.

Soil parameter	Value
Sand (%)	41.9
Silt (%)	33.5
Clay (%)	24.5
Texture class	Loam
CEC ($cmol_c$ kg^{-1})	12.9
Bulk Density (g cm^{-3})	1.4
Saturated paste pH	8.31
EC_e (dS m^{-1})	2.64
SAR (mmol l^{-1})$^{0.5}$	4.34
Soluble Na^+ (mg L^{-1})	251
Soluble Ca^{2+} (mg L^{-1})	196
Soluble Mg^{2+} (mg L^{-1})	36.2
Soluble Cl^- (mg L^{-1})	486
Soluble SO_4^{2-} (mg L^{-1})	979

in the study. Sorghum was planted every year in mid-May and was harvested in the first week of November (approximately 150–160 days after planting). Switchgrass was initially transplanted in May of 2017 and harvested once a year in every November after reaching physiological maturity. Both sorghum and switchgrass plots were fertilized once at the beginning of the experiment at 120:120:120 kg ha^{-1} of N: P_2O_5:K_2O in the form of urea (46–0–0), monoammonium phosphate (11–52-0) and sulfate of potash (0–0-50).

Irrigation water was applied as flood irrigation and a total of 0.61 m (applied at m^3/m^2 area) of either fresh or treated wastewater to both switchgrass and sorghum for each cropping season. A total of 7–8 irrigations were scheduled in 3–4-week intervals per cropping season. Freshwater was sourced from the Rio Grande River, which is the main surface water source in the region. Treated wastewater was collected from a local wastewater treatment facility in a 500-gallon tank and was transported to the research site for application. Random samples of fresh and wastewater were collected during the irrigation season and were analyzed for their chemical properties (Table 2). Methods of water analysis are detailed in Chaganti et al. (2021). One additional site was used as a native background control (BC) with no history of agronomic management for the last 20 years, which was located at an adjacent field with similar soil type.

2.2. Soil sampling and preparation

Soil samples were collected from individual experimental plots at the end of growing season of 2018, which was approximately-three years after initiation of the experimental treatments. Three soil samples were collected from individual treatment plot at 0–15 cm soil depths using a hand-held auge. Samples were transported to lab, air dried, ground, sieved through a 2 mm sieve and were homogenized. A subsample was immediately stored at −80 °C until microbial DNA extraction. Remaining samples were stored at room temperature until carbon pool analysis.

2.3. Soil carbon analysis

A subsample of airdried sample was used for SOC and soil inorganic carbon (SIC) analysis using standard protocols (Nelson and Sommers, 1996). Samples were first analyzed for total carbon (TC) and total nitrogen (TN) contents (mg kg^{-1}) using dry combustion method on a total elemental analyzer instrument (Elementar Inc.). Another set of sub samples were acid treated (5 % HCl) in combustion vessels until all reactions ceased and then analyzed for TC using the same dry combustion method. This analysis was reported as SOC-content and was used for obtaining SIC-content after deducting from TC-content. Total elemental analyzer measurements were calibrated using a set of two primary standards and two verified soil standards as check references for setting quality check thresholds. A subsample from the air-dried sample was oven dried at 105 °C for 24 hr to determine the % moisture in air dried sample, which was used for moisture correcting the final concentrations of carbon on dry mass basis.

Total microbial biomass-carbon (MBC) was determined using a modified chloroform-fumigation extraction method (Scott-Denton et al., 2006). Briefly, a set of airdried subsamples were fumigated for approximately 48 hr in airtight containers. Another set of samples without fumigation were used as non-treated controls. Both sample sets were extracted using 0.5 M K_2SO_4 after shaking for 2 hr and analyzed using a wet combustion-based C/N analyzer (Shimadzu Inc.,). MBC was estimated as the difference between K_2SO_4 extracted SOC in the fumigated versus non-fumigated (Joergensen et al., 2011). Microbial biomass to organic carbon ratio (MBOCR) was determined by using the final dry mass concentration of SOC and MBC from individual soil samples.

Table 2
Chemical characterization of fresh water and treated wastewater used in the study. Error values represent standard deviations for five samples.

Property	Freshwater	Wastewater
pH	6.78 ± 0.1	6.86 ± 0.03
ECiw (dS m^{-1})	0.69 ± 0.1	1.75 ± 0.4
SAR	2.62 ± 0.3	5.76 ± 0.7
SAR Adj (mmol/L)	2.85 ± 0.3	5.90 ± 0.7
Na^+ (mg L^{-1})	107 ± 6.4	286 ± 46
DOC (mg L^{-1})	23.31 ± 1.8	28.41 ± 1.4
NH_4^+ (mg L^{-1})	3.35 ± 2.2	7.02 ± 3.4
K^+ (mg L^{-1})	12.9 ± 2.9	25.2 ± 3.0
Mg^{2+} (mg L^{-1})	16.5 ± 1.1	26.6 ± 1.6
Ca^{2+} (mg L^{-1})	101 ± 11	140 ± 14
F^- (mg L^{-1})	0.35 ± 0.1	1.30 ± 0.97
Cl^- (mg L^{-1})	80 ± 12	221 ± 60
NO_3^- (mg L^{-1})	4.75 ± 0.6	59 ± 15
PO_4^{3-} (mg L^{-1})	4.84 ± 3.8	6.42 ± 0.8
SO_4^{2-} (mg L^{-1})	124 ± 20	246 ± 33
CO_3^{2-} + HCO_3^- (mg L^{-1})	118 ± 15	90 ± 7.5

2.4. Microbial community assessment

Microbial DNA from frozen soil sample was extracted using PowerLyser PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. Quality and concentration of extracted DNA were determined spectrophotometrically using a nano-spectrophotometer (GE Health Sciences Inc.,). Prokaryotic diversity was estimated by sequencing the V4 region of the 16S rRNA genes amplified by primers S-D-Arch-0519-a-S-15, 5′-CAGCMGCCGCGGTAA-3′, and S-D-Bact-0785-b-A-18, 5′-TACNVGGG-TATCTAATCC-3′ (Klindworth et al., 2012) and fungal diversity by sequencing the ITS2 marker with primers ITS7 5′- GTGAATCATC-GAATCTTTG-3′ and ITS4 5′- TCCTCCGCTTATTGATATGC −3′ (Ihrmark et al., 2012; White et al., 1990). Paired-end sequence data were generated on an Illumina MiSeq instrument (Illumina, San Diego, CA) as described in the Illumina 16S metagenomic sequencing library preparation protocol. The raw sequencing reads were processed with a combination of QIIME 1.9.1 (Caporaso et al., 2010) and USEARCH 8.0.1 (Edgar, 2010) software packages. Individual ITS sequence tags were compared to the UNITE fungal ITS sequence database (Abarenkov et al., 2010) and individual 16S sequences were compared to the Silva database 128 (Quast et al., 2012) using UCLUST in order to pick referenced-based (prokaryotes) operational taxonomic units (OTUs) at 97 % similarity. The OTU abundance datasets were further normalized using cumulative sum scaling (CSS) transformation (Paulson et al., 2013) available on the QIIME platform. All the sequence data have been deposited in the NCBI Genbank database under project numbers PRJNA871212.

2.5. Statistical analysis

Experimental data were analyzed to compare treatment impacts on soil chemical and microbial parameters. The statistical model was designed to compare individual treatment factors of crop systems (SO vs SG), two irrigation water sources (FW vs TW) and soil amendment (GA vs NA), and their interactions through a three-way factorial ANOVA. Additionally, the mean differences (based on least mean square difference) were estimated among the individual treatments for comparing the differences among the treatments for SOC, SIC, TC, MBC and MBOCR. ANOVA and mean difference analyses were performed in SAS software (SAS Inc.). The OTU abundance with taxonomy classification was used to prepare a graphical depiction of prokaryotic diversity among the experimental variables. A two-way permutation multivariate analysis of variance (PERMANOVA) was used to test the significant differences in community structure between the experimental treatments based on Bray-Curtis distance measured between the groups (Anderson, 2001). Canonical correspondence analysis (CCA) was performed using PAST 3.1 software on OTU abundance data with corresponding soil biochemical data as environmental variables for ordination axes. The implementation of CCA in PAST software follows the eigen analysis algorithm (Legendre and Legendre, 1998) with each environmental variable plotted as correlations with abundance scores.

3. Results

3.1. Impact of experimental treatments on soil carbon pools and microbial biomass

Mean content of SOC and TC were significantly different ($p < 0.01$) between the crop factor (Table S1). Crop × water interaction factor significantly impacted the SOC content, but there was no significant difference between the amendment factor (GA vs NA) or among any other interactions (Table S1). Higher SOC and TC content was observed in the sorghum (So) plots, which recorded 6.58 and 13.58 g kg^{-1} SOC and TC, respectively, compared to the switch grass (Sg) plots, which recorded 4.74 and 12.66 g kg^{-1} SOC and TC, respectively (Fig. 1). Between the water factor, mean SOC and TC contents were significantly ($p < 0.05$) higher in the TW-plots, which recorded 6.10 and 13.47 g kg^{-1} SOC and TC, respectively, compared to the freshwater (FW) irrigated plots, which recorded 5.38 and 12.77 g kg^{-1} SOC and TC, respectively (Fig. 1). Highest mean SOC and TC content was observed in the treatment So_TW_GA (7.65 and 14.29 g kg^{-1}, respectively) and lowest in the treatment Sg_FW_GA (4.86 and 12.09 g kg^{-1}) (Fig. 1). Highest mean SOC content was observed in the So_TW plots at 7.48 g kg^{-1} and lowest in the Sg_TW-plots at 4.73 g kg^{-1}. Mean content of SOC and TC in BC-plots were 4.58 and 12.74 g kg^{-1}, respectively.

Mean content of SIC was significantly different ($p < 0.01$) between the crop factor, but there was no significant difference between other factors and interactions (Table S1). Somewhat opposite trends were seen for SIC contents compared to SOC and TC in response to the treatments. Mean content of SIC was significantly higher in the Sg-plots, which recoded 8.00 g kg^{-1} compared to the So-plots, which recorded 6.99 g kg^{-1} (Fig. 1). Highest mean SIC contents were observed in the BC-plots at 8.15 g kg^{-1}, followed by the treatment Sg_TW_NA at 7.96 g kg^{-1} and the lowest in the treatment So_TW_GA at 6.63 g kg^{-1} (Fig. 1).

Microbial biomass carbon (MBC) was significantly higher in BC-plots (1.15 g kg^{-1}) compared to all experimental plots (Fig. 2), but there was no significant difference between the treatment factors (Table S1). The MBC level in the experimental plots ranged between 0.55 and 0.70 g kg $^{-1}$, which corresponded to 3.9 to 9.72 % of TC, respectively. However, the microbial biomass to organic carbon ratio (MBOCR) metric which represented MBC as a percentage of SOC, was significantly different between the crop factor (Table S1), as consistently higher ratios were noted in the Sg-plots compared to So-plots (Fig. 2). other factors did not significantly influence the MBOCR (Table S1). The highest MBOCR was noted in the BC-plots at around 0.25 and lowest in the treatment So_TW_NA at around 0.07. The relative proportion of organic to inorganic carbon (OICR) in the treatments was significantly influenced by crop, by water source ($p < 0.05$) and their interactions (Table S1). Pearson correlation analysis between the analysis parameters indicated a positive significant correlation between TC and SOC, and between TC and OICR (Table 3 and Figure S1). A significant negative correlation was observed between SOC and SIC (Table 3 and Figure S1). Additionally, significant negative correlation was observed between the SOC and MBC and between CNR and OICR and a significant positive correlation was observed between the SOC and TN (Table 3).

3.2. Microbial community structure under different treatments

Diversity of prokaryotes was mostly higher in all treatment plots compared to the BC-plots. The Shannon diversity index for prokaryotic community in BC plots was at 5.42 ± 0.17compared to above 6 in most treatment plots, with highest recorded in the treatment So_TW_NA at 6.24 ± 0.18 (Table S2). The Shannon diversity index for fungal community in BC plots was at 3.4 ± 0.35, and in treatment plots it ranged between 3.41 ± 0.1 and 3.86 ± 0.07, and there was no significant differences the treatments (Table S2). Two-way permutation multivariate analysis of variance (PERMANOVA) indicated that prokaryotic community composition was significantly different ($p \leq 0.05$) between the two crop treatments (Table 4). Within the So-plots, the prokaryotic community was significantly altered by the W × A interaction effect (Table 4), but there were no significant differences within Sg-plots. The fungal community was not significantly altered between the experimental factors.

Canonical correspondence analysis (CCA) further validated that prokaryotic community structure in most treatment-plots shifted significantly compared to BC plots (Figure S2). A separate analysis leaving out the BC_treatment showed that the prokaryotic community largely separated by TW-treatments, and by crop-amendment interaction within the TW-treatment plots (Fig. 3). Among the soil metadata used in this analysis, MBC, TC, SAR and TN were the major soil parameters that diverged along the community structure shifts. Soil parameters of SAR, pH, TC and TN largely aligned with TW-treatment, whereas MBC aligned largely with FW-treatments.

The relative abundance of bacterial phyla indicated that Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes and Bacteroidetes constituted the major phyla in all the treatments (Fig. 4). Relative abundance of Proteobacteria, Acidobacteria and Bacteroidetes increased in all treatment plots compared to the BC-plots. Whereas Actinobacteria

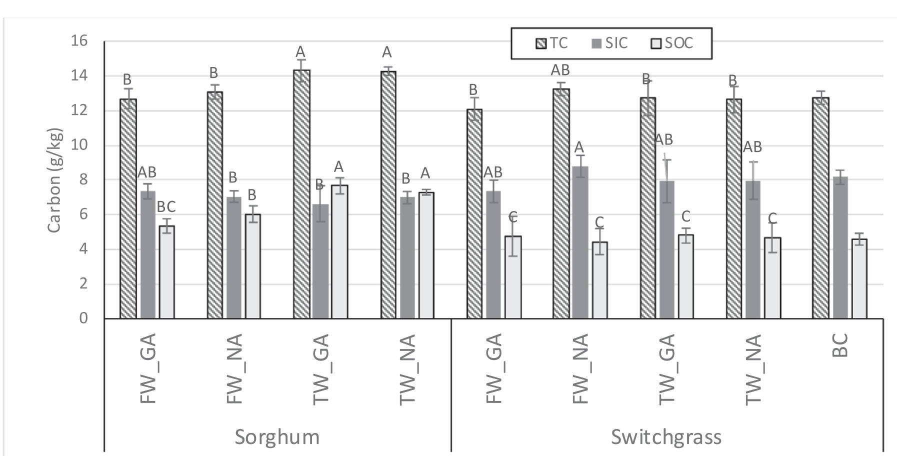

Fig. 1. Mean content of total carbon (TC), soil inorganic carbon (SIC) and soil organic carbon (SOC) under different treatments. Error bars denote standard deviations. Letters above the bars represent mean differences. Different letters represent significant difference based on LSD test at $p < 0.05$.

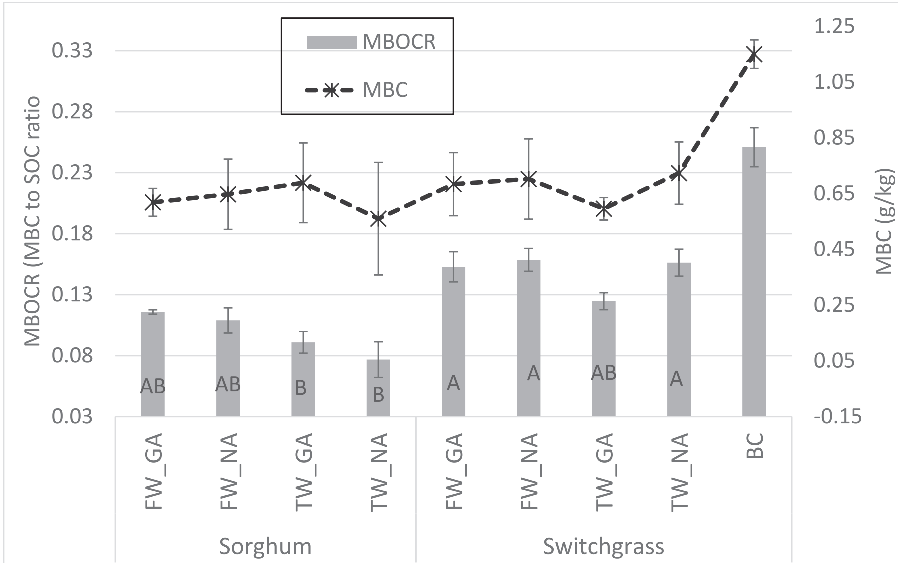

Fig. 2. Mean content of microbial biomass-carbon (MBC) and microbial biomass to organic-carbon ratio (MBOCR) ratio under different treatments. Error bars denote standard deviations. Letters within the bars represent mean differences. Different letters represent significant difference based on LSD test at p < 0.05.

Table 3
Pearson correlation between the individual parameters.

Parameters[a]	TC	SIC	SOC	TN	CNR	MBC	MBOCR
SIC	0.20						
SOC	0.60**	−0.67**					
TN	0.41**	−0.18	0.46**				
CNR	0.33*	−0.60**	0.73**	−0.26			
MBC	−0.34*	0.05	−0.30*	−0.35*	−0.07		
MBOCR	−0.51**	0.40**	−0.72**	−0.47**	−0.42**	0.85**	
OICR	0.33*	−0.85**	0.94**	0.39*	0.73**	−0.24	−0.65**

*$p < 0.05$ and ** $p < 0.01$.
[a] TC = total carbon, SIC = soil inorganic carbon, SOC = soil organic carbon, CNR = carbon to nitrogen ratio, MBC = microbial biomass carbon, OICR = organic to inorganic carbon ratio, MBOCR = microbial biomass to organic carbon ratio.

Table 4
Permanova analysis results for prokaryotic and fungal community composition between the experimental treatments. Water and amendment factors were tested within the individual crop treatments.

Crop Factor	Prokaryotic	Fungal
Total sum of squares:	0.4558	0.4442
Within-group sum of squares:	0.4021	0.4189
F:	2.936	1.325
p (same):	0.0498	0.1656

	Source	Switchgrass Sum of squares	Mean square	F	p	Sorghum Sum of squares	Mean square	F	p
Prokaryotes	Water	0.017	0.017	1.534	0.216	0.043	0.043	2.785	0.088
	Amendment	0.016	0.016	1.457	0.240	0.023	0.023	1.502	0.244
	Interaction	0.007	0.007	0.622	0.531	0.080	0.080	5.143	0.014
	Residual	0.089	0.011			0.125	0.016	0.569	
	Total	0.130				0.272			
Fungal	Amendment	0.021	0.021	1.066	0.368	0.017	0.017	0.925	0.493
	Soil	0.020	0.020	1.033	0.399	0.014	0.014	0.750	0.700
	Interaction	0.033	0.033	1.678	0.083	0.012	0.012	0.680	0.791
	Residual	0.156	0.020			0.146	0.018		
	Total	0.230				0.189			

decreased in all the treatments compared to the BC-plots. Significant shifts between the treatments were not observed at phylum level. However, differences at OTU level were evident, as several species within the taxonomic-orders Solirubrobacterales and Rhodospirillales decreased significantly in So and Sg-plots compared to BC-plots (Figure S3). Whereas several species within the family *Saprospiraceae* and phylum *Gemmatimonadetes* increased significantly in So- and Sg-plots compared to BC-plots. Several bacterial OTUs were differentially

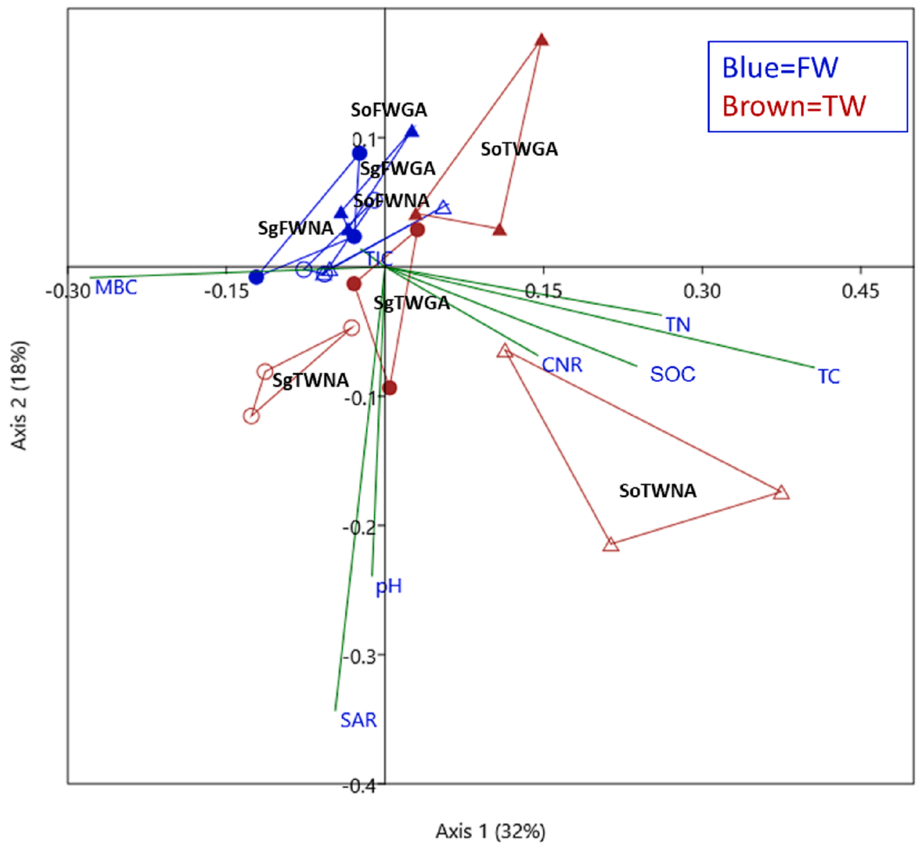

Fig. 3. Scatter plot showing the canonical correspondence analysis (CCA) of distance matrix of prokaryotic OTU abundance with soil parameters, as influenced by experimental treatments. Several soil parameters were used as environmental variables for correspondence correlation based on eigenvalue estimates.

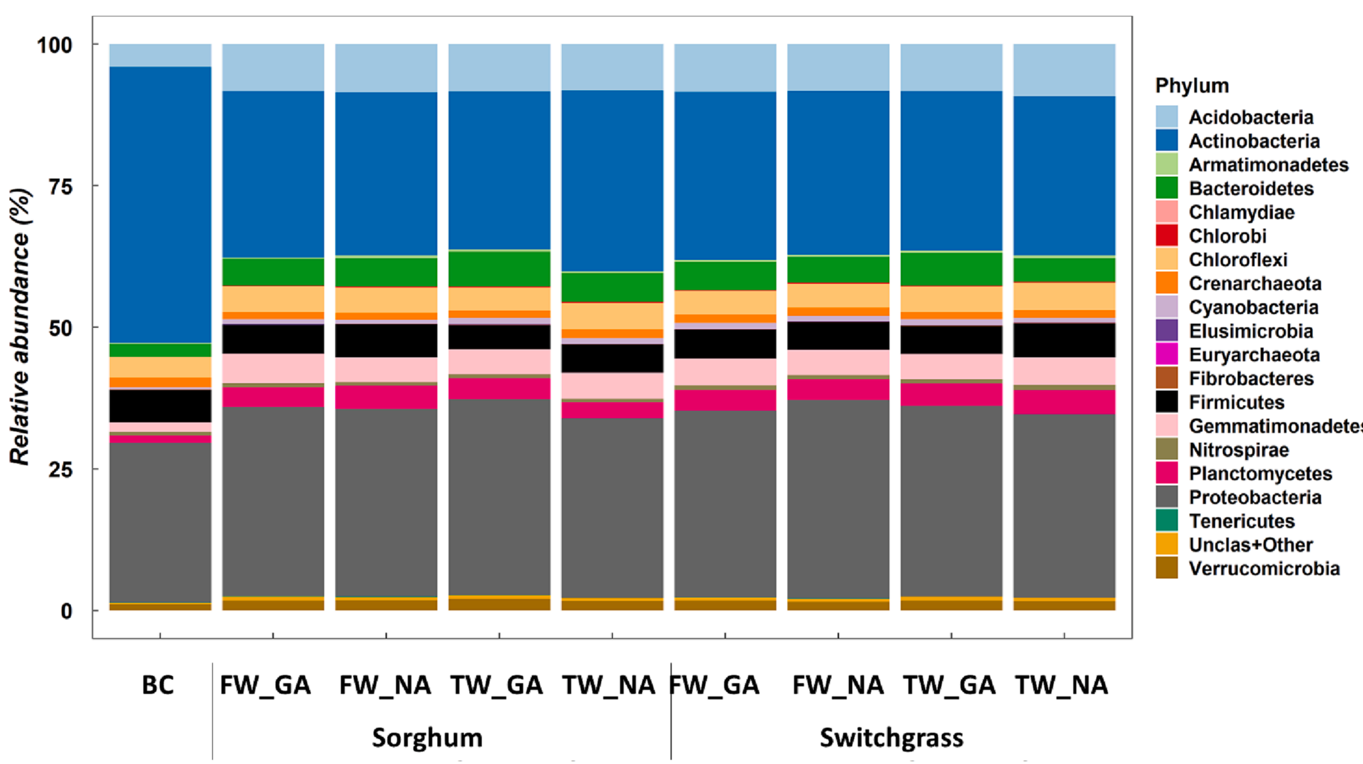

Fig. 4. Relative abundance of Bacterial phyla under experimental treatments.

abundant between the two treatments of So_TW_GA vs Sg_FW_NA. Several species within family *Saprospiraceae (*phylum *Bacteroidetes*), genus *Flavisolibacter (*phylum *Bacteroidetes),* order *Solirubrobacterales (*phylum*Actinobacteria)*, genus *Rubrobacter* and family *Xanthomonadaceae* were at higher abundance in the treatment So_TW_GA compared to the treatment Sg_FW_NA (Figure S3). Whereas, several species with genus *Rubrobacter*, order *Solirubrobacterales*, genus *Pseudonocardia* and genus *Jiangella* were at lower abundance in the treatment So_TW_GA compared to the treatment Sg_FW_NA. Among the fungal phyla (Fig. 5). *Ascomycota, Zygomycota* and *Unclassified taxa* were the major groups in all treatments. *Ascomycota* constituted up to a relative abundance between 60 and 82 %. In most treatments, *Ascomycota* increased compared to background sites, whereas unclassified fungi and *Basidiomycota* significantly decreased (Fig. 5). Phylum Glomeromycota, which represents most arbuscular mycorrhizal fungi constituted<1 % of relative abundance, which increased in most treatments compared to BC plots. Major OTUs detected in the experimental treatments were *Funneliformis mosseae, Claroideoglomus etunicatum, Septoglomus viscosum, Glomus cubense and Rhizophagus fasciculatus* (Figure S3). Two OTUs detected in BC plots *were Rhizophagus fasciculatus* and *Septoglomus viscosum.*

4. Discussion

Results of this field study revealed the relationships between microbial community responses, SOC and SIC stocks and divergent impacts of TW and crop treatment interactions on soil carbon dynamics. Water and crop treatments significantly altered SOC and SIC contents compared to background native plots (BC treatment), confirming that both pools of carbon were sensitive to agronomic treatments and contributed to soil carbon-fluxes in these arid-saline soils. As a result, major differences in TC contents were observed between the treatments but were largely influenced by changes in SOC. Contents of TC and SOC were higher in several TW treatments compared to the respective FW treatments and BC plots, which supports the study hypothesis that TW irrigation increased the SOC content. However, a crop-water interaction effect was more prominent, as higher SOC was mostly seen in the So_TW treatments compared to the Sg_TW treatments. Highest SOC and TC was recorded in the treatment So_TW_GA, suggesting that gypsum amended bioenergy sorghum systems under TW irrigation is a potential climate-smart agronomic system for increasing soil carbon sequestration in these soils. One of the drivers for this effect by TW-irrigation could be higher soil organic matter (SOM) contribution through root-microbe interactions induced by higher nutritional value of TW, as both N and dissolved organic carbon contents were higher compared to FW (Table 2). Nitrogen value of treated TW was noted as a major factor driving microbial community in a previous study (Frenk et al., 2015). Microbial diversity, which is an indicator of higher root-microbe interactions (Zhang et al., 2017), was slightly higher (total Shannon diversity for prokaryotes and fungi) in GA amended compared to their respective NA amended plots which received TW, which suggest the positive effect of TW on microbial community only when used in combination with gypsum amendment. Similar trends of increasing alpha and beta diversity were observed in another study with significant changes to microbial community composition under wastewater application (Dang et al., 2019).

According to PERMANOVA analysis, the prokaryotic community was largely different between the water and crop treatment factors, but there were no major shifts in fungal diversity and composition. This suggests that prokaryotes were more responsive to agronomic treatments while fungi were relatively stable and were not as responsive. Studies have noted similar trends in arid soils as fungi were not impacted by minor modifications in salinity stress or nutrient fluxes (Kamble et al., 2014; Rath et al., 2016). It was also interesting to note that prokaryotic community structure was significantly different between the sorghum and switchgrass, suggesting that the differences in SOC and SIC contents between the crop treatments could be influenced by microbial community changes. It is well established that microbial community is a strong driver of SOC sequestration and generally elicits positive impacts in low-carbon soils (Bastida et al., 2021). It was also interesting to note within CCA-ordination analysis that the prokaryotic community of TW-treated plots diverged from FW-plots (Fig. 3), as water-factor showed somewhat contrasting trends for SOC and SIC contents (Fig. 1). These

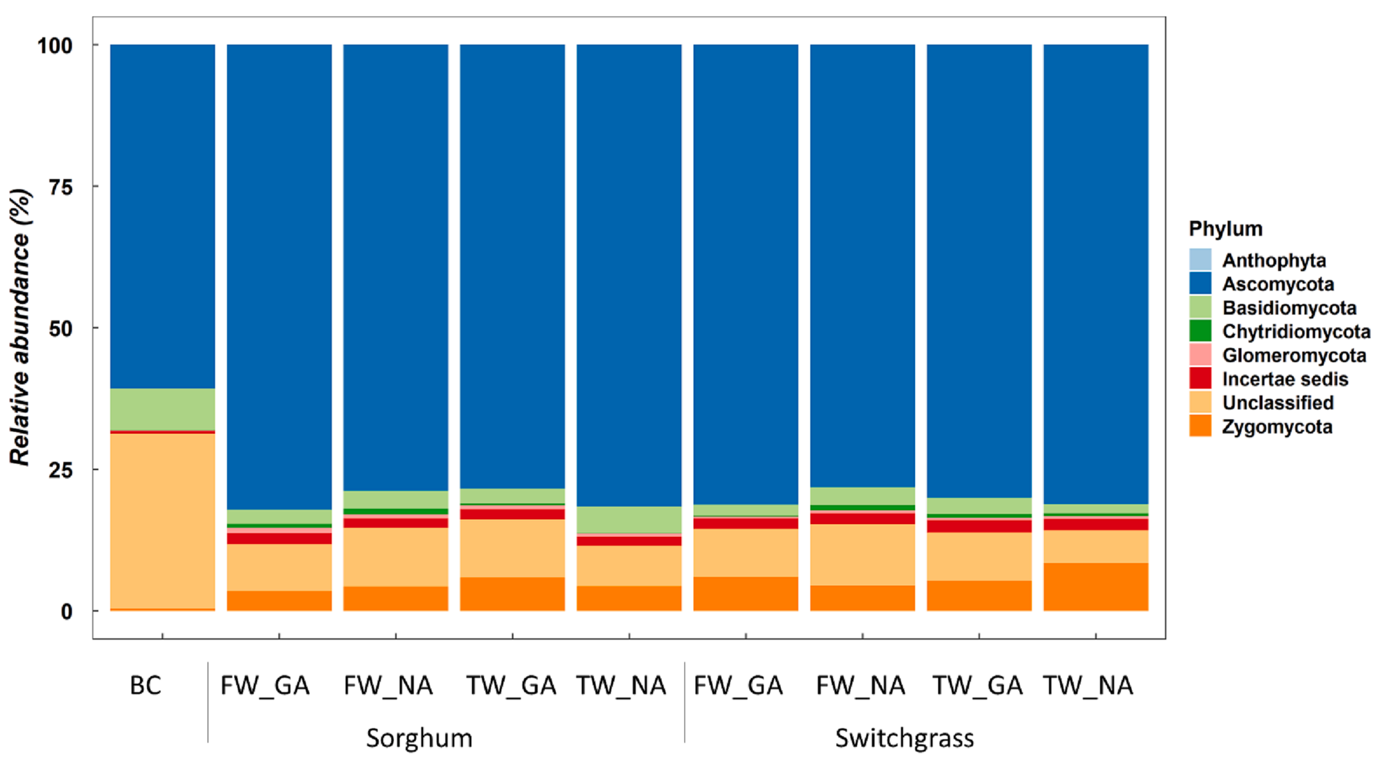

Fig. 5. Relative abundance of Fungal phyla under experimental treatments.

results support the assumption that the resultant microbial community structure in TW contributed to higher organic carbon sequestration. There is not much evidence for direct impacts of specific microbial groups on soil carbon sequestration in arid soils (Hartman et al., 2017). Generally, salt stress-induced changes to the microbial community were noted to negatively impact metabolic growth and overall soil respiration and diminish carbon decomposition potential (Setia, 2013). However, nutrient rich TW probably induced positive feedback loops between root-microbe interactions and increased microbial metabolism. Increasing root-microbe interactions was shown to increase SOC in a low carbon soil (Somenahally et al. 2020) and could also enhance organic carbon stabilization processes (Kästner et al., 2021). Several taxa were enriched in So_TW-GA treatment compared to BC, as higher relative abundance was observed within *Proteobacteria* and *Ascomycota* (Figs. 4 and 5). Whereas *Actinobacteria* decreased in the So_TW-GA treatment compared to BC treatment. These taxa are considered copiotrophs, which demonstrate higher growth rates in response to nutrient pulses in arid soils (Chen et al., 2021; Vallejos et al., 2022). Particularly, members of *Actinobacteria* are typically well-represented in arid soils and showed enhanced tolerance to desiccation (Neilson et al., 2012; Šťovíček et al., 2017). These results suggest some reorganization among copiotrophic taxa in response to experimental treatments, which may have impacted SOC and SIC turnover. Studies have reported these taxa as a major group of microbes involved in SOM decomposition in arid soils (Mavi and Marschner, 2012). One study indicated that a copiotroph-dominated bacterial community may contribute to SOM accumulation in soils (Wang et al., 2022) confirming the assumption that higher microbial growth rate can return higher microbial residue derived SOM to the soil (Shao et al., 2021). Our results confirm the nutritional value of TW as a major driver for enhancing the copiotroph-dominated bacterial community, which probably contributed to a higher rate of organic carbon accumulation in some treatments. However, it must be noted that gypsum amendment may have mitigated negative effects of sodium in TW-treatments, similar to the results seen in another study (Angin et al., 2005).

A significant quantity of TC in the surface soil was constituted within the inorganic pool, with mean SIC contents ranging between 6.6 g kg^{-1} to 8.8 g kg^{-1}, which corresponded to 46 and 66 % of the TC, respectively. These contents are within the range typically observed in saline soils within arid climates (Viscarra Rossel et al., 2016), which substantiates the importance of SIC stocks in arid soils and that even smaller changes could significantly impact carbon storage potential in arid lands (Raza et al., 2021). Results indicated disparate trends between SIC and SOC in response to several experimental treatments. For example, SIC contents were significantly lower in So-plots compared to Sg-plots, and in some TW treatments within So-plots compared to FW treatments and BC plots. Results of this study indicated that the native SIC was mostly conserved in Sg-treatments, whereas some quantity of SIC appeared to be lost in the So_TW plots, which recorded the highest content of SOC, suggesting contrasting trends between the two pools in response to this treatment. There is some evidence connecting the SOC and SIC cycling (Yang et al., 2010) and several studies reported a negative relationship between the two carbon pool changes (Zhao et al., 2019; Zhao et al., 2016). As discussed earlier, the microbial community structure was significantly different in the treatment So_TW_GA compared to others, which suggest the potential role of responsive microbial community in diminishing SIC content. There is some evidence to support that microbes are responsible for recycling some SIC into SOC pool in saline coastal soils (Shao et al., 2022), and another study showed potential SIC conversion to SOC through chemoautotrophic and heterotrophic pathways in arid soils (Liu, et al., 2021). Other studies noted microbial activity resulted in loss of SIC from the surface soil (Bughio et al., 2016), as microbial respiration and other metabolic processes could alter soil pH, pCO_2 and carbon-mineral complexes, which can destabilize carbonate mineral phases and promote dissolution (Jin and Evans, 2007; Liu et al., 2018; Monger et al., 1991). It is probable that some of the dissolved carbonates may have just leached down the soil profile and were not lost as CO_2 emissions from the system (López-Ballesteros et al., 2017). However, field observations are lacking for direct impacts of microbial activity on long term impacts on SIC stocks (Ferdush and Paul, 2021). Particularly, it is important to understand whether saline soil microbial community shifts towards copiotroph-dominated community structure, which potentially resulted in SOC sequestration, caused any permanent loss of SIC stocks from the surface soil.

5. Conclusions

This study presented many novel results validating the potential role of microbial community responses on SOC and SIC contents. Results showed that a wastewater-irrigated bioenergy sorghum cropping system amended with gypsum significantly increased SOC content and resulted in net increase of TC content. This combination of agronomic practices appeared to be a promising land-use practice for achieving higher soil carbon sequestration in arid saline soils. However, this treatment resulted in some decline of SIC, revealing divergent effects of land use practices on SIC and SOC -pool fluxes. Significant shifts in microbial community structure were observed in response to many treatments and a copiotroph-dominated prokaryotic community was assembled under TW irrigation, but no negative impacts were observed on microbial biomass and microbial diversity. Increases in soil organic matter by TW irrigation without significantly impacting the soil microbial community is appealing to the value of recycling TW irrigation for sustainable agriculture in arid climate. However, long-term effects on salinity and toxicity concerns must be evaluated before widely considering TW as a reliable source of irrigation (Hashem and Qi, 2021). Establishing temporal thresholds of salinity buildup and soil health metrics will be valuable information to guide mitigation strategies to prevent negative consequences under long-term irrigation. It was also clear that both SOC and SIC carbon pools were sensitive to land use management, which underscores the need for considering both forms of carbon while assessing soil carbon sequestration in arid saline-soils. Potential linkage between organic and inorganic carbon cycling warrants further investigations under different scenarios of carbon sequestration in arid soils, particularly for delineating SIC stock responses over long term.

CRediT authorship contribution statement

Anil C. Somenahally: Conceptualization, Methodology, Writing – original draft. **Javid McLawrence:** Methodology. **Vijayasatya N. Chaganti:** Investigation, Writing – review & editing. **Girisha K. Ganjegunte:** Conceptualization, Investigation, Writing – review & editing. **Olabiyi Obayomi:** Software, Writing – review & editing. **Jeff A. Brady:** Software, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

This study was supported by USDA Project No. 2017-68007-26318, through the National Institute for Food and Agriculture, Agriculture and Food Research Initiative. Part of Dr. Somenahally's salary was supported by USDA-NIFA Hatch project (Accession No. 1024085 and Project number: TEX0-1-9603) and USDA-NIFA McIntire-Stennis project (Accession No. 1017765 and Project number: TEX09754). Part of Dr.

Ganjegunte's salary was supported by USDA-NIFA Hatch project (Accession No. 1001806 and Project number: TEX0-1-9162).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110227.

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