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ORIGINAL PAPER

Effects of seawater irrigation on soil microbial community structure and physiological function

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Abstract Irrigation with diluted seawater would be an alternative water resource which can play an important role under scarce resources of freshwater for promoting agricultural production in coastal areas. *Salvadora persica* Linn. was irrigated with different concentrations of seawater (0, 10, 20, 40, 60, 80 and 100 % seawater), and their effect on plant growth, nutrient contents in soil and plants, shift in soil microbial community structure (phospholipid fatty acid; PLFA) and community-level physiological profiling (CLPP, Biolog ECO MicroPlate) were studied. Plant dry matter was significantly increased with all seawater treatments, and highest increase was at 20 % seawater treatment. Sodium and chloride contents were significantly increased, whereas ratios of K/Na and Ca/Na were significantly decreased in plants with seawater irrigation. Soil electrical conductivity (EC), available K and Na were significantly increased with increasing the concentration of seawater. Total PLFA concentration and PLFA profile of soils were used as indices of total microbial biomass and community composition, respectively. The concentrations of total PLFA, gram-positive, gram-negative and actinomycetes biomarker PLFAs were significantly reduced at 20, 40, 80 and 40 % concentrations of seawater, respectively. The application of different concentrations of seawater induced a clear shift in the soil microbial community structure toward the bacterial abundance. The microbial community structure and community-level physiological profiling in seawater irrigation treatments had significantly differentiated. It can be concluded that irrigation with different concentrations of seawater had significant impact on soil chemical and microbial properties which is attributed due to the salinity stress.

Keywords Community-level physiological profiling · Phospholipid fatty acid · Halophytes · Nutrients · Seawater agriculture

Introduction

Soil salinity is one of the world's oldest and most serious problem in the arid and semiarid regions and a major threat to agricultural production and ecosystem sustainability. Seawater intrusion into coastal aquifers is one of the causes for salinity problems in coastal regions. In India, about 30 million ha of coastal land remains barren and uncultivable because of higher soil salinity (Singh and Surendra 1994). Higher concentrations of salts in soil solution have harmful effects on plant growth and crop yield, and they can be lethal to plants (Munns 2002). Salt-tolerant plants have the ability to minimize these harmful effects by adopting anatomical, morphological and physiological characteristics such as an extensive root system, succulent stem and salt-secreting glands on the leaf surface (Ungar 1991). Availability of water is reduced in saline soils due to lower osmotic potential in soil water created by high salt concentration. In addition to salt-induced water stress, salt-stressed plants also experience negative effects on growth and development due to nutrient imbalance and ion toxicity (Hardikar et al. 2011). The uptake of nutrient by plants has

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been frequently studied under saline soil conditions (Patel and Pandey 2007; Patel et al. 2010; Hardikar et al. 2011), but the relationship between uptake of plant nutrient and soil salinity is rather multifaceted and remains poorly understood (Tozlu et al. 2000). An investigation of growth and nutrient uptake of plants by seawater irrigation can provide insights into the mechanisms that plants are tolerant of salt stress.

Increasing salt concentration in soil could have various effects on the soil physical, chemical and biological characteristics. Coastal soils are often at high risk of salinization because either they are fed by tidal water or irrigation with saline water results in salt concentrations built up over time because of evaporation. Plenty of information is available on the effects of salinity on the physical and chemical characteristics of soil (Ramoliya et al. 2006; Patel and Pandey 2007; Patel et al. 2010); however, soil microbial activity in saline soils is scantily studied. Soil microbial communities and their activities are significantly affected by salinity (Pankhurst et al. 2001; Baumann and Marschner 2013) which are known to alter by biotic and abiotic stresses. The extent of changes in the microbial community structure is difficult to study, since this usually involves isolation and identification of bacteria that can grow in pure culture on agar plates (Bååth et al. 1992), but minor part of total microbial community could be cultured. One way to overcome this problem is to investigate the phospholipid fatty acid (PLFA) profile of soil microbial community (Chaudhary et al. 2012). Since soil microbes are characterized by the PLFA biomarker, an altered PLFA profile in a soil would exhibit a shift in microbial community composition.

Microbial community changes in sediments have been explored where an increase in salinity had no effect on terminal restriction fragment length polymorphism (T-RFLP) patterns of 16S rRNA (Bernhard et al. 2005; Edmonds et al. 2009), whereas decreased microbial activity is determined by PLFA (Baumann and Marschner 2013). Soil salinity significantly affects microbial activity, growth as well as microbial community composition also (Hollister et al. 2010; Baumann and Marschner 2013). Particularly, the fungal biomass estimated by PLFA (Pankhurst et al. 2001) or ergosterol analysis (Sardinha et al. 2003) was strongly declined under saline soils which result in increased bacteria/fungi ratio under soil salinization. Higher attention has been paid to use of culture-independent (like PLFA) methods for microbial communities studies in ecosystem functioning.

There are inherent difficulties in analyzing microbial species richness and abundance, and it has been suggested that the creation of functional species equivalents based on functional classification of microbial communities. The functional classification of microbial communities based on carbon substrate utilization pattern (Biolog ECO MicroPlate) has been frequently used for examining of community-level physiological functions (Garland and Mills 1991). Potential microbial functional diversity is defined as the number and type carbon substrate sources used by the microbial community (Goodfriend 1998). Functional diversity is a valuable parameter for comparison of differences in ecosystems, habitats, or sites. The objective of present study was to ascertain the functional relationships among soil microbial communities from different concentration of seawater irrigated soil.

Salvadora persica Linn. (Salvadoraceae) is evergreen perennial halophyte and is one of the dominant halophyte species in coastal area of Gujarat (India), where the salt concentration of the soil would inhibit the growth of most other crops. It is considered an important crop plant for saline coastal areas due its oil content, pharmaceutical application, animal feed and energy values, small but edible fruits, and many others. Seeds from *S. persica* plants contain 30–40 % of oil (Gupta and Saxena 1968) with high percentages of lauric and myristic acid. Plant extracts of *Salvadora* have showed antifungal, antibacterial and antiviral properties and also have a variety of other medicinal uses (Zodape and Indusekhar 1997). Though, the results on the physiology of salt tolerance of *S. persica* and cultivation are well documented (Gururaja Rao et al. 1999, 2000, 2001), but soil microbial community change with seawater irrigation has not been previously reported. Therefore, this work was structured around the hypothesis that seawater irrigation with different dilutions to agricultural soil has direct effects on nutrient content, microbial community structure and function through increased soil salinity. In present study, we have applied the phospholipid fatty acid (PLFA) profiling and Biolog ECO MicroPlate approaches in order to gain an insight into the soil microbial community structure and functions in *S. persica* grown with different salinity levels.

Materials and methods

Soil and experimental design

Soil (fine montmorillonitic hyperthermic Vertic Halaquepts) was collected (0–20 cm) in July 2011 from agricultural farmers field of Bhavnagar, Gujarat, India (N21°40′24 and E72°09′54). Annual average long-term rainfall and temperature are 539 mm and 27.8 °C, respectively. The cation exchange capacity and $CaCO_3$ content in soil were 35.52 cmol kg^{-1} and 14.1 %, respectively. The textural composition of soil was 54 % sand, 14 % silt and 32 % clay with sandy clay loam texture. Field collected soil was passed

through 2 mm sieve, homogenized and used for the pot experiment.

Earthen pots were filled with 24 kg (dry weight basis) soil. *Salvadora* (*Salvadora persica*) seeds were collected from coastal region (Dholera) of Bhavnagar district of Gujarat, India, and three seeds were sown in each pot at a depth of 1 cm in the month of October 2011. At 4–5 leaves stage, one healthy plant per pot was retained and allowed to grow. Plants were fertilized with 11.75 mg P kg^{-1} and 60 mg N kg^{-1} soil through KH_2PO_4 and urea, respectively, and placed outdoors under natural photoperiod, temperature, and humidity conditions to mimic the natural existing environmental conditions. During the study period, monthly averaged temperature and relative humidity were ranged between 19.4 (January) to 32.6 °C (May) and 41 (March) to 80 % (August), respectively. Pot experiment was arranged in completely randomized design. Pots were moved around every week to reduce the variations due border effect.

The plants were treated with various concentration of saline water prepared by diluting 100 % seawater with municipal freshwater. The treatments consisted of different concentration of seawater, i.e., 0, 10, 20, 40, 60, 80 and 100 %, and each treatment was replicated three times. Application of seawater started after 1 month of growth as per treatment. Plants were harvested after 11 months (August 2012) of growth. The rainfall received in the months of Oct 2011, May 2012, June 2012, July 2012 and August 2012 was 8.1, 0.7, 53, 90 and 79 mm, respectively, whereas there was no rain in other months during the study period.

Growth parameters and nutrient analysis

After harvesting of plants, dry biomass was estimated after drying of biomass to a constant weight at 65 °C in hot air oven. The plant samples were washed, dried at 65 °C in oven and ground by stainless steel ball mill (Retsch, Germany). The nitrogen (N) content in plant samples was determined by the Elemental analyzer (Elementar, Vario Micro Cube, Germany). Phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), sulfur (S) and chloride (Cl) concentrations were determined by wavelength-dispersive X-ray Fluorescence Spectrometer (WDXRF) (Bruker AXS, S4 Pioneer). The XRF has an advantage for simultaneous measurement of the elements present in plant samples with direct analysis (Queralt et al. 2005). Pellet of plant powder can hardly be done without addition of a binder; we prepared a powder pellet by mixing 3 g of plant powder with 1 g of boric acid (binder) and homogenized (Saini et al. 2002). These prepared plant sample pallets were pressed at 20 t to obtain a cylindrical pellet (40 mm, diameter) in aluminum cups. Certified reference material (CRM) of plant samples were used for calibration and quality check of the analysis. For calibration, the CRMs used were SRM 1515 apple leaves, and SRM 1573a tomato Leaves with elements Na, K, Ca, Mg, B, Mn, Cl, Fe, P, Cu, S, Zn and Mo (Patidar et al. 2013). These CRMs were supplied by National Institute of Standards and Technology (NIST), USA.

Biochemical analysis of soil was carried out after the harvest of *Salvadora*. Soil subsamples were air-dried, ground in wooden mortar and pestle, and passed through 2 mm sieve. Soil pH and electrical conductivity (EC) were determined using 1: 2.5 (w: v) soil: water ratio. Soil organic C was determined by the Walkley-Black wet oxidation method (Nelson and Sommers 1982). Available N was determined by alkaline-permanganate method (Subbiah and Asija 1956), while available P was extracted with 0.5 M $NaHCO_3$ (Olsen et al. 1954) and estimated by spectrophotometer (Murphy and Riley 1962). Available K and Na were extracted with neutral normal NH_4OAc (Hanway and Heidel 1952) and determined by flame photometer.

Phospholipid fatty acid (PLFA) profiles

Phospholipid fatty acids from soil were extracted in three steps using the methods of Bardgett et al. (1996) and Frostegård et al. (1993). In summary, lipids were extracted from soil using citrate buffer, chloroform and methanol at a ratio of 0.8:1:2 and then fractionated into neutral-, glycol- and phospholipids on silicic acid column. The phospholipids were then subjected to alkaline methanolysis and were analyzed by Agilent 6850 gas chromatograph (GC) system (Agilent technologies Inc.) equipped with an Agilent Ultra 2 capillary column and a flame ionization detector (FID-GC). The measurement was done with MISystem, version 6.0 (MIDI Inc., Newark, DE), using the TSBA 6 method. Methyl nonadecanoate (19:0) used as an internal standard, which was used for calculation of PLFAs (Zelles 1999). The summed masses of PLFAs (nmol PLFA g^{-1} soil) reported for typical of fungi (18:2ω6,9), gram-negative bacteria (cy17:0, 16:1ω7, 16:1ω5, 18:1ω5, 18:1ω7, 17:1ω9, cy19:0), gram-positive bacteria (i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, i19:0, a19:0) and actinomycetes (10Me16:0, 10Me17:0, 10Me18:0) were used as signature PLFA biomarkers for these microbial groups. The ratio of fungal to bacterial PLFAs (F/B) was used as an indication of changes in microbial biomass (Bååth 2003). The ratio of saturated (S) and monounsaturated (M) PLFAs (S/M) was used as an indicator of physiological or nutritional stress in bacterial communities (Kieft et al. 1997; Bossio and Scow 1998). Total bacterial, fungal and actinomycetes PLFAs were calculated using both absolute concentration (nmole per g^{-1} soil) and relative abundances (mole percent of total PLFA).

Community-level physiological profiles (CLPP)

Community-level physiological profile was characterized using Biolog ECO MicroPlate (Biolog Inc., San Jose, CA, USA). Each ECO MicroPlate consists of three replicate wells of 31 carbon substrates and a control (without carbon substrate), total 96 wells. The 31 carbon substrates in ECO MicroPlate were subdivided into six categories: polymers, carbohydrates, carboxylic acids, amino acids, amines and phenolic compounds, as described by Choi and Dobbs (1999). Moist soil samples (3 g) were added to 27 mL of sterile 0.85 % NaCl solution, shaken on a rotary shaker and left to settle. Cleared suspension was further diluted to ten times, and then, 150 µl was inoculated into each well in ECO MicroPlate. Then these microplates were incubated for 10 days at 30° C in incubator. The well absorbance values were read at 590 nm with a microplate reader (SpectraMax Plus, Molecular Devices, CA, USA) at 24-h intervals. The optical density data of each well were corrected by subtracting optical density of control well (corrected data). Average well color development (AWCD) was calculated according to Garland and Mills (1991), $\text{AWCD} = \Sigma(S - C)/N$ where S is absorbance of color developed in substrate well, C is the absorbance value of the control well, and N is the number of substrates (31). The optical density at 96 h of incubation time was used for non-metric multidimensional scaling (NMS) analysis. Before NMS analysis, corrected data were transformed by dividing each value by AWCD of the plate (Garland and Mills 1991).

Statistical analysis

The experiment was performed in a completely randomized design involving seven treatments with three replications. All statistical analyses were carried out using SigmaPlot 12.0 (Systat Software, Inc., San Jose, CA, USA) and PC-ORD software (McCune and Mefford 2006). The PLFAs (molar %) was used for non-metric multidimensional scaling (NMS) plots. A second matrix containing summed values for the taxonomic groups and stress indicators was used to construct a joint plot overlaid on the NMS. The transformed data of ECO MicroPlate were also used for NMS analysis, and a second matrix containing C substrate categories (polymers, carbohydrates, carboxylic acids, amino acids, amines and phenolic) was used to construct a joint plot overlaid on the NMS. The data were examined using NMS with the Sorensen distance measures. The NMS scores were then loaded into SigmaPlot 12.0 for ANOVA analysis, if treatment resulted in significant differences ($p < 0.05$).

Results and discussion

Plant biomass, nutrient content and soil characteristics

The effect of salt stress (seawater irrigation) on *Salvadora* plants was expressed on dry matter production (Table 1). Plant dry matter was significantly increased with all seawater treatments, and highest increase was at 20 % seawater treatment. Numerous halophytes were not only able to tolerate salinity, but growth was also stimulated by NaCl treatment (Yousif et al. 2010). In agreement with previous studies, 75–100 % seawater caused a yield reduction in *Salicornia persica*, *Sarcocornia fructicosa* and *Helianthus tuberosus* L. (Zhao et al. 2008; Ventura et al. 2011); our studies also demonstrated that *Salvadora* dry matter yield was decreased at 100 % seawater compared to low concentration of seawater (Table 1); however, still this yield was higher than control treatment (0 % seawater) because highest EC of soil in present study was 2.43 dS m^{-1} which was lower than that of 6.1 dS m^{-1} where *Salvadora* exhibited a reduction in growth (Ramoliya et al. 2004). *Salvadora* tolerate the salinity by maintaining osmotic balance and ion homeostasis (Parida et al. 2016). Salinity-induced higher accumulation of organic metabolites such as amino acids, reducing sugars and polyphenols was observed (Parida et al. 2016).

Mineral analysis of aboveground biomass of *Salvadora* was performed for several elements as depicted in Table 2. The concentrations of N, K, S, Ca and Cl were much greater than that of P, Na and Mg. The concentrations N, P, K, S, Ca and Mg were not significantly influenced by the seawater treatments; however, increased salinity is reported to reduce (Ramoliya et al. 2004; Vigo et al. 2005). The increase in concentration of Na and Cl was significant at 40 and 60 % seawater treatment, respectively, and above concentration. This response of facultative halophytes (*Salvadora*) reflects the utilization of metabolically

Table 1 Effect of seawater irrigation on dry weight of *Salvadora* (*Different letters* denote significant differences among treatments at $p < 0.05$ level)

Seawater concentrations (%)	Plant weight (gm pot^{-1})
0	14.52c
10	23.20ab
20	25.82a
40	23.46ab
60	24.58ab
80	25.27a
100	21.98b

Table 2 Effect of seawater irrigation on nutrient content of *Salvadora* (*Different letters* denote significant differences among treatments at $p < 0.05$ level)

Seawater concentrations (%)	N (%)	P (%)	K (%)	Na (%)	S (%)	Ca (%)	Mg (%)	Cl (%)	K/Na	Ca/Na	Mg/Na
0	1.05a	0.11a	1.71a	0.39d	1.43a	3.17a	0.68a	2.12c	4.32ab	8.07a	1.74a
10	1.12a	0.13a	1.78a	0.40d	1.66a	3.35a	0.69a	2.75b	4.51a	8.35a	1.70a
20	1.51a	0.10a	1.51a	0.43 cd	1.44a	2.99a	0.59a	2.50bc	3.55bc	7.09ab	1.41a
40	1.22a	0.09a	1.76a	0.46b	1.54a	3.45a	0.74a	2.45bc	3.82ab	7.57ab	1.60a
60	1.29a	0.11a	1.60a	0.53a	1.64a	3.04a	0.70a	3.17a	2.97c	5.69b	1.30a
80	1.53a	0.10a	1.39a	0.49abc	1.98a	3.50a	0.76a	3.00ab	2.84c	7.15ab	1.54a
100	1.21a	0.09a	1.58a	0.52ab	1.72a	3.09a	0.70a	3.00ab	3.10c	6.07b	1.38a

Table 3 Effect of seawater irrigation on soil characteristics (*Different letters* denote significant differences among treatments at $p < 0.05$ level)

Seawater concentrations (%)	EC (dS m^{-1})	pH	OC (%)	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
0	0.87d	9.89a	0.35a	32.08a	8.07a	615.84e	1685.34d
10	0.89d	9.84a	0.37a	32.08a	7.83a	659.52de	1801.00 cd
20	0.93d	9.93a	0.35a	36.75a	7.61a	816.75 cd	1860.48c
40	1.37c	9.79a	0.29a	30.92a	7.04a	794.92 cd	1890.22c
60	1.50bc	9.77a	0.36a	40.25a	10.21a	821.12c	2052.15b
80	1.67b	9.73a	0.27a	39.08a	9.15a	978.36b	2121.54b
100	2.43a	9.91a	0.38a	39.08a	8.67a	1209.84a	2372.69a

undesirable ions for osmotic adjustment since it accumulates preferentially in the vacuoles (Marschner 1995). The K/Na ratio decreased (from 4.51 to 2.97) with increase in salinity. Similarly, Ca/Na ratio was also significantly decreased (from 8.35 to 6.07) with seawater treatments. These results show the halophytic behavior of *Salvadora* and accumulated Na as a primary osmoticum and decreased K concentration (Maggio et al. 2000). These results are consistent with those reported for *Salicornia* and *Sarcocornia* (Ventura et al. 2011), for *Salvadora oleoides* (Hardikar et al. 2011) and for Olive (Vigo et al. 2005). The decreased ratios K/Na and Ca/Na are due to the antagonistic effects of Na with K and Ca (Vigo et al. 2005). *S. persica* confirmed in group B and/or group C plant (Ramoliya et al. 2004) as per Marschner's (1995) classification on the basis of K and Na uptake. These plants have the ability to substitute Na for K. Sodium has a positive effect on growth of plants in group B plants, whereas group C plants contain very little K that can be substituted with Na without a negative effect on growth. However, the increase in seawater concentration did not have any noticeable effect on the Mg/Na ratio.

Using seawater to irrigate *Salvadora*, EC of soil was significantly increased at 40 % and above concentration of seawater and highest being observed at 100 % seawater (Table 3) as observed by the Kowalski and Palada (1994) by irrigating vegetable crops with different dilution of seawater. This indicates that continuous application of seawater will definitely increase the salt concentration in soil. Results in this study also demonstrated that increased EC of soil resulted from seawater irrigation indicating that salinization of soils would occur; however, increase was not much higher because experimental pots were kept outdoor (rain fed conditions). This was because EC of soil came into equilibrium, i.e., the total soluble salts applied to the soil mass by seawater irrigation was equivalent to that leached from the soil mass by rainfall and excessive irrigation (Zhao et al. 2008), and results were also supported by Tang and Liu (2004). There was no significant effect of seawater on pH and OC of experimental soil. Similarly, N and P contents of soils were also not influenced by the seawater irrigation. However, as expected available (NH_4OAc extractable) K and Na significantly increased with increasing the concentration of seawater. Similarly, Ghadiri et al. (2006) also observed increased EC of soil by irrigation of barley with Caspian seawater and recommended that seawater supplementary irrigation of salt-tolerant plants in light textured soils and at the later stages of plant growth. The K and Na concentrations were significantly increased at 20 % and above concentration of seawater, and reached maximum at 100 % seawater.

Soil microbial community structure

Phospholipid fatty acid profiling has been used extensively for characterization of soil microbial community structure because phospholipids are present in the membranes of living cells of microbes and break down rapidly in soils which are directly related to the viable soil microbial biomass (Zelles 1999; Baumann and Marschner 2013). To obtain the information on shift in microbial community structure, soils were extracted for PLFA and total 36 PLFAs were observed. Microbial biomass was measured using the concentration of PLFAs (Fig. 1). Total microbial biomass was significantly reduced with 20 % and higher concentrations of seawater in comparison with control treatment (29 nmole g^{-1} soil) and reached to minimum at 100 % seawater (15 nmole g^{-1} soil). In view of the fact that the irrigation with seawater has significantly increased the salt concentration (EC) of soil (Table 3) resulted in reduction of microbial biomass (Pankhurst et al. 2001). Similarly, reduced microbial biomass and reduced rate of soil respiration were observed in saline soil compared to non-saline soil (Pankhurst et al. 2001; Baumann and Marschner 2013). Similar to total microbial biomass, biomass of gram-positive bacteria was also decreased with seawater treatment, but significant reduction was observed at 40 % and higher concentrations, whereas similar influence of seawater was also expressed at 80 % concentration in case of gram-negative bacteria and total bacterial biomass, which support the observations of Rajaniemi and Allison (2009) who reported that the gram-negative bacteria were more salt tolerant in a salinity gradient experiment. Microbial biomass of actinomycetes was significantly decreased at 40 % and higher concentrations of seawater in comparison with control treatment (1.8 nmole g^{-1} soil) and reached to minimum at 100 % seawater (0.3 nmole g^{-1} soil) which confirm the observation of Zahran (1997) who noted that actinomycetes represent only a small fraction of the microbial flora of saline soils; they are be less tolerant to salt stress. However, microbial biomass of fungi was not statistically influenced by the seawater irrigation treatments. Fungus has tolerated the existing increased salinity. This finding also substantiates with observation of Kamble et al. (2014) who reported higher fungal biomass (estimated by 18:2ω6,9) in high saline soils and suggested that fungi would be more important in saline soils.

The non-metric multidimensional scaling (NMS) was conducted with molar per cent of PLFAs and a three-dimensional solution was recommended. Collectively, these differences in PLFA profiling suggested a marked influence of salinity on the shift in soil microbial community structure (Rajaniemi and Allison 2009). The NMS analysis showed that the first axis explained 30 % of the variability, the second axis 24 %, and the third axis 32 %, or 86 % of the total variability (Fig. 2). The fungal PLFA biomarker was negatively correlated ($r = -0.80$ and $r = -0.43$, respectively) with axis one and axis two. The gram-negative and gram-positive biomarker PLFAs negatively correlated with axis one ($r = -0.73$) and axis two ($r = -0.56$), respectively. Similarly, molar ratio of total bacterial PLFAs negatively correlated with axis one ($r = -0.64$) and axis two ($r = -0.18$). Axis two and axis

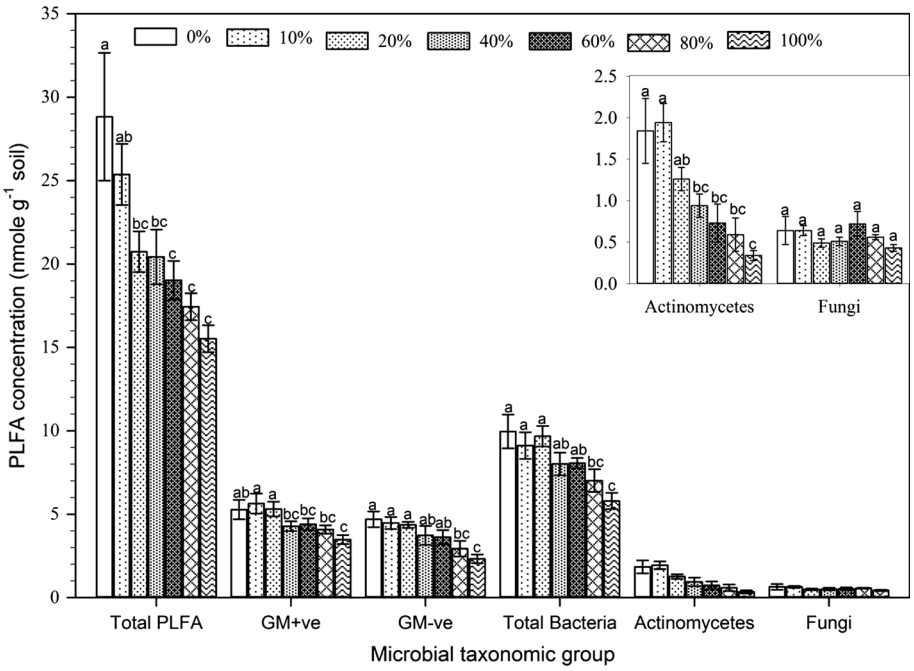

Fig. 1 PLFA (nmol g^{-1}) concentrations in soil samples irrigated with different dilutions of seawater (*Different letters* denote significant differences among treatments at $p < 0.05$ level)

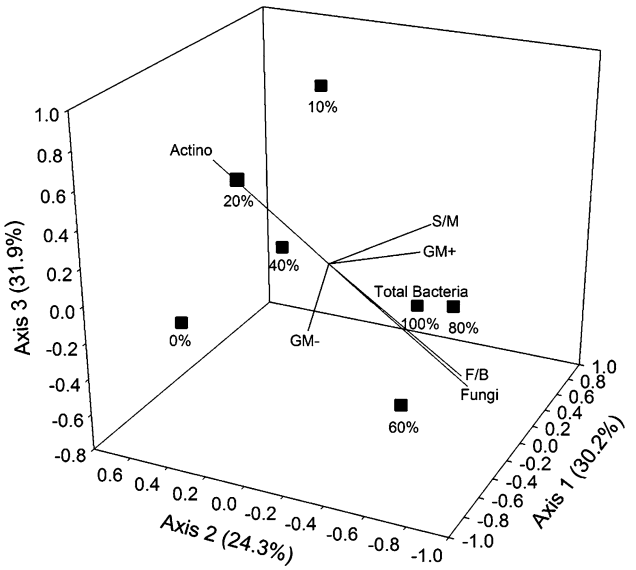

Fig. 2 NMS representation of soil samples distance based on molar % of PLFA profile

three were positively correlated ($r = 0.52$ and $r = 0.53$, respectively) with actinomycetes biomarkers. Correlation among microbial PLFAs showed that F/B ratio was negatively correlated with axis two ($r = -0.67$) and axis three ($r = -0.52$). The stress indicator, S/M ratio was positively correlated ($r = 0.83$) with axis one.

The NMS scores of PLFA profiles were analyzed using ANOVA. The NMS scores significantly separated the microbial community structure of seawater irrigated soil in comparison with control soil (Fig. 2). On the basis of NMS scores, control treatment was significantly separated from 10 (axis one), 40, 60, 80 and 100 % (axis two), and 20 % (axis 3) of seawater concentration. The microbial community of 10 % seawater was significantly differed from 20 and 60 % (axis 1), and 60, 80 and 100 % (axis tree) of seawater. The 40, 60, 80 and 100 % seawater irrigated soils were significantly differed from 20 % seawater on the basis of scores of axis three, whereas 40 % seawater irrigated soil was separated from 60, 80 and 100 % seawater by the scores of axis two. The 60 % seawater irrigated soil differed from 100 % concentration by the scores of axis one, whereas 60 and 80 %, and 80 and 100 % seawater irrigated soils have similar microbial community structure.

Relative abundance of gram-positive biomarker PLFAs significantly increased with seawater irrigation compared to control treatment. Similarly, abundance of gram-negative biomarker PLFAs also increased up to 60 % seawater irrigation (Fig. 3). The molar percent of actinomycetes PLFA biomarkers significantly decreased with seawater irrigation. There was no significant difference molar percent of fungal biomarker due to seawater irrigation (Fig. 3). These differences PLFA profiling make the microbial community structural shift toward bacterial dominance with seawater irrigation (Fig. 2). Further, Baumann and Marschner (2013) also reported that salinity treatment had significantly influenced soil microbial community structure and characterized by a high abundance of the PLFA a15:0 which is a biomarker for gram-positive bacteria. Similarly, under salinity, microbial community also shifted toward the higher relative abundance of gram-negative bacteria (Rajaniemi and Allison 2009). Soil

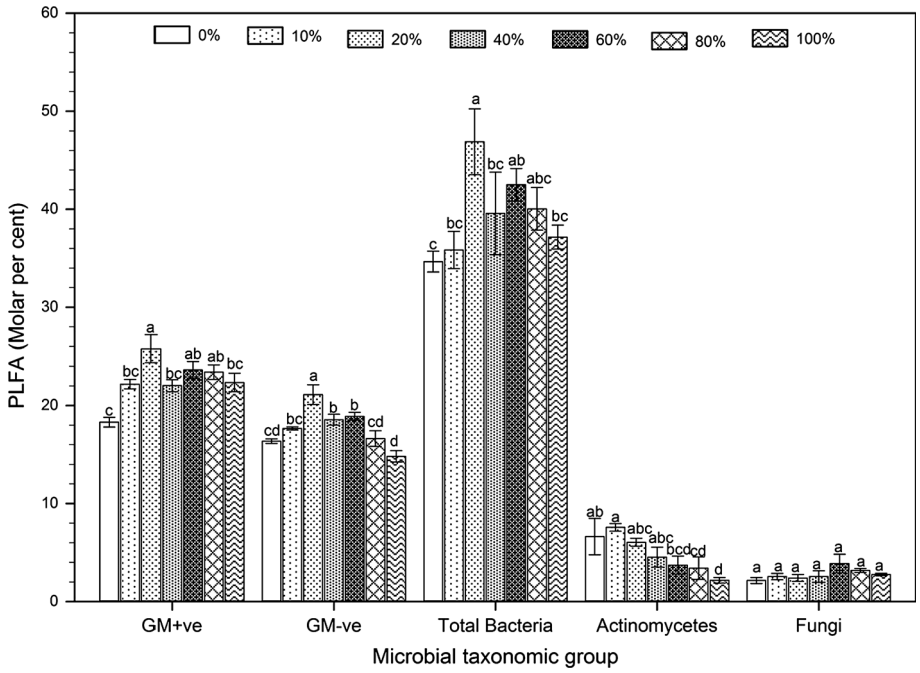

Fig. 3 Comparisons of PLFAs (molar %) of gram-positive, gram-negative, total bacteria, actinomycetes and fungi in soil (*Different letters* denote significant differences among treatments at $p < 0.05$ level)

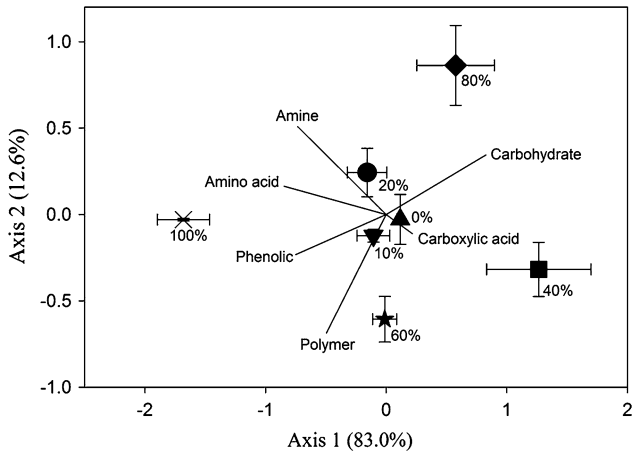

Fig. 4 NMS analysis of substrate utilization patterns after 96-h incubation of Biolog ECO MicroPlate

bacterial communities have ability to change in order to tolerate osmotic stress caused by salinity and altered community composition under salinity stress (Kamble et al. 2014). The PLFA analysis did show significant differences in molar ratios indicative to gram-negative and gram-positive bacteria, while increased salinity causes changes in the composition of the soil microbial community.

Soil microbial community-level physiological profiling

Biolog ECO MicroPlates are frequently used as a culture-dependent approach to distinguish the catabolic potential of microbial community. The NMS analysis of transformed (normalized) values of Biolog ECO MicroPlates of the 31 carbon substrates indicated significant differences in their utilization pattern between different concentrations of seawater treatments (Fig. 4). Studies have indicated that there were significant effects of soil salinity on soil microbial community structure and catabolic capacity (Nelson and Mele 2007; Yu et al. 2012). The 83 and 13 % of the variability was explained by the axis one and axis two, respectively, by NMS analysis. Among the substrate categories, polymer, phenolic, amino acid and amine were negatively correlated ($r = -0.50$, -0.75, -0.85 and -0.73, respectively) with axis one, whereas carbohydrate was positively correlated ($r = 0.82$). Axis two was negatively ($r = -0.69$) with polymer and positively ($r = 0.51$) correlated with amines. The NMS showed a good discrimination (by scores of axis one) of 100 % concentration of seawater soil from other treatments as well as 40 % seawater concentration from other treatments. NMS scores of axis two differentiated 80 % seawater concentration from other treatments; further, this axis also differentiated the 60 % seawater concentration from 0, 10 and 20 % concentration. This indicated that irrigation of soil with different concentrations of seawater has significantly affected microbial community function.

Salt stress induces increased carbohydrate utilization by microbial community is probably due to increased osmotic pressure in root tissue exerted by the salt and the subsequent release of carbohydrates (Nelson and Mele 2007). The exudation of amino acids most likely increased with the salt stress from plant roots (Nelson and Mele 2007) may be explained by the direction of the arrows for NMS joint plot (Fig. 4). The γ-hydroxybutyric acid is known to increase in plants when there is deficiency of oxygen (Breitkreuz et al. 2003); similarly, the present study also not observed strong relationship with carboxylic acids. Though microbial activities were decreased under saline conditions, it is evident that considerable microbial diversity and activity persisted as observed in by carbon utilization pattern. This showed a good adaptability of microbes to soil environment. Once soil environmental conditions changed, microbial community would consecutively alter the composition, or transform the substrates (Yu et al. 2012).

Conclusion

It is concluded that seawater irrigation induced soil salinity not only influences the chemical characteristics of soils but also greatly affects soil microbial community which are important for biogeochemical cycling of nutrients. Irrigation with seawater dilutions had profound effects on different soil microbial groups and has also affected soil microbial community structure and community-level physiological profiling. In present study, we have demonstrated the possibility of diluted seawater irrigation for cultivating *Salvadora* in coastal agriculture. Plant biomass was significantly increased with seawater treatments and highest being observed at 20 % seawater dilution. This study also indicated that PLFA and CLPP techniques are effective in terms of distinguishing microbial community structure and physiological functions in seawater irrigated soils.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

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