



## Nutrients, microbial community structure and functional gene abundance of rhizosphere and bulk soils of halophytes



Doongar R. Chaudhary<sup>a,b,\*</sup>, Ritesh K. Gautam<sup>a</sup>, Basit Yousuf<sup>a,b</sup>, Avinash Mishra<sup>a,b</sup>, Bhavanath Jha<sup>a,b,\*</sup>

<sup>a</sup>Discipline of Marine Biotechnology and Ecology, CSIR – Central Salt and Marine Chemicals Research Institute, G.B. Bhavnagar, Gujarat 364 002, India

<sup>b</sup>Academy of Scientific and Innovative Research (AcSIR), CSIR, New Delhi, India

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### ABSTRACT

Salt marshes occupy the transition zone between terrestrial and marine ecosystem and provide habitat for salt tolerant plant species-halophytes. Dominant halophytes such as *Salicornia brachiata*, *Aeluropus lagopoides* and *Suaeda maritima* play significant role in carbon sequestration, nutrient mineralization, nutrient cycling and improving micro-environment. There are scanty reports on the rhizosphere microbial communities of halophytes. Whether the rhizosphere of halophytes harbors unique microbial communities or has species specificity is yet to be established. In the present study, we have determined microbial community structure and functional genes abundance as well as major nutrients and three key enzyme activities in both rhizosphere and bulk soil of three halophytes (*Salicornia*, *Aeluropus* and *Suaeda*) growing on salty marsh of coastal Gujarat, India. The  $\text{NH}_4\text{-N}$  and available K were significantly higher in the rhizosphere than in the bulk soil in all the cases. The rhizosphere of *Suaeda* was characterized by significantly higher concentrations of organic carbon, total N,  $\text{NH}_4\text{-N}$  and available P as compared to other two halophytes. The rhizospheric soil had significantly higher concentrations of total PLFA, GM+ve, GM–ve and total bacterial PLFA biomarkers compared to the bulk soil. Among halophytes, significantly higher concentrations of total PLFA, GM+ve, GM–ve, total bacterial and actinomycetes biomarker PLFAs were recorded in *Suaeda* and *Aeluropus* in comparison to *Salicornia*. The NMS analysis of PLFA profiles revealed a significant difference in microbial community structure in *Salicornia* compared to *Aeluropus* and *Suaeda*. Similarly, rhizosphere microbial community structure of *Salicornia* was significantly different from *Suaeda* and *Aeluropus*. In rhizosphere soils, significantly higher abundance (molar%) of GM–ve, GM +ve, total bacterial and actinomycetes PLFAs were observed in *Aeluropus* and *Suaeda* compared to *Salicornia*. Activities of  $\beta$ -glucosidase and urease were significantly higher in rhizosphere than in the bulk soil. The abundance of 16S rRNA, *cbbL* and *nifH* genes, detected by the quantitative RT-PCR was significantly varied among soils and halophytes. The results exhibited high bacterial diversity, abundance of  $\text{CO}_2$  and N-fixing bacteria, which can be explored further for their role in C and N cycling for sustainable agriculture and environment management.

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### 1. Introduction

The rhizosphere is the volume of soil adjacent to the plant roots that plays a significant role in plant health and soil fertility which differ significantly from root free soil (bulk soil) (Sørensen, 1997; Hinsinger et al., 2009). Actively growing roots secrete a diverse array of organic root exudates that stimulate the growth of microbial populations present in the rhizosphere, resulting in a

microbial community structure at soil–root interface (i.e., rhizosphere) (Hinsinger et al., 2009). The existence of steep chemical, microbiological, and physical gradients along the rhizosphere to the bulk soil is well documented. Chief among these are substantial differences in soil pH (Gahoonia and Nielsen, 1991; Chiu et al., 2002), cation-exchange capacity (Chiu et al., 2002), nutrient availability (Gahoonia and Nielsen, 1991; Chiu et al., 2002; Meo et al., 2003; Yanai et al., 2003; Collignon et al., 2011), organic carbon (Chiu et al., 2002; Chaudhary et al., 2012a), and microbial community (Chaudhary et al., 2012). Nutrient availability in the rhizosphere depends on the difference between element output fluxes, principally by plant uptake, and element input fluxes, principally by mass flow, mineral weathering and organic matter

\* Corresponding authors. Tel.: +91 278 2564761; fax: +91 278 2567562.

E-mail addresses: [drchaudhary@csmcri.org](mailto:drchaudhary@csmcri.org) (D.R. Chaudhary), [bjha@csmcri.org](mailto:bjha@csmcri.org) (B. Jha).

mineralization (Hinsinger et al., 2009). It is also well established that the release of organic substances and exudates from roots are processes which influence the nutrient availability in the rhizosphere (Koranda et al., 2011). Plant root exudates and microbial enzymes directly influence the mineralization of organic matter (Collignon et al., 2011; Koranda et al., 2011). Root exudates also enhance nutrient availability through their use as carbon substrates for rhizospheric microbes which enhance the nutrient release and uptake by plant (Sørensen, 1997). Interestingly, the accumulation of nutrients is generally observed in the rhizosphere soil compared to bulk soil of plants (Meo et al., 2003; Yanai et al., 2003). However, little is known about the impact of halophytic plants on the availability of nutrients in rhizosphere and bulk soil.

The plant species and root zone can also affect the structural and functional diversity of rhizosphere microbial community due to variations in root exudation and rhizodeposition in different zones (Sørensen, 1997; Garbeva et al., 2008). Analyses of microbial communities using both culture-dependent and culture-independent methods showed clear effects of the rhizosphere on species composition (Söderberg et al., 2002; Garbeva et al., 2008; Chaudhary et al., 2012). As soil microbes are often limited by energy in soils, root exudates such as organic acids, sugars and amino acids may stimulate the growth of microbial populations and the activities of extracellular enzymes capable of influencing biogeochemical cycling of C, N, P and S (Stevenson and Cole, 1999; Fontaine and Barot, 2005; Ai et al., 2012). It is, therefore, important to understand the influence of plant roots on the composition of microbial community in the rhizosphere and the bulk soil of crops. Established molecular methods, such as phospholipid fatty acid (PLFA), offer valuable insights into the microbial communities and several studies indicate that plants have a strong effect on rhizosphere microbial community composition (Garbeva et al., 2008; Koranda et al., 2011; Ai et al., 2012; Chaudhary et al., 2012). Phospholipid fatty acids are a major component of microbial membrane and are of interest as biomarkers because they are derived from viable cells (upon cell death the phosphate group is rapidly cleaved) and they provide information on a range of microbial properties: total biomass, physiological stress, and functional community composition (Green and Scow, 2000). In several instances, a relatively high abundance of GM–ve bacterial species was found in rhizosphere preferably growing on plant labile C, while GM+ve bacteria may be dominant in bulk soil (Bird et al., 2011; Koranda et al., 2011; Chaudhary et al., 2012). It also evident that different plant species select different bacterial communities and these plant specific enrichments can be increased by repeated cultivation of the same plant species in the same field (Smalla et al., 2001). In general, soil enzyme activities are lower in bulk soil than in the rhizosphere, as a result of microbial activity induced by root exudates and release of enzymes from roots (Zhang et al., 2011, 2012a). Plants roots release 17% of the photosynthate captured and most of which is available to the microbial community (Nguyen, 2003). However, most of investigations have been conducted at bulk soil scale of agricultural crops and as a result, there is virtually no information on rhizosphere effects on extracellular enzyme activities and microbial community structure in rhizospheric soils of halophytes.

Quantitative real-time PCR (qPCR) has recently been applied to environmental microbiology and now allows detection and quantification of microbial gene or transcript numbers from environmental samples. Nitrogen and carbon fixation are important processes that facilitate nitrogen uptake and carbon sequestration in to biological systems. The dinitrogen reduction process is mediated by the enzyme complex nitrogenase and responsible gene *nifH* or *nifD* or *nifK* is an ideal tool for analyzing the nitrogen fixing community in soil (Jasrotia and Ogram, 2008; Warttiainen et al., 2008) thus, it is important to monitor the size and

activity of N fixing population in coastal ecosystem. The key enzyme of the carbon cycle is the ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO) (Ellis, 1979). The most distributed type of RubisCO is the form I, which occurs in plants as well as in photo- and chemoautotrophic bacteria (Selesi et al., 2007). This gene is commonly used in microbial ecology studies on CO<sub>2</sub> fixation in terrestrial ecosystems (Selesi et al., 2007; Yousef et al., 2012; Xiao et al., 2013). These analyses revealed high and unprecedented *cbbL* diversity in the environment and significant role for microbial autotrophy in the sequestration of soil C. Densities of total bacteria, N- and C-fixer were estimated by quantifying the 16S rRNA, *nifH* and *cbbL* genes.

Soil salinity and irrigated agriculture have coexisted since ancient times, and ever since the problem of salinity in agriculture has become a challenge. Salt marshes are intertidal ecosystems, backed up against the land on one side while open to the sea on the other (Wiegert et al., 1981). The vegetation growing in these areas are very diverse in some part of world, while in other regions they are dominated by a few plant species, usually halophytic grasses (Isacch et al., 2006). Halophytes, the vegetation of saline habitats, are a specialized plant group, characterized by the possession of great osmotic tolerance (Glenn et al., 1999). The halophytes function as significant sources of carbon for organic matter through re-mineralization in coastal ecosystem function as a net sink of CO<sub>2</sub> from the atmosphere, and perform important role in carbon cycling (Sousa et al., 2010a,b). About half of the higher plant families consist of halophytes where the plant family Chenopodiaceae contributes the largest number of halophytes species (~550) followed by family Poaceae (grasses), Fabaceae (legumes) and Asteraceae (composites) contributing fewer than 5% of halophyte species (Glenn et al., 1999). *Salicornia brachiata* Roxb., *Aeluropus lagopoides* (L.) Trin. ex Thw. and *Suaeda maritima* L. Dum are dominant species in the coastal area of Gujarat. In this study, we compared microbial community structure and biogeochemical functional capacity of halophytes (*Salicornia*, *Suaeda* and *Aeluropus*) from rhizosphere and bulk soil to determine whether plant species alter the microbial component of the marsh ecosystem. Since, the root systems of these halophytes differ substantially in morphology and total biomass (Sousa et al., 2010a,b; Zhang et al., 2012b), we hypothesized that the microbiota associated with their rhizospheres would differ in both community composition and biogeochemical functions.

Most of the above mentioned studies on rhizosphere and bulk soils were mainly focused on annual field crops. No information is available for that in halophytic plants, so far which are continuously growing under hyper-salinity condition of inter-tidal soil proximity. The objective of this study was to investigate the nutrients, enzymes, microbial community and functional gene abundance in the rhizosphere and bulk soils of halophytes.

## 2. Materials and methods

### 2.1. Study site and sampling

Adjacent populations of *Salicornia*, *Suaeda* and *Aeluropus* were located at inter-tidal soil of Methla village (21°09'N 658; 072°00'E 278), 80 km far from Bhavnagar (Bay of Khambhat), Gujarat, India. The soil is classified according to the United States Department of Agriculture (USDA) soil taxonomy as Fine, mixed, (calcareous), hyperthermic, Typic Salorthids. In March 2011, a total of three plots (replicate), each 3 m × 3 m, were marked within monospecific populations of each halophyte species. The random sampling method was used to ensure representative sampling from the different halophyte plants. Plants were dug with a shovel and placed on a large plastic sheet followed by roots were shaken vigorously to separate soil not tightly adhering to the roots (bulk

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