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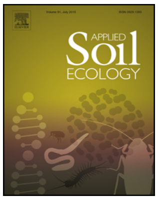

Spatial microbial community structure and biodiversity analysis in "extreme" hypersaline soils of a semiarid Mediterranean area

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ABSTRACT

In recent years specific attention has been paid on the biotechnological potential of microorganisms in extreme soils, in particular in saline soils. Salinity is one of the most widespread soil degradation processes on the Earth, and saline soils can be defined as extreme soils or border line habitats in which several factors, as high salt content, may limit the growth of organisms. In this study, the physical, chemical and microbiological soil properties were investigated in the shallower horizon of natural salt-affected soils in Sicily (Italy). The main aim of the research was to evaluate the structure and diversity of bacterial and archaeal communities by terminal-restriction fragment length polymorphism (T-RFLP) according to arbitrary different classes of vegetation and salt crust cover in soils. Furthermore, the structure of microbial communities was assessed considering the heterogeneity of physical–chemical properties of the habitat under investigation, as a function of vegetation, crust cover, and salinity classes. The results provided information on the type of distribution of different microbial community composition and diversity as a function of both vegetation and crust cover as well as salinity classes. In particular, the archaeal community showed a richness and diversity significantly affected by the spatial gradients of soil salinity, conversely, the bacterial one showed a decreasing trend with increasing gradient of soil salinity. The T-RFLP cluster analysis showed the formation of two groups for both bacterial and archaeal community, significantly ($p < 0.05$) influenced by sand and silt content, electrical conductivity (EC_e), vegetation cover percentage, salt crust and for by texture composition. In particular, the discriminant analysis obtained for the different salt crust classes for archaeal community stressed the membership of one of the two clusters to the class with the lower salt crust percentage (0–40%).

1. Introduction

A definition of "extreme environment" completely acceptable for all ecosystems is not easy to find, but it is true in some habitats where environmental conditions such as pH, temperature, pressure, nutrients or saline concentrations can be extremely high or low and only limited numbers of species (that may grow at high cell densities) are well adapted to those conditions (Ventosa, 2006). Hypersaline ecosystems are distributed globally and represent a wide range of types (Terry et al., 2000; Oren, 2002; Ventosa et al., 2008; Hollister et al., 2010). The majority of studies published on the microbiology of hypersaline ecosystems focus on aquatic communities (Oren, 2002), whereas far fewer have attempted to characterize hypersaline soils and sediments (David et al., 2005; Dong et al., 2006; Ventosa et al., 2008; Hollister et al., 2010).

We can qualify soils as "extreme" when they support only colonization by organisms presenting a specific and common adaptation (Dion and Nautiyal, 2008). Microorganisms in these habitats may share a strategy and have developed multiple adaptations for maintaining populations and cope eventually to extreme conditions.

Salinity is one of the most widespread soil degradation processes and saline soils can be defined as extreme soils or border line soil in which uneven temporal and spatial water distribution and localized high concentration of salts occur, characterizing restricted habitats where most of the present micro-organisms are salt tolerant (halotolerant), or halophilic (that

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require salt for maintaining their membrane integrity and enzyme stability and activity). The differences between saline and hypersaline soils are not well defined by chemical parameters. Soils could be considered hypersaline when salt concentration exceeds certain thresholds (Dion and Nautiyal, 2008). According to Amoozegar et al. (2003) soil can be defined saline when the concentration of salt is higher than 0.2% (w:v), also a soil is saline when the electrical conductivity (EC_e) of a saturated paste is greater than 4 dS m^{-1}, (Richards, 1954). The two international soil classification systems introduced higher minimum thresholds of EC_e to classify a soil horizon as saline. In fact, the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014) considers the reference value 15 dS m^{-1} of EC_e in defining the salic horizon, while the USDA Soil Taxonomy (Soil Survey Staff, 2014) fixed the threshold at 30 dS m^{-1}.

Microorganisms are affected by the salt concentration and generally in hypersaline environment can be found from moderately halophilic Bacteria to extremely halophilic Bacteria and Archaea communities. Very little information exists regarding the diversity of microorganisms isolated by hypersaline soils (Dion and Nautiyal, 2008; Hollister et al., 2010). Halophilic and halotolerant Bacteria and Archaea communities are essential for the biogeochemical processes in soils of extreme environments (Ma and Gong 2013). The main mechanisms of tolerance of the microbial communities have been attributed to enzymes with unique structural features that provides them to sustain high salt conditions. Soil halophiles enzymes results to be potentially useful for a variety of applications, including restoration of conditions in salt-affected soils, remediation of pollutants, industrial bio catalysis, food processing, washing, biosynthetic processes, synthesis of exopolysaccharides, compatible solutes, carotenoids (Ventosa et al., 2008). Many studies have been focused on the isolation and characterization of halophilic Bacteria and Archaea communities in saline and hypersaline soils (Quesada et al., 1982; Ventosa et al., 1998) and biotechnological applications are under investigation (Ghazanfar et al., 2010; Keshri et al., 2013; Arora et al., 2014; Tsiamis et al., 2014; Canfora et al., 2014). However, more extensive studies on the ecology, structure, diversity, and functionality of organism occurring in natural saline soils are needed. Naturally salt-affected soils have a biotechnological potential in their microbial communities. These, in fact, represent not only a gene reserve for potential biotechnological applications in the improvement and conservation of saline environments, but they can serve as model systems for exploring relationships between diversity and activity at the soil level. The naturally saline soils differ from other saline soils because are not generated by any anthropic degradation processes, and the salinity is “genetic” (due to the parent material and/or pedogenesis).

In depth knowledge of autochthonous microbial community structure of natural hypersaline soils is basic to identify microbial candidate genes of target microorganisms that have evolved and adapted to live in saline soils. This could allow to detect, monitor and understand the effects of human-induced and/or climate changes induced by natural catastrophes. A naturally saline soil is also a mutable environment where precipitation and fluctuation of climatic conditions can strongly impact the distribution of salts creating sometimes a heterogeneous landscape. Space and scale are recognized as fundamental factors in studying microbial structure analysis.

In Italy, most of salt-affected soils are in the central-south and insular areas. Even if today a quantification of the total area with salt-affected soils is uncertain, recent exploratory surveys (Dazzi and Lo Papa, 2013) have put in evidence that these areas appear prevailingly distributed in the low Po Valley, in long stretches of the Tyrrhenian and Adriatic coasts, along the coast in Apulia, Basilicata, Calabria and Sardinia and in wide stretches in Sicily.

Traditional microbiology using methods culture-dependent has been dealing only with a minor proportion of the actual soil microbiota. Only culture-dependent methods of molecular ecology of microorganisms have provided the possibility of analyzing microbial communities in their entirety. DNA-based technique has become a powerful tool for studying diversity and composition of soil bacterial communities in cultivation-independent ways.

Terminal-restriction fragment length polymorphism (T-RFLP) analysis is based on the detection of a single restriction fragment in each sequence amplified directly from the DNA soil sample and is capable of surveying dominant members comprising at least 1% of the total community. Terminal restriction fragment (TRF) patterns obtained by using T-RFLP technique are generated and analyzed in a series of steps that combine PCR, restriction enzyme digestion and electrophoresis on automated sequencer. T-RFLP can be used to examine microbial community structure and community dynamics in response to changes in different environmental parameters, or, to study bacterial population composition in natural habitats. It has been applied to studies of complex microbial communities in diverse environments such as soil (Lueders and Friedrich, 2003; Fierer et al., 2003; Kuske et al., 2003; Mengoni et al., 2006).

In this study, attention has been focused on the structure of soil microbial community, both bacterial and archaeal communities, in Mediterranean naturally salt-affected soils under semiarid climate. In particular, aims of this study were: (i) to evaluate the characteristics and the distribution of physical and chemical properties of the shallower soil horizons; (ii) to evaluate the genetic structure and diversity of bacterial and archaeal communities by T-RFLP approach and (iii) to correlate microbial genetic diversity with selected soil physical and chemical properties.

2. Materials and methods

2.1. Study area

It was considered a natural area in Piana del Signore (Italian for “Plain of Lord”), an alluvial flat land in Southern Sicily (Fig. 1S) where the geomorphology has been modeled by the river Gela. In the basin of this river the prevalent lithology is made up by Messinian evaporites belonging to the Gessoso-Solfifera geological formation, in which many types of saline rocks crop out (Gypsum, Carbonates and Marls, having frequently chloride and sulfide rock inclusions). The area we surveyed is 12.3 hectares wide and lies about 1 km far from the coastline and 1.2 km from the river Gela estuary.

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Vegetation is structured as a scattered mosaic of patches whose distribution is related to the flooding pattern and duration. The plant association is defined as Junco subulati-Sarcocornietum fruticosae Brullo, 1988 belonging to the Thero-Salicornietea Br.-Bl. & R.Tx. ex A. & O. Bolòs, 1950 class, in which most plants are halophilous pioneer swards typical of salt marshes. The community is constituted by *Sarcocornia fruticosa* (L.) A. J. Scott, Suaeda vera J. F. Gmelin, Juncus subulatus Forssk., *Juncus bufonius* L., *Phragmites australis* (Cav.) Steud subsp. australis, Symphyotrichum squamatum (Spreng.) G.L. Nesom, *Polypogon monspeliensis* (L.) Desf., *Spergularia maritima* (All.) Chiov., Monerma cylindrica (Willd.) Coss. & Durieu. Zones covered by vegetation alternate with zones having complete absence of plants. The area is temporarily flooded in the autumn and winter seasons, with longer permanence of water in those zones where salt crusts form on the soil surface afterwards it becomes drier. Salt crusts with thickness of 1–3 mm appear above the soil surface during the dry season, from June to September. Climate is semiarid