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# Microbial activity and functional diversity in Psamment soils in a forested coastal dune-swale system

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ABSTRACT

The aim of the study was to examine soil microbial activity and functional diversity in different parts of coastal landscapes influenced by recurring saltwater intrusion in the Ravenna area (Italy). For this reason, seven profiles were selected in the San Vitale Pinewood, in low-lying interdune spot and next to dune crests and swales. Soils were classified as Typic Psammaquent, Typic Ustipsamment, Aquic Ustipsamment and Sodic Psammaquent. Chemical, physical and biochemical properties of soil horizons, such as microbial biomass metabolic quotient and enzyme activity, were determined to examine the effects of soil salinity and sodicity levels on microbial activity and functional diversity. The various soils and horizons could be split into distinct groups based on Differential Function Analysis of their properties. Cellulase, xylosidase and arylsulfatase showed a peak of their activity in surface horizons of sodic soils. α-glucosidase activity also was high in deeper horizons of those soils. Moreover, functional diversity, evaluated through calculation of Shannon's diversity index, was higher in the surface and deeper horizons of saline soils than non-saline soils. Conversely, soil with shallow water-table showed similar enzyme activity to soil located in the highest spots of the dune system. However, the highest values of specific activity (per unit of organic carbon) recorded in the deep horizons of the Typic Ustipsamment soil suggested more efficient hydrolytic activity of organic substrates due to oxygenation of soil. In conclusion, hydromorphic conditions in these soils influence the efficiency of organic substrate hydrolysis while soil salinity and sodicity increase both biochemical activity and functional diversity of microbial communities.


## 1. Introduction

The European Environment Agency has recognized that the problem of saltwater intrusion due to groundwater over-exploitation is one of the major threats to coastal area freshwater resources in Europe (Scheidleger et al., 2004). Saltwater has invaded the coastal aquifer along the southern Adriatic coast of the Po Plain in Italy. The area is subject to subsidence (Teatini et al., 2005), the water tables are below sea level and saltwater has replaced freshwater in the aquifer (Antonellini et al., 2008; Giambastiani et al., 2007). The saltwater intrusion in the dune system of the Ravenna coast has produced hydromorphic soil conditions. Water-saturated zones are typical for hydromorphic soils, which are characterized by groundwater close to the soil surface (20–100 cm) (Buscaroli et al., 2009). The diffusion of oxygen from the atmosphere into the water-saturated zones is limited, and anoxic environments are formed that usually allow the activity of methanogenic archaea bacteria (Fiedler and Sommer, 2000; Kammann et al., 2001).

Enzymatic activity, primarily that of oxidoreductases, and the intensity of $CO_2$ emission have shown a positive correlation with the degree of soil hydromorphism and a negative correlation with the degree of soil salinization (Kazeev et al., 2004). Sorokin et al. (2006) reported slow decomposition of soil organic matter (SOM) in hydromorphic soil because of a low coefficient of microbiological mineralization and the lack of nitrogen (coefficient of oligotrophy). When hydromorphic conditions occur, because of saltwater intrusion by subsidence phenomena, a saline-sodic condition may be established. Saline-sodic landscapes are subjected to modified hydrologic processes which can alter soil chemistry, carbon and nutrient biogeochemical cycling, and SOM decomposition (Nelson et al., 1996). Saline-sodic soils are subjected to some processes which affect the soil microbial biomass and microbial activity, changing the nature and delivery of soil nutrients (Rietz and Haynes, 2003; Wong et al., 2010). In fact, salt-affected soils usually have low organic matter contents primarily due to poor plant growth leading to low inputs of organic materials into the soil (Wong et al., 2010). In general, the effects of salinity on microbial activity have been attributed to adverse effects similar to those on plant health. Salinity is a major factor in controlling microbial abundance, diversity, composition and functions (Borneman et al., 1996; Matsuguchi and Sakai, 1995; Omar et al., 1994; Polonenko et al., 1981). Osmotic effects caused by increasing

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salt concentration and specific ion toxicity may cause nutritional imbalance for microbial growth and enzyme synthesis (Batra and Manna, 1997). A combined depressive effect of salinity and low pH on microbial biomass has been reported in previous studies (Pankhurst et al., 2001). This is most likely due to a shift in community structure, from one dominated by fungi to one dominated by prokaryotic microorganisms consisting mainly of bacteria which may be less active, competitive and diverse (Pankhurst et al., 2001; Sadinha et al., 2003).

Among the great variety of hydromorphic soils only organic soils (Histosols) have been regularly studied for microbial activity in order to evaluate greenhouse gas fluxes (Jungkunst and Fiedler, 2007). Conversely, little information exists on microbial activity from mineral soils with hydromorphic properties such as Aquents (Soil Survey Staff, 2010) or Gleysols (IUSS Working Group WRB, 2006) despite the fact that they are common in most climate zones (FAO, 2001). In this study we examined the variations of biochemical properties in surface and subsurface soils within a landscape that may well have been affected by salt water intrusion caused by over-extraction of groundwater in the coastal area of Ravenna, Italy. In particular, the aim of the study was to assess the effect of hydromorphic and saline-sodic conditions on enzyme activity and functional diversity of soil microorganisms involved in SOM decomposition processes.

## 2. Materials and method

### 2.1. Site description and climatic features

The San Vitale Pinewood is a Site of Community Importance (SIC) of about 1222 ha and represents a remnant of pinewoods where man has settled, on beach-ridge sands deposited after the twelfth century (Fig. 1). The Pinewood developed on sand deposits (Regione Emilia Romagna, 2010) from sediments of an old branch of the Po River Delta (Bondesan et al., 1995). The dune and inter-dune system formed over 500 to 1000 years and almost parallels the coastline. The area is near the Adriatic Sea and the Pialassa Baiona lowlands, which likely promotes the intrusion of saltwater into aquifer. The San Vitale Pinewood area falls into pedological unit number 118 (Fig. 1) of the Emilia–Romagna soil map (Regione Emilia Romagna, 2010); these soils are characterized by sand and calcareous soil deposited as sand bars and vegetated by old-growth forest.

The warm Mediterranean climate of the area is typical of mild coastal environments, with about 600 mm of rainfall from March to December an annual mean temperature of 13 °C.

### 2.2. Soil sampling and vegetation cover

Seven soil profiles were described and sampled in the Pinewood. Soil profiles were taken along two east–west transects, positioned in a northern area (PW1, PW2, PW3, PW4) and in a southern area (PW6, PW7, PW9) (Fig. 1).

The profile locations were chosen in order to intercept differences in both microtopography (dune-inter-dune system) and water table depth (Fig. 1).

According to the parameters indicated by Richardson et al. (2001), sites PW1, PW3, PW7 and PW9 are in low-lying inter-dune areas at sea level. The areas of PW3 and PW7 are covered by woods and swamp forests with poplar (*Populus nigra* L.), buck thorn (*Frangula alnus* Mill.), elm (*Ulmus minor* Mill.) and alder trees (*Alnus glutinosa* L. ). The area of PW9 is mostly covered by halophytic vegetation such as sea rush (*Juncus maritimus* Lam. and *Juncus acutus* L.).

Profile PW2 is located next to dune crests in an area characterized by plantations of umbrella and maritime pines (*Pinus pinea* L., *Pinus Pinaster* Aiton) with brushwood rich in common hawthorn (*Crataegus monogyna* Jacq.), ivy (*Hedera helix* L.) and dog rose (*Rosa canina* L.) (Piccoli et al., 1991). Profiles PW4 and PW6 are located between the crests of the dune and a wide swale area covered by thermophilic submediterranean forests (evergreen oak — *Quercus ilex* L., with wet association of poplar — *Populus alba* L. and ash — *Fraxinus ornus* L., *F. oxycarpa* Bieb.). The water table is within 1 m of the surface at profiles PW4 and PW6.

Table 1 shows the morphological descriptions of the seven profiles. The horizons are described according to morphological characteristics. Air-dried samples of soil from each horizon were passed through a 2 mm sieve before processing for chemical analysis and microbiological assays. The horizons A1 and A2 are considered as surface soil, while the AC, ACg, C and Cg horizons as deep soil.

### 2.3. Soil classification

The survey was carried out in the San Vitale pinewood, along two transects located at right angles to the dune system, highlighting a close relationship between microtopography, horizon morphological properties and salinity, enabling taxonomic discrimination at subgroup level, within the Ustipsamments/Psammaquents complex (Soil Survey Staff, 2010).

Profiles PW2, PW4 and PW6, located in the highest spots of the dune system, are occasionally (PW4 and PW6 classified as Aquic Ustipsamments according to Soil Survey Staff, 2010) or not at all affected by the water table (PW2 classified as Typic Ustipsamment according to Soil Survey Staff, 2010).

On the contrary, profiles PW1, PW3, PW7 and PW9, located in swale area, are periodically saturated and with particular reference to the chemical characteristics of the aquifer, PW1 is classified as Typic Psammaquents, while PW3, PW7 and PW9 are classified as Sodic Psammaquents (Soil Survey Staff, 2010).

### 2.4. Soil chemical properties

Contents of total organic carbon ($C_{org}$), total extractable carbon ($C_{extr}$), humic acid ($C_{HA}$) and fulvic acid ($C_{FA}$) fractions were determined by wet oxidation at 160 °C with $K_2Cr_2O_7$ 1/3 M, according to the method of Springer and Klee (1954). $C_{extr}$ was extracted using a solution of NaOH 0.1 M and $Na_4P_2O_7$ 0.1 M incubated at 65 °C for 24 h. The $C_{HA}$ fraction was separated from $C_{FA}$ by precipitation of the total extract at pH<2; $C_{FA}$ was separated from the non-humified organic material (CNH) by solid chromatography using polyvinylpyrrolidone (PVP) resin according to the method described by Ciavatta et al. (1990). The data resulting from the carbon analysis were used to compute the following humification indices: degree of humification $DH = (C_{HA} + C_{FA}) * 100 / C_{extr}$ and humification rate $HR = (C_{HA} + C_{FA}) / C_{org}) * 100$ (Ciavatta et al., 1990). Soil pH and electrical conductivity (EC) were determined in the 1:2.5 soil:water ratio. The soil cation exchangeable capacity (CEC) was determined in $BaCl_2$ 0.1 M followed by a re-exchange with aqueous $MgSO_4$ solution (0.1 M) by inductively coupled plasma optical emission spectrometer (Arcos, Spectro). In order to minimize matrix effect, the exchangeable cations ($K^+$, $Na^+$, $Mg^{2+}$, $Ca^{2+}$) were determined in diluted solutions using AAS (Perkin Elmer). The sodium exchangeable percentage (ESP) was calculated as exchangeable-$Na^+$/CEC *100.

### 2.5. Microbial biomass and respiration

Microbial biomass carbon ($C_{mic}$) was estimated using the fumigation extraction (FE) method (Vance et al., 1987). The $C_{org}$ contained in the extracts was determined after oxidation with 0.4 N $K_2Cr_2O_7$ at 100 °C for 30 min. Microbial biomass was calculated as follows: $C_{mic} = ExC/k_{EC}$ where ExC is the difference between the organic C extracted from fumigated soils and the organic C extracted from not-fumigated soils and $k_{EC} = 0.38$ (Joergensen and Brookes, 1990).