Dissolved organic matter dynamics and microbial activity in salt-affected soils

A thesis submitted in fulfilment of the degree of
Doctor of Philosophy in Soil Science

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Abstract

Salt-affected soils (comprising saline and sodic soils) contain excessive amounts of salts and cover over 10 % of the world's arable land. They are a serious land-degradation problem because a) salinity causes poor plant growth and low microbial activity due to osmotic stress, ion toxicity and imbalanced nutrient uptake and b) plant growth in sodic soils is limited by poor soil structure and aeration. As a consequence of the poor plant growth, salt-affected soils have low organic matter content. Therefore, to minimise soil degradation, it is important to understand the processes in salt-affected soils particularly those involved in nutrient cycling.

Dissolved organic matter (DOM) is the most labile portion of soil organic matter pools and affects many biogeochemical processes such as nutrient cycling, translocation and leaching, microbial activity and mineral weathering. Even though it only comprises a small portion of the total organic matter (< 1 %), it can be used to determine changes in soil C dynamics prior to detection in the total SOM pool. Salinity and sodicity influence organic matter turnover by affecting the amount of plant material entering the soil as well as the rate of decomposition. While the effects of salinity and sodicity on soil microorganisms and soil organic matter turnover have been studied separately, little is known about their interaction. Therefore the objective of this thesis was to determine the interactive effect of salinity and sodicity on soil microbial activity and dissolved organic matter dynamics in soils of different texture.

Four non-saline and non-sodic soils differing in texture (4, 13, 24 and 40 % clay, termed S-4, S-13, S-24 and S-40) were collected from Monarto near South Australia. The water content resulting in maximum respiration in the soils was

assessed by adjusting the soils to different water content and measuring the respiration for two weeks at 25 ºC. The soils were leached with a combination of NaCl and $CaCl_2$ stock solutions to induce different levels of salinity ($EC_{1:5)}$ ranging from 0 to 10 dS m^{-1} and sodium absorption ratio [SAR< 3 (non-sodic) and ≥20 (sodic)] in various experiments. Wheat residue and in one experiment glucose were added as a nutrient source for soil microbes. Respiration was measured continuously throughout the experiments and dissolved organic C, dissolved organic N, total dissolved N (TDN), specific ultra-violet absorbance (SUVA), microbial biomass, electrical conductivity, pH and SAR were analysed at different times during the experiments.

The concentration of dissolved organic carbon (DOC) and nitrogen (DON) is influenced by the type of extractant used. To determine which extractant is the most useful for the experiments described in this thesis, different textured soils were incubated with wheat residue for two weeks and DOC and DON were extracted with water, 0.5M K_2SO_4 or 2M KCl at a 1:5 ratio. Irrespective of soil texture, the concentrations of DOC and DON extracted with 0.5M K_2SO_4 or 2M KCl were more than twice than those extracted with water. Therefore, for the experiments described in this thesis dissolved organic C and N were extracted with a 1:5 soil: water ratio.

In the first experiment, a sand and a sandy clay loam were adjusted to similar EC levels ($EC_{1:5}$ 0.5, 1.3, 2.5 and 4.0 dS m^{-1} in the sand and $EC_{1:5}$ 0.7, 1.4, 2.5 and 4.0 dS m^{-1} in the sandy clay loam) and combined with two sodium absorption ratios: SAR < 3 and 20. The soils were incubated at the water content optimal for microbial activity (6.4 g 100 g $soil^{-1}$ for the sand and 15.6 g 100 g $soil^{-1}$ for the sandy clay loam). This experiment showed that at a similar EC, cumulative respiration was more strongly affected by EC in the sand than sandy clay loam which may have been due to their different water content and therefore, differential osmotic potential. Further, the concentration of DOC, DON and SUVA were significantly higher at EC 0.5 or 0.7 at SAR 20 than at higher EC levels indicating that high SAR in combination with low EC is likely to increase the risk of DOC and DON movement downwards within the soil profile in the salt-affected soils which may cause further soil degradation.

To assess the impact of multiple drying and wetting on microbial biomass and DOC concentration in salt-affected soils, the loamy sand was adjusted to two levels of $EC_{1:5}$ (1.0 and 2.5 dS m^{-1}) and SAR (< 3 and 20) and then exposed to 1-3 drying and rewetting cycles each consisting of 1 week drying and 1 week moist incubation. The flush in respiration after rewetting was lower in saline and saline-sodic soils than in soil without added salt. At the low EC, the solubility of organic matter was higher at SAR 20 compared to SAR < 3 suggesting that loss of C via DOC leaching may be increased in sodic soils, irrespective of the drying and wetting cycles.

For the study on the effect of sodicity (SAR < 3 and >20) and salinity ($EC_{1:5}$ 1.0 and 5.0 dS m^{-1}) on DOM sorption, four soils of different texture (4, 13, 24 and 40 % clay) were shaken overnight at 4°C with solutions containing 0, 23, 43, 58, 86 and 128 mg C L^{-1} extracted from wheat residue. Sorption was calculated from the difference between initial DOM concentration and that after shaking. The experiment showed that high SAR (>20) only decreased DOC sorption at low EC (1.0 dS m^{-1}) which can be explained by the high electrolyte concentration causing flocculation of DOC at high EC (5.0 dS m^{-1}). DOC sorption was greatest in the soil with 24 % clay across all concentrations of DOC added whereas DOC sorption did

not differ greatly between the soils with 4, 13 and 40 % clay which suggested that sorption of DOC was not directly related to clay concentration, but instead was a function of CEC (highest in the soil with 24 % clay) and concentration of Fe and Al (highest in the soils with 4 and 13 % clay).

The study to examine how different forms of C (wheat straw and glucose, added at 2.5 mg C g^{-1}) with and without added inorganic N affect the response of microbial activity and biomass to increasing $EC_{1:5}$ (0.1 to 10 dS m^{-1}) showed that respiration and microbial biomass C decreased with increasing EC, but the decrease was smaller with glucose than with wheat straw. Addition of N to glucose and wheat straw to bring the C/N ratio to 20 significantly decreased cumulative respiration and microbial biomass C at a given EC. Thus, addition of easily available C can enhance microbial tolerance to salinity whereas high N addition rates may have an adverse impact on microbial activity.

In the last experiment, salt was added to the four soils to achieve EC values between 0.4 and 5.0 dS m^{-1} with two levels of SAR : < 3 and >20 together with the optimal water content for microbial activity, which resulted in three osmotic potential ranges in all four soils (> -0.55, -0.62 to -1.62 and -2.72 to -3.0 MPa). This experiment confirmed that salt stress has similar effects on soil microbes in soils of different texture and water content when expressed as osmotic potential whereas the soil microbes appear to be more sensitive to salts in lighter textured soils when EC is used as measure of salinity. Therefore, osmotic potential needs to be considered when comparing saline soils with different water holding capacity.

The results of the study showed increasing salinity adversely affects microbial activity and therefore increases DOC and DON concentration, whereas an increased

DOC and DON concentration in response to sodicity was observed only at low EC. Thus, both salinity and sodicity can result in increased loss of C and N through high concentration of DOM in leachates which may lead to further soil degradation and reduce C sequestration. The study also confirmed that soil texture and water content play an important role in determining the response of microbes to salt stress due to their effect on the salt concentration in the soil solution. Therefore, osmotic potential is a better measure for evaluating stress to microbes in the salt-affected soils than EC. Further, the study also highlighted that addition of a readily available and easily decomposable source of energy improves the ability of microbes to tolerate salinity whereas N addition has no or a negative impact on microbial activity and growth.

Declaration

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Manpreet Singh Mavi

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Chapter 1:

INTRODUCTION

Introduction

Land degradation is a major impediment to sustaining crop production in many arid and semi-arid regions of the world (Bossio et al., 2007). Low rainfall and high potential evapotranspiration in these regions promote the upward movement of salts in the soil solution which adversely affects soils physical, chemical and biological properties (Rengasamy, 2006). Therefore, it is important to understand the processes in salt-affected soils particularly those involved in nutrient cycling.

Salt-affected soils (comprising saline and sodic soils) which are characterised by excessive amounts of salts are a serious land-degradation problem. They cause poor plant growth and low microbial activity through osmotic stress and imbalanced nutrient uptake. Salt-affected soils occur within the boundaries of more than 100 countries and vary in their nature and properties. According to a report published by FAO in 2000, the total global area of salt-affected soils was 831 million hectares (Martinez-Beltran and Manzur, 2005). Salt-affected soils deteriorate as a result of changes in soil reaction (pH) and in the proportions of certain cations and anions present in the soil solution and on the exchange sites. These changes lead to osmotic and ion-specific effects as well as to imbalances in plant nutrition, which may range from deficiencies in several nutrients to toxic concentrations of others like sodium (Na). Such changes have a direct impact on the activity of plant roots and soil microbes, and ultimately on crop growth and yield (Grattan and Grieve, 1999; Mengel and Kirkby, 2001; Naidu and Rengasamy, 1993).

In Australia, saline soils have been estimated to cover over 17 million hectares, while sodicity affects approximately 340 million ha (NLWRA, 2001). Sodicity is caused by high levels of exchangeable Na adsorbed on the surfaces of soil particles. Increasing sodicity causes aggregates to disperse and the dispersed particles fill in the soil pores, causing decreased infiltration and permeability to water, and the formation of surface crusts and seals. Worldwide, a soil is considered to be sodic if exchangeable sodium percentage (ESP) is > 15. But within Australia, a soil is considered sodic when the ESP exceeds 6 (Isbell, 1998) because lower electrolyte levels in Australian systems causes the soils to disperse at a lower ESP. Generally, saline soils can be ameliorated with the use of high quality irrigation water or rainfall, which leaches soluble salts from the profile.

Soil organic matter (SOM) is a complex and dynamic soil component that exerts a major influence on the properties of soil: physical (stabilisation of soil structure and porosity), chemical (cation exchange and pH buffering) and biological (provision of substrate and nutrients for microbes) and plant nutrient availability in the ecosystem. Multiple factors influence the soil organic matter content of salt-affected soils, including litter input and high sodicity, salinity, pH and water content (Nelson and Oades, 1996). Salinity and sodicity have an impact on SOM turnover by affecting the amount of plant material entering the soil as well as rate of decomposition. Increasing sodicity may increase the loss of dissolved organic carbon (DOC) from the soil by dissolving organic matter (DOM), or by converting it either to a more dispersed or decomposable form (Wong et al., 2008). On the other hand, decreasing sodicity and increasing total electrolyte concentration has been found to decrease the concentration of organic carbon in solution due to flocculation (Skene and Oades, 1995).

Dissolved organic matter is the most labile portion of organic matter in soil. Even though it only comprises a small portion of the total organic matter (< 1 %), it can be used to determine changes in soil C dynamics under changing environmental conditions prior to detection in the total SOM pool. DOM is a primary source of mineralizable C, N, P, and S and is an important contributor to nutrient availability and cycling because it is immediately available to soil microbes (Haynes, 2005; Kuiters and Mulder, 1993). DOM also plays an important role in soil development, influences the potential for leaching of nutrients and may have detrimental impacts on water quality (Qualls and Haines, 1991). To minimise soil degradation and to ameliorate salt-affected soils, it is essential that factors and processes that alter DOM concentrations are well understood particularly for soils with poor structural stability and low inputs of soil organic matter such as salt-affected soils.

Numerous studies demonstrate the effects of salinity and/or sodicity on soil biological processes and organic matter dynamics, but often with contradictory results (Laura, 1976; Nelson et al., 1996; Pathak and Rao, 1998; Rietz and Haynes, 2003; Setia et al., 2011; Tripathi et al., 2006; Wong et al., 2008). The extent and direction of the observed effects of high salt concentrations on microbial activity, biomass and community structure are not consistent and seem to depend on the different soil properties (such as soil pH, anion composition, texture and soil organic matter content) and levels of salinity and sodicity (Li et al., 2006; Muhammad et al., 2008). Therefore, advancing our knowledge on organic matter dynamics in diverse soils and climatic conditions is necessary for understanding nutrient leaching, emissions of CO_2 to the atmosphere and soil erosion (Peinemann et al., 2005).

In spite of the extent of salt-affected soils, information is still sparse, particularly on the interactive effect of different levels of salinity and sodicity on microbial and organic matter dynamics in soils of varying texture. This literature review will focus on different aspects of salt-affected soils, dissolved organic matter dynamics and the effect of salinity and sodicity on microbial activity and DOM, leading to identification of knowledge gaps addressed in this thesis.

Chapter 2:

LITERATURE REVIEW

Literature Review

2.1 Salt-affected soils

2.1.1 Characterization and distribution

Salt-affected soils are classified as saline, sodic or saline-sodic on the basis of EC (electrical conductivity), SAR (sodium absorption ratio) and pH (Brady and Weil, 2002). Soils with EC_e (saturated extract) >4 dS m^{-1} and SAR >13 are classified as saline-sodic (US Salinity Laboratory Staff, 1954). Saline soils have an EC_e >4 dS m^{-1} (SAR< 13) and contain Na^+, $Mg^{2+,}$ and Ca^{2+} as dominant cations and Cl^- and SO_4^{2-} are the dominant anions. Salinity adversely affects soil microorganisms and plants by decreasing the osmotic potential of the soil solution.

Soils can be naturally saline (primary salinity), such as from salt accumulation resulting from weathering of parent materials, or submergence of the soil under seawater, or from natural salt deposits and rainfall. Although rainwater usually contains low salt concentrations, the small amount added over millions of years such as in Australian soils, which are mostly very old, can result in substantial salt accumulation. In contrast, secondary salinity refers to salinisation resulting from human activity, especially land development and agriculture (Ghassemi et al., 1995).

In Australia, soil salinity can be classified as dryland salinity (associated with ground water) or dry saline land salinity (not hydrologically connected to a saline ground water table). Approximately 5.7 million hectares of farmland in Australia is now at high risk from dryland salinity and this may rise to 17 million hectares by 2050 (NLWRA, 2001). Dryland salinity commonly occurs at the foot of slopes and on valley floors where the water table is shallow. This salinity is related to widespread removal of deep-rooted perennial native vegetation and its replacement with shallow-rooted annual crops and pastures, which increases water infiltration and leads to rising groundwater tables (Hatton et al., 2003). Water tables of less than 2 meters depth in valley floors can result in the development of dryland salinity (Rengasamy, 2006).

On the other hand, dry saline land or transient salinity is not associated with the ground water table and involves surface and sub-surface soil salinity. Dry saline land occurs in semi-arid parts of South Australia within duplex or texture contrast soils on upper or middle slopes, particularly when there is an impermeable clay rich sodic B horizon below a sandy/loamy A horizon. This impermeable horizon restricts movement of water into the B horizon, and consequently water will accumulate above this layer, forming a perched water table. During summer, the water will evaporate, which leads to accumulation of salt in the top soil and sub soil layers (Rengasamy, 2002); the salts are leached into the deeper soil layers with the rainfall in autumn and winter. In southern Australia, it is estimated that 16 % of cropping area is affected by dryland salinity, whereas up to 67 % could potentially be affected by transient salinity (Kelly and Rengasamy, 2006).

In sodic soils, Na^+ is a major cation on the exchange sites of the soil particles. These soils are characterized by SAR >13 and EC< 4 dS m^{-1} according to USDA although the Australian classification system (Isbell, 1998) defines sodic soils as having an ESP >6.

The ESP is measured according to the following equation:

$$\mathbf{ESP = (Na_{exch}/CEC) \times 100}$$

where Na_{exch} is the amount of exchangeable Na^+ and CEC is the cation exchange capacity, both expressed in cmol kg^{-1} soil.

The ESP of a soil describes the amount of exchangeable sodium in the soil relative to the other exchangeable cations present. However, for simplicity of measurement, sodium adsorption ratio (SAR) of soil extracts or soil solution is frequently used to describe the sodicity level of the soil. SAR is approximately equivalent to ESP for saturation extracts (Richards, 1954) but approximately half the value of ESP for 1:5 soil:water ratio (Rengasamy et al., 1984).

$$\mathbf{SAR = [Na^+] / [Ca^{2+} + Mg^{2+}]^{\frac{1}{2}}}$$

where Na^+, Ca^{2+} and Mg^{2+} concentrations in the soil solution are in mmol L^{-1}.

Sodic soils occur both naturally and as a result of human activities, occupying approximately 23 % of the arable land in Australia (Rengasamy, 2002). Soils can be naturally sodic or can become sodic by the application of irrigation water with high SAR and EC. Due to poor soil structure, these soils are prone to erosion and plants grow poorly, which can lead to loss of organic matter from top soil, resulting in reduced farm production and income (Sumner et al., 1998).

Globally, nearly 1 billion hectares of the agricultural land is affected by salinity and sodicity (Daggar, 2009). According to the FAO Land and Plant Nutrition Management Service, over 6 % of the world land area is affected by either salinity or sodicity. Although salt-affected soils are more widespread in arid and semi-arid regions of the world but they can be found in all the continents of the world and in every climatic region (Table 1).

Table 1. Regional distribution of salt-affected soils, in million hectares (Mha)

Regions	Total area	Saline soils		Sodic soils	
	Mha	Mha	%	Mha	%
Africa	1,899	39	2.0	34	1.8
Asia, the Pacific and Australia	3,107	195	6.3	249	8.0
Europe	2,011	7	0.3	73	3.6
Latin America	2,039	61	3.0	51	2.5
Near East	1,802	92	5.1	14	0.8
North America	1,924	5	0.2	15	0.8
Total	12,781	397	3.1	434	3.4

Source: FAO Land and Plant Nutrition Management Service

Geology, climate, topography and human activities are some of the important factors responsible for accumulation of salts in soil. Geological formations consisting of salt rich sediments can provide large amounts of salts to be transported by surface or ground water. Geomorphology has substantial effects on salt levels, increasing them within closed basin or lowland areas, where groundwater rises due to poor drainage system. Climatic factors such as low precipitation and high evaporation can increase the concentration of salts in soils, surface and ground water and contribute to salinity problems. Improper use of land and over-irrigation with water containing high levels of soluble salts are human-induced factors causing secondary salinity.

There is no accurate estimation of the economic damage worldwide resulting from salinity or sodicity, but losses of more than US$12 billion per year have been suggested (Ghassemi et al., 1995). Also, a study by Rengasamy (2002) estimated that transient salinity is costing the Australian farming economy in the vicinity of $1330 million per year.

2.1.2 Effect of salinity and sodicity on physical and chemical properties of soils

Saline soils may have a high pH if they are dominated by carbonates and bicarbonates of Na^+ or Ca^{2+} ions (Sardinha et al., 2003). However, in Australia saline soils usually have lower pH because sodium chlorides and sulphates are predominant (50-80 %) in most saline soils (Rengasamy, 2006). High concentration of salts in the soil solution prevents soil particles from dispersing and promotes flocculation. Therefore, vegetation in saline soils is not limited by poor aggregation or aeration but by detrimental effect of high salt concentrations on plant growth (Brady and Weil, 2002 ; Gupta and Abrol, 1990; Rengasamy, 2002). High concentrations of salt in the soil solution result in low osmotic potential (see Section 2.1.3), ion competition and ion imbalance (see Section 2.1.4).

In sodic soils on the other hand, the high sodium concentration on the exchange sites of the soil particles in combination with low salt concentrations in the soil solution leads to dispersion and degrades the soil structure. After wetting, the swelling and dispersion of clay particles are the major reasons for the deterioration of the soil structure (Rengasamy et al., 1984). Soil clay particles have a negative charge and therefore attract cations. This zone of positive cations around clay particles is known as ‘diffuse double layer’. Within this diffuse double layer, the concentration of cations is higher near the surface of clay particles and decreases with distance. The clay particles disperse when the diffuse double layer is thickened by large hydrated ions like Na^+ or due to electrostatic repulsion (Shainberg, 1992). Clay particles move through the soil profile clogging pore spaces and thus reducing water infiltration and nutrient movement within the soil. Therefore, sodic soils are very wet immediately after rain or irrigation and become very dry when water dries out through evaporation within a few days. Moreover, the solubility of sodium, aluminium and boron are increased due to the high pH and cause ion toxicity along with water-logging (Naidu and Rengasamy, 1993). Thus the lack of structural stability in these soils may result in soil hardening by seal and crust formation at the soil surface causing poor root development and plant growth with high risk of soil erosion (Sumner et al., 1998).

Sodic behaviour can also be exhibited in soils at very low ESP or SAR levels, when the electrolyte concentration is below the critical flocculation concentration (CFC) (Quirk and Schofield, 1955). Rengasamy et al. (1984) suggested that above $EC_{1:5}$ 0.6 dS m^{-1}, Red Brown Earths of South Australia remain flocculated irrespective of the percentage of sodium on the exchange complex (Figure 1). As a result, soil permeability and structure can be maintained through the application of water at the appropriate electrolyte level, depending on the degree of sodium saturation. While the extent of dispersion is due to high levels of Na^+ in a soil, complementary divalent cations like Ca^{2+} have the potential to promote flocculation (Keren and Ben-Hur, 2003). However, this needs further investigation in a range of soils with different salinity and sodicity levels.

2.1.3 Water potential in salt-affected soils

Water moves in soil from sites with high potential energy to sites with low potential energy to attain equilibrium with its surroundings (Hillel, 1998). It is subject to several forces in the soil environment, the combined effects of which result in a deviation in potential energy relative to the reference state referred as the soil water potential (ψ). Therefore, soil water potential is a measure of the difference in potential energy between the water in the soil relative to the energy of pure free water. The energy of soil water can be changed by changing the pressure of the water (pressure potential), concentration of solutes in the water (osmotic potential), or by the adhesive and cohesive forces that bind water to solid surfaces and in capillaries of a matrix (matric potential) and by the position of the water in a gravitational field (gravitational potential). The total potential is the sum of the component potentials:

Soil water potential (ψ) = $\psi_p + \psi_o + \psi_m + \psi_g$

where ψ_p= pressure potential; ψ_o= osmotic potential; ψ_m= matric potential; ψ_g= gravitational potential

Among the different components of soil water potential, osmotic and matric components are considered to be most important for availability of water to plants and microbes.

In soils, osmotic potential results from interaction of salts with soil water (Papendick and Campbell, 1981). Increasing concentrations of salt in soil solution attract water molecules, which restricts the availability of the soil water to plants and microorganisms. Therefore, high concentrations of dissolved salt in soil solution cause severe osmotic stress to soil microbes and reduce water uptake by the cells. In soils with very low osmotic potential , water moves from the cells to the lower osmotic potential of the soil solution (Brady and Weil, 2002). Osmotic adjustment is a fundamental response of plants and microbes to salinity (Griffin, 1969; Wyn Jones and Gorham, 1983) for their survival and growth which occurs through uptake of solutes and/ or synthesis of organic compounds. This solute accumulation lowers the osmotic potential in the cell and helps to maintain turgor of the cells (Wright et al., 1997).

Figure 1. Relationship between dispersion, ESP and EC (Adapted from Rengasamy et al. 1984) CFC= critical flocculation concentration.

The osmotic potential of the soil water can be estimated using the equation given by Richards (1954):

Osmotic potential (MPa) = $-0.036 \times \mathrm{EC}\ (\mathrm{dS\ m^{-1}}) \times O_{act}/O_{ref}$

where O_{act} is actual moisture content (g g^{-1}) of the soil and O_{ref} is the reference water content (g g^{-1}) of the 1:5 soil-water mixture.

As non-saline soils dry, the dominant component of water stress is matric potential, resulting from interaction of water with soil particles. In a wet but unsaturated soil, water is loosely bound to the soil solids and exists as thick water films around soil particles. As the soil dries, the water films around aggregates become thinner and disconnected, which decreases water availability to microbes and plants resulting in matric stress (Brady and Weil, 2002 ; Ilstedt et al., 2000). As a result, substrate and nutrient diffusion is also restricted which becomes the main stress in a very dry soil, even at low osmotic potential (Rattray et al., 1991; Stark and Firestone, 1995).

The concentration of salts in the soil solution also depends on the water content of the soil. At a given salt content of the soil, the osmotic potential of the soil decreases with decreasing water content due to increased concentration of salts in the solution. This may be particularly important when comparing the effect of salinity in soils of different texture. The water retention capacity of a fine-textured soil is greater than a coarse-textured soil, therefore at a given $EC_{1:5}$ and matric potential, the osmotic potential of the soil solution is lower in the coarse-textured soil. Thus, osmotic potential of soil solution may be a more appropriate parameter than EC for assessing the effect of salts on plant growth (Ben-Gal et al., 2009). However, this factor has not been studied in detail with respect to microbial activity and DOC in soils of varying texture.

2.1.4 Influence of salinity and sodicity on plants

High salt concentrations in the soil solution (low osmotic potential) affect plant growth and survival by reducing seed germination, root growth, evapotranspiration, photosynthesis, protein synthesis and enzyme activity, thereby causing reduced yield (Qadir and Schubert, 2002). The effects of salinity and sodicity on plant physiology and morphology have been reported in many studies (Akilan et al., 1997; Allen et al., 1994; Mäser et al., 2002; Munns et al., 2006); osmotic effects dominate in saline and saline-sodic soils, while poor soil structure dominates in sodic soils.

In dry saline soils, plants need to overcome both low matric and osmotic potentials to take up water and thus a large amount of energy is needed to survive. Rengasamy (2006) showed that in the absence of salt, plants can continue to take up water from a loam soil at 5 % water content, but when the soil salinity increased to $EC_{1:5} > 1$ dS m^{-1}, plants ceased water uptake at 18 % water content as total water potential (matric + osmotic) decreased to stress level (-1.5 MPa). However, mature plants or salt-tolerant species can adjust to higher salinity by making osmotic adjustments.

In salt-affected soils, sodium (Na^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) are the dominant cations and chloride (Cl^-), sulphate (SO_4^{2-}), carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) are the dominant anions. Excess concentration of these cations and anions in plants can lead to ion imbalance and ion toxicity (Naidu et al., 1992). However, Rengasamy (2010) and Tavakkoli et al. (2010) showed that the ion effect dominates only at low salinity levels whereas the osmotic effect becomes more important at higher salinity. Nevertheless, the accumulation of ions such as Na^+ and Cl^- may have a toxic effect on plant roots while Cl^- accumulation in the leaf tissue over time reduces photosynthesis and respiration (Naidu and Rengasamy, 1993). This situation may be further compounded by the enhanced toxicity of other macro- and micro-nutrients, such as Zn, Mg and B (Curtin and Naidu, 1998). An increase in the concentration of exchangeable Na^+ in the sodic soils decreases the concentration of Ca^{2+} and Mg^{2+}, whereas high Cl^- reduces the concentration of anions like NO_3^-, thus reducing their uptake by plants (Munns and Tester, 2008).

Sodicity adversely affects nutrient and water uptake by plants due to poor soil structure. Adequate soil water content is often difficult to maintain in areas affected by sodicity due to water-logging at the surface. On the other hand, the formation of surface crusts reduces seed germination and plant establishment (So and Aylmore, 1993). When the soil pH increases above 9, B, carbonate and bicarbonate toxicity as well as deficiency in Fe, Mn, Cu, Zn and P may cause nutritional imbalance in plants (Rengasamy, 2002).

2.1.5 Effect of salinity and sodicity on soil microorganisms

Apart from the physical and chemical properties, salinity and sodicity have an adverse effect on soil biochemical processes. Salinity affects soil microorganisms by decreasing osmotic potential, which kills sensitive microbial genotypes and increases the metabolic burden in tolerant genotypes. Thus, the physiology and composition of the microbial community is altered by salt stress (Sardinha et al., 2003; Wichern et al., 2006; Zahran, 1997). Salt tolerance in microbial species can be due to two main mechanisms. Firstly, in species adapted to highly saline environments such as salt lakes, Na^+ and Cl^- or NH_4^+ or SO_4^{2-} are accumulated which is possible because their enzymes function despite the high salt concentrations in the cytosol (Killham, 1994). Other microbes produce osmoregulatary compounds like amino acids in bacteria and polyols in fungi (Killham and Firestone, 1984; Oren, 2001; Schimel et al., 1989). The synthesis of osmoregulatary compounds requires large amounts of energy (30-110 Adenosine-

Tri-Phosphate) and is therefore a metabolic burden for microbes (Oren, 1999). Thus, microorganisms under salinity stress are able to utilize less energy for other metabolic functions and growth, which reduces their substrate use efficiency (Wichern et al., 2006).

Salinity has been found to negatively influence the activity of soil microbes (Pankhurst et al., 2001; Pathak and Rao, 1998; Setia et al., 2011; Tripathi et al., 2006). On the contrary, some studies have reported increased rates of carbon and nitrogen mineralization with increasing salinity (Chandra et al., 2002; Laura, 1976; Wong et al., 2008). It has been shown that the effect of salinity on soil microorganisms also depends on the type of salts in soil solution; e.g. sodium chloride has been found to be more toxic than calcium chloride (Agarwal et al., 1971; Garcia and Hernandez, 1996). However, Setia et al. (2010) reported similar effect of Ca^{2+} and Na^{+} on soil respiration. Accordingly, Pathak and Rao (1998) found significant carbon dioxide evolution even at $EC_e = 97$ dS m^{-1}.

Further, increased availability of organic substrates has been shown to help microbes to counteract some of the negative effect of salts (Wichern et al., 2006). Although the effects of chemical composition of the organic substrates on decomposition have been addressed in non-salt-affected soils (Thuries et al., 2002; Vanlauwe et al., 1994), it is not clear how microbial activity and biomass in salt-affected soils respond to differences in substrate availability and composition.

Studies have found decreased microbial biomass (Sardinha et al., 2003; Tripathi et al., 2006; Yuan et al., 2007) and microbial diversity (Ibekwe et al., 2010; Nelson and Mele, 2007) with increasing salinity and generally, fungi are reported to be more sensitive to salinity than bacteria (Chowdhury et al., 2011; Pankhurst et al., 2001; Sardinha et al., 2003). On the other hand, some studies have reported no effect of salinity on microbial biomass and community (Sarig and Steinberger, 1994) or increased microbial biomass with salinity and sodicity (Wong et al., 2008). Nelson et al. (1996) found no significant effect of salinity and sodicity on total microbial biomass, but significant effects of both on the microbial biomass derived from added plant residues, which could explain the discrepancies between studies pointed out here.

Further, Nelson et al. (1996) found that sodicity increased C mineralization because it makes the SOM more accessible to microorganisms whereas Setia et al. (2011) found a negative effect of sodicity on CO_2 emission due to poor aeration by clay dispersion and toxicities of ions. On the other hand, Pathak and Rao (1998) found that sodicity had no effect on C mineralization.

Increasing salinity may also reduce N mineralization rates (Jackson and Vallaire, 2009; Pathak and Rao, 1998). However, ammonification has been found to be less affected by salinity than nitrification (Irshad et al., 2005; Laura, 1974; Pathak and Rao, 1998). Among the nitrifiers, *Nitrobacter* species are more affected by salinity than *Nitrosomonas* (Johnson and Guenzi, 1963). On the other hand, an increase in mineralization of N with salt additions has been reported in some studies (Broadbent and Nakashima, 1971; Singh et al., 1969).

Accordingly, the inconsistency of reports on the effects of salinity and sodicity on soil microbes stresses the need for further experiments with different soils to elucidate the effects of the interaction between salinity and sodicity on soil microbial activity, biomass and organic matter decomposition.

2.2 Dissolved organic matter dynamics in soils

2.2.1 Pools and Role of Soil Organic matter

Soil organic matter (SOM) is the largest organic C pool in the terrestrial biosphere and is a major ecosystem N and P reserve. It is the third largest carbon (C) pool after oceans (38,000 Pg C) and geologic pools (5000 Pg C) and is estimated to contain approximately 1576 Pg of C to a depth of one meter (Kolahchi and Jalali, 2007). The distribution of soil organic carbon (SOC) largely follows a gradient similar to biomass accumulation, increasing with increasing precipitation and decreasing temperature. Primarily SOC content is determined by the balance between C input and C output from decomposition by soil microorganisms.

Soil organic matter is defined as the non-living component of organic matter in soil (Trumbore, 1997) which is derived from plants and animals. In soils, a continuous and degradable supply of SOM is essential because it acts as a source of energy for microbial biomass and provides a reservoir of nutrients to sustain soil fertility and plant growth. The microbial biomass converts the nutrients held in plant, animal and microbial residues into plant-available forms through mineralisation (Baldock and Nelson, 1999) and thus plays a significant part in nutrient cycling in the ecosystem.

Further, SOM is also important for maintaining structural stability in soil because it prevents dispersion and disaggregation (Barzegar et al., 1997; Nelson et al., 1997). Components of SOM like aromatic humic material, polysaccharides, microbial mucilage and organic polymers as well as dead roots and hyphae improve soil aggregation at a range of scales (Tisdall and Oades, 1982). In addition, SOM also contributes to the soil's cation exchange capacity, pH buffering capacity and its ability to complex cations (Krull et al., 2004).

SOM may be divided into two major components: stable and active forms. Stable organic matter consists of humus and inert organic matter with low turnover rates of 100 to more than 1000 years. The size of the active organic matter pool has been estimated using measurable entities such as: particulate organic matter (POM), dissolved organic matter and microbial biomass, with turnover rates varying from weeks or months to a few years (Baldock, 2002). The active soil C pools are frequently used as indicators of SOM dynamics (Alvarez et al., 1998) because changes caused by environmental and management stresses are detected earlier in the active C pools than in SOM as a whole. Tipping et al. (2012) demonstrated the importance of DOM for understanding SOM turnover by applying the DyDOC model to an extensive experimental dataset. Moreover, Kaiser and Kalbitz (2012) showed that DOM can be used as an indicator for processes controlling the turnover of soil organic matter in the soil.

2.2.2 Sources and fluxes of DOM

Dissolved organic matter (DOM) is the most mobile and dynamic non-living organic matter fraction and affects many biogeochemical processes such as nutrient translocation and leaching, microbial activity and mineral weathering (Kalbitz et al ., 2000; McDowell, 2003). Since carbon represents the bulk of the organic matter (>50 %), DOM is often quantified by measuring dissolved organic carbon (DOC). Soil solutions contain varying amounts of DOM, which originates from plant litter, humus, microbial biomass, root exudates and organic amendments (Figure 2). Earlier studies have concluded that DOC production is a function of microbial activity in the soil (Bolan et al. 2011; Guggenberger and Zech, 1993; McDowell and Likens, 1988), and therefore it is likely that its production is controlled by factors controlling biological activity. Indeed, some studies reported a positive correlation between DOC and soil respiration (Andersson et al., 1994; Marschner and Bredow, 2002) while in others it was not (Beauchamp et al., 1980; Cook and Allan, 1992; Davidson et al., 1987; Ganthier et al., 2010).

Dissolved organic matter is defined operationally as a continuum of organic molecules of different sizes and structures that pass through a filter of 0.45 μm pore size (Thurman, 1985; Zsolnay, 2003). In the case of studies involving soils, the terms water soluble organic matter (WSOM) or water extractable organic matter (WEOM) are also used, which represent the fraction of the soil organic matter extracted with water or dilute salt solution that passes a 0.45 μm filter (Zsolnay, 2003). Generally, 70-90 % of DOM is present as aromatic humic molecules which are recalcitrant and not readily degraded by microbes (Guggenberger and Zech, 1994; Kalbitz et al., 2003). Only small proportions of DOM can be identified chemically, mostly low molecular weight substances such as organic acids, sugars, amino acids (Herbert and Bertsch, 1995). Although DOM comprises only a small part of soil organic matter (< 1 %), it affects many processes in soil and water including environmental problems such as soil and water pollution and global warming (Kalbitz and Kaiser, 2003).

The turnover of dissolved organic C, N and P in soils is a major pathway of element cycling (Kalbitz et al., 2000) and therefore plays an important role in terrestrial C, N and P budgets (Neff et al., 2000). DOC is an important source of C for microbes and presents a potentially important pathway of ecosystem C loss from soil along with other nutrients via runoff and leaching into surface water bodies and groundwater (Baldock and Skjemstad, 1999; Stevenson and Cole, 1999) which can have detrimental effects on water quality (EPA South Australia, 1998). Moreover, leaching can reduce the amount of DOM available for mineralization within the soil and reduce soil nutrient cycling and fertility (Kalbitz et al., 2000; Marschner and Kalbitz, 2003).

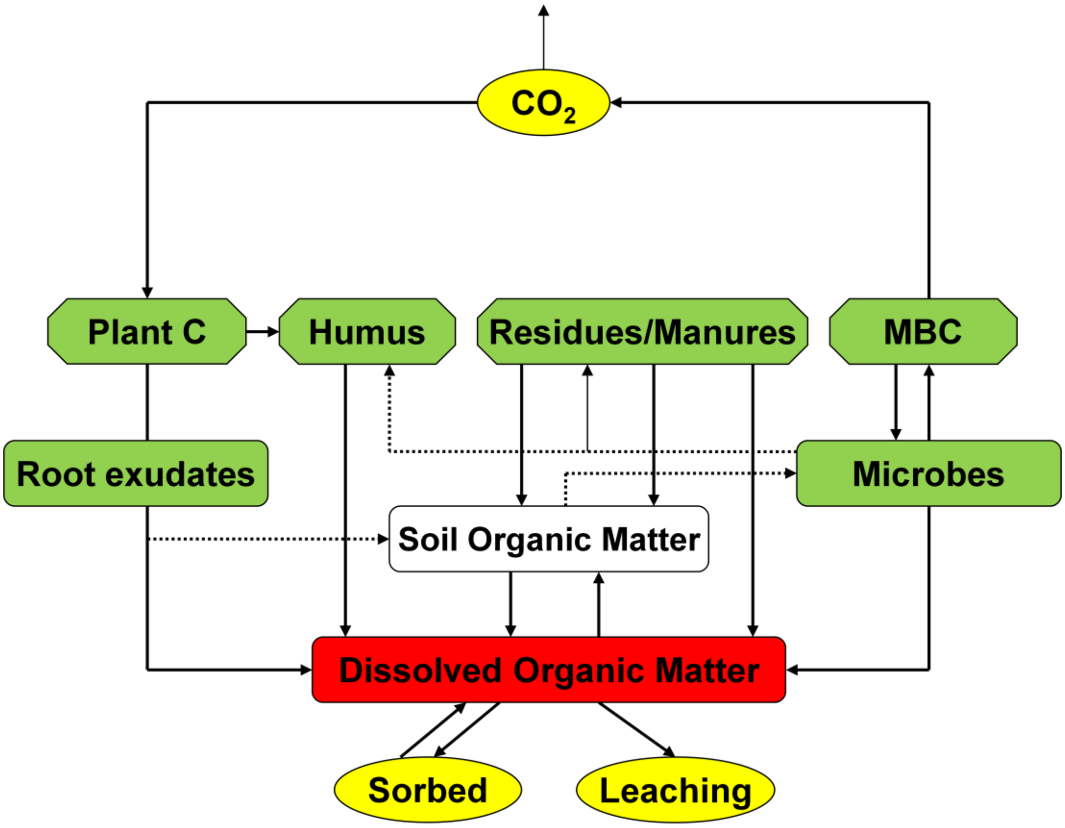

Figure 2. Dissolved organic matter (DOM) dynamics in soil

Further, DOM is a potential source of the stabilized carbon in subsoils (Kalbitz and Kaiser, 2008; Schmidt et al., 2011) although this accumulation is not infinite (Guggenberger and Kaiser, 2003; Kalbitz and Kaiser, 2008). Studies have shown that the contribution of DOC to soil carbon pools down to 60 cm could range from 25 % of the total carbon stock (Neff and Asner, 2001) to 73-89 % of mineral soil carbon (Michalzik et al., 2003). The retention and mobility of DOC in soils is controlled primarily by its sorption to mineral surfaces (Kramer et al., 2012; McDowell and Likens, 1988; Ussiri and Johnson, 2004). A number of processes for DOC sorption in soils have been postulated: ligand exchange, cation exchange, anion exchange, physical adsorption, inner sphere complexation, pi-donor-acceptor interactions, cation bridging, hydrogen bonding and van der Waals forces (Chorover et al., 2004; Gu et al., 1994; Jardine et al., 1989; Keiluweit and Kleber, 2009; Mikutta et al., 2009; Sollins et al., 1996). Several studies have shown the effect of soil properties such as clay and organic carbon content, surface charge and electrolyte concentration, oxalate-extractable Fe and Al and pH on DOC sorption (Guggenberger and Kaiser, 2003; Kahle et al., 2003; Kalbitz and Kaiser, 2008; Kogel-Knabner et al., 2008; Rashad et al., 2010). However, most of these studies were with non-saline and non-sodic soils from temperate climates or forest ecosystems.

Ninety percent of N in soil is found in organic forms and this fraction plays an important role in plant nutrition (Goulding et al., 2000; Kelly and Stevenson, 1996). Dissolved organic nitrogen (DON) fluxes in soil are primarily regulated by the abundance of C and N in soil organic matter because they regulate microbial activity, which in turn is affected by microbial N demand (Murphy et al., 2000). Similarly Satti et al. (2003) found that N mineralization was regulated by the quality of C in the litter and by total N in the soil. Thus, the dynamics of DON is closely related to those of DOC since their formation involves similar processes (McGill and Cole, 1981; Qualls and Haines, 1991).

Despite intensive research in the last decade, our knowledge of the formation and fate of DOM in soils and its response to changing environmental conditions is still fragmentary and often inconsistent. Most of the information available today is the result of studies on temperate soils and forest ecosystems. Thus, future research on DOM dynamics should be extended to different soils and those under different land uses.

2.2.3 Impact of soil texture on microbial activity and DOM

Soil texture plays a significant role in residue decomposition and microbial community structure and activity (Franzluebbers et al., 1996; Hassink, 1994; Sugihara et al., 2010). In coarse-textured soils, the turnover of microbial biomass C and N is faster than in fine-textured soils because C in fine-textured soils is bound to clay particles and thus is less accessible for degradation by microbes and enzymes (Berg and McClaugherty, 2003; Franzluebbers et al., 1996; Hassink, 1994). Therefore, clay minerals play an important role in the stabilization of soil organic matter in the soils (Amato and Ladd, 1992; Oades, 1988).

Further, the capacity of soil to adsorb DOC was found to be positively correlated with soil clay content (Nelson et al., 1993; Shen, 1999). Apart from clay concentration, clay type may also affect the capacity of soil to bind organic matter, with smectite tending to have a greater protective effect than illite or kaolinite (Nelson et al., 1999). Clays with higher specific surface area and cation exchange capacity (CEC) adsorb more C (Kahle et al., 2003; Ransom et al., 1998). However, Plante et al. (2006) observed no effect of soil texture on decomposition of organic matter. Therefore, for a wider applicability of the results of the present study, soils with different texture were used in the experiments designed to study DOM dynamics in salt-affected soils.

2.2.4 Influence of drying and rewetting on soil microbes and DOM

In a Mediterranean climate, many soils experience drying and rewetting phases which alter soil microbial and nutrient dynamics (Davidson, 1992). During summer, top soils may undergo multiple drying and wetting events when dry periods are interrupted by occasional rainfall. These drying and rewetting events could be exacerbated in the future as the annual soil water content will decrease by almost 25 % in the Mediterranean and subtropical regions (Meehl et al., 2007). As described in Section 2.1.3, drying of soil causes the matric potential to become more negative (Harris, 1980), and reduces the thickness of the water film surrounding soil aggregates, inhibiting diffusive transport of substrates to microbes (Stark and Firestone, 1995). Microbes may compensate for the low water potential in the surrounding medium by accumulation of solutes in the cell (Halverson et al., 2000; Harris, 1980). This process is very energy-demanding and therefore a metabolic burden for the microbes (Harris, 1980; Schimel et al., 2007).

Rewetting of dry soils results in a flush of microbial activity which usually lasts for 2-5 days (Austin et al., 2004; Wu and Brookes, 2005). This wetting pulse may be attributed to increased substrate availability to microbes from release of osmolytes accumulated during drying phase, cell lysis and breakdown of aggregates releasing previously protected organic matter (Denef et al., 2001; Fierer and Schimel, 2003; Halverson et al., 2000; Kieft et al., 1987). Generally, the size of the pulse in microbial activity upon wetting decreases with the frequency of dry-wet cycles (Jager and Bruins, 1975; Mikha et al., 2005; Prieme and Christensen, 2001) which has been explained by a decrease in labile organic matter over time (Birch, 1958) and/or by a shift in bacterial community composition (Fierer et al., 2003).

In non-saline and non-sodic soils, DOC concentrations increase following drying and rewetting (Chittleborough et al., 1992; Kalbitz and Knappe, 1997; Zsolnay et al., 1999). Lundquist et al. (1999) gave three possible explanations for the increase in DOC during rewetting cycles: (i) reduced microbial utilization of DOC in dry periods, (ii) enhanced turnover of microbial biomass and condensation of microbial products by rewetting, and (iii) disruption of soil aggregates making previously sequestered carbon more available as DOC.

Most studies on effects of drying and rewetting on microbial activity and DOC have been carried out in non-saline soils. Chowdhury et al. (2011) reported that saline soils exposed to drying and rewetting cycles may be less prone to loss of C than the non-saline soils. Although several studies have addressed the effects of moisture on microbial activity and DOC dynamics, with varying results in different soil environments, there seem to be very few studies in salt-affected soils.

2.2.5 Effect of salinity and sodicity on solubility of DOM and nutrient loss

As outlined above, salinity has been found to negatively influence the size and activity of soil microbial biomass and biochemical processes essential for maintenance of soil organic matter (Chowdhury et al., 2011; Yuan et al., 2007). Sokoloff (1938) and Nelson et al. (1996) found that sodicity increased SOC mineralization because it makes the SOM more accessible to microorganisms. Therefore, sodicity may increase the potentially leachable SOC in the soil. Over time, soil carbon stores become depleted as organic matter is mineralized by microorganisms, while plant inputs decrease due to stresses caused by increasing salinity and sodium content of soil (Wong et al., 2008).

Leaching of DOM may be particularly important for sodic soils, because Na-organic complexes are highly soluble and mobile, and easily lost by leaching (Naidu and Rengasamy, 1993; Wong et al., 2008) and runoff. The high solubility of organic matter in sodic soils represents a major pathway by which C and N can be lost (Peinemann et al., 2005). Conversely, increasing salinity causes soils to flocculate, offsetting the effects caused by sodicity (Shainberg and Letey, 1984) and decreasing the concentrations of organic carbon released (Skene and Oades, 1995).

Thus, the conflicting results of various investigations on the effect of salts on microbial and organic matter dynamics suggest that more studies are needed to understand the mechanisms governing the dynamics of DOM and nutrient leaching in these degraded environments.

2.3 Effect of addition of C and N on microbial activity and DOM

In salt-affected soils, physico-chemical and biological problems arising from lack of organic matter are usually evident in the form of low microbial activity, low cation exchange capacity, poor soil aggregation and low water and nutrient holding capacity (Liang et al., 2003). Tejada and Gonzalez (2005) demonstrated that an increase in organic matter content of saline soils increases soil structural stability and microbial biomass, and decreases soil bulk density. It has also been reported that application of organic matter to saline soils can accelerate sodium leaching, decrease the exchangeable sodium percentage and electrical conductivity and increase aggregate stability (El-Shakweer et al., 1977). Different organic amendments have been applied to improve salt-affected soils but the results have varied depending on salt composition and quantity (Lax et al., 1994; Rao and Pathak, 1996). Previous studies have demonstrated that application of manures significantly increased the yield of crops grown on saline and sodic soils (Gaffar et al., 1992; Tahir et al., 1991). Liang et al. (2005) confirmed the view that incorporation of manure is an effective low-input agro-technological approach to enhancing soil fertility and minimizing phytotoxicity induced by secondary salinisation. Further, the chemical composition of organic amendments added to soil determines the effect that they will have on C and nutrient dynamics in soil. The rates of decomposition vary widely due to differences in water content, temperature, pH and the availability of nutrients like N and P for the microbes. Amending soil with high C/N and C/P organic matter causes immobilization of N and P (Gregorich et al., 2000) and therefore reduces leaching of N and P. In general, monomers like glucose are decomposed within hours to few days in soil whereas polymers are decomposed more slowly due to their complex structure (Oades, 1988). Thus, the effects of chemical composition of the substrate on decomposition have been addressed in non-salt-affected soils (Thuries et al., 2002; Vanlauwe et al., 1994) but it is not clear how carbon form (easily available or recalcitrant) affects the response of microbes in salt-affected soils.

In soil, apart from C, availability of N is another factor regulating microbial growth and activity. Addition of N may increase (Henriksen and Breland, 1999; Recous et al., 1995) or decrease microbial activity (Janssens et al., 2010; Kowalenko et al., 1978; Sall et al., 2003; Soderstrom et al., 1983) or have no effect (Allison and Klein, 1962; Hobbie and Vitousek, 2000; Prescott, 1995). The different effects may be due to the ratio of available C to N, the original N availability of the soil as well as the effects of N addition on soil properties such as pH.

Further, N addition can also influence DOC through its role as a main limiting nutrient in terrestrial ecosystems, and due to the role of labile organic matter in N immobilisation (Aber, 1992; Zech et al., 1994). Pregitzer et al. (2004) showed strongly increased DOC export from a North American forest soil following long-term N additions, but other studies have shown little or no response (Gundersen et al., 1998; McDowell et al., 2004). Neither N deposition (Fowler et al., 2001) nor surface water nitrate concentrations (Davies et al., 2005) support the hypothesis of N as a driver of DOC increase, although these observations do not exclude the possibility that long-term soil N-enrichment could impact on DOC production.

2.4 Aims of the study

The lack of consistency in the results of various investigations on the effect of salinity and sodicity on soil microbes and DOM suggest that the factors governing the dynamics of DOM and nutrient leaching in these soils are not fully understood

Therefore the present study has the following aims:

- To examine the interactive effect of salinity and sodicity on microbial respiration and DOM dynamics in soils with a range of textures (Chapter 3).
- To investigate the influence of drying and wetting on microbial activity, biomass and DOC in saline and saline-sodic soils (Chapter 4).
- To evaluate the effect of salinity and sodicity on DOC sorption in soils with diverse clay contents (Chapter 5)

- To study how different forms of C: easily available (glucose) and less available (wheat straw), with and without added inorganic N, affect the response of microbial activity and biomass to increasing EC (Chapter 6).
- To determine the effect of osmotic potential in combination with sodicity on microbial activity and DOM dynamics in different soils (Chapter 7).

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CHAPTER 3:

SALINITY AND SODICITY AFFECT SOIL RESPIRATION AND DISSOLVED ORGANIC MATTER DYNAMICS DIFFERENTIALLY IN SOILS VARYING IN TEXTURE

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Salinity and sodicity affect soil respiration and dissolved organic matter dynamics differentially in soils varying in texture

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ABSTRACT

The individual effects of salinity and sodicity on organic matter dynamics are well known but less is known about their interactive effects. We conducted a laboratory incubation experiment to assess soil respiration and dissolved organic matter (DOM) dynamics in response to salinity and sodicity in two soils of different texture. Two non-saline non-sodic soils (a sand and a sandy clay loam) were leached 3–4 times with solutions containing different concentrations of NaCl and $CaCl_2$ to reach almost identical electrical conductivity ($EC_{1:5}$) in both soils ($EC_{1:5}$ 0.5, 1.3, 2.5 and 4.0 dS m^{-1} in the sand and $EC_{1:5}$ 0.7, 1.4, 2.5 and 4.0 dS m^{-1} in the sandy clay loam) combined with two sodium absorption ratios: SAR < 3 and 20. Finely ground wheat straw residue was added (20 g kg^{-1}) as substrate to stimulate microbial activity. Cumulative respiration was more strongly affected by EC than by SAR. It decreased by 8% at EC 1.3 and by 60% at EC 4.0 in the sand, whereas EC had no effect on respiration in the sandy clay loam. The apparent differential sensitivity to EC in the two soils can be explained by their different water content and therefore, different osmotic potential at the same EC. At almost similar osmotic potential: −2.92 MPa in sand (at EC 1.3) and −2.76 MPa in the sandy clay loam (at EC 4.0) the relative decrease in respiration was similar (8–9%). Sodicity had little effect on cumulative respiration in the soils, but DOC, DON and specific ultra-violet absorbance (SUVA) were significantly higher at SAR 20 than at SAR < 3 in combination with low EC in both soils (EC 0.5 in the sand and EC 0.7 and 1.4 in the sandy clay loam). Therefore, high SAR in combination with low EC is likely to increase the risk of DOC and DON leaching in the salt-affected soils, which may lead to further soil degradation.

1. Introduction

Land degradation by salts is a major threat to sustainable crop production in many arid and semi-arid regions of the world (Bossio et al., 2007). Low rainfall and high potential evapotranspiration in these regions promote the upward movement of salts in the soil solution which adversely affects soils physical, chemical and biological properties (Rengasamy, 2006). Worldwide more than 831 million hectares of land is salt-affected (Martinez-Beltran and Manzur, 2005) and this area is likely to increase in the future because of secondary salinisation due to irrigation and clearing of native vegetation (Pannell and Ewing, 2006). Therefore, it is important to understand the processes in salt-affected soils particularly those involved in nutrient cycling.

Salt-affected soils are classified as saline, sodic and saline-sodic on the basis of EC (electrical conductivity), SAR (sodium absorption ratio) and pH (Brady and Weil, 2002). Soils with EC_e (saturated extract) > 4 dS m^{-1} and SAR > 13 are classified as saline-sodic (US Salinity Laboratory Staff, 1954). Saline soils have an EC_e of the saturation extract > 4 dS m^{-1} (SAR < 13) and contain Na^+, Mg^{2+}, and Ca^{2+} as dominating cations and Cl^- and SO_4^{2-} are the dominant anions. The effects of excessive salts in the soil solution include reduced water uptake due to low osmotic potential (Harris, 1980), high pH, and ion competition limiting nutrient uptake (Keren, 2000) which not only reduce plant growth but also have a negative influence on the size and activity of soil microbial biomass and biochemical processes essential for maintenance of soil organic

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matter (Rietz and Haynes, 2003; Tripathi et al., 2006; Yuan et al., 2007). Some microbes respond to low osmotic potential by accumulating osmolytes to retain water whereas sensitive microbes die (Hagemann, 2011). In sodic soils ($EC_e < 4$ and $SAR > 13$ according to USDA classification, but $SAR > 3$ in the Australian classification system (Isbell, 2002)), Na is the dominant cation on the exchange sites of the soil particles. Increasing Na saturation on the exchange sites results in dispersion of organic matter and clay particles, thus destroying aggregates and soil structure.

Oades (1988) showed that high electrolyte concentration plays a major role in linking organic matter to clays. Therefore, high clay content and increasing electrolyte concentration will make organic matter less accessible to microbes for decomposition in salt-affected soils (Nelson et al., 1997). Hence, clay dispersion is negatively correlated with EC and positively correlated with SAR and pH (Nelson et al., 1998).

Dissolved organic matter (DOM which includes C, N and other organically bound nutrients) is the most mobile and dynamic non-living organic matter fraction. It comprises only a small part of soil organic matter (< 1%); nevertheless, it is a primary source of mineralizable C, N and P and affects many processes in soil such as nutrient translocation and leaching, microbial activity, mineral weathering and nutrient availability (Evans et al., 2005; Kalbitz et al., 2000; Zsolnay, 2003). The concentration of dissolved organic carbon (DOC) can decrease as a result of sorption, precipitation or mineralisation by soil microorganisms. The ease with which DOC can be degraded by microbes is related to its content of aromatic C compounds (Marschner and Kalbitz, 2003). High solubility of organic matter due to sodicity can cause loss of DOM by leaching (Peinemann et al., 2005). Conversely, increasing salinity causes soils to flocculate, offsetting the effects caused by sodicity (Shainberg and Letey, 1984).

Contradictory results have been reported on the effect of salinity and sodicity on soil respiration and microbial biomass (Laura, 1976; Nelson et al., 1996; Pathak and Rao, 1998; Rietz and Haynes, 2003; Sarig et al., 1993; Wong et al., 2008). These contradictory observations may be due to differences in soil properties, especially the levels of salinity and soil pH (Muhammad et al., 2008), but there are few studies that have assessed the interaction of salinity and sodicity on soil microbial activity and organic matter decomposition in soils of different texture.

Based on the findings reported in the literature outlined above, we hypothesised that (1) salinity would decrease microbial activity but increase DOC concentration because of decreased organic matter decomposition, and (2) sodicity would increase microbial activity and DOC concentration because of increased soil organic matter solubility.

2. Materials and methods

2.1. Soils

Two non-saline and non-sodic soils, of sand and sandy clay loam texture were collected from A horizon (0–30 cm) of a soil near Monarto, South Australia (35°05′ S and 139°06′ E) (Table 1). The area has a dry Mediterranean climate, and the average temperature is 30.1 °C in summer and 15.9 °C in winter with mean annual rainfall of 352 mm. Samples were thoroughly mixed, air dried, passed through a 2 mm sieve and stored air-dry at room temperature. Textures were assigned according to the Australian Soil and Land Survey Field Handbook (The National Committee on Soil and Terrain, 2009).

2.2. Soil characterization

Table 1
Physical and chemical properties of the soils.

Soil property	Unit	Sand	Sandy clay loam
Sand	%	95	67.70
Silt	%	1.30	8.30
Clay	%	3.70	24.0
$EC_{(1:5)}$	dS m^{-1}	0.05	0.10
$SAR_{(1:5)}$		0.55	0.92
$pH_{(1:5)}$ soil:water		7.47	8.41
Bulk density	g cm^{-3}	1.75	1.43
Water holding Capacity	g $g^{-1}soil^{-1}$	0.08	0.26
Cation exchange capacity	cmolc(+) kg^{-1}	6.12	
$CaCO_3$	%	1.74	28.70
Total N	%	0.05	0.24
Total Organic C	%	0.49	1.71

Soil pH and EC were measured in a 1:5 soil:water suspension after 1 h end-over-end shaking at 25 °C. The sodium adsorption ratio (SAR) was determined in a 1:5 soil:water extract. Calcium and Mg was measured using atomic absorption spectrophotometer, Na by flame photometry (Rayment and Higginson, 1992). The $SAR_{1:5}$ was calculated by the equation (Richards, 1954). The water holding capacity (WHC) was measured using a sintered glass funnel connected to a 100 cm water column ($\Psi m = -10$ kPa). Soil was placed in rings in the sintered glass funnel, thoroughly wetted and allowed to drain for 48 h. Dry weight of the soil was determined after oven drying at 105 °C for 24 h. Bulk density was measured by the clod method (Blake, 1965). The osmotic potential of the soil water was estimated using the equation given by Richards (1954):

$$\text{Osmatic potential(OP)} = -0.036 \times \text{EC} \times \left[\frac{O_{act}}{O_{ref}}\right]$$

where O_{act} is actual moisture content (g g^{-1}) of the soil and O_{ref} is the reference water content (g g^{-1}) of the 1:5 soil–water mixture.

Particle size was analysed by the hydrometer method (Bouyoucos, 1936) and organic carbon was analysed by dichromate oxidation (Walkley and Black, 1934). Total C and N were measured on a CNS Leco-2000 analyser. The aromaticity of the DOC in the soil–water suspension was estimated by determining the specific ultra-violet absorbance (SUVA in l mg DOC^{-1} m^{-1}) in the 0.45 μm filtered soil–water suspension at 254 nm (Chin et al., 1997) in a UV spectrophotometer using a quartz cuvette.

2.3. Extraction of dissolved organic carbon (DOC) and nitrogen (DON)

The concentration of dissolved organic C and N is influenced by the extractant used. Compared to extraction with water, extractants such as K_2SO_4 and KCl may extract more (Haynes, 2005; Madou and Haynes, 2006) or less DOC (Bolan et al., 1996; Haney et al., 1999). To assess which extractant is the most suitable for the soils used here, a preliminary experiment was conducted (water, 0.5 M K_2SO_4 and 2 M KCl) in three texturally different soils (loamy sand, sandy clay loam and sandy clay). The soils were amended with mature wheat residue 2% (w/w), incubated for two weeks at constant temperature (25 °C) and then extracted with different extractants. Irrespective of soil texture, the concentrations of DOC and DON extracted with 0.5 M K_2SO_4 or 2 M KCl were more than twice than those extracted with water. The DOC and DON concentrations in the water extract were in a similar range as reported in other studies (Andersson et al., 2000; Chantigny, 2003). Therefore, for this study dissolved organic carbon (DOC) and nitrogen (DON) was extracted with a 1:5 soil:water ratio, shaking end-over-end for 1 h, centrifuged at 4000 rpm for 10 min and passed through a 0.45 μm filter under vacuum. The concentration of DOC and DON in the suspensions was measured as non-purgeable organic C and N in a Formacs TOC/TN analyser after acidification of the sample and purging of the inorganic C. The filtered samples were stored in a refrigerator at 4 °C if analysis was to be within 10 days. If there was a longer delay, samples were immediately frozen at −5 °C and then defrosted at 4 °C prior to analysis.

2.4. *Experimental design*

2.4.1. *Soil preparation*

Eight salt solutions with different EC and SAR were prepared using combinations of 1 M NaCl and 1 M $CaCl_2$ stock solutions. The EC of the solutions were 7.5, 20, 40 and 60 dS m^{-1} which were combined with solutions with two SAR values; < 3 and 20. The experimental soils (approximately 300 g) were placed on a funnel with a filter paper and leached 3–4 times with these solutions. At each leaching event, about 60–80 ml of the solution was added after which soils were dried at 25–30 °C in a fan-forced oven for 72–96 h, mixed thoroughly after drying to break the clods and then analysed for EC and SAR. The drying maximizes the soil-solution contact. This process was repeated until the desired combination of EC and SAR ($EC_{1:5}$ levels of 0.5, 1.3, 2.5 and 4.0 dS m^{-1} in the sand and 0.7, 1.4, 2.5 and 4.0 dS m^{-1} in the sandy clay loam soils in combination with SAR < 3 or 20) were achieved. These $EC_{1:5}$ levels correspond to EC_e 7, 17, 33 and 54 dS m^{-1} in the sand and 8, 15, 27 and 44 dS m^{-1} in the sandy clay loam. After adjustment of EC and SAR, the soils were kept dry at room temperature until the onset of the experiment. Electrical conductivity, pH, and SAR after 10 and 42 days of incubation remained unchanged in both soils.

2.4.2. *Pre-incubation*

A preliminary study showed that the water content resulting in maximum respiration was 80% WHC for the sand (6.4 g·100 g $soil^{-1}$) and 60% WHC for the sandy clay loam (15.6 g·100 g $soil^{-1}$). These water contents were achieved by adding the required amount of reverse osmosis water. The wetted soils were pre-incubated for 14 days at 25 °C. A pre-incubation of 14 days was selected on the basis of Butterly et al. (2009), who found that microbial activity stabilized within 10 days after rewetting air-dry non-saline soil. Throughout the pre-incubation and the subsequent incubation period, reverse osmosis water was added on a mass basis to maintain the target water content.

2.4.3. *Incubation*

Mature wheat straw (C:N ratio 47:1, as a nutrient source for soil microbes), was ground, sieved to 0.25–2 mm, then added at 20 g kg^{-1} soil and thoroughly mixed into the soils. The pre-incubated soil with residue (20 g) was added to polyvinyl cores (PVC, radius 1.85 cm, height 5 cm) with a nylon mesh base (0.75 mm, Australian Filter Specialist) and packed to the bulk density of the soil in the field. The cores were placed individually into 1 L glass jars and the jars sealed with gas tight lids equipped with septa to allow quantification of the CO_2 concentration in the headspace. The jars were incubated in the dark at 22–25 °C and CO_2 release was measured over 42 days. Separate sets of samples for DOC, DON, EC, SAR, pH and SUVA were destructively harvested after 10 days and at the end of the experiment after 42 days with 3 replicates per treatment (EC × SAR combination) and sampling time.

2.4.4. *Soil respiration*

Soil respiration was measured by quantifying headspace CO_2 concentrations within each jar using a Servomex 1450 infra-red gas analyzer (Servomex, UK). The CO_2 concentration was measured every 1 or 2 days during the first 8–10 days and then every 3–5 days until the end of the experiment. For each measurement period, an initial measurement of the CO_2 concentration in the headspace was taken immediately after sealing the jars. The closed jars were incubated for a defined duration and a second measurement of CO_2 concentration in the headspace was taken. After the second measurement, the jars were opened to refresh the headspace in the jars using a fan. The CO_2 evolved from each sample was the difference between the initial and final CO_2 concentrations for each measurement period. The infra-red gas analyzer was calibrated using known amounts of CO_2 injected into glass jars similar to those used for the samples. The relationship between CO_2 concentration and detector response was determined by linear regression. This relationship was used to calculate the CO_2 concentration in the jars with soils. The CO_2 concentration (ml) for each measurement period was multiplied by the gas volume of the jars to obtain the mg of CO_2–C respired during each measurement period and divided by the soil dry weight. The following equation was used to convert CO_2 (ml) to mg CO_2–C per g of soil per day.

$$X = \frac{D \times 1000 \times \text{Atomic weight of carbon}}{Y_{(\text{std})} \times \text{Time(days)} \times \text{Mass of soil(g)}}$$

Where, X = mg CO_2–C/g soil/day, D = $D_1 - D_0$ (D_1 = CO_2 (ml) at time T_1 and D_0 = CO_2 (ml) at time T_0). $Y_{(\text{std})}$ = volume of 1 mol of CO_2 at given temperature calculated from ideal gas law (volume of 1 mol of CO_2 at 25 °C and 1 atmospheric pressure is 24465.3 ml). The values for each measurement period were added to calculate cumulative respiration over the 42 days incubation period.

2.4.5. *Statistical analysis*

The data were analysed using Genstat 10 (GenStat® for Windows10.0, VSN Int. Ltd, UK, 2005). Soil respiration, DOC, DON and SUVA data were subjected to a two-way analysis of variance (ANOVA) and Tukey test was used to determine significant differences ($P < 0.05$) between treatments and soils.

3. Results

In the sand, cumulative respiration decreased significantly with increasing salinity after 10 and 42 days (Fig. 1). Cumulative CO_2–C was lowest at EC 4.0 + SAR 20, being 60% lower than at EC 0.5 + SAR 20. Cumulative respiration was more strongly affected by EC than by SAR. It decreased by 8, 24 and 42% at EC 1.3, EC 2.5 and EC 4.0 respectively. SAR 20 increased cumulative respiration after 42 days of incubation by 9% at EC 0.5 and by 2.3% at EC 1.3. However, cumulative respiration was not affected by SAR at higher EC. In the sandy clay loam, cumulative respiration was not significantly affected by EC or SAR (Fig. 1).

Apart from EC 4.0 in the sandy clay loam, the DOC concentration was higher in the sand than sandy clay loam (Fig. 2). In both soils, DOC decreased with time, but increased with increasing salinity and was higher at SAR 20 than at SAR < 3. In the sand, the DOC concentration increased by 20% at EC 2.5 and by 38% at EC 4.0 respectively, but SAR 20 increased DOC compared to SAR < 3 by 18% only at EC 0.5. In the sandy clay loam, DOC was more strongly affected by EC and SAR. The DOC concentration was highest at EC 4.0 + SAR 20, being 142% higher than in EC 0.7 + SAR 20 (Fig. 2). Compared to SAR < 3, SAR 20 significantly increased DOC at EC 0.7 and EC 1.4. In both soils, DOC concentrations were highest at EC 4.0 and lowest at EC 0.5/0.7. DON showed a similar pattern as DOC (data not shown). The DON concentration were lower than those of DOC ranging from 20 to 36 μg DON g $soil^{-1}$ in the sand and from 8 to 33 μg DON g $soil^{-1}$ in the sandy clay loam.

SUVA values ranged from 1.22 to 3.43 l mg DOC^{-1} m^{-1} in the sand and from 1.32 to 4.13 l mg DOC^{-1} m^{-1} in the sandy clay loam (Fig. 3). In the sand, SUVA was not significantly affected by EC or SAR except at EC 0.5 + SAR 20 where it was higher after 10 and 42

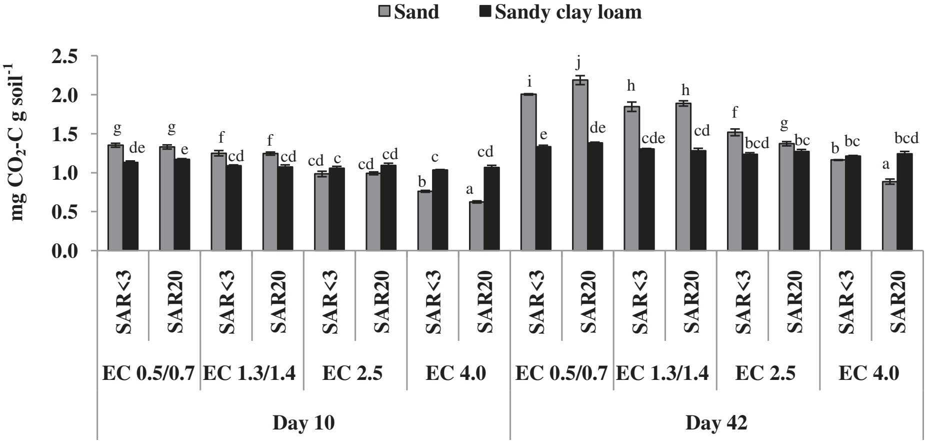

Fig. 1. Cumulative respiration at $EC_{1:5}$ 0.5/0.7, 1.3/1.4, 2.5 and EC 4.0 in combination with $SAR < 3$, and 20 after 10 and 42 days in the sand and sandy clay loam. Vertical lines indicate standard deviation of the mean ($n = 3$). Values with different letters differ significantly at $p < 0.05$ across different treatments and soils.

days of incubation. However, in the sandy clay loam, SUVA decreased significantly with increasing EC. At EC 0.7 and EC 1.4, SUVA was significantly higher with SAR 20 than with $SAR < 3$. Although the $EC_{1:5}$ values were quite similar in the two soils, the osmotic potential ranged from −0.92 to −9.23 MPa in the sand and −0.48 to −2.76 MPa in the sandy clay loam (Fig. 4). The osmotic potential at EC 0.5 and EC 1.3 in the sand was very similar to that at EC 1.4 and EC 4.0 in the sandy clay loam. At a similar osmotic potential of −2.92 MPa (EC 1.3 in the sand) and −2.76 MPa (EC 4.0 in the sandy clay loam), the percent decrease in respiration was approximately the same in the sand and the sandy clay loam.

4. Discussion

Salinity affected soil respiration and DOC irrespective of SAR, whereas sodicity only had an effect at low EC levels. The study also highlighted that soil texture and water content play an important role in determining the response of microbes to salt stress.

4.1. *Salinity effect*

The results confirmed the hypothesis that salinity would decrease microbial activity but increase DOC because of decreased soil organic matter decomposition. The decrease in respiration with increasing salinity can be explained by the low osmotic potential (Figs. 1 and 4) (Chowdhury et al., 2011b). The low osmotic potential induced by high salinity will reduce microbial activity and thus transformation of organic substrates (Mamilov et al., 2004; Wichern et al., 2006). Some studies show that soil respiration is positively correlated with amount of water-extractable C (Burford and Bremner, 1975; Marschner and Bredow, 2002) while in others it was not (Beauchamp et al., 1980; Cook and Allan, 1992; Davidson et al., 1987). Nevertheless, most of DOM (70–90%) is present as aromatic humic molecules which are recalcitrant and not readily degraded by microbes (Guggenberger and Zech, 1994; Kalbitz et al., 2003). Although high salt concentrations in the soil solution can decrease the amount of water-extractable C (Kieft et al., 1987), salinity increased the concentration of DOC (Fig. 2) and DON suggesting that this is due to reduced ability of the stressed microbes to utilize readily available labile organic matter from the added residues. The SUVA is a measure of the aromaticity and thus of DOC degradability (Weishaar et al., 2003). Aromatic structures (hydrophobic) are more recalcitrant than aliphatic structures (hydrophilic) and therefore degradable by microbes. Moreover, aromatic structures have a higher affinity for clay minerals which protect them against degradation (Qualls and Richardson, 2003). In this study, SUVA values decreased significantly with increasing salinity in the sandy clay loam. This suggests that at low salinity soil microbes utilized the easily degradable hydrophilic DOC thereby increasing the concentration of the

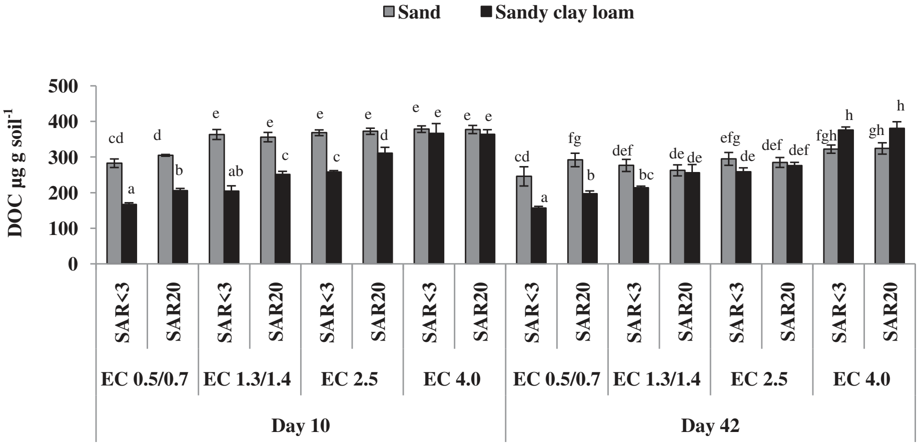

Fig. 2. Dissolved organic carbon (DOC) at $EC_{1:5}$ 0.5/0.7, 1.3/1.4, 2.5 and 4.0 in combination with $SAR < 3$, and 20 after 10 and 42 days in the sand and sandy clay loam. Vertical lines indicate standard deviation of the mean ($n = 3$). Values with different letters differ significantly at $p < 0.05$ across different treatments and soils.

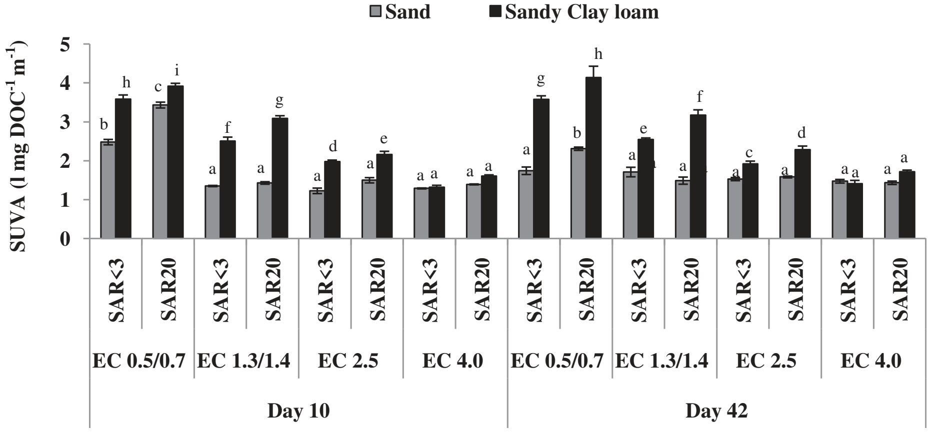

Fig. 3. SUVA at $EC_{1:5}$ 0.5/0.7, 1.3/1.4, 2.5 and 4.0 in combination with SAR $<$ 3, and 20 after 10 and 42 days in the sand and sandy clay loam. Vertical lines indicate standard deviation of the mean ($n = 3$). Values with different letters differ significantly at $p < 0.05$ across different treatments and soils.

aromatic DOC fraction. Moreover, the high electrolyte concentration at high salinity may have precipitated only aromatic DOC compounds, whereas low molecular weight DOC (hydrophilic) remained soluble (Romkens and Dolfing, 1998).

4.2. *Sodicity effect*

We hypothesised that sodicity would increase microbial activity and DOC due to increased soil organic matter solubility. This hypothesis was only partly confirmed as DOC was higher at SAR 20 compared to SAR $<$ 3 only at low EC. On the other hand, SAR had little effect on respiration at any EC level. Hence, although sodicity in combination with low EC increased DOC concentrations, this did not result in increased respiration except for EC 0.5 in the sand. Sodicity causes dispersion of clay and soil organic matter (Backstrom et al., 2004; Norrstrom and Bergstedt, 2001) and Nelson et al. (1996) and Laura, 1976 suggested that this may lead to higher CO_2 emission due to the greater C availability. The lack of effect of high SAR on respiration and DOC at higher EC (2.5 and 4.0) in the present study can be explained by the high electrolyte concentration (Na, Ca, Mg) which blocks functional groups on the organic matter and cross-links polymers to create dense inflexible molecules which are more stable to biological attack and play a major role in binding organic matter to clays (Oades, 1988).

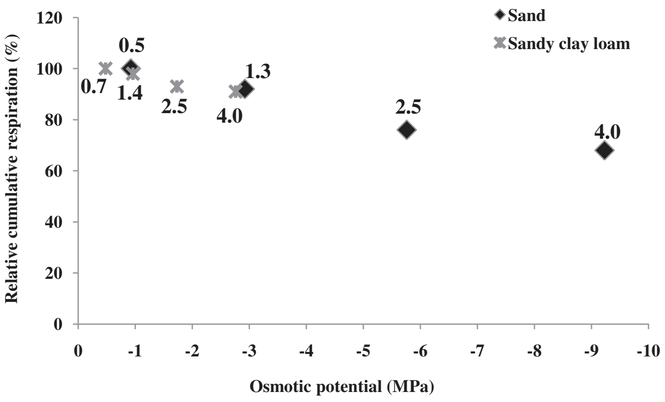

Fig. 4. Relative change in cumulative respiration in the two soils in relation to EC and calculated osmotic potential (OP). Values next to the symbols indicate EC values in the respective soils.

4.3. *Differential response to increasing EC in the two soils*

In contrast to the sand, the cumulative respiration in the sandy clay loam was not significantly affected by EC. This can be explained by comparing the osmotic potential at the different EC levels in the two soils (Fig. 4). The osmotic potential ranged from −0.92 to −9.23 MPa in the sand but only from −0.48 to −2.76 MPa in the sandy clay loam. Thus, at a given EC, the osmotic potential which the soil microbes were exposed to was considerably lower in the sandy clay loam than the sand. Both soils were incubated at optimal water content for respiration which was 64 g kg soil^{-1} in the sand and 156 g kg soil^{-1} in the sandy clay loam. The difference in water content in the two soils is due to difference in texture. The sand with a coarse texture (only 4% clay) can retain less water than the sandy clay loam (24% clay). Thus, the same amount of salt, as measured by EC, was dissolved in a greater amount of water in the sandy clay loam than in the sand. Therefore, in the present study, the lack of adverse affect of salinity on microbial activity in the sandy clay loam can be explained by the difference in water content in the two soils. This apparent differential sensitivity to EC in soils with different texture is in agreement with other studies (Chowdhury et al., 2011a). On the other hand, at a similar osmotic potential (−2.92 MPa in the sand at EC 1.3 and −2.76 MPa at EC 4.0 in the sandy clay loam) the percent decrease in respiration was approximately the same in the two soils.

5. Conclusions

The results show that increasing salinity adversely affects microbial activity and therefore increases DOC and DON concentration, whereas an increased DOC and DON concentration in response to sodicity was observed only at low EC. Therefore, high SAR in combination with low EC increases the risk of DOC and DON leaching and further soil degradation. In addition, the results of the experiment also indicate that soil texture and water content play an important role in determining the response of microbes to salt due to their effect on the salt concentration in the soil solution. Therefore, osmotic potential (a measure of the salt concentration in the soil solution) may be a better measure for evaluating stress faced by microbes in the salt-affected soils than EC.

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CHAPTER 4:

DRYING AND WETTING IN SALINE AND SALINE-SODIC SOILS-EFFECTS ON MICROBIAL ACTIVITY, BIOMASS AND DISSOLVED ORGANIC CARBON

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REGULAR ARTICLE

Drying and wetting in saline and saline-sodic soils—effects on microbial activity, biomass and dissolved organic carbon

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Abstract

Aims There are few studies on the interactive effect of salinity and sodicity in soils exposed to drying and wetting cycles. We conducted a study to assess the impact of multiple drying and wetting on microbial respiration, dissolved organic carbon and microbial biomass in saline and saline-sodic soils.

Methods Different levels of salinity ($EC_{1:5}$ 1.0 or 2.5) and sodicity (SAR<3 or 20) were induced by adding NaCl and $CaCl_2$ to a non-saline/non-sodic soil. Finely ground wheat straw residue was added at 20 g kg^{-1} as substrate to stimulate microbial activity. The constant moist (CM) treatment was kept at optimum moisture content for the length of the experiment. The drying and rewetting (DW) treatments consisted of 1 to 3 DW cycles; each DW cycle consisted of 1 week drying after which they were rewet to optimum moisture and then maintained moist for 1 week.

Results Drying reduced respiration more strongly at EC2.5 than with EC1.0. Rewetting of dry soils produced a flush in respiration which was greatest in the soils without salt addition and smallest at high salinity (EC2.5) suggesting better substrate utilisation by microbes in soils without added salts. After three DW events, cumulative respiration was significantly increased by DW compared to CM, being 24% higher at EC1.0 and 16% higher at EC2.5 indicating that high respiration rates after rewetting may compensate for the low respiration rates during the dry phase. The respiration rate per unit MBC was lower at EC2.5 than at EC1.0. Further, the size of the flush in respiration upon rewetting decreased with each ensuing DW cycle being 50–70% lower in the third DW cycle than the first.

Conclusions Both salinity and sodicity alter the effect of drying and rewetting on soil carbon dynamics compared to non-saline soils.

Keywords Dissolved organic C · Drying and wetting · Microbial activity · Microbial biomass · Salinity · Sodicity

Introduction

In Mediterranean climate, surface soils may experience large fluctuations in water content which have the potential to significantly alter soil microbial activity and growth as well as nutrient dynamics. During summer, top soils may undergo multiple drying and wetting events when dry periods are interrupted by occasional rainfall. These drying and rewetting events

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could be exacerbated in the future as the annual soil water content will decrease by almost 25% in the Mediterranean and subtropical regions (Meehl et al. 2007). Drying of soil causes the matric potential to become more negative (Harris 1980), limiting the availability of water to microbes. Moreover, drying of soil reduces the thickness of the water film surrounding soil aggregates inhibiting diffusive transport of substrates to microbes (Stark and Firestone 1995). Microbes may compensate for the low water potential in the surrounding medium by accumulation of solutes in the cell (Harris 1980; Halverson et al. 2000). This process is very energy-demanding and therefore a metabolic burden for the microbes (Harris 1980; Schimel et al. 2007).

Rewetting of dry soils results in a flush of microbial activity which usually lasts for 2–5 days (Austin et al. 2004; Fierer and Schimel 2003; Wu and Brookes 2005). This wetting pulse may be attributed to increased substrate availability to microbes from release of osmolytes accumulated during drying phase, cell lysis and breakdown of aggregates releasing previously protected organic matter (Kieft et al. 1987; Halverson et al. 2000; Denef et al. 2001; Fierer and Schimel 2003). Generally, the size of wetting pulse decreases with the frequency of dry-wet cycles (Jager and Bruins 1975; Prieme and Christensen 2001; Mikha et al. 2005). The decrease in the size of the rewetting pulse has been explained by a decrease in labile organic matter over time (Birch 1958) and/or by a shift in bacterial community composition (Fierer et al. 2003).

Worldwide more than 831 million hectares of land is salt-affected (Martinez-Beltran and Manzur 2005). Therefore, understanding of processes in salt-affected soils is important, particularly those involved in nutrient cycling. Although the effects of drying and rewetting are well documented for non-salt affected soils, there are only two studies in the salt-affected soils (Chowdhury et al. 2011; Nelson et al. 1996). Salt-affected soils are classified as saline, sodic and saline-sodic on the basis of EC (electrical conductivity), SAR (sodium absorption ratio) and pH (Brady and Weil 2002). Soils with EC_e (saturated extract) >4 dS m^{-1} and $SAR>13$ are classified as saline-sodic (US Salinity Laboratory Staff 1954). Saline soils have an EC_e of the saturation extract >4 dS m^{-1}. Increasing salt concentration in the soil solution results in decreasing osmotic potential (Harris 1980) which reduces water uptake by plants and microbes due to low osmotic potential; further stressors include high pH and ion competition limiting nutrient uptake (Keren 2000). Such changes also have a negative effect on the size and activity of soil microbial biomass and biochemical processes essential for maintenance and turnover of soil organic matter (Rietz and Haynes 2003; Tripathi et al. 2006; Yuan et al. 2007). Some microbes respond to low osmotic potential by accumulating osmolytes to retain water (Hagemann 2011), whereas sensitive microbes die. This situation is exacerbated when saline soils dry as both osmotic and matric potential become more negative.

In sodic soils ($SAR>13$), Na^+ is the dominant cation on the exchange sites of the soil particles rather than Ca^{2+} and Mg^{2+}. High Na^+ saturation of the exchange sites results in dispersion of organic matter and clay particles, leading to breakdown of aggregates and soil structure. The dispersed clay particles can clog pores resulting in hardsetting of the soil upon drying, decreased water infiltration and permeability and reduced root growth (Sumner 1993; Qadir and Schubert 2002).

Dissolved organic matter (DOM) is the most mobile and dynamic non-living organic matter fraction. It comprises only a small part of soil organic matter (<1% of soil organic C). Nevertheless, it is a primary source of mineralizable C, N and P and affects many soil processes such as nutrient translocation and leaching (Qualls and Haines 1991), microbial activity and mineral weathering (Kuiters and Mulder 1993). This is particularly significant for sodic soils, because the increased solubility of organic matter can enhance C and N loss by leaching (Peinemann et al. 2005). However, increasing salinity causes soils to flocculate, offsetting the effects caused by sodicity (Shainberg and Letey 1984). Previous studies indicated that wet-dry cycles change the concentration as well as the chemical characteristics of DOC (Lundquist et al. 1999; Christ and David 1996; Kalbitz et al. 2000; Blodau et al. 2004).

This study was conducted to assess the impact of multiple drying and wetting on soil respiration, dissolved organic carbon (DOC) and microbial biomass in saline and saline-sodic soils. We hypothesised that compared to constantly moist soils, (1) drying will decrease respiration rate more strongly in saline and saline-sodic soils due to the lower water potential, and (2) rewetting of dry soils will produce a greater respiration flush in saline soils due to release of osmolytes and in saline-sodic soils due to the greater solubility of organic matter.

Materials and methods

Soil properties

A non-saline and non-sodic loamy sand (sand 85%, silt 2.5% and clay 12.5%, pH 8.2, $EC_{1:5}$ 0.09, SAR 1.2, water holding capacity 160 mg g^{-1}, bulk density 1.56 g cm^{-3}, organic carbon 11.4 mg kg^{-1}, microbial biomass C 43 mg kg^{-1}) was collected from the A horizon (0–30 cm) of a soil near Monarto (35º05′ S and 139º06′ E), South Australia. The area has a dry Mediterranean climate, and the average temperature is 30.1°C in summer and 15.9°C in winter with mean annual rainfall of 352 mm. The soil was air dried, passed through a 2 mm sieve and stored air-dry at room temperature before the start of the experiment. The EC and pH were measured in a 1:5 soil:water suspension after 1 h end-over-end shaking at 25°C. The sodium adsorption ratio (SAR) was also determined in a 1:5 soil:water extract. Calcium and Mg^{2+} concentrations were measured by atomic absorption spectrophotometer, Na^+ by flame photometry (Rayment and Higginson 1992). The $SAR_{1:5}$ was calculated by the equation (Richards 1954). The water holding capacity (WHC) was measured using a sintered glass funnel connected to a 100 cm water column ($\Psi m = -10$ kPa). Soil was placed in rings in the sintered glass funnel, thoroughly wetted and allowed to drain for 48 h. Dry weight of the soil was determined after oven drying at 105°C for 24 h. Bulk density was measured by the clod method (Blake 1965). Particle size was analysed by the hydrometer method (Bouyoucos 1936) and organic carbon was analysed by dichromate oxidation (Walkley and Black 1934). Microbial biomass C was determined by the fumigation extraction method (Vance et al. 1987). Organic C in the extracts was determined after dichromate digestion by titrating with $0.033M$ acidified ferrous ammonium sulphate (Anderson and Ingram, 1993). Microbial biomass C is calculated from the difference between the extracted carbon from chloroform fumigated and non-fumigated samples. No multiplication factor was used because the relationship between actual microbial biomass and that derived by this method in these soils is not known. Dissolved organic C (DOC) was extracted with a 1:5 soil:water ratio, shaking end-over-end for 1 h, followed by centrifuging at 4000 rpm for 10 min and passing through a 0.45 μm filter under vacuum. The concentration of DOC in the suspensions was measured as non-purgeable organic C and N in a Formacs TOC/TN analyser after acidification of the sample and purging of the inorganic C. The filtered samples were stored in a refrigerator at 4°C if analysed within 10 days. If the analysis was delayed, samples were immediately frozen at −5°C and then defrosted at 4°C prior to analysis. The aromaticity of the DOC in the soil–water suspension was estimated by determining the ultra-violet absorbance in the 0.45 μm filtered soil–water suspension at 254 nm (Chin et al. 1997) using a quartz cuvette. The ultra-violet absorbance is expressed in l mg DOC^{-1} m^{-1}.

Soil preparation

Four salt solutions with different EC and SAR combinations were prepared. The ECs of the solutions were 10 and 30 dS m^{-1} with SAR<3 and 20. The experimental soils (approximately 300 g) were placed on a filter paper in a funnel and repeatedly leached with these solutions. At each leaching event, about 60–80 mL of the solution was added. Then the soils were dried at 25–30°C in a fan-forced oven for 72–96 h, mixed thoroughly to break the clods and then analysed for EC and SAR. The drying process was necessary to maximize soil-solution contact. This process was repeated 3–4 times to achieve the desired combination of EC and SAR ($EC_{1:5}$ 1.0 and 2.5 dS m^{-1} in combination with SAR<3 or 20). These $EC_{1:5}$ levels correspond to EC_e 12 and 31 dS m^{-1} calculated using the equation (Rengasamy 2006):

$$ECe = (14 - 0.13 \times clay\,\%) \times EC_{1:5}$$

After adjustment of EC and SAR, the soils were kept dry at room temperature until the onset of the experiment. Electrical conductivity, pH, and SAR did not change during incubation.

Soil incubation

The incubation experiment consisted of five salt treatments [EC1.0 SAR<3, EC1.0 SAR20, EC2.5 SAR<3, EC2.5 SAR20 and control (without salt addition)] and two water content treatments [drying and rewetting (DW) and constantly moist (CM)] with four replicates per sampling date. Air dry soils were wet to 75% WHC which resulted in maximal respiration in this soil in a preliminary experiment (data not shown) and incubated for 7 days at 22–25°C before the experiment started. A pre-incubation of 7 days was selected on the basis of Butterly et al. (2009), who found that microbial activity stabilized within 7–10 days after rewetting air-dry non-saline soil. Throughout the pre-incubation and the subsequent measurement period, reverse osmosis water was added on a weight basis to maintain the target water content. After the pre-incubation, the soils were amended with 20 g kg^{-1} mature wheat straw (C:N ratio 47:1) as a nutrient source for soil microbes. The residue was ground, sieved to 0.25–2 mm and thoroughly mixed into the soils. Then 25 g of soil with residue was added to polyvinyl cores (PVC) with a radius of 1.85 cm and height of 5 cm and a nylon mesh base (0.75 mm, Australian Filter Specialist) and packed to the bulk density of the soil in the field. The cores were placed individually into 1 L glass jars and the jars sealed with gas tight lids equipped with septa to allow quantification of the CO_2 concentration in the headspace. The jars were incubated in the dark at 22–25°C and CO_2 release was measured over 56 days. All treatments were incubated for 14 days at constant moisture (75% of WHC) before the start of the first drying cycle (Fig. 1). Separate set of cores for each treatment were destructively harvested for DOC and MBC at each sampling time: before the beginning of first drying 14 days after addition of residues and then at on 1 d after 1st (day 23) and 3rd rewetting event (day 51) (Fig. 1).

Soil drying during the drying period was achieved by placing small pouches containing self-indicating silica gel (BDH chemicals) into the glass jars and which were changed at 1, 2 and 3 d when jars were vented after measuring CO_2 concentration in the head space (Butterly et al. 2009). The DW treatments were dried for 1 week after which they were rewet to 75% WHC. There were one to three DW events, sampling for DOC and MBC occurred only after the first or third rewetting (Fig. 1). The final sampling was on day 56 when the soils in 1 DW and 3 DW had been incubated moist for 35 and 7 days, respectively.

Soil respiration

Soil respiration was measured by quantifying headspace CO_2 concentrations within each jar using a Servomex 1450 infra-red gas analyser (Servomex, UK). The CO_2 concentration was measured every 1 or 2 days during the initial incubation period from day 1 to 14 after residue addition. During the drying and wetting phase CO_2 was measured daily at the start of drying or wetting, but longer intervals were used when the respiration rates were low. For each measurement period, an initial measurement of the CO_2 concentration in the headspace was taken immediately after sealing the jars. The closed jars were incubated for a defined duration and a second measurement of CO_2 concentration in the headspace was taken. After the second measurement, the jars were opened to refresh the headspace in the jars using a fan. The CO_2 evolved from each sample is the difference between the initial

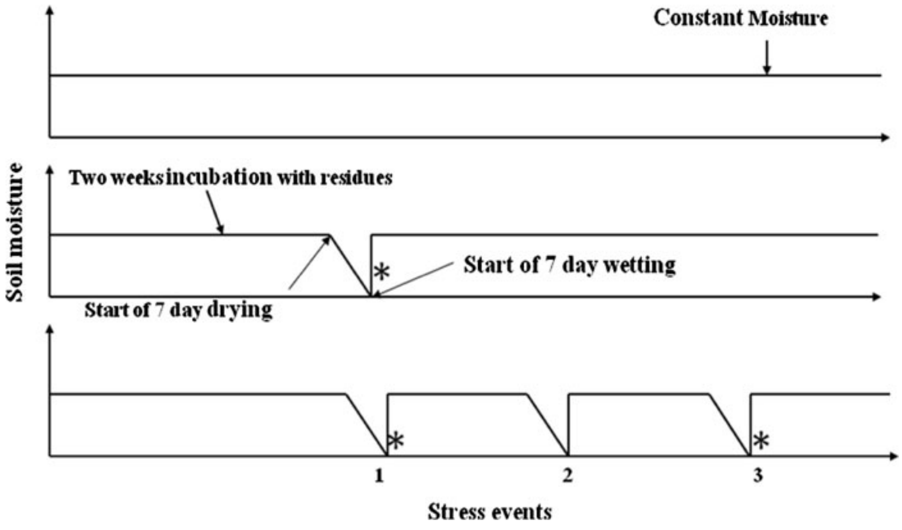


Fig. 1 Experimental design. *Indicate sampling time for DOC and microbial biomass C (1 day after rewetting) after the first and third drying period

and final CO_2 concentrations for each measurement period. The infra-red gas analyser was calibrated using known amounts of CO_2 injected into glass jars similar to those used for the samples. The relationship between CO_2 concentration and detector response was determined by linear regression. This relationship was used to calculate the CO_2 concentration in the jars with soils. The CO_2 concentration for each measurement period was multiplied by the gas volume of the jars to obtain the mL of CO_2–C respired during each measurement period and divided by the soil dry weight. The following equation was used to convert CO_2 (mL) to mg CO_2–C per g of soil per day.

$$X = \frac{D \times 1000 \times A}{Y(std) \times T \times M}$$

Where, X = mg CO_2–C g^{-1} soil day^{-1}, D = D_1−D_0 (D_1=CO_2 (mL) evolved from soil during time T_1 and D_0 = CO_2 (mL) evolved at time T_0), A = atomic weight of C. $Y_{(std)}$ = volume of 1 mol of CO_2 at given temperature calculated from ideal gas law (volume of 1 mol of CO_2 at 25°C and 1 atmospheric pressure is 24465.3 mL), T = time (days) and M = mass of soil (g). The values for each measurement period were added to calculate the cumulative respiration over the 56 days incubation period.

Statistical analysis

The data were analysed using Genstat 10 (GenStat® for Windows10.0, VSN Int. Ltd, UK, 2005). Soil respiration, DOC, microbial biomass C and SUVA data were subjected to a two-way analysis of variance ANOVA (soil treatment × moisture) at a given time point and Tukey test was used to determine significant differences ($P<0.05$) between the soil treatments and the moisture levels. Regressions between respiration rate and DOC or microbial biomass C were calculated with SPSS.

Results

Respiration

In the constantly moist (CM) treatment, the respiration rate declined gradually over time whereas in the drying-wetting treatments, the respiration rate fluctuated with changes in soil water content (Fig. 2). As the soils dried, the respiration rate decreased and CO_2 was no longer detectable 2–3 days after drying started. Both in CM and DW, respiration rates were lowest at high salinity and highest in the soils without added salts. Irrespective of sodicity, at first drying cycle, relative to soils without added salts, the extent of reduction in respiration by salinity was similar in CM and DW soils. During the second and third drying cycle, relative to soils without added salts, the extent of reduction in respiration at EC2.5 in DW soils was 11–16% greater than in CM, but at EC1.0, the average respiration rate was reduced more in CM than in DW. During the first drying phase, compared to CM, the average respiration rate in the DW soils was 68–74% lower in all the treatments. But during the third drying phase, compared to CM soils, the average respiration rate was reduced by 30% at EC1.0, by 46% in soils without salt additions and by 70% at EC2.5, irrespective of SAR.

The rewetting after 1 week of dry incubation produced a flush in respiration within 1 day in DW treatments which was greatest in the soils without salt addition and smallest at EC2.5, where the maximal respiration was 60% lower than in the soils without salt addition (Fig. 3a). The flush of respiration decreased with each DW cycle. At the third DW cycle, the rewetting flush was 50–70% lower than in the first cycle. At the first rewetting, SAR did not influence respiration rates in DW or CM. After the third rewetting, respiration rates were significantly higher at SAR20 than at SAR<3 at both EC levels and in both soil water treatments. However, the increase in respiration rate due to high SAR was 13–20% lower in DW than in CM (Fig. 3b). Respiration rates in DW declined in the week of moist incubation but remained higher than in CM.

Cumulative respiration was highest in the soil without salt additions followed by EC1.0 and EC2.5 in both moisture treatments (Table 1). After three DW events, cumulative respiration was significantly increased by DW compared to CM soils, being 24% higher at EC1.0 and 16% higher at EC2.5. Compared to SAR<3, SAR20 significantly increased cumulative respiration at both EC1.0 and 2.5 in DW but only at EC1.0 in CM. At the end of the experiment, there were no significant difference in cumulative respiration between 1 DW and 3 DW.

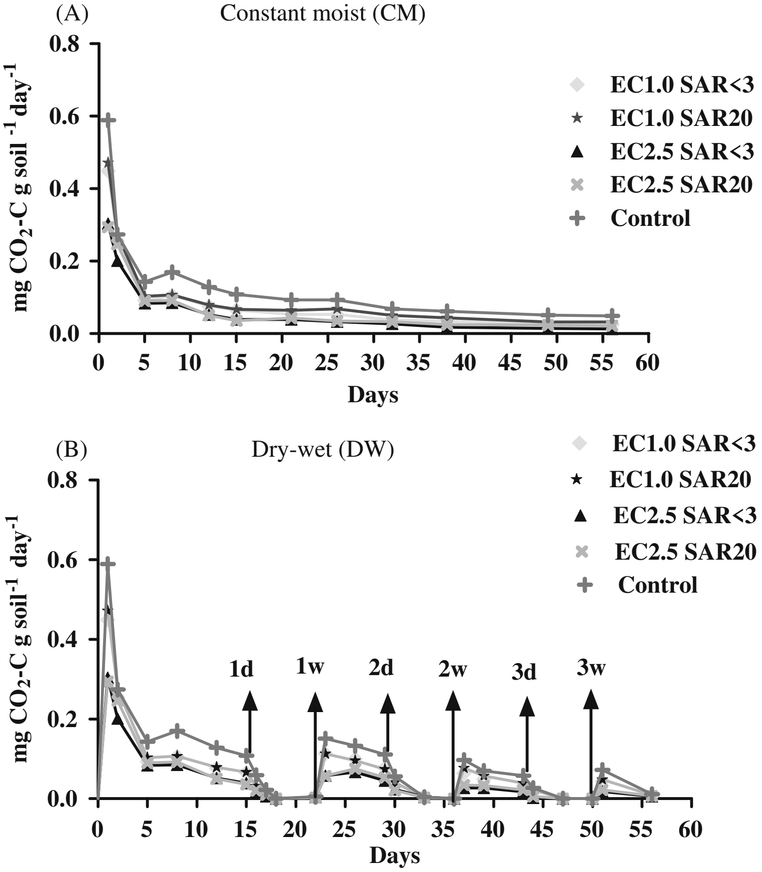


Fig. 2 Respiration rates in the constant moist (**a**) and dry–wet (**b**) soils (n=4). *Arrows* indicate the onset of drying (d) and wetting (w) cycles

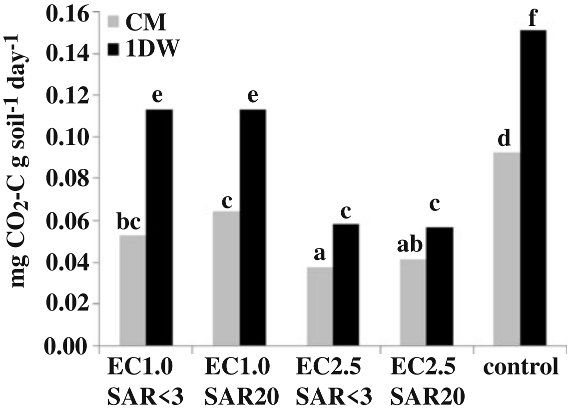
Dissolved organic C

In both CM and DW, DOC measured 1 day after the rewetting in DW decreased over time in almost all treatments but its concentration was always higher in DW (157–293 μg g soil^{-1}) compared to CM (130–234 μg g soil^{-1}). One day after the first rewetting (day 23), DOC concentrations in DW were 33–50% higher than in CM (Fig. 4a). Less DOC was released after the third DW (day 51), but DOC concentrations were still significantly higher in DW (Fig. 4b). The DOC concentration increased with increasing EC and SAR in both CM and DW. However, at SAR20 compared to CM soils the relative increase in DOC was 6–11% lower at both EC levels in DW. A quadratic relationship best explained relationship between DOC and respiration rate after the first (R^2=0.25, P<0.05) and the third DW (R^2=0.84, P=0.001) events. Respiration rate increased strongly beyond DOC concentrations of 220–245 μg g soil^{-1}. After the first rewetting (day 23), SUVA decreased with increasing EC but increased with increasing SAR (Table 2). Compared to CM, SUVA was lower in DW in all soils with added salt. However, in the soil without added salts, compared to CM, SUVA increased by 37% after the first DW and by 9% after the third DW event.

Microbial biomass

In both moisture treatments, MBC 1 day after rewetting was higher in soils with salt addition than without added salt. One day after the first rewetting (day 23), MBC was 47% higher at EC2.5 than at EC1.0 in both CM and DW and in DW, SAR 20 significantly increased MBC by 36% compared to SAR<3 at EC1.0 (Fig. 5a). One day after the third rewetting (day 51), MBC was significantly lower in DW than in CM. There were no differences between the salt treatments in DW, whereas MBC increased significantly with increasing EC and SAR in the CM soils (Fig. 5b).

(A) After first rewetting (day 23)



(B) After third rewetting (day 51)

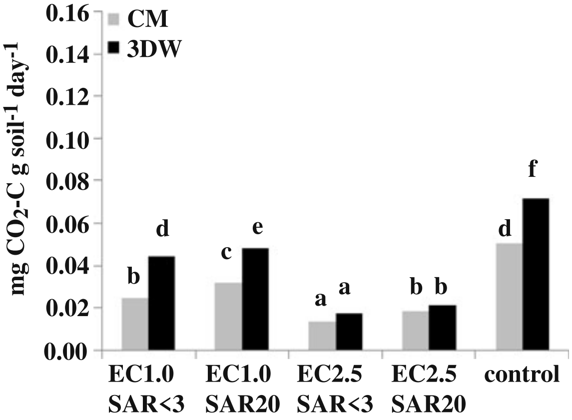
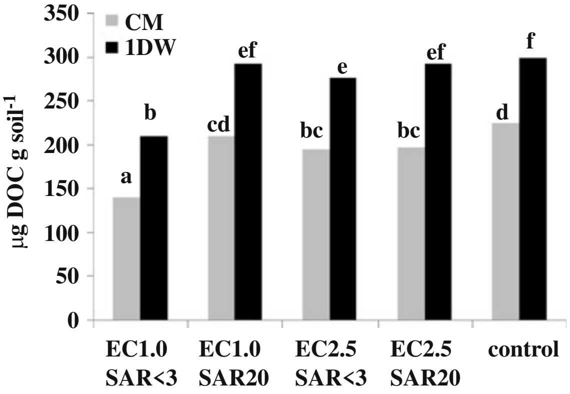


Fig. 3 Respiration rate in soils with $EC_{1:5}$ 1.0 and 2.5 in combination with SAR<3 and 20 in constantly moist (CM) and dry–wet (DW) treatments after first (**a**) and after third rewetting (**b**) (n=4). Values with *different letters* differ significantly (p<0.05) at a given sampling time

(A) After first rewetting (day 23)



(B) After third rewetting (day 51)

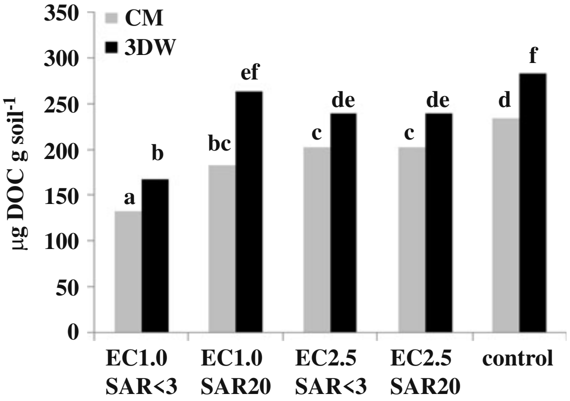


Fig. 4 Dissolved organic carbon (DOC) in soils with $EC_{1:5}$ 1.0 and 2.5 in combination with SAR<3 and 20 in constantly moist (CM) and dry–wet (DW) treatments after first (**a**) and after third rewetting (**b**) (n=4). Values with *different letters* differ significantly (p<0.05) at a given sampling time

However, on day 56, compared to CM, DW increased MBC by 17–30% in soils with salt addition and by 42% in soils without salt additions (Fig. 5c). MBC was linearly negatively correlated with respiration rate after first DW (R^2=0.59, P=0.001), but there was no significant relationship between the two parameters after third DW.

Table 1 Cumulative respiration (mg CO_2–C g $soil^{-1}$) in soils with $EC_{1:5}$ 1.0 and 2.5 in combination with SAR<3 and 20 in constantly moist (CM) and dry–wet (DW) treatments (n=4). Values with different letters differ significantly (p<0.05) at a given sampling time

Treatments	After first DW phase		After third DW phase		At the end of the experiment	
	CM	1 DW	CM	3 DW	1 DW	3 DW
EC1.0 SAR<3	1.11 b	1.25 cd	1.31 d	1.63 f	1.55 c	1.63 cd
EC1.0 SAR20	1.22 c	1.32 d	1.46 e	1.74 g	1.66 de	1.75 e
EC2.5 SAR<3	0.81 a	0.84 a	0.94 a	1.09 b	1.08 a	1.09 a
EC2.5 SAR20	0.86 a	0.88 a	1.02 ab	1.19 c	1.16 ab	1.19 b
Control	1.53 e	1.64 f	1.91 h	2.28 i	2.21 f	2.29 f

Table 2 SUVA (l mg DOC^{-1} m^{-1}) in soils with EC$_{1:5}$ 1.0 and 2.5 in combination with SAR<3 and 20 in constantly moist (CM) and dry–wet (DW) treatments (n=4). Values with different letters differ significantly (p<0.05) at a given sampling time

Treatments	After first rewetting		After third rewetting	
	CM	1 DW	CM	3 DW
EC1.0 SAR<3	2.93 bc	2.19 abc	3.21 c	2.17 a
EC1.0 SAR20	9.34 e	7.64 d	8.65 e	6.92 d
EC2.5 SAR<3	1.74 a	1.22 a	1.96 a	1.65 a
EC2.5 SAR20	3.33 c	1.80 ab	3.02 bc	2.36 ab
Control	8.31 de	11.42 f	8.50 e	9.26 e

Discussion

The results show that drying reduces the ability of the microbes in saline and saline-sodic soils to tolerate low water potential. The higher respiration flush upon rewetting in soils without added salts indicate that microbes in non-saline soils are better able to utilise the substrates released than those in saline and saline-sodic soils. However, lower respiration rate per unit MBC at EC2.5 indicate that the substrate was utilized more effectively at high salinity.

Drying phase

The results indicate that our first hypothesis (compared to constantly moist soils, drying will decrease respiration rate more strongly in saline and saline-sodic soils due to the lower water potential) can be partially accepted. Drying of soils decreased respiration rates compared to CM (Fig. 2) which is most likely due to the limited availability of water and substrate to the microbes at low water potential (Sommers et al. 1981; Franzluebbers et al. 1994; Pulleman and Tietema 1999; Voroney 2007). At the same water content (matric potential), soils with high salt concentration have lower osmotic and therefore water potential (Harris 1980). But contrary to our first hypothesis, during the first drying cycle, the relative decrease in respiration rate due to drying compared to CM was similar in all salt treatments. This suggests that respiration responds to the decrease in water content relative to CM and not to the absolute changes in water potential. This is in agreement with Chowdhury et al. (2011) who exposed naturally saline soils to one DW cycle. Moreover, the relative increase in MBC after rewetting compared to CM (Fig. 5a) was similar in all salt treatments suggesting that the stress imposed by the drying did not differ substantially among salt treatments. However, in partial agreement with our hypothesis, at the third drying, compared to constantly moist soils, drying decreased respiration rate more strongly at EC2.5 than at EC1.0 at both SAR levels. This can be explained by the lower MBC after the 3 DW cycle (Fig. 5b) where the microbes were stressed three times as compared to having been stressed by low water content only once after the 1 DW. This suggests that the stress induced by multiple DW cycles reduces the ability of the microbes in saline and saline-sodic soils to tolerate low water potential. Nelson et al. (1996) also reported that drying and wetting increases the effect of salinity on soil microbes.

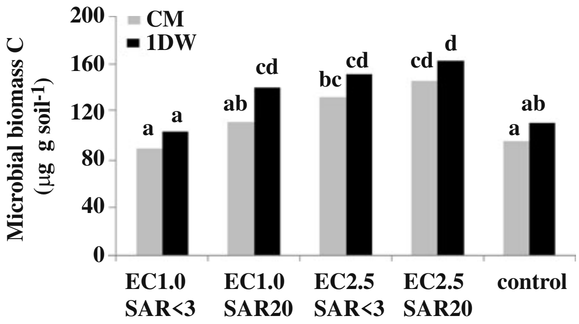
Rewetting phase

Phase I (after the first DW)

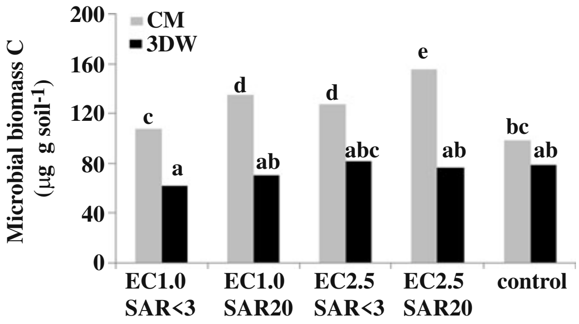
Previous studies in the non-saline soils have suggested that accumulation of osmolytes during the drying phase and their rapid release upon rewetting as well as the release of previously protected organic matter (Denef et al. 2001) can explain the flush in respiration after rewetting (Kieft et al. 1987; Halverson et al. 2000; Fierer and Schimel 2003). Similarly, in this study, at first rewetting, irrespective of sodicity, there was a flush in respiration which was highest in the treatment without added salts and smallest in high salinity treatments (Fig. 3a). This indicates that the microbes in non-saline soils are better able to utilise the substrates released by rewetting than those in saline and saline-sodic soils.

Increased substrate availability upon rewetting is also indicated by the higher DOC concentration in the DW treatments compared to CM (Fig. 4). The lack

(A) After first rewetting (day 23)



(B) After third rewetting (day 51)



(C) At the end of the experiment (day 56)

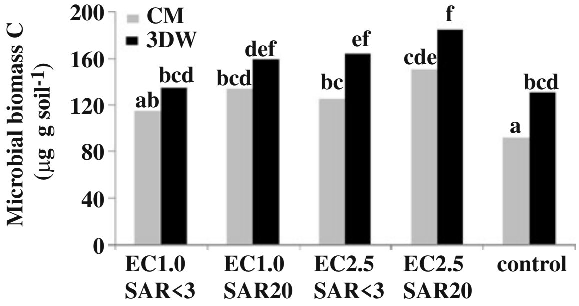


Fig. 5 Microbial biomass carbon (MBC) in soils with $EC_{1:5}$ 1.0 and 2.5 in combination with SAR<3 and 20 in constantly moist (CM) and dry–wet (DW) treatments after first (**a**) and after third rewetting (**b**) and at the end of the experiment (**c**). (n=4). Values with *different letters* differ significantly (p<0.05) at a given sampling time

of diffusion during drying may result in accumulation of microbial substrate. This together with cell lysis and greater accessibility and solubility of C due to breakdown of aggregates may contribute to the higher DOC concentrations after rewetting dry soil (Prechtel et al. 2000; Lundquist et al. 1999). Further, compared to CM, lower SUVA in DW in the salt treatments indicates cell lysis during rewetting of dry soil which may have released high proportions of hydrophilic compounds (low SUVA), whereas DOC in continuously moist soils contains a higher proportion of hydrophobic compounds (Christ and David 1996). Thus, not only was more DOC available upon rewetting, but it was also more easily degradable.

Sodicity (SAR20) increased DOC, particularly at EC1.0 (Fig. 4) which can be explained by soil colloid dispersion which occurs at high SAR and low salt concentrations in the soil solution, leading to greater solubility of organic matter (Backstrom et al. 2004; Norrstrom and Bergstedt 2001). However, in contrast to Nelson et al. (1996) and Laura (1976) who suggested that sodicity may lead to higher CO_2 release due to the greater C availability, cumulative respiration after the first rewetting was not increased by SAR (Fig. 3). On the other hand, MBC was higher with SAR20 than SAR<3 (Fig. 5), indicating that C released by dispersion was utilised by the microbes for growth. High salt concentrations in the soil solution can decrease the amount of water-extractable C (Kieft et al. 1987). Therefore, the reduced effect of sodicity on DOC at EC2.5 can be explained by the higher concentration of salts in the soil solution causing flocculation of organic matter.

Compared to the soil without added salt, microbial biomass at both moisture treatments was higher with EC2.5 at both SAR levels and in EC1.0 at SAR20 (Fig. 5a). This occurred although DOC concentrations were similar as in the soil without added salt, suggesting that the substrate was used more efficiently for biomass growth in the soils with added salt. The relative increase in MBC upon rewetting compared to CM was similar in all salt treatments suggesting that salinity and sodicity do not affect the response to DW. However, with EC2.5, the increase in respiration rate upon rewetting was lower than without added salt and at EC1.0 (Fig. 3a). Thus, at EC2.5 respiration rate per unit MBC was lower, also indicating that substrates were utilized more effectively. Therefore, our second hypothesis (rewetting of dry soils will produce greater flush in respiration due to release of osmolytes in saline soils and greater solubility of organic matter in saline-sodic soils) has to be rejected.

Phase-II (after the third DW)

Upon the third rewetting, the respiration rates were lower than after the first DW in all treatments (Fig. 3b), confirming earlier studies that the flush in respiration decreases with the number of DW cycles

(Birch 1958; Jager and Bruins 1975; Prieme and Christensen 2001; Fierer and Schimel 2003; Mikha et al. 2005). On the other hand, DOC concentrations and the aromaticity of the DOC were similar after the first and third DW (Fig. 4, Table 2), whereas in contrast to the first DW, MBC was lower in the DW treatments than that in CM (Fig. 5b). Thus, in this study, the lower respiration rates and lower MBC after the third DW cannot be explained by lower substrate availability. This suggests that after multiple DW events, microbes are less capable of utilising the substrate that becomes available upon rewetting. The relative differences between EC1.0 and EC2.5 and soil without added salt were similar after the first and the third DW indicating that exposure to multiple DW do not change the effect of salinity on respiration upon rewetting.

In contrast to the first DW cycle, respiration rate were higher at SAR20 to SAR<3 in both CM and DW soils (Fig. 3b). This occurred although DOC concentrations were increased by sodicity only at EC1.0. However, sodicity increased the aromaticity of the DOC only at EC1.0. This suggests that at EC1.0, the greater respiration rates with SAR20 can be explained by higher DOC concentration, whereas at EC2.5, the higher respiration rates are due to a greater proportion of easily degradable compounds in the DOC. The finding that high SAR increased respiration rates on day 51 in both CM and DW treatments suggests that this was a function of time and not specifically induced by DW. The decomposition products present at this later stage may be less likely to be bound to soil clays in which a large proportion of the exchange sites were occupied by Na compared to those produced earlier.

The recovery of MBC towards the end of the experiment in the DW treatments to concentrations exceeding those in CM at EC1.0 and in the soil without added salt (Fig. 5c) suggests that after rewetting, the surviving microbes in the DW treatments were able to utilize the substrate released upon rewetting. Further, cumulative respiration was significantly higher in the soils with three DW cycles than in CM in all salt treatments (Table 2) indicating that the high respiration rates after rewetting may compensate for the low respiration rates during the dry phase (Lundquist et al. 1999; Miller et al. 2005). The lack of significant differences between cumulative respiration between one and three DW soils can be explained by the decrease in the size of the rewetting respiration flush with each ensuing DW cycle.

It should be noted that we induced salinity and sodicity just before the onset of the experiment as in several previous studies (Nelson et al. 1996: Pathak and Rao 1998; Wong et al. 2008; Chowdhury et al. 2011). This has the advantage that salinity and sodicity effects can be studied without confounding factors such as differential soil texture or organic matter content which are likely to occur in saline-sodic soils collected in the field. However, the rapid increase in EC and SAR may not have allowed the microbial community to adjust to the new conditions (Khan et al. 2008; Wong et al. 2008) leading to an overestimation of the salt effect.

Conclusions

Sodicity in combination with low EC increased organic matter solubility and substrate utilisation by microbes and this was little affected by drying and rewetting cycles. The lower flush in respiration after rewetting in saline and saline-sodic soils than in soil without added salt indicates reduced loss of CO_2 from these soils but loss of C via DOC leaching may be increased in sodic soils. On the other hand, the lower respiration rate per unit MBC at high salinity suggests that substrate was utilized more effectively. Thus, both salinity and sodicity alter the effect of drying and rewetting on soil carbon dynamics.

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CHAPTER 5:

SORPTION OF DISSOLVED ORGANIC MATTER IN SALT-AFFECTED SOILS: EFFECT OF SALINITY, SODICITY AND TEXTURE

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Sorption of dissolved organic matter in salt-affected soils: Effect of salinity, sodicity and texture

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HIGHLIGHTS

- We studied the interactive effect of salinity and sodicity on DOC sorption in soils varying in texture.
- DOC losses from saline–sodic soils will be lower than sodic soils due to cation bridging at high electrolyte concentration.
- DOC sorption in salt-affected soils is more strongly controlled by CEC and Fe/Al concentration than by clay concentration.

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ABSTRACT

Loss of dissolved organic matter (DOM) from soils can have negative effects on soil fertility and water quality. It is known that sodicity increases DOM solubility, but the interactive effect of sodicity and salinity on DOM sorption and how this is affected by soil texture is not clear. We investigated the effect of salinity and sodicity on DOM sorption in soils with different clay contents. Four salt solutions with different EC and SAR were prepared using combinations of 1 M NaCl and 1 M $CaCl_2$ stock solutions. The soils differing in texture (4, 13, 24 and 40% clay, termed S-4, S-13, S-24 and S-40) were repeatedly leached with these solutions until the desired combination of EC and SAR ($EC_{1:5}$ 1 and 5 dS m^{-1} in combination with SAR <3 or >20) was reached. The sorption of DOC (derived from mature wheat straw) was more strongly affected by SAR than by EC. High SAR (>20) at EC1 significantly decreased sorption in all soils. However, at EC5, high SAR did not significantly reduce DOC sorption most likely because of the high electrolyte concentration of the soil solution. DOC sorption was greatest in S-24 (which had the highest CEC) at all concentrations of DOC added whereas DOC sorption did not differ greatly between S-40 and S-4 or S-13 (which had higher concentrations of Fe/Al than S-40). DOC sorption in salt-affected soil is more strongly controlled by CEC and Fe/Al concentration than by clay concentration per se except in sodic soils where DOC sorption is low due to the high sodium saturation of the exchange complex.

1. Introduction

Dissolved organic matter is the most mobile and dynamic non-living organic matter fraction and affects many biogeochemical processes such as nutrient translocation and leaching, microbial activity and mineral weathering (McDowell, 2003). Dissolved organic carbon (DOC) along with other nutrients can be lost from soil via runoff and leaching into surface water bodies and groundwater (Baldock and Skjemstad, 2000; Stevenson and Cole, 1999) which can have detrimental effects on water quality (EPA South Australia, 1998). Moreover, leaching can reduce the amount of DOM available for mineralization within the soil, reduce soil nutrient cycling (Kalbitz et al., 2000; Marschner and Kalbitz, 2003) and may lead to further soil degradation (Mavi et al., 2012). The retention and mobility of DOC in soils is controlled primarily by its sorption to mineral surfaces (McDowell and Likens, 1988; Ussiri and Johnson, 2004). A number of DOC sorption processes in soils have been postulated: ligand exchange, cation and anion exchange (Arnarson and Keil, 2000; Gu et al., 1994), inner sphere complexation (Chorover et al., 2004; Mikutta et al., 2009), pi–donor–acceptor interactions (Keiluweit and Kleber, 2009), cation bridging, hydrogen bonding and van der Waals forces (Jardine et al., 1989; Mikutta et al., 2007; Sollins et al., 1996). Several studies have shown the effect of soil properties such as clay and organic carbon contents, surface charge and electrolyte concentrations, oxalate-extractable Fe and Al and pH on DOC sorption (Guggenberger and Kaiser, 2003; Kahle et

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al., 2003; Kalbitz and Kaiser, 2008; Kögel-Knabner et al., 2008; Rashad et al., 2010). However, most of these studies were with non-saline and non-sodic soils from temperate climates or forest ecosystems.

Clay minerals play an important role in the stabilisation of soil organic matter (Hassink, 1997; Tisdall and Oades, 1982) by interacting with organic matter to form complexes making organic molecules less susceptible to biodegradation (Amato and Ladd, 1992; Oades, 1988). The capacity of the soil to adsorb DOC was found to be positively correlated with soil clay content in some studies (Nelson et al., 1993; Shen, 1999) whereas in others it was not (Kahle et al., 2003; Kaiser and Zech, 2000). Apart from clay concentration, clay type may also affect the capacity of soil to bind organic matter. Clays with higher specific surface area and cation exchange capacity (CEC) adsorb more C (Kahle et al., 2003; Nelson et al., 1997; Ransom et al., 1998).

Salt affected soils occur predominantly in arid and semi-arid regions where rainfall is insufficient to leach salts from the root zone. Salt-affected soils are classified as saline, sodic and saline–sodic on the basis of EC (electrical conductivity), SAR (sodium absorption ratio) and pH (Brady and Weil, 2002). Saline soils have an EC_e (saturated extract) >4 dS m^{-1} whereas soils with EC_e >4 dS m^{-1} and SAR >13 are classified as saline–sodic (US Salinity Laboratory Staff, 1954). Increasing salt concentration in the soil solution results in decreasing osmotic potential (Harris, 1980) which reduces water uptake by plants; further stressors include high pH and ion competition limiting nutrient uptake (Keren, 2000). Low osmotic potential also has negative effects on microbial activity and therefore on soil biogeochemical processes essential for maintenance and turnover of soil organic matter (Rietz and Haynes, 2003; Yuan et al., 2007; Mavi and Marschner, 2012). In sodic soils (SAR >13), Na^+ is the dominant cation on the exchange sites of the soil particles causing dispersion of organic matter and clay particles which may lead to breakdown of aggregates and soil structure. The dispersed clay particles can clog pores, cause hardsetting of the soil upon drying, decreased water infiltration and permeability and reduced plant growth (Qadir and Schubert, 2002; Sumner, 1993).

Further, organic compounds are more susceptible to losses in dissolved or colloidal forms, because of reduced binding to soil minerals (Nelson et al., 1996; Peinemann et al., 2005). On the other hand, dispersion of soil particles may also increase the effective surface area for binding of organic matter. In saline–sodic soils, dispersion of clays and organic matter may be lower than in sodic soils because high salt concentrations cause flocculation of the soil particles (Shainberg and Letey, 1984). Indeed, Nelson et al. (1998) showed that dispersion of clays and organic matter is negatively correlated with EC and positively correlated with SAR and pH. However, the effect of salinity on DOC sorption in sodic soils has not been studied systematically, particularly how the salinity and sodicity affect DOC sorption in soils of different clay contents.

Due to the importance of DOM for nutrient cycling, it is essential to better understand the processes that alter DOM concentration and dynamics. Although globally nearly 1 billion ha of agricultural soils are salt-affected (Daggar, 2009), studies on DOM dynamics in these soils are sparse. Therefore, this study was conducted to investigate the interactive effect of salinity and sodicity on DOC sorption in soils varying in clay content (4 to 40%). We hypothesised that (1) high salinity (electrolyte concentration) will favour DOC sorption in contrast to low salinity; (2) high SAR will decrease DOC sorption because of the high concentration of sodium on the exchange complex; and (3) DOC sorption will increase with increasing clay content.

2. Materials and methods

2.1. Soils

Four non-saline and non-sodic soils differing in texture (4, 13, 24 and 40% clay; Table 1) were collected from A horizons (0–30 cm) at several sites covering an area of approximately 10 km^2 near Monarto (35°05′ S and 139°06′ E) located 80 km east of Adelaide in South Australia. The area has a dry Mediterranean climate, and the average temperature is 30.1 °C in summer and 15.9 °C in winter with mean annual rainfall of 352 mm. Samples were thoroughly mixed, air dried, passed through a 2 mm sieve and stored air-dry at room temperature. Based on their clay content, they are denoted as S-4, S-13, S-24 and S-40. X-ray diffraction analysis of the soils showed that the dominating mineral was quartz followed by (in order of decreasing contribution) feldspar > illite > kaolinite > anatase > smectite. Regarding the clay mineralogy, all soils contained illite and kaolinite, but smectite was only detected in S-24. The CEC was highest in S-24 whereas the concentrations of water-soluble Fe and Al were higher in S-4 and S-13 than in the other two soils.

2.2. Soil preparation

Four salt solutions with different EC and SAR were prepared using combinations of 1 M NaCl and 1 M $CaCl_2$ stock solutions. The ECs of the solutions were 10 and 50 dS m^{-1} combined with two SAR values: <3 and >20. The soils (approximately 300 g) were placed on a filter paper in a funnel and repeatedly leached with these solutions. At each leaching event, about 60–80 mL of the solution was added, then the soils were dried at 25–30 °C in a fan-forced oven for 72–96 h, mixed thoroughly to break the clods and analysed for EC and SAR. The drying process was necessary to maximise the soil-solution contact. This process was repeated until the desired combination of EC and SAR ($EC_{1:5}$ 1 and 5 dS m^{-1} in combination with SAR <3 or >20; Table 3) was achieved. These $EC_{1:5}$ levels correspond to EC_e 13 and 67 dS m^{-1} in S-4; 12 and 63 dS m^{-1} in S-13; 11

Table 1
Physical and chemical properties of the soils.

Soil property	Unit	Sand	Loamy sand	Sandy clay loam	Sandy clay
		S-4	S-13	S-24	S-40
Sand	%	95.0	85.0	67.7	52.5
Silt	%	1.3	2.5	8.3	7.5
Clay	%	3.7	12.5	24.0	40.0
$EC_{1:5}$	dS m^{-1}	0.10	0.09	0.10	0.12
$SAR_{1:5}$		1.3	1.3	1.8	2.5
$pH_{1:5}$		7.5	8.2	8.3	8.5
Bulk density	g cm^{-3}	1.75	1.56	1.43	1.33
Water holding capacity	%	8.1	16.4	26.0	32.1
Cation exchange capacity	cmolc(+) kg^{-1}	5.2	14.7	37.0	28.3
Water-soluble Fe	mg kg^{-1}	21.4	21.2	0.8	0.5
Water-soluble Al	mg kg^{-1}	33.6	44.3	0.5	0.9
Total N	%	0.05	0.24	0.19	0.19
Total C	%	0.62	1.82	2.50	2.43

Table 2
Chemical composition of the undiluted DOC solution.

pH	DOC	DON	Al	Fe	Ca	Mg	K	Na
1:5	mg L^{-1}							
5.77	1200	74	0.1	0.1	13.9	15.1	219.5	19.7

and 55 dS m^{-1} in S-24; 9 and 44 dS m^{-1} in S-40 calculated using the equation (Rengasamy, 2006):

$$EC_e = (14.0 - 0.13 \times clay\%) \times EC_{1:5}.$$

After adjustment of EC and SAR, the soils were kept dry at room temperature until the onset of the experiment.

2.3. DOC extraction

DOC was extracted from finely ground mature wheat straw by shaking 30 g straw with 900 mL of deionized water for 1 h. The suspension was then passed through 0.45 μm filter. The composition of the undiluted DOC solution is given in Table 2.

2.4. Batch sorption experiment

Six different DOC concentrations varying from 0 to 128 mg C L^{-1} were used. The DOC concentrations were chosen based on a preliminary experiment with the soils which showed that highest sorption was achieved at about 130 mg C L^{-1}. The DOC stock solution was diluted with deionized water to obtain six different DOC concentrations: 0, 23, 43, 58, 86 and 128 mg C L^{-1}. Thirty mL of the DOC solutions was added to 3 g dry soil, thus the concentrations of DOC per unit soil weight were 230, 430, 580, 860 and 1280 mg kg^{-1} soil. The suspensions were shaken end-over-end overnight at 4 °C, followed by centrifuging at 4000 rpm for 10 min. The supernatant was passed through a 0.45 μm filter under vacuum.

2.5. Analyses and calculations

The water-soluble Fe and Al concentrations in the soils and wheat extracts were determined by inductively coupled plasma emission spectrometry. The EC and pH were measured in a 1:5 soil:water suspension after 1 h end-over-end shaking at 25 °C. The sodium adsorption ratio (SAR) was also determined in a 1:5 soil:water extract. Calcium and Mg concentrations were measured by atomic absorption spectrophotometry, and Na by flame photometry (Rayment and Higginson, 1992). The $SAR_{1:5}$ was calculated using the Richards equation (Richards, 1954):

$$SAR_{1:5} = \left[Na^{+}\right] \Big/ \left[Ca^{2+} + Mg^{2+}\right]^{1/2}$$

where Na^{+}, Ca^{2+} and Mg^{2+} concentrations in the soil solution are in mmol L^{-1}.

Table 3
Expected and achieved $EC_{1:5}$ and $SAR_{1:5}$ in soils S-4, S-13, S-24 and S-40.

Expected $EC_{1:5}$	Achieved $EC_{1:5}$	Expected $SAR_{1:5}$	Achieved $SAR_{1:5}$
S-4		S-4	
EC1	1.08	SAR <3	1.38
EC1	1.20	SAR >20	27.2
EC5	5.07	SAR <3	1.69
EC5	4.91	SAR >20	30.9
S-13		S-13	
EC1	1.10	SAR <3	1.51
EC1	1.30	SAR >20	25.3
EC5	4.92	SAR <3	1.91
EC5	5.10	SAR >20	31.1
S-24		S-24	
EC1	1.20	SAR <3	2.12
EC1	1.31	SAR >20	22.0
EC5	4.90	SAR <3	1.94
EC5	5.01	SAR >20	30.1
S-40		S-40	
EC1	1.10	SAR <3	2.84
EC1	1.26	SAR >20	23.1
EC5	5.09	SAR <3	2.67
EC5	5.04	SAR >20	28.3

The water holding capacity (WHC) was measured gravimetrically (Klute, 1986) and bulk density was measured by the clod method (Blake, 1965). Particle size distribution was analysed by the hydrometer method (Bouyoucos, 1936). Textures were assigned according to the Australian Soil and Land Survey Field Handbook (The National Committee on Soil and Terrain, 2009). Soil organic carbon was analysed by dichromate oxidation (Walkley and Black, 1934). The concentration of DOC in the filtered (0.45 μm) supernatants was measured as non-purgeable organic C and N in a Formacs TOC/TN analyser after acidification of the sample and purging of the inorganic C. The filtered samples were stored in a refrigerator at 4 °C and analysed within 10 days. The aromaticity of the DOC in the filtered supernatants was estimated by determining the specific ultra-violet absorbance (SUVA in l mg DOC^{-1} m^{-1}) at 254 nm (Chin et al., 1997) using a quartz cuvette; SUVA was calculated by dividing absorbance by the DOC concentration.

The mass of DOC sorbed was interpreted using equilibrium concepts in the initial mass isotherm given by Nodvin et al. (1986):

$$RE = mX_i - b$$

where; RE is the amount of DOC released or removed from solution (mg C g $soil^{-1}$) and X_i is the initial DOC concentration (mg C g $soil^{-1}$). The slope of the linear regression (m) is the partition coefficient and a measure of the affinity of the soil to the DOC. The intercept of the equation (b) indicates the amount of DOC released from the soil when a solution with no DOC is added. The null point DOC concentration was estimated from the intercept of the plot of the equilibrium DOC concentration (mg C L^{-1}) and the distribution coefficient (K_d, cm^3 g^{-1}) was obtained from the slope by:

$$K_d = (m/1 - m)(Vol_{solution}/Mass_{soil}).$$

2.6. Statistical analysis

The experiment consisted of 4 soils, 4 EC and SAR combinations and 6 DOC concentrations with 4 replicates. The data were analysed using Genstat 10 (GenStat® for Windows10.0, VSN Int. Ltd, UK, 2005). A three-way (soils × salt treatment × DOC added) analysis of variance (ANOVA) was carried out (Table 4) and Tukey post-hoc tests were used to determine significant differences ($P<0.05$) between DOC sorption among different soils at a given salt treatment and DOC added.

Table 4
Significance of different factors as indicated by the analysis of variance.

Source of variation	DOC sorbed	SUVA
	F value	
DOC added	<0.001	<0.001
Soil	<0.001	<0.001
Salt treatment	<0.001	<0.001
DOC added × soil	<0.001	<0.001
DOC added × salt treatment	<0.001	<0.001
Soil × salt treatment	<0.001	<0.001
DOC added × soil × salt treatment	0.002	<0.001

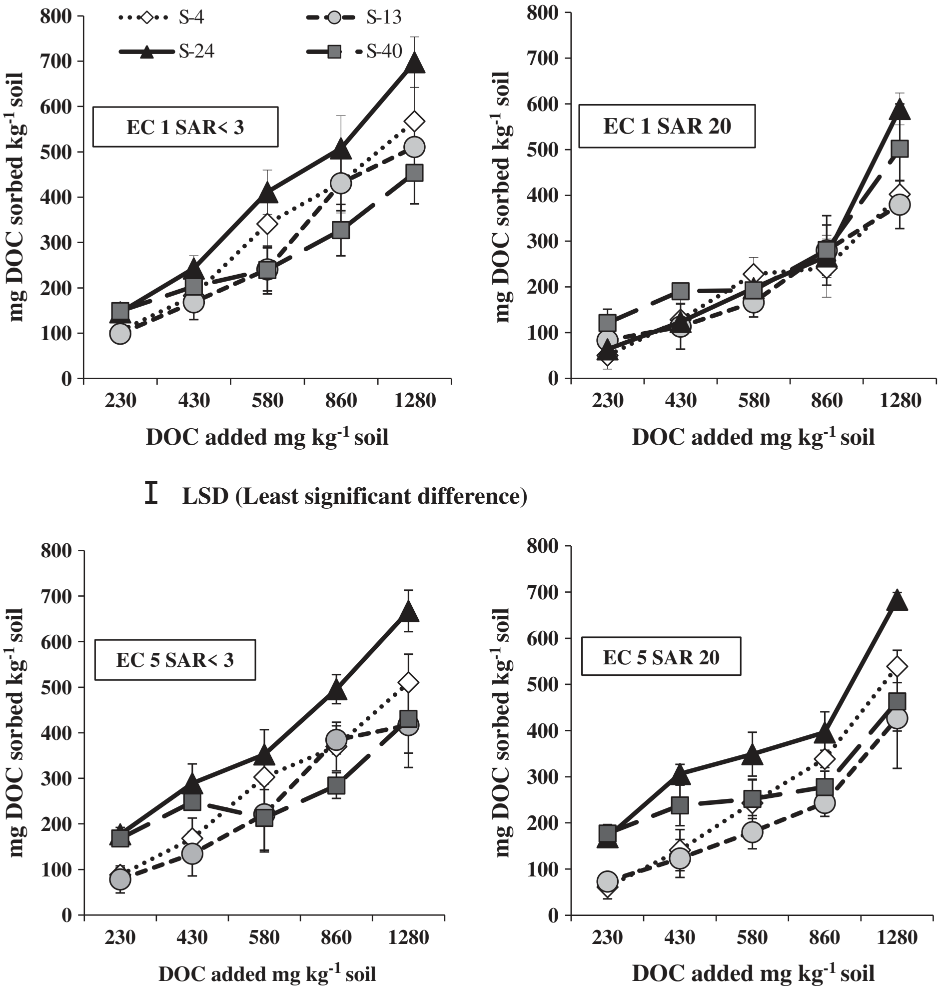

Fig. 1. DOC sorption at $EC_{1:5}$ 1 and 5 in combination with SAR <3 and >20 for soils S-4, S-13, S-24 and S-40. Error bars indicate standard deviation of the mean (n = 4).

3. Results

The amount of DOC sorbed increased with increasing concentration of added DOC in all soils and salinity/sodicity treatments (Fig. 1). In most salinity/sodicity treatments, the concentration of sorbed DOC was highest in S-24 except at EC1 SAR >20 where sorption did not differ among soils. At EC1, sorption of DOC was significantly lower at SAR >20 than at SAR <3 in all soils except at higher concentration of added DOC in S-40. On the other hand, DOC sorption was not affected by SAR at EC5.

In general, irrespective of the salt treatment, the proportion of added DOC sorbed was higher in the fine textured soils (S-24 and S-40) than in coarse textured soils (S-4 and S-13) (Fig. 2). In S-4, the percentage of added DOC sorbed increased with increasing concentration of DOC added up to 580–860 mg kg^{-1} in all salinity/sodicity treatments. In contrast, irrespective of SAR, the percentage of added DOC sorbed decreased with increasing concentration of DOC added at both EC levels in S-40 and at EC5 in S-24. The percentage of added DOC sorbed was not significantly affected by EC but the SAR had an effect at the lower EC. At EC1, the percentage of added DOC sorbed was lower at SAR >20 than at SAR <3 in all soils. The percentage of added DOC sorbed was not affected by SAR at EC5.

The coefficient of determination (R^2) of the linear regression between DOC added and total DOC retained or released ranged between 0.85 and 0.97 in all soils indicating that the initial mass isotherm was an appropriate model for analysing the results (Table 5). The m and K_d values were highest for S-24 and intercept (b) and $DOC_{null\ point}$ were higher in S-13 and S-24 than in S-4 and S-40 suggesting a higher desorption capacity in the former soils. Only at SAR <3, the slope of the linear regression (m) was greater at EC1 than EC5 in all soils. The intercept (b) was higher at EC1 than EC5 only in S-4 and S-13. With the exception of highest concentration of added DOC in S-40, the slope m was lower at SAR >20 than at SAR <3 at EC1 whereas there were no differences in m between SAR levels at EC5. The intercept b was higher at SAR >20 compared to SAR <3 at EC1 but not at EC5.

The SUVA values were higher in the light textured soils than in the heavier textured soils at EC1 SAR >20, but not in the other salt treatments (Fig. 3). SUVA was significantly higher at SAR >20 than at SAR <3 at EC1 at the lower DOC addition rates whereas SAR had no effect on SUVA at EC5.

4. Discussion

This study showed that high SAR only decreases DOC sorption at low EC which can be explained by the high electrolyte concentration at high EC causing binding of DOC to soil particles most likely due to cation bridging. The results also show that sorption of DOC was not directly related to clay concentration, but instead a function of CEC and concentration of Fe and Al.

4.1. Effect of salinity (EC)

The concentration and type of electrolyte on the exchange complex has been shown to significantly affect the DOC sorption capacity of a soil (Rashad et al., 2010; Reemtsma et al., 1999; Skyllberg and

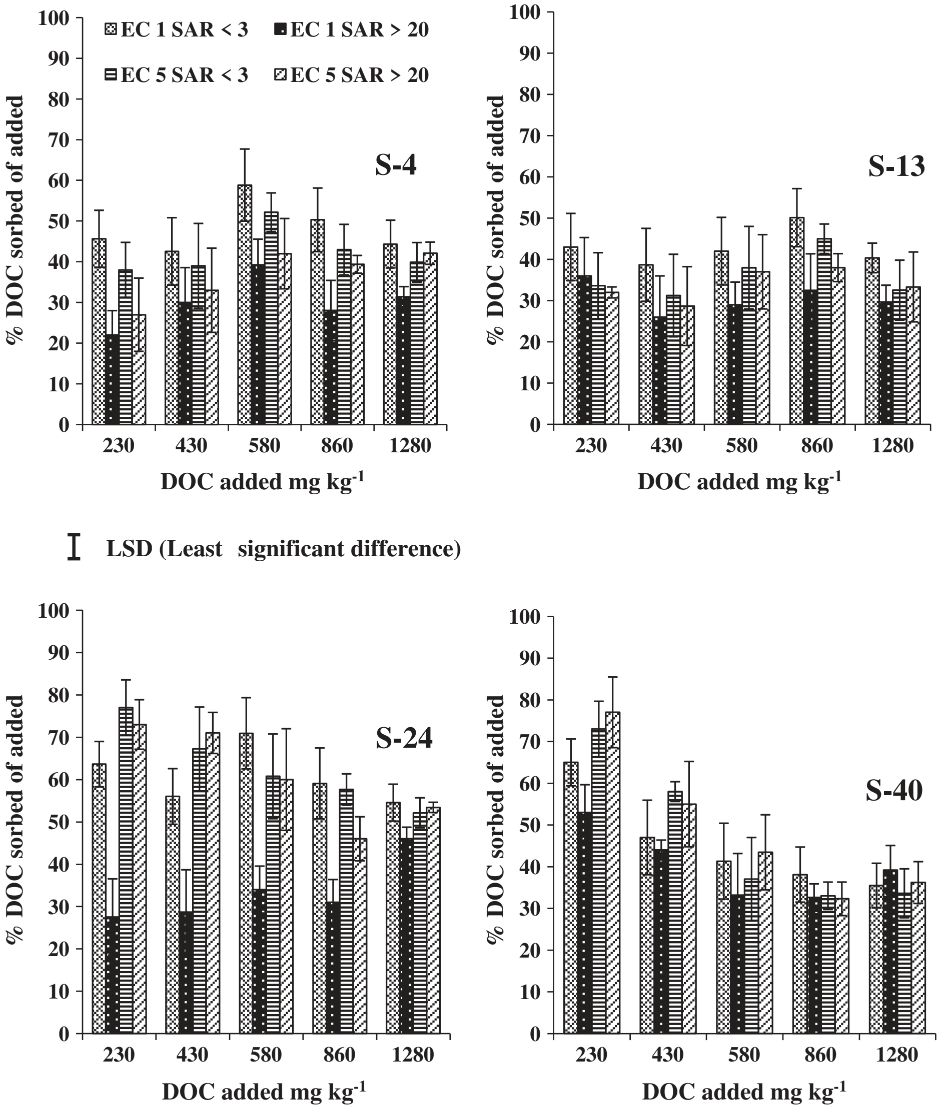

Fig. 2. Percentage DOC sorbed of added at $EC_{1:5}$ 1 and 5 in combination with SAR <3 and >20 for soils S-4, S-13, S-24 and S-40. Error bars indicate standard deviation of the mean (n=4).

Magnusson, 1995). Cations in the soil solution can reduce the solubility of organic molecules by providing the bridge to cross-link and bind them to negatively charged exchange sites (Guggenberger and Zech, 1994; Tipping and Woof, 1991). Therefore, our first hypothesis was that high salinity (electrolyte concentration) will favour DOC sorption. However, this was not confirmed in all cases (Fig. 1). Moreover, Gu et al. (1994) and Davis (1982) reported that increasing ionic strength (from 10 mM to 100 mM $CaCl_2$) had little or no effect on organic matter sorption. However, DOC sorption was higher at EC5 than at EC1 at SAR >20 in the heavier textured soils and this effect was limited to low concentrations of DOC added. This suggests that in saline–sodic soil more DOC was sorbed than in sodic soil possibly due to higher cation concentration in the former. Indeed, Mikutta et al. (2007) also reported that the larger sorption of organic matter to soil particles was due to the formation of cation bridges at the mineral surfaces.

4.2. Effect of sodicity (SAR)

In this study, SAR had a greater effect on sorption of DOC than EC which indicates that our second hypothesis (high SAR will decrease DOC sorption) can be accepted. However, the SAR effect was modulated by EC. At EC1 compared to SAR <3, SAR >20 significantly decreased sorption and resulted in lower slope (m) and K_d values in all soils, except at the highest concentration of added DOC in S-40 (Fig. 1, Table 5). This suggests that a higher proportion of the exchange sites occupied by monovalent cation (Na^+) reduces DOC sorption, which is in agreement with other studies (Bäckström et al., 2004; Norrström and Bergstedt, 2001). This also highlights the importance of divalent cations such as Ca^{2+} for DOC sorption (Baldock and Skjemstad, 2000; Mikutta et al., 2007; Varadachari et al., 2000). However, at EC5, high SAR did not significantly reduce DOC sorption, because the higher electrolyte concentration in the soil solution (Ca, Mg, Na) blocks functional groups on the organic matter and cross-links polymers to create dense in-flexible molecules which then bind to clays (Oades, 1988) and this overrides the repulsive effect of a high proportion of Na^+ on the exchange sites of the clay particles. The finding that at EC1, high SAR decreased DOC sorption mainly at low DOC addition rates suggests that at higher DOC concentration, the higher ionic strength of the solutions causes cross-linking and binding as described above for the high EC. Moreover, a large proportion of the sorption sites occupied by organic matter may also over-ride the repulsive effect of high Na saturation.

Table 5
DOC sorption isotherm parameters at $EC_{1:5}$ 1 and 5 in combination with SAR <3 and >20 for soils S-4, S-13, S-24 and S-40 (n = 4).

		EC1 SAR <3	EC1 SAR >20	EC5 SAR <3	EC5 SAR >20
Soil-4					
m (slope)	Unitless	0.46	0.31	0.40	0.34
b (intercept)	mg C g^{-1}	0.26	0.28	0.23	0.22
K_d	cm^3 g^{-1}	8.87	4.53	6.64	7.54
DOC null point	mg C L^{-1}	58.0	85.0	58.0	52.0
R^2 (n = 4)		0.96	0.93	0.94	0.95
Soil-13					
m (slope)	Unitless	0.43	0.30	0.36	0.31
b (intercept)	mg C g^{-1}	0.35	0.47	0.31	0.34
K_d	cm^3 g^{-1}	7.51	4.43	5.63	4.93
DOC null point	mg C L^{-1}	86.0	154.0	86.0	104.0
R^2 (n = 4)		0.93	0.93	0.89	0.91
Soil-24					
m (slope)	Unitless	0.55	0.44	0.49	0.51
b (intercept)	mg C g^{-1}	0.32	0.45	0.44	0.43
K_d	cm^3 g^{-1}	12.22	8.18	9.23	10.41
DOC null point	mg C L^{-1}	57.0	102.0	92.0	83.0
R^2 (n = 4)		0.96	0.92	0.94	0.97
Soil-40					
m (slope)	Unitless	0.33	0.31	0.30	0.36
b (intercept)	mg C g^{-1}	0.22	0.30	0.25	0.27
K_d	cm^3 g^{-1}	4.99	5.63	4.21	4.56
DOC null point	mg C L^{-1}	67.0	83.0	82.0	82.0
R^2 (n = 4)		0.91	0.89	0.85	0.88

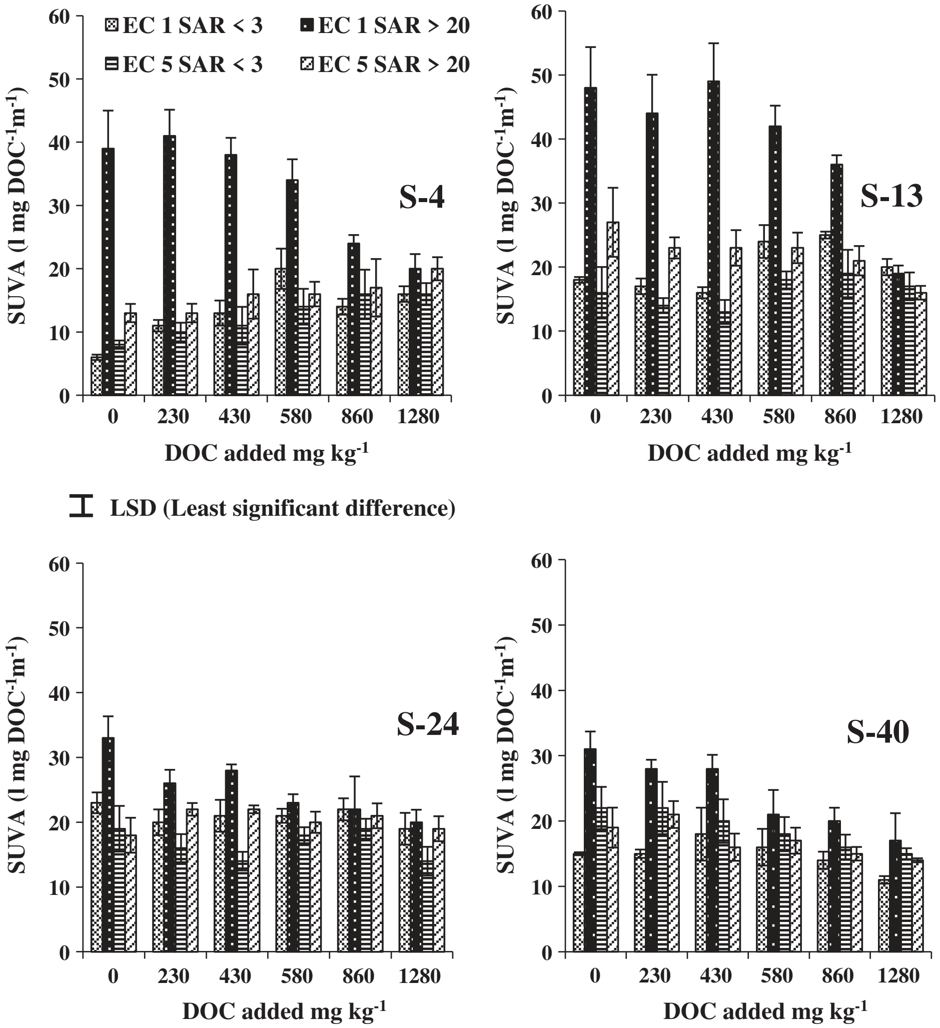

Fig. 3. SUVA (l mg DOC^{-1} m^{-1}) at $EC_{1:5}$ 1 and 5 in combination with SAR <3 and >20 for soils S-4, S-13, S-24 and S-40. Error bars indicate standard deviation of the mean (n = 4).

4.3. Texture effect on DOC sorption

Generally, the sorption capacity of a soil for organic matter is related to the surface area of the soil which in turn is related to its clay content (Nelson et al., 1997) because most clays have a net negative charge, small size and large surface area (Oades, 1988). Thus, our third hypothesis was that DOC sorption will increase with increased clay content in saline and saline–sodic soils. However, this was not the case. Except at EC1 and SAR >20, DOC sorption was greatest in S-24 at all concentrations of DOC added whereas DOC sorption did not differ consistently between S-4, S-13 and S-40 (Fig. 2). The high DOC sorption capacity of S-24 can therefore not be due to the clay content alone; other soil properties must also play a role.

The higher DOC sorption of S-24 can be explained by its higher CEC which was 30% higher than in S-40 (Table 1) and is probably due to the higher smectite content of S-24. Compared to illite and kaolinite, smectite are expandable clay minerals with smaller particle size, larger surface area and higher cation exchange capacity (Nelson et al., 1997; Wattel-Koekkoek et al., 2001). Similarly, Nelson et al. (1997) and Ransom et al. (1998) reported that organic matter is preferentially sequestered by soil sediments rich in smectite.

Despite the differences in clay content and CEC between S-4, S-13 and S-40, they differed little in DOC sorption. This may be due to the higher water-soluble Fe and Al concentrations in S-4 and S-13 compared to S-40 (Table 1). Iron and Al oxides bind DOC more strongly than clay minerals (Kaiser and Zech, 2000), particularly when they are amorphous (Jardine et al., 1989; Kaiser et al., 1996; Moore et al., 1992). And clays covered with Fe/Al oxides have a higher sorption capacity than those without Fe/Al oxides (Kaiser et al., 1996; Kalbitz et al., 2005; Mikutta et al., 2007). Exposed amorphous Fe and Al oxides are likely to be the main contributors to the Fe and Al concentrations in the water extract (Darke and Walbridge, 2000) shown in Table 1. Further, native organic matter may already be bound to the potential DOC sorption sites (Hassink, 1997; Kaiser and Zech, 2000; Shen, 1999). Thus, the high concentration of native organic carbon in S-40 may have also impeded sorption of DOC whereas with the lower organic matter content in S-4 and S-13 more potential binding sites would be available to bind the added DOC. The high native organic matter content of S-40 may also explain that DOC sorption was little affected by EC and SAR at high concentrations of DOC added.

5. Conclusions

The results of this study showed that in the salt-affected soils used here, it is not the clay content per se, but the CEC and the Fe and Al concentrations that most strongly influence DOC sorption. The results further indicate that DOC loss from saline–sodic soils will be lower than from sodic soils due to cation bridging at high electrolyte concentration. These findings suggest that increasing the electrolyte concentration in sodic soils by liming or irrigation with saline water may reduce nutrient loss via leaching and increase organic matter sequestration.

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CHAPTER 6:

MICROBIAL RESPONSE IN SALINE AND NON-SALINE SOILS TO ADDITION OF CARBON AND NITROGEN

Microbial response to addition of carbon and nitrogen in saline and non-saline soils

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Abstract. Addition of carbon and nitrogen to soil can enhance microbial tolerance to salinity, but it is not known how carbon form affects microbial tolerance and if this is modulated by N addition. Therefore, an incubation study was conducted to assess the effect of C form and N availability on microbial activity and biomass in a soil at different salinity levels. A non-saline soil was adjusted to five salinity levels [$EC_{1:5}$ 0.1, 2.5, 5.0, 7.5 and 10 dS m^{-1}] using a combination of $CaCl_2$ and NaCl. The soils were amended with 2.5 mg C g^{-1} as glucose or mature wheat straw (C/N ratio 47:1) and with NH_4Cl to glucose to achieve a C/N ratio of 47 (similar to that of wheat straw) or to glucose and wheat straw to achieve a C/N ratio of 20. Soil respiration was measured over 30 days. Microbial biomass C and N (MBC, MBN), dissolved organic C (DOC) and total dissolved N (TDN) were measured on day 30. Cumulative respiration and MBC decreased with increasing EC, less so with glucose than with wheat straw. MBC was more sensitive to EC than cumulative respiration, irrespective of C source. Addition of N to glucose and wheat straw to bring the C/N ratio to 20 significantly decreased cumulative respiration and MBC at a given EC. This study showed that in the short-term, addition of a readily available and easily decomposable source of energy improves the ability of microbes to tolerate salinity. The results also suggest that in saline soils, irrespective of the C substrate, N addition has no or a negative impact on microbial activity and growth.

Additional keywords: Dissolved organic C, total dissolved N, microbial activity, microbial biomass, salinity.

Introduction

In arid climates, salinity is a major limiting factor for crop production and contributor to land degradation. Low rainfall and high potential evapotranspiration in these regions promote the upward movement of salts in the soil solution, which adversely affects soil physical, chemical and biological properties (Rengasamy 2006). Worldwide more than 397 million ha of land is affected by salinity (FAO/AGL 2000) and this area is likely to increase in the future because of secondary salinisation due to irrigation and clearing of native vegetation (Pannell and Ewing 2006).

Saline soils generally have low organic matter and N concentration (Asmalodhi *et al.* 2009). The effects of high salt concentrations in the soil solution include low water uptake by plants due to low osmotic potential (Harris 1980), high pH, and ion competition limiting nutrient uptake (Keren 2000). These effects not only reduce plant growth but also have a negative impact on the size and activity of soil microbial biomass and biochemical processes (Rietz and Haynes 2003; Tripathi *et al.* 2006; Yuan *et al.* 2007; Mavi *et al.* 2012). Some microbes respond to low osmotic potential by accumulating osmolytes to retain water (Hagemann 2011) whereas sensitive microbes die.

Previous studies have shown the adverse affects of salinity on C and N mineralisation and release of nutrients (Frankenberger and Bingham 1982; Nelson *et al.*

1996; Pathak and Rao 1998; Conde *et al.* 2005). Addition of organic matter is considered to be a viable option for ameliorating salt-affected soils (Garcia *et al.* 2000). Increased availability of substrate has been shown to help the microbes to counteract some of the negative effect of salts (Wichern *et al.* 2006). Although the effects of chemical composition of the substrate on decomposition have been addressed in non-salt affected soils (Vanlauwe *et al.* 1994; Thuries *et al.* 2002), it is not clear how carbon form (easily available or recalcitrant) affects the response of microbes to salinity.

In soil, apart from C, availability of N is another factor regulating microbial growth and activity. Addition of N may increase (Recous *et al.* 1995; Henriksen and Breland 1999) or decrease microbial activity (Kowalenko *et al.* 1978; Soderstrom *et al.* 1983; Sall *et al.* 2003; Janssens *et al.* 2010) or have no effect (Allison and Klein 1962; Prescott 1995; Hobbie and Vitousek 2000). The different effects may be due to the ratio of available C to N, the N availability of the soil and s the effects of N on soil properties such as pH.

To optimize amelioration strategies for salt-affected soils, it is important to understand how C and N availability affect microbial activity and growth. Two incubation studies were conducted in which glucose and wheat straw with and without inorganic N were added to a soil with different salinity levels with the aim to investigate how different forms of C: easily available (glucose) and less available (wheat straw) with and without added inorganic N affect the response of microbial activity and biomass to increasing EC.

Materials and methods

A non-saline sandy clay loam (sand 67.7%, silt 8.3% and clay 24%, pH 8.3, $EC_{1:5}$ 0.09 dS m^{-1}, $SAR_{1:5}$ 1.8, water holding capacity 0.3 g g $soil^{-1}$, bulk density 1.42 g cm^{-3}, total organic C 17.1 g kg^{-1}, total N 2.4 g kg^{-1} was collected from the A horizon (0-30 cm) of a soil near Monarto (35º05′ S and 139º06′ E), South Australia. The area has a dry Mediterranean climate and the average temperature is 30ºC in summer and 16ºC in winter with a mean annual rainfall of 352 mm. The soil was air dried, passed through a 2 mm sieve and stored air-dry at room temperature before the start of the experiments.

To adjust the EC, two salt solutions with EC 40 and 80 dS m^{-1} were prepared using a combination of $CaCl_2$ and NaCl. To adjust the EC, each time approximately 300 g of soil was placed on a filter paper in a funnel and repeatedly leached with these solutions. At each leaching event, about 60-80 mL of the solution was added. Then the soils were dried at 25-30ºC in a fan-forced oven for 72-96 h, mixed thoroughly to break the clods and then analysed for EC. The drying process was necessary to maximize soil-solution contact. This process was repeated 3-4 times to achieve the desired levels of EC. After adjustment of EC, the soils were kept dry at room temperature until the onset of the experiment.

Air dry soils were wet to 60% WHC, which resulted in maximal respiration in this soil in a preliminary experiment (data not shown) and incubated for 7 days at 22-25ºC before the start of the experiment. A pre-incubation of 7 days was selected on the basis of Mavi and Marschner (2012) and Mavi *et al.* (2012), who found that microbial activity stabilized within 7-10 days after rewetting air-dry non-saline soil. Throughout the pre-incubation and the subsequent measurement period, reverse osmosis water was added to maintain the target water content.

Experiment 1

The aim of this experiment was to determine the relationship between microbial activity and EC and how this is affected by the form and rate of C additions. The EC was adjusted to $EC_{1:5}$ 0.1, 5, 10, 15 and 20 dS m^{-1} and sodium absorption ration (SAR) was maintained at < 3 (non-sodic) in all treatments. These $EC_{1:5}$ levels correspond to EC_e 1.1, 54, 108, 163 and 217 dS m^{-1} calculated using the equation (Rengasamy 2006):

$$ECe\ (dS\ m^{-1}) = (14.0 - 0.13 \times soil\ clay\ \%) \times EC_{1:5}\ (dS\ m^{-1})$$

After the pre-incubation, the soils with different salinity levels were amended with either glucose or mature wheat straw (C/N ratio 47:1; ground, sieved to 0.25-2 mm) at 2.5 or 5.0 mg C g $soil^{-1}$ and thoroughly mixed into the soils.

Experiment 2

The aim of the second experiment was to assess the effect of C form and N availability on microbial activity and growth at different salinity levels. Based on the results of the first experiment, the EC was adjusted to five salinity levels [$EC_{1:5}$ 0.1, 2.5, 5.0, 7.5 and 10 dS m^{-1}] and SAR < 3 was kept in all the samples. After the pre-incubation, soils with different salinity levels were amended with 2.5 mg C g^{-1} as glucose or mature wheat straw in combination with no N addition or N added as NH_4Cl. Sufficient N was added to acchieve C/N ratios of 47 similar to wheat straw (N added to glucose treatment only) or 20 (N added to both glucose and wheat straw treatments). Thus, there were five C+N treatments (i) glucose (ii) wheat straw (iii) glucose + 0.05 mg N g $soil^{-1}$ [glucose + N

(C/N 47)] (iv) glucose + 0.125 mg N g soil^{-1} [glucose + N (C/N20)] and (v) wheat straw + 0.071 mg N g soil^{-1} [wheat straw + N (C/N20)].

Incubation

After amending the soils with C and N and mixing them thoroughly, 20 g of soil was added to polyvinyl chloride cores (PVC) with a radius of 1.85 cm and height of 5 cm and a nylon mesh base (0.75 mm, Australian Filter Specialist) and packed to the bulk density of the soil in the field. The cores were placed individually into 1 L glass jars and the jars sealed with gas tight lids equipped with septa to allow quantification of the CO_2 concentration in the headspace. There were 4 replicates per EC and C treatment. The jars were incubated in the dark at 22-25ºC and CO_2 release was measured over 50 days in the first experiment and over 30 days in the second experiment.

Soil analysis

The EC and pH were measured in a 1:5 soil:water suspension after 1 h end-over-end shaking at 25ºC. The water holding capacity (WHC) was measured using a sintered glass funnel connected to a 100 cm water column ($\Psi m = -10$ kPa). Soil was placed in rings in the sintered glass funnel, thoroughly wetted and allowed to drain for 48 h. Dry weight of the soil was determined after oven drying at 105°C for 24 h. Bulk density was measured by the clod method (Blake 1965). Particle size was analysed by the hydrometer method (Bouyoucos 1936), available N (Subbiah and Asija 1956) and organic carbon were analysed by dichromate oxidation (Walkley and Black 1934). In experiment 2, microbial biomass C (MBC) and microbial biomass N (MBN) were determined at the end of the experiment by the fumigation extraction method (Vance *et al.* 1987), C and N concentrations in the extracts were determined by Formacs TOC/TN analyser. No multiplication factor was used because the relationship between actual microbial biomass and that derived by this method in these soils is not known. Dissolved organic C (DOC) and total dissolved N (TDN) were extracted at the end of Experiment 2 with a 1:5 soil: water ratio, shaking end-over-end for 1 h, followed by centrifuging at 4000 rpm for 10 minutes and passing through a 0.45 µm filter under vacuum. The concentration of DOC and TDN in the suspensions was measured as non-purgeable organic C and N in a Formacs TOC/TN analyser after acidification of the sample and purging of the inorganic C. The filtered samples were stored in a refrigerator at 4ºC if analysed within 10 days. If the analysis was delayed, samples were immediately frozen at -5°C and then defrosted at 4°C prior to analysis.

Soil respiration

Soil respiration was measured by quantifying headspace CO_2 concentrations within each jar using a Servomex 1450 infra-red gas analyser (Servomex, UK). The CO_2 concentration was measured every 1 or 2 days during the initial incubation period but longer intervals (3-5 days) were used when the respiration rates were low. For each measurement period, an initial measurement of the CO_2 concentration in the headspace was taken immediately after sealing the jars. The closed jars were incubated for a defined duration and a second measurement of CO_2 concentration in the headspace was taken. After the second measurement, the jars were opened to refresh the headspace in the jars using a fan. The CO_2 evolved from each sample is the difference between the initial and final CO_2 concentrations for each measurement period. The infra-red gas analyser was calibrated using known amounts of CO_2 injected into glass jars similar to those used for the samples. The relationship between CO_2 concentration and detector response was determined by linear regression. This relationship was used to calculate the CO_2 concentration in the jars with soils. The CO_2 concentration for each measurement period was multiplied by the gas volume of the jars to obtain the volume of CO_2-C respired during each measurement period and divided by the soil dry weight. The following equation was used to convert CO_2 (mL) to mg CO_2-C per g of soil per day.

$$X = \frac{D \times 1000 \times A}{Y_{(std)} \times T \times M}$$

Where, X = mg CO_2-C g^{-1} soil day^{-1}, D = CO_2 (mL) evolved from soil during time T, A= atomic weight of C. $Y_{(std)}$ = volume of 1 mol of CO_2 at 25 ºC and 1 atmospheric pressure (24465 mL), T= time (days) and M= mass of soil (g). The values for each measurement period were added to calculate the cumulative respiration over the 50 and 30 days of incubation period respectively.

Statistical analysis

The data were analysed using two-way analysis of variance ANOVA [C+N treatment × EC levels] in Genstat 10 (GenStat® for Windows10.0, VSN Int. Ltd, UK, 2005) and Tukey post-hoc test was used to determine significant differences ($P < 0.05$). Regressions between EC and measured parameters were calculated with SPSS.

Results

Experiment 1

Respiration was detected only up to EC10. Cumulative respiration was higher with 5 mg C than with 2.5 mg C and decreased with increasing salinity from EC0.1 to EC10, irrespective of the source and amount of added C (Table 1). The relative decrease in cumulative respiration with increasing EC was greater with wheat straw than with glucose. With wheat straw, cumulative respiration compared to EC0.1 decreased by 44 % at EC5 and by 80 % at EC10, whereas with glucose it decreased by 11 % and 71 % at EC5 and EC10, respectively. The percent decrease in cumulative respiration was similar at both rates of added C. Based on the above results, the second experiment was conducted with salinity levels from EC0.1 to EC10 and with a C rate of 2.5 mg C g soil^{-1}.

Experiment 2

Soil respiration

Cumulative respiration was highest in the non-saline soil (EC0.1) and decreased with increasing salinity in all treatments (Fig. 1). With increasing EC, the occurrence of the maximal respiration rate was delayed by one to three days with the delay becoming longer as the EC increased (data not shown). At all EC levels, cumulative respiration was lower with wheat straw than with glucose. Generally, irrespective of N addition, the decrease in cumulative respiration with increasing EC was less with glucose than with wheat straw. With glucose alone, compared to EC0.1 cumulative respiration decreased by 12 % at EC2.5 and by 62 % at EC10, whereas with wheat straw alone, the decrease in cumulative respiration was 28 % and 90 % at EC2.5 and EC10, respectively. At EC0.1, compared to glucose alone, addition of N to glucose had no significant effect on cumulative respiration. However, at EC2.5 to EC7.5, addition of N to glucose to bring the C/N ratio to 20 significantly decreased cumulative respiration and delayed the occurrence of maximal respiration rates. This decrease did not occur when the N concentration was adjusted to a C/N of 47. The addition of N with wheat straw significantly decreased cumulative respiration compared to wheat straw alone up to EC5. Cumulative respiration was significantly negatively correlated with EC in both glucose (R^2=0.42; $P < 0.001$) and wheat straw (R^2=0.26; $P < 0.001$) treatments, but the slope was greater with wheat straw than with glucose (Table 3).

Dissolved organic C (DOC)

The DOC concentration increased with increasing salinity in all treatments (Table 2). At EC0.1, DOC was similar with glucose alone and wheat straw alone, but from EC2.5 to EC10, DOC was significantly higher with wheat straw alone. With glucose alone, the DOC concentration did not change up to EC5, but then increased with increasing EC. On the other hand with wheat straw alone, the DOC concentration increased with increasing EC over the entire range of ECs. Addition of N to glucose to bring the C/N ratio to 47 decreased the DOC concentration only at EC0.1, whereas adding N to glucose to bring it to C/N 20 increased the DOC concentration at EC ≥5. In contrast, decreasing the C/N ratio of wheat straw to 20 decreased DOC concentrations at all EC levels. The DOC concentration was positively correlated with EC; the relationship was similar with glucose and wheat straw (Table 3).

Total dissolved N (TDN)

The TDN concentrations were significantly lower in treatments without added N than in those with added N (Table 2). In general, irrespective of the C source, salinity increased the TDN concentration in treatments with added N up to EC5. At a given adjusted N concentration, TDN concentrations were lower with wheat straw than with glucose. Irrespective of the substrate, TDN was not significantly correlated with EC.

Microbial biomass C (MBC)

Increasing salinity decreased MBC in all C treatments (Fig. 2a). MBC was always significantly higher with glucose alone compared to wheat straw alone. The percentage decrease in MBC with increasing EC was smaller with glucose alone than with wheat straw alone, e.g. at EC5, MBC was decreased by 22 % with glucose, but by 44 % with wheat straw. This is also evident in the greater slope of the regression between EC and MBC with wheat straw compared to glucose (Table 3). At a given EC, addition of N to glucose or wheat straw to bring the C/N ratio to 20 decreased MBC, whereas addition of N to glucose to bring the C/N ratio to 47 had no effect on MBC compared to glucose alone. There was a negative relationship between MBC and EC with wheat straw (R^2=0.64, $P < 0.001$) and glucose (R^2=0.86, $P < 0.001$) (Table 3).

Microbial biomass N (MBN)

The MBN concentration was higher at EC0.1 than in the saline soils, but there was no clear difference among the saline soils (Fig. 2b). Addition of N increased MBN in the glucose treatments, with the addition of more N (to bring the C/N ratio to 20) resulting in higher MBN than adjusting the C/N ratio to 47. Microbial biomass N did not differ between glucose alone and wheat straw alone, but with the addition of N, MBN was significantly higher with glucose than with wheat straw. With wheat straw, MBN was significantly negatively correlated with EC in wheat straw (R^2=0.31; $P < 0.001$) but not with glucose. Irrespective of the substrate, the slope of EC with MBN was lower than with MBC (Table 3).

The MBC/MBN ratio did not differ between glucose alone and wheat straw alone at EC0.1 and EC2.5, but the ratio was significantly higher with glucose alone than with wheat straw alone at the higher EC levels (Fig. 2c). The MBC/MBN ratio was significantly higher in treatments without added N than with N addition, irrespective of salinity and source of added C. With N addition, there were no differences in the MBC/MBN ratio among the C treatments or EC levels.

Discussion

Effect of C addition

The results of the study confirm that low osmotic potential induced by increasing salinity reduces microbial activity and biomass (Pankhurst *et al.* 2001; Mamilov *et al.* 2004; Yuan *et al.* 2007; Chowdhury *et al.* 2011), irrespective of the C source. The greater slope of the regression between EC and MBC compared to that of EC and cumulative respiration, both with wheat straw and glucose, indicated that MBC was more sensitive to salinity than cumulative respiration. Thus under saline conditions, C is utilised preferentially for energy (respiration) rather than growth, resulting in poor C utilisation efficiency. This may be due to the high energy demand for the synthesis of osmolytes (Oren 1999). The increased concentrations of DOC and TDN with increasing salinity also suggests reduced ability of the microbes to utilize available substrates for growth which is in agreement with Mavi *et al.* (2012). Increasing EC affected MBC and DOC more strongly than MBN and TDN suggesting that EC affects C utilisation more than N utilisation.

Cumulative respiration and MBC concentrations were generally higher with glucose than with wheat straw, which can be explained by several factors (i) in glucose all C is available whereas only a small proportion of wheat C is readily available, (ii) the soluble glucose is more evenly distributed in the soil than the particulate wheat straw and thus accessible by a greater proportion of the soil microbes, and (iii) glucose can be used as a substrate by the vast majority of soil organisms (Anderson and Domsch 1978) whereas decomposition of wheat straw macromolecules such as lignin, hemi-cellulose and cellulose, requires specialized enzymes. Synthesis of these enzymes is energy-consuming and they are produced by only a fraction of the microbial biomass (Wu *et al.* 1993).

The percent decrease in cumulative respiration and MBC with increasing EC was 2-3 times greater with wheat straw than with glucose alone, indicating that the readily available C in glucose increased the ability of the microbes to tolerate low osmotic potential. This is in agreement with Pathak and Rao (1998) and Wichern *et al.* (2006), who reported that increased substrate availability, can help microbes to tolerate salinity. The generation of osmolytes requires large amounts of energy (Oren 1999) which can be readily produced from the easily available C from glucose (Hagemann 2011). Further, since glucose C can be utilised by the majority of soil microbes, the death of a few salinity sensitive genotypes will have little impact on activity and growth of the microbial biomass as a whole. To utilise wheat straw C on the other hand, microbes have to divert part of the energy generated to synthesis of enzymes such as cellulases and ligninases and thus have less energy available for synthesis of osmolytes. Additionally, the ability to decompose cellulose and lignin is restricted to a small proportion of the microbial community. The death or reduced activity of salt-sensitive genotypes will therefore have a greater impact than among the glucose utilisers.

It should be noted however, that the differences among the C sources found in this study where the duration of the experiments was 30-50 days may differ from the long-term effects. It is conceivable that with wheat straw a low level of microbial activity and growth is maintained for a longer period of time even at higher salinity levels than with glucose where the available C is quickly depleted.

Effect of N addition

The addition of N may increase (Recous *et al.* 1995; Henriksen and Breland 1999; Conde *et al.* 2005), decrease (Kowalenko *et al.* 1978; Nohrstedt *et al.* 1989; Bowden *et al.* 2004; Craine *et al.* 2007; Ramirez *et al.* 2010) or have no effect on microbial activity (Prescott 1995; Hobbie and Vitousek 2000). In the present study, compared to glucose alone, the low N (0.05 mg N g soil^{-1}) addition to glucose to achieve a C/N ratio of 47 had no effect on cumulative respiration, MBC and DOC. Compared to wheat straw alone, addition of N to glucose to achieve a C/N ratio of 47 resulted in significantly higher cumulative respiration and decreased DOC which suggests that more of the added C was utilised by the microbes. However, the finding that MBC was not increased indicates that easily available glucose C is mainly used for energy production and not for growth.

At the higher N addition rates to achieve a C/N ratio of 20 (0.071-0.125 mg N g soil^{-1}) cumulative respiration and MBC were significantly lower both with wheat straw and glucose compared to wheat straw and glucose alone with a greater decrease in glucose than with wheat straw. The reduction of microbial activity and growth with N addition is in agreement with other studies (Hopkins *et al.* 2008; Treseder 2008; Vuelvas-Solorzano *et al.* 2009). A lack of a positive growth effect by the high N addition may be due to the fact that even in treatments without added N, the MBC/MBN ratio varied between 10-20 which indicates that N uptake was already sufficient in treatments with no added N and low N addition rate. The negative effect of the high N rates on microbial activity and growth can be explained by: (i) reaction of ammonia and amino compounds with polyphenols and metabolites to form recalcitrant compounds (Fog 1988; Agren *et al.* 2001); (ii) inhibition of the activity and production of lignin degrading enzymes by ammonia (Carreiro *et al.* 2000); (iii) protection of N containing compounds from biological oxidation by interacting with soil minerals (Baldock and Skjemstad 2000), and (iv) changes in microbial community structure due to N addition (Treseder 2008; Baumann *et al.* 2009).

With glucose, the DOC concentration was not affected by the higher rate of N addition up to EC2.5, but then increased suggesting that less of the added glucose C was utilised by the microbes which is supported by the lower respiration and biomass. With wheat straw on the other hand, high N addition decreased DOC indicating reduced decomposition of the wheat straw and thus less release of water-soluble C. Similarly, Chantigny *et al.* (1999) reported decreased water-soluble C with high N additions.

The higher MBN and TDN concentrations in treatments with added N than without N indicate assimilation of inorganic N and synthesis of soluble N compounds.

Vega-Jarquin *et al.* (2003) also found that microbes in the saline soils are capable of immobilising inorganic N.

Conclusions

This study showed that high concentrations of easily available C can increase the ability of microbes to tolerate high salinity. Although only respiration was measured in the present study, the high microbial activity may also increase mineralisation and solubilisation of nutrients and thereby enable microbes to cope better with salt stress. However, the greater activity and biomass resulted in increased immobilisation of N which would only become available when the microbial biomass turns over. On the other hand, a smaller fraction of the microbial biomass was able to utilise the less readily available C in wheat straw which appears to be more sensitive to salinity than the glucose-utilising microbes. Further, the study suggested that, at least in soils with a microbial C/N ratio of around 20, addition of N will have no or a negative effect on microbial activity and growth irrespective of EC but may increase microbial N immobilisation and TDN concentrations.

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Table 1. Cumulative respiration (mg CO_2-C g soil^{-1}) after 50 days in soils with $EC_{1:5}$ 0.1, 5 and 10 amended with glucose and wheat straw at the rate 2.5 or 5 mg C g^{-1}. Values with different letters differ significantly ($p < 0.05$) across different treatments and EC levels (n=4)

Treatment	Rate of C addition (mg C g^{-1})	EC 0.1	EC 5	EC 10
Glucose	2.5	1.02 h	0.91 g	0.29 c
	5	1.50 j	1.32 i	0.38 d
Wheat straw	2.5	0.56 e	0.26 c	0.11 a
	5	0.72 f	0.41 d	0.16 b

Table 2. DOC and TDN (µg g soil^{-1}) after 30 days in soil with $EC_{1:5}$ 0.1, 2.5, 5, 7.5 and 10 amended with glucose and wheat straw with and without N (n=4)

Treatment	EC 0.1	EC 2.5	EC 5	EC 7.5	EC 10
	Dissolved organic carbon (DOC)				
Glucose	181	190	205	275	301
Wheat straw	184	240	297	335	382
Glucose + N (C/N 47)	118	164	215	256	289
Glucose + N (C/N 20)	128	185	260	336	360
Wheat straw + N (C/N 20)	122	170	211	242	255
	lsd (0.05)= 29				
	Total dissolved nitrogen (TDN)				
Glucose	21	24	30	29	24
Wheat straw	23	34	60	64	58
Glucose + N (C/N 47)	419	512	581	586	548
Glucose + N (C/N 20)	906	1229	1301	1267	1355
Wheat straw + N (C/N 20)	587	709	773	800	770
	lsd (0.05)=31				

Table 3. Relationship between salinity (EC) and cumulative respiration, MBC DOC and MBN ($P < 0.001$)

Parameter	**Wheat residue**	**R^2**	**Glucose**	**R^2**
Cumulative respiration	Y = -0.01x + 0.57	0.26	Y= -0.007x + 0.63	0.42
MBC	Y= -7.97x + 376	0.64	Y= -5.33x + 83	0.86
DOC	Y= 4.01x + 133	0.75	Y= 4.10x + 124	0.74
MBN	Y= -1.22x + 68	0.31	Y= -0.92x + 101	ns

ns: not significant

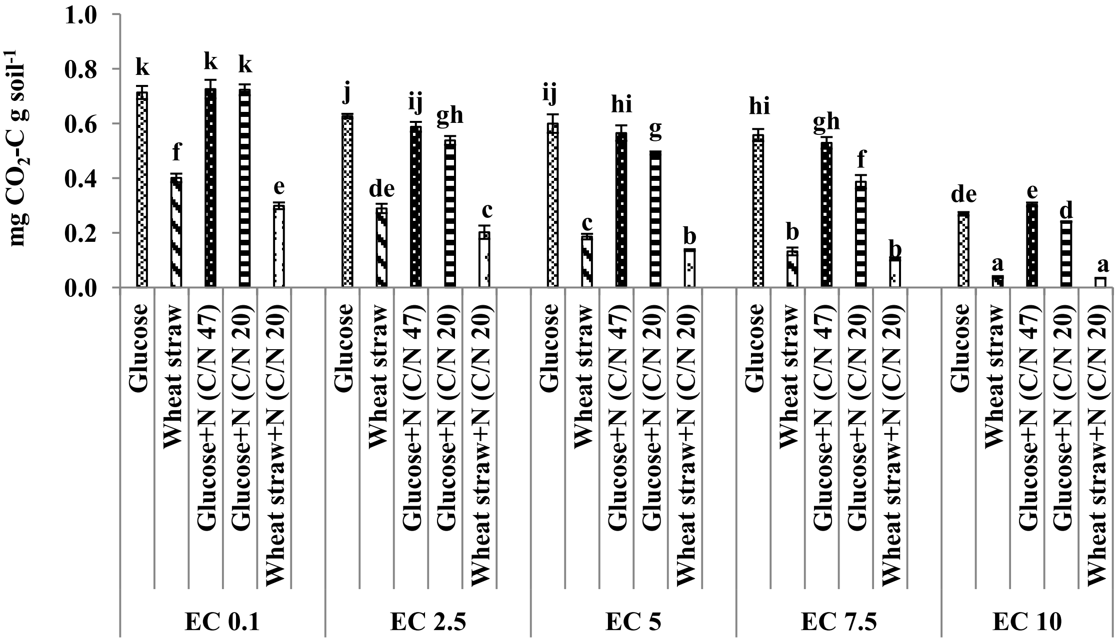

Fig. 1. Cumulative respiration ($\mathrm{mg\ CO_2\text{-}C\ g\ soil^{-1}}$) after 30 days in soil with $EC_{1:5}$ 0.1, 2.5, 5, 7.5 and 10 amended with glucose and wheat straw with and without N. Vertical lines indicate standard deviation of the mean (n=4). Bars with different letters differ significantly ($p < 0.05$).

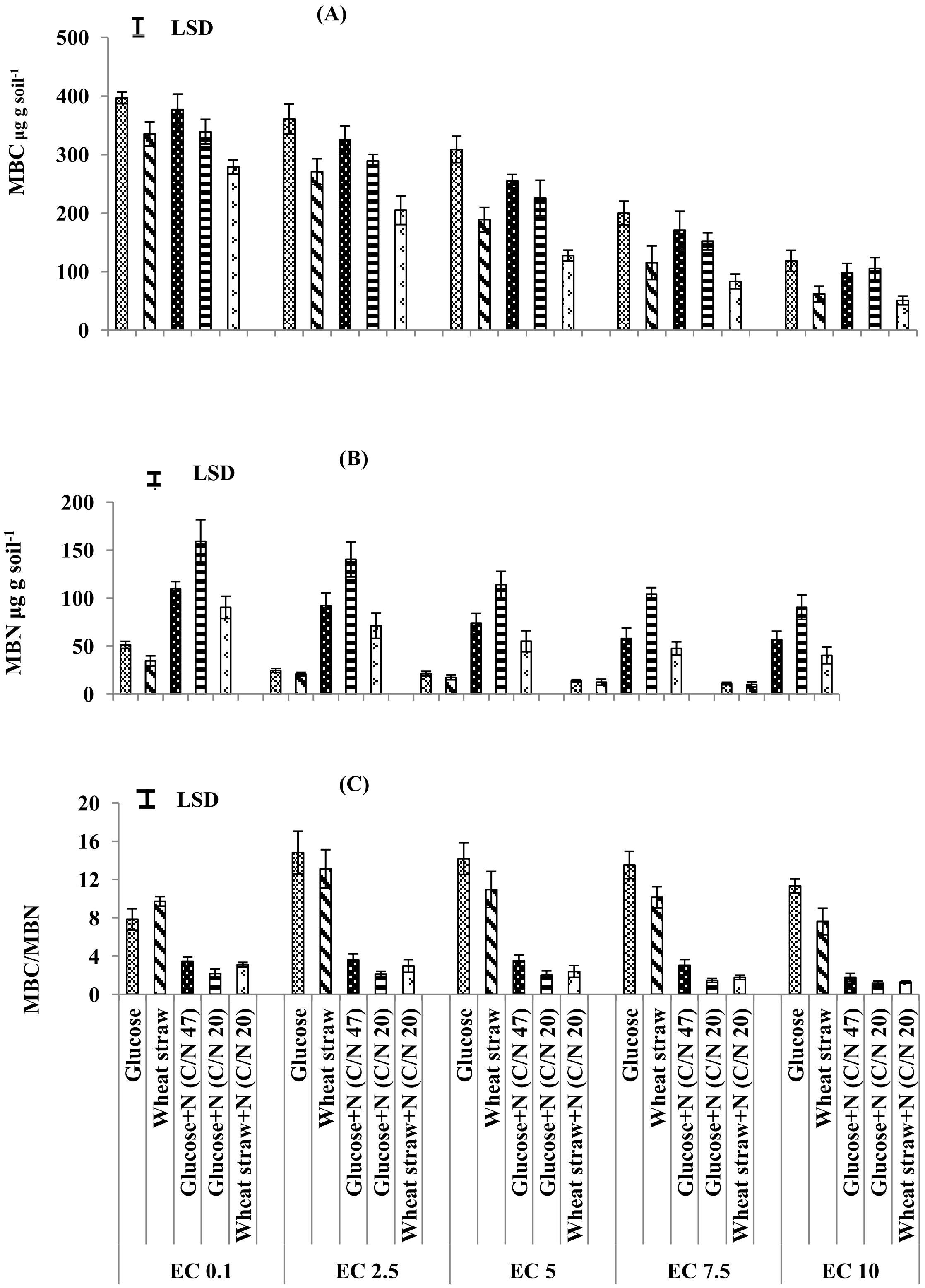

Fig. 2. MBC (A), MBN (B) and MBC/MBN (C) ratio after 30 days in soil with $EC_{1:5}$ 0.1, 2.5, 5, 7.5 and 10 and treatments glucose and wheat straw with and without N (n=4).

CHAPTER 7:

OSMOTIC POTENTIAL IS A BETTER PARAMETER THAN ELECTRICAL CONDUCTIVITY TO ASSESS SALINITY EFFECTS ON ORGANIC MATTER DYNAMICS IN SALT-AFFECTED SOILS OF DIFFERENT TEXTURE

Osmotic potential is a better parameter than electrical conductivity to assess salinity effects on organic matter dynamics in salt-affected soils of different texture

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Abstract

A few recent studies have indicated that osmotic potential of soil solution may be a more appropriate parameter than electrical conductivity (EC) for assessing the effect of salts on plant growth and soil biomass. However, this has not been examined in detail with respect to microbial activity and dissolved organic matter in soils of different texture. Four non-saline and non-sodic soils differing in texture (4, 13, 24 and 40 % clay, termed S-4, S-13, S-24 and S-40) were leached with NaCl and $CaCl_2$ solutions resulting in $EC_{1:5}$ between 0.4 and 5.0 dS m^{-1} with two levels of sodicity [sodium absorption ratio; $SAR_{1:5}$< 3 (non-sodic) and >20 (sodic)]. After adjusting the water content to levels optimal for microbial activity, which differed among the soils, we achieved three ranges of osmotic potential in all soils: > -0.55, -0.62 to -1.62 and -

2.72 to -3.0 MPa. Finely ground wheat straw residue was added (20 g kg^{-1}) as substrate to stimulate microbial activity.

At a given $EC_{1:5}$, cumulative respiration was lower in the lighter textured than the heavier-textured soils, whereas there was little difference in response to decreasing osmotic potential among soils. In all soils, cumulative respiration and osmotic potential decreased to a similar extent with a greater decrease on day 40 than on day 10. Cumulative respiration was higher at $SAR_{1:5}$ 20 than $SAR_{1:5} < 3$ only at osmotic potentials between -0.62 and -1.62 MPa on day 40. Concentrations of dissolved organic C (DOC) and N (DON) increased compared to the controls without salt addition at the lowest osmotic potential (-2.72 to -3.0 MPa) in all soils and at both sampling times. At both sampling times, $SAR_{1:5}$ 20 increased DOC and DON concentrations compared to $SAR_{1:5} < 3$ at osmotic potentials between -0.62 and -1.62 MPa in all soils except S-40. The aromaticity of the dissolved organic C decreased with decreasing osmotic potential in all soils and it was higher at $SAR_{1:5}$ 20 compared to $SAR_{1:5} < 3$. Thus, by specifically adjusting the osmotic potential to similar levels in soils of different texture, we confirmed that when comparing soils of varied texture, osmotic potential is a better parameter to evaluate the effect of salinity on microbial activity than $EC_{1:5}$. In addition, the study also showed that salt-affected soils pose an increased risk of C and N loss due to high concentration of dissolved organic matter (DOM) in leachates.

Key Words: Dissolved organic matter; Osmotic potential; Salinity; Sodicity; Soil respiration; Soil texture

1. Introduction

Salt-affected soils (comprising saline and sodic soils) contain excessive amounts of salts which are a serious land-degradation problem which affects over 6 % of the world area (FAO-AGL 2000). Plant growth and microbial activity are low in salt-affected soils due to osmotic stress, poor soil structure and imbalanced nutrient uptake (Grattan and Grieve, 1999; Mengel and Kirkby, 2001). Thus it is imperative to understand the processes involved in nutrient dynamics in salt-affected soils.

Soil organic matter (SOM) is important for maintaining structural stability in sodic soils because it prevents dispersion and disaggregation (Barzegar et al., 1997; Nelson et al., 1997). Dissolved organic matter (DOM), which is the most mobile and dynamic organic matter fraction, is frequently used as an indicator for SOM dynamics (Alvarez et al., 1998) because changes caused by environmental and management stresses are detected earlier in this fraction than in SOM as a whole. Dissolved organic carbon (DOC), which represents the main component of DOM, along with other nutrients can be lost from soil via runoff and leaching into surface water bodies and groundwater (Baldock and Skjemstad, 1999; Stevenson and Cole, 1999), which can have detrimental effects on water quality (EPA South Australia, 1998). Moreover, leaching can reduce the amount of DOC available for mineralization within the soil and reduce soil nutrient cycling and fertility (Kalbitz et al., 2000; Marschner and Kalbitz, 2003).

Salinity has been found to negatively affect the size and activity of soil microbial biomass and biochemical processes essential for maintenance of soil organic matter (Chowdhury et al., 2011; Mavi and Marschner 2012; Rietz and Haynes, 2003; Tripathi et al., 2006; Yuan et al., 2007). On the other hand, sodicity increases solubility of the organic matter and thus C mineralization and potential leachable C in the soil (Nelson et al., 1996; Sokoloff, 1938). The high solubility of organic matter in sodic soils represents a major pathway by which C and N can be lost (Peinemann et al., 2005; Wong et al., 2008; Mavi et al., 2012). Conversely, increasing salinity causes soils to flocculate, offsetting the effects caused by sodicity (Shainberg and Letey, 1984).

In soils, osmotic potential results from interaction of salts with soil water (Papendick and Campbell, 1981). Increasing concentrations of salt in the soil solution attract water molecules which restrict the availability of the soil water to plant and microorganisms. Therefore, high concentrations of dissolved salt in soil solution cause severe osmotic stress to soil microbes (Brady and Weil, 2002). Further, the concentration of salts in the soil solution also depends on the water content of soil. At a given salt content of soil, the osmotic potential of soil decreases with decreasing water content due to increased concentration of salts in the solution. This may be particularly important when comparing the effect of salinity in soils of different texture. The water retention capacity of a fine-textured soil is greater than a coarse-textured soil, therefore at a given $EC_{1:5}$, the osmotic potential of the soil solution may be lower in the coarse-textured soil. However, this factor has not been investigated in detail with respect to microbial activity and dissolved organic matter in soils varying in texture. Therefore, this study was conducted with the aims (1) to confirm whether osmotic potential is a better measure than EC for evaluating the stress to microbes in salt-affected soils of different texture; and (2) to investigate the interactive effect of osmotic potential and sodicity on microbial activity and DOM dynamics in soils.

2. Materials and Methods

2.1. Soil collection and characterization

Four non-saline and non-sodic soils differing in texture (4, 13, 24 and 40 % clay; Table 1) were collected from the A horizon (0-30 cm) at several sites near Monarto (35º04´ S and 139º07´ E) located 60 km east of Adelaide in South Australia. The area has a dry Mediterranean climate, and the average temperature is 30.1 ºC in summer and 15.9 ºC in winter with mean annual rainfall of 352 mm. Samples from each soil were thoroughly mixed, air dried, passed through a 2 mm sieve and stored air-dry at room temperature. Based on their clay content, they are denoted as S-4, S-13, S-24 and S-40.

Textures were assigned according to the The National Committee on Soil and Terrain, (2009). Soil pH and EC were measured in a 1:5 soil: water suspension after 1 h end-over-end shaking at 25 ºC. The sodium adsorption ratio (SAR) was determined in a 1:5 soil: water extract. Calcium and Mg concentrations were measured using atomic absorption spectrophotometry and Na by flame photometry (Rayment and Higginson, 1992). The $SAR_{1:5}$ was calculated by the Richards equation (Richards, 1954). The total water-soluble iron and aluminium concentration in the soils were determined by using inductively coupled plasma emission spectrometry. The water holding capacity (WHC) was measured using a sintered glass funnel connected to a 100 cm water column ($\Psi m = -10$ kPa). Soil was placed in rings in the sintered glass funnel, thoroughly wetted and allowed to drain for 48 h. The water content of the soil was determined by weighing before and after oven drying at 105 °C for 24 h. Bulk density was measured by the clod method (Blake, 1965). The osmotic potential of the soil water was estimated using the equation given by Richards (1954):

$$\mathbf{Osmotic\ potential}\ (\mathbf{MPa}) = -\mathbf{0.036} \times \mathbf{EC}\ (\mathbf{dS\ m^{-1}}) \times \left[\frac{\mathbf{O_{act}}}{\mathbf{O_{ref}}}\right]$$

where O_{act} is actual water content (g g^{-1}) of the soil and O_{ref} is the reference water content (g g^{-1}) of the 1:5 soil-water mixture.

Particle size was analysed by the hydrometer method (Bouyoucos, 1936) and organic carbon content was analysed by dichromate oxidation (Walkley and Black, 1934). Total C and N were measured on a CNS Leco-2000 analyser. The concentration of DOC and DON in the filtered (0.45 µm) supernatants was measured as non-purgeable organic C and N in a Formacs TOC/TN analyser after acidification of the sample and purging of the inorganic C. The filtered samples were stored in a refrigerator at 4 ºC before analysis. The aromaticity of the DOC in the soil-water suspension was estimated by determining the specific ultra-violet absorbance (SUVA in l mg DOC^{-1} m^{-1}) in the 0.45 µm filtered soil-water suspension at 254 nm (Chin et al., 1997) in a UV spectrophotometer using a quartz cuvette.

2.2. Soil preparation

For this experiment, different textured soils (S-4, S-13, S-24 and S-40) were each brought to three different ranges of osmotic potential in combination with two SAR levels. Eight salt solutions with different EC and SAR were prepared using combinations of NaCl and CaCl2 stock solutions. The solutions had EC's of 5, 20, 40 and 60 dS m^{-1} and SAR values of: < 3 and 20. The soils (approximately 300 g) were placed on a funnel with a filter paper and leached 3-4 times with these solutions. At each leaching event, about 60-80 ml of the solution was added after which soils were dried at 25-30 ºC in a fan-forced oven for 72-96 h, mixed thoroughly after drying to break the clods and then analysed for EC and SAR. This process was repeated 3-4 times until the desired combinations of $EC_{1:5}$ levels and $SAR_{1:5} < 3$ or 20 were achieved (Table 2). After adjusting the water content (see below) according to the soil texture, these $EC_{1:5}$ levels correspond to osmotic potentials (MPa) ranging from -0.06 to -3.0 MPa (Table 2). After adjustment of EC and SAR, the soils were kept dry at room temperature until the onset of the experiment.

2.3 Incubation

Air dry soils were wet to 85 % WHC for S-4 (0.068 g g soil^{-1}), 75 % for S-13 (0.123 g g soil^{-1}), 60 % for S-24 (0.156 g g soil^{-1}) and 50 % for S-40 (0.165 g g soil^{-1}). Their water contents resulted in maximal respiration in the respective soils as determined in a preliminary experiment (data not shown). The soils were pre-incubated for 10 days at 22-25 ºC before the start of the experiment. Ten days were chosen on the basis of earlier experiments (Mavi and Marschner, 2012; Mavi et al., 2012), which showed that microbial activity stabilized within 7-10 days after rewetting air-dry non-saline soil. Throughout the pre-incubation and the subsequent measurement period, deionised water was added to maintain the target water content. At this water content, there were three ranges of osmotic potential: > -0.55, -0.62 to -1.62 and -2.72 to -3.0 MPa.

Mature wheat straw (C:N ratio 47:1, as a nutrient source for soil microbes), was ground, sieved to 0.25-2 mm, then added at 20 g kg^{-1} soil and thoroughly mixed into the soils. The pre-incubated soil with residue (20 g) was added to cores (PVC, radius 1.85 cm, height 5 cm) with a nylon mesh base (0.75 mm, Australian Filter Specialist) and packed to the bulk density of the soil in the field. The cores were placed individually into 1 L glass jars and the jars sealed with gas tight lids equipped with septa. The jars were incubated in the dark at 22-25 ºC and CO_2 release was measured over 40 days. Respiration was quantified by measuring headspace CO_2 concentrations at regular intervals using a Servomex 1450 infra-red gas analyser (Servomex Group, UK): daily in the first 10 days, every 3-4 days thereafter. After each measurement, the jars were opened to equilibrate the CO_2 to ambient concentrations and then resealed. The CO_2 evolved from each sample was calculated as the difference between the initial (immediately after resealing of the jars) concentration and that at the end of the measuring interval. Separate sets of samples were destructively harvested after 10 days and at the end of the experiment after 40 days and analysed for DOC, DON, EC, SAR and SUVA.

2.4. Statistical analysis

The experiment consisted of 4 soils, 4 osmotic potential (OP) and 2 SAR treatments with 4 replicates. The data were analysed using Genstat 10 (GenStat® for Windows10.0, VSN Int. Ltd, UK, 2005). A three-way (soil × OP × SAR) analysis of variance (ANOVA) was carried out and Tukey post-hoc tests were used to determine significant differences ($P<0.05$) between the measured parameters among different soils at a given OP and SAR combination.

3. Results

Cumulative respiration decreased with increasing $EC_{1:5}$ in all soils (Fig 1a and b). In general, cumulative respiration was higher in soils S-4 and S-13 compared to soils S-24 and S-40. At the same $EC_{1:5}$, cumulative respiration compared to the soil without salt addition was decreased more in the lighter textured soils (S-4 and S-13) compared to the heavier textured soils (S-24 and S-40). Therefore, a similar decrease in cumulative respiration was reached at higher EC in the heavier textured soils compared to soils S-4 and S-13. For example, compared to the control, cumulative respiration was decreased by 20 % at $EC_{1:5}$ 1.3 in soil S-4 but at $EC_{1:5}$ 5 in soil S-40.

Cumulative respiration decreased with decreasing osmotic potential in all soils with a greater decrease on day 40 than on day 10 (Fig 1a and b). Particularly on day 40, the decrease in cumulative respiration with decreasing osmotic potential was greater in the lighter textured soils, but the differences among soils were small compared to those when cumulative respiration was plotted against EC. Sodicity affected cumulative respiration only on day 40 where it was greater at $SAR_{1:5}$ 20 than at $SAR_{1:5} < 3$ at osmotic potentials between -0.62 and -1.62 MPa whereas $SAR_{1:5}$ had no effect at the lowest osmotic potential.

In all soils and at both sampling times, the DOC concentrations were increased compared to the controls without salt addition at the lowest osmotic potential (-2.72 to -3.0 MPa) (Fig 2). However, the DOC concentration was differentially affected at osmotic potential between -0.62 and -1.62 MPa at both sampling times where, compared to the controls, the DOC concentration was lower in S-4 and S-13, but higher in S-24 and S-40. At both sampling times, the DOC concentrations were higher with $SAR_{1:5}$ 20 compared to $SAR_{1:5} < 3$ at osmotic potentials between -0.62 and -1.62 MPa in all soils except S-40. In soil S-40, $SAR_{1:5}$ 20 significantly increased DOC only at osmotic potential -0.62 MPa on day 40.

Similarly to the DOC concentrations, the DON concentrations were increased compared to the controls without salt addition at the lowest osmotic potential (-2.72 to -3.0 MPa) (Fig. 3). At osmotic potential between -0.62 and -1.62 MPa at both sampling times, the DON concentration was lower compared to the controls in S-4 and S-13, but higher in S-24 and S-40. Compared to $SAR_{1:5} < 3$, $SAR_{1:5}$ 20 significantly increased DON concentrations at osmotic potential between -0.62 to -1.62 MPa at both sampling times in S-4, S-13 and S-24 but not in S-40.

At both sampling times, SUVA decreased with decreasing osmotic potential, more so in soils S-24 and S-40 than in the two lighter textured soils (Fig 4). SUVA increased from day 10 to day 40. Only at osmotic potentials between -0.55 and -1.62 MPa, SUVA was higher at $SAR_{1:5}$ 20 compared to $SAR_{1:5} < 3$.

4. Discussion

4.1. Microbial response to osmotic potential and EC

Recent studies have suggested (Ben-Gal et al., 2009; Chowdhury et al., 2011; Mavi et al., 2012), that osmotic potential may be a more appropriate measure of salt stress for plants and soil microbes than the $EC_{1:5}$ because it is a function of the salt concentration in the soil solution and it takes differences in water content among soils into account. The EC1:5 on the other hand is measured in a defined soil: water ratio which is similar for all soils irrespective of their water holding capacity. This may lead to underestimation of the salt stress to microbes particularly in soils with low water holding capacity. The ECe which is measured in the saturated soil extract is a better measure of salt stress than the $EC_{1:5}$, but may also not adequately reflect the salt stress to microbes in soils when soils of diverse texture are at different percentages of their water-holding capacity. Therefore, this study was specifically designed to have similar osmotic potential ranges in soils of varied texture which is a more refined approach than in previous studies where the EC was adjusted and only afterwards were differences in osmotic potential used to explain the differences among soils. By adjusting the osmotic potential to similar levels in the present study, it was possible to observe some general effects of salt stress on microbial activity although some differences remained between light and heavier textured soils.

4.2. Osmotic potential effect

Compared to the controls without salt addition, the parameters measured in this study were affected at the lowest osmotic potentials (about -3 MPa) in all soils and this effect was stronger on day 40 than on day 10. At the lowest osmotic potential compared to the control, cumulative respiration and SUVA were lower whereas DOC and DON concentrations were higher. Reduced microbial activity at low osmotic potential, that is high salt concentration in the soil solution, has been shown previously (Chowdhury et al., 2011; Mamilov et al., 2004; Mavi et al., 2012; Pankhurst et al., 2001; Setia et al., 2011; Wichern et al., 2006) and can be explained by death of salt-sensitive microbes and the greater energy demand for salt tolerance mechanisms such as the synthesis of osmolytes (Oren, 1999). The higher DOC and DON concentrations can be explained by the reduced decomposition rates which resulted in more C remaining in solution compared to the controls which is in agreement with Mavi et al. (2012). The low SUVA is a consequence of the high DOC concentrations because of the dilution effect.

4.3. Sodicity effect

In the present study, compared to $SAR_{1:5} < 3$, high $SAR_{1:5}$ increased cumulative respiration on day 40 and DOC/DON and SUVA at both sampling times. Sodicity results in dispersion of clay and organic matter because the high proportion of the monovalent sodium ions on the surfaces of the soil particles prevents binding of clay particles and organic matter (Bäckström et al., 2004; Norrström and Bergstedt, 2001). However, in all soils, high sodicity ($SAR_{1:5}$ 20) affected the measured parameters only at medium osmotic potential (-0.62 to -1.62 MPa). The lack of effect of sodicity at lower osmotic potential in the present study can be explained by the flocculation of soil at high electrolyte concentration which decreases the thickness of the diffusive cloud around soil particles increasing the attractive forces (Rengasamy and Sumner, 1998).

Further, the effect of sodicity on cumulative respiration became apparent only on day 40, which can be explained by the high availability of soluble carbon in the first days after residue addition. Even if a greater proportion of this soluble C was adsorbed at $SAR_{1:5} < 3$ compared to $SAR_{1:5}$ 20, the remaining C concentration in the soil solution was sufficient for high respiration rates. Moreover, it may take some time for organic compounds to bind to clays and other soil particles. Between day 10 and day 40, soluble C from the residues would have been decomposed and organic compounds bound to soil particles at low $SAR_{1:5}$ whereas they remained in solution at $SAR_{1:5}$ 20.

Despite the higher cumulative respiration rates at $SAR_{1:5}$ 20 compared to $SAR_{1:5} < 3$, DOC and DON concentrations were higher at $SAR_{1:5}$ 20 suggesting that the release of organic compounds and lack of binding of added C was greater than what the soil microbes were able to decompose. Therefore, in this case, the ability of the microbes to decompose the available C seems to have been compromised by the salt concentration in the soil solution (Rietz and Haynes, 2003; Wichern et al., 2006). Although sodicity increased DOC concentrations, it also increased SUVA. Thus the proportion of aromatic C in the DOC was increased by sodicity. There are at least two explanations of this observation: (i) soil microbes may have utilised aliphatic C preferentially, and (ii) aliphatic C may be preferentially bound to the few remaining binding sites on the soil particles.

4.4. Effect of soil texture

Even when adjusted to the same osmotic potential, some differences remained between light and heavier textured soils especially at the medium osmotic potential range (-1.25 to -1.62 MPa). Compared to the controls without salt addition, DOC and DON concentrations were lower in the light textured soils at this medium osmotic potential whereas they were higher in the heavier textured soils. This could be due to the lower soil organic matter content and the higher Fe and Al concentration and respiration rates of the light textured soils. There may have been more un-occupied potential organic matter binding sites in the lighter textured soils due to their inherent lower soil organic matter content (Hassink, 1997) and higher Fe and Al concentration (Kaiser and Zech, 2000). Further, the lighter textured soils had overall greater cumulative respiration, thus more soluble C was respired compared to the heavier textured soils.

5. Conclusions

This study confirmed that salt stress has similar effects on soil microbes in soils of different texture and water content when expressed as osmotic potential whereas the soil microbes appear to be more salt sensitive in lighter textured soils when $EC_{1:5}$ is used as measure of salinity. Therefore, the osmotic potential also needs to be considered when the water content of a saline soil fluctuates. The results also showed that both salinity and sodicity can result in increased DOC and DON concentrations in leachates which may adversely affect the C sequestration potential of salt-affected soils.

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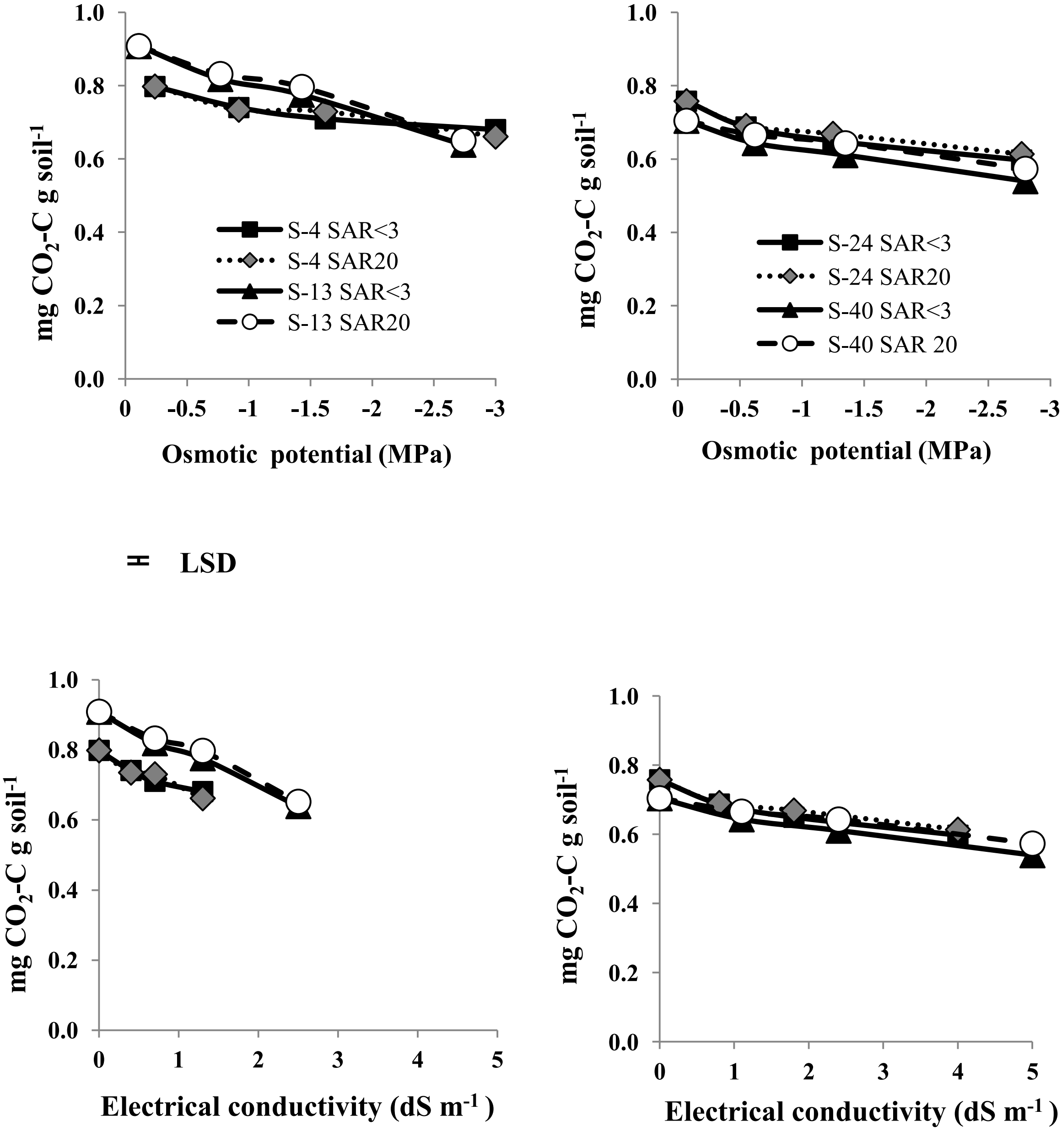


Fig. 1a. Cumulative respiration after 10 days in relation to osmotic potential and electrical conductivity ($EC_{1:5}$) in combination with $SAR_{1:5} < 3$ and 20 in soil S-4, S-13, S-24 and S-40. Bar indicates least significant difference (LSD) of the mean (n=4)

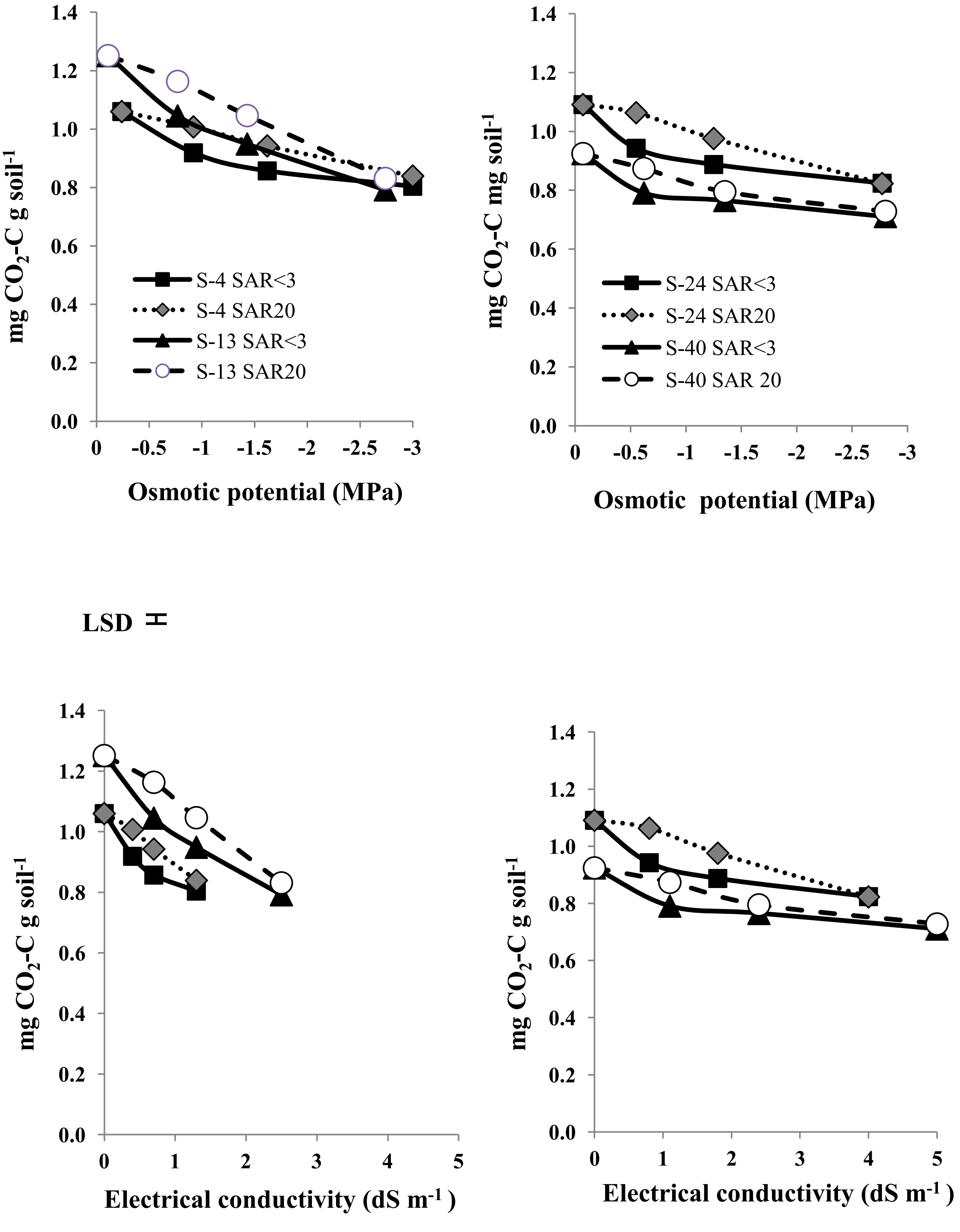


Fig. 1b. Cumulative respiration after 40 days in relation to osmotic potential and electrical conductivity ($EC_{1:5}$) in combination with $SAR_{1:5}$< 3 and 20 in soil S-4, S-13, S-24 and S-40. Bar indicates least significant difference (LSD) of the mean (n=4)

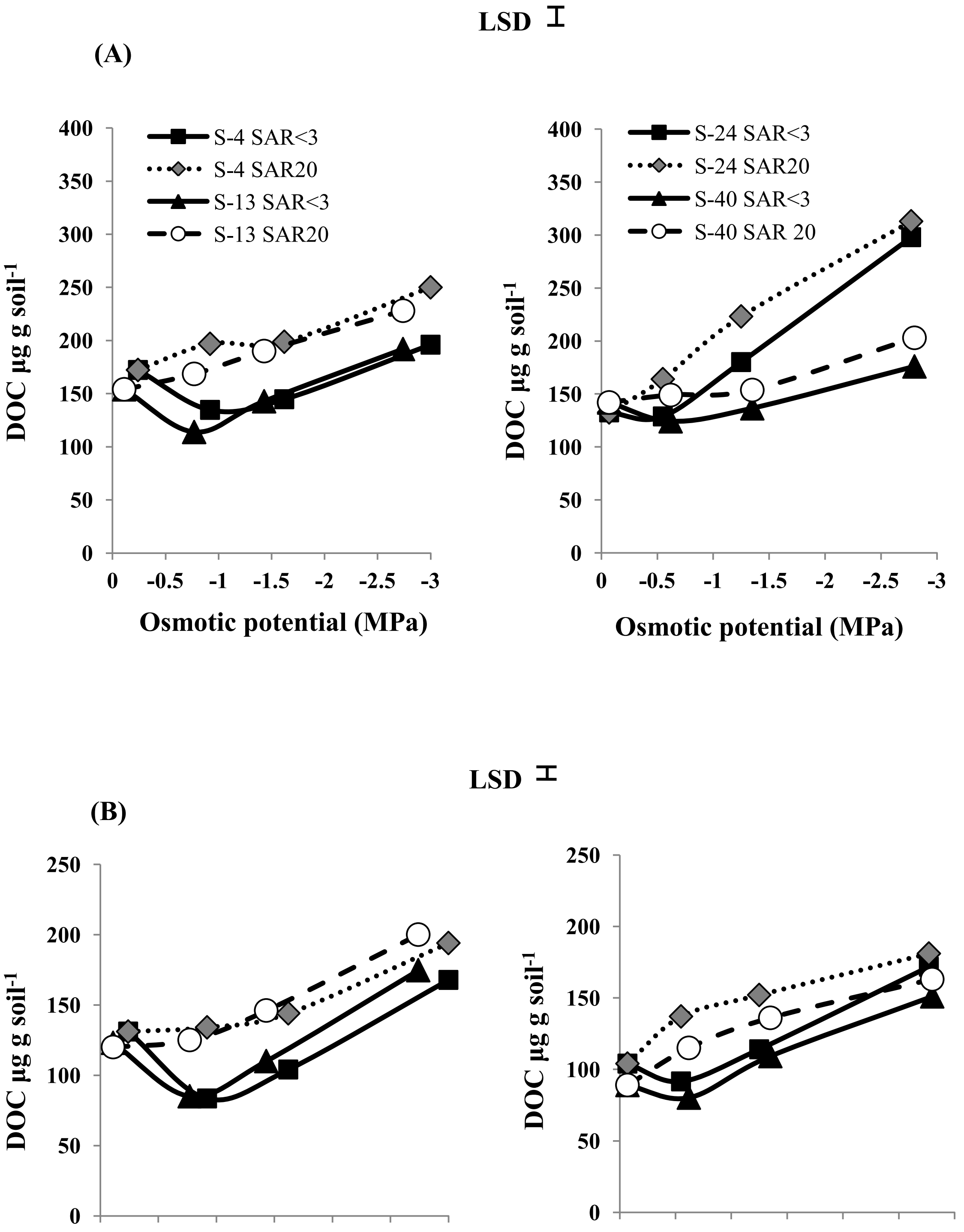
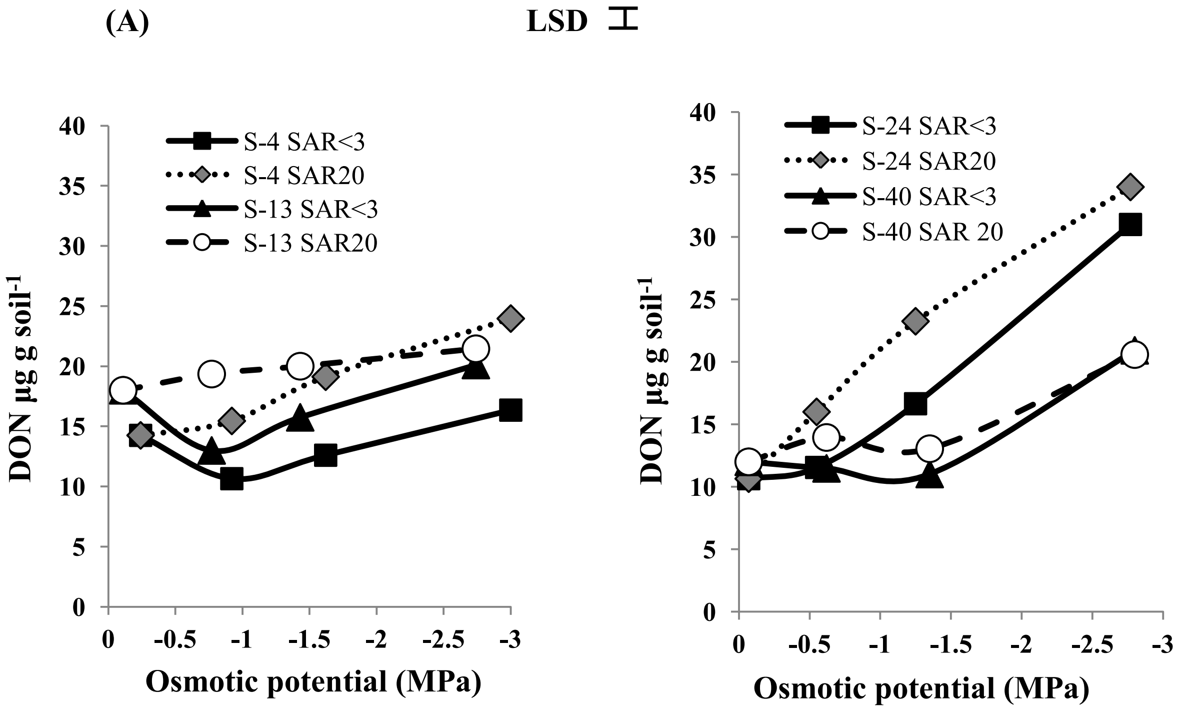


Fig. 2. Dissolved organic carbon (DOC) after (a) 10 and (b) 40 days in relation to osmotic potential in combination with $SAR_{1:5} < 3$ and 20 in soils S-4, S-13, S-24 and S-40. Bars indicate least significant difference (LSD) of the mean (n=4)



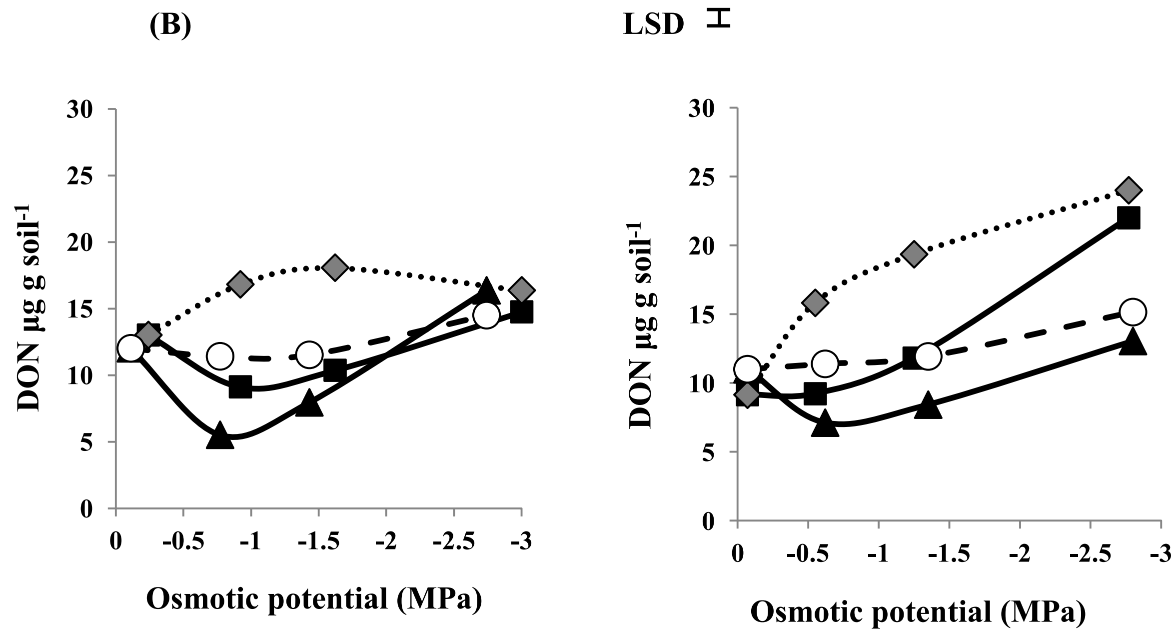


Fig. 3. Dissolved organic nitrogen (DON) after (a) 10 and (b) 40 days in relation to osmotic potential in combination with $SAR_{1:5}$< 3 and 20 in soil S-4, S-13, S-24 and S-40. Bars indicate least significant difference (LSD) of the mean (n=4)

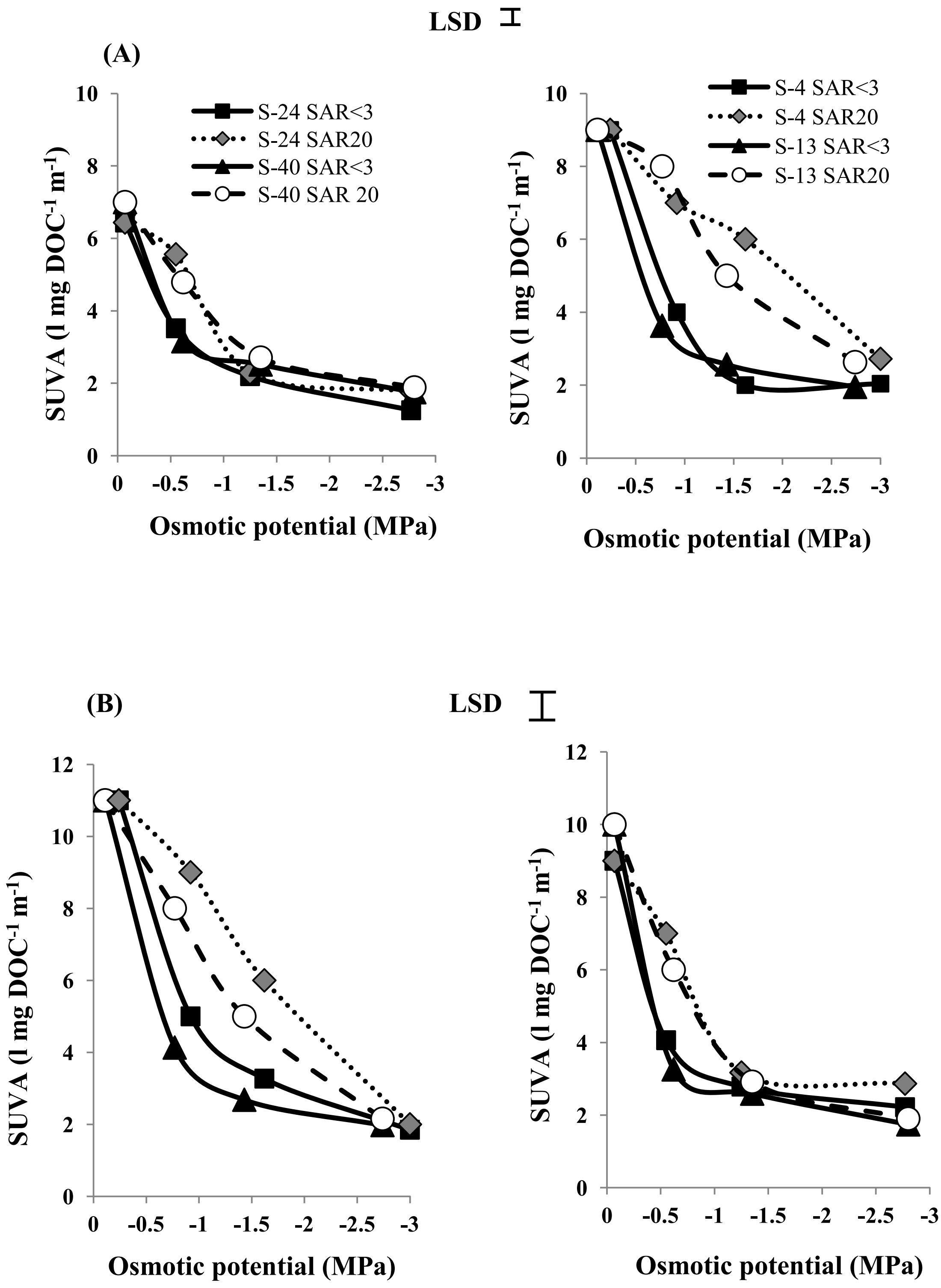


Fig. 4. Specific UV absorbance (SUVA) after (a) 10 and (b) 40 days in relation to osmotic potential in combination with $SAR_{1:5}$< 3 and 20 in soil S-4, S-13, S-24 and S-40. Bars indicate least significant difference (LSD) of the mean (n=4)

Table 1. Physical and chemical properties of the soils

Soil property	Unit	Sand S-4	Loamy sand S-13	Sandy clay loam S-24	Sandy clay S-40
Sand	%	95.0	85.0	67.7	52.5
Silt	%	1.3	2.5	8.3	7.5
Clay	%	3.7	12.5	24.0	40.0
$EC_{(1:5)}$	dS m^{-1}	0.1	0.1	0.1	0.1
$SAR_{(1:5)}$		1.3	1.3	1.8	2.5
$pH_{(1:5)}$		7.5	8.2	8.3	8.5
Bulk density	g cm^{-3}	1.8	1.6	1.4	1.3
Water holding capacity	g g $soil^{-1}$	0.1	0.2	0.3	0.3
Cation exchange capacity	cmolc(+) kg^{-1}	5.2	14.7	37.0	28.3
Fe	mg kg^{-1}	21.4	21.2	0.8	0.5
Al	mg kg^{-1}	33.6	44.3	0.5	0.9
Total N	%	0.1	0.2	0.2	0.2
Total C	%	0.6	1.8	2.5	2.4

Table 2. Adjusted osmotic potential (OP) corresponding to different $EC_{1:5}$ levels in soils S-4, S-13, S-24 and S-40

Electrical conductivity ($EC_{1:5}$) dS m^{-1}	Osmotic potential (OP) MPa
S-4	
Control	-0.24
EC 0.4	-0.92
EC 0.7	-1.62
EC 1.3	-3.00
S-13	
Control	-0.07
EC 0.7	-0.77
EC 1.3	-1.43
EC 2.5	-2.74
S-24	
Control	-0.06
EC 0.8	-0.55
EC 1.8	-1.25
EC 4	-2.77
S-40	
Control	-0.11
EC 1.1	-0.62
EC 2.4	-1.35
EC 5	-2.80

CHAPTER 8:

CONCLUSIONS AND FUTURE RESEARCH

Conclusions and future research

Salt-affected soils can be found on 831 million hectares world-wide, predominantly in arid and semi-arid regions where rainfall is insufficient to leach salts from the root zone. Therefore, one of the greatest soil management challenges is ameliorating areas degraded by excessive salts for enhancing crop production to feed the growing population with minimal risks to the environment. Addition of organic matter is a viable option for maintaining stability in salt-affected soils because it prevents dispersion, disaggregation and enhances microbial captivity. Previous studies have demonstrated the individual effects of salinity and sodicity on soil biological processes and organic matter dynamics (Laura, 1976; Nelson et al., 1996; Pathak and Rao, 1998; Rietz and Haynes, 2003; Setia et al., 2011; Tripathi et al., 2006; Wong et al., 2008) but often with contradictory results. Salinity has been found to negatively influence the activity of soil microbes (Chowdhury et al., 2011; Rietz and Haynes, 2003) whereas some studies have reported increased rates of carbon and nitrogen mineralization with increasing salinity (Chandra et al., 2002; Laura, 1976; Wong et al., 2008). Nelson et al. (1996) found that sodicity increased C mineralization because it makes the SOM more accessible to microorganisms. On the other hand, Pathak and Rao (1998) found that sodicity had no effect on C mineralization. Therefore, the lack of consistency in the results of these studies is most likely due to the different soil properties and levels of salinity and sodicity used in the various experiments (Muhammad et al., 2008). However, the interactive effect of defined levels of salinity and sodicity on soil microbial activity and organic matter decomposition has not been investigated in detail in different soils. Therefore, the experiments presented in the thesis examined the interactive effect of salinity and sodicity on the dynamics of microbial activity and biomass and dissolved organic matter (DOM) in soils differing in texture.

The results presented in Chapters 3 and 7 showed that salinity had an adverse effect on microbial activity (respiration) due to the decrease in osmotic potential which led to increased concentration of dissolved organic C (DOC) and N (DON). This indicates a reduced ability of the stressed microbes to utilize readily available labile organic matter from the added residues. On the other hand, SAR had little effect on respiration at any EC level in sand and sandy clay loam soil. However, sodicity in combination with low EC increased DOC and DON concentrations suggesting an increased risk of loss of C and N through leaching in sodic soils. In addition, the results also indicated that soil texture and water content play an important role in determining the response of microbes to salt due to their effect on the salt concentration in soil solution. Therefore, osmotic potential (a measure of the salt concentration in soil solution) may be a better measure for evaluating the stress faced by microbes in salt-affected soils than EC measured in a 1:5 soil: water ratio.

The experiment in Chapter 4 was carried out to assess the impact of multiple drying and wetting in saline and saline-sodic soils. The results showed that drying and rewetting cycles had little effect on the impact of sodicity at low EC; irrespective of the water treatment sodicity increased organic matter solubility and substrate availability. The results also showed that salinity reduces the ability of microbes to utilise the substrates released by rewetting compared to microbes in non-saline soils. The lower flush in respiration after rewetting in saline and saline-sodic soils than in soil without added salt indicated reduced loss of CO_2 from these soils but loss of C via DOC leaching may be increased in sodic soils. On the other hand, the lower respiration rate per unit microbial biomass C (MBC) at high salinity suggested more effective substrate utilization. Thus, both salinity and sodicity altered the effect of drying and rewetting on soil carbon dynamics.

The retention and mobility of DOC in soils is controlled primarily by its sorption to mineral surfaces (McDowell and Likens, 1988; Ussiri and Johnson, 2004). The experiment described in Chapter 5 on sorption of DOC indicated that saline-sodic soils sorb more DOC than sodic soils and high EC favours the binding of aromatic carbon particularly at low concentration of added DOC. The results also showed that DOC sorption in salt-affected soil is more strongly controlled by CEC and Fe/Al concentration than by clay concentration per se except in sodic soils where DOM sorption was low due to the high sodium saturation of the exchange complex. These findings suggest that increasing the electrolyte concentration in sodic soils by liming or irrigation with saline water may reduce nutrient loss via leaching and increase organic matter sequestration.

The experiment on the effect of different forms of C (wheat straw and glucose) and inorganic N on microbial activity and biomass (Chapter 6) showed that, compared to glucose, wheat straw was decomposed more slowly and the decomposer community was more sensitive to salinity than glucose-utilising microbes. Thus, high concentrations of easily available C can increase the ability of microbes to cope with salt stress. Further, the study also suggested that, at least in soils with a microbial C/N ratio of around 20, addition of N will have no or a negative effect on microbial activity and growth irrespective of EC but may increase microbial N immobilisation and DON concentrations.

In the last experiment described in Chapter 7 confirmed that salt stress has similar effects on soil microbes in soils of different texture and water content when expressed as osmotic potential whereas soil microbes appear to be more sensitive to salinity in lighter textured soils when $EC_{1:5}$ is used as measure of salinity. Therefore, osmotic potential needs to be considered when comparing saline soils with different water holding capacity.

Thus the experiments of the study showed that both salinity and sodicity can result in increased C and N concentrations in leachates, the former through reduced decomposition and the latter due to increased organic matter solubility; and may lead to further soil degradation and reduce C sequestration. The study also emphasised that soil texture and water content play an important role in determining the response of microbes to salt stress and addition of easily decomposable source of energy can improve the ability of microbes to tolerate salinity.

Limitation of the research

Although the results of the study will increase our understanding of the interactive effect of salinity and sodicity on microbial activity and organic matter dynamics in salt-affected soils, a limitation of the current study was that, as in several previous studies (Chowdhury et al., 2011; Nelson et al., 1996; Pathak and Rao, 1998; Wong et al., 2008), the salt was added to previously non-saline non-sodic soils to achieve different level of salinity and sodicity in all experiments. This was done to have defined EC and SAR combinations without differences in other soil properties such as texture and native organic matter content as would have been the case in field-collected salt-affected soils. The rapid increase in EC and SAR in soils with added salts may not have allowed the microbial community to adjust to the new conditions leading to an overestimation of the salt effect (Khan et al., 2008; Setia et al., 2011; Wong et al., 2008). Another limitation was that substrate in the form of residues or glucose was added to increase microbial activity which would have been very low in the unamended soil. This allowed on the one hand to clearly detect the impact of salinity and sodicity on microbial activity and DOC, but may also have overestimated their effect because the microbes stimulated by the freshly added substrates may be more sensitive to salinity and sodicity than the less active microbes decomposing the native organic matter. However, even in the field, substrates are added continuously through litter fall, root exudates and dead roots. Thus the addition of residues or glucose does to some extent represent the situation in the field.

Suggestions for future research

The present study showed that increasing salinity and sodicity can enhance dissolved C and N losses from salt-affected soils. However, further research is needed to answer some of the questions which may have important implications for organic matter dynamics in these degraded environments:

- In the studies presented in this thesis, DOC was obtained by shaking the soil in water. However, as DOC moves through the soil profile it may be subject to decomposition, mineralisation or sorption. To investigate the role of these processes, soil cores could be leached with DOM solutions or *in situ* studies of leaching and subsurface nutrient flow from salt-affected soils amended with organic residues and inorganic fertilizers could be undertaken after rainfall events to ascertain dissolved C, N and P leaching in the degraded soils.

  Additional treatments could include residues and manures varying in C/N ratio to understand the fate and nature of the DOM released from different organics. To follow the fate of the C and N from the residues, 13C and 15N labelled residues could be used.

- One important limitation of DOM analysis is attributed to its high complexity, low concentration and high polarity (Schmidt et al., 2009). Improved analytical methods to characterize specific compounds or functional groups in DOM and reliable predictability of the fluxes of DOM are now available, such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS which is capable of resolving complex molecular mixtures and provides information about the exact elemental composition of individual compounds) or nuclear magnetic resonance (NMR) can be used to understand the complexities involving DOM dynamics in soils.
- In the experiments described here, only two contrasting levels of sodicity were used (non-sodic and sodic). It would be important to investigate the impact of sodicity on microbial activity and DOM in greater detail by either imposing a greater range of SARs or by collecting various sodic soils from the field.
- Particularly salinity varies seasonally, being higher in summer when the saline water rises to the soil surface and lower in winter when rain leaches the salts from the top soil. Thus for a more accurate assessment of the effect of salt-affected soil on CO_2 emissions and DOM leaching it would be useful to measure these parameters in the field over the course of the year accompanied by controlled environment studies in which the water content and the salt concentration of the soil fluctuates in a similar manner as in the field.

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