# Soil microbial activity and community structure as affected by osmotic and matric potential.

A thesis submitted in fulfilment of the degree of Doctor of Philosophy,
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Dedicated to my father, Dr. A. B. M. Habibur Rahman Chowdhury

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## Abstract

Salinization of soils is a serious land degradation problem, causing poor plant growth and low microbial activity due to osmotic stress, ion toxicity and imbalanced element uptake. In arid, semi arid or seasonally arid (Mediterranean) regions, low or fluctuating matric potential causes further stress to soil microorganisms in saline soil by decreasing the osmotic potential as salts in the soil solution become more concentrated, as well as by reducing diffusion and thus substrate availability. Soil properties such as soil texture, water retention characteristics and organic matter content also influence soil microbial activity and community structure and the effect of salinity and matric potential on soil microorganisms. While the effects of low matric and low osmotic potential on soil microorganisms have been studied separately, little is known about their interaction. The objective of this thesis was to determine the interaction between soil matric and osmotic potential on soil microbial activity and community structure.

Most experiments described in this thesis were carried out with two non-saline soils (sand and sandy loam) differing in nutrient status, microbial biomass and community composition. Osmotic stress was induced by application of different rates of NaCl. In all experiments, pea residues were added to increase substrate availability and thus microbial activity. Respiration was measured throughout the experimental period (usually 14 days); microbial community structure was measured by phospholipid fatty acid (PLFA) analysis and PLFA patterns were compared by multivariate analysis.

The soils were air-dried after collection and an experiment was carried out to determine how quickly microbial activity stabilises after rewetting. Respiration rates in three non-saline and four saline soils stabilised seven to ten days after rewetting of the air dry soil. Therefore the soils used in this study were pre-incubated for 10 days before the experiments were started.

To investigate the effect of adaptation to matric and osmotic stress, the sandy loam was incubated for 14 days at different matric or osmotic potential (adaptation) or at optimal water content (no adaptation). Then matric and osmotic potential were adjusted in the treatments with no adaptation, whereas the potentials were maintained in the adapted

treatments. Cumulative respiration after 14 days decreased with decreasing osmotic or matric potential with no differences between adapted and non-adapted treatments indicating that prior exposure to low matric and osmotic stress does not increase tolerance compared to a sudden decrease in osmotic and matric potential.

The study in which the effect of matric and osmotic stress was compared, both soils showed a greater decrease in cumulative respiration at a given water potential (osmotic + matric) due to matric stress compared to osmotic stress. In the sand, a large proportion of the decrease in cumulative respiration at a given water potential may be due to concomitant low osmotic potential, whereas in the sandy loam the contribution of osmotic potential was small. Decreasing osmotic and matric potential had little effect on microbial biomass (sum of PLFAs), but changed microbial community structure. Compared to bacteria, fungi were less tolerant to decreasing osmotic potential, but more tolerant to decreasing matric potential.

The study on the combined effect of matric and osmotic potential showed that cumulative respiration at a given soil water content decreased with decreasing osmotic potential, but the effect of decreasing water content differed between the two soils, respiration in the sand being more affected. Cumulative respiration decreased with decreasing water potential but was poorly related to EC or water content alone. In both soils, the microbial biomass (sum of PLFAs) was affected by the interaction of EC and water content, with the EC having the greater effect.

To investigate the recovery of microbial activity after rewetting of soil, the two soils were incubated for 14 days at different water content and then adjusted to optimal water content and respiration measured for 65 days. Rewetting of the soils caused a flush in respiration rate, with the flush being greater the lower the water content before rewetting. Cumulative respiration of previously dried soils increased at a greater rate compared to the constant moist treatment, indicating recovery. But even after 50 days, cumulative respiration remained lower in the previously dry soils.

To investigate the effect of drying and rewetting (DRW) in saline soil, the salinised sandy loam was exposed to 1-3 DRW cycles each consisting of 1 week drying and 1 week moist incubation. The size flush in respiration decreased with increasing number of DRW

cycles and was negatively related to the EC of the soil. Microbial community structure was affected by DRW and salinity.

To investigate the effect of the length of the dry period on the size of the flush in respiration after rewetting, a non-saline and four saline sandy loam soils from the field differing in EC were maintained dry for 1-5 days, maintained at the achieved water content for 4 days and then rewet. Rewetting induced a flush in respiration only if the WP of the soils was previously decreased at least 3-fold compared to the constantly moist soil.

The study showed that in order to understand microbial biomass and activity in saline soils, both osmotic and matric potential must be considered, particularly at low water contents when the salt concentration in the soil solution increases. Hence, the EC is a poor indicator of the stress microbes are exposed to in saline environments because, as the water content changes, microbes will be subjected to different osmotic and matric potentials even though the measured EC changes little. Low matric potential may be more detrimental than a corresponding low osmotic potential at optimal soil water content because of the reduced diffusion of substrates to the microbes at low matric potential. Thus, they may be unable to synthesise osmoregulatory compounds to maintain cell water content.

Furthermore, microorganisms previously exposed to low potential (either matric or osmotic) do not appear to be more tolerant to low potential than those from optimal conditions. This suggests that the high metabolic burden for synthesis of osmoregulatory compounds does not allow microbes to tolerate further decreases in potential particularly when diffusion of substrates is limited by low water content.

# Declaration

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# Chapter 1

## Introduction and Review of Literature

# 1 Introduction and Review of Literature

## 1.1 Introduction

Saline soils are characterised by high concentrations of soluble salts in soil solution and have been identified as one of the most important environmental problems world-wide. Saline soils can occur naturally, but can also be created and expanded by human activities. Globally there are around 1000 million hectares of saline soil (FAO, 2008) and about 100 million ha (5%) of the total arable land area has been damaged by high salt concentrations (Lambers, 2003). Of the irrigated land area, twenty million hectares (about 20% of the total) are salt affected (Ghassemi et al., 1995). The extent of saline areas is increasing due to mismanagement and exploitation of agricultural land to meet the demands of increasing population.

In Australia, saline soils cover more than 17 million hectares (Rengasamy, 2006). Many Australian soils are naturally saline due to the weathering of the regolith and deposition processes in combination with the arid climate. A large proportion of the saline soils in Australia are due to rising saline water tables causing accumulation of salts in the upper soil layers (Rengasamy, 2006). The hydrology of the landscape has been altered by the increased groundwater intrusion into the root zone that has followed widespread replacement of native vegetation with crops, pasture and irrigation (Gilfedder and Walker, 2001). The presence of salt in Australian soils causes annual agricultural production losses of more than $130 million (Lambers, 2003). Predictions suggest that 5.7 million ha of land in Australia is at high risk of developing dryland salinity; this may rise to 17 million ha by 2050 (NLWRA, 2001). It is also predicted that the climate will become significantly warmer in Australia in the future (Hughes, 2003). The number of extreme rainfall and drought events is also expected to increase, leaving the country drier by the end 21$^{st}$ century (Hughes, 2003). This will lead to greater shortages of available water for plants.

High concentrations of salt in soil can have a detrimental impact on its physical, chemical and biological properties (Rengasamy, 2006) and unbalance the ecosystem. Salts in soil cause osmotic stress to plants and soil biota by decreasing the osmotic potential of the soil solution. Salinity can stress or even kill soil microorganisms which drive

nutrient cycling (Wichern et al., 2006). Key ecological functions such as nitrogen transformation are reduced or delayed if a large proportion of a microbial population dies (Schimel et al., 2007). Salinity changes microbial community structure as microbial genotypes differ in their tolerance to low osmotic potential (Gennari et al., 2007; Mandeel, 2006). Tolerant microorganisms accumulate solutes in the cell to counteract the low soil osmotic potential (Killham, 1994). Some microorganisms selectively exclude solutes such as sodium and chloride and accumulate other ions necessary for metabolism, especially ammonium. Others produce organic osmolytes (Oren, 2001) which help counteract the potential gradient between soil solution and cell cytoplasm: amino acids in bacteria and polyols in fungi (Killham and Firestone, 1984; Schimel et al., 1989). Salinity can also cause nutrient imbalances as sodium and chloride may compete with nutrient cations and anions in uptake and key metabolic functions. Together with the low osmotic potential, reductions in soil microbial activity in saline soil result in poor plant growth, which reduces crop yields and organic matter input into the soil (Sumner et al., 1998); these lead to environmental problems such as erosion and eutrophication (Rengasamy, 2006).

Dry soil (low soil matric potential) and fluctuating matric potential are further stressors for soil biota and plants in a Mediterranean climate such as in Southern Australia. Soils dry during summer; occasional rainfall rewets the soil, causing marked drying and rewetting cycles. Both drying and rewetting cause stress to soil microorganisms (Fierer and Schimel, 2002). Decreases in matric potential reduce available water as the water films around aggregates become thinner and disconnected and water is held more tightly to aggregate surfaces and in smaller pores (Ilstedt et al., 2000). Soil microorganisms need more energy for water uptake and become substrate-limited as substrate and nutrient diffusion are restricted (Stark and Firestone, 1995). Studies in non-saline soils have shown that some microbial genotypes maintain their viability by changes in membrane properties and proteins or formation of sheaths, capsules, slimes or resting structures (Potts, 1994). In other studies, decreasing matric potential has been shown to induce accumulation of osmolytes (amino acids and polyols) similar to those found in response to salt stress (Schimel et al., 2007). Decreasing matric potential can change microbial community structure (DeGrood et al., 2005; Reichardt et al., 2001). As the soils dry, microorganisms will also experience decreases in osmotic potential, as osmotic potential decreases with decreasing matric potential due to the increasing salt concentration in the remaining soil solution.

Thus soil microorganisms respond to low matric or osmotic potential (induced by drying or the presence of salts) in a similar manner by accumulating osmolytes to retain water. There are many studies on the effect of either matric or osmotic stress on soil microorganisms, but there are no published studies on the interactive effect of matric potential and osmotic potential on microbial activity and community structure or on nutrient availability. This thesis is the first approach which compares the effect of these two stresses on microbial activity and community structure. An understanding of these interactions is needed to assess nutrient turnover in saline soils that is crucial for their sustainable use and rehabilitation. Studying these interactions is also timely as climate change is likely to result in long dry periods interrupted by occasional rainfall events (West et al., 1994) in arid and semi- arid areas such as Southern Australia.

This literature review will present a general discussion of osmotic and matric potentials in salt-affected and dry soils and their effects on soil microorganisms and will identify knowledge gaps to be addressed in this thesis.

## 1.2 Importance of microorganisms in nutrient cycling

Nutrient cycling can be defined as the movement of nutrients within and between the biotic (soil microorganisms and plant roots) or abiotic (soil solution and minerals) entities in the soil environment (Brady and Weil, 1999). Although soil microorganisms account for only 1-5% of the organic matter content in soil (Killham, 1994; Nsabimana et al., 2004), they are the most important component as they are the drivers of the most of the movement within the nutrient cycle (Gil-Sotres et al., 2005).

A large number of microorganisms reside naturally in soil, performing a wide range of functions and maintaining soil productivity. Bacteria, actinomycetes and fungi are the dominant soil microorganisms both in numbers and biomass (Sumner, 2000). As organic materials enter the soil they are colonized by a range of microorganisms. Soil microorganisms break down the residues thereby releasing plant-available nutrients (Ashman and Puri, 2002). Moreover microorganisms also affect nutrient availability by solubilisation, chelation, oxidation and reduction (Marschner and Rengel, 2007; Sylvia et al., 1999). These processes are important because most plants are only able to take up

inorganic forms of nutrients. Nitrate (through nitrification), sulfate (through sulfur oxidation), and to some extent phosphate ions (through phosphorus mineralization) can be found in soils mainly through microbial processes (Tan, 2000).

A number of climatic and biotic factors affect nutrient cycling in soil (Jordan, 1985), including moisture, temperature and rate of decomposition (Tan, 2000). The microbial community varies in structure with soil type, organic matter content and environmental factors (Yang and Crowley, 2000) . Stressors, such as high concentrations of heavy metals or salts, fire and low or high water content, can not only kill sensitive microorganisms but also reduces the activity of surviving organisms because of the metabolic burden imposed by the need for stress tolerance mechanisms (Harris, 1981; Oren, 1999; Schimel et al., 2007). In a hot, dry climate, low soil water content and salinity are the most important stressors for soil microbial communities. Key ecological functions such as nitrogen transformation and the decomposition of hard-to-degrade plant residues which are carried out by specific microbes are affected by salinity and drought (Pankhurst et al., 2001; Pathak and Rao, 1998; Pulleman and Tietema, 1999; Wichern et al., 2006; Yuan et al., 2007). Therefore salt stress and drought may affect nutrient cycling and nutrient availability, resulting in reduced crop yield.

## 1.3 Soil water potential

The soil water potential (ψ) is the energy of a defined mass / volume of water in the soil relative to the energy of pure free water. Soil water potential (ψ) is the sum of components of forces that affect the energy state of water in soil (Papendick and Campbell, 1981):

$$\psi = \psi_\pi + \psi_m + \psi_g + \psi_p + \psi_\Omega$$

where, $\psi_\pi$ = osmotic potential, $\psi_m$ = matric potential, $\psi_g$ = gravitational potential, $\psi_p$ = pressure potential, $\psi_\Omega$ = overburden potential.

Soil microorganisms need to overcome these combined potentials to take up water from soil solution (Griffin, 1969). Therefore the potential of the soil water is more important to

soil organisms than soil water content (Wood, 1995). In saline and dry soils, osmotic and matric components contribute more to water flow and availability for physiological processes than the gravitational potential, $\psi_g$ (due to elevation differences from the reference), pressure potential, $\psi_p$ (applied air or hydraulic pressure) and overburden potential $\psi_\Omega$ (weight of overlying material).

The osmotic potential ($\psi_\pi$) which is the result of interactions of salts with soil water is important in saline soils (Papendick and Campbell, 1981). Water molecules are attracted to electrostatically charged cations such as hydrogen, sodium potassium and calcium. Therefore, increasing concentrations of salt in soil solution restrict the availability of the soil water to plant roots and microorganisms. Moreover, the osmotic potential of the soil solution generally changes with soil water content, reducing water content by evapo-transpiration concentrates the salts in the remaining solution. High concentrations of dissolved salt in soil solution cause severe osmotic stress to soil microbes as this leads to constraints in the uptake of water by cells and in extreme cases, water moves from the cells to the lower osmotic potential of the soil solution (Brady and Weil, 1999).

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

$$\Psi_\pi = -0.036\ EC_{meas}\ (\theta_{ref}/\theta_{act})$$

where, $\Psi_\pi$ = the soil osmotic potential (MPa) at the actual moisture content, $\theta_{act}$ of the soil and $EC_{meas}$ = the measured electrical conductivity (dS $m^{-1}$) of an extract with a water content $\theta_{ref}$ (= 5 g $g^{-1}$ for a 1:5 soil : water mixture).

The osmotic potential of the soil solution can also be calculated by using the van't Hoff equation (after Singh and Kanehiro, 1969):

$$\pi = icRT = -\Psi_\pi$$

where, $\pi$ = osmotic pressure in Pa, i = van't Hoff factor (2 for sodium chloride, potassium chloride; 3 for calcium chloride, magnesium chloride; and 4 for aluminium chloride, ferric

chloride), c = solute concentration in mol $m^{-3}$ or mmol $L^{-3}$, T = absolute temperature (K), R = gas constant (8.314 J $mol^{-1}$ $K^{-1}$).

Soil water matric potential ($\psi_m$) arises from the interaction of soil water with solid surfaces (Papendick and Campbell, 1981). Water in a wet but unsaturated soil is loosely bound to the soil solids and exists as thick water films around soil particles and in all but the largest pores. However, in a drier soil the remaining water is retained in the smallest pores and in a thin water film around the particles by strong adhesion forces (Brady and Weil, 1999). Water is less available and slower to move in a drier soil because the adhesion forces between the water molecules and soil particles and charged ions increases with decreasing water content. Soil microorganisms and plants need to overcome the force of this interaction to take up water. The relationship between soil water matric potential and water content of a soil can be established through the moisture retention curve of that soil; this can be approximated by the following equation (Hillel, 1980):

$$\Psi = a.\ \theta^{-b}$$

where, $\Psi$ = water potential; a, b = empirical constants; $\theta$ = water content.

Most of the water in soil is held on surfaces and in micro-pores and cannot easily be removed by growing plants or by active microorganisms. The amount of clay in a soil largely determines the proportion of micro-pores in that soil and its surface area. At a given water potential, a clay soil holds more water than loam or sand. Similarly, at a certain water content, clay soils hold water more strongly than the coarse-textured ones; thus soil texture has a major influence on soil water retention. Therefore, water potential is a measure of water availability as it indicates the effect on microbial activity better than water content, particularly if soils of different texture are compared. In most existing studies on the effect of salt and water stress on soil microorganisms, only one soil type was used. However, soil properties such as texture, the shape of the water retention curve and organic matter content may influence the effect of salinity and matric potential on soil microorganisms and nutrient turnover (Griffin, 1980). Therefore, most experiments described in this thesis were carried out with two non-saline soils (sand and sandy loam) differing in nutrient status, microbial biomass and community composition.

## 1.4 Salt-affected soils

### 1.4.1 Types and distribution

Saline soils are characterised by high concentrations of soluble cations such as sodium, calcium and magnesium and anions such as chloride, sulphate, carbonate and bicarbonate in the soil solution and on exchange sites (Rengasamy, 2010). These can cause osmotic stress and ion toxicity as well as nutritional imbalance to soil biota and plants (Abrol et al., 1988; Fitzpatrick et al., 2001). The United States salinity laboratory staff defined a soil as saline when the electrical conductivity of the saturation extract ($EC_e$) is > 4 dS $m^{-1}$ and the pH is < 8.5 (Richards, 1954). A soil becomes sodic when it has a significant proportion of sodium ions compared to calcium and magnesium ions on the exchange sites (ANZECC, 2000; SalCon, 1997); this causes deterioration of soil structure including hydraulic properties. Sodicity is expressed as exchangeable sodium percentage (ESP) of the soil or the sodium adsorption ratio (SAR) of a soil water extract.

Sodium adsorption ratio is calculated as:

$$SAR = [Na^+] / [Ca^{2+} + Mg^{2+}]^{½}$$

where, the concentrations of $Na^+$ (sodium), $Ca^{2+}$ (calcium), and $Mg^{2+}$ (magnesium) are in mmol $L^{-1}$.

Exchangeable sodium percentage is calculated as:

$$ESP = (\text{exchangeable } Na^+ / \text{cation exchange capacity}) \times 100$$

Sodic soils have high SAR (> 15) and often a high pH (> 8.5) (Richards, 1954). In Australia a soil with ESP > 6, is termed sodic (Isbell, 1996), instead of ESP > 15, as defined by United States salinity laboratory staff (Richards, 1954). The lower limit of ESP in Australia is based on high content of sodium salts and low concentrations of soluble salts especially low calcium content combined with widespread structural degradation of Australian soils (Rengasamy and Olsson, 1991). Soils that have detrimental concentrations of neutral soluble salts and sodium ions are called saline-sodic soil [$EC_e$ > 4 dS $m^{-1}$, ESP >

6] (Isbell, 1996). Plant growth and microbial activity in these soils can be adversely affected by both excess salts and excess sodium level. This project focuses on saline soils.

Soils can be naturally saline, or they can become saline due to changes in the catchment hydrological balance or the application of saline irrigation water (Ghassemi et al., 1995). According to Fitzpatrick et al. (2001), the type of salinity depends on three important features: hydrological status (presence or absence of ground water), natural (primary) or induced (secondary) status and soil chemical status (predominant type of soluble salt, sodicity). Gradual weathering of parent rock such as basalt and marine sandstone causes accumulation of salts in the soil profile. In arid and semiarid areas in Australia these salts accumulate in the surface soil due to high evapotranspiration and limited rainfall (McBride, 1994). Dryland salinity is developed by rising saline groundwater (Rengasamy, 2002). Wide-spread removal of native deep-rooted plants and trees followed by cultivation of crops and pastures decreases plant interception of water and increases infiltration through the soil profile. Evapotranspiration and capillarity during spring and summer causes dissolved salts to move upwards into the root zone of plants. Generally, where the water table rises to within two metres of the soil surface, salinity affects the root zone of plants (NLWRA, 2001).

Transient salinity is less well known, but more wide spread. It is not groundwater-related, but is the result of a perched water table over impermeable subsoil (e.g., a layer of sodic and / or clay-rich soil) which is common in duplex soils (Rengasamy, 2002). Water and salts that have accumulated over millennia in Australian soils remain in the soil profile above the subsoil. After winter rains, the salts are leached out of the topsoil but accumulate above the subsoil. Transient salinity is always accompanied by high EC in the subsoil (Figure 1), and can also be expressed at the surface as so-called ‘magnesia patches’. In the cropping areas of southern Australia, it is estimated that 16% of the area is affected by dryland salinity, whereas up to 67% could potentially be affected by transient salinity (Kelly and Rengasamy, 2006).

Irrigation-induced salinity is less widespread in Australia, but salt concentrations can be very high (Rengasamy, 2010). The use of low quality saline and saline-sodic groundwater, drainage water or waste water can increase the concentration of salts in irrigated soil (Gardner, 2004). Further, the frequency of irrigation and the cropping pattern

may affect the salt concentration and composition in cultivated soil (Boivin et al., 2002; Herrero and Pérez-Coveta, 2005).



Figure 1. Different types of dryland salinity found in the Australian landscape (Rengasamy, 2002).

### 1.4.2 Effects of salinity and sodicity on soil physical and chemical properties

Soil salinity, i.e. high concentrations of soluble salts in soil solution affects physical and chemical soil processes by altering soil water dynamics, physical stability and nutrient movement in soil. Various salts in different concentrations are present in saline soil. Saline soils are located generally in dry climates such as in arid and semiarid areas. Generally calcium salts are predominant in saline soils with high pH (Chen and Barak, 1982; Sardinha et al., 2003); however, in Australia sodium salt is predominant (50-80%) in most saline soils (Rengasamy, 2006).

A 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

destroys the soil structure. After wetting, the swelling and dispersion of clay particles is the major reason for deterioration of the soil structure (Rengasamy, 2006). Clay particles leach down through the soil profile and develop a layer of clay with few pore spaces thus reducing water infiltration and hydraulic conductivity. Changes in hydraulic conductivity affect nutrient movement into the ground water. Poor soil structure also induces water logging in alkaline-sodic soils. Solubility of sodium, aluminium and boron are increased due to the high pH and cause ion toxicity along with waterlogging. 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. The lack of structural stability in these soils may result in soil hardening by seal and crust formation at the soil surface. This causes poor root development and plant growth. Moreover, the lack of plant cover increases the risk of erosion by wind and water and pollution of water bodies (Rengasamy, 2006).

### 1.4.3 Effects of salinity and sodicity on plants

Salinity inhibits plant growth directly because of the low osmotic potential of the soil solution, specific ion toxicity and may induce nutrient deficiency if salt ions inhibit the uptake or metabolic functioning of essential cations or anions by competition at uptake sites in the roots or binding sites within the cells (Marschner, 1995). Presence of excess salt in the soil solution affects plant growth and survival by reducing root growth, evapotranspiration, photosynthesis, protein synthesis and enzyme activity, thereby causing stunting and reduced yield (Chhabra, 1996; Staples et al., 1984). There are many studies of the effect of salt on plant physiology and of plant mechanisms of salt tolerance (e.g., Leopold and Willing, 1984; Mäser et al., 2002; Munns et al., 2006; Unger, 2008; Zhu, 2001).

Low osmotic potential is the main effect of soluble salts and dominates in saline and saline-sodic soil, resulting in reduced plant growth (Shaw, 1999). The ability of plants to take up adequate water for growth is decreased due to the presence of high amounts of dissolved salts in the root zone (Keren, 2000; Rengasamy, 2010). Therefore, plants growing in saline soils show drought symptoms (Keren, 2000). Plants exposed to osmotic stress are stunted and yellowish-green (chlorotic) (Keren, 2000; Shainberg and Oster, 1978). In places where both salinity and drought are present, plants need to overcome

both low matric and osmotic potentials to take up water, and more energy is needed to survive. When soils dry out due to evapotranspiration, the salt concentration in the soil solution increases thereby decreasing the osmotic potential of the soil water. 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). Thus, the effect of salinity on plants is also affected by seasonal variation in water content of saline soils. In a Natrixeralf under wheat in Southern Australia, the soil water content varied between 18 and 35% with highest contents in winter (June) and lowest in summer (November). Correspondingly, the water potential varied between -1.1 MPa in summer and -0.6 MPa in winter (Rengasamy 2010).

In saline soils, plants expend more energy on osmotic adjustment by accumulating organic and inorganic solutes thereby lowering the osmotic potential inside their cells to counteract the low osmotic potential of the soil solution (Fitzpatrick et al., 2001). The energy demand for this adjustment is one factor that reduces growth. There are substantial differences in salt tolerance among the crop species (Table 1). Salinity can also delay or prevent seed germination (Bell, 1999; Oster et al., 1996). Ghollarata and Raiesi (2007) reported 95 and 91% reduction in dry weight of roots and shoots, respectively of Berseem clover (*Trifolium alexandrinum* L.) when irrigated with saline water of 10 dS $m^{-1}$ compared to distilled water. Salt stress during germination, vegetative growth or reproductive phases can cause severe yield reduction (Eynard et al., 2005). Kelly and Rengasamy (2006) found that an osmotic potential below -1.0 MPa reduces the yield of crop to uneconomic levels.

Ion toxicity or *specific ion effects* are due to excess concentrations of sodium, calcium, magnesium, potassium, chloride, sulphate, bicarbonate and boron in the soil solution of saline soils (Naidu et al., 1992). Increasing the concentration of certain ions in plants can lead to ion imbalance and ion toxicity (Munns and Tester, 2008). At low salinity, the *ion effect* of salt is predominant, whereas when the salinity increases, the osmotic effect becomes more important (Munns et al., 2006; Rengasamy, 2010; Tavakkoli et al., 2010). Phytotoxic boron concentrations are often predominant in highly saline soil in arid and semi-arid irrigated areas (Shouse et al., 2006). At high pH (greater than 8.5), high concentrations of carbonate and bicarbonate can also lead to calcium, zinc and copper

deficiency in sodic soils resulting in nutritional imbalance (Naidu et al., 1992; Rengasamy, 2002).

Poor structure in sodic soil adversely affects plant nutrient uptake and water supply to the plant (Qadir and Schubert, 2002; Rengasamy et al., 2003). In sodic soils where the B horizon has a high bulk density, root growth is limited to the surface layers which can increase drought susceptibility (Curtin and Naidu, 1998). On the other hand, these soils are prone to waterlogging. Therefore, root growth can be reduced due to lack of oxygen (So and Aylmore, 1993) and increased susceptibility of plant roots to pathogens (Naidu and Rengasamy, 1993).

Table 1. Relative salt tolerance of certain crop plants (Arshad, 2008)

| Crop type | Tolerance Level | Electrical conductivity (dS $m^{-1}$) of saturated soil extract, $EC_e$ | |
|---|---|---|---|
| | | Threshold $EC_e$[a] | 50% Yield $EC_e$[a] |
| Field crops: | | | |
| Barley | High | 8 | 18 |
| Cotton | High | 8 | 17 |
| Sugar beet | High | 7 | 15 |
| Sorghum | Moderate | 5 | 12 |
| Wheat | Moderate | 9 | 12 |
| Cow peas | Moderate | 5 | 9 |
| Peas | Low | 3 | 5 |
| Corn | Low | 2 | 6 |
| Rice | Low | 3 | 4 |
| Vegetables and fruits: | | | |
| Broccoli | Moderate | 3 | 8 |
| Spinach | Moderate | 2 | 9 |
| Tomato | Moderate | 2 | 8 |
| Bean | Low | 1 | 4 |
| Strawberry | Low | 1 | 3 |
| Pineapple | Low | 1 | 3 |

[a] Threshold is the salinity below which crop yields generally are not decreased significantly; 50% Yield $EC_e$ refers to the salt concentration at which crop yield is 50% of normal yield attainable at threshold $EC_e$ values.

### 1.4.4 Effect of salinity on soil microorganisms

Salinity affects soil microorganisms mainly through decreasing osmotic potential, which kills sensitive microbial genotypes, increases the metabolic burden in tolerant genotypes and alters the structure and activity of the microbial community (Wichern et al., 2006). Additionally, microorganisms are limited by the reduced input of available substrates due to poor plant growth.

To minimize the effect of low osmotic potential in saline soils, tolerant microorganisms accumulate solutes in the cell to counteract the concentration gradient between cell cytoplasm and soil solution (Killham, 1994). Some microorganisms selectively accumulate ions necessary for metabolism especially ammonium. Others produce osmoregulatory compounds (Oren, 2001): amino acids in bacteria and polyols in fungi (Killham and Firestone, 1984; Schimel et al., 1989). These osmoregulatory compounds are referred to as 'compatible solutes'. They have no net charge under physiological conditions and are compatible with cellular function at high concentration (Sleator and Hill, 2002). The percentage of total cell carbon and nitrogen in the cytoplasm as osmoregulatory compounds is high, so less carbon and nitrogen is incorporated into cell membranes and cell walls. Synthesizing osmoregulatory compounds requires large amounts of energy: 30-110 ATP (adenosine tri phosphate) compared to 30 ATP for cell wall synthesis (Oren, 1999). Tolerance to salt is thus a substantial metabolic burden for microorganisms, less energy is available for other metabolic pathways and growth and substrate use efficiency are lower (Wichern et al., 2006).

Soil microbial community structure is changed due to salinity as microbial genotypes differ in tolerance to osmotic stress (Badran, 1994; Pankhurst et al., 2001). For example, fungi are more sensitive to salinity than bacteria (Pankhurst et al., 2001; Wichern et al., 2006). The study by Sardinha et al. (2003) found decreasing ergosterol concentration with increasing salinity which indicates fungal abundance decreased with salinity. Bacterial dominance in highly saline soil is also reflected by the low biomass C/N (carbon to nitrogen) ratio (Jenkinson, 1988; Paul and Clark, 1989). Therefore, the bacteria / fungi ratio may be increased due to salinity. This will affect nutrient cycling because, compared to bacteria, fungi have a greater enzymatic capacity to decompose complex compounds such as lignin and plant polysaccharides as well as a higher carbon

incorporation efficiency and thus a higher potential for carbon sequestration (Bailey et al., 2002; Harper and Lynch, 1985). High salinity also reduces the microbial biomass (Rietz and Haynes, 2003; Sardinha et al., 2003; Tripathi et al., 2006; Wichern et al., 2006) and decreases microbial diversity and species richness (Ibekwe et al., 2010; Nelson and Mele, 2007). On the other hand, some studies reported that there was no effect of salinity on soil microbial biomass and community structure (Sarig et al., 1993; Sarig and Steinberger, 1994). Increases in microbial cell number and microbial carbon and nitrogen with increasing salinity are also reported (Polonenko et al., 1981; Sarig et al., 1993). Wong et al. (2008) found an initial short term increase in soil microbial biomass with increasing salinity and sodicity of a non saline soil. They suggested this was due to an increase in readily available organic matter content due to dispersion of soil aggregates in sodic soil and dissolution or hydrolysis of soil organic matter. Killham et al. (1990) suggested that microbial biomass can increase with increasing osmotic potential due to their osmotic adaptation at less than -1.0 MPa. Thus, the effect of salinity on microbial biomass and community structure is controversial. The varying effects may be due to the salinity level, soil type and microbial community composition as well as water content. This stresses the importance of conducting experiments with different soil types under controlled conditions and the consideration of water potential instead of EC only.

Changes in microbial activity with increasing salinity have been reported. Saline soils have been found to have lower microbial activity than non saline soils (Naidu and Rengasamy, 1993; Pankhurst et al., 2001). García et al. (1994) reported decreased carbon mineralization as a measure of microbial activity with increasing salinity in a range of soils ($EC_{1:10}$ = 0.2 – 2.86 dS $m^{-1}$). Low microbial activity in saline soils was also shown in studies by Malik and Haider (1977), Pathak and Rao (1998), Rietz et al. (2001) and Wichern et al. (2006). The addition of salt or salt mixtures to non-saline soil resulted in a non-linear reduction of microbial activity between -0.05 and -3 MPa (Johnson and Guenzi, 1963; Singh and Kanehiro, 1969). However, mineralization can occur at high salinity; Pathak and Rao (1998) found significant carbon dioxide evolution at $EC_e$ = 97 dS $m^{-1}$. Microbial activity in saline soil is also affected by the composition of dissolved salts in soil solution; sodium chloride being the most toxic (Frankenberger and Bingham, 1982; García and Hernández, 1996). However, the adverse effect of sodium chloride on soil microorganisms is reduced when readily available organic substrates are present (McCormick and Wolf, 1980). Stressed microbial communities in highly saline soils use the carbon resources inefficiently

due to increased respiratory activity; therefore a greater proportion of available carbon is lost as carbon dioxide per unit of microbial biomass (Rietz and Haynes, 2003). However, some studies reported contradictory results of increased rates of carbon and nitrogen mineralization with increasing salinity (Birch, 1958; Chandra et al., 2002; Laura, 1973, 1976; Nelson et al., 1996; Sokoloff, 1938).

Salinity can also affect enzyme activity. Frankenberger and Bingham (1982) suggested three possible mechanisms by which excess salt in soil solution can affect enzyme activity: (i) inactivation of the enzyme after it is released from the cell, (ii) decrease in solubility of the protein which results in the alteration of the ionic conformation of the active centre of enzymes (*salting-out effect*), and (iii) specific ion toxicities which cause nutritional imbalance and therefore affect enzyme synthesis. Soil enzyme activities decreased with increasing salinity up to $EC_e$ = 40.8 dS $m^{-1}$ (Batra and Manna, 1997). Salinity up to $EC_{1:5}$ = 9.48 dS $m^{-1}$ decreased the activity of β-glycosidase, protease and phosphatase enzymes, thereby affecting the carbon, nitrogen and phosphorus cycles (García and Hernandez, 1996). Similar to microbial activity, the inhibiting effect on enzymes varies with the type and amount of salt in soil solution, sodium chloride being the most toxic salt (Frankenberger and Bingham, 1982).

Soil microorganisms play a key role in nitrogen mineralisation and salinity has been shown to substantially alter N transformations in soil. Nitrogen mineralisation is reduced (Pathak and Rao, 1998; Rietz and Haynes, 2003; Sardinha et al., 2003; Yuan et al., 2007), while ammonification is less affected than nitrification (García and Hernandez, 1996; Laura, 1974; McClung and Frankenberger Jr, 1987) due to salinity resulting in an accumulation of ammonium (Pathak and Rao 1998, Laura 1974). Among the nitrifying bacteria, Johnson and Guenzi (1963) indicated that *Nitrobacter* sp are more affected by salinity than *Nitrosomonas* as they possess lower species diversity. On the other hand, nitrogen mineralization may also increase after addition of salt to soil (Broadbent and Nakashima, 1971). Singh and Kanehiro (1969) found that increased nitrogen mineralization may be due to autolysis of the microbial biomass as a result of the low osmotic potential (Wichern et al., 2006; Broadbent, 1965).

Thus, the effect of salinity on microbial biomass, community structure and activity is controversial. The varying effects may be due to the salinity level, soil type and microbial

community composition as well as water content. This stresses the importance of conducting experiments with different soil types under controlled conditions and the consideration of osmotic and matric potential instead of EC only.

## 1.5 Effect of soil water content and matric potential on soil microorganisms

Soil water is an important factor for nutrient cycling because water within the soil acts as the medium for nutrient transport and microbial motility and is crucial for metabolism. Changes in water content in soil affect matric potential, osmotic potential, substrate availability and diffusion of nutrients and gases (Drenovsky et al., 2004; Griffiths et al., 2005; Schimel et al., 2007).

Soils are subject to variation in soil moisture through seasonal cycles of rainfall and irrigation. Arid and semiarid regions have long dry periods followed by wetting through episodic rainfall (Gleeson et al., 2008). The intensity of the drying and rewetting cycles depend on climate, soil type, crop and tillage system (Oliveira et al., 2005). Climate change can increase the intensity and length of dry periods which may cause extreme and irregular water stress for soil microorganisms and plants (Borken and Matzner, 2009). Both drying and repeated drying and rewetting of soils are important features of dry regions which affect soil microorganisms (Fierer et al., 2003).

### 1.5.1 Low water content

Soils lose water through percolation and evapotranspiration, therefore these processes decrease matric potential. As soils dry out, the water films around aggregates become thinner and water availability decreases because the water is held more tightly to the aggregate surfaces (Ilstedt et al., 2000). As a result, at very low matric potentials microbes become substrate-limited as nutrients cannot move by diffusion through these thin and sometimes discontinuous water films (Stark and Firestone, 1995). This can be more severe in coarse-textured soils because they have few small pores and clay particles which hold water at low matric potential (Brady and Weil, 1999). At a given water potential, fine-textured soils have a higher water content than coarse-textured soils. Stark and

Firestone (1995) found that in a silt loam soil, substrate limitation was the main limiting factor for the activity of nitrifying bacteria at matric potential greater than -0.6 MPa, whereas when the matric potential decreased below -0.6 MPa, cell dehydration was the main limiting factor. Griffin and Quail (1968) found that the movement of bacteria decreased rapidly when the diffusion pathway was disrupted at low matric potential. Wong and Griffin (1976) reported that bacterial movement ceased when the soil matric potential decreased from -0.02 MPa to -0.1 MPa. Although some microorganisms can thrive at low osmotic potential, the combination with low matric potential reduces their activity through decreased nutrient movement and solute diffusion. The minimum osmotic potential for growth of *B. subtilis* is - 1.7 MPa at adequate water supply but at matric potential less than -0.1 MPa, its movement becomes negligible at this osmotic potential (Scott, 1957).

In common with the effects of low osmotic potential, microorganisms accumulate osmoregulatory compounds or other salts to counteract the effect of low matric potential (Schimel et al., 2007; Csonka and Hanson, 1991). Other genotypes may maintain their viability by the formation of desiccation-resistant spores or cysts (Gould et al., 1977). Adapted or tolerant microorganisms are able to control the concentration of intracellular compounds to overcome the changes in soil water potential. The accumulation of osmoregulatory compounds upon drying can be substantial: they can make up to 40% of cell carbon and 30% of cell nitrogen (Schimel et al., 2007). In a grassland ecosystem this is equivalent to at least 3-6% of annual net primary production and 10-40% of net nitrogen mineralisation (Schimel et al., 2007) to survive a single drying event. Hence, the ability to survive in dry soil may be increased at high substrate availability (Griffin, 1980).

Osmoregulatory compounds may inhibit enzyme activity, as enzyme confirmation is changed by the reduced hydration (Lanyi et al., 1979; Skujins and McLaren, 1967). In a Jarrah forest in Western Australia enzyme activity (phosphatase) was negatively correlated with soil water content (Grierson and Adams, 2000). Enzymes play an essential role in converting organic nutrients into inorganic forms available to plants. Therefore, a decrease in enzyme activity, and thus decomposition rate, will decrease nutrient cycling and availability to plants. Decreasing matric potential decreases carbon and nitrogen mineralisation (Pulleman and Tietema, 1999), cell numbers and the capacity of soil bacteria to decompose carbon substrates (Griffiths et al., 2003). Sommers et al. (1981) suggested that water potential influences microbial decomposition in two phases: initially

there was a rapid decrease in decomposition rate between -0.03 and -1.0 MPa and then decomposition rate decreased linearly with decreasing water potential.

Decreasing soil water content changes the size of the microbial biomass (Bottner, 1985; Campbell et al., 1973). A large proportion of microorganisms die (He et al., 1997), mainly the active soil microbes (Bottner, 1985; Drenovsky et al., 2004; Griffiths et al., 2003) which are more susceptible to cell lysis due to their thinner cell walls. Moreover, gram negative bacteria are found to be more sensitive than gram positive bacteria to drastic changes in water potential (Harris, 1981; Nesci et al., 2004). Generally, fungal activity is greater than that of bacteria in water stressed soil, except at very low potentials (Harris, 1981). Fungi may be able to survive at lower matric potentials than bacteria because their hyphae bridge air gaps and transport water and substrates (Killham, 1994). Wilson and Griffin (1975) reported that bacterial activity was dominant at potentials above -0.6 to -0.8 MPa whereas the activity of fungi and actinomycetes was dominant at -0.8 to -2.0 MPa. Total respiration was severely reduced at -3.0 MPa. Many fungi and actinomycetes are metabolically inactive, but remain viable at very low matric water potential such as -4.0 to -10.0 MPa (Sommers et al., 1981).

Low matric potential can also change microbial community structure, but the effect on the bacteria / fungi ratio is controversial. Some fungi can tolerate low water potential (Harris, 1981) and drought can result in a relative increase in fungal biomass (DeGrood et al., 2005; Reichardt et al., 2001). However, a decrease in fungal biomass or an increase in the bacteria / fungi ratio with decreasing soil water content have also been reported (Frey et al., 1999; Williams and Rice, 2007). This may be due to differences in community structure, as individual fungal species differ in response to matric potential (Klamer and Hedlund, 2004; McLean and Huhta, 2000). Nitrifiers are less tolerant to drought than the ammonifiers, thus ammonium may accumulate in dry soil (Paul and Clark, 1989).

### 1.5.2 Drying and rewetting

Soil water potential changes very rapidly when dry soil is wetted by rainfall or irrigation. In general, drying is a slow process which allows soil microorganisms to accumulate osmoregulatory compounds but the rewetting of a dry soil represents a sudden

shock to soil microorganisms as the water potential increases very rapidly (Smith, 1979). The soil matric potential can increase from less than -20 MPa to zero upon rapid wetting of a dry soil (Evans et al., 1975).

When the soil is rewetted, the sudden increase in water potential causes an influx of water into the cells resulting in increased turgor pressure in cells that may inactivate soil microorganisms or cause cell lysis or death (Brown, 1979; Harris, 1981; Luard, 1982; Salema et al., 1982). Tolerant microorganisms rapidly release the intracellular organic solutes to avoid the risk of cell lysis (Halverson et al., 2000; Reed et al., 1986). Other adaptation mechanisms are polymerization of organic solutes to counteract the sudden change in osmotic potential or catabolism of solutes to carbon dioxide (Berrier et al., 1992). A study with two California grassland soils showed that rewetting a dry soil to field capacity from -2.8 and -6.9 MPa caused the release of 17-70% of soil microbial biomass carbon (Kieft et al., 1987). A single drying and rewetting cycle can decrease the microbial biomass by one third (Bottner, 1985; Wu and Brookes, 2005). On the other hand, some studies found large “flushes” in respiration after rewetting with no change in the size of microbial biomass, suggesting that there was no cell lysis (Fierer and Schimel, 2002; Mikha et al., 2005). Wu and Brookes (2005) found that only 28-40% of the respiration flushes after wetting of a dry soil were from cell lysis; the majority were from non-biomass soil organic matter which also includes microbial metabolites. Therefore, although the microbial biomass is markedly altered by drying and rewetting cycles, non-biomass soil organic matter may be the major source of carbon which is mineralized during drying and rewetting. Drying and rewetting cycles can alter the structure of macro- and micro-aggregates and break bonds between soil particles and make physically protected organic matter available (Degens and Sparling, 1995; Denef et al., 2001), thereby increasing the concentration of available substrate in the soil solution (Fierer and Schimel, 2002). In another study, the initial labile substrate pool for microbes after rewetting was found to be of microbial origin (released osmoregulatory compounds and cell lysis) but later the available substrate were found to derive from plant material (Saetre and Stark, 2005).

The increase of available substrate stimulates the activity of surviving microorganisms; which peaks within the first 24 hours after rewetting (Fierer and Schimel, 2002; Kieft et al., 1987). The quality of organic matter, the properties of the soil biota as well as the size of the organic pool and microbial biomass control the size of flush (Butterly

et al., 2009, 2010; Turner et al., 2003; VanGestel et al., 1993). The increase of the wetting flush has been found to decrease with the frequency of drying and rewetting cycles in a wide range of soils (Fierer and Schimel, 2002; Jager and Bruins, 1975; Mikha et al., 2005; Prieme and Christensen, 2001). The size of the rewetting flush may be smaller because of a decrease in available organic matter content over time (Birch, 1958), stabilization of soil aggregates, changes in microbial biomass size and physiology or by adaptation of the microbial community. Aggregates may become more stable and slake-resistant after the first two drying and rewetting cycles (Denef et al., 2001). The organic matter content may have two opposing effects on microbial activity during drying and rewetting. On the one hand, organic matter may alleviate drying stress through retention of water (He et al., 1997). On the other, high organic matter content is often associated with high microbial biomass and therefore may result in greater flushes following rewetting (Bloem et al., 1992; Franzluebbers et al., 2000).

Microbial community composition changes with drying and rewetting as microorganisms differ in their resistance to water stress (Fierer et al., 2003; Schimel et al., 2007). Fungi and gram-positive bacteria are more tolerant to fluctuating matric potential as they possess stronger cell walls (Schimel et al., 2007). Steenwerth et al. (2005) reported a change in microbial community composition by drying and rewetting without a change in biomass size. Fast growing microorganisms may be less resistant to drying and rewetting cycles than slow growing microorganisms (Fierer and Schimel, 2002; Fierer et al., 2003; VanGestel et al., 1993). There are some reports indicating that soil microorganisms can adapt to drying and rewetting in ecosystems where drying and rewetting is a regular event (Kieft et al. 1987, Fierer et al. 2003). Fierer et al. (2003) observed that the soil microbial community in a soil under oak was altered in response to drying and rewetting while the microbial community in a soil under grass was largely unaffected. At the study site, the grassland soils may be exposed to drying and rewetting events more frequently than the oak soils as they have less vegetation cover.

Frequent drying and rewetting may lead to a decrease in carbon sequestration as more soil organic matter becomes accessible to microbial attack. Where the microbial biomass is the source of a rewetting flush, frequent drying and rewetting can potentially reduce the carbon mineralization and increase carbon sequestration (Fierer and Schimel,

2002). On the other hand, the turnover rates of immobilized nutrients such as phosphorus and sulphur can be accelerated by rewetting dry soil (Lodge et al., 1994).

Drying and rewetting can also have long term effects on microbial functioning. It was found that 48 days after rewetting a dry soil, the bacterial and archaeal community structure was different from that of the continuously moist incubated soil and the capacity of the microbial community to decompose a fungicide was reduced (Pesaro et al., 2004).

Thus, drying and rewetting cycles can induce short and long term changes in nutrient availability. This is particularly critical in saline soils where the soil fertility is already low due to salt stress.

## 1.6 Interactive effects of osmotic and matric potential on microorganisms and plants

Both low matric and low osmotic potential cause a decrease in the free energy of water and affect microbes by decreasing the water potential in the surrounding environment (Shalhevet, 1993). Therefore, both types of potential will have the same effect on intracellular water activity and thus the physiological effects are theoretically equivalent (Griffin and Luard, 1979; Stark and Firestone, 1995). However, low matric potential may be more stressful to soil microorganisms than low osmotic potential. Kroeckel and Stolp (1984) found that low matric potential was inhibitorier than low osmotic potential for respiration and nitrogen fixation of *Azotobacter*. Griffin (1980) stated that a combination of matric potential -1.3 MPa and osmotic potential of -0.2 MPa is more stressful for microbial activity than the combination of matric potential -0.2 MPa and osmotic potential -1.3 MPa. Fungal germination and growth was found to be more sensitive to matric than osmotic stress in media (Brownell and Schneider, 1983; Griffin, 1980; Magan, 1988; Magan et al., 1995; Ramirez et al., 2004). Mycelial extension of *Alternaria alternate*, *Microdochium bolleyii* and other fungi have been found to be more sensitive to matric stress than to osmotic stress (Adebayo and Harris, 1971; Magan et al., 1995). The stronger negative effect of low matric potential compared to low osmotic potential may be the consequence of the restricted diffusion and mass flow of substrates and to the movement of organisms

themselves (Adebayo and Harris, 1971) and thus reduced ability of the microbes to synthesize osmoregulatory compounds to counteract the low potential outside the cells.

The effect of drying on plants and their physiological adjustment in saline soils have been studied extensively (e.g. Blum, 1988; Leopold and Willing, 1984; Mäser et al., 2002; Munns et al., 2006; Shannon et al., 1994; Unger, 2008). When saline soils dry by evapotranspiration, the osmotic and matric potential of soil solution decrease simultaneously, exposing plants and microorganisms to increasing levels of both osmotic and matric stress (Shalhevet, 1993).

Shalhevet (1993) suggested that the decrease in matric and osmotic potential can have additive effects on plants. However, some studies where plants were grown at low matric potential at different low osmotic potentials showed that salt in soil can mitigate the negative effects of low matric potential. Plants in soils with low matric potential may survive longer in a salt-stressed condition than in non-salt-stressed condition as growth was slow in salt-stressed soil and less water was depleted for growth than in non-salt-stressed plants (Mc Cree and Richardson, 1987; McCree, 1986; Richards, 1992; Shalhevet, 1993). Salt stress can also precondition plants to low soil water potential by osmotic adjustment, increasing their ability to survive as the soil dries (Shalhevet, 1993). Glenn and Brown (1998) found that salts in the soil solution enhanced the growth of the xerohalophyte *Atriplex canescens* (Pursh.) in soil with low matric potential by increasing the number of days to wilting and increasing the efficiency of water use from soil through sodium uptake for osmotic adjustment.

Microorganisms and plants have similar cellular responses to decreasing osmotic potential as they produce similar osmoregulatory compounds to compensate the stress (Csonka, 1989). Therefore, it could be hypothesised that microbial communities subjected to salt stress would be more tolerant to drought stress. On the other hand, as soils dry, salts become more concentrated. Because producing osmoregulatory compounds is energetically expensive (Oren, 1999) and poses a stress for microbial metabolism, the already stressed microorganisms in saline soils may be less tolerant to the additional stress caused by low matric potential. Moreover, saline soils have low organic matter content (Pankhurst et al., 2001); substrate and energy limitation may therefore limit the capacity of microbes to tolerate an additional stress.

## 1.7 Resistance and resilience of microorganisms to stress

Stress causes physiological challenges and threatens microbial function and survival by altering soil micro-climate and resources (Schimel et al., 2007). Microorganisms in soil are subject to many natural and anthropogenic stresses which may affect their function (Philippot et al., 2008). The degree to which such functions are affected after a stress is termed resistance, and the rate and extent of recovery after a stress is defined as resilience (Pimm, 1984). For resistance, microorganisms alter the allocation of resources from growth to survival pathways, but under extreme stress they become dormant or die. If several stresses occur consecutively, the impact of the later stresses may be lower if they induce similar physiological changes (Philippot et al. 2008).

Functional resistance and resilience of the soil microbial activity to applied stress vary widely in a wide range of soil and land management types (Kuan et al., 2007) and higher functional resistance is associated with more diverse microbial community structure. Factors that affect soil resilience and resistance are species composition, diversity, food web structure, soil type and vegetation, climate, land use and stress regime (Orwin et al., 2006; Griffiths et al. 2000; Girvan et al. 2005). Polonenko et al. (1981) observed rapid recovery of microbial activity after removal of salt stress by leaching. But the microbial population developed during recovery was different from that in non- stressed soil.

Various parameters have been used to assess microbial resistance and resilience in soils. The “metabolic quotient” (respiration to the soil microbial biomass ratio, $qCO_2$) is commonly used to determine the effect of stress on soil microbial population (Wong et al., 2008; Dilly, 2001; Wichern et al., 2006). The study by Sarig and Steinberger (1994) found that soil which was previously subjected to osmotic stress had a higher $qCO_2$, indicating microbial stress. Yuan et al. (2007) found an increase in $qCO_2$ with increasing $EC_{1:5}$ and reported $qCO_2$ in other stressed soils such as those subjected to increasing salinity, low soil pH and increased heavy metal concentration. Griffiths et al. (2001) used functional stability to quantify the differences in biological status between soils. Functional stability is expressed compared to the activity (production of $CO_2$ through respiration) in control treatment:

Resistance = % change from control = [(control $CO_2$- treated $CO_2$) / control $CO_2$] × 100%

Resilience is calculated as the change in resistance over time.

Cook and Orchard (2008) expressed resilience of microorganisms to matric water stress as function of water content and time. There are several other indices used in the literature for comparison of the effect of stresses on microorganisms (Orwin and Wardle, 2004). Which of the indices is the most suitable depends on the stressor and the response variable studied.

## 1.8 Aim of this study

Salinity is a stressor for soil microorganisms which causes cell death and decreases microbial activity. In the Mediterranean climate of Southern Australia, soil microorganisms in saline soils are also exposed to water stress: low matric potential and drying and rewetting cycles. The overview above shows that the effects of osmotic and matric potential have been studied extensively, but generally as separate, unrelated entities. The conflicting results of the studies on the effect of salinity and water stress on soil microorganisms reported in this chapter are most likely the result of neglecting the influence of decreasing matric potential on the salt concentration in different soils. Indeed, most studies only report $EC_{1:5}$, $EC_e$, % salt, or g water $kg^{-1}$ soil, % of water holding capacity (WHC) rather than the actual osmotic and matric potentials of the soil solution which are the true measures of water availability. Comparisons between different soils are problematic because they differ in water-holding capacity and retention curves. These issues need to be taken into consideration if the effects of matric and osmotic potential on soil microorganisms are to be understood. This thesis aims to address these knowledge gaps.

The overall objective of the project is to determine how soil microbial activity and community composition are affected by the interaction between soil matric and osmotic potential. To fulfil the objective, this project specifically aimed to:

- Determine the time until microbial activity becomes stable after rewetting of air dry soil in a range of non-saline and saline soils. In addition, to assess the effect of adaptation of soil microorganisms on sensitivity to different matric and osmotic potentials (Chapter 2).

- Quantify and compare the effects of osmotic and matric potential on soil microbial activity and community structure in two soils of different texture (Chapter 3).

- Investigate the interactive effects of osmotic and matric potential on microbial activity, size and composition in two soils of different texture (Chapter 4).

- Determine the activity, size and composition of the microbial biomass after different levels of matric stress and rewetting of soil in order to determine the recovery of microbial activity (Chapter 5).

- Examine the influence of repeated drying and rewetting cycles on saline soil microorganisms (Chapter 6).

- Determine the effect of length of dry period on microbial activity in rewetted saline soils (Chapter 7).

## 1.9 References


Abrol, I.P., Yadav, J.S.P., Massoud, F.I., 1988. Salt-affected soils and their management. Food and Agricultural Organization of the United Nations, Rome.

Adebayo, A.A., Harris, R.F., 1971. Fungal growth responses to osmotic as compared to matric water potential. Soil Science Society of America Journal 35, 465-469.

ANZECC, 2000. Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand.

Arshad, M., 2008. Soil salinity and salinization, In: Chesworth, W. (Ed.), Encyclopedia of Soil Science Dordrecht, Springer, pp. 699-704.

Ashman, M.R., Puri, G., 2002. Essential soil science: A Clear and Concise Introduction to Soil Science. Blackwell Science, Hoboken, New Jersey.

Badran, R.A.M., 1994. Cellulolytic activity of some cellulose-decomposing fungi in salinized soils. Acta Mycologica (Poland) 29, 245-251.

Bailey, V.L., Smith, J.L., Bolton, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology and Biochemistry 34, 997-1007.

Batra, L., Manna, M.C., 1997. Dehydrogenase activity and microbial biomass carbon in salt-affected soils of semiarid and arid regions. Arid Soil Research and Rehabilitation 11, 295-303.

Bell, D.T., 1999. Australian trees for the rehabilitation of waterlogged and salinity-damaged landscapes. Australian Journal of Botany 47, 697-716.

Berrier, C., Coulombe, A., Szabo, I., Zoratti, M., Ghazi, A., 1992. Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. European Journal of Biochemistry 206, 559-565.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9-31.

Bloem, J., de Ruiter, P.C., Koopman, G.J., Lebbink, G., Brussaard, L., 1992. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. Soil Biology and Biochemistry 24, 655-665.

Blum, A., 1988. Plant breeding for stress environments. CRC Press, Boca Raton, Florida.

Boivin, P., Favre, F., Hammecker, C., Maeght, J.L., Delariviere, J., Poussin, J.C., Wopereis, M.C.S., 2002. Processes driving soil solution chemistry in a flooded rice-cropped vertisol: analysis of long-time monitoring data. Geoderma 110, 87-107.

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15, 808-824.

Bottner, P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with $^{14}C$- and $^{15}N$-labelled plant material. Soil Biology and Biochemistry 17, 329-337.

Brady, N.C., Weil, R.R., 1999. The nature and properties of soil 12th ed. Prentice-Hall Inc. Upper Saddle River, New Jersey.

Broadbent, F.E., 1965. Effect of fertilizer nitrogen on the release of soil nitrogen. Soil Science Society of America Journal 29, 692.

Broadbent, F.E., Nakashima, T., 1971. Effect of added salts on nitrogen mineralization in three California soils. Soil Science Society of America Journal 35, 457-460.

Brown, A.D., 1979. Physiological problems of water stress, In: Shilo, M. (Ed.), Strategies of microbial life in extreme environments. Wiley-VCH, Dahlem konferenzen, Berlin, pp. 65-81.

Brownell, K.H., Schneider, R.W., 1983. Roles of matric and osmotic components of water potential and their interaction with temperature in the growth of *Fusarium oxysporum* in synthetic media and soil. Phytopathology 75, 53-57.

Butterly, C.R., Bunemann, E.K., McNeill, A.M., Baldock, J.A., Marschner, P., 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biology and Biochemistry 41, 1406-1416.

Butterly, C.R., Marschner, P., McNeill, A.M., Baldock, J.A., 2010. Rewetting $CO_2$ pulses in Australian agricultural soils and the influence of soil properties. Biology and Fertility of Soils, 1-15.

Campbell, C.A., Biederbeck, V.O., Warder, F.G., Robertson, G.W., 1973. Effect of rainfall and subsequent drying on nitrogen and phosphorus changes in a dryland fallow loam. Soil Science Society of America Proceedings 37, 909-915.

Chandra, S., Joshi, H. C., Pathak, H., Jain, M. C., Kalra, N., 2002. Effect of potassium salts and distillery effluent on carbon mineralization in soil. Bioresource Technology 83, 255-257.

Chen, Y., Barak, P., 1982. Iron nutrition of plants in calcareous soils. Advances in Agronomy 35, 240.

Chhabra, R., 1996. Soil salinity and water quality. A. A. Balkema Brookfield, USA.

Cook, F.J., Orchard, V.A., 2008. Relationships between soil respiration and soil moisture. Soil Biology and Biochemistry 40, 1013-1018.

Csonka, L.N., 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiology and Molecular Biology Reviews 53, 121-147.

Csonka, L.N., Hanson, A.D., 1991. Prokaryotic osmoregulation: genetics and physiology. Annual Reviews in Microbiology 45, 569-606.

Curtin, D., Naidu, R., 1998. Fertility constraints to plant production, In: Sumner, M.E., Naidu, R. (Eds.), Sodic Soil: distribution, management and environmental consequences. Oxford University Press: New York, pp. 107–123.

Degens, B.P., Sparling, G.P., 1995. Repeated wet-dry cycles do not accelerate the mineralization of organic C invilved in the macro-aggregation of a sandy loam soil. Plant and Soil 175, 197-203.

DeGrood, S.H., Claassen, V.P., Scow, K.M., 2005. Microbial community composition on native and drastically disturbed serpentine soils. Soil Biology and Biochemistry 37, 1427-1435.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology and Biochemistry 33, 1599-1611.

Dilly, O., 2001. Metabolic and anabolic responses of arable and forest soils to nutrient addition. Journal of Plant Nutrition and Soil Science 164, 29-34.

Drenovsky, R.E., Elliott, G.N., Graham, K.J., Scow, K.M., 2004. Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities. Soil Biology and Biochemistry 36, 1793-1800.

Evans, R.A., Kay, B.L., Young, J.A., 1975. Microenvironment of a dynamic annual community in relation to range improvement. Hilgardia 43, 79-102.

Eynard, A., Schumacher, T.E., Lindstrom, M.J., Malo, D.D., 2005. Effects of agricultural management systems on soil organic carbon in aggregates of Ustolls and Usterts. Soil and Tillage Research 81, 253-263.

FAO, 2008. FAO Land and Plant Nutrition Management Service. Available at http://www.fao.org/ag/agl/agll/spush

Fierer, N., Schimel, J.P., 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777-787.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Influence of drying–rewetting frequency on soil bacterial community structure. Microbial Ecology 45, 63-71.

Fitzpatrick, R.W., Rengasamy, P., Merry, R.H., Cox, J.W., 2001. Is dryland soil salinisation reversible. National Dryland Salinity Program. Web site http://www.ndsp.gov.au.

Frankenberger Jr, W.T., Bingham, F.T., 1982. Influence of Salinity on Soil Enzyme Activities. Soil Science Society of America Journal 46, 1173-1177.

Franzluebbers, A.J., Haney, R.L., Honeycutt, C.W., Schomberg, H.H., Hons, F.M., 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Science Society of America Journal 64, 613-623.

Frey, S.D., Elliott, E.T., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology and Biochemistry 31, 573-585.

García, C., Hernandez, T., 1996. Organic matter in bare soils of the Mediterranean region with a semiarid climate. Arid Land Research and Management 10, 31-41.

García, C., Hernández, T., Costa, F., 1994. Microbial activity in soils under mediterranean environmental conditions. Soil Biology and Biochemistry 26, 1185-1191.

Gardner, W.K., 2004. Changes in soils irrigated with saline groundwater containing excess bicarbonate. Australian Journal of Soil Research 42, 825-831.

Gennari, M., Abbate, C., La Porta, V., Baglieri, A., Cignetti, A., 2007. Microbial Response to $Na_2SO_4$ Additions in a Volcanic Soil. Arid Land Research and Management 21, 211-227.

Ghassemi, F., Jakeman, A.J., Nix, H.A., 1995. Salinisation of Land and Water Resources. Human causes, extent, management and case studies. University of New South Wales Press, Sydney.

Ghollarata, M., Raiesi, F. 2007. The adverse effects of soil salinization on the growth of *Trifolium alexandrinum* L. and associated microbial and biochemical properties in a soil from Iran. Soil Biology and Biochemistry 39, 1699-1702.

Gilfedder, M., Walker, G., 2001. Dryland salinity risk: a review of assessment methods. Natural Resource Management 4, 2-9.

Gil-Sotres, F., Trasar-Cepeda, C., Leiros, M.C., Seoane, S., 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biology and Biochemistry 37, 877-887.

Girvan, M.S., Campbell, C.D., Killham, K., Prosser, J.I., Glover, L.A., 2005. Bacterial diversity promotes community stability and functional resilience after perturbation. Environmental Microbiology 7, 301-313.

Gleeson, D.B., Herrmann, A.M., Livesley, S.J., Murphy, D.V., 2008. Influence of water potential on nitrification and structure of nitrifying bacterial communities in semiarid soils. Applied Soil Ecology 40, 189-194.

Glenn, E.P., Brown, J.J., 1998. Effects of soil salt levels on the growth and water use efficiency of *Atriplex canescens* (Chenopodiaceae) varieties in drying soil. American Journal of Botany 85, 10.

Gould, G.W., Measures, J.C., Wilkie, D.R., Meares, P., 1977. Water relations in single cells. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 278, 151-166.

Grierson, P.F., Adams, M.A., 2000. Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (*Eucalyptus marginata* Donn ex Sm.) forest in south-western Australia. Soil Biology and Biochemistry 32, 1817-1827.

Griffin, D.M., 1969. Soil water in the ecology of fungi. Annual Review of Phytopathology 7, 289-310.

Griffin, D.M., 1980. Water potential as a selective factor in the microbial ecology of soils, In: Parr, J.F., Gardner, W.R., Elliott, L.F. (Eds.), Water potential relations in soil microbiology. Soil Science Society of America, Madison Wisconsin pp. 141-151.

Griffin, D.M., Luard, E.J., 1979. Water stress and microbial ecology, In: Shilo, M. (Ed.), Strategies of microbial life in extreme environments, Dahlem Konferenzen, Berlin, pp. 49-63.

Griffin, D.M., Quail, G., 1968. Movement of bacteria in moist, particulate systems. Australian Journal of Biological Sciences 21, 579-582.

Griffiths, B.S., Bonkowski, M., Roy, J., Ritz, K., 2001. Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. Applied Soil Ecology 16, 49-61.

Griffiths, B.S., Hallett, P.D., Kuan, H.L., Pitkin, Y., Aitken, M.N., 2005. Biological and physical resilience of soil amended with heavy metal-contaminated sewage sludge. European Journal of Soil Science 56, 197-205.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J., Ruiter, P.C.d., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity & ecosystem function relationship. Oikos 90, 279-294.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2003. Physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69, 6961-6968.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64, 1630-1637.

Harper, S.H.T., Lynch, J.M., 1985. Colonization and decomposition of straw by fungi. Transactions of the British Mycological Society 85, 655-661.

Harris, R.F., 1981. Effect of water potential on microbial growth and activity, In: Parr, J.F., Gardner, W.R., Elliott, L.F. (Eds.), Water potential relations in soil microbiology. Soil Science Society of America, Madison Wisconsin, pp. 23–95.

He, Z.L., Wu, J., O 'Donnell, A.G., Syers, J.K., 1997. Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. Biology and Fertility of Soils 24, 421-428.

Herrero, J., Pérez-Coveta, O., 2005. Soil salinity changes over 24 years in a Mediterranean irrigated district. Geoderma 125, 287-308.

Hillel, D.J., 1980. Fundamentals of Soil Physics. Academic Press, New York.

Hughes, L., 2003. Climate change and Australia: trends, projections and impacts. Austral Ecology 28, 423-443.

Ibekwe, A.M., Poss, J.A., Grattan, S.R., Grieve, C.M., Suarez, D., 2010. Bacterial diversity in cucumber (*Cucumis sativus*) rhizosphere in response to salinity, soil pH, and boron. Soil Biology and Biochemistry 42, 567-575.

Ilstedt, U., Nordgren, A., Malmer, A., 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biology and Biochemistry 32, 1591-1599.

Isbell, R.F., 1996. The Australian Soil Classification. CSIRO Publishing, Collingwood, Victoria.

Jager, G., Bruins, E.H., 1975. Effect of repeated drying at different temperatures on soil organic matter decomposition and characteristics, and on the soil microflora. Soil Biology and Biochemistry 7, 153-159.

Jenkinson, D.S., 1988. Determination of microbial biomass carbon and nitrogen in soil, In: Wilson, J.R. (Ed.), Advances in nitrogen cycling in agricultural ecosystems. Commonwealth Agricultural Bureau, International., Wallingford, pp. 368-386.

Johnson, D.D., Guenzi, W.D., 1963. Influence of Salts on Ammonium Oxidation and Carbon Dioxide Evolution from Soil. Soil Science Society of America Journal 27, 663.

Jordan, C.F., 1985. Nutrient cycling in tropical forest ecosystems. John Wiley and Sons, Chichester.

Kelly, J., Rengasamy, P., 2006. Diagnosis and Management of Soil Constraints: Transient Salinity, Sodicity and Alkalinity, The University of Adelaide and Grains Research and Development Corporation, Canberra, Australia, pp. 1-32.

Keren, R., 2000. Salinity, In: Sumner, M.E. (Ed.), Handbook of Soil Science. CRC Press. Boca Raton., pp. G3-G25.

Kieft, T.L., Soroker, E., Firestone, M.K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biology and Biochemistry 19, 119-126.

Killham, K., 1994. Soil ecology. Cambridge University Press, Cambridge.

Killham, K., Firestone, M.K., 1984. Salt stress control of intracellular solutes in Streptomycetes indigenous to saline soils. Applied and Environmental Microbiology 47, 301-306.

Killham, K., Schimel, J.P., Wu, D., 1990. Ecophysiology of the soil microbial biomass and its relation to the soil microbial N pool. Soil Use and Management 6, 86-88.

Klamer, M., Hedlund, K., 2004. Fungal diversity in set-aide agricultural soil investigated using terminal-restriction fragment length polymorphism. Soil Biology and Biochemistry 36, 983-988.

Kroeckel, L., Stolp, H., 1984. Influence of soil water potential on respiration and nitrogen fixation of Azotobacter vinelandii. Plant and Soil 79, 37-49.

Kuan, H.L., Hallett, P.D., Griffiths, B.S., Gregory, A.S., Watts, C.W., Whitmore, A.P., 2007. The biological and physical stability and resilience of a selection of Scottish soils to stresses. European Journal of Soil Science 58, 811-821.

Lambers, H., 2003. Dryland salinity: a key environmental issue in southern Australia. Plant and Soil 257, 5-7.

Lanyi, J.K., Avron, M., Bayley, S.T., Brock, T.D., Brown, A.D., Fitt, P.S., Griffin, D.M., Horowitz, N.H., Kushner, D.J., Larsen, H., Norkrans, B., Truper, H.G., Weber, J., 1979. Life at low water activities: group report, In: M, S. (Ed.), Strategies of microbial life in extreme environments. Dahlem Konferenzen, Berlin., pp. 125-135.

Laura, R.D., 1973. Effects of sodium carbonate on carbon and nitrogen mineralisation of organic matter added to soil. Geoderma 9, 15-26.

Laura, R.D., 1974. Effects of neutral salts on carbon and nitrogen mineralization of organic matter in soil. Plant and Soil 41, 113–127.

Laura, R.D., 1976. Effects of alkali salts on C and N mineralization of organic matter in soil. Plant and Soil 44, 587-596.

Leopold, A.C., Willing, R.P., 1984. Evidence for toxicity effects of salt on membranes, In: Staples, R.C., Toenniessen, G.H. (Eds.), Salinity Tolerance in Plants: Strategies for Crop Improvement. John Wiley and Sons, New York, pp. 67-76.

Lodge, D.J., McDowell, W.H., McSwiney, C.P., 1994. The importance of nutrient pulses in tropical forests. Trends in Ecology and Evolution 9, 384-387.

Luard, E.J., 1982. Effect of osmotic shock on some intracellular solutes in two filamentous fungi. Microbiology 128, 2575.

Magan, N., 1988. Effects of water potential and temperature on spore germination and germ-tube growth in vitro and on straw leaf sheaths. Transactions of the British Mycological Society 90, 97-107.

Magan, N., Challen, M.P., Elliot, T.J., 1995. Osmotic, matric and temperature effects on in vitro growth of Agaricus bisporus and A. bitorquis strains. Mushroom Science 14, 773–780.

Malik, K.A., Haider, K., 1977. Decomposition of carbon-14-labelled plant material in saline-sodic soils, Soil Organic Matter Studies. Proceedings of a Symposium organized by IAEA, FAO and Agrochimica. , Vienna (International Atomic Energy Agency), pp. 215-225.

Mandeel, Q.A., 2006. Biodiversity of the genus Fusarium in saline soil habitats. Journal of basic microbiology 46, 480-494.

Marschner, H., 1995. Functions of mineral nutrients: macronutrients, Mineral nutrition of higher plants. Academic Press Limited; London.

Marschner, P., Rengel, Z., 2007. Nutrient cycling in terrestrial ecosystems. Springer, Verlag, Germany.

Mäser, P., Gierth, M., Schroeder, J.I., 2002. Molecular mechanisms of potassium and sodium uptake in plants. Plant and Soil 247, 43-54.

McBride, M.B., 1994. Environmental chemistry of soils. Oxford University Press, New York (USA).

McClung, G., Frankenberger Jr, W.T., 1987. Nitrogen mineralization rates in saline vs. salt-amended soils. Plant and Soil 104, 13-21.

McCormick, R.W., Wolf, D.C., 1980. Effect of sodium chloride on CO2 evolution, ammonification, and nitrification in a Sassafras sandy loam. Soil Biology and Biochemistry 12, 153-157.

McCree, K.J., 1986. Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. Functional Plant Biology 13, 33-43.

McCree, K.J., Richardson, S.G., 1987. Salt increases the water use efficiency in water stressed plants. Crop science (USA) 27, 543-547.

McLean, M.A., Huhta, V., 2000. Temporal and spatial fluctuations in moisture affect humus microfungal community structure in microcosms. Biology and Fertility of Soils 32, 114-119.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biology and Biochemistry 37, 339-347.

Munns, R., James, R.A., Läuchli, A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany 57, 1025.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Plant Biology 59, 651.

Naidu, R., Rengasamy, P., 1993. Ion interactions and constraints to plant nutrition in Australian sodic soils. Australian Journal of Soil Research 31, 801-819.

Naidu, R., Rengasamy, P., deLacy, N.J., Zarcinas, B.A., 1992. Soil solution composition of some sodic soils, In: Naidu, R., Sumner, M.E., Rengasamy, P. (Eds.), Australian Sodic Soils. CSIRO, Canberra, Australia, pp. 155-162.

Nelson, D.R., Mele, P.M., 2007. Subtle changes in rhizosphere microbial community structure in response to increased boron and sodium chloride concentrations. Soil Biology and Biochemistry 39, 340-351.

Nelson, P.N., Ladd, J.N., Oades, J.M., 1996. Decomposition of $^{14}C$-labelled plant material in a salt-affected soil. Soil Biology and Biochemistry 28, 433-441.

Nesci, A., Etcheverry, M., Magan, N., 2004. Osmotic and matric potential effects on growth, sugar alcohol and sugar accumulation by Aspergillus section Flavi strains from Argentina. Journal of Applied Microbiology 96, 965-972.

NLWRA, 2001. Dryland Salinity. Australia's dryland salinity assessment 2000. Commonwealth of Australia, Canberra.

Nsabimana, D., Haynes, R.J., Wallis, F.M., 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Applied Soil Ecology 26, 81-92.

Oliveira, R.S., Dawson, T.E., Burgess, S.S.O., Nepstad, D.C., 2005. Hydraulic redistribution in three Amazonian trees. Oecologia 145, 354-363.

Oren, A., 1999. Bioenergetic aspects of halophilism. Microbiology and Molecular Biology Reviews 63, 334-348.

Oren, A., 2001. The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems. Hydrobiologia 466, 61-72.

Orwin, K.H., Wardle, D.A., 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology and Biochemistry 36, 1907-1912.

Orwin, K.H., Wardle, D.A., Greenfield, L.G., 2006. Ecological consequences of carbon substrate identity and diversity in a laboratory study. Ecology 87, 580-593.

Oster, J.D., Shainberg, I., Abrol, I.P., 1996. Reclamation of salt affected soil, In: Agassi, M. (Ed.), Soil erosion, conservation and rehabilitation. Marcel Dekker, New York, pp. 315–352.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biology and Fertility of Soils 33, 204-217.

Papendick, R.I., Campbell, G.S., 1981. Theory and measurement of water potential., In: Parr J.F., Gardner W.R., .Elliott L.F. (Eds.), Water potential relations in soil microbiology. Soil Science Society of America Special Publication Number 9, Madison, Wisconsin, pp. 1-22

Pathak, H., Rao, D.L.N., 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biology and Biochemistry 30, 695-702.

Paul, E.A., Clark, F.E., 1989. Soil Microbiology and Biochemistry. Academic Press, Inc. San Diego, California.

Pesaro, M., Nicollier, G., Zeyer, J., Widmer, F., 2004. Impact of soil drying-rewetting stress on microbial communities and activities and on degradation of two crop protection products. Applied and Environmental Microbiology 70, 2577.

Philippot, L., Cregut, M., Chèneby, D., Bressan, M., Dequiet, S., Martin Laurent, F., Ranjard, L., Lemanceau, P., 2008. Effect of primary mild stresses on resilience and resistance of the nitrate reducer community to a subsequent severe stress. FEMS Microbiology Letters 285, 51-57.

Pimm, S.L., 1984. The complexity and stability of ecosystems. Nature 307, 321-326.

Polonenko, D., Mayfield, C., Dumbroff, E., 1981. Microbial responses to salt-induced osmotic stress. Plant and Soil 59, 269-285.

Potts, M., 1994. Desiccation tolerance of prokaryotes. Microbiology and Molecular Biology Reviews 58, 755.

Prieme, A., Christensen, S., 2001. Natural perturbations, drying-wetting and freezing-thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soils. Soil Biology and Biochemistry 33, 2083-2091.

Pulleman, M., Tietema, A., 1999. Microbial C and N transformations during drying and rewetting of coniferous forest floor material. Soil Biology and Biochemistry 31, 275-285.

Qadir, M., Schubert, S., 2002. Degradation processes and nutrient constraints in sodic soils. Land Degradation and Development 13, 275-294.

Ramirez, M.L., Chulze, S.N., Magan, N., 2004. Impact of osmotic and matric water stress on germination, growth, mycelial water potentials and endogenous accumulation of sugars and sugar alcohols in Fusarium graminearum. Mycologia 96, 470.

Reed, R.H., Warr, S.R.C., Kerby, N.W., Stewart, W.D.P., 1986. Osmotic shock-induced release of low molecular weight metabolites from free-living and immobilized cyanobacteria. Enzyme and Microbial Technology 8, 101-104.

Reichardt, W., Briones, A., De Jesus, R., Padre, B., 2001. Microbial population shifts in experimental rice systems. Applied Soil Ecology 17, 151-163.

Rengasamy, P., 2002. Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. Australian Journal of Experimental Agriculture 42, 351-361.

Rengasamy, P., 2006. Soil salinity and sodicity, In: Stevens, D., Kelly, J., McLaughlin, M., Unkovich, M. (Eds.), Growing crops with reclaimed wastewater. CSIRO Publishing, Collingwood, Australia, pp. 125-138.

Rengasamy, P., 2010. Soil processes affecting crop production in salt-affected soils. Functional Plant Biology 37, 613-620.

Rengasamy, P., Chittleborough, D., Helyar, K., 2003. Root-zone constraints and plant-based solutions for dryland salinity. Plant and Soil 257, 249-260.

Rengasamy, P., Olsson, K.A., 1991. Sodicity and soil structure. Soil Research 29, 935-952.

Richard, L.A., 1954. Determination of the properties of saline and alkali soils, Diagnosis and improvement of saline and alkali soil. United States Department of Agriculture Handbook 60, Washington, DC, pp. 7-53.

Richards, R.A., 1992. Increasing salinity tolerance of grain crops: Is it worthwhile? Plant and Soil 146, 89-98.

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biology and Biochemistry 35, 845-854.

Rietz, D.N., Haynes, R.J., Chidoma, S., 2001. Effects of soil salinity induced under irrigated sugercane in the Zimbabwean lowveld on soil microbial activity. Proceedings of the South African Sugar Technologists Association 75, 68-74.

Saetre, P., Stark, J.M., 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. Oecologia 142, 247-260.

SalCon, 1997. Salinity management handbook, Queensland Department of Natural Resources. Report DNRQ97109, Brisbane, Australia.

Salema, M.P., Parker, C.A., Kidby, D.K., Chatel, D.L., Armitage, T.M., 1982. Rupture of nodule bacteria on drying and rehydration. Soil Biology and Biochemistry 14, 15-22.

Sardinha, M., Müller, T., Schmeisky, H., Joergensen, R.G., 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Applied Soil Ecology 23, 237-244.

Sarig, S., Roberson, E.B., Firestone, M.K., 1993. Microbial activity-soil structure: response to saline water irrigation. Soil Biology and Biochemistry 25, 693-697.

Sarig, S., Steinberger, Y., 1994. Microbial biomass response to seasonal fluctuation in soil salinity under the canopy of desert halophytes. Soil Biology and Biochemistry 26, 1405-1408.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386-1394.

Schimel, J.P., Jackson, L.E., Firestone, M.K., 1989. Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. Soil Biology and Biochemistry 21, 1059-1066.

Scott, W.J., 1957. Water relations of food spoilage microorganisms, In: Stewart, G.F. (Ed.), Advances in Food Research. Academic Press, New York, pp. 83-127.

Shainberg, I., Oster, J.D., 1978. Quality of irrigation water. International Irrigation Information Center Bet Dagan, Illinois.

Shalhevet, J., 1993. Plants under water and salt stress, In: Fowden, L., Mansfield, T., Stoddart, J. (Eds.), Plant adaptation to environmental stress Chapman and Hall, New York pp. 133–154.

Shannon, M.C., Grieve, C.M., Francois, L.E., 1994. Whole-plant response to salinity, In: Wilkinson, R.E. (Ed.), Plant-environment interactions. Marcel Dekker, New York, pp. 199–244.

Shaw, R.J., 1999. Soil salinity, electrical conductivity and chloride, In: Peverill, K.I., Sparrow, L.A., Reuter, D.J. (Eds.), Soil analysis: An interpretation manual. CSIRO Publishing, Melbourne, Victoria, Australia, pp. 129–145.

Shouse, P.J., Goldberg, S., Skaggs, T.H., Soppe, R.W.O., Ayars, J.E., 2006. Effects of shallow groundwater management on the spatial and temporal variability of boron and salinity in an irrigated field. Vadose Zone Journal 5, 377.

Singh, B.R., Kanehiro, A.S., 1969. Effect of Chloride Salts on Ammonium Nitrogen Release in Two Hawaiian Soils. Soil Science Society of America Journal 33, 557.

Skujins, J.J., McLaren, A.D., 1967. Enzyme reaction rates at limited water activities. Science 158, 1569-1570.

Sleator, R.D., Hill, C., 2002. Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiology Reviews 26, 49-71.

Smith, D.C., 1979. Is a lichen a good model of biological interactions in nutrient-limited environments?, In: Shilo, M. (Ed.), Strategies of microbial life in extreme environments, Dahlem Konferenzen, Berlin, pp. 291-304.

So, H.B., Aylmore, L.A.G., 1993. How do sodic soils behave? The effects of sodicity on soil physical behavior. Australian Journal of Soil Research 31, 761-777.

Sokoloff, V.P., 1938. Effect of neutral salts of sodium and calcium on carbon and nitrogen in soils. Journal of Agricultural Research 57, 201–216.

Sommers, L.E., Gilmour, C.M., Wildung, R.E., Beck, S.M., 1981. The effect of water potential on decomposition processes in soils, In: Parr, J.F., Gardner, W.R., Elliot, L.F.

(Eds.), Water Potential Relations in Soil Microbiology. Soil Science Society of America, Madison Wisconsin, pp. 97–117.

Staples, R.C., Toenniessen, G.H., Rockefeller, F., 1984. Salinity tolerance in plants. Wiley New York.

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218-221.

Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Scow, K.M., Rolston, D.E., 2005. Response of microbial community composition and activity in agricultural and grassland soils after a simulated rainfall. Soil Biology and Biochemistry 37, 2249-2262.

Sumner, M.E., 2000. Handbook of Soil Science. CRC Press, Boca Raton.

Sumner, M.E., Rengasamy, P., Naidu, R., 1998. Sodic soils: a reappraisal, In: Sumner, M.E., Naidu, R. (Eds.), Sodic soils: distribution, properties, management and environmental consequences. Oxford University Press, New York. pp. 3-17.

Sylvia, D.M., Fuhrmann, J.J., Hartel, P., Zuberer, D.A., 1999. Principles and applications of soil microbiology. Prentice Hall, New Jersey.

Tan, K.H., 2000. Environmental Soil Science. Marcel Dekker New York.

Tavakkoli, E., Rengasamy, P., McDonald, G.K., 2010. The response of barley to salinity stress differs between hydroponic and soil systems. Functional Plant Biology 37, 621-633.

Tripathi, S., Kumari, S., Chakraborty, A., Gupta, A., Chakrabarti, K., Bandyapadhyay, B.K., 2006. Microbial biomass and its activities in salt-affected coastal soils. Biology and Fertility of Soils 42, 273-277.

Turner, B.L., Driessen, J.P., Haygarth, P.M., McKelvie, I.D., 2003. Potential contribution of lysed bacterial cells to phosphorus solubilisation in two rewetted Australian pasture soils. Soil Biology and Biochemistry 35, 187-189.

Unger, P.W., 2008. Soil water and its management, In: Chesworth, W. (Ed.), Encyclopedia of Soil Science, Springer, Dordrecht, Netherlands, pp. 699-704.

VanGestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass and activity in soils with fluctuating water contents. Geoderma 56, 617-626.

West, N.E., Stark, J.M., Johnson, D.W., Abrams, M.M., Wight, J.R., Heggem, D., Peck, S., 1994. Effects of climatic change on the edaphic features of arid and semiarid lands of western North America. Arid Land Research and Management 8, 307-351.

Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100-108.

Williams, M.A., Rice, C.W., 2007. Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Applied Soil Ecology 35, 535-545.

Wilson, J.M., Griffin, D.M., 1975. Water potential and the respiration of microorganisms in the soil. Soil Biology and Biochemistry 7, 199-204.

Wong, P.T.W., Griffin, D.M., 1976. Bacterial movement at high matric potentials--II. In fungal colonies. Soil Biology and Biochemistry 8, 219-223.

Wong, V., Dalal, R., Greene, R., 2008. Salinity and sodicity effects on respiration and microbial biomass of soil. Biology and Fertility of Soils 44, 943-953.

Wood, M., 1995. Environmental soil biology. Blackie Academic and Professional, Glasgow.

Wu, J., Brookes, P.C., 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biology and Biochemistry 37, 507-515.

Yang, C.H., Crowley, D.E., 2000. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Applied and Environmental Microbiology 66, 345-351.

Yuan, B.C., Xu, X.G., Li, Z.Z., Gao, T.P., Gao, M., Fan, X.W., Deng, H.M., 2007. Microbial biomass and activity in alkalized magnesic soils under arid conditions. Soil Biology and Biochemistry 39, 3004-3013.

Zhu, J.K., 2001. Plant salt tolerance. Trends in Plant Science 6, 66-71.

# CHAPTER 2

## Effect of rewetting of air-dry soil and adaptation to low matric and osmotic potential

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# Effects of rewetting of air-dry soil and adaptation to low matric and osmotic potential.

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## Abstract

When conducting incubation experiments to measure soil respiration in response to a given treatment in soils of different texture, it is important to have baseline information about the soils and to ensure that the effect of the storage or pre-incubation conditions on the results are minimized. Three experiments were conducted with up to seven soils (3 non-saline, 4 saline) to investigate (i) the relationship between soil water content or water potential and soil respiration, (ii) the time required until soil respiration is stabilised after rewetting of air-dry soil, and (iii) if pre-incubation at a given matric or osmotic potential changes the response of soil respiration to matric or osmotic potential. The texture of the soils varied from sand to sandy loam. Cumulative respiration showed an optimum curve in response to differential soil water content in all 7 soils, but the maximal cumulative respiration was reached at different water content in the soil, being 45 g $kg^{-1}$ for the non-saline sand and 200 g $kg^{-1}$ for the non-saline sandy loam. However, when expressed against water potential, maximal cumulative respiration was achieved between 0 and -1 MPa in all soils. Rewetting of air-dry soil induced a flush of respiration in the first 1-2 days, but respiration rates stabilised 7-8 days after rewetting in all soils. Pre-incubation at low matric or osmotic potential for two weeks did not significantly affect soil respiration at low matric or osmotic potential in the following two weeks compared to soils which had been pre-incubated at optimal matric potential and high osmotic potential. In conclusion, when comparing the response of respiration to water availability among soils of different texture it is important to consider water potential and not water content. Furthermore, an adaptation period of 2 weeks does not seem to increase the tolerance of soil respiration to low osmotic or matric potential compared to non-adapted soils.

**Keywords**: adaptation, cumulative respiration, preincubation, water content, water potential.

## Introduction

Microbial activity is strongly affected by soil water content, increasing with increasing soil water content from dry to moist soil but then decreasing again (Harris, 1981; Killham, 1994). The increase is due to greater water and nutrient availability as the pores become increasingly water-filled. Beyond the optimal soil water content however, further increases in water content decrease microbial activity due to limited gas exchange and thus anaerobic conditions.

After collection, soils are often air-dried and then sieved to remove large particles and stones. The soils are then stored air-dry before being used for experiments. Microbial activity is very low in air-dry soil due to lack of water and nutrients. Many microorganisms survive by becoming dormant or by forming spores. Therefore, air-drying also minimizes carbon (C) loss during storage. In Mediterranean climate such as that in Southern Australia, top soils often dry during early summer and remain air-dry for several months. Hence, air-drying and storage of air-dry soil mimics the conditions in the field.

Rewetting of air-dry soil causes a flush of respiration which is due to the release of previously accumulated osmolytes in microbial cells and of organic matter within aggregates (Kieft et al., 1987; Halverson et al., 2000; Denef et al., 2001; Fierer and Schimel, 2003). This flush occurs within a few minutes after rewetting, with a peak in the first day, but then decreases rapidly due to consumption of the labile C (Kieft et al., 1987; Fierer and Schimel, 2003). The size of the flush depends on the C content of the soil and also the size of the microbial biomass, being greater in soils with high C content and/or microbial biomass C (Butterly et al., 2009; Butterly et al., 2010). Respiration rates comparable to those in moist soil are often reported to be found 5-10 days after rewetting (Kieft et al., 1987; Fierer and Schimel, 2003; Mikha et al., 2005). Compared to continuously moist soil, rewetting also causes a change in microbial community structure, particularly in soils with higher C availability (Butterly et al., 2009; Butterly et al., 2010).

This flush in respiration after rewetting of air-dry soil may mask treatment effects that are to be investigated in an experiment. Therefore, it is important to rewet the soils before imposing any treatments to allow microbial activity to return to equilibrium. The time

until this equilibrium is reached may vary with soil type, hence it is important to test this for any soil to be used.

Water availability is not only a function of the matric potential but also, particularly in saline soils, of osmotic potential. Low osmotic potential, caused by high salt concentrations in the soil solution draws water out of the cells and thus inhibits microbial activity. Whereas sensitive cells are killed at low osmotic potential, tolerant microorganisms accumulate osmolytes (Oren, 2001; Killham and Firestone, 1984; Schimel et al., 1989; Beales, 2004).

Microbial communities may be more tolerant to a stressor such as low osmotic or matric potential if they are given time to adapt. During this adaptation period, sensitive genotypes die, whereas tolerant genotypes survive and become dominant. In previous studies, microbial activity recovered within two days of exposure to high heavy metal concentrations (Diaz-Ravina and Baath, 1996) or a pH change (Pettersson and Baath, 2004), indicating that a new, more tolerant community has developed. However, it is not known how quickly microbial communities adapt to low matric or osmotic potential.

In the field, microbial communities are able to adapt to low osmotic or low matric potential because they are usually exposed to these stressors for weeks, months or even years. Hence, sensitivity to a low water potential may be overestimated in experiments where microbial communities are suddenly exposed to the stressor and not given time to adapt. Therefore, it needs to be investigated if pre-incubation of a soil at low osmotic or matric potential makes the microbial communities more resistant to these stresses than if the stress is imposed suddenly.

The aims of the experiments described in this chapter were to: (i) determine the soil water content that results in maximal cumulative respiration for the soils to be used in the experiments described in this thesis, (ii) determine the time until respiration reached constant rates after rewetting of air-dry soil in the soils to be used in the subsequent experiments, and (iii) assess the effect of adaptation on sensitivity to different matric and osmotic potential.

## Materials and Methods

Seven soils were used, three non-saline and four saline soils. A non-saline sand, a non-saline sandy loam and the four saline sandy loam soils were collected from Monarto, South Australia (35° 05´ S and 139° 06´ E). Another non-saline sandy loam was collected from Mount Bold, South Australia (38.11ºS 138.69ºE). The particle size analysis of each soil was determined by hydrometer method (Day, 1965). The EC and pH were measured in a 1:5 soil: water suspension after 30 minutes settlement following 1 hour end-over-end shaking at 25°C. Total organic carbon (TOC) was determined by dichromate oxidation described by Walkley and Black (1934). Water holding capacity (WHC) was determined using suction and pressure techniques (Klute, 1986) at -0.01MPa. Each soil was placed in cores of 1 cm diameter and 0.5 cm height in 4 replicates using the appropriate amount of air-dry soil to maintain the bulk density according to their texture. Soils were thoroughly wetted with NaCl solutions according to their EC. Separate pressure plates were used for different EC's. Soils were allowed to drain for 7 days at -0.01MPa pressure in pressure chambers. Oven dry weight of soil was determined after drying at 105°C for 24h. The properties of the soils are given in Table 1.

Table 1. Properties of the soils used in the experiments. Texture is indicated in the soil names: S stands for sand, SL for sandy loam.

| | Sand | Silt | Clay | EC 1:5 dS $m^{-1}$ | pH 1:5 | TOC (g $kg^{-1}$) | Water holding capacity (g $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Monarto | | | | | | | |
| SM | 91.3 | 5.0 | 3.7 | 0.15 | 7.7 | 6.9 | 67 |
| SLM1 | 60.0 | 21.3 | 18.8 | 0.19 | 9.3 | 16.4 | 357 |
| SLM2 | 70.0 | 15.0 | 15.0 | 0.76 | 9.3 | 10.1 | 356 |
| SLM3 | 65.0 | 16.2 | 18.8 | 1.62 | 9.5 | 2.6 | 314 |
| SLM4 | 60.0 | 21.0 | 19.0 | 2.82 | 9.0 | 4.7 | 314 |
| SLM5 | 60.0 | 21.0 | 19.0 | 4.07 | 8.9 | 5.0 | 406 |
| Mount Bold | | | | | | | |
| SLMB | 57.5 | 25.0 | 17.5 | 0.68 | 5.2 | 36.5 | 364 |

**Determination of the water retention curves**

The water retention curves of the soils were determined using suction and pressure techniques (Klute, 1986). Matric potential was estimated from the moisture retention curve using the following equation (Hillel, 1980):

$$\Psi = a.\ \theta^{-b}$$

where, $\Psi$ = water potential; a, b = empirical constants; $\theta$ = water content.

For the four saline soils, the water retention curves were determined by equilibrating the soils with saline solutions according to their EC. The saline solutions of the appropriate EC were prepared with NaCl salt using the following equation (Robert Murray, personal communication):

$$y=1857x$$

where, y= NaCl concentration, g $L^{-1}$ and x= EC of solution, dS $m^{-1}$.

The electrical conductivity was determined in a 1:5 soil: water mixture ($EC_{1:5}$) after 1 hour end-over-end shaking. The $EC_{1:5}$ was converted to the EC of a saturated paste ($EC_e$) using the following equation (Rengasamy, 2006):

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

The osmotic potential was estimated using the following equation (after Richards, 1954):

$$\Psi_\pi = -0.036\ EC_{meas}\ (\theta_{ref}/\theta_{act})$$

where, $\Psi_\pi$ = the soil osmotic potential (MPa) at the actual moisture content, $\theta_{act}$ of the soil and $EC_{meas}$ = the measured electrical conductivity (dS $m^{-1}$) of an extract with a water content $\theta_{ref}$ (= 5 g $g^{-1}$ for a 1:5 soil : water mixture).

**Respiration measurements**

The soils (25 g, oven dry basis) were added to PVC cores (diameter 3.7 cm, height 5 cm) with a nylon mesh base (0.75 µm, Australian Filter Specialist) and packed to a bulk density (http://www.pedosphere.com/resources/bulkdensity/triangle_us.cfm) according to their texture : 1.55 g $cm^{-3}$ (sand) and 1.46 g $cm^{-3}$ (sandy loam). The cores were placed immediately into 1 L glass incubation jars and sealed with gas tight lids equipped with septa to allow headspace sampling. The glass jars were incubated in the dark at 22-25°C. Respiration was quantified by measuring headspace carbon dioxide ($CO_2$) concentrations every 24h using a Servomex 1450 infra-red gas analyser (Servomex Group, Crowborough, England). After each measurement, the jars were opened to equilibrate the $CO_2$ to ambient concentrations and then resealed. The $CO_2$ concentrations were measured immediately after resealing the jars. The $CO_2$ evolved from each sample was calculated as the difference between the initial (after resealing of the jars) and the $CO_2$ concentrations after 24h.

**Experiment 1**

The aim of Experiment 1 was to determine the soil water content that results in maximal cumulative respiration for the soils to be used in the experiments described in this thesis.

All seven soils mentioned above were used. The soils were incubated moist for 10 days. This incubation time was chosen based on previous experiments conducted in our lab which had shown that respiration rates stabilized 7-10 days after rewetting of air-dry soil in a range of different soils. The water content was based on a subjective assessment: the soils were not too dry and not too wet. Following this pre-incubation, the soils were dried by spreading them in a large glass dish and placing the dish in fan forced oven at 25°C for up to three hours until they had reached the desired water content. Then, pea (*Pisum sativum* L.) straw (C/N 26, water soluble C 27 g $kg^{-1}$), ground and sieved (0.25 - 2 mm), was mixed into the soils (2% w/w) to provide a readily-available nutrient source. Respiration was measured until the respiration rates remained stable for at least 2 days, which was between 5 and 10 days after addition of the pea straw. There were 2 replicates per treatment. The water content and corresponding matric potential is shown in Table 2.

Table 2. Water content and corresponding matric potential in the seven soils in Experiment 1.

| % of WHC | Water content (g $k^{-1}$ soil) | | | | | | | Matric potential (MPa) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SM | SLMB | SLM1 | SLM2 | SLM3 | SLM4 | SLM5 | SM | SLMB | SLM1 | SLM2 | SLM3 | SLM4 | SLM5 |
| 70 | 47 | 255 | 250 | 250 | 220 | 220 | 283 | 0.00 | -0.07 | -0.07 | -0.07 | -0.14 | -0.14 | -0.14 |
| 60 | 40 | 219 | 214 | 214 | 189 | 189 | 243 | -0.09 | -0.09 | -0.10 | -0.08 | -0.19 | -0.19 | -0.19 |
| 50 | 34 | 182 | 179 | 178 | 157 | 157 | 203 | -0.18 | -0.11 | -0.12 | -0.10 | -0.23 | -0.28 | -0.23 |
| 40 | 27 | 146 | 143 | 143 | 126 | 126 | 162 | -0.27 | -0.13 | -0.14 | -0.12 | -0.72 | -0.82 | -0.35 |
| 30 | 20 | 109 | 107 | 107 | 94 | 94 | 122 | -1.66 | -0.72 | -0.66 | -0.49 | -1.34 | -1.37 | -1.12 |
| 25 | 13 | 73 | 71 | 71 | 63 | 63 | 81 | -3.32 | -2.11 | -1.60 | -1.58 | -1.96 | -1.92 | -1.89 |
| 20 | 10 | 55 | 54 | 54 | 47 | 47 | 61 | -4.15 | -2.81 | -2.06 | -2.13 | -2.27 | -2.20 | -2.28 |
| 10 | 7 | 36 | 36 | 36 | 31 | 31 | 41 | -4.98 | -3.50 | -2.53 | -2.67 | -2.58 | -2.47 | -2.67 |

**Experiment 2**

The aim of Experiment 2 was to determine the time until respiration reached constant rates after rewetting of air-dry soil in the soils to be used in the subsequent experiments.

All seven soils mentioned above were used. The soils were rewet to the water content that resulted in maximal respiration (determined in Experiment 1) and respiration measured for 18 days. By then respiration rates had remained constant for at least 10 days. There were 2 replicates per treatment.

**Experiment 3**

The aim of Experiment 3 was to assess the effect of adaptation on sensitivity to different matric and osmotic potential. The non-saline sandy loam was used for this experiment.

The general treatment structure is shown in Table 3.

Table 3. Design for Experiment 3

| Preincubation<br>10 days | Adaptation period<br>14 days | Measurement period<br>14 days |
|---|---|---|
| Optimal water content | Non-adapted soils: optimal water content (200g $kg^{-1}$) | Different water content and EC |
| Optimal water content | Adapted soils: different water content and EC | Different water content and EC (same as in adaptation period) |

Two sets of soils were exposed to different conditions in the adaptation period (14 days): (1) non-adapted: incubated at optimal soil water content and (2) adapted: incubated at different water content and EC. After this adaptation period, the non-adapted soils were adjusted to the same water content and EC as the adapted soils while the water content and EC of the adapted soils was maintained. Pea straw (2% w/w) was added and respiration measured over 14 days (measurement period).

The EC levels used were $EC_e$ 0.7, 6.0, 7.7, 17.3, 34.3, 42.0 dS/cm (referred to as EC0.7, EC6.0, EC7.7, EC17.3, EC34.4 and EC42.0) which were achieved by adding 0, 1.5, 2, 5, 10 and 12.5 g NaCl $kg^{-1}$ soil. The soil water content was maintained constant at 200 g $kg^{-1}$ which is optimal for this soil.

The soil water content was adjusted to 90, 100, 120, 130, 150 and 200 g $kg^{-1}$ (referred to as WC90, WC100, WC120, WC130 and WC200) as described for Experiment 1. The matric and osmotic potentials in the different treatments are shown in Table 4.

Table 4. $EC_e$ and water content in treatments of Experiment 3 with corresponding osmotic and matric potential.

| $EC_e$ (dS $m^{-1}$) | NaCl added (g $kg^{-1}$) | Osmotic potential (MPa) | Water content (g $kg^{-1}$) | Matric potential (MPa) |
|---|---|---|---|---|
| 0.7 | 0 | -0.06 | 200 | -0.10 |
| 6.1 | 1.5 | -0.46 | 150 | -0.13 |
| 7.7 | 2.0 | -0.59 | 130 | -0.14 |
| 17.3 | 5.0 | -1.32 | 120 | -0.31 |
| 34.3 | 10.0 | -2.63 | 100 | -1.08 |
| 41.8 | 12.5 | -3.21 | 90 | -1.46 |

There were 3 replicates per treatment. Significant differences between different treatments in respiration rate and cumulative respiration were assessed by 2-way ANOVA (adaptation x EC or water content) and Tukey test with $\alpha = 0.05$ (GenStat® for Windows 8.0, VSN Int. Ltd, UK, 2005).

## Results

The moisture retention curves are presented in Figure 1 for 5 soils; the other sandy loam soils from Monarto had similar retention curves to those shown. Whereas the five sandy loams have quite similar retention curves, the soil water content of the sand decreased more rapidly, reaching very low matric potentials at higher water contents than in the sandy loam soils.

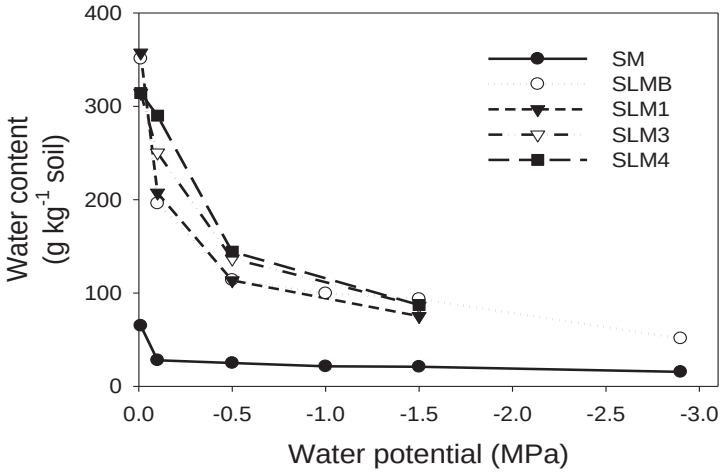


Figure 1. Water retention curves of a sand and four sandy loam soils.

Table 5. Parameters for logarithmic equations of water retention curves of the soils. Matric potential (MPa) is inserted into the equation in positive values. Water content is in g $100g^{-1}$ soil.

| Soil | r2 | Constant | b1 |
|---|---|---|---|
| SM | 0.87 | 2.11 | -0.80 |
| SLMB | 0.98 | 9.86 | -5.13 |
| SLM1 | 0.99 | 8.50 | -5.74 |
| SLM2 | 0.97 | 7.74 | -5.72 |
| SLM3 | 0.96 | 11.25 | -4.69 |
| SLM4 | 0.88 | 12.33 | -4.76 |
| SLM5 | 0.95 | 13.15 | -6.38 |

**Experiment 1**

Cumulative respiration was affected by water content. In the sandy loam soils, cumulative respiration increased with increasing water content initially, but then decreased (Figure 2 A, for 5 soils, the other sandy loam soils from Monarto had similar retention curves to those shown).

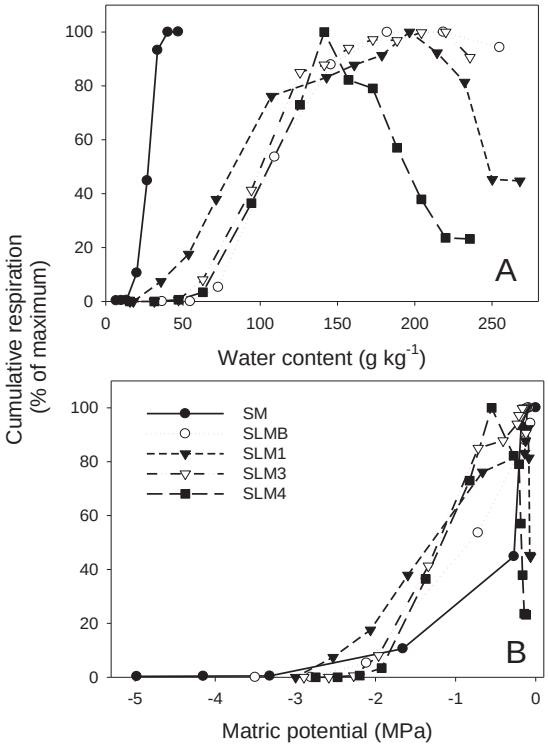


Figure 2. Cumulative respiration in percentage of maximum in a sand and four sandy loam soils as a function of water content (A) and matric potential (B).

In the sand, there was no decrease in cumulative respiration at the higher water contents. The response of cumulative respiration to different water content varied between soils with some being more affected than others. The water content that resulted in maximal respiration was much lower in the sand than in the sandy loam soils. And among the sandy loam soils, it was lowest in SLM4.

The water content that resulted in maximal cumulative respiration was 45 g $kg^{-1}$ for the non-saline sand and 200 g $kg^{-1}$ for the non-saline sandy loam (Figure 2). In the five sandy loam soils it ranged from 140 to 240 g $kg^{-1}$. These water contents are referred to as optimal water content in this thesis.

However, when cumulative respiration is plotted against matric potential, the response is very similar between the five soils (Figure 2B).

**Experiment 2**

Respiration rates were high on the first day after rewetting but then declined rapidly in all seven soils (Figure 3). The respiration rates stabilised after 7-8 days and then remained constant until day 18 when the experiment was stopped.

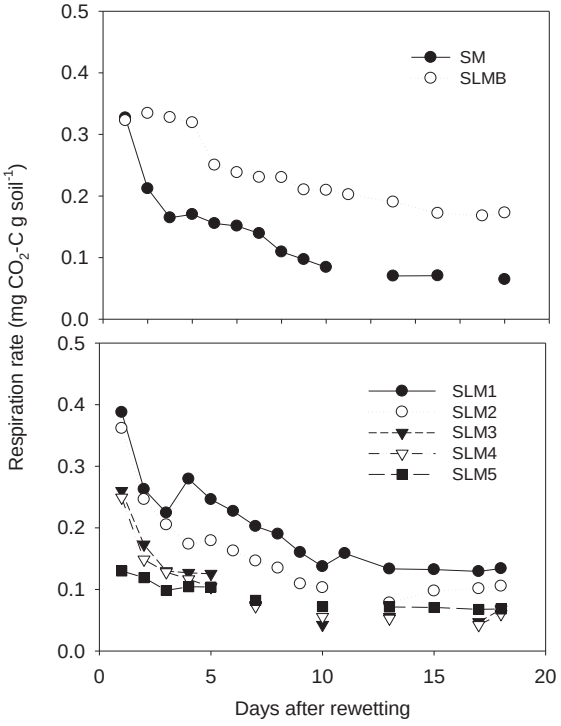


Figure 3. Respiration rates in the seven soils over 38 days after rewetting.

**Experiment 3**

The matric potential ranged between -0.10 and -1.46 MPa, while the osmotic potential ranged between -0.05 and -3.21 MPa (Table 4). Respiration rates and cumulative respiration were strongly affected by EC and water content, both decreasing with increasing EC and decreasing water content (Figures 4, 5).

For example, respiration rate on day 1 compared the non-saline soils was less than 30% at EC42.0 and compared to the optimal soil water content, 20% at WC90 (Figure 4). In contrast to the strong effect of EC and water content on respiration, the effect of adaptation on respiration rate was small and transient. Significant differences in respiration

rate between adapted and non-adapted soils were detected only during the first 3 days. On day 1, respiration rates were higher in adapted compared to non-adapted soils with generally greater differences at lower EC and higher water content. Compared to day 1, respiration rates on day 2 were lower in adapted soils but remained similar in non-adapted soils. There were no significant differences in respiration rates between adapted and non-adapted soils on day 2. The same is true for the respiration rates on day 3 in the soils incubated at different water content. In the soils with different ECs, respiration rates on day 3 were significantly higher in non-adapted soils than in adapted soils up to EC7.7 but not at higher EC levels. The decrease in respiration rates from day 2 to day 3 was greater in adapted than in non-adapted soils.

Cumulative respiration after 14 days was similar in adapted and non-adapted soils and strongly affected by EC and water content (Figure 5). Cumulative respiration compared to the non-saline soils was 40% at EC42.0 and compared to the optimal soil water content 13% at WC90.

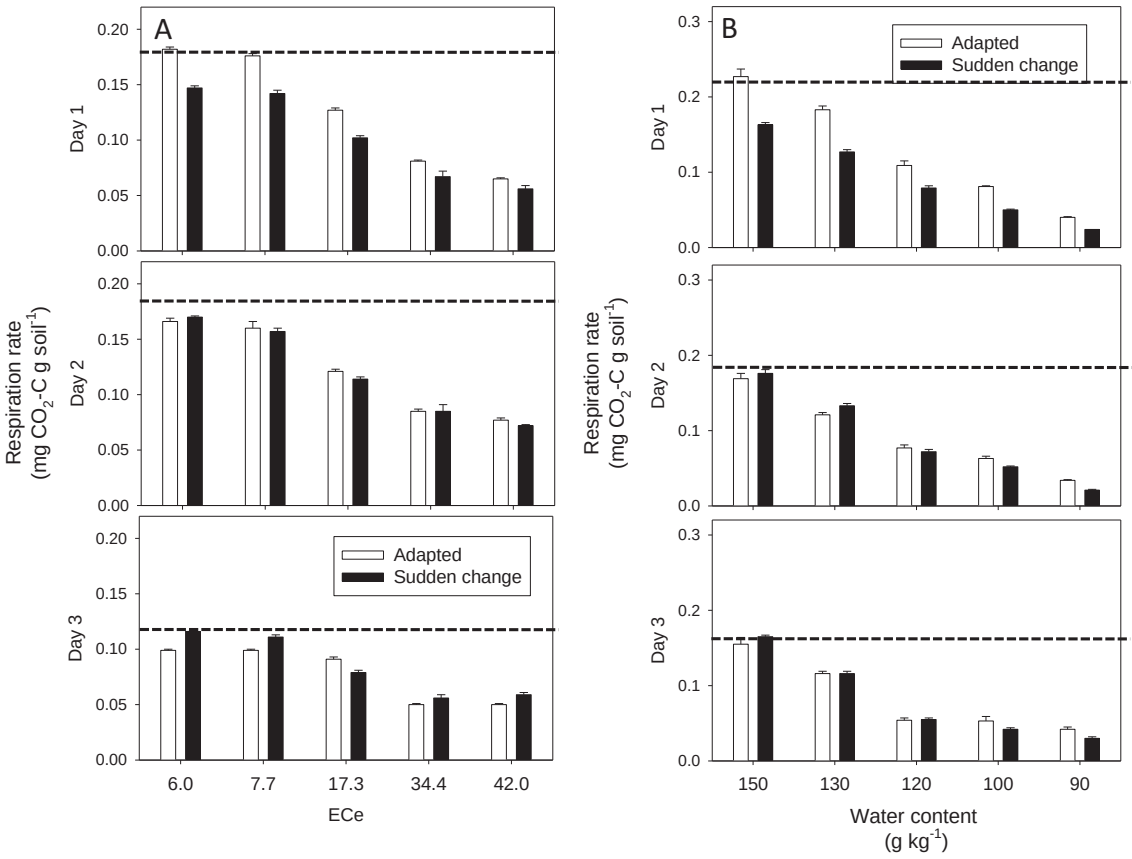


Figure 4. Respiration rates in the first 3 days after residue addition and, for the non-adapted soils, the change in osmotic (A) or matric potential (B). Dashed line indicates respiration rates of the control (optimal water content, no salt addition).

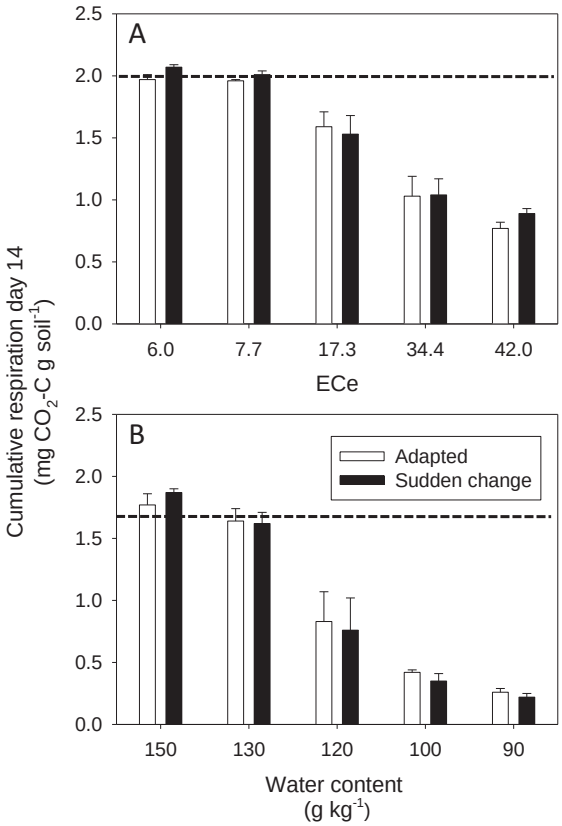


Figure 5. Cumulative respiration 14 days after residue addition and, for the non-adapted soils, the change in osmotic (A) or matric potential (B). Dashed line indicates respiration rates of the control (optimal water content, no salt addition).

## Discussion

The strong response of soil respiration to soil water content (Figure 2) is in agreement with many previous studies (as reviewed by e.g. Harris, 1981; Killham, 1994) and can be explained by the changes in water and oxygen ($O_2$) availability. In dry soil, water films around aggregates are very thin and water is held tightly on to aggregate surfaces resulting in a lack of water for metabolic activity and diffusion of substrates to the microbes limits their activity (West et al., 1989; Ilstedt et al., 2000). As the water content increases, the thickness of the water film around aggregates increases and small pores become water-filled. The water content optimal for microbial activity is reached when the soil contains enough water to allow diffusion of substrates to the microbes and enzymes

away from them, but large pores are still air-filled to allow gas exchange, i.e. removal of $CO_2$ from respiration and supply of $O_2$ since most soil microorganisms are aerobic (Ilstedt et al., 2000). If the water content increases further, gas exchange is impaired because diffusion of gases is $10^3$-$10^4$ times lower in water than in air (Armstrong, 1979). Hence, anaerobic conditions develop, limiting the activity of aerobe microorganisms. Differential anaerobic microbial communities develop using a succession of alternative electron acceptors, from nitrate at high redox potential over manganese and iron to low molecular weight compounds such as acetic acid at very low redox potential from which methane is produced (Marschner, 1995; Liesack et al., 2000).

The differential response of the different soils to changes in water content is related to their texture. Coarse textured soils have few small pores in which water is retained, but many large pores to allow gas exchange. Therefore water and not oxygen availability governs microbial activity and higher water contents are required for optimal activity in coarse textured soils. Fine textured soils on the other hand, have many small pores to retain water, but very few large pores for gas exchange. Thus, in fine textured soils, oxygen availability limits microbial activity and lower water contents are required for optimal activity.

Due to these differences in water retention properties, it is important to determine the water content that results in optimal microbial activity for each soil to ensure maximal activity in the control soils and/or during pre-incubation before the start of an experiment. Moreover, it is not the water content per se but the availability of the water to microorganisms that determines their activity; water availability is best reflected in water potential because it is a measure of how much energy is required to remove water from the soil. Therefore, in order to compare the effect of water availability on microbial activity in soils, it is necessary to relate microbial activity to water potential not water content (Figure 2B). This is particularly important when comparing different soils since the matric potential at a given soil water content varies between soils (Figure 1). Thus, in order to expose microbes in different soils to the same water stress (matric potential), they have to be adjusted to different water contents, higher water content in coarse soils, lower in fine textured soils (Figure 2B).

Respiration was also strongly affected by EC (osmotic potential) in the sandy loam (Figure 4, 5) showing that not only matric potential, but also osmotic potential affects microbial activity. The negative effect of salinity on microbial activity is in agreement with many other studies (Pathak and Rao, 1998; Rietz and Haynes, 2003; Yuan et al., 2007), but in most of these studies, only the EC is given. However, the EC is a poor measure of salinity stress to microbes because it does not necessarily reflect the salt concentration in the soil solution. At the same $EC_{1:5}$ or $EC_e$, the salt concentration in the soil solution is most likely higher in a coarse textured soil than in a fine textured soil because the former has a lower water content. Thus the same amount of salt (measured as EC) is contained in a smaller amount of water, increasing its concentration and thus decreasing the osmotic potential.

The flush in respiration after rewetting of air-dry soil followed by a rapid decrease within 2-4 days (Figure 3) is in agreement with many previous studies (e.g. Kieft et al., 1987; Fierer and Schimel, 2002; Xiang et al., 2008; Butterly et al., 2009) and can be explained by the release of easily decomposable organic compounds upon rewetting and their depletion. The stabilisation of the respiration rates after about 8 days after rewetting is also in accordance with previous studies (Butterly, unpublished). These steady-state respiration rates may be somewhat higher than in soils that have been stored moist because they contain more C which would have been decomposed during storage in moist soils. Additionally, the community composition of the air-dried and rewet soils may differ from those in moist soils (Hamer et al., 2007; Butterly et al., 2009). Nevertheless, it can be assumed that the microbial community in the air-dried and rewet soils is established and in equilibrium within 8 days after rewetting. Thus, any effect of a treatment imposed after this period can be attributed to the treatment itself and is not confounded by the previous drying and rewetting.

We had hypothesized that a sudden decrease in matric or osmotic potential may not allow soil microbes to adapt and therefore have a more detrimental effect than in soils where the microbes had time to adapt. Surprisingly, cumulative respiration of adapted and non-adapted soils was the same after the 14-day experimental period (Figure 5). Thus, prior exposure to low matric or osmotic potential for 14 days did not result in greater tolerance to low matric and osmotic potential compared to soils where the potential was adjusted just prior to the start of the respiration measurements. However, there were some

small and transient differences in the first 3 days after adding the residues (Figure 4). On the first day after addition of the residues, respiration rates were higher in the adapted soils compared to the non-adapted soils which had just experienced a change in water potential. However, on day 2 after addition of residues, the respiration rates in the adapted soils were lower than on day 1 whereas they remained stable in the non-adapted soils. On day 3, the respiration rates declined in adapted and non-adapted soils with a smaller decrease in the non-adapted soils. Thus, the adapted microbes could respond more quickly to the increased substrate availability than the microbes which had were exposed to a change in water potential immediately before residue addition. However, the slower decrease in respiration rates in the first three days in the non-adapted soils suggests that a tolerant community developed quickly and could utilise the substrates, whereas the easily decomposable compounds had already been decomposed in the adapted soils. Thus, in the adapted soils, the increase in respiration was rapid but short-lived whereas it was delayed but more sustained in the non-adapted soils. This differential time course explains why there were no differences in cumulative respiration between adapted and non-adapted soils after 14 days.

Therefore, we have to decline the hypothesis that a sudden decrease in matric or osmotic potential may not allow soil microbes to adapt and therefore have a more detrimental effect than in soils where the microbes had time to adapt. The results suggest, that although a sudden change in water potential transiently reduces microbial activity, microbes can adapt to decreases in water potential within a few days and then utilise available substrates to the same extent as adapted microbes.

However, it should be noted that due to the lack of easily available substrates, microbial activity was very low during the adaptation period in all treatments, therefore it is likely that only a small proportion of the microbial community was active and those that were active in the adapted soils may not have been able to accumulate osmolytes. After addition of the pea straw, many of the previous inactive microbes would become active. These freshly activated microbes in the adapted soils may therefore experience a sudden stress and the need to accumulate osmolytes similar to the microbes in the non-adapted soils. Therefore, to ascertain if adaptation increases tolerance to low water potential, easily available substrates should be added during the adaptation period to allow a greater proportion of the microbial population to be active and hence, adapt.

## Conclusion

These experiments provided the following information that is important for the other experiments reported in this thesis:

- For the seven soils to be used, the water retention curve was determined which allows adjusting the soils to a desired matric potential. This will enable comparison between soils of different texture because the basis for this comparison will be matric potential, hence the actual work needed to withdraw water from the soil and not water content.

- The determination of the water content (matric potential) that results in maximal activity for each soil is important to ensure that matric potential is not limiting microbial activity during the pre-incubation and in the control treatments.

- The constant respiration rates 8 days after rewetting of air-dry soil show that a per-incubation period of 10 days is sufficient to achieve a stable microbial activity; it can be assumed that the microbial community has recovered from the effect of storage and rewetting. In the experiments described in this thesis, any treatments will be imposed after a pre-incubation of 10 days.

- Since adapted and non-adapted microbial communities do not seem to differ substantially in their response to low water potential, differential water potentials will be imposed at the start of the experimental period in most experiments.

## References

Armstrong, W., 1979. Aeration in higher plants. Advances in Botanical Research 7, 225-232.

Beales, N., 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: A review. Comprehensive Reviews in Food Science and Food Safety 3, 1-20.

Butterly, C.R., Bunemann, E.K., McNeill, A.M., Baldock, J.A., Marschner, P., 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass

during repeated drying and rewetting of soils. Soil Biology & Biochemistry 41(7), 1406-1416.

Butterly, C.R., Marschner, P., McNeill, A.M., Baldock, J.A., 2010. Rewetting CO2 pulses in Australian agricultural soils and the influence of soil properties. Biology and Fertility of Soils 46(7), 739-753.

Day, P. R., 1965. Particle fractionation and particle size analysis. In: C.A. Black, D.D. Evans, L.E. Ensminger, J.L. White and F.E. Clark, Editors, *Methods of Soil Analysis. Part 1. Physical and Mineralogical Properties*, American Society of Agronomy, Madison, WI, USA, pp. 545–567.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology and Biochemistry 33, 1599-1611.

Diaz-Ravina, D., Baath, E., 1996. Development of metal tolerance in soil bacterial communities exposed to experimentally increased metal levels. Applied and Environmental Microbiology 62, 2970-2977.

Fierer, N., Schimel, J.P., 2002. Effect of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777-787.

Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67, 798-805.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64, 1630-1637.

Hamer, U., Unger, M., Makeschin, F., 2007. Impact of air-drying and rewetting on PLFA profiles of soil microbial communities. Journal of Plant Nutrition and Soil Science 170, 259-264.

Harris, R.F., 1981. Effect of water potential on microbial growth and activity. Water potential relations in soil microbiology. J. F. Parr and W. R. Gardner. Madison, Soil Science Society America Special Edition #9, 23-95.

Hillel, D., 1980. Fundamentals in soil physics. New York, Academic Press.

Ilstedt, U., Nordgren, A., Malmer, A., 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biology and Biochemistry 32, 1594-1599.

Kieft, T.L., Soroker, E., Firestone, M.K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biology and Biochemistry 19, 119-126.

Killham, K., 1994. Soil Ecology. Cambridge, Cambridge University Press.

Killham, K., Firestone, M.K., 1984. Salt stress control of intracellular solutes in Streptomycetes indigenous to saline soils. Applied and Environmental Microbiology 47, 301-306.

Klute, A., 1986. Water retention: laboratory methods. Methods of soil analysis, Part 1. A. Klute. Madison, Soil Science Society of America 635-660.

Liesack, W., Schnell, S., Revsbech, N.P., 2000. Microbiology of flooded rice paddies. FEMS Microbiology Reviews 24, 625-645.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. London, Academic Press.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biology and Biochemistry 37, 339-347.

Oren, A., 2001. The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implication of the functioning of salt lake ecosystems. Hydrobiologia 466, 61-72.

Pathak, H., Rao, D.L.N., 1998. Carbon and nitrogen mineralisation from added organic matter in saline and alkali soils. Soil Biology and Biochemistry 30, 695-702.

Pettersson, M., Baath, E., 2004. Effects of the properties of the bacterial community on pH adaptation during recolonisation of a humus soil. Soil Biology and Biochemistry 36, 1383-1388.

Rengasamy, P., 2006. Soil salinity and sodicity. Growing crops with reclaimed wastewater. D. Stevens, CSIRO, 125-138.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. Soil Science 78(2), 7-33.

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biology and Biochemistry 35, 845-854.

Schimel, J.P., Scott, W.J., Killham, K., 1989. Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. Applied and Environmental Microbiology 55, 1635-1637.

Walkley, A. and Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science 37, 29–38.

West, A.W., Sparling, G.P., Speir, T.W., 1989. Microbial activity in gradually dried or rewetted soils as governed by water and substrate availability. Australian Journal of Soil Science 27, 747-757.

Xiang, S.R., Doyle, A., Holden, P.A., Schimel, J.P., 2008. Drying and rewetting effects on C and N mineralisation and microbial activity in surface and subsurface Californian grassland soils. Soil Biology and Biochemistry 40, 2280-2289.

Yuan, B.C., Li, Z.Z., Liu, H., Gao, M., Zhang, Y.Y., 2007. Microbial biomass and activity in salt-affected soils under arid conditions. Applied Soil Ecology 35, 319-328.

# CHAPTER 3

## Response of microbial activity and community structure to decreasing soil osmotic and matric potential

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# CHAPTER 4

## Soil microbial activity and community composition : Impact of changes in matric and osmotic potential

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# CHAPTER 5

## Recovery of soil respiration after drying

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## Recovery of soil respiration after drying.

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**Abstract**

*Aim*: Soils are exposed frequently to drying and wetting events and previous studies have shown that rewetting results in a strong but short-lived flush of microbial activity. The aim of this study was to determine the effect of the water content during the dry period on the size and duration of the flush and on the rate of recovery.

*Methods*: Two soils (a sand and a sandy loam) were maintained at different water contents (WC) 30, 28 and 25 g water $kg^{-1}$ soil (sand) and 130, 105 and 95 g water $kg^{-1}$ soil (sandy loam) for 14 days, then rewet to the water content at which respiration was optimal [WC 35 (sand), WC200 (sandy loam)] and maintained at this level until day 68. Ground pea straw (C/N 26) was added and incorporated on day 1. The controls were maintained at the optimal water content throughout the 68 days.

*Results*: Respiration rates during the dry phase (days 1-14) decreased with decreasing water content. The flush of respiration after rewetting peaked on day 15 in the sandy loam and on day 16 in the sand; it was greatest in the soils that had been maintained at the lowest water content [WC25 (sand) and WC95 (sandy loam)]. Cumulative respiration during the remainder of the incubation period in which all soils were maintained at optimal water content increased more strongly in all the soils that had been dry compared to the constantly moist control. On the final day of the dry period (day 14), cumulative respiration in the dry soils was 29-65% (sand) and 67-94% (sandy loam) of the constantly moist control whereas on day 68 it was 80-84% (sand) and 86-96% (sandy loam). The greater

increase in cumulative respiration in the previously dry soils can be explained by the reduced decomposition rates during the dry period which resulted in higher substrate availability on day 14 compared to the constantly moist control. Microbial community structure assessed by phospholipid fatty acid analyses changed over time in all treatments but was less affected by water content than respiration; it differed only between the highest and the lowest water content. These differences were maintained throughout the incubation period in the sandy loam and transiently in the sand.

*Conclusions*: The soil water content during the dry phase affects the size of the flush in microbial activity upon rewetting and that microbial activity in previously dried soils may not be fully restored even after 54 days of moist incubation, suggesting that drying of soil can have a significant and long-lasting impact on microbial functioning.

**Keywords**: drying and rewetting; respiration rate; water potential; microbial community composition.

## Introduction

Top soils often dry out during the summer, particularly in Mediterranean climates. The soils experience drying and rewetting events as the dry periods may be interrupted by occasional rainfall events.

As a soil dries, water is held in increasingly smaller pores and the water films around aggregates become thinner and disconnected (Ilstedt et al. 2000); decreasing water availability and increasing the energy required to withdraw water. Moreover, substrate and nutrient diffusion are restricted and microbes become substrate-limited (Stark and Firestone 1995). Water availability can be expressed as water potential, i.e. with low (more negative) potential indicating high energy requirement. The water potential is the sum of various potentials; in soils, the most important potentials are matric potential (a measure of how strongly the water is held onto soil surfaces) and osmotic potential (a function of the concentration of soluble salts in the soil solution).

Decreasing water content also affects the osmotic potential, because the salt concentration in the remaining soil solution increases as the water content decreases. Hence, microorganisms will also experience decreases in osmotic potential as the soil dries (Chowdhury et al. 2011).

Stressors, such as low water potential, impose a metabolic burden on the surviving microbes due to the need for stress tolerance mechanisms (Harris 1980; Oren 1999; Schimel et al. 2007). In response to low water potential, some microbes accumulate osmolytes which prevent the movement of water out of the cells (Oren 2001).

Rewetting dry soil induces a flush of respiration that usually occurs within a few hours after rewetting and is followed by a rapid decrease in respiration rates (Butterly et al. 2009; Franzluebbers et al. 1994; Kieft et al. 1987; Mikha et al. 2005). The flush of respiration upon rewetting has been explained by increased substrate availability due to release of the osmolytes accumulated during the dry phase, cell lysis and breakdown of aggregates which releases previously protected organic matter (Denef et al. 2001; Fierer and Schimel 2003; Halverson et al. 2000; Sparling et al. 1985). In a soil in which the size and activity of the microbial biomass was manipulated by the addition of different substrates, the size of the flush after rewetting was greatest in the treatment with the largest and most active biomass (Butterly et al. 2009). Drying and rewetting may also change microbial community composition (Butterly et al. 2009; Hamer et al. 2007; Schimel et al. 2007; Wilkinson et al. 2002; Williams 2007), which suggests that some microbial species or groups are more susceptible to drying and rewetting stress than others. For example, fast-growing organisms are more likely to die in dry soil and are more susceptible to rewetting than slow-growing microbes (Bottner 1985; Van Gestel et al. 1993). Thus, after drying and rewetting events, slower growing microbes may dominate (Bottner 1985; Cortez 1989). In a forest soil, rewetting of dry soil induced a shift towards gram-positive bacteria and an increase in bacteria/fungi ratio (Hamer et al. 2007). The effect of single and multiple drying and rewetting cycles on the size of the flush upon rewetting has been studied extensively (e.g., Butterly et al. (2009); Mikha et al. (2005); Van Gestel et al. (1993)), however in most studies, the water content of the soils during the dry phase was reduced to very low levels: air-dry or until the soils did not lose any more water at room temperature.

Little is known about the impact of the water content during the dry phase on the size of the flush upon rewetting and how long after rewetting it takes for microbial activity (measured by cumulative respiration) to return to the level of the constantly moist control.

Recovery after stress/disturbance is an important feature in ecosystem sustainability. Recovery can be partial or complete and the length of time required for recovery varies. For example, after soil disinfection, the capacity to decompose glucose and chitin was fully restored after 12 weeks (Wada and Toyota 2007). But after heating of soil to a temperature experienced during forest fire (200°C for one hour), bacterial density and activity were still less than the controls even after 15 weeks incubation (Diaz-Ravina et al. 1996). Bacterial community composition had recovered 56 d after gamma-irradiation, whereas the fungal community did not (McNamara et al. 2007). Substrate-induced respiration recovered within six days after rewetting of air-dry soil, but the rate of degradation of the fungicide metalaxyl-M and the insecticide lufenuron was still reduced even after 34 days (Pesaro et al. 2004). Microbial biomass C and N recovered completely after drying and rewetting within 24 d moist incubation in a soil with low soil organic matter content, but had recovered to only 50% of the moist control in a soil with high soil organic matter content (Hamer et al. 2007), which is in contrast to the study by De Nobili et al. (2006) who found that recovery after rewetting of dry soil was more rapid in soil with higher SOM. Orwin et al. (2006) reported that recovery after drying and rewetting was not correlated with resource availability (soil C, N, P).

The aim of this study was to determine the effect of soil water content during the dry period on the magnitude of the flush of respiration after rewetting and the time for recovery in two soils, a sand and a sandy loam. We tested the following hypotheses: (1) the flush in respiration will be greatest in the treatment with the lowest water content during the dry period; and (2) the time to recovery will be shortest and the extent of recovery greatest in the treatment with the highest water content during the dry period.

## Materials and Methods

Two soils from Monarto (35° 05´ S and 139° 06´ E) and Mount Bold (38° 11´ S and 138° 69´ E), South Australia differing in texture were used in the study: a sand (sand

91.3%, silt 5%, clay 3.7%, pH 7.7, EC1:5 150 µS/cm, N 0.09%, C 0.69%, water holding capacity 6.7%) and a sandy loam (sand 57.5%, silt 25%, clay 17.5%, pH 5.2, EC 1:5 68 µS/cm, N 0.33%, C 3.65%, water holding capacity 36.4%). After collection, the soils were air-dried and sieved to <2mm.

The moisture retention curves of the soils were determined with suction and pressure techniques (Klute 1986) and given in (Chowdhury et al. 2011).The osmotic potential at a given water content was estimated using the equation of Richard (1954).

Previous experiments had shown that maximal respiration occurred at matric potential -0.03 MPa in the sand (35 g water $kg^{-1}$ soil) and -0.10 MPa in the sandy loam (200 g water $kg^{-1}$ soil) (Chowdhury et al. 2011).The air-dry soils were pre-incubated at these optimal water contents for 10 days at 25°C before the experiment was begun. Ten days was chosen on the basis of several experiments with a range of soils (including the two used in the present study) which showed that respiration rates stabilized 7-10 days after rewetting air-dry soil (unpublished data). At the end of the pre-incubation, the soils were dried to the desired water content in a fan forced oven at 25°C. The selection of water contents was based on previous experiments (Chowdhury et al. 2011), ranging from the optimal to that at which respiration was decreased by 30-50%. The sand was adjusted to 30, 28 and 25 g water $kg^{-1}$ soil, the sandy loam to 130, 105 and 95 g water $kg^{-1}$ soil. The control soils were maintained at the optimal water content (35 and 200 g water $kg^{-1}$ soil for the sand and the sandy loam, respectively). In the following, the treatments are referred to as WC35, WC30, WC28 and WC25 (sand) and WC200, WC130, WC105 and WC95 (sandy loam).

After adjusting the water content, pea (*Pisum sativum*) straw (C/N 26, ground and sieved to 0.25 - 2 mm) was mixed into the soils (2% w/w) to provide a readily available nutrient source. The amended soils were then added into PVC cores and the cores were placed into glass jars (as described below) for respiration measurement (day 0). Respiration was measured over 68 days. On day 14, water was added to the dried soils to bring the water content of all treatments to the optimal water content. This water content was maintained until day 68.

*Analyses*

Pre-incubated sand (40 g) or sandy loam (30 g), was added to PVC cores (diameter 3.7 cm, height 5 cm) with a nylon mesh base (0.75 µm, Australian Filter Specialist) and packed to a bulk density according to their texture (http://www.pedosphere.com/resources/bulkdensity/triangle_us.cfm): 1.55 g $cm^{-3}$ (sand) and 1.46 g $cm^{-3}$ (sandy loam). The cores were placed immediately into 1 L glass incubation jars and sealed with gas tight lids equipped with septa to allow headspace sampling. The glass jars were incubated in the dark at 22-25 °C. Respiration was quantified by measuring headspace $CO_2$ concentrations at regular intervals using a Servomex 1450 infra-red gas analyser (Servomex Group, Crowborough, England): daily in the first 20 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. The water content was maintained by weighing the cores and adding reverse osmosis water.

The percentage of C remaining of the added pea straw was calculated from cumulative respiration and the amount of C added on day 0. This calculation assumes that only the added pea residues are decomposed and that native SOM does not contribute to the respiration.

The microbial community structure was assessed on days 5, 14, 20 and 68 by phospholipid fatty acids (PLFA) analysis (based on (Frostegård et al. 1993)). Phospholipid fatty acids are components of cell membranes and rapidly dephosphorylated upon cell death; therefore PLFAs represent the living biomass (White 1995). The PLFA patterns provide a coarse measure of microbial community structure. Microbial groups such as bacteria and fungi, but not species or genotypes, differ in PLFA composition of their membranes; the so-called signature fatty acids can be used as a measure of abundance of microbial groups: Gram-positive bacteria i15:0 and i16:0; Gram-negative bacteria 16:1ω7c and 18:1ω7; actinomycetes 10ME-17:0 and 10ME-18:0, and fungi 18:2ω6, 18:1ω9 and 18:3ω6 (Kandeler 2007; Zak et al. 2000; Zelles et al. 1995).

Freeze-dried soil (4 g) was extracted with a one-phase solvent of chloroform, methanol and citrate buffer (1:2:0.8 v/v/v). The lipid-containing phase was collected and dried under a stream of nitrogen at 40°C. The PLFAs were separated from other fatty acids using silicic acid columns (Supelclean LC-Si-SPE Tubes, Supelco). The columns were washed sequentially with chloroform, acetone, and methanol, collecting the methanol fraction which contains the PLFAs. After alkaline methanolysis the organic phase was collected in dichloromethane. An internal standard (methylnonadecanoate, 19:0) was added to each sample.

The fatty acid methyl esters were separated in a gas chromatograph with a flame ionization detector (GC-FID) (HP 6890) using an SP-2560 fused silica capillary column (75 m, 180 μm × 0.14 μm film thickness; Supelco, Sigma-Aldrich, Australia) with helium as carrier gas. The injector temperature was 250°C and the detector temperature was 260°C. The temperature program was as follows: after an initial temperature of 140°C, the temperature was ramped at 4°C/min to 240°C, and then held for 15 min.

The individual PLFA peaks were identified by comparing retention times with peaks of Supelco 37 standard mixture (Supelco, Bellefonte, PA) and peaks identified by GC-MS (gas chromatograph combined with mass selective detector HP 5973) using the same column and temperature program conditions and carrier gas as described above. Electron energy in electron impact was 70 eV. Mass spectrometer peak identification was based on comparison with the software library NIST02.L. The amounts of individual PLFAs are expressed in μg/g soil.

*Statistical analysis*

Significant differences between different treatments in a given soil over time in cumulative respiration, sum of PLFAs, sum of bacterial and fungal PLFAs were assessed by 2-way ANOVA and Tukey test with $P \leq 0.05$. (GenStat® for Windows 8.0, VSN Int. Ltd, UK, 2005). Regressions between matric or water potential and relative cumulative respiration were calculated in MS Excel.

For statistical comparison of the microbial community structure, PLFA patterns, transformed as log (x+1), were analysed by Primer E software (Primer-E Ltd, Plymouth

Marine Laboratory, Plymouth, UK) and plotted using non-metric multi-dimensional scaling (MDS). The PLFA data were (x+1) transformed to balance the contributions of fatty acids by down-weighing the dominant fatty acids and increasing the weighting of rare fatty acids (Clarke and Warwick 2001). MDS plots with a 2D stress < 0.2 are considered to represent a good reflection of the overall structure of the communities. Significant differences in microbial community structure between treatments were determined by PERMANOVA ($P \leq 0.1$).

## Results

### *Potentials*

During the first 14 days (dry period), the matric potential in the sand ranged from -0.16 to -0.44 MPa and the water potential from -1.04 to -1.62 MPa (Table 1). In the sandy loam the matric potential ranged from -0.10 to -1.30 MPa and the water potential from -0.19 to -1.56 MPa. The osmotic potential was higher in the sand than in the sandy loam, ranging from -0.88 to -1.18 MPa, compared to only -0.09 to -0.26 MPa in the sandy loam.

### *Respiration rates*

In the sand in the first 24 hours after adjusting the soil water content and incorporating the pea straw (day 0), respiration rates decreased with decreasing water content (Figure 1A). At the lowest water content (WC25) respiration rates were 72% lower than in the control WC35. On day 14, respiration rates had decreased in all treatments and were still lowest at WC25, although the difference between WC25 and WC35 was less than on day 1 (32%).

Respiration rates in the sand peaked two days after adjusting all treatments to the optimal soil water content (day 16). On day 16, respiration rates were highest in WC25 and WC28, being more than 30% greater than in the constantly moist treatment WC35. After day 16 respiration rates declined over time in all treatments, with the decline being greatest in WC35. Compared to WC35, respiration rates at WC25 were 82% higher on day 20 and

62% higher on day 32. At the end of the experiment (day 68), respiration rates were low in all treatments.

Respiration rates in the sandy loam on day 1 were also lowest at the lowest water content (WC95), being 45% of that at the highest water content (WC200) (Figure 1 B). Respiration rates decreased from day 1 to day 14, but the decrease was greatest in WC200; thus on day 14, respiration rates at WC95 were 22% higher than in WC200. In contrast to the sand, respiration rates peaked one day after rewetting (day 15). On the first (day 15) and second day (day 16) after rewetting, respiration rates were more than two-fold higher in WC95 than in WC200. Respiration rates decreased over time, but, as in the sand, the decrease was greater in WC200. The relative difference between WC200 and WC95 decreased over time, with the respiration rates in WC95 being 70% higher on day 20 but only 10% higher on day 36. On day 68 respiration rates were low in all treatments.

*Cumulative respiration*

Cumulative respiration in the sand on day 1 decreased with water content; at WC25 it was only 28% of WC35 (Figure 2 A). The relative differences among the treatments remained the same during the 14 day dry period. On day 15 [1 day after adjusting to the optimal water content (35 g water $kg^{-1}$ soil)], cumulative respiration decreased with water content; in WC25, it was only 28% of that at the highest water content (WC35). On the second day after rewetting, relative cumulative respiration in WC25 had increased, but was still only 36% of WC35. However by day 20, relative cumulative respiration in WC25 had increased to 51% of WC35. The relative cumulative respiration increased over time particularly in WC25, so that on day 68, all treatments that had been exposed to drying had reached 80% of the cumulative respiration of WC35.

Of the C added with the pea straw (assuming no decomposition of native SOM), only 70% remained on day 16 in WC35 whereas 89% remained in WC25. This difference became smaller over time, on day 36, 58% and 71% remained at WC35 and WC25, respectively. By the end of the experiment, the difference was even smaller, 49% and 59% remained at WC35 and WC25, respectively.

To assess respiration per unit biomass, cumulative respiration at given sampling time was divided by the sum of PLFAs at this date. Cumulative respiration per unit PLFA was always highest in WC35 (0.18 mg $CO_2$-C μg PLFA on day 1), but the difference to the dried treatments decreased over time. On day 1 cumulative respiration per unit PLFA in WC25 was only 21% of that in WC35, whereas it was 76% on day 68.

Similar responses were found in the sandy loam: cumulative respiration on day 1 decreased with water content; at WC95, it was only 45% of that at WC200 (Figure 2 B). But unlike the sand, cumulative respiration in the sandy loam increased more strongly in the dry treatments (WC95, WC105 and WC130) than in WC200. Thus, the relative differences between the moist control and the drying treatments decreased during the 14 day dry period. On day 14, cumulative respiration in WC95 was 67% of WC200. The difference to WC200 continued to decrease during the moist incubation. By day 20, cumulative respiration in WC95 was 80% of WC200, and by day 68 it was 86%. In WC105 and WC130, cumulative respiration was less than 10% lower than in WC200.

Of the C added, 63% remained on day 14 in WC200 whereas 75% remained in WC95. Even more pronounced than in the sand, the differences between the moist and the dried soil decreased over time. On day 32, 53% of the added C had been decomposed in WC200, compared to 60% in WC95. On day 68, 36% remained in WC200 and 45% in WC95.

Cumulative respiration per unit PLFA was lower in the sandy loam than in the sand (0.04 mg $CO_2$-C μg PLFA on day 1 in WC200), and, except for day 1, there were no significant differences in this parameter among the treatments (data not shown).

*Correlation between cumulative respiration and matric or water potential*

When analysed separately, cumulative respiration (in percentage of the constantly moist control) in the sand and the sandy loam was positively correlated with matric and water potential, i.e. cumulative respiration decreased with decreasing matric or water potential. However the relationship was strongest on day 1 ($r^2$=0.91 in both soils). On day 32, the correlation coefficient was 0.51 in the sand and 0.73 in the sandy loam. On day 68, there was no correlation between matric or water potential and cumulative respiration in

the sand, whereas in the sandy loam cumulative respiration was correlated with both potentials ($r^2$ = 0.77). To assess if the relationship between relative cumulative respiration and potentials can be applied across both soils, the data of the two soils was combined. Relative cumulative respiration was weakly correlated with matric potential on day 1 but not on the other days ($r^2$=0.17) (Figure 3). However, it was correlated with water potential at all sampling times ($r^2$=0.56-0.73) with no clear trend over time.

*Microbial community structure*

On day 20 (6 days after rewetting), the sum of PLFAs was 4.3 (sand) and 15.0 μg $g^{-1}$ soil (sandy loam). The abundance of bacterial fatty acids was 1.8 (sand) and 6.5 μg $g^{-1}$ soil (sandy loam) and that of fungi was 1.9 (sand) and 3.8 μg $g^{-1}$ soil (sandy loam). In both soils, the sum of total, bacterial and fungal PLFA did not change over time and there were no clear trends among the treatments (data not shown). The Permanova results of the PLFA patterns indicate that the microbial community structure differed between the highest and the lowest water content on days 14 and 20 in the sand and up to day 68 in the sandy loam (Figure 4). In all treatments and both soils, microbial community structure on day 68 differed from that on day 14.

## Discussion

This study showed that microbial activity is reduced by low water content and recovers after rewetting, but it remained below that of the moist control 54 days after rewetting. Whereas microbial activity during the dry phase and after rewetting was strongly affected by water content, microbial community structure differed only between the lowest and the highest water content.

*Respiration during the dry phase and flush after rewetting*

In the first 14 days, the decrease in respiration rates was greatest in the moist control which is probably due to the strong depletion of labile substrates in the first few days after residue addition compared to the soils with lower water content where the initial respiration rates were lower (Figure 1). Thus, the smaller difference among treatments

between respiration rates on day 14 compared to day 1 are due to the smaller decrease in respiration rates in the soils with lower water content and does not necessarily indicate adaptation of the microbes to low water content. Indeed, the relative difference in cumulative respiration between treatments was similar on days 1 and 14 in the sand indicating the lack of adaptation (Figure 2). However in the sandy loam, the relative difference between the dry soils and the moist control was greater on day 1 (29-55% lower) than on day 14 (6-33% lower). This suggests that the microbes in the sandy loam were able to adapt to the lower water content.

In agreement with our first hypothesis, the magnitude of the rewetting flush was greatest in the soils with lowest water content (Figure 1). This may be explained by the substrate availability upon rewetting which is likely to be greatest in the treatment with the lowest water content because: (i) microbes have accumulated more osmolytes during the dry period compared to the soils with the higher water content and these osmolytes are released/and or metabolised upon rewetting; and (ii) very dry aggregates may be more vulnerable to breakdown upon rewetting because the influx of water is greater due to the strong gradient in potential towards the centre of the aggregates.

The rewetting flush occurred on the first day after rewetting in the sandy loam but was delayed by two days in the sand (Figure 1). At WC30 and 28 in the sand, this may be due to the lower water potential compared to WC130 and WC103 in the sandy loam (Table 1). However, although the WC25 in the sand and WC95 in the sandy loam had approximately same water potential (-1.62 and -1.56 MPa), the response to rewetting was delayed only in the sand. And this delay occurred although a higher percentage of the added C remained on day 14 in the sand (92%) than in the sandy loam (75%).

The delayed response to rewetting in the sand may be explained by three factors: (i) a microbial biomass which was 3-4 fold lower than in the sandy loam; this would not only limit the concentration of osmolytes released from the cells upon rewetting, but also the capacity of the surviving microbes to utilise the substrates immediately; (ii) fewer small pores than in the sandy loam, which may still contain water when the large pores are already drained, allowing microbes within them to survive in otherwise dry soil ; and (iii) the lower osmotic potential which could have resulted not only in reduced water availability but also ion toxicities and element imbalances.

*Recovery of respiration during moist incubation*

Although respiration rates decreased over time during the moist incubation in all treatments, the decline was less in the previously dried soils (Figure 1), presumably due to increased substrate availability as decomposition rates in the first 14 days were lower. On day 14, at least 10% more of the C added with the pea straw was still available at the lowest water content compared to the moist control. At the end of the experiment (day 68), after the previously dried soils had been incubated for 54 days at optimal water content, cumulative respiration had recovered to between 80 and 96% of the moist control in both soils, with the recovery being greater in the sandy loam (Figure 2). By then, respiration rates were very low and similar in all treatments; therefore a full recovery of the dry treatments is unlikely even if the moist incubation were to be continued for longer.

The second hypothesis (i.e. the time to recovery will be shortest and the extent of recovery greatest in the treatment with the highest water content during the dry period) was based on the premise that a lower water stress during the dry period would allow microbes to recover more rapidly and to a greater extent than microbes exposed to more severe drying stress. However, this hypothesis is only supported by the cumulative respiration in the sandy loam where WC130 reached similar values as in the moist control after 20 days whereas cumulative respiration in WC95 was still 14% lower than the moist control on day 68 (Figure 2). However in the sand, cumulative respiration in all dried treatments was similar on day 68 (17-20% lower than the control) and the rate of increase in cumulative respiration was greater in WC25 (increasing from 32% of the control on day 15 to 80% of the control on day 68) than in WC30 (increasing from 67% of the control on day 15 to 84% of the control on day 68). This suggests that in the sand, the impact of water stress in all drying treatments was similar; indeed, water potential varied only between -1.25 and -1.62 MPa. In contrast, water potential in the sandy loam varied from -0.31 to -1.56 MPa. Despite the smaller differences in water potential between the drying treatments in the sand, the amount of C added remaining on day 14 in WC25 was 10% lower than in WC30, indicating that the microbes were less active during the dry period. A similar difference in substrate utilisation between WC95 and WC130 was recorded in the sandy loam. Thus, although the greater rate of recovery in WC25 can be attributed to the higher substrate availability, a stronger increase in cumulative respiration in WC95 should also have occurred in the sandy loam. However, whereas only 75% of the added C remained on day 14 in WC95 in

the sandy loam, 92% remained in WC25 in the sand. This greater percentage of the C remaining from the added residues in the sand may have included a greater amount of easily available compounds which could lead to higher recovery rates in the sand.

Although differential substrate availability can help explain the results of the present experiment, the importance of substrate availability in recovery after drying and rewetting is controversial. Whereas De Nobili et al. (2006) found that recovery after rewetting of soil was more rapid in soil with higher soil organic matter content, the opposite was reported by Hamer et al. (2007) and in the study by Orwin et al. (2006) recovery after drying and rewetting was not correlated with resource availability (soil C, N, P).

*Response of the microbial community structure to water content*

Whereas microbial activity (respiration rates and cumulative respiration) differed among the treatments in both soils, the abundance of bacteria or fungi was not affected by differential extent of drying in either the sand or the sandy loam, and microbial community structure differed only between the highest and the lowest water content (Figure 4). This suggests that, in contrast to microbial activity, community structure is less sensitive to water content. Moreover, differences in response to drying and subsequent recovery cannot be explained by differences in community structure. However, it should be noted that PLFA is only a relatively coarse measure of community structure because it does not provide information on species or genotype level. For this, DNA-based methods are required. It is possible that such methods could reveal changes in relative abundance of certain species, e.g. within bacteria, fungi or functional groups such as nitrifying bacteria.

In many previous studies, microbial communities in soils exposed to one or several drying and rewetting cycles differed from those in constantly moist soils (Butterly et al. 2009; Gordon et al. 2008; Hamer et al. 2007; Schmitt and Glaser 2011), but not always (Butterly et al. 2009; Griffiths et al. 2003). Of these studies, most used fatty acid analysis, only Griffiths et al. (2003) assessed bacterial community structure by DNA-based methods. In the studies that reported changes in community structure, the soils were dried to very low water contents - lower than in the present study. Therefore, the difference in community structure between the highest and the lowest water content here is in agreement with those previous studies. Interestingly, these differences in community

structure between the highest and the lowest water content in the present study remained throughout the recovery period in the sandy loam although microbial community structure changed over time in all treatments (Figure 4). Similarly, Pesaro et al. (2004) found that bacterial and archaeal community structure in dried and rewetted soil differed from that in the constantly moist control even after 34 d of moist incubation.

*Relationship between cumulative respiration and water potential*

Cumulative respiration was positively correlated with matric and water potential in both soils, however, the relationship became less strong over time after rewetting which also shows microbial activity recovered in the previously-dried soils (Figure 3). When the data of both soils was combined, cumulative respiration was not related to matric potential, whereas it was positively correlated with water potential (matric+osmotic) throughout the experiment. The poor relationship to matric potential can be explained by the relatively low osmotic potential in the sand, particularly at low water contents. This shows that it is important to consider water potential and not just matric potential when comparing the effect of water stress on microbes in different soils.

## Conclusions

This study showed that the flush in respiration after rewetting of dried soil is greatest in soils that had been exposed to the lowest water content which is most likely due to greater substrate availability upon rewetting. The results also suggest that, even after 54 days at optimal water content recovery after drying may not be complete, particularly in soils that have been exposed to very low water potentials. This indicates that drying of soil can have a significant and long-lasting impact on microbial function. Both extent of stress and recovery after rewetting is soil type dependent. In fine-textured soils, the fraction of microbes located in small pores may be exposed to less severe water stress than those in larger pores and can, therefore, recover more rapidly after rewetting. This needs to be verified using a greater number of soils. This study also highlighted the importance of considering water potential rather than water content or matric potential when comparing water stress between soils.

In this study, the abundance of broad groups (e.g. bacteria or fungi) was little affected by the extent of drying, suggesting that the large differences in respiration were due to modulation of the activity per cell and not changes in community structure. However, it cannot be ruled out that microbial community structure on a finer scale, e.g. genotypes, may change in response to drying or during the following moist period.

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**References**

Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with C-14-labeled and N-15-labelled plant material. Soil Biol Biochem 17: 329-337.

Butterly C R, Bunemann E K, McNeill A M, Baldock J A, Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biol Biochem 41: 1406-1416.

Chowdhury N, Marschner P, Burns R G (2011) Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant Soil. in press.

Clarke K R, Warwick R M (2001) Change in marine communities: an approach to statistical analysis and interpretation. Primer-E, Plymouth.

Cortez J (1989) Effect of drying and rewetting on mineralization and distribution of bacterial constituents in soil fractions. Biol Fertil Soils 7: 142-151.

De Nobili M, Contin M, Brookes P C (2006) Microbial biomass dynamics in recently air-dried and rewetted soils compared to others stored air-dry for up to 103 years. Soil Biol Biochem 38: 2871-2881.

Denef K, Six J, Bossuyt H, Frey S D, Elliott E T, Merckx R, Paustian K (2001) Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol Biochem 33: 1599-1611.

Diaz-Ravina M, Prieto A, Bååth E (1996) Bacterial activity in a forest soil after soil heating and organic amendments measured by the thymidine and leucine incorporation techniques. Soil Biol Biochem 28: 419-426.

Fierer N, Schimel J P (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Sci. Soc. Am. J. 67: 798-805.

Franzluebbers K, Weaver R W, Juo A S R, Franzluebbers A J (1994) Carbon and nitrogen mineralization from cowpea plant parts decomposing in moist and in repeatedly dried and wetted soil. Soil Biol Biochem 26: 1379-1387.

Frostegård A, Bååth E, Tunlid A (1993) Shifts in the strucutre of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol Biochem 25: 723-730.

Gordon H, Haygarth P M, Bardgett R D (2008) Drying and rewetting effects on soil microbial community composition and nutrient leaching. Soil Biol Biochem 40: 302-311.

Griffiths R I, Whiteley A S, O'Donnell A G, Bailey M J (2003) Physiological and community responses of established grassland bacterial populations to water stress. Appl Environ Microbiol 69: 6961-6968.

Halverson L J, Jones T M, Firestone M K (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci Soc Am J 64: 1630-1637.

Hamer U, Unger M, Makeschin F (2007) Impact of air-drying and rewetting on PLFA profiles of soil microbial communities. J Plant Nutrit Soil Sci 170: 259-264.

Harris R F (1980) Effect of water potential on microbial growth and activity. In Water potential relations in soil microbiology. pp 23-95. Soil Science Society America, Madison.

Ilstedt U, Nordgren A, Malmer A (2000) Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biol Biochem 32: 1594-1599.

Kandeler E (2007) Physiological and biochemical methods for studying soil biota and their function. In Soil Microbiology, ecology, and biochemistry. Ed. E A Paul. pp 53-84. Elsevier.

Kieft T L, Soroker E, Firestone M K (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol Biochem 19: 119-126.

Klute A (1986) Water retention: laboratory methods. In Methods of soil analysis, Part 1. Ed. A Klute. pp 635-660. Soil Science Society of America, Madison.

McNamara N P, Griffiths R I, Tabouret A, Beresford N A, Bailey M J, Whiteley A S (2007) The sensitivity of a forest soil microbial community to acute gamma-irradiation. Appl Soil Ecol 37: 1-9.

Mikha M M, Rice C W, Milliken G A (2005) Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biol Biochem 37: 339-347.

Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Molec Biol Rev 63: 334-348.

Oren A (2001) The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implication of the functioning of salt lake ecosystems. Hydrobiologia 466: 61-72.

Orwin K H, Wardle D A, Greenfield L G (2006) Context-dependent changes in the resistance and resilience of soil microbes to an experimental disturbrance for three primary plant chronosequences. Oikos 112: 196-208.

Pesaro M, Nicollier G, Zeyer J, Widmer F (2004) Impact of soil drying-rewetting stress microbial communities and activities and on degradation of two crop protection products. Appl Environ Microbiol 70: 2577-2587.

Richard L A (1954) Determination of the properties of saline and alkali soils. United States Department of Agriculture Handbook 60, Washington, DC. pp. 7-53.

Schimel J P, Balser T C, Wallenstein M (2007) Microbial stress response physiology and its implications for ecosystem function. Ecology 88: 1386-1394.

Schmitt A, Glaser B (2011) Organic matter dynamics in a temperate forest soil following enhanced drying. Soil Biol Biochem 43: 478-489.

Sparling G P, Whale K N, Ramsay A J (1985) Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air-dried soils. Aust J Soil Res 23: 613-621.

Stark J M, Firestone M K (1995) Mechanisms for soil moisture effects on the activity of nitrifying bacteria. Appl Environ Microbiol 61: 218-221.

Van Gestel M, Merckx R, Vlassak K (1993) Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow-growing microorganisms in soils from different climates. Soil Biol Biochem 25: 109-123.

Wada S, Toyota K (2007) Repeated applications of farmyard manure enhance resistance and resilience of soil biological functions against soil disinfection. Biol Fertil Soils 43: 349-356.

White D C (1995) Chemical ecology: possible linkage between macro- and microbial ecology. Oikos 74: 177-184.

Wilkinson S C, Anderson J M, Scardelis S P, Tisiafouli M, Taylor A, Wolters V (2002) PLFA profiles of microbial communities in decomposing conifer litter subject to moisture stress. Soil Biol Biochem 34: 189-200.

Williams M A (2007) Resonse of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. Soil Biol Biochem 39: 2750-2757.

Zak D R, Pregnitzer K S, Curtis P S, Holmes W E (2000) Atmosperic CO2 and the composition and function of soil microbial communities. Ecol Appl 10: 47-59.

Zelles L, Rackwitz R, Bai Q Y, Beck T, Beese F (1995) Discrimination of microbial diversity by fatty acid profiles of phospholipids and lipopolysaccharides in differently cultivated soils. Plant Soil 170: 115-122.

Table 1 Water content and matric, osmotic and water potential in the sand and the sandy loam maintained during the first 14 days of incubation.

| Sand | | | | Sandy loam | | | |
|---|---|---|---|---|---|---|---|
| Water content | Potential | | | Water content | Potential | | |
| | Matric | Osmotic | Water | | Matric | Osmotic | Water |
| (g $kg^{-1}$) | (MPa) | | | (g $kg^{-1}$) | (MPa) | | |
| 35 | -0.16 | -0.88 | -1.04 | 200 | -0.10 | -0.09 | -0.19 |
| 30 | -0.18 | -1.07 | -1.25 | 130 | -0.14 | -0.17 | -0.31 |
| 28 | -0.25 | -1.10 | -1.34 | 105 | -0.90 | -0.23 | -1.13 |
| 25 | -0.44 | -1.18 | -1.62 | 95 | -1.30 | -0.26 | -1.56 |

Figure 1 Respiration rates on days 1 to 68 in the sand (A) and the sandy loam (B) maintained at different water contents from day 1 to 14, rewet on day 14 and then maintained at optimal water content [(WC35 (sand) and WC200 (sandy loam)] until day 68 (n=4). Lines indicate standard error. Arrow indicates rewetting.

Figure 2 Cumulative respiration [expressed in mg $CO_2$-C g $soil^{-1}$ from the onset of the respiration measurements (day 0) to the given day] on days 1, 14, 15, 16, 20, 32 and 68 in the sand (A) and the sandy loam (B) maintained at different water contents from day 1 to 14, rewet on day 14 and then maintained at optimal water content [(WC35 (sand) and WC200 (sandy loam)] until day 68 (n=4). Lines indicate standard error. Arrow indicates rewetting.

Figure 3 Relationship between cumulative respiration (% of constantly moist control) in the sand and the sandy loam and matric potential (A) and water potential (B) on days 1, 14 and 32 (n=4).

Figure 4 Non-metric multi-dimensional scaling plots of the microbial community structure assessed by phospholipid fatty acid analysis on days 5, 14, 20 and 68 in the sand (A) and the sandy loam (B) maintained at different water contents from day 1 to 14, rewet on day 14 and then maintained at optimal water content [(WC35 (sand) and WC200 (sandy loam)] until day 68. Symbols represent means of 3 replicates for a given water content and sampling day, numbers indicate sampling days. Symbols close to each other indicate similar community composition whereas communities represented by symbols that are far apart from another differ in composition.

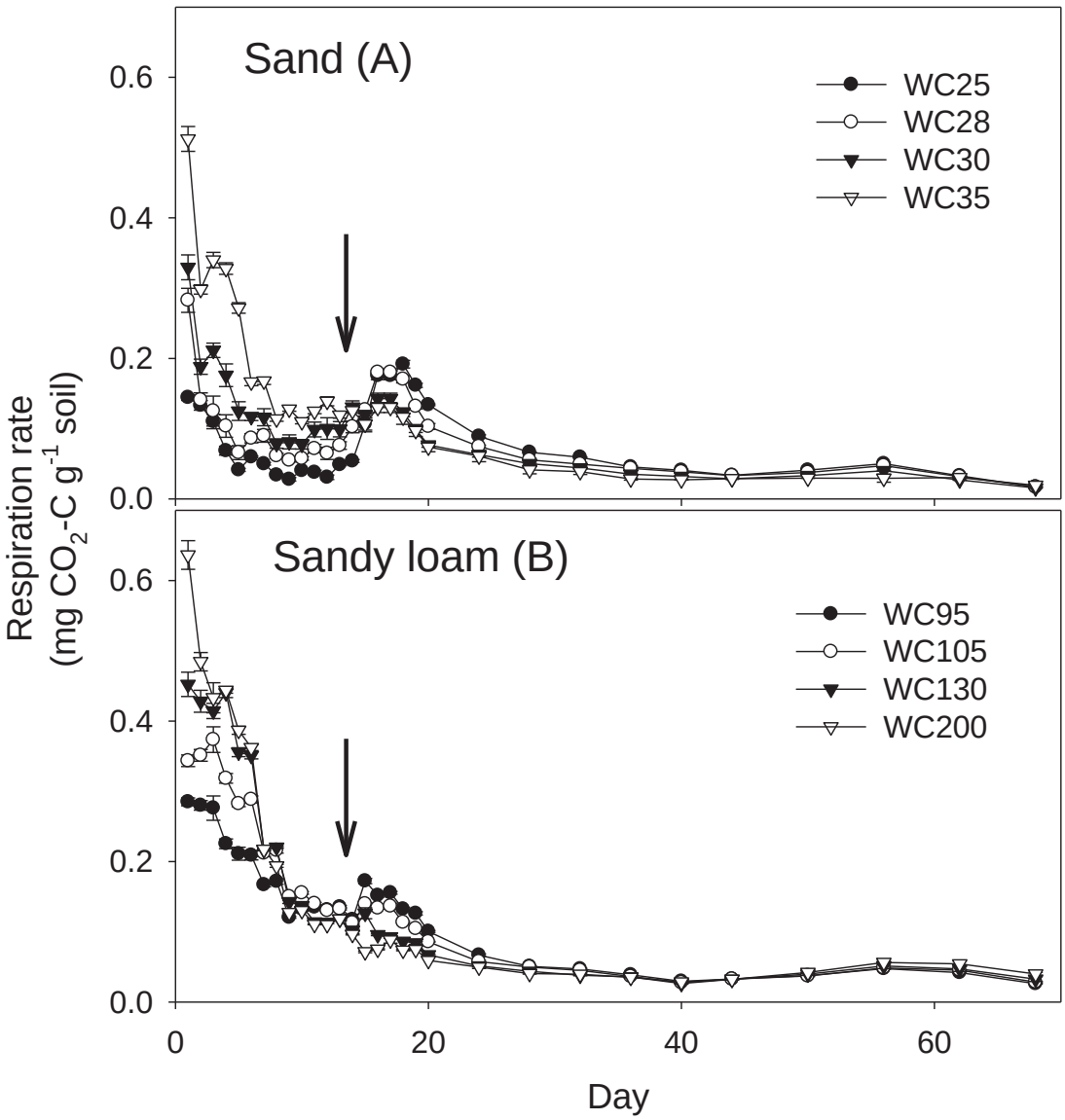

Sand (A)
WC25
WC28
WC30
WC35
Sandy loam (B)
WC95
WC105
WC130
WC200
Respiration rate
(mg CO2-C g-1 soil)
0.0
0.2
0.4
0.6
0
20
40
60
Day

Figure 1

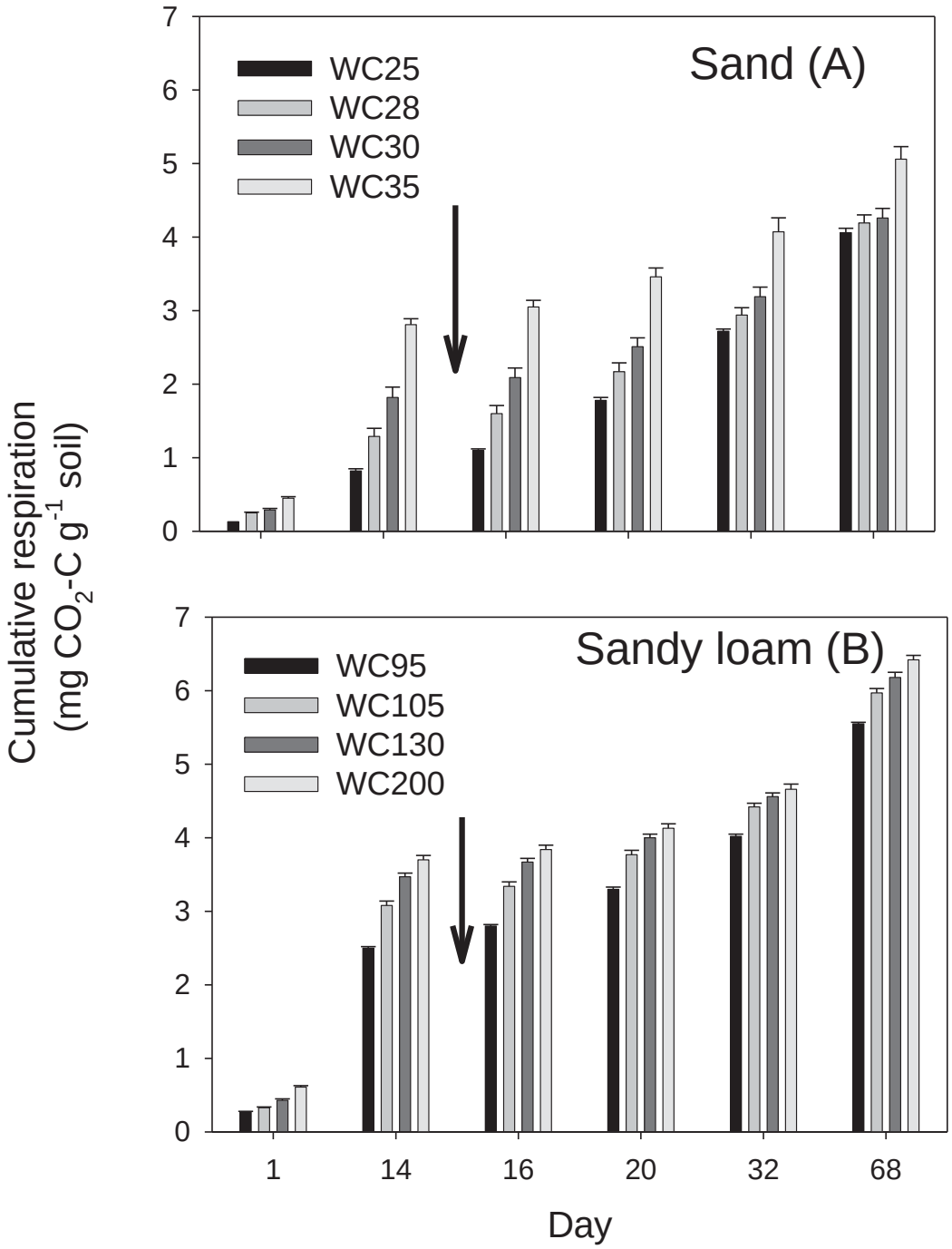

Sand (A)
WC25
WC28
WC30
WC35
Sandy loam (B)
WC95
WC105
WC130
WC200
Cumulative respiration
(mg CO2-C g-1 soil)
0
1
2
3
4
5
6
7
1
14
16
20
32
68
Day

Figure 2

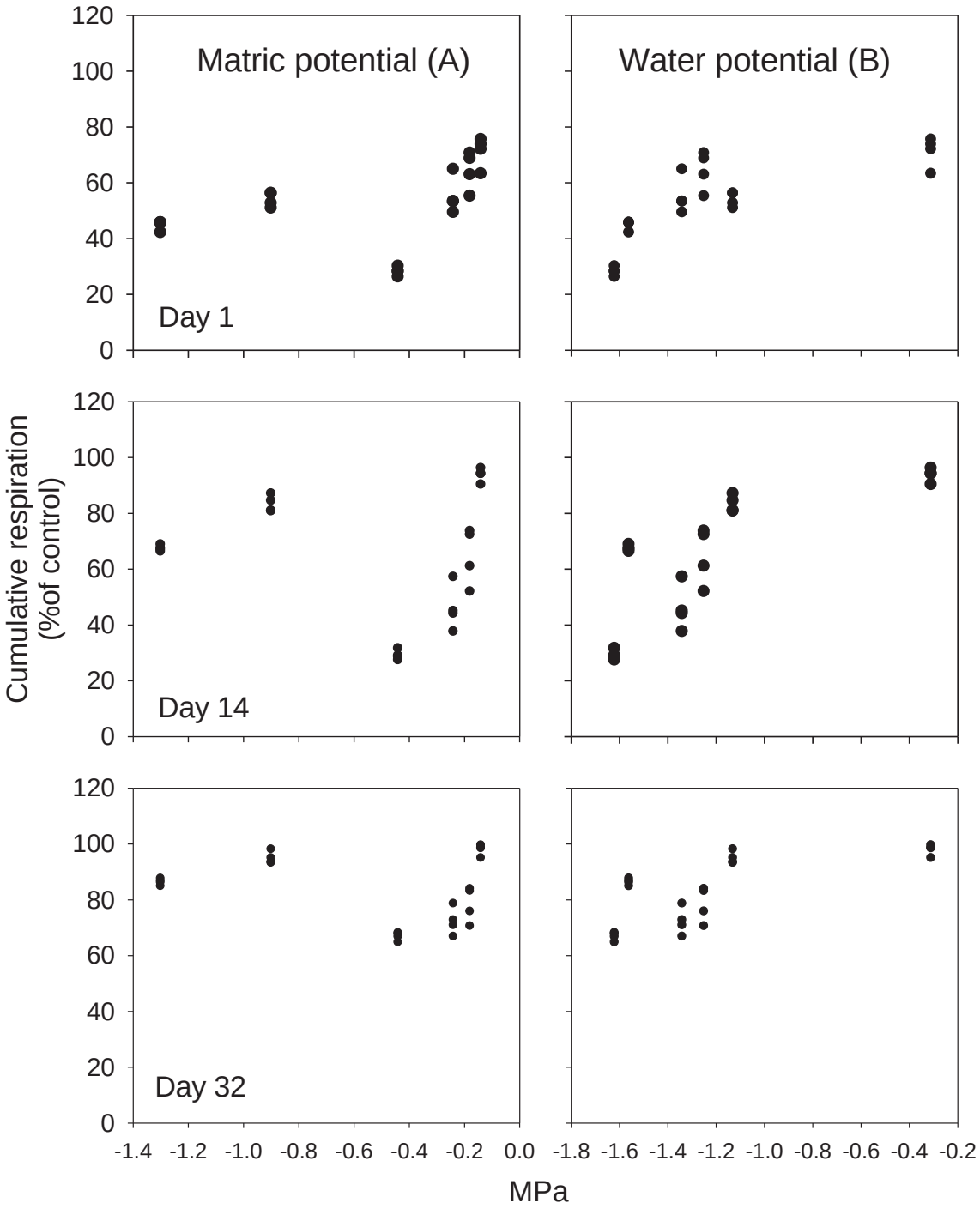
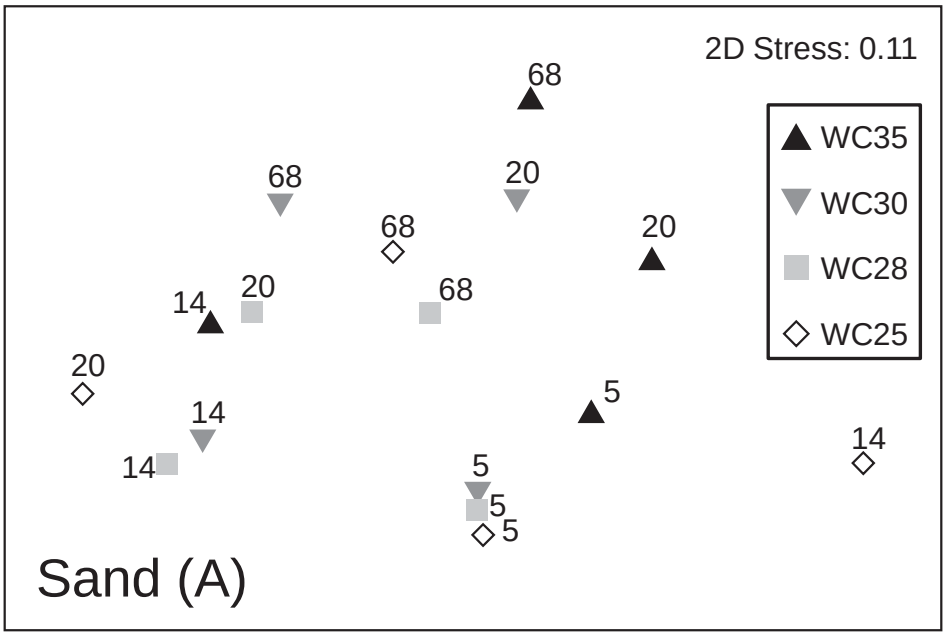

Matric potential (A)
Water potential (B)
Day 1
Day 14
Day 32
Cumulative respiration (%of control)
MPa
120
100
80
60
40
20
0
-1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2 0.0
-1.8 -1.6 -1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2

Figure 3


2D Stress: 0.11
WC35
WC30
WC28
WC25
Sand (A)

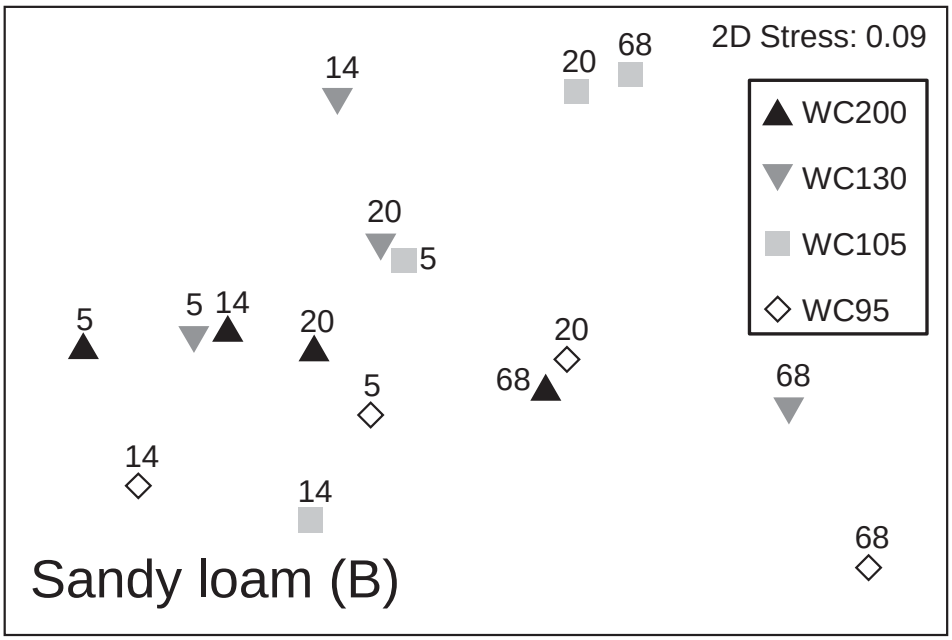

2D Stress: 0.09
WC200
WC130
WC105
WC95
Sandy loam (B)

Figure 4

# CHAPTER 6

## Microbial activity and community composition in saline and non-saline soils exposed to multiple drying and rewetting events

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I hereby certify that the statement of contribution is accurate.

Signed Date

Nakatani A S.

Contributed to planning of experiment, performed analysis on samples and interpreted data.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

Setia, R.

Contributed to planning of experiment, performed analysis on samples and interpreted data.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

Marschner P

Supervised development of work, data interpretation and manuscript evaluation.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

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# Microbial activity and community composition in saline and non-saline soils exposed to multiple drying and rewetting events.

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## Abstract

*Background and aims*: The effects of DRW have been studied extensively in non-saline soils, but little is known about the impact of DRW in saline soils. An incubation experiment was conducted to determine the impact of 1-3 drying and re-wetting events on soil microbial activity and community composition at different ECe levels (ECe 0.7, 9.3, 17.6 dS $m^{-1}$).

*Methods*: A non-saline sandy loam was amended with NaCl to achieve the three EC levels 21 days prior to the first DRW; wheat straw was added 7 days prior to the first DRW. Each DRW event consisted of one week drying and one week moist (50% of WHC). After the last DRW, the soils were maintained moist until the end of the incubation period (63 days after addition of the wheat straw). A control was kept moist (50% of WHC) throughout the incubation period.

*Results*: Respiration rates on the day after rewetting were similar after the first and the second DRW, but significantly lower after the third DRW. After the first and second DRW, respiration rates were lower in EC17.6 compared to the lower EC levels, whereas salinity had little effect on respiration rates after the third DRW or at the end of the experiment when respiration rates were low. Compared to the continuously moist control, respiration rates were about 50% higher on d15 and d29. On d44, respiration rates were about 50%

higher at EC9.7 than at the other two EC levels. Salinity had little effect on microbial biomass or community composition.

*Conclusions*: Microbes in moderately saline soils may be able to utilise substrates released after multiple DRW events better than microbes in non-saline soil. However, at high EC (EC17.6), the low osmotic potential reduced microbial activity to such an extent that the microbes were not able to utilise substrate released after rewetting of dry soil.

**Keywords**: drying and rewetting, phospholipid fatty acid analysis, respiration, salinity

## Introduction

In summer, particularly in Mediterranean climates, soils experience drying and rewetting (DRW) events as the dry periods may be interrupted by occasional rainfall events. In such DRW events, microbes are exposed to dual stressors: the decreasing water potential in drying soils and the sudden increase in water potential upon rewetting. Within a few hours after rewetting, a flush of respiration occurs which is followed by a rapid decrease in respiration rates (Butterly et al. 2009b; Franzluebbers et al. 1994; Kieft et al. 1987; Mikha et al. 2005). The flush of respiration upon rewetting is thought to be due to increased substrate availability from released osmolytes accumulated during the dry phase, cell lysis and breakdown of aggregates and exposure of previously protected organic matter (Denef et al. 2001a; Fierer and Schimel 2003; Halverson et al. 2000; Sparling et al. 1985). The size of the flush in microbial activity decreases with increasing number of DRW events (e.g. (Butterly et al. 2009b; Mikha et al. 2005; Van Gestel et al. 1993), which has been explained by a reduced microbial biomass or activity, changes in physiological state of the microbes as well as by a reduction in SOM release or availability (Denef et al. 2001a; Fierer and Schimel 2002; Mikha et al. 2005; Wu and Brookes 2005). Drying and rewetting may also change microbial community composition (Butterly et al. 2009b; Hamer et al. 2007; Schimel et al. 2007; Wilkinson et al. 2002; Williams 2007), indicating that some microbial species or groups are more susceptible to the drying and rewetting stress than others.

Natural or human-induced salinity is wide-spread: globally, salinity is sufficiently high to cause osmotic stress and yield reductions on $10^8$ ha (5%) of arable land (Lambers 2003). The low osmotic potential in these soils kills sensitive microbes whereas others can adapt by accumulating osmolytes (Oren 2001), such as amino acids or polyols (Beales 2004; Killham and Firestone 1984; Schimel et al. 1989). The synthesis of such osmolytes, which can occur within a few hours after exposure to low osmotic potential (Hagemann 2011), consumes large amounts of energy: up to 3 times more than required for cell wall synthesis (Oren 1999). Hence, salinity reduces microbial biomass size and activity (Tripathi et al. 2006; Wichern et al. 2006). Salinity not only affects microbial activity and growth, but also microbial community composition (Pankhurst et al. 2001; Gros et al. 2003; Gennari et al. 2007; Nelson and Mele 2007; Llamas et al. 2008) indicating differential tolerance to low osmotic potenial among microbial species or groups (Llamas et al. 2008; Mandeel 2006).

Although the effect of DRW has been studied extensively in non-saline soils, there are no published reports on the impact of DRW in saline soils. The microbes in saline soils may respond to DRW differently to those in non-saline soils because they have already experienced low water potential. The aim of this study was to assess the effect of one to three DRW events on microbial activity, biomass and activity in soils with different levels of salinity. In view of the literature cited above, we pose the following two hypotheses: (1) rewetting of dry soil will cause a greater flush in respiration in saline compared to non-saline soils because of the accumulation of osmolytes under saline conditions, and (2) DRW will have less effect on the microbial community composition in saline soils because the microbes are already exposed to low water potential.

## Materials and Methods

### *Soil sampling and amendment*

A non-saline sandy loam soil (air-dried and sieved to <2mm) from Mount Bold (38° 11´ S and 138° 69´ E), South Australia was used (sand 57.5%, silt 25%, clay 17.5%, pH 5.2, EC 1:5 68 µS/cm, N 0.33%, C 3.65%, water holding capacity 36.4%). Previous experiments had shown that in this soil, respiration was maximal at 50% WHC.

The air-dry soil was pre-incubated at the desired ECe at 50% of WHC for 10 days at 25°C before the experiment was begun. A pre-incubation of 10 days was selected on the basis of previous experiments with this and other soils that showed that respiration rates stabilise 8 days after rewetting of air-dry soil. The different levels of ECe (ECe 0.7, 9.3, 17.6 dS $m^{-1}$) were achieved by adding different amounts of NaCl to the soils and mixing thoroughly. At 50%WHC, these ECe values correspond to osmotic potentials of -0.1, -0.8 and -1.5 MPa for ECe 0.7, 9.3, 17.6 dS $m^{-1}$, respectively [calculated using the equation from Richard (1954)]. The $EC_{1:5}$ was converted to ECe using the equation ECe= (14.0-0.13× clay %) × $EC_{1:5}$ (Rengasamy 2006). At the end of the pre-incubation, wheat (*Triticum aestivum*) straw with a C/N ratio of 122 ground and sieved (0.25 - 2 mm) was mixed into the soils (2% w/w), to provide a readily available nutrient source.

*Soil incubation*

Thirty g (dry weight basis) of pre-incubated soil was added to PVC cores with a diameter of 3.7 cm and height of 5 cm and a nylon mesh base (0.75 µm, Australian Filter Specialist) and adjusted to a bulk density of 1.46 g $cm^{-3}$ which is the normal bulk density for soils of this texture. The cores were immediately placed into 1 L glass incubation vessels and sealed with gas tight lids equipped with septa to allow quantification of the $CO_2$ concentration in the headspace. The glass jars were incubated in the dark at 22-25 °C for 63 days.

After addition of the wheat straw, all cores were incubated at 50% WHC for 7 d prior to imposing DRW treatments. Within each ECe level, soils were exposed to 1, 2 and 3 DRW events (referred to 1DRW, 2DRW and 3DRW hereafter, Figure 1). To impose drying, cotton pouches (60 mm x 60 mm) containing 8 g self-indicating silica gel (BDH Chemicals) were added to each jar and changed at 1, 2 and 4 d when jars were vented after measuring $CO_2$ concentration in the headspace (Butterly et al. 2009b). Drying-rewetting events consisted of 1 week drying period followed by one week incubation at 50% of WHC. The control treatment consisted of soils kept at 50% of WHC throughout the incubation period.

Soils were sampled for microbial community composition on day 7, 7 d after rewetting in the first and second DRW (days 21 and 35) and at the end of the incubation

period 63. The EC was determined in a 1: 5 soil: water ratio and did not change during the incubation period. Soil for phospholipid fatty acid extraction was first frozen at -20°C and then freeze-dried. There were four replicates for each treatment at each sampling date.

*Analyses*

Respiration was quantified by measuring headspace $CO_2$ concentrations at regular intervals using a Servomex 1450 infra-red gas analyser (Servomex Group, Crowborough, England): daily in the first 20 days and every 3-4 days thereafter. After each measurement, the jars were opened to equilibrate the $CO_2$ to ambient concentrations, resealed and headspace $CO_2$ concentrations measured again. 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. The water content was maintained by weighing the cores and adding reverse osmosis water.

Microbial community composition was assessed by phospholipid fatty acid (PLFA) analysis (based on (Frostegard et al. 1993). Phospholipid fatty acids are components of cell membranes and rapidly dephosphorylated upon cell death; therefore PLFAs represent the living biomass (White 1995). Microbial groups such as bacteria and fungi, but not species or genotypes, differ in PLFA composition of their membranes; the so-called signature fatty acids can be used as a measure of abundance of the active fraction of microbial groups and PLFA patterns provide a coarse measure of microbial community composition. Freeze-dried soil (4 g) was extracted with a one-phase solvent of chloroform, methanol and citrate buffer (1:2:0.8 v/v/v). The lipid-containing phase was collected and dried under a stream of nitrogen at 40°C. The PLFAs were separated from other fatty acids using silicic acid columns (Supelclean LC-Si-SPE Tubes, Supelco). The columns were washed sequentially with chloroform, acetone, and methanol, collecting the the methanol fraction which contains the PLFAs. After alkaline methanolysis the organic phase was collected in dichloromethane. An internal standard (methylnonadecanoate, 19:0) was added to each sample.

The fatty acid methyl esters were separated in a gas chromatograph with a flame ionization detector (GC-FID) (HP 6890) using an SP-2560 fused silica capillary column (75 m, 180 μm × 0.14 μm film thickness; Supelco, Sigma-Aldrich, Australia) with helium as

carrier gas. The injector temperature was 250°C and the detector temperature was 260°C. The temperature program was as follows: after an initial temperature of 140°C, the temperature was ramped at 4°C/min to 240°C, and then held for 15 min.

The individual PLFA peaks were identified by comparing retention times with peaks of Supelco 37 standard mixture (Supelco, Bellefonte, PA). Unknown peaks were identified by GC-MS (gas chromatograph combined with mass selective detector HP 5973) using the same column and temperature program conditions and carrier gas as described above. Electron energy in electron impact was 70 eV. Mass spectrometer peak identification was based on comparison with the software library NIST02.L.

The amounts of individual PLFAs are expressed in μg/g soil. Signature PLFAs were used as indicators for specific microorganisms groups: Gram-positive bacteria i15:0 and i16:0; Gram-negative bacteria 16:1ω7c and 18:1ω7; and fungi 18:2ω6, 18:1ω9 and 18:3ω6 (Kandeler 2007; Zak et al. 2000; Zelles et al. 1995).

*Statistical analysis*

Significant differences between different treatments at a given time point in respiration rate, cumulative respiration, sum of PLFAs, sum of bacterial and fungal PLFAs were assessed by 2-way ANOVA (EC x moisture treatment) and Tukey test with $P \leq 0.05$. (GenStat® for Windows 8.0, VSN Int. Ltd, UK, 2005).

For statistical comparison of the microbial community composition, PLFA patterns, transformed as log (x+1), were analysed by Primer E software (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK) and plotted using non-metric multi-dimensional scaling (MDS). The PLFA data were (x+1) transformed to balance the contributions of fatty acids by down-weighing the dominant fatty acids and increasing the weighting of rare fatty acids (Clarke and Warwick 2001). Significant differences in microbial community composition between treatments were determined by PERMANOVA ($P \leq 0.1$).

## Results

*Respiration*

One day after the first rewetting event (d15), respiration rates were 2-3 fold higher in 1DRW than in M (Figure 2). In 1DRW, respiration rates were 30% higher at EC0.7 and EC9.3 than at EC17.6 whereas in M, respiration rates were 30% higher at EC9.3 and EC17.6 than at EC0.7.

One day after the second rewetting event (d29), respiration rates were lower than after the first rewetting event in M and 1 DRW which had not been exposed to the second DRW. Respiration rates in 2DRW were similar to those in 1DRW on d15 (2.5-4 fold higher than M) and lowest in M at all EC levels. In 1DRW and 2DRW, respiration rates were 30% higher at EC0.7 and EC9.3 than at EC17.6 whereas in M, there were no differences among EC levels.

The respiration rates after the third rewetting event in 3DRW (d43) were lower than after the second rewetting event (2DRW, 3DRW). The respiration rates were higher in 2DRW than in 3DRW at EC0.7 and than in 3DRW and M at EC9.3, but there were no significant differences among moisture treatments at EC17.6. At the end of the experiment (d63), respiration rates were very low with no differences between treatments.

On the day before the first rewetting event (d14), cumulative respiration was significantly lower in 1DRW than in M and within a given moisture treatment, lower in EC17.6 than in the two lower EC levels (Figure 3). One week after the first rewetting (d21), cumulative respiration was 17-30% higher in 1DRW than in M at the two lower EC levels but not at EC17.6. In both moisture treatments, cumulative respiration was higher at EC0.7 and EC9.3 than at the highest EC level.

On day 35 (one week after the second rewetting of DRW2 and DRW3), cumulative respiration at EC0.7 and EC9.3 was 12-35% higher in 1DRW and 2 DRW than in M, whereas there were no significant differences between the DRW treatments and M at the highest EC level. At all EC levels, cumulative respiration was higher in 1DRW than in 2DRW.

On day 49 (one week after the third rewetting of DRW3), cumulative respiration at EC0.7 compared to M was about 30% higher in 1DRW and 2DRW and 10% higher in 3DRW. At EC9.3, cumulative respiration was significantly higher than M only in 1DRW and 2DRW being 28 and 22% higher, respectively. At EC17.6, there were no significant differences in cumulative respiration among the moisture treatments.

At the end of the experiment (d63), there were no significant differences between M and the DRW treatments at EC17.6. At EC0.7, only 1DRW had a higher cumulative respiration (12%) than M, whereas at EC9.2 cumulative respiration of 1DRW and 2DRW was significantly higher than in M with an increase by about 20%.

*Microbial biomass and community composition*

At the end of week 1, before the first DRW, there were no significant differences in microbial biomass (sum of PLFAs), and the concentrations of bacterial and fungal fatty acids between the 3 EC levels (Table 1). Microbial biomass and the concentration of bacterial and fungal fatty acids decreased over time in all treatments; generally, they were 50% lower at the end of the experiment than on d7. Throughout the experiment, microbial biomass and the concentration of bacterial fatty acids were little affected by either moisture treatment or EC. On d35 compared to M, the concentration of fungal fatty acids in 1DRW was significantly higher at EC0.7 and higher in 1DRW and 2DRW at EC17.6.

The B/F ratio increased from 1.4 to 1.6 over time and was generally higher in M than in the DRW treatments. Salinity had no significant effect on the B/F ratio.

Microbial community composition was more strongly affected by the moisture treatment than the other two main effects (EC and time), with time having the least effect. At all EC levels and all sampling dates, the community composition in M differed from that in the DRW treatments, but there were no differences among the DRW treatments.

On d7, the microbial community composition in EC0.7 differed from that at the higher EC levels. On d21, the microbial community composition in 1DRW was not affected by EC whereas in M, the community composition at EC0.7 differed from those at the higher

EC levels (Figure 4). On d35, the microbial community composition was not affected by EC in 1DRW and 2DRW, but differed between the three EC levels in M (Figure 4).

On d63, EC had no effect on microbial community composition in 1DRW and 2DRW, but in 3DRW and M there were significant differences between EC0.7 and the higher EC levels (Figure 4).

## Discussion

The results of this experiment show that rewetting of dry soil causes a flush in respiration in saline soils, but at high salinity the size of the flush is smaller than in non-saline soils in absolute terms and relative to the continuously moist soil. This suggests that low osmotic potential restricts the ability of microbes to utilise the substrates released during rewetting.

The size of the rewetting flush was lower after the third DRW event compared to the first two (Figure 2). Other studies also found a decrease in the size of the rewetting flush with increasing number of DRW events (Butterly et al. 2009b; Fierer and Schimel 2002; Mikha et al. 2005; Van Gestel et al. 1993), although in these, the flush gradually decreases with number of DRW events, whereas in our study, the size of the flush was similar the first two events and strongly reduced at the 3. DRW event. Explanations for the decrease in flush size include a decrease in microbial biomass size and lack of exposure of fresh organic material at later DRW events. A smaller microbial biomass would release less osmolytes and may decompose released substrates at a lower rate (Wu and Brookes 2005). In the present study, microbial biomass (sum of PLFA) decreased with time (Table 1), which would support the hypothesis that the decreased flush size after the third DRW was due to a smaller biomass. However, microbial biomass decreased over time to a similar extent in the moist control which suggests that the viable microbial biomass, as detected by PLFA (White 1995), is not decreased by DRW but rather by substrate limitation. Moreover, whereas microbial biomass decreased by about 50% from the start to the end of the experiment, the size of the respiration flush after the third rewetting (d43) was only 25% of that after the first two rewetting events. This suggests that the decrease in the size of the flush after the third rewetting cannot be explained by a reduced microbial

biomass alone. It should be noted however, that in the present study, the viable microbial biomass was measured, not as in most other studies, the total microbial biomass. The viable microbial biomass represents only a fraction of the total biomass and was measured 7 days after rewetting which may have allowed recovery. It cannot be ruled out that the non-active microbial biomass decreased more strongly in the DRW treatments than in the moist control as particularly the first DRW events would have caused cells to burst and the released substrates to be decomposed by the active fraction of the biomass.

The exposure of fresh organic material upon rewetting is due to the breakdown of aggregates with the sudden influx of water (Denef et al. 2001a). This occurs particularly after first rewetting, whereas later, most aggregates are already destroyed and rewetting does not result in significant exposure of fresh organic material (Denef et al. 2001b; Fierer and Schimel 2002). It is possible that a greater fraction of aggregates were broken down in the first two rewetting events than in the third, however the soil was sieved to <2mm which suggests that there were few macro-aggregates present even at the start of the experiment.

The lack of difference in size of the flush after rewetting between the first and second rewetting event in the present study may be due to the addition of wheat straw which would provide a readily available nutrient source to the microbes surviving the rewetting stress. In the previous studies which showed a decrease in the size of the flush between the first and second rewetting, no substrates where added at the start of the incubation (Butterly et al. 2009b; Fierer et al. 2003; Mikha et al. 2005). In soils, microbial activity is usually limited by C availability (De Nobili et al. 2001; Hoyle et al. 2008), therefore in the soils without substrate addition, the flush of microbial activity would be based on nutrients released from the microbial biomass or exposure of previously protected organic matter.

In agreement with many previous studies, respiration rates and cumulative respiration were negatively affected by salinity (Figures 2, 3), e.g., Chowdhury et al. (2011 a, b); Pathak and Rao (1998); Setia et al. (2011). This is in contrast to the small and inconsistent effects of salinity on microbial biomass and community composition (Table 1, Figure 4) and suggests that microbial activity is more affected by salinity than soil microbial

size or composition. Hence in the present study, the reduced activity in saline soils is mainly due to a lower activity per unit biomass.

Other studies, mainly using saline soils collected from the field, found that microbial community composition in saline soils differed from that in non-saline soils (Abed et al. 2007; Pankhurst et al. 2001). The lack of strong and consistent effect of salinity on microbial community composition in the present study could be due to the fact that salinity was induced only two weeks before the start of the experiment and substrate in the form of wheat straw was added to all soils. In the field, salinity develops more slowly and is accompanied by an often strong reduction in plant growth and thus reduced organic matter content. The lower substrate availability in saline soils may therefore be mainly responsible for the salinity effect on community composition in field-collected soils.

The 1. hypothesis (rewetting of dry soil will cause a greater flush in respiration in saline compared to non-saline soils because of the accumulation of osmolytes under saline conditions) cannot be unequivocally denied or confirmed. The flush in respiration caused by rewetting was about 50% lower at EC17.9 compared to the lower EC levels on d15 and d29 which would be in contrast to the hypothesis (Figure 2). However on d44, the respiration rate was 50% higher at EC9.3 than at the other two EC levels. These results suggest that with high salinity (EC17.9), microbial activity is so severely reduced by the low osmotic potential that it cannot respond to an increase in substrate availability after rewetting to a similar extent as microbes in soils with lower salinity. Moreover, the osmotic potential in the soil with EC17.9 may have been too low even after rewetting (osmotic potential -1.5 MPa) that fewer, if any, osmolytes were released upon rewetting. At EC9.3, the size of the respiration flush was greater relative to the control only after the third rewetting, but then the increase was substantial with the flush being about 50% greater than at EC0.7. This indicates that microbes in moderately saline soils are better able to withstand multiple DRW events than those in non-saline soils which could be due to a greater availability of substrates after rewetting either from released osmolytes or from substrate remaining from the added wheat straw. The latter is unlikely because cumulative respiration and hence substrate utilisation was similar at EC9.3 and EC0.7 throughout the experiment. The two salinity treatments did not differ in size of the microbial biomass (Table 1). Thus, the relative greater flush at EC9.3 after the third DRW event cannot be explained by a greater biomass. After the first two rewetting events, a greater release of

osmolytes at EC9.3 may have been masked by the high substrate availability from the added wheat straw. At those earlier rewetting events, the remaining wheat residues may still have contained sufficiently high amounts of relatively easily decomposable compounds. After the third rewetting event on the other hand, the osmolytes released by the microbes at EC9.3 could have represented an easily decomposable nutrient source in a soil which otherwise contained only relatively recalcitrant organic substrates. It should be noted however, that we did not determine the release of osmolytes directly and only use the size of the flush relative to the moist control as indirect measure of substrate availability.

In several previous studies, DRW changed microbial community composition (Butterly et al. 2009b; Hamer et al. 2007; Schimel et al. 2007; Wilkinson et al. 2002; Williams 2007), although that was not always the case (Butterly et al. 2009a). We hypothesised that DRW will have less effect on the microbial community composition in saline soils because the microbes are already exposed to low water potential. This hypothesis was not supported by the results, because although microbial community composition of the DRW treatments generally differed from that of the continuously moist control, this was the case at all EC levels. Thus, the microbial community in saline soils is altered by DRW to a similar extent as that in non-saline soil.

There were no consistent differences in microbial community composition among DRW treatments. This suggests that one DRW event is sufficient to change microbial community composition and that additional DRW events do not further alter the community. The effect of one DRW was long-lasting: on d63, the community composition in 1DRW remained different from that of the continuously moist control even though this treatment had been kept moist for 49 days.

## Conclusions

The results suggest that microbes in moderately saline soils may be able to withstand multiple DRW events better than those in non-saline soils and are better able to utilise substrates released at later rewetting events. However, at high EC (EC17.6), the low osmotic potential reduced microbial activity so strongly that the microbes could not take advantage of substrate released after rewetting of dry soil.

It has been suggested, that multiple DRW events could result in significant losses of organic C from soils due to the flush in respiration after rewetting, if this is not compensated by low activity during the dry period (Wu and Brookes 2005; Xiang et al. 2008). Our results suggest that although C is also lost upon DRW in saline soils, the increase in total C lost compared to the constantly moist soil is smaller at high EC compared to the non-saline soil. This, together with the overall lower respiration rates in the saline soils suggests that saline soils have a greater potential to store C in semi-arid climates than non-saline soils.

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## References

Abed R M M, Kohls K, De Beer D (2007) Effect of salinity changes on the bacterial diversity, photosynthesis and oxygen consumption of cyanobacterial mats from an intertidal flat of the Arabian Gulf. Environ Microbiol 9: 1384-1392.

Beales N (2004) Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: A review. Compreh Rev Food Sci Food Safety 3: 1-20.

Butterly C R, Buenemann E K, McNeill A M, Baldock J A, Marschner P (2009a) Phosphorus and carbon dynamics during repeated drying and rewetting of soils with different microbial biomass. Soil Biol Biochem 41: 1406-1416.

Butterly C R, Bunemann E K, McNeill A M, Baldock J A, Marschner P (2009b) Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biol Biochem 41: 1406-1416.

Chowdhury N, Marschner P, Burns R G (2011a) Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant Soil (in press).

Chowdhury N, Marschner P, Burns R G (2011 b) Soil microbial activity and community composition: impact of changes in matric and osmotic potential. Soil Biology and Biochemistry 41(7): 1406-1416.

Clarke K R, Warwick R M (2001) Change in marine communities: an approach to statistical analysis and interpretation. Primer-E, Plymouth.

De Nobili M, Contin M, Mondini C, Brookes P C (2001) Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol Biochem 33: 1163-1170.

Denef K, Six J, Bossuyt H, Frey S D, Elliott E T, Merckx R, Paustian K (2001a) Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol Biochem 33: 1599-1611.

Denef K, Six J, Paustian K, Merckx R (2001b) Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry-wet cycles. Soil Biol Biochem 33: 2145-2153.

Fierer N, Schimel J P (2002) Effect of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biol Biochem 34: 777-787.

Fierer N, Schimel J P (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Sci Soc Am J 67: 798-805.

Fierer N, Schimel J P, Holden P A (2003) Influence of drying-rewetting frequency on soil bacterial community structure. Microb Ecol 45: 63-71.

Franzluebbers K, Weaver R W, Juo A S R, Franzluebbers A J (1994) Carbon and nitrogen mineralization from cowpea plant parts decomposing in moist and in repeatedly dried and wetted soil. Soil Biol Biochem 26: 1379-1387.

Frostegård A, Bååth E, Tunlid A (1993) Shifts in the strucutre of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol Biochem 25: 723-730.

Gennari M, Abbate C, La Porta V, Baglieri A (2007) Microbial response to $Na_2SO_4$ additions in a volcanic soil. Arid Land Research and Management 21: 211-227.

Gros R, Poly F, Jocteur-Monrozier L, Faivre P (2003) Plant and soil microbial community responses to solid waste leachates diffusion on grassland. Plant and Soil 255: 445-455.

Hagemann M (2011) Molecular biology of cyanobacterial salt acclimation. FEMS Microbiol Rev 35: 87-123.

Halverson L J, Jones T M, Firestone M K (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci Soc Am J 64: 1630-1637.

Hamer U, Unger M, Makeschin F (2007) Impact of air-drying and rewetting on PLFA profiles of soil microbial communities. J Plant Nutr Soil Sci 170: 259-264.

Hoyle F C, Murphy D V, Brookes P C (2008) Microbial response to the addition of glucose in low-fertility soils. Biol Fertil Soils 44: 571-579.

Kandeler E (2007) Physiological and biochemical methods for studying soil biota and their function. In: Soil Microbiology, ecology, and biochemistry. Ed. E A Paul. pp 53-84. Elsevier.

Kieft T L, Soroker E, Firestone M K (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol Biochem 19: 119-126.

Killham K, Firestone M K (1984) Salt stress control of intracellular solutes in *Streptomycetes* indigenous to saline soils. Appl Environ Microbiol 47: 301-306.

Lambers H (2003) Dryland salinity: a key environmental issue in southern Australia. Plant Soil 257: v-vii.

Llamas D P, Gonzales M D, Gonzales C I, Lopez G R, Marquina J C (2008) Effects of water potential on spore germination and viability of Fusarium species. J Industr Microbiol Biotechnol 35: 1411-1418.

Mandeel Q A (2006) Biodiversity of the genus *Fusarium* in saline soil habitats. J Basic Microbiol 46: 480-494.

Mikha M M, Rice C W, Milliken G A (2005) Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biol Biochem 37: 339-347.

Nelson D R, Mele P M (2007) Subtle changes in rhizosphere microbial community structure in response to increased boron and sodium chloride concentrations. Soil Biol Biochem 39: 340-351.

Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 63: 334-348.

Oren A (2001) The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implication of the functioning of salt lake ecosystems. Hydrobiologia 466: 61-72.

Pankhurst C E, Yu S, Hawke B G, Harch B D (2001) Capacity of fatty acid profiles and substrate utilisation patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations win South Australia. Biol Fertil Soils 33: 204-217.

Pathak H, Rao D L N (1998) Carbon and nitrogen mineralisation from added organic matter in saline and alkali soils. Soil Biol Biochem 30: 695-702.

Rengasamy P (2006) Soil salinity and sodicity. In Growing crops with reclaimed wastewater. Ed. D Stevens. pp 125-138. CSIRO.

Richard L A (1954) Determination of the properties of saline and alkali soils. United States Department of Agriculture Handbook 60, Washington, DC. pp. 7-53.

Schimel J P, Balser T C, Wallenstein M (2007) Microbial stress response physiology and its implications for ecosystem function. Ecology 88: 1386-1394.

Schimel J P, Scott W J, Killham K (1989) Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. Appl Environ Microbiol 55: 1635-1637.

Setia R, Marschner P, Baldock J S, Chittleborough D J, Verma V (2011) Relationships between carbon dioxide emission and soil properties in salt affected landscapes. Soil Biol Biochem 43: 667-674.

Sparling G P, Whale K N, Ramsay A J (1985) Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air-dried soils. Aust J Soil Res 23: 613-621.

Tripathi S, Kumari S, Chakraborty A, Gupta A, Chakraborty K, Bandyapadhyay B K (2006) Microbial biomass and its activities in salt-affected coastal soils. Biol Fertil Soils 42: 273-277.

Van Gestel M, Merckx R, Vlassak K (1993) Soil drying and rewetting and the turnover of 14C-labelled plant residues: first order decay rates of biomass and non-biomass 14C. Soil Biol Biochem 25: 125-134.

White D C (1995) Chemical ecology: possible linkage between macro- and microbial ecology. Oikos 74: 177-184.

Wichern J, Wichern F, Joergensen R G (2006) Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137: 100-108.

Wilkinson S C, Anderson J M, Scardelis S P, Tisiafouli M, Taylor A, Wolters V (2002) PLFA profiles of microbial communities in decomposing conifer litter subject to moisture stress. Soil Biol Biochem 34: 189-200.

Williams M A (2007) Resonse of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. Soil Biol Biochem 39: 2750-2757.

Wu J, Brookes P C (2005) The proportional mineralisation of microbial biomass and organic matter by air-drying and rewetting of a grassland soil. Soil Biol Biochem 37: 507-515.

Xiang S R, Doyle A, Holden P A, Schimel J P (2008) Drying and rewetting effects on C and N mineralisation and microbial activity in surface and subsurface Californian grassland soils. Soil Biol Biochem 40: 2281-2289

Zak D R, Pregnitzer K S, Curtis P S, Holmes W E (2000) Atmosperic CO2 and the composition and function of soil microbial communities. Ecol Appl 10: 47-59.

Zelles L, Rackwitz R, Bai Q Y, Beck T, Beese F (1995) Discrimination of microbial diversity by fatty acid profiles of phospholipids and lipopolysaccharides in differently cultivated soils. Plant Soil 170: 115-122.

Table 1 Sum of total phospholipid fatty acids, bacterial and fungal fatty acids and bacteria/fungi ratio prior to the first drying (d7) and seven days after rewetting (d21 and 35) and at the end of the incubation period (d63) for soils maintained moist throughout (M) and soils exposed to 1-3 DRW events (1DRW, 2DRW, 3DRW) with different levels of salinity (ECe 0.7, 9.3, 17.6 dS $m^{-1}$) (n=4, values at the same sampling date followed by different letters are significantly different, P≤ 0.05).

| Day | Treatment | ECe | Total PLFA | | Bacterial fatty acids | | Fungal fatty acids | | B/F ratio | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | μg $g^{-1}$ | | | | | |
| 7 | M | 0.7 | 23.3 | a | 8.9 | a | 7.7 | a | 1.2 | a |
| | | 9.3 | 24.6 | a | 9.9 | a | 7.7 | a | 1.3 | a |
| | | 17.6 | 26.5 | a | 10.1 | a | 8.5 | a | 1.2 | a |
| 21 | M | 0.7 | 29.1 | ab | 10.8 | ab | 7.6 | ab | 1.4 | a |
| | | 9.3 | 23.5 | a | 9.0 | a | 5.9 | a | 1.5 | a |
| | | 17.6 | 22.0 | a | 9.1 | a | 5.3 | a | 1.8 | a |
| | 1DRW | 0.7 | 41.9 | b | 16.4 | b | 12.9 | b | 1.3 | a |
| | | 9.3 | 38.9 | ab | 15.6 | ab | 12.6 | b | 1.2 | a |
| | | 17.6 | 30.0 | ab | 11.6 | ab | 9.9 | ab | 1.4 | a |
| 35 | M | 0.7 | 20.9 | abc | 7.8 | abc | 5.0 | abc | 1.6 | b |
| | | 9.3 | 17.2 | ab | 6.7 | ab | 4.4 | ab | 1.6 | b |
| | | 17.6 | 12.6 | a | 4.8 | a | 3.5 | a | 1.4 | ab |
| | 1DRW | 0.7 | 33.2 | c | 13.3 | c | 9.6 | d | 1.4 | ab |
| | | 9.3 | 20.7 | abc | 8.7 | abc | 6.1 | abcd | 1.4 | ab |
| | | 17.6 | 29.3 | bc | 11.5 | bc | 10.2 | d | 1.1 | a |
| | 2 DRW | 0.7 | 24.2 | abc | 9.4 | abc | 7.3 | abcd | 1.3 | ab |
| | | 9.3 | 27.1 | bc | 10.9 | bc | 8.5 | bcd | 1.3 | ab |
| | | 17.6 | 24.9 | abc | 9.7 | abc | 8.8 | cd | 1.2 | a |
| 63 | M | 0.7 | 33.1 | c | 11.9 | b | 9.8 | d | 1.2 | a |
| | | 9.3 | 21.4 | abc | 9.3 | ab | 4.5 | ab | 2.1 | c |
| | | 17.6 | 9.9 | a | 4.4 | a | 1.9 | a | 2.2 | c |
| | 1DRW | 0.7 | 22.6 | bc | 9.2 | ab | 5.8 | bc | 1.6 | b |
| | | 9.3 | 24.7 | bc | 10.5 | b | 6.2 | bc | 1.7 | b |
| | | 17.6 | 26.3 | bc | 10.9 | b | 7.4 | bcd | 1.5 | ab |
| | 2 DRW | 0.7 | 19.1 | ab | 7.7 | ab | 4.8 | abc | 1.6 | b |
| | | 9.3 | 21.8 | abc | 9.1 | ab | 5.8 | bc | 1.6 | ab |
| | | 17.6 | 27.5 | bc | 11.2 | b | 7.9 | cd | 1.4 | ab |
| | 3 DRW | 0.7 | 16.6 | ab | 6.7 | ab | 4.4 | ab | 1.5 | ab |
| | | 9.3 | 22.2 | abc | 9.1 | ab | 6.2 | bc | 1.5 | ab |
| | | 17.6 | 17.3 | ab | 7.3 | ab | 4.9 | abc | 1.5 | ab |

Figure 1 Experimental design with dark rectangles indicating moist incubation (50% of WHC) and white rectangles indicating dry periods. Arrows show rewetting events and numbers indicate sampling dates microbial biomass and community composition.

Figure 2 Respiration rates on the first day after rewetting, d15, 29 and 43, and at the end of the incubation period (d63) for soils maintained moist throughout (M) and soils exposed to 1-3 DRW events (1DRW, 2DRW, 3DRW) with different levels of salinity (ECe 0.7, 9.3, 17.6 dS $m^{-1}$) (n=4).'R' indicates treatments that were rewet one day prior.

Figure 3 Cumulative respiration before the first rewetting (d14), seven days after rewetting, d21, 35 and 49, and at the end of the incubation period (d63) for soils maintained moist throughout (M) and soils exposed to 1-3 DRW events (1DRW, 2DRW, 3DRW) with different levels of salinity (ECe 0.7, 9.3, 17.6 dS $m^{-1}$) (n=4).

Figure 4 Multiple dimensional scaling plots of microbial community composition based on phospholipid fatty acid analysis seven days after the first and second DRW event (d21 and 35) and at the end of the incubation period (d63) for soils maintained moist throughout (M) and soils exposed to 1-3 DRW events (1DRW, 2DRW, 3DRW) with different levels of salinity (ECe 0.7, 9.3, 17.6 dS $m^{-1}$) (symbols are averages of 4 replicates).

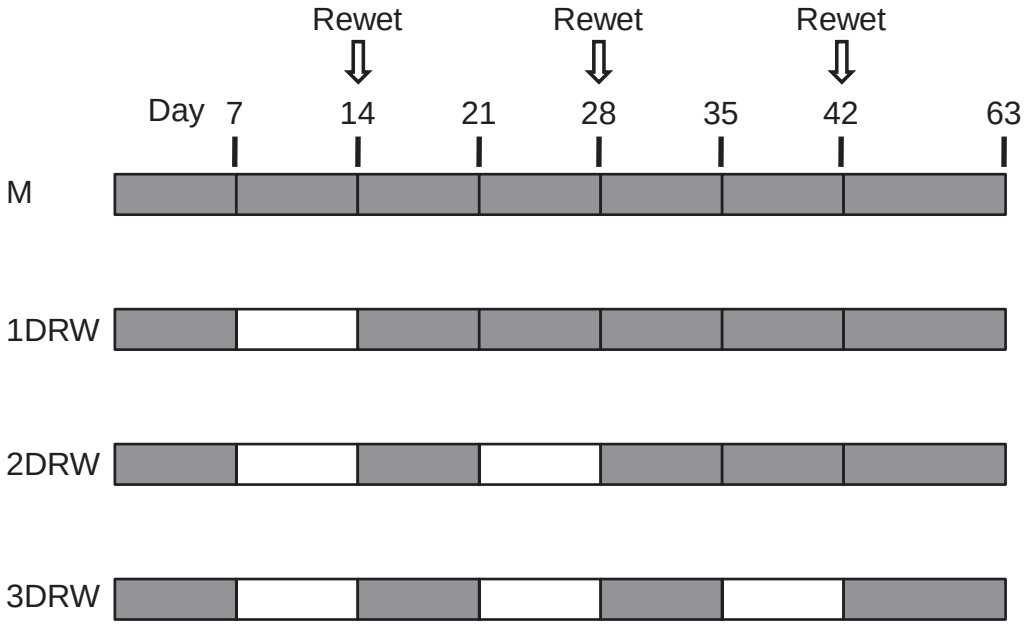


Figure 1

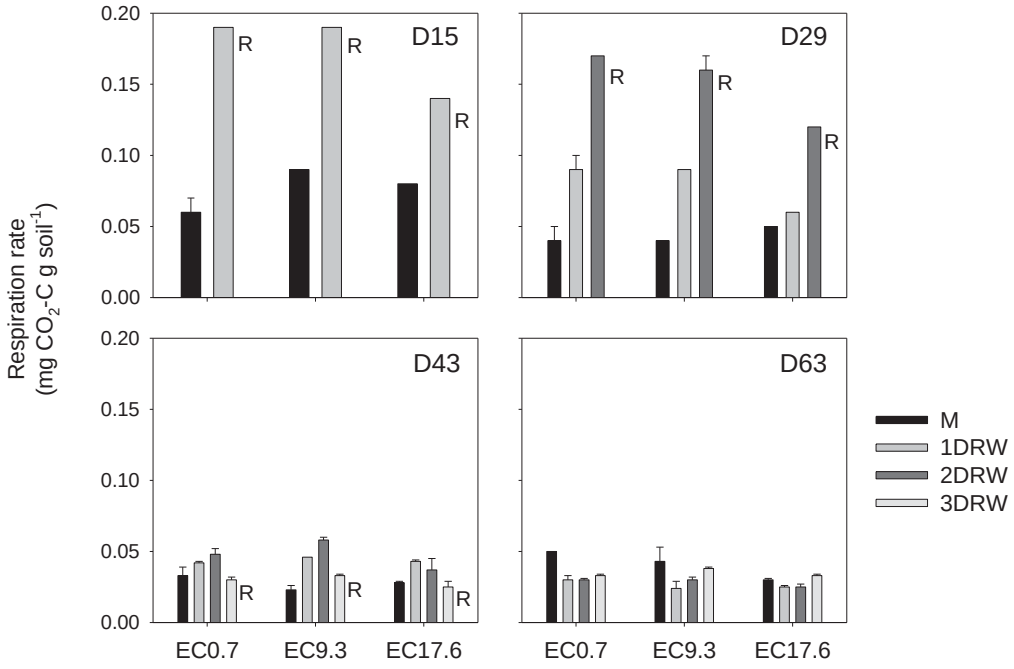
Respiration rate
(mg $CO_2$-C g $soil^{-1}$)
0.20
0.15
0.10
0.05
0.00
D15
D29
D43
D63
R
EC0.7
EC9.3
EC17.6
M
1DRW
2DRW
3DRW

Figure 2

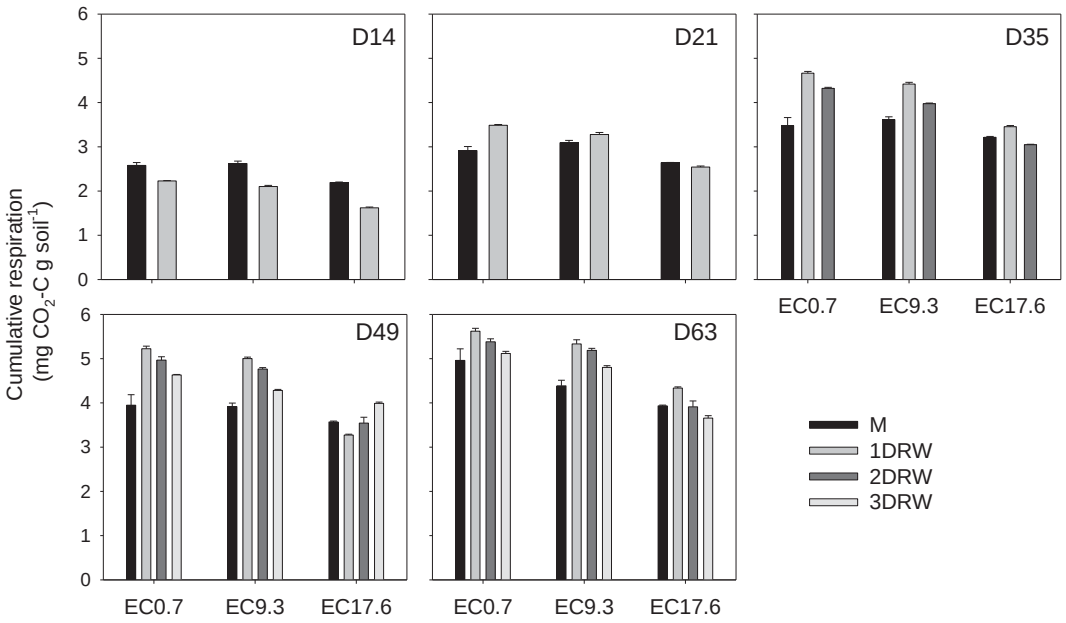
D14
D21
D35
D49
D63
Cumulative respiration
(mg $CO_2$-C g $soil^{-1}$)
0
1
2
3
4
5
6
EC0.7
EC9.3
EC17.6
M
1DRW
2DRW
3DRW

Figure 3

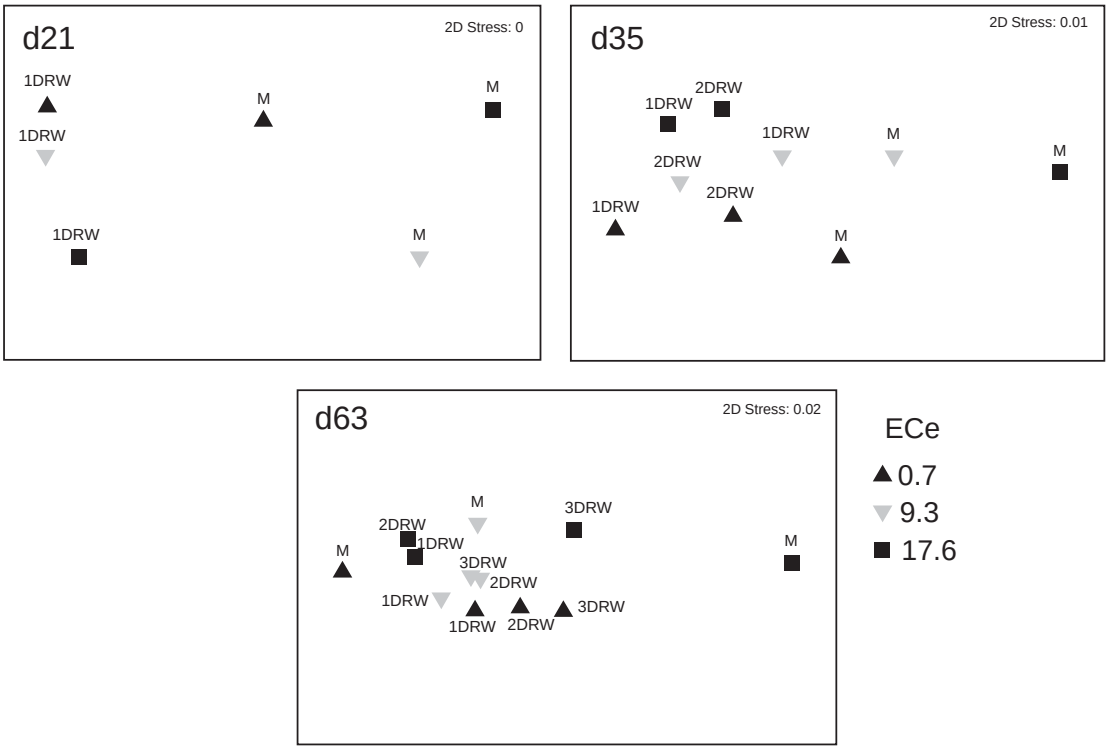
d21
2D Stress: 0
1DRW
1DRW
1DRW
M
M
M
d35
2D Stress: 0.01
1DRW
2DRW
1DRW
2DRW
1DRW
2DRW
M
M
M
d63
2D Stress: 0.02
M
2DRW
1DRW
M
3DRW
3DRW
2DRW
1DRW
1DRW
2DRW
3DRW
M
ECe
0.7
9.3
17.6

Figure 4

# CHAPTER 7

## The extent of drying influences the flush of respiration after rewetting in non-saline and saline soils

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I hereby certify that the statement of contribution is accurate.

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Contributed to planning of experiment and performed analysis on samples.
I hereby certify that the statement of contribution is accurate.

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Marschner, P.

Supervised development of work, data interpretation and manuscript evaluation.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

## The extent of drying influences the flush of respiration after rewetting in non-saline and saline soils.

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### Abstract

Drying and rewetting are common events in soils during summer, particularly in Mediterranean climate where soil microbes may be further challenged by salinity. Previous studies in non-saline soils have shown that rewetting induces a flush of soil respiration, but little is known about how the extent of drying affects the size of the respiration flush or how drying and rewetting affects soil respiration in saline soils. Five sandy loam soils, ranging in ECe from 2 to 48 dS $m^{-1}$ (EC2, EC9,EC19, EC33 and EC48), were kept at soil water content optimal for respiration (M) or dried for 1, 2, 3, 4 or 5 days (referred to 1D, 2D, 3D, 4D and 5D) and maintained at the achieved water content for 4 days. Then the soils were rewet to optimal water content and incubated moist for 5 days. Water potential (WP) decreased with increasing drying time; in the 5D treatment, WP ranged between -15 and -30 MPa, with the lowest potentials in soil EC33. In moist and dry conditions, respiration rates were highest in soil EC2 and decreased with increasing EC up to soil EC19. The soils EC19, EC33 and EC48 had similar respiration rates. Respiration rates decreased with increasing time of drying; when expressed relative to M, the decline was similar in all soils. Rewetting of soils that had been dried for 1 or 2 days did not result in an increase in respiration rate compared to M. When soils were dried for 3 or more days, rewetting induced a flush in respiration within one day. The flush in respiration was greatest in 5D and smallest in 3D, and greater in EC2 than in the saline soils. Cumulative respiration was highest in soil EC2 and declined with increasing EC to soil EC19; there were no significant differences in cumulative respiration among soils EC19, EC33 and EC48. In soil EC2, cumulative respiration at the end of the 5 day moist incubation was little affected by the drying treatments: compared to M, cumulative respiration was decreased by only 12% in

5D. In the saline soils, cumulative respiration was 24-34% lower in 5D compared to M. In soils EC2 and EC9, the drying treatments did not affect microbial biomass C (MBC) at the end of the 5 day moist incubation. However, in soils EC19, EC33 and EC48, MBC was generally higher in the drying treatments compared to M, particularly in 5D, where MBC was more than 2 to 4 fold higher. In conclusion, rewetting induced a flush in respiration only if the WP of the soils was previously decreased at least 3-fold compared to M. Hence, only marked increases in WP induce a flush in respiration upon rewetting. The consistent decrease in respiration during the dry phase relative to M among the soils which differed in original WP indicates that respiration is more affected by the relative decrease in WP than by the absolute WP reached during drying. The smaller flush in respiration upon rewetting of saline soils suggests that these soils may be less prone to lose C when exposed to drying and rewetting compared to non-saline soils.

**Keywords:** drying and rewetting, microbial biomass, respiration, salinity, water potential.

## Introduction

Soil water availability to plants and microbes is a function of the amount of energy required to withdraw water. This can be expressed as water potential, with more energy being required by plants and microbes to take up water as the water potential becomes more negative (decreases). The water potential is the sum of various potentials, with matric potential (a measure of how strongly the water is held onto soil surfaces) and osmotic potential (a function of the concentration of soluble salts in the soil solution) being particularly important.

During summer, top soils may experience drying and rewetting cycles when dry periods are interrupted by occasional rainfall events. Previous studies using non-saline soils have shown that rewetting of dry soils induces a flush of respiration. As the soil dries, water is lost from increasingly smaller pores and the water films around aggregates become thinner and disconnected. Water availability decreases (water potential becomes more negative) because the remaining water is held more tightly to the aggregate surfaces (Ilstedt et al., 2000). In addition to the low water availability, microbes become substrate-limited because diffusion is restricted (Stark and Firestone, 1995). Moreover, the

increasing salt concentration in the remaining soil solution results in decreased osmotic potential (Chowdhury et al., 2011 a, b).

In order to maintain cell turgor and metabolic functions at low water potential, some microbes accumulate osmolytes (Oren, 1999; Hagemann, 2011). The accumulation or synthesis of osmolytes requires energy and is therefore a metabolic burden to the surviving microbes (Harris, 1980; Oren, 1999; Schimel et al., 2007).

In non-saline soils a flush of respiration usually occurs within a few hours after rewetting of dry soil, which lasts for 1-2 days after which respiration rates decline to levels similar to those in continuously moist soil (Kieft et al., 1987; Franzluebbers et al., 1994; Mikha et al., 2005; Butterly et al., 2009). Several mechanisms induced by rewetting result in increased substrate availability and thus explain the flush in respiration, namely release of the osmolytes accumulated during the dry phase, cell lysis and breakdown of aggregates which releases previously protected organic matter (Sparling et al., 1985; Halverson et al., 2000; Denef et al., 2001; Fierer and Schimel, 2003). The size of the flush upon rewetting in response to single and multiple drying and rewetting cycles has been studied extensively (e.g. (Van Gestel et al., 1993; Mikha et al., 2005; Butterly et al., 2009)), however in most studies, the water content of the soils during the dry phase was very low (air-dry or until the soils did not lose any more water at room temperature).

As outlined above, the effects of drying and rewetting are well described for non-saline soil. However, saline soils cover large proportions of land [$10^8$ ha (5%) of arable land (Lambers, 2003)], mainly in arid and semi-arid regions of the world; thus, they too may experience drying and rewetting cycles. Salinity reduces soil respiration (Setia et al., 2011; Chowdhury et al., 2011 a, b) and may change microbial community composition (Pankhurst et al., 2001; Gros et al., 2003; Gennari et al., 2007) due to differential tolerance to low osmotic potential among microbial genotypes (Mandeel, 2006; Llamas et al., 2008). Fungi have been reported to be more sensitive to salinity than bacteria (Pankhurst et al., 2001; Wichern et al., 2006; Chowdhury et al., 2011 b). The greater metabolic burden of microbes in saline soils compared to those in non-saline soils may change the effects of drying and rewetting on microbial activity. Microbes in saline soils may be more affected by the decreasing matric potential because of the additional low osmotic potential and thus a

greater metabolic burden for the synthesis of osmolytes. Upon rewetting, the flush in respiration may be greater than in non-saline soils because more osmolytes are released.

The aims of this study were to assess the effect of drying and rewetting on soil respiration and microbial biomass in soils with different levels of salinity (non-saline to highly saline). Furthermore, the extent of drying was varied by drying the soils for 1-5 days. We hypothesised that (i) compared to the moist control soil, drying will decrease respiration rates more strongly in the saline soils because of the lower WP during the dry phase compared to non-saline soil, (ii) the flush in respiration will be greater in soils that were dried more strongly compared to moderately dried soils, and (iii) the flush in respiration will be greater in saline soils due to the greater release of osmolytes.

## Materials and Methods

*Soil characterisation*

Five sandy loam soils (one non-saline, four saline) were collected from various locations in Monarto, South Australia (35° 05´ S and 139° 06´ E). After collection from 0-10 cm depth, the soils were air-dried and sieved to <2mm (Table 1). The United States Salinity Laboratory Staff defined a soil as saline when the electrical conductivity of the saturation extract ($EC_e$) is > 4 dS $m^{-1}$ , therefore soil EC2 is considered to be non-saline, while the other soils (EC9, EC19, EC33 and EC48) are saline.

The water retention curves of the soils were determined using suction and pressure techniques (Klute, 1986). Matric potential was estimated from the moisture retention curve using the following equation (Hillel, 1980).

The $EC_{1:5}$ was converted to ECe using the equation ECe = (14.0-0.13× clay %) × $EC_{1:5}$ (Rengasamy, 2006). The osmotic potential of the soil water was estimated using the equation: $\Psi_\pi = -0.036\ EC_{meas}\ (\theta_{ref}/\theta_{act})$ (Richards, 1954). Where $\Psi_\pi$ = the soil osmotic potential (MPa) at the actual moisture content, $\theta_{act}$ of the soil and $EC_{meas}$ = the measured electrical conductivity (dS $m^{-1}$) of an extract with a water content $\theta_{ref}$ (= 5 g $g^{-1}$ for a 1:5 soil:

water mixture). Water potential (WP) refers to the sum of osmotic (OP) and matric potential (MP).

The relationship between soil respiration and water content for each soil was determined in a preliminary experiment in which the water content was varied between 10 and 70% water holding capacity, corresponding to a matric potential of -2.7 to -0.1 MPa. In all soils, respiration rates were highest at 50% WHC, which corresponds to a matric potential of between -0.12 and -0.28 MPa.

*Experimental set-up*

Air-dry soils were wet to this matric potential to 50% WHC and pre-incubated for 10 d at 25°C before the experiments were begun. Ten days was selected on the basis of several previous studies in our lab using a wide range of soils which showed that microbial respiration stabilized between 7 and 10 days after rewetting air-dry non-saline soil (data not shown).

After this pre-incubation, pea (*Pisum sativum* L.) straw (C/N 26, water soluble C 27 g $kg^{-1}$), ground and sieved (0.25 - 2 mm), was mixed into the soils (2% w/w) to provide a readily-available nutrient source. The soils (30g) were then added to PVC cores (diameter 3.7 cm, height 5 cm) with a nylon mesh base (0.75 µm, Australian Filter Specialist) and packed to a bulk density of 1.46 g $cm^{-3}$ which is typical for sandy loam soils (http://www.pedosphere.com/resources/bulkdensity/triangle_us.cfm). The cores were placed in large plastic containers and incubated in the dark at 22-25 °C for 7 days during which the soil water content was maintained by weight. Then, the cores were placed individually into 1 L glass incubation jars and sealed with gas tight lids equipped with septa to allow headspace sampling. The glass jars were placed in the same room as for the previous incubation periods. Sets of each soil were kept either at optimal water content or dried and rewet (Figure 1). Drying was achieved by placing small pouches containing self-indicating silica gel (BDH Chemicals) into the glass jars. To ensure rapid drying, the silica was exchanged daily with a second quantity that was regenerated at 110°C overnight (Butterly et al., 2009). Soils were dried for 1, 2, 3, 4 or 5 days (referred to 1D, 2D, 3D, 4D and 5D) and maintained at the achieved water content for 4 days. Then the soils were rewet to optimal water content (50% WHC) and incubated for 5 days while maintaining this

water content. Thus, depending on the length of the drying phase, the experimental period ended on day 18, 19, 20, 21 and 22 after addition of pea residues for 1D, 2D, 3D, 4D and 5D, respectively (Figure 1). For the continuously moist treatment (M) maintained at 50%WHC, the experimental period ended on day 22. The water content was maintained by weighing the cores and adding reverse osmosis water if required. There were three replicates per moisture treatment and soil.

*Measurements*

Respiration was quantified by measuring headspace $CO_2$ concentrations every 24h using a Servomex 1450 infra-red gas analyser (Servomex Group, Crowborough, England). After each measurement, the jars were opened to equilibrate the $CO_2$ to ambient concentrations and then resealed. The $CO_2$ concentrations were measured immediately after resealing the jars. The $CO_2$ evolved from each sample was calculated as the difference between the initial (after resealing of the jars) and the $CO_2$ concentrations after 24h.

Microbial biomass C was determined on day 7 and at the end of the experimental period by fumigation-extraction (Vance et al., 1987) as described in Anderson and Ingram (1993) using 5 g soil. Briefly, one set of samples were fumigated with ethanol free chloroform for 24 h at 25°C in a sealed desiccator. Non-fumigated set of samples were stored at 8°C. After fumigant removal, both fumigated and non-fumigated soils were extracted with freshly prepared 0.5 M $K_2SO_4$ at 1:4 ratio and filtered. Dissolved organic carbon in the extracts was determined after dichromate digestion by titrating with 0.033 M 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.

*Statistical analysis*

Significant differences between different treatments at a given time point in respiration rate, cumulative respiration, microbial biomass were assessed by 2-way

ANOVA (soil x moisture treatment) and Tukey test with $P \leq 0.05$. (GenStat® for Windows 11.0, VSN Int. Ltd, UK, 2005).

Regressions between total potential and respiration parameters or microbial biomass were calculated with SPSS.

## Results

### *Potentials*

Matric potential decreased linearly in all soils with increasing drying time, reaching values less than -2 MPa in the 5D treatment (Table 2).Except for soil EC2, OP and WP decreased exponentially with increasing drying time. In 5D, OP and WP ranged between -15 and -30 MPa, with the lowest potentials in soil EC33. In soil EC2 MP was the dominant potential, whereas OP dominated in the saline soils, contributing between 70 and 91% to WP.

### *Respiration*

In moist and dry conditions, respiration rates were highest in soil EC2 and decreased with increasing EC up to soil EC19. The soils EC19, EC33 and EC48 generally had similar respiration rates.

Drying for 1 day reduced average respiration rates in the dry phase compared to M significantly but by only 10-20% (Figure 2). Longer drying periods, which resulted in lower water potentials, reduced average respiration rates in the dry phase compared to M, by 46-57% in 2D, 66-77% in 3D, 81-87% in 4D; in 5D the average respiration during the dry phase very low. There was a quadratic relationship between WP and respiration rate during the dry phase (Figure 3, $r^2=0.61$, $P<0.001$).

In all soils, rewetting of soils that had been dried for only 1 day and where WP was decreased by only 20-30% compared to M, did not increase respiration rates compared to those in the dry phase or in M (Figure 4). The increase in respiration rate after rewetting in

2D was gradual and small; thus there was no flush in respiration after only moderate drying although WP was approximately 2 fold lower in 2D compared to M. In all other drying treatments, rewetting induced a flush in respiration within one day with higher rates being maintained for about 3 days. The flush in respiration was greatest in 5D and smallest in 3D, where the maximum respiration rate was about 30% lower than in 5D. The increase in maximal respiration rate in 5D compared to 1D was greater in soil EC2 (90% increase) compared to the saline soils where the maximal respiration rate in 5D was 40-70% higher in soils EC9, EC19 and EC33 and only 30% higher in soil EC48. The maximal respiration rate after rewetting increased with magnitude of change in WP after rewetting (Figure 5). In the saline soils (EC ≥9), the two factors were linearly correlated ($r^2$=0.54, P=0.001). By day 4 after rewetting, respiration rates were similar to those in M in the saline soils, whereas they remained higher in soil EC2 until day 5 after rewetting.

Cumulative respiration was highest in soil EC2 and declined with increasing EC to soil EC19; there were no significant differences in cumulative respiration among soils EC19, EC33 and EC48 (Table 3). In soil EC2, cumulative respiration was significantly affected by the drying treatments, but compared to M, cumulative respiration was decreased by only 12% in 5D. In the saline soils, cumulative respiration was 24-34% lower in 5D compared to M. There was a quadratic relationship between cumulative respiration and water potential (Figure 3, $r^2$=0.71, P<0.001).

*Microbial biomass carbon*

In the unamended soils, MBC was higher in soils EC2 and EC9 with 67 and 45 mg $kg^{-1}$ compared to the soils EC19, EC33 and EC48 where it ranged only between 3 and 6 mg $kg^{-1}$ (Table 1). Seven days after residue addition and before the drying treatments started, MBC ranged from 172-192 mg C $kg^{-1}$ with no significant differences among the soils. At the end of the incubation period, MBC had decreased by ≥50% in the saline soils, particularly in M, with the greatest decrease in soil EC48, where MBC was up to 6 fold lower than on day 7. In soils EC2 and EC9, the drying treatments did not affect MBC at the end of the incubation (Table 3). On the other hand in soils EC19, EC33 and EC48, MBC was significantly higher in 4D and 5D compared to M; in 5D, MBC was more than 2 fold higher in soils EC19 and EC33 and more than 4 fold higher in soil EC48. Extractable C

was always highest in soil and lowest in soil EC19 (Table 2). In soils EC19, EC33 and EC48, extractable C was lower in 5D than in M.

Cumulative respiration was positively correlated with MBC ($r^2$=0.27, P=0.003), whereas respiration rate during the dry phase was not.

## Discussion

In agreement with previous studies (Kieft et al., 1987; Franzluebbers et al., 1994; Mikha et al., 2005; Butterly et al., 2009), rewetting of dry soils induced a flush of respiration. However, the intensity of the flush was affected by both the extent of drying and soil salinity.

*Respiration rates during the dry phase*

Our first hypothesis (compared to the moist control soil, drying will decrease respiration rates more strongly in the saline soils because of the lower WP during the dry phase compared to non-saline soil) has to be rejected. Relative to M, average respiration rates in the dry phase decreased to a similar extent in all soils (Figure 2 although OP and WP were substantially lower in the saline soils than in soil EC2, particularly in EC19, EC33 and EC48. For example in 3D, WP was -1.7 MPa in EC2, but ranged from -8.7 to -12.8 MPa in EC19, EC33 and EC48. However, the decrease in WP in the drying treatments relative to M was quite similar in all soils. This indicates that not the absolute WP determines the negative effect of drying on soil respiration, but the relative decrease compared to the moist soil. Indeed, the 2-fold decrease in WP from M to 2D decreased respiration in the dry phase by about 50% and the 3-fold decrease in WP in 3D decreased respiration by about 75%. Nevertheless, the quadratic relationship between respiration rate during the dry phase and WP (Figure 3) suggests that respiration rates are very low at WP < -10 MPa, irrespective of the initial potential of the soils.

*Respiration after rewetting*

In agreement with our second hypothesis (the flush in respiration will be greater in soils that were dried more strongly compared to moderately dried soils), weak drying (1D and 2D) did not result in a flush in respiration after rewetting and did not increase respiration rates compared to M (Figure 4). In these treatments, rewetting increased WP by 20-30% in 1D and 2-fold in 2D. Thus, such moderate increases in WP had little effect on microbial activity, possibly because the rewetting did not result in a strong increase in substrate availability. The reasons for a lack of increased substrate availability could be (i) little accumulation of osmolytes during the dry phase and therefore no substantial release upon rewetting, (ii) little aggregate breakdown upon rewetting, and/or (iii) less substrate remaining of the added residues compared to the treatments with stronger drying because of continuing decomposition during the dry phase. The latter is unlikely to be the case in the present study because extractable C at the end of the incubation period was higher in 1D and 2D compared to 4D and 5D (Table 2). However, it should be noted that the extractable C was determined 5 days after rewetting, therefore it cannot be ruled out that extractable C was lower in 1D and 2D immediately after rewetting. Moreover, extractable C may not be easily decomposable: dissolved organic C may contain a significant proportion of poorly decomposable compounds (Qualls, 2005; McDowell and Koopmanns, 2006).

When rewetting increased WP more than 2-fold (in 3D, 4D and 5D), it induced a strong flush in respiration in all soils, with the absolute and relative increase compared to M greatest in soil EC2 and 5D (Figure 4). Therefore our third hypothesis (the flush in respiration will be greater in saline soils due to the greater release of osmolytes) has to be rejected. The strong increase in respiration upon rewetting in 5D may be explained by the fact that compared to the other drying treatments in a given soil, 5D resulted in the lowest WP and lowest respiration rates during the dry phase and rewetting induced the greatest increase in WP (Table 2). Due to the low relative WP in the dry phase, it can be assumed that accumulation of osmolytes in 5D was greater than in the other treatments and that the strong increase in WP upon rewetting induced a rapid and strong release of these osmolytes. This, together with a possible release of previously protected organic matter would have resulted in a strong increase in substrate availability for the surviving microbes. The increase in respiration rate upon rewetting was greatest in soil EC2, which suggests that microbes in non-saline soils are better able to utilise the released substrates than

those in saline soils, where, even in moist soils, the WP was low. This is in agreement with our previous studies in which, after addition of plant residues, respiration rates decreased with increasing salinity (Setia et al., 2011; Chowdhury et al., 2011 a, b).

The results further indicate that high respiration rates after rewetting may compensate to some extent, the low respiration rates during the dry phase. The differences among the moisture treatments were greater for the respiration rates during the dry phase (Figure 2) than for cumulative respiration at the end of the incubation period (Table 3). Moreover, respiration rates during drying were more strongly affected by WP than cumulative respiration at the end of the experiment (Figure 3). Compared to WP -1MPa or higher, respiration rates at -10 MPa, were 6-7 fold lower, whereas cumulative respiration was only about 3-fold lower. This can be explained by the higher maximal respiration rate after rewetting in 3D, 4D and 5D compared to 1D and 2D.

*Microbial biomass*

The similar concentration of MBC seven days after residue addition indicates that microbes in saline soils are capable of rapidly utilising substrates added to the soil. Indeed, the increase in MBC after addition of residues was greater in soils EC19, EC33 and EC48 where MBC in the unamended soil ranged between 3 and 6 mg $kg^{-1}$ compared to 67 and 45 mg $kg^{-1}$ in soils EC2 and EC9 (Table 1). The strong increase in MBC is unlikely to have been accompanied by high respiration rates as respiration rates were lower in the saline soils than in soil EC2 in this experiment (Figure 4) and in previous experiments with salinized soils (Chowdhury et al., 2011 a, b) and saline soils from the field (Setia et al., 2011). Thus, respiration per unit MBC was lower in saline soils, indicating that substrates were utilised more effectively.

However, in the period from day 7 to the end of the incubation, MBC decreased more strongly in the saline soils than in soil EC2 in all moisture treatments, with the greatest decrease in M (Table 3). This indicates that once the easily available (water-soluble) C compounds from the residues are depleted, a large proportion of the microbial biomass in the saline soils died, possibly due to a lack of microbes capable of decomposing more recalcitrant C compounds. In previous experiments with non-saline soils to which salt was added, fungi appeared to be more sensitive to low osmotic potential

than bacteria (Chowdhury et al. 2011 a, b). Other studies have also found a lower absolute or relative abundance of fungi in saline compared to non-saline soils (Pankhurst et al., 2001; Wichern et al., 2006). Since the more recalcitrant compounds in plant residues are thought to be mainly decomposed by fungi (Killham, 1994), a low abundance of fungi could limit the ability of the microbial community to survive once the easily decomposable compounds are depleted. A further reason for the strong decline of MBC in the saline soils may be the greater energy requirement for osmotic adjustment than in soil EC2.

At the end of incubation period, MBC was little affected by moisture treatment in soils EC2 and EC9, but in the more saline soils, microbial biomass C was higher in 5D than in M (Table 3). This is most likely due to the higher substrate availability in 5D. In this treatment, respiration rates during the dry phase were very low (Figure 2). Although rewetting induced a flush in respiration, cumulative respiration at the end of the incubation period was lower in 5D than in 1D and M. Thus, it can be assumed that, compared to M or moderate soil drying (1D), more easily available C from the added pea residues was still available due to the lack of decomposition during the dry phase. The difference in MBC between soils EC2 and EC48 were smallest in 5D, which suggests that the greater availability of relatively easily decomposable compounds in 5D may have improved the ability of the microbes to tolerate low water potential and/or recover after rewetting. Furthermore, the strong drying in 5D may have selected for microbial genotypes with a high tolerance to low water potential.

## Conclusions

The results of this study suggest that rewetting results in a flush in respiration even in highly saline soils. However, the flush in respiration upon rewetting occurs only if the WP of the soils was decreased at least 3-fold during the dry phase, i.e. rewetting increases WP by a factor of 3 or more. Hence, only marked increases in WP induce a flush in respiration upon rewetting. The consistent decrease in respiration during the dry phase relative to M among the soils which differed in original WP indicates that respiration is more affected by the relative decrease in WP than by the absolute WP reached during drying if the WP remains above -10MPa in the dry phase.

The lower flush in respiration upon rewetting of saline soils suggests that these soils may be less prone to lose C when exposed to drying and rewetting compared to non-saline soils. Furthermore, although microbes in saline soils are able to efficiently convert easily available compounds from added residues into microbial biomass, they appear to have a limited ability to utilise more recalcitrant compounds. Both factors, high C use efficiency and low rates of decomposition of recalcitrant compounds could increase C storage in these soils.

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**References**

Anderson, J.M., Ingram, J.S.L., 1993. Tropical soil biology and fertility. Wallingford, CAB International.

Butterly, C.R., Bunemann, E.K., McNeill, A.M., Baldock, J.A., Marschner, P., 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biology & Biochemistry 41, 1406-1416.

Chowdhury, N., Marschner, P., Burns, R.G., 2011a. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil in press.

Chowdhury, N., Marschner, P., Burns, R.G., 2011 b. Soil microbial activity and community composition: impact of changes in matric and osmotic potential. Soil Biology and Biochemistry 41(7): 1406-1416.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology & Biochemistry 33, 1599-1611.

Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67, 798-805.

Franzluebbers, K., Weaver, R.W., Juo, A.S.R., Franzluebbers, A.J., 1994. Carbon and nitrogen mineralization from cowpea plant parts decomposing in moist and in repeatedly dried and wetted soil. Soil Biology & Biochemistry 26, 1379-1387.

Gennari, M., Abbate, C., La Porta, V., Baglieri, A., 2007. Microbial response to $Na_2SO_4$ additions in a volcanic soil. Arid Land Research and Management 21, 211-227.

Gros, R., Poly, F., Jocteur-Monrozier, L., Faivre, P., 2003. Plant and soil microbial community responses to solid waste leachates diffusion on grassland. Plant and Soil 255, 445-455.

Hagemann, M., 2011. Molecular biology of cyanobacterial salt acclimation. FEMS Microbiology Reviews 35(1), 87-123.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64, 1630-1637.

Harris, R.F., 1980. Effect of water potential on microbial growth and activity. Water potential relations in soil microbiology. Soil Science Society America, Madison. pp 23-95.

Hillel, D., 1980. Fundamentals in soil physics. New York, Academic Press.

Ilstedt, U., Nordgren, A., Malmer, A., 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biology & Biochemistry 32, 1594-1599.

Kieft, T.L., Soroker, E., Firestone, M.K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biology & Biochemistry 19, 119-126.

Killham, K., 1994. Soil Ecology. Cambridge, Cambridge University Press.

Klute, A., 1986. Water retention: laboratory methods. Methods of soil analysis, Part 1. A. Klute. Soil Science Society of America, Madison. pp 635-660.

Lambers, H., 2003. Dryland salinity: a key environmental issue in southern Australia. Plant and Soil 257, v-vii.

Llamas, D.P., Gonzales, M.D., Gonzales, C.I., Lopez, G.R., Marquina, J.C., 2008. Effects of water potential on spore germination and viability of Fusarium species. Journal of Industrial Microbiology & Biotechnology 35, 1411-1418.

Mandeel, Q.A., 2006. Biodiversity of the genus Fusarium in saline soil habitats. Journal of Basic Microbiology 46, 480-494.

McDowell, R.W., Koopmanns, G.F., 2006. Assessing the bioavailability of dissolved organic phosphorus in pasture and cultivated soil treated with different rates of nitrogen fertiliser. Soil Biology & Biochemistry 38, 61-70.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biology & Biochemistry 37, 339-347.

Oren, A., 1999. Bioenergetic aspects of halophilism. Microbiology and Molecular Biology Review 63, 334-348.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilisation patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations win South Australia. Biology and Fertility of Soils 33, 204-217.

Qualls, R.G., 2005. Biodegradability of fractions of dissolved organic carbon leached from decomposing leaf litter. Environment, Science and Technology 39, 1616-1622.

Rengasamy, P., 2006. Soil salinity and sodicity. In: Growing crops with reclaimed wastewater. Stevens, D., Kelly, J., McLaughlin, M. J., Unkovich, M. (ed). CSIRO Publishing, Canberra. pp 125-138.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. Soil Science, 7-33.

Schimel, J.P., Balser, T.C., Wallenstein, M., 2007. Microbial stress response physiology and its implications for ecosystem function. Ecology 88, 1386-1394.

Setia, R., Marschner, P., Baldock, J.S., Chittleborough, D.J., Verma, V., 2011. Relationships between carbon dioxide emission and soil properties in salt affected landscapes. Soil Biology & Biochemistry 43, 667-674.

Sparling, G.P., Whale, K.N., Ramsay, A.J., 1985. Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air-dried soils. Australian Journal of Soil Research 23, 613-621.

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil moisture effects on the activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218-221.

Van Gestel, M., Merckx, R., Vlassak, K., 1993. Soil drying and rewetting and the turnover of $^{14}$C-labelled plant residues: first order decay rates of biomass and non-biomass $^{14}$C. Soil Biology & Biochemistry 25, 125-134.

Vance, C.P., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703-707.

Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100-108.

Table 1. Properties of the soils used in the study.

| Soil | Sand | Silt | Clay | EC 1:5 | ECe | pH 1:5 | TOC | MBC | Water holding capacity |
|---|---|---|---|---|---|---|---|---|---|
| | (%) | | | (dS $m^{-1}$) | | | (g $kg^{-1}$) | (mg $kg^{-1}$) | (g $kg^{-1}$) |
| EC2 | 60.0 | 21.3 | 18.8 | 0.19 | 2 | 9.3 | 16.4 | 67 | 357 |
| EC9 | 70.0 | 15.0 | 15.0 | 0.76 | 9 | 9.3 | 10.1 | 45 | 356 |
| EC19 | 65.0 | 16.2 | 18.8 | 1.62 | 19 | 9.5 | 2.6 | 6 | 314 |
| EC33 | 60.0 | 21.0 | 19.0 | 2.82 | 33 | 9.0 | 4.7 | 3 | 314 |
| EC48 | 60.0 | 21.0 | 19.0 | 4.07 | 48 | 8.9 | 5.0 | 4 | 406 |

Table 2. Osmotic, matric and water potential after drying for 1-5 days in soils differing in ECe.

| Soil | M | 1D | 2D | 3D | 4D | 5D |
|---|---|---|---|---|---|---|
| | | | Matric potential (MPa) | | | |
| EC2 | -0.12 | -0.14 | -0.42 | -1.50 | -2.09 | -2.70 |
| EC9 | -0.10 | -0.12 | -0.29 | -1.41 | -2.07 | -3.02 |
| EC19 | -0.23 | -0.72 | -1.30 | -1.91 | -2.42 | -2.71 |
| EC33 | -0.28 | -0.79 | -1.31 | -1.85 | -2.24 | -2.55 |
| EC48 | -0.23 | -0.26 | -0.64 | -1.05 | -1.68 | -2.15 |
| | | | Osmotic potential (MPa) | | | |
| EC2 | -0.09 | -0.11 | -0.14 | -0.22 | -0.31 | -0.56 |
| EC9 | -1.01 | -1.24 | -1.59 | -2.35 | -3.25 | -7.38 |
| EC19 | -3.23 | -4.04 | -5.26 | -5.26 | -12.77 | -20.37 |
| EC33 | -4.66 | -5.75 | -7.47 | -7.47 | -16.41 | -27.25 |
| EC48 | -4.71 | -5.46 | -6.51 | -6.51 | -10.33 | -14.15 |
| | | | Water potential (MPa) | | | |
| EC2 | -0.21 | -0.25 | -0.56 | -2.40 | -2.40 | -3.26 |
| EC9 | -1.11 | -1.36 | -1.88 | -3.76 | -5.32 | -1040 |
| EC19 | -3.46 | -4.76 | -6.56 | -9.65 | -15.19 | -23.08 |
| EC33 | -4.94 | -6.54 | -8.77 | -12.80 | -18.65 | -29.80 |
| EC48 | -4.94 | -5.72 | -7.14 | -8.66 | -12.01 | -16.30 |

Table 3. Cumulative respiration from day 8 to the end of the incubation period and microbial biomass C and $K_2SO_4$-extractable C (non-fumigated soil) in soils differing in ECe dried for 1-5 days, followed by a 4-day dry incubation and rewetting, at the end of the 5-day moist incubation period (n=3).

| | M | 1D | 2D | 3D | 4D | 5D |
|---|---|---|---|---|---|---|
| | Cumulative respiration (mg $CO_2$-C $g^{-1}$) | | | | | |
| EC2 | 1.30 | 1.31 | 1.21 | 1.17 | 1.22 | 1.15 |
| EC9 | 0.95 | 0.96 | 0.77 | 0.84 | 0.73 | 0.72 |
| EC19 | 0.67 | 0.58 | 0.48 | 0.52 | 0.50 | 0.47 |
| EC33 | 0.56 | 0.55 | 0.42 | 0.50 | 0.45 | 0.41 |
| EC48 | 0.66 | 0.53 | 0.44 | 0.52 | 0.41 | 0.44 |
| | lsd=0.07 | | | | | |
| | Microbial biomass C (mg $kg^{-1}$) | | | | | |
| EC2 | 101 | 127 | 164 | 119 | 184 | 159 |
| EC9 | 82 | 105 | 99 | 80 | 156 | 115 |
| EC19 | 80 | 96 | 88 | 128 | 116 | 47 |
| EC33 | 84 | 92 | 84 | 120 | 122 | 52 |
| EC48 | 47 | 33 | 33 | 70 | 94 | 16 |
| | lsd=34.3 | | | | | |
| | $K_2SO_4$-extractable C (mg $kg^{-1}$) | | | | | |
| EC2 | 242 | 262 | 260 | 180 | 176 | 182 |
| EC9 | 276 | 294 | 260 | 242 | 220 | 227 |
| EC19 | 205 | 190 | 197 | 122 | 132 | 199 |
| EC33 | 213 | 230 | 187 | 201 | 142 | 229 |
| EC48 | 418 | 352 | 325 | 300 | 283 | 378 |
| | lsd=42.8 | | | | | |

Figure 1. Experimental design. Grey rectangles indicate moist incubation, white rectangles dry period with D showing days of drying and rectangles without D incubation at the water content reached during the drying phase.

Figure 2. Respiration rates during the dry phase in soils differing in ECe dried for 1-5 days (n=3, bars indicate standard error).

Figure 3. Relationship between water potential during the dry period in soils differing in ECe dried for 1-5 days and respiration rates during the dry phase or cumulative respiration at the end of the 5-day moist incubation period. Symbols are averages of each treatment for a given soil (n=3).

Figure 4. Respiration rates in soils differing in ECe dried for 1-5 days at the end of the dry phase (day -1), on the day the soils were rewet (day 0) and in the following 5-day moist incubation period (n=3, bars indicate standard error).

Figure 5. Relationship between the magnitude of change in water potential at rewetting and maximal respiration rate after rewetting in soils differing in ECe dried for 1-5 days. Symbols are averages of each treatment for a given soil (n=3).

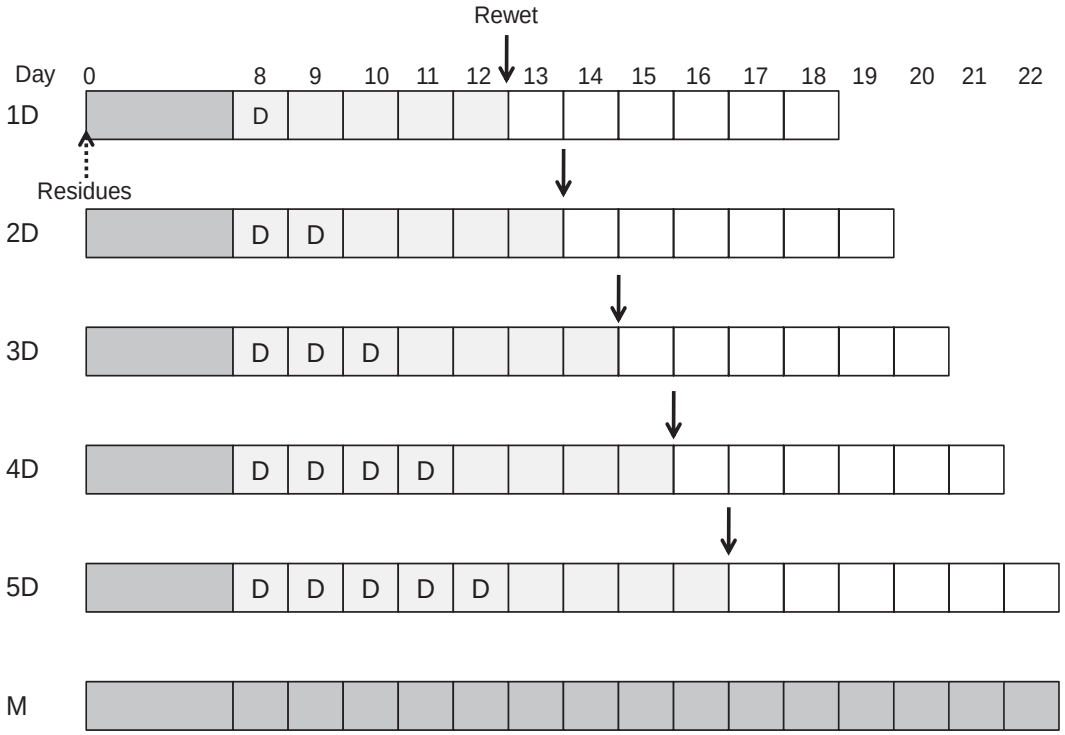
Rewet
Day
0
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
1D
D
Residues
2D
D
D
3D
D
D
D
4D
D
D
D
D
5D
D
D
D
D
D
M

Figure 1

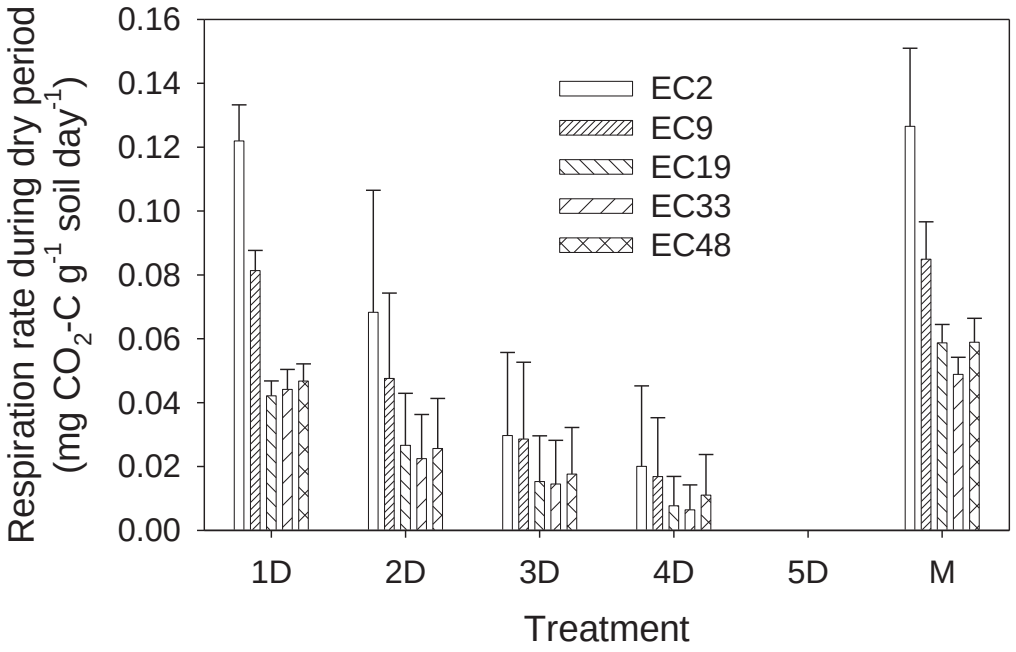
Respiration rate during dry period
(mg $CO_2$-C $g^{-1}$ soil $day^{-1}$)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
EC2
EC9
EC19
EC33
EC48
1D
2D
3D
4D
5D
M
Treatment

Figure 2

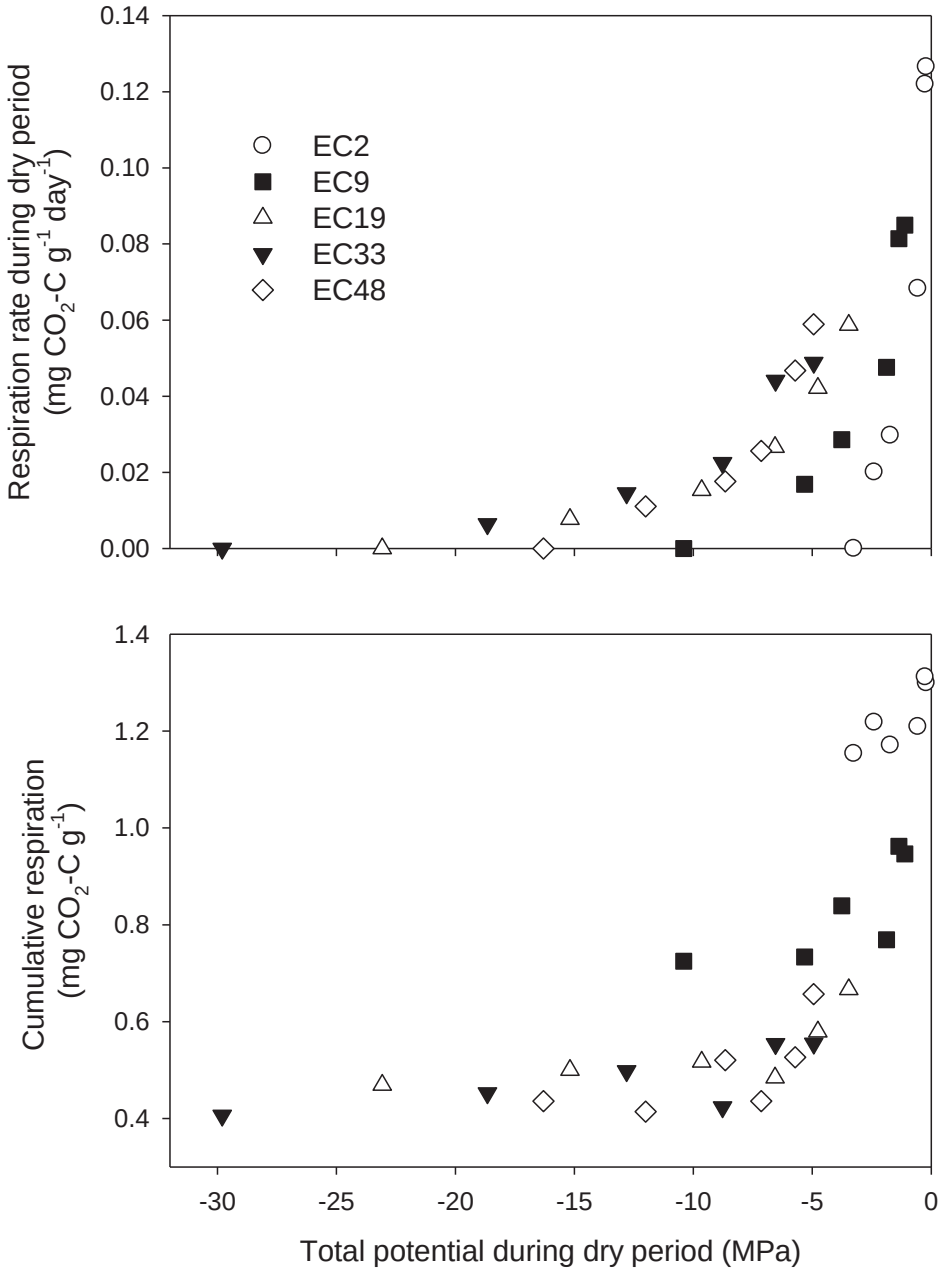
Respiration rate during dry period
(mg CO2-C g-1 day-1)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
EC2
EC9
EC19
EC33
EC48
Cumulative respiration
(mg CO2-C g-1)
1.4
1.2
1.0
0.8
0.6
0.4
-30
-25
-20
-15
-10
-5
0
Total potential during dry period (MPa)

Figure 3

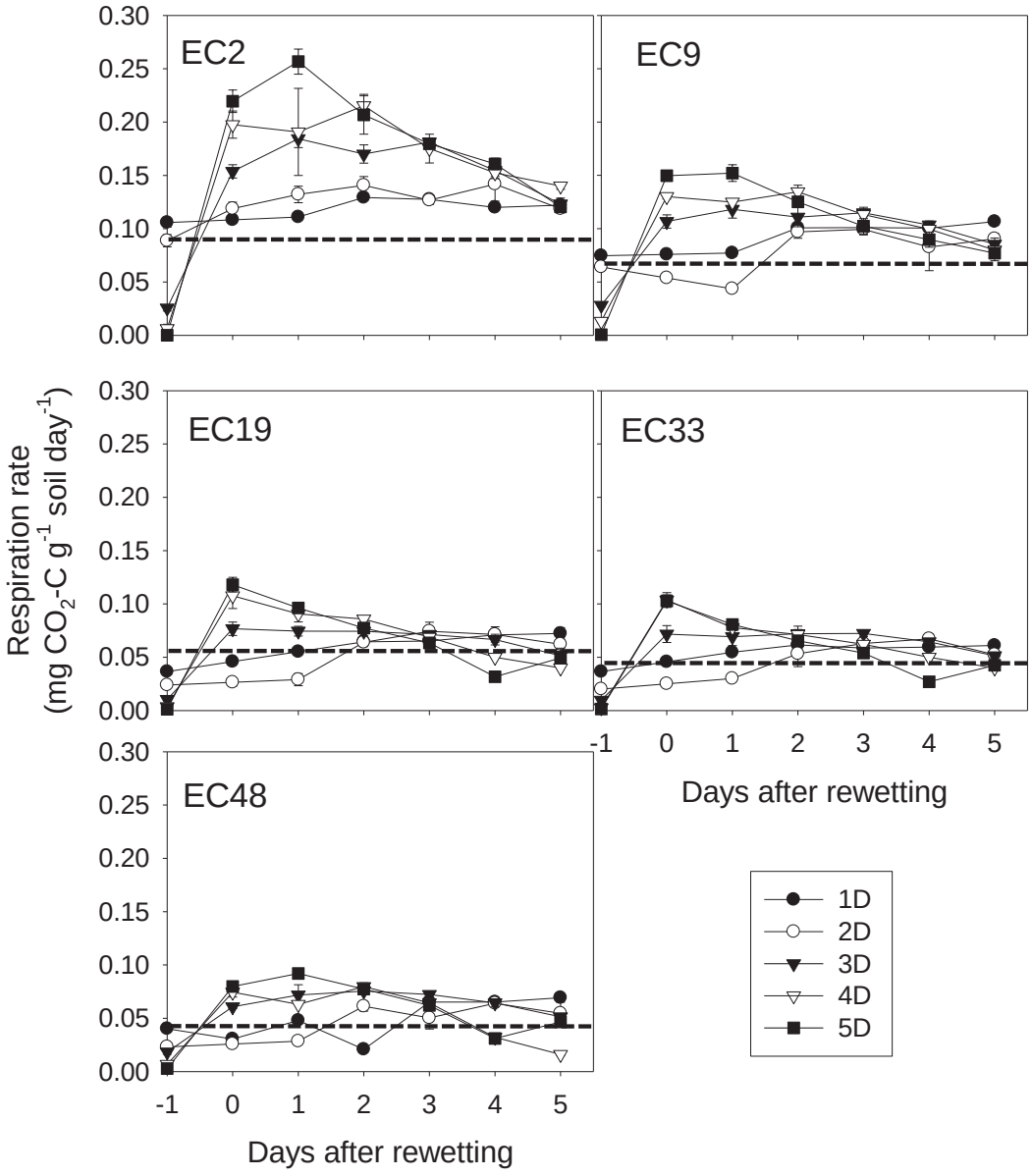
EC2
EC9
EC19
EC33
EC48
Respiration rate
(mg CO2-C g-1 soil day-1)
Days after rewetting
Days after rewetting
1D
2D
3D
4D
5D

Figure 4

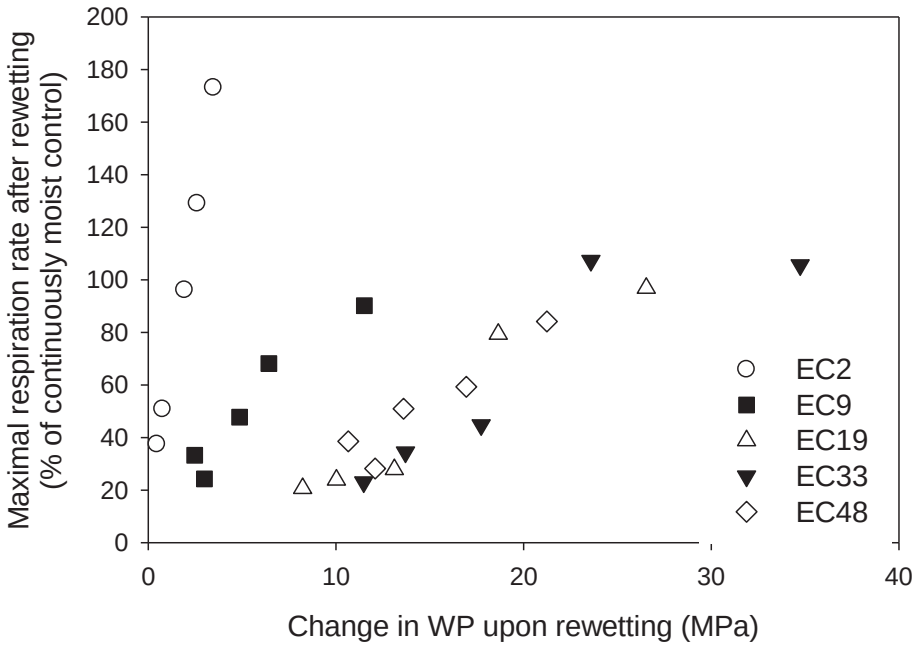
Maximal respiration rate after rewetting
(% of continuously moist control)
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
Change in WP upon rewetting (MPa)
EC2
EC9
EC19
EC33
EC48

Figure 5

# Chapter 8

## Conclusions and Future Research

# 8 Conclusions and Future Research

Crop growth is often poor on salt-affected soils which cover a large area world-wide. If these soils could be ameliorated so that crop growth is enhanced, they could play an important role in increasing food production for the growing human population. One prerequisite for successful amelioration is an understanding of the processes (chemical, physical and biological) in salt-affected soils. While there has been substantial progress in knowledge of many chemical and physical processes in these soils, less is known about the biological properties. Among the latter, activity and community structure of soil microorganisms are critical for nutrient availability and plant growth. A number of studies have assessed microbial activity and community structure in salt-affected soils (e.g. Pathak and Rao, 1998; Pankhurst et al., 2001; Rietz and Haynes, 2003; Tripathi et al., 2006; Wichern et al., 2006; Gennari et al., 2007), but usually without consideration of the impact of soil water content on salt concentration in the soil solution.

Salt-affected soils are mostly found in arid and semi-arid regions where the soil water content varies seasonally and soils are often exposed to drying and rewetting events. Therefore, it is important to assess the relationship between the impact of salinity on soil microbes and soil water content. This relationship was addressed in a number of experiments presented in this thesis.

The main findings of the experiments presented in this thesis were:

- Water potential is a better measure of the impact of salinity and soil water availability on soil microbes than EC or soil water content, particularly when comparing soils of different texture.

- The negative effect of salinity on soil microbes may be exacerbated at low soil water content.

- Rewetting of non-saline or saline soils induces a flush of respiration only if the soil was previously dried to water potentials at least three-fold lower than the water potential for maximal microbial activity.

- High salinity may reduce the ability of soil microbes to utilise substrates released after rewetting of dry soil.

- Microbial activity was more sensitive to low water potential than microbial biomass or community composition.

Water potential is a measure of the energy required to withdraw water from the soil, thus it is a better measure of water availability to plants and microbes than water content or salinity measured by EC. This was confirmed in a number of experiments described in this thesis where microbial activity was more closely related to water potential (osmotic and/or matric) than to EC or gravimetric water content. Relating microbial activity to water potential also revealed that the response of microbes in soils of different texture to decreasing water potential is quite similar although they may differ substantially in EC or gravimetric water content.

Water potential and its components osmotic and matric potential decrease as the soil dries or the EC increases. Hence, it becomes increasingly difficult for microbes to withdraw water from the soil and water may even be drawn out of the soils if the potential in the soil is lower than that in the cells. To avoid the latter, microbes may accumulate osmolytes which help maintaining cell turgor and thus metabolic activity (Oren, 2001; Schimel et al., 2007; Hagemann, 2011).

The comparison of the impact of low osmotic or low matric potential (Chapter 3, (Chowdhury et al., 2011a)) suggested that low matric potential may be more detrimental than a corresponding low osmotic potential at optimal soil water content. This may be due to restricted diffusion of substrates and thus a reduced ability of the microbes to synthesise osmolytes to help maintain cell water content. The study also showed that decreasing soil water content concentrates the salts and thus decreases the osmotic potential. Thus, microbes in dry soils are exposed to two stressors, particularly in coarser textured soils due to their lower water-holding capacity.

The study in which salinised soils were exposed to different water contents (Chapter 4, (Chowdhury et al., 2011 b)), confirmed that in order to understand microbial biomass and activity in saline soils, water potential (osmotic + matric potential) must be

considered, particularly at low water contents. The EC is a poor indicator of the stress microbes are exposed to in saline environments because although the measured EC remains the same, microbes will be subjected to different osmotic and matric potentials as the water content changes. Despite differences in water content and EC between the two soils used in that study, the response of microbes to decreasing water potential was similar and could be separated into two strategies. A decrease in WP up to -2MPa killed a proportion of the microbial community, but the remaining microbes adapted and maintained their activity per unit biomass. At lower WP however, the adaptation mechanisms were not sufficient and although the microbes survived, their activity per unit biomass was reduced.

The three studies related to drying and rewetting (Chapters 5, 6 and 7) showed that when exposed to a single drying and rewetting event, the soil water content (or water potential) during the dry phase affects the size of the flush in respiration upon rewetting in non-saline and saline soils. Rewetting induced a flush in respiration only if the water potential of the soil was previously decreased at least 3-fold compared to the water potential for maximal microbial activity. In other words, only marked increases in water potential induce a flush in respiration upon rewetting. Interestingly in the experiment with non-saline and saline soils from the field (Chapter 7), there was a consistent decrease in respiration during the dry period relative to optimal water potential although the soils differed in original water potential and therefore also in water potential during the dry phase. This indicates that respiration was more affected by the relative decrease in WP than by the absolute WP reached during drying. The flush in respiration upon rewetting was smaller in the saline soils compared to the non-saline soil which suggests that saline soils may be less prone to lose C when exposed to drying and rewetting compared to non-saline soils.

Despite the flush of microbial activity upon rewetting and higher respiration rates compared to the continuously moist soil for approximately 1 week after rewetting, cumulative respiration in previously dried soils may not reach those of continuously moist soil, even after 54 days of moist incubation (Chapter 5), suggesting that drying of soil can have a significant and long-lasting impact on microbial functioning.

The experiment where salinised soil with different EC was exposed to one to three drying and rewetting events (Chapter 6), microbial activity after the third rewetting event

was higher relative to the moist control in the soil with ECe 9.3 dS $m^{-1}$ compared to the soil with ECe 0.7 dS $m^{-1}$. This indicates that microbes in moderately saline soils may be able to withstand multiple DRW events better than those in non-saline soils and those microbes in the soil with ECe 9.3 dS $m^{-1}$ were better able to utilise substrates released at the third rewetting. However, at high EC (ECe 17.6 dS $m^{-1}$), the low osmotic potential reduced microbial activity to such an extent that the microbes could not take advantage of any substrate released after rewetting of dry soil. This is in agreement with the experiment with a single drying and rewetting event with saline soils from the field (Chapter 7) where respiration rates upon rewetting in soils with ECe ≥19 dS $m^{-1}$ were very low.

Finally, low water potential consistently had a stronger effect on microbial activity (soil respiration) than on microbial biomass or community composition. Low matric potential appeared to have a more negative effect on bacteria, whereas low osmotic potential was more detrimental to fungi; but in general the relative changes in microbial biomass or community composition were smaller than those on microbial activity. Thus, microbial genotypes appear to adjust their activity per cell in response to low water potential, but this has only a moderate impact on their competitive ability.

The results described in this thesis will increase the knowledge of biological properties of salt-affected soil, however a point of critique could be that in most experiments, salt was added to previously non-saline soil. This was done to avoid potential differences in soil physical and chemical properties among soils with different EC collected from the field which may affect the size of the microbial community, nutrient availability and thus the microbial response to changes in matric potential. Indeed, the saline soils collected from the field and used in the study described in Chapter 7, differed substantially in total organic C and microbial biomass C as well as in EC. Adding different amounts of salt to previously non-saline soil will minimize the influence of other soil properties on microbial activity and community structure. However, the response of microbes freshly exposed to salinity may differ from that of soils that were exposed to a given EC for longer periods of time. The results shown in Chapter 2 suggest that a 14-day exposure to different EC values did not affect the response of microbial activity to a given EC compared to non-adapted soils. In the field, however, microbes may be exposed to a given EC for several weeks, months or even years which may induce changes in microbial community composition by favouring adapted species. Nevertheless, the fact that low respiration was

found at ECe ≥17- 19 dS $m^{-1}$ in salinised and saline soils from the field suggests that the results obtained from salinised soils are representative for saline soils in the field.

The experiments described here answered a number of questions regarding microbial activity and community composition in saline soils in response to varying water content, but there are a number of research questions that could be addressed in future studies:

1. How does substrate availability affect the response of microbes in saline soils to varying water content? In the studies described here, pea residues with a low C/N ratio were added at 2% (w/w). Both amount and C/N ratio of substrate addition could be varied to test the hypothesis that high substrate availability increases the tolerance of microbes to low water potential.

2. Do root exudates affect the response of microbes in saline soils to varying water content? Root exudates comprise mainly easily available compounds (Neumann, 2007) and rhizosphere communities differ from those in the surrounding bulk soil (Butler et al., 2003; Marschner et al., 2004; de Ridder-Duine et al., 2005; Marschner, 2005). Thus, the effect of low water potential on microbial communities in the rhizosphere may differ from that on bulk soil communities which were studied in the experiments described in this thesis. To assess this, soil respiration and microbial community composition in rhizosphere soil from salt-tolerant plants (e.g. *Atriplex* sp.) grown in soils with different EC and corresponding soil from unplanted pots could be measured at different water contents.

3. How does decreasing or fluctuating water potential affect community composition of microbial groups such as bacteria, fungi and archaea? The phospholipid fatty acid analysis used in the experiments described here only allows separating broad groups (bacteria, fungi) and a coarse assessment of microbial community composition. The community composition of bacteria, fungi and archaea, DNA based methods such as terminal restriction length polymorphism (TRFLP) or denaturing gradient gel electrophoresis (DGGE) could be assessed in experiments similar to those used in this thesis. The community composition of archaea may be particularly interesting because some archaea are extremophiles, i.e. tolerant to

harsh conditions such as low water content or high salinity (Woese, 1987; Oren, 2001).

4. How does microbial activity in saline soils in the field vary over seasons and are fluctuations in activity related to changes in water potential? For the experiments described here, soils were sieved, air-dried and then rewet before starting the experiments. Sieving will destroy macro-aggregates and air-drying may kill some microbial genotypes. Therefore, the results obtained with disturbed soil may differ substantially from those in the field. For the field study, soil respiration could be measured placing a chamber over the soil surface and measuring soil $CO_2$ flux several times during the year, focussing on times where the soil water content changes, e.g. early and late summer. These measurements would be accompanied by determination of EC and water content to calculate osmotic and matric potential.

## References


Butler, J.L., Williams, M.A., Bottomley, P.J., Myrold, D.D., 2003. Microbial community dynamics associated with rhizosphere carbon flow. Applied and Environmental Microbiology 69, 6793-6800.

Chowdhury, N., Marschner, P., Burns, R.G., 2011a. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil in press.

Chowdhury, N., Marschner, P., Burns, R.G., 2011 b. Soil microbial activity and community composition: impact of changes in matric and osmotic potential. Soil Biology and Biochemistry 41(7), 1406-1416.

de Ridder-Duine, A.S., Kowalchuk, G.A., Klein Gunnewiek, P.J.A., Smant, W., Van Veen, J.A., De Boer, W., 2005. Rhizosphere bacterial community composition in natural stands of Carex avenaria (sand sedge) is determined by bulk soil community composition. Soil Biology and Biochemistry 37, 349-357.

Gennari, M., Abbate, C., La Porta, V., Baglieri, A., 2007. Microbial response to $Na_2SO_4$ additions in a volcanic soil. Arid Land Research and Management 21, 211-227.

Hagemann, M., 2011. Molecular biology of cyanobacterial salt acclimation. Fems Microbiology Reviews 35(1), 87-123.

Marschner, P., 2005. Microbial community structure and function in the rhizosphere. Biotechnological applications of microbes. A. Varma and G. K. Podila. New Dehli, IK International, 43-65.

Marschner, P., Crowley, D.E., Yang, C.H., 2004. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant and Soil 261, 199-208.

Neumann, G., 2007. Root exudates and nutrient cycling. Nutrient cycling in terrestrial ecosystems. P. Marschner and Z. Rengel. Berlin, Springer, 123-157.

Oren, A., 2001. The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implication of the functioning of salt lake ecosystems. Hydrobiologia 466, 61-72.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilisation patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations win South Australia. Biology and Fertility of Soils 33, 204-217.

Pathak, H., Rao, D.L.N., 1998. Carbon and nitrogen mineralisation from added organic matter in saline and alkali soils. Soil Biology and Biochemistry 30, 695-702.

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biology and Biochemistry 35, 845-854.

Schimel, J.P., Balser, T.C., Wallenstein, M., 2007. Microbial stress response physiology and its implications for ecosystem function. Ecology 88, 1386-1394.

Tripathi, S., Kumari, S., Chakraborty, A., Gupta, A., Chakraborty, K., Bandyapadhyay, B.K., 2006. Microbial biomass and its activities in salt-affected coastal soils. Biology and Fertility of Soils 42, 273-277.

Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100-108.

Woese, C.R., 1987. Bacterial evolution. Microbiological Reviews 51, 221-271.