THE UNIVERSITY OF ADELAIDE

Response of microbial activity and biomass to changes in soil salinity and water content

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I dedicate this thesis to my grandmother Yueying Wang

my greatest ever incentive for completing this work

TABLE OF CONTENTS

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ABSTRACT

Salinization is a serious land degradation problem because osmotic stress and toxic ions cause poor plant growth and low soil microbial activity. The effect of salinity on soil microbes has been studied previously, but usually at constant salinity. However, in the field salinity may vary over time. Another factor influencing the effect of salinity on soil microbes is the soil water content. The osmotic potential, which is a measure of the salt concentration in the soil solution, increases as soils dry. The aim of the experiments described in this thesis was to assess how soil microbial activity and microbial biomass respond to changes in soil salinity and soil water content. One non-saline and four saline soils from Monarto, South Australia (35° 05´ S and 139° 06´ E) were used in the experiments. Soils were air-dried after collection. In some experiments, salinity was induced by adding certain amount of NaCl (dissolved in RO water), or decreased by leaching. Preliminary experiments were carried out to quantify the salts or water needed to reach the desired salinity. Pea (*Pisum sativum L.*) straw (C/N=26) was used as available substrate in most experiments except for experiments in Chapter four, where glucose was used. Soil CO_2 release (respiration as measure of microbial activity) was measured daily throughout each experimental period, microbial biomass was determined at different times in each experiment by fumigation extraction.

The experiments described in Chapter 2 (Soil Biology and Biochemistry 53, 50-55, 2012) were conducted to investigate the response of soil microbial activity and biomass to increasing salinity. The electrical conductivity of the saturation extract (EC_e) of five different soils was adjusted to 3 to 119 dS m^{-1} by adding NaCl. After 15 days, cumulative respiration and microbial biomass were negatively correlated with EC. Irrespective of the original soil EC, cumulative respiration at a given adjusted EC was similar, suggesting that microbes from originally saline soils are not more tolerant to increases in salinity than those from originally non-saline soils.

The experiment described in Chapter 3 (Biology and Fertility of Soils 49, 367-371, 2013) was designed to investigate the response of soil microbial activity and biomass to decreasing salinity. Three

saline soils were used, the EC_e was decreased by leaching to EC_e 6 to 32 dS m^{-1}. At a given adjusted EC, irrespective of the original EC, cumulative respiration recovered to the same level as in the soils which had originally lower EC. This was also true for microbial biomass C, except for the soil with the highest original EC, where microbial biomass C did not fully recover.

The aim of the two experiments in Chapter 4 (Soil Biology and Biochemistry 65, 322-328, 2013) was to investigate the response of soil microbial activity and biomass to changes of salinity. In both experiments, one non-saline soil and two saline soils were used. Every 5 days, soil cores were dipped into a salt solution (contained glucose as available substrate) to increase or maintain the EC or salinity was decreased by leaching. In Experiment 1, soil salinity was increased or reduced between EC 1, 11, and 31 dS m^{-1} repeatedly over six 5-day cycles. In Experiment 2, soil salinity was increased over four 5-day cycles from 1 or 11 to 31 dS m^{-1} either abruptly (within one cycle) or gradually (over at least 2 cycles). The results showed that soil microbes can respond quickly to changes in EC with respect to activity and growth when they are supplied with easily available C. A previous exposure to high EC did not limit the ability of the soil microbes to respond to a subsequent decrease in salinity. Compared to the originally saline soils, microbial activity and biomass in the originally non-saline soil were higher, less affected by EC increases and recovered more quickly after the EC was decreased. A gradual EC increase did not result in greater respiration or microbial biomass compared to an abrupt increase.

In semi-arid and Mediterranean ecosystems, surface soils frequently experience dry and rewet events. The experiment in Chapter 5 (Soil Biology and Biochemistry 43, 2265-2272, 2011) aimed to determine the effect of the length of the dry period on the size of the flush in respiration after rewetting. One non-saline and four saline soils were used. The length of the dry period varied between 1 and 5 days which resulted in different water contents, being lowest with the longest dry period. At the end of the dry period, soils were rewet to optimal water content. The experiment showed that rewetting induced a flush in respiration only if the water potential of the soils was previously decreased at least 3-fold compared to optimal water content.

Chapter 6 (submitted Biology and Fertility of Soils) includes three experiments with the aim to test the hypotheses that: (i) the osmotic potential to which the microbes were previously exposed influences their activity at a different osmotic potential and (ii) the response is modulated by the speed of the changes in osmotic potential were tested. Three soils were used, a non-saline soil and two saline soils where the EC_e was adjusted to 10 and 30 dS m^{-1}. In Experiment 1, the relationship between soil water content and respiration was determined. Water contents for optimal, medium and low respiration were chosen for the following experiments. There were five treatments for each soil In Experiment 2, and each treatment included two periods: maintained at optimum water content; from optimum to medium water content; maintained at medium water content; slow change from low to medium water content; rapid change from low to medium water content. In Experiment 3, the effect of the speed by which the water content was changed on soil respiration was further investigated. In both Experiments 2 and 3, respiration at the target water content was higher at medium or high water content when the soils had a low water content before, indicating that the response of microbial activity to a certain water content (osmotic potential) is influenced by the previous water content. However, microbial activity was less related to the speed of changes, as cumulative respiration was not consistently different between rapid and slow drying.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Nan Yan April 2014

PUBLICATIONS ARISING FROM THIS THESIS

1. Yan, N., Marschner, P., 2012. Response of microbial activity and biomass to increasing salinity depends on the final salinity, not the original salinity. Soil Biology & Biochemistry 53, 50-55.

2. Yan, N., Marschner, P., 2013. Microbial activity and biomass recover rapidly after leaching of saline soils. Biology and Fertility of Soils 49, 367-371.

3. Yan, N., Marschner, P., 2013. Response of soil respiration and microbial biomass to changing EC in saline soils. Soil Biology & Biochemistry 65, 322-328.

4. Chowdhury, N., Yan, N., Islam, M.N., Marschner, P., 2011. The extent of drying influences the flush of respiration after rewetting in non-saline and saline soils. Soil Biology & Biochemistry 43, 2265-2272.

CHAPTER 1

Introduction and Literature Review

1 Introduction and Literature Review

1.1 Introduction

Soil salinization, which is the accumulation of excess salts, is a serious land degradation issue in more than 100 countries. Over 900 Mha of land throughout the world are affected by salinity or sodicity or both corresponding to about 10% of total arable land (Szabolcs, 1989). Salinization is found on ≥ 5% of the land area in Africa and up to 22% of arable land in West Asia. Salinization affects 1-3 Mha land in Europe and 17 western states of the United States of America (Chhabra, 1996; Ladeiro, 2012; UNEP, 2007). It is estimated that at least ten hectares of arable land are lost world-wide every minute, three of which from soil salinization (Buringh, 1978). Salinization reduces crop value and yield, destroys species habitats, and causes severe socioeconomic and environmental problems in the long term. Annual global income losses due to salinization are estimated at over 12 $\times 10^9$ US $ (Ghassemi et al., 1995).

In Australia, one of the driest continents in the world, about 30% of the land area is affected by salinization (Rengasamy, 2006b). Two forms of salinity are recognised in Australia: primary salinity, which originates mainly from deposition of oceanic salt by rain and wind and is stored in the soil or groundwater and secondary salinity, which is the salinization of land and water resources due to human activities, including irrigation salinity and dryland salinity. Both forms of salinity are due to rising water tables bringing dissolved salts in the root zone of plants. The National Land and Water Resources Audit estimates that in Australia, nearly 5.7 million hectares are at risk or affected by dryland salinity; a figure that could triple to 17 million hectares in 50 years (NLWRA, 2001); and more than $130 million of agriculture production is lost annually from salinity (NDSP, 2004).

High concentrations of salts in the soil solution adversely influence plant growth and soil microorganisms mainly through three mechanisms: 1) osmotic effects, which reduce water uptake by plants and microbes; 2) specific ion effects (toxicity or ion imbalance); and 3) changes in soil physical

and chemical properties. Through its negative effect on plants and soil microbes, salt accumulation can impair nutrient cycling.

Many studies have been conducted on the effects of salinity on soil microbes, including microbial activity (Chowdhury et al., 2011a), biomass (Laura, 1974; Rietz and Haynes, 2003; Sarig and Steinberger, 1994), and community structure (Gennari et al., 2007; Gros et al., 2003; Pankhurst et al., 2001). However, in the field, soil salinity is seldom constant over time or uniform in space: in coastal soils, soil salinity fluctuates seasonally, due to the intrusion of seawater into the groundwater (Tripathi et al., 2006). In irrigated soils, salinity varies with the quality of the irrigation water. Amount and distribution of rainfall events, especially in arid and semi-arid areas, also affect soil salinity and consequently influence soil microbial activity. For example, Mamilov et al. (2004) observed over 5 times higher soil respiration rates in saline soil after a wet year compared to a year with less rain. This emphasises the importance of water content for the effect of salinity because the concentration of salt in the soil solution determines the strength with which the water is held in the soil solution (osmotic potential). The salt concentration in the soil solution increases as the water content decreases because the salt is concentrated in the remaining soil solution.

Many studies have explored the effects of salinity and soil water content on soil microorganisms, but usually separately, little is known about how soil microbial activity and microbial biomass respond to changes in soil salinity and soil water content. Since soil salinity and water content are not constant in the field, it is important to understand the effect of changes in salinity and water content on soil microorganisms for the sustainable use and rehabilitation of saline soils.

This literature review covers the following topics: the role of microbes in soils, the concept of soil water potential, soil salinity and its effect on microbes and plants, concluding with the aims of this study.

1.2 Soil microorganisms

In general, soil microorganisms constitute less than 1% (w/w) of the soil mass, but they play a key role in soil properties and processes. Soil microbes include bacteria, archaea, fungi, protozoa and viruses (Tate, 2000). Microorganisms participate in oxidation, nitrification, ammonification, nitrogen fixation, and other processes which lead to decomposition of soil organic matter and transformation of nutrients (Amato and Ladd, 1994), they can also store C and nutrients in their biomass which are mineralized after cell death by surviving microbes (Anderson and Domsch, 1980). Our understanding of these processes increased considerably in recent years with advances in molecular and analytical methodologies (Fierer et al., 2005; Gessner et al., 2010).

1.2.1 Importance of soil microorganisms for nutrient cycling

Nutrient cycling is the flux of nutrients within and between the various biotic or abiotic pools in which nutrients occur in the soil environment (Brady and Weil, 2002). Microorganisms have a major impact on the cycling of elements, most of which are essential for the growth of living organisms. Bacteria, archaea and fungi, in particular, are crucial for the cycling of several important inorganic nutrients in soils. Through oxidation, ammonification and other metabolic processes, organic materials are decomposed, releasing essential inorganic plant nutrients to the soil. Nitrate (through nitrification), sulfate (through sulfur oxidation), phosphate (through phosphorus mineralization) are present in soils primarily due to the action of microorganisms. Therefore, microbes are essential to maintaining a productive and valuable soil system. Disturbance of the soil environment, such as land use change or soil cultivation, can shift microbial communities and can have detrimental effects on soil nutrient cycling (French et al., 2009).

1.2.2 Microbial activity and its contribution to atmospheric CO_2

The pool of organic C in soils is the largest terrestrial carbon store on earth, containing twice as much C than the atmospheric pool (Lal et al., 1995). According to Schimel et al. (1994), soils contain two thirds of the world's terrestrial C. However, the soil also has the potential to be a large C source,

because the rate of net organic C accumulation or loss is a function of inputs and outputs (Figure.1). Output occurs through mineralization. The emission of CO_2 from soils, which includes respiration from soil organisms and roots, contributes approximately 10% to atmospheric CO_2 (Raich and Potter, 1995). Organic C input can be as plant and microbial residues and secretions, dead animals, organic amendments such as compost or manures. Areas at the bottom of slopes may also receive substantial organic C input through material eroded from other parts of the landscape. Microbes also play an essential role in the formation of humic substances which are stable forms of organic C and critical for organic C sequestration in soils (Burns et al., 1986).

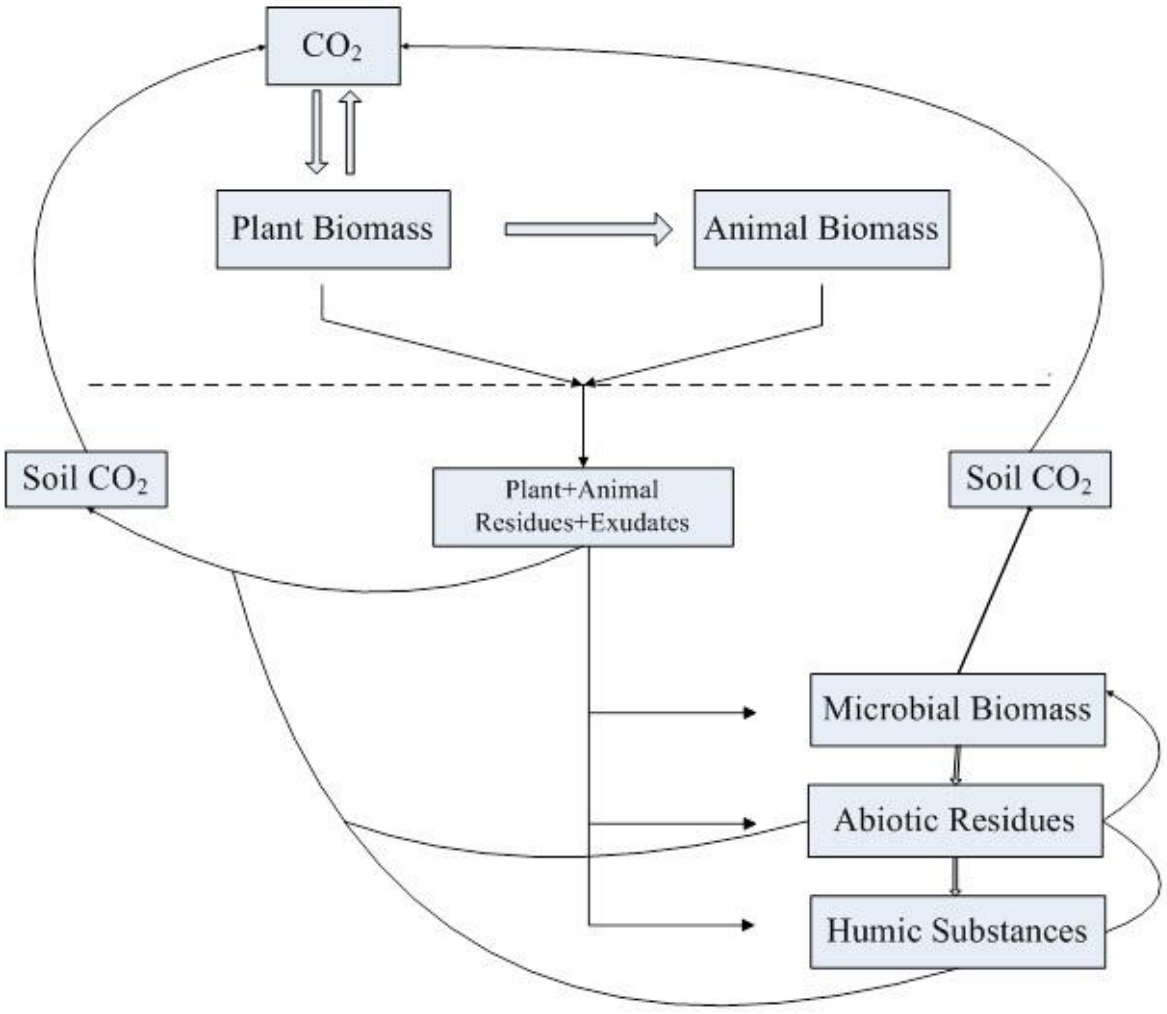

Figure 1. Conceptual model of carbon cycle emphasizing transfers between major soil organic matter pools (Tate, 2000).

1.3 Soil water content

1.3.1 Forms of water in soils and the water potential

Substantial volumes of water are stored in soils. For example, 1ha of medium textured soil (1m deep) with a water content at field capacity of 20% can store 8.0 ×10^5 L water (Or and Wraith, 2000). Plants and organisms rely heavily on water in soils and water is essential for nutrient cycling. However, soil water content varies both in time and in space which not only influences water availability to plants and microbes but also has a major effect on the rate of diffusion of solutes and gases (Adl, 2003).

The status of soil water can be described in two ways: the soil water content, which indicates how much water is present, and soil water potential, which relates to the energy level by which the water is held in the soil. The water potential is the amount of pressure that needs to be applied to transport a solution of known molarity from a referenced elevation to that of pure water (McKenzie, 2002). Processes dealing with water balance are usually more related to water content; whereas processes related to water movement are mainly related to soil water potential (Warrick and Or, 2007).

Soil water potential is the sum of matric, osmotic and gravitational potential; matric potential is expressed in negative values. The attraction of water to the soil solids provides a matric force and consequently results in matric potential. The osmotic potential is attributed to the presence of solutes in the soil solution, inorganic salts or organic compounds cluster the water molecules around the aggregates and hence reduce the freedom movement of the water. The gravitational potential is induced by gravity (attraction to the earth centre), it acts on soil water the same as it does on any other body (Brady and Weil, 2002). The relationship between matric potential and water content can be measured using pressure plates. Matric potential is the dominant component of water potential in non-saline soils. Osmotic potential can be measured by displacement technique (Sands and Reid, 1980), but also calculated based on measured electrical conductivity and water content using the following formula:

$$O_s = -0.036\ EC_{meas}\theta_{ref}/\theta_{act}$$

Where O_s= the soil osmotic potential (MPa) at the actual moisture content (θ_{act}, g g^{-1}) of the soil and EC_{meas}= the measure electrical conductivity (dS m^{-1}) of the extract at the reference water content (θ_{ref}, g g^{-1}) of the 1:5 soil/water mixture (Richards, 1954).

1.3.2 Relationship between soil water content and water potential

The greater amount of water a soil holds, the lower (less negative) the water potential and vice versa. But the relationship between water content and water potential varies with soil texture. For example, a clay soil holds much more water at a given potential than does a loam or sand and, at a given moisture content, the water is held much more strongly in the clay. Furthermore, well-structured soils usually have more large pores (>0.06mm) in which water is not held as tightly as in small and medium size pores resulting in lower potential (Brady and Weil, 2002). Hence, in order to understand whether the water moves and determine if it is available for plants or microbes, both the amount of water present and energy status should be considered.

1.3.3 Effect of soil water content on microorganisms

The water content of soils controls microbial activity and is a major factor that determines the rates of mineralization (Paul et al., 2003). Water is not only an essential transport medium for substrates, it is also an important participant in hydrolysis process. However, excess soil water content results in limited O_2 diffusion because O_2 diffusion in water is much lower (about 10^4 times) than in air which will reduce the activity of aerobic microorganisms (Kozlowski, 1984; Skopp et al., 1990), but could increase the activities of anaerobes. Lack of water reduces microbial activity and growth (Bottner, 1985; Kieft et al., 1987), C and N mineralization (Pulleman and Tietema, 1999; Sleutel et al., 2008) and shifts microbial community structure (Hueso et al., 2012; Sorensen et al., 2013). Cells retain sufficient water for cell turgor and metabolism by maintaining a higher osmotic potential (more negative) in the cytoplasm than that of the surrounding environment (Martin et al., 1999). When encountering low water content, soil microbes have to accumulate organic and inorganic compounds which increase the osmotic potential inside their cells to counteract the high osmotic potential (more negative) of the soil

solution. The organic or inorganic compounds are termed osmolytes. Examples of organic osmolytes are glutamate and proline in bacteria, inorganic osmolytes are various salts, such as KCl. Organic osmolytes generally have low molecular weight (Boot et al., 2013), however, synthesis of osmolytes requires large amounts of energy and may therefore reduce growth compared to high water availability (Harris, 1981; Oren, 1999; Wichern et al., 2006). Further as soils dry out, substrate supply becomes increasingly limited because the pores drain and water films around aggregates become thinner and disconnected (Ilstedt et al., 2000; Stark and Firestone, 1995).

It has been suggested that fungi, Gram-positive bacteria and archaea can better tolerate high matric potential than Gram-negative bacteria because they have stronger cell walls (Fierer et al., 2003; Martin et al., 1999; Schimel et al., 2007; Vasileiadis et al., 2012).

1.3.4 Effect of fluctuating water content

Soil moisture and the distribution of water within a soil profile vary with seasonal cycles of rainfall, irrigation periods (farm lands) and temperature. In semi-arid and Mediterranean ecosystems, surface soils frequently experience long dry periods followed by a relatively rapid wetting (Fierer and Schimel, 2002). The effects of drying and rewetting on soil microbial processes have been studied (Griffiths et al., 2003; Herron et al., 2009; Ilstedt et al., 2000; Schimel et al., 2007; Xiang et al., 2008). The concentration of available substrate and microbial activity peak in the first 24h after rewetting (Fierer and Schimel, 2003). This is because, upon rewetting, cells of sensitive microbes lyse, whilst other microbial genotypes release the organic solutes they accumulated during the dry phase (Halverson et al., 2000). Furthermore, soil aggregates break down and their previously protected organic matter is exposed and can then be decomposed. Microbial biomass, activity and nitrification decrease with increasing number of dry and rewetting cycles (Mikha et al., 2005; Nelson et al., 1996; Wu and Brookes, 2005). The decrease in microbial biomass with increasing number of drying and rewetting cycles may be due to the higher microbial biomass turnover (Van Gestel et al., 1993) and the loss of C during the flush in respiration upon rewetting (Fierer and Schimel, 2003). However, the response of microbial activity to drying and rewetting varies with soil type (Jin et al., 2013) which may be

due to the interaction of soil moisture and soil type, aggregation and the concentration of potentially bioavailable soil organic matter (Anderson and Ingram, 1993). However, drying and rewetting can also kill some microbes, delay bacterial growth (Goransson et al., 2013) and change microbial community structure which, in turn, could influence nutrient cycling (Fierer et al., 2003; Schimel et al., 2007). Butterly et al. (2009) found that drying and rewetting induced a reduction in fungi and an increase in Gram-positive bacteria. However, this is not always the case, 6-10 drying-rewetting cycles reduced bacterial growth but not that of fungi (Bapiri et al., 2010).

1.4 Saline soils

1.4.1 Forms of salt-affected soils

A soil that contains excess salts so as to impair its productivity is called a salt-affected soil. Salt in the soil can influence soil processes through the salt concentration in the soil solution (salinity) which determines the osmotic potential and the concentration of sodium on the exchange complex of the soil (sodicity) which influences soil structural stability. Salinity can, over time, lead to sodicity.

The major soluble salts in soils are the cations Na^+ (sodium), Ca^{2+} (calcium), Mg^{2+} (magnesium) and K^+ (potassium), and the anions Cl^- (chloride), SO_4^{2-} (sulfate), HCO_3^- (bicarbonate), CO_3^{2-} (carbonate) and NO_3^- (nitrate) (Shi and Wang, 2005).

The salt content of soil can be estimated by electrical conductivity of a saturated soil paster (EC_e) or a more dilute suspention of soil in water e.g. a 1:5 soil: water extract ($EC_{1:5}$)(Richards, 1954). The $EC_{1:5}$ can be converted to EC_e using the equation (Hazelton and Murphy, 2007):

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

From the EC values, the total salt concentration (in milli equivalent per litre) can be calculated using the formula:

$$\text{Salt concentration (me/l)} = \text{EC } (\mu\text{s/m})/100$$

The exchangeable sodium percentage (ESP) is a measure of sodicity and is calculated from the equation:

$$\text{ESP} = \frac{\text{Exchangeable sodium, cmolc/kg}}{\text{Cation exchange capacity, cmolc/kg}} \times 100\%$$

The sodium-adsorption-ratio (SAR) is a good indicator of the sodium status (Brassard et al., 2008). It is widely used because it is more easily measured than ESP. SAR is defined by Richards (1954) as:

$$\text{SAR} = \frac{Na^{+}}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

Where the concentrations of Na^{+}, Ca^{2+} and Mg^{2+} in the soil solution are in me/l.

There are several classification systems for salt-affected soils in the world, for example the USDA system, the USSR system and the Australian system (Chhabra, 1996). In this review the USDA system which classifies soils in three distinct categories (saline, sodic and saline-sodic soil) is used.

Saline soils have an electrical conductivity of the saturated paste (EC_e) >4 dS m^{-1}, ESP <15 or SAR <13 and pH <8.5. Sodic soils have an ESP >15 or SAR >13. Soils that have both detrimental levels of neutral soluble salts (EC_e >4 dS m^{-1}) and a high proportion of sodium ions (ESP >15 or SAR >13) are classified as saline-sodic soils (Table 1) (Brady and Weil, 2002; CISEAU et al., 2005).

Table 1. Classification of salt-affected soils

Salt-affected soil classification	EC_e (dS m^{-1})	pH	Sodium adsorption ratio	Soil physical condition
Saline	>4.0	< 8.5	<13	Normal
Saline-sodic	>4.0	< 8.5	>13	Normal
Sodic	<4.0	> 8.5	>13	Poor

1.4.2 Causes of salinity

Salt-affected soils can be classified according to how the salinity developed: primary salinity which occurs naturally where the soil parent material is rich in soluble salts, or geochemical processes

result in salt-affected soil. Secondary salinity is salinization of land and water resources due to human activities.

Human activities which can induce salinization include poor irrigation management; insufficient drainage; improper cropping patterns and rotations; and chemical contamination (Oldeman et al., 1990; UNEP, 2007). Dryland salinity which is wide-spread in Australia (5.7Mha) is due to land use change (NLWRA, 2001). Since the European settlement in Australia, native perennial deep rooted vegetation was replaced by shallow rooted crops and pasture species and soil organic matter content decreased (George et al., 1997; Hajkowicz and Young, 2005; Strehlow et al., 2005). This resulted in reduced evapotranspiration and soil water storage capacity and therefore increased flow of water through the soil profile which raised the groundwater level so that the saline ground water is now close to the soil surface causing dryland salinity (NLWRA, 2001).

The large extent of salt-affected soils in Australia can be explained partly by the fact that this is the driest continent of the world. Due to the climatic conditions, 87% of the average annual rainfall (420 mm) is lost into the atmosphere through evaporation and transpiration (Chartres, 1993). Therefore, salts entering the soil from rain or irrigation are accumulated in the upper horizons and not leached. Another reason for primary salinity is the development of Australia landscape, and deposition of ocean salts by wind and rain (Northcote, 1972; Peck, 1993). Land use change, as mentioned before, also contributes to the large extent of salt-affected soils.

It is predicted that the human population will reach 8 billion in 2025. To avoid or minimize food shortage, saline soils have to be rehabilitated and managed to meet the food demand of an ever growing human population (Ladeiro, 2012). Changing farming and land use system or vegetation management play an important role in managing water sources and salinity (NDSP, 2004). For example, to reduce dryland salinity, planting of deep-rooted perennial vegetation such as trees for fruit, nuts and oil or native species such as *Acacia spp* (Thrall et al., 2009). Groundwater levels can be controlled by drains, subsurface drains, and pumping (NDSP, 2004).

1.4.3 Effects of salinity on plants and microbes

High concentrations of soluble salts affect plants and microbes via two primary mechanisms: osmotic effect and specific ion effects.

a) Osmotic effect

Soluble salts increase the osmotic potential (more negative) of the soil water, drawing water out of cells which may kill microbes and roots through plasmolysis. The high osmotic potential also makes it more difficult for roots and microbes to remove water from the soil (Oren, 1999). Plants and microbes can adapt to low osmotic potential by accumulating osmolytes, however, synthesis of osmolytes requires large amounts of energy (Oren, 1999; Wichern et al., 2006).

b) Specific ion effects:

At high concentrations, certain ions, including Na^+, Cl^-, and HCO_3^-, are toxic to many plants (Chhabra, 1996).

These two mechanisms reduce plant growth and nutrient uptake. Yield of most crops is reduced at EC_e 2-45 dS m^{-1}, but the growth of very tolerant crops such as barley, cotton and sugar beet is only slightly reduced at EC_e 10-20 dS m^{-1} (Arshad, 2008; Ayers and Westcot, 1976).

Many studies showed that salinity reduces microbial activity, microbial biomass and changes microbial community structure (Andronov et al., 2012; Batra and Manna, 1997; Pathak and Rao, 1998; Rousk et al., 2011; Setia et al., 2011b). Salinity reduces microbial biomass mainly because the osmotic stress results in drying and lysis of cells (Batra and Manna, 1997; Laura, 1974; Pathak and Rao, 1998; Rietz and Haynes, 2003; Sarig et al., 1996; Sarig and Steinberger, 1994; Yuan et al., 2007a). Some studies showed that soil respiration decreased with increasing soil EC (Adviento-Borbe et al., 2006; Wong et al., 2009; Yuan et al., 2007b). Setia et al. (2010) found that soil respiration was reduced by more than 50% at $EC_{1:5} \geq 5.0$ dS m^{-1}. However, Rietz and Haynes (2003) reported that soil respiration was not significantly correlated with EC, but as EC increased, the metabolic quotient increased. The sensitivity of soil enzyme activities to salinity varies: activities of urease, alkaline phosphatase, â-

glucosidase were strongly inhibited by salinity (Frankenberger and Bingham, 1982; Pan et al., 2013), whereas dehydrogenase and catalase were less affected (Garcia and Hernandez, 1996).

As explained above, microorganisms have the ability to adapt to or tolerate stress caused by salinity by accumulating osmolytes (Del Moral et al., 1987; Quesada et al., 1982; Sagot et al., 2010; Zahran et al., 1992). Fungi tend to be more sensitive to salt stress than bacteria (Gros et al., 2003; Pankhurst et al., 2001; Sardinha et al., 2003; Wichern et al., 2006), thus the bacterial/fungi ratio is likely to be increased in saline soils. Inevitably, differences in salinity tolerance among microbes results in changes in community structure compared to non-saline soils (Gros et al., 2003; Pankhurst et al., 2001). Proline and glycine betaine are the main organic osmolytes and potassium cations are the most common inorganic solutes used as osmolytes accumulated by salinity tolerant microbes (Csonka, 1989). However, the synthesis of organic osmolytes requires high amounts of energy (Killham, 1994, Oren 2001). Accumulation of inorganic salts as osmolytes can be toxic therefore it is confined to halophytic microbes which evolved salt tolerant enzymes to survive in highly saline environments.

1.5 Aims of this study

In most studies on soil microbes in saline soils, only the effect of salinity was considered, not the combined and interactive effect of salinity and water content on osmotic potential. As explained above, the salt concentration in the soil solution (osmotic potential) is the main factor influencing microbial response to salinity. The osmotic potential is influenced by salinity measured in a given soil: water ratio and the soil water content. Therefore, it is important to consider osmotic potential in studies on microbes in saline soils. Further in the field, soil salinity and water content are not constant in time nor space. Soil microbial activity, biomass and community structure in saline soils change seasonally (Cao et al., 2011; Moss et al., 2006; Sarig and Steinberger, 1994). But in these studies, soil samples were collected at different times from fields with halophytes (i.e. *Tamarix chinensis* and *Reaumuria negeoensis*), thus other factors may have induced the observed changes - such as temperature and

plant growth. Therefore, experiments in controlled conditions are needed to better understand the effect of fluctuating salinity and soil water content on soil microbes.

The research detailed in this thesis aims to address these knowledge gaps and specifically to:

1. Investigate the response of soil microbial activity and biomass in non-saline and saline soils to increasing salinity (Chapter 2).

2. Determine the responses of microbial activity and biomass in saline soils collected from the field and subject to decreasing salinity (leaching) (Chapter 3).

3. Assess the response of soil microbial activity and microbial biomass in saline and non-saline soil to fluctuating salinity (Chapter 4).

4. Determine the effect of drying and rewetting on soil respiration and biomass in saline soils (Chapter 5).

5. Test the hypotheses that the osmotic potential to which the microbes were previously exposed influences their activity at a different osmotic potential and that the response is modulated by the speed of the changes in osmotic potential (Chapter 6).

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CHAPTER 2

Response of microbial activity and biomass to increasing salinity depends on the final salinity, not the original salinity

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STATEMENT OF AUTHORSHIP

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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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Response of microbial activity and biomass to increasing salinity depends on the final salinity, not the original salinity

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ABSTRACT

Salinization is a global land degradation issue which inhibits microbial activity and plant growth. The effect of salinity on microbial activity and biomass has been studied extensively, but little is known about the response of microbes from different soils to increasing salinity although soil salinity may fluctuate in the field, for example, depending on the quality of the irrigation water or seasonally. An incubation experiment with five soils (one non-saline, four saline with electrical conductivity (EC_e) ranging from 1 to 50 dS m^{-1}) was conducted in which the EC was increased to 37 EC_e levels (from 3 to 119 dS m^{-1}) by adding NaCl. After amendment with 2% (w/w) pea straw to provide a nutrient source, the soils were incubated at optimal water content for 15 days, microbial respiration was measured continuously and chloroform-labile C was determined every three days. Both cumulative respiration and microbial biomass (indicated by chloroform-labile C) were negatively correlated with EC. Irrespective of the original soil EC, cumulative respiration at a given adjusted EC was similar. Thus, microorganisms from previously saline soils were not more tolerant to a given adjusted EC than those in originally non-saline soil. Microbial biomass in all soils increased from day 0 to day 3, then decreased. The relative increase was greater in soils which had a lower microbial biomass on day 0 (which were more saline). Therefore the relative increase in microbial biomass appears to be a function of the biomass on day 0 rather than the EC. Hence, the results suggest that microbes from originally saline soils are not more tolerant to increases in salinity than those from originally non-saline soils. The strong increase in microbial biomass upon pea straw addition suggests that there is a subset of microbes in all soils that can respond to increased substrate availability even in highly saline environments.

1. Introduction

Salt accumulation is a serious global land degradation problem with more than 800 Mha of land affected world-wide by either salinity or sodicity (Yadav et al., 2011). In Australia, salt-affected soils are distributed across 357 Mha (Rengasamy, 2006b), and another 15 Mha are predicted to become saline in the next 50 years.

Saline soils are characterized by an electrical conductivity of the saturated paste (EC_e) >4 dS m^{-1}, an exchangeable sodium percentage (ESP) <15 and a pH <8.5 (US Salinity Laboratory Staff, 1954). Salinity causes land degradation because it has adverse effects on soil microorganisms and plants mainly via (i) low water availability (low osmotic potential), and, (ii) ion toxicity due to high concentrations of Na^+, Cl^-, and HCO_3^- causing yield decline, plant death and reducing soil fertility.

Soil microorganisms play an important role in nutrient cycling and fertility of soils by mineralization, solubilization and also immobilization of nutrients. Salinity can stress, or even kill soil microorganisms (Wichern et al., 2006) mainly due to the low osmotic potential of the soil solution (Chowdhury et al., 2011a; Setia et al., 2010; Yuan et al., 2007b), which results in drying and lysis of cells. To adjust to the low osmotic potential in saline soils, salt-tolerant microbes lower the osmotic potential within the cell thus preventing water loss and retaining cell turgor and metabolism. This can be achieved by taking up salts (only the most salt-tolerant microbes, e.g. in salt lakes), or by synthesis of osmolytes, for example, betaine and malic acid (Killham, 1994) which is energy-demanding and therefore poses a great metabolic burden (Oren, 2001).

The effect of salinity on microbial activity and biomass in soils has been studied extensively with varying results. Sarig and Steinberger (1994) and Wong et al. (2008) showed that the size of the microbial biomass was not affected by soil salinity, but most other studies found that salinity depresses microbial biomass (Batra and Manna, 1997; Elgharably and Marschner, 2011; Laura,

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1974; Pathak and Rao, 1998; Rietz and Haynes, 2003; Rousk et al., 2011; Sarig et al., 1996; Sarig and Steinberger, 1994). Most studies showed that soil respiration decreased with increasing soil EC (Adviento-Borbe et al., 2006; Asghar et al., 2012; Chowdhury et al., 2011a; Elgharably and Marschner, 2011; Setia et al., 2010; Wong et al., 2009; Yuan et al., 2007b). However, Rietz and Haynes (2003) found that soil respiration was not significantly correlated with EC. Some studies found that the bacteria–fungi ratio increased with increasing osmotic potential, due to a greater sensitivity of fungi to salinity compared to bacteria (Chowdhury et al., 2011a; Pankhurst et al., 2001; Sardinha et al., 2003). But Wichern et al. (2006) found that fungi were more tolerant to salinity than bacteria.

In the field, salinity is not constant. In irrigated systems, changes in irrigation water salinity will affect the salt concentration in the soil (Boivin et al., 2002; Herrero and Perez-Coveta, 2005). Further, as soils dry during summer, the salt concentration in the remaining soil solution will increase. The fluctuating salinity results in changes in osmotic potential and may influence activity and growth of microorganisms (Chowdhury et al., 2011b; Setia et al., 2010; Wichern et al., 2006). However, little is known about the response of soil microbial activity and biomass to increasing salinity. We hypothesized that microbes in already saline soils are less affected by increasing salinity than microbes in non-saline soils because they have already developed tolerance mechanisms, e.g. accumulated osmolytes. The response of the microbes would be further affected by the level of the imposed EC.

2. Materials and methods

2.1. Soils

Five soils (one non-saline, four saline) were collected from 0 to 10 cm depth under natural vegetation in Monarto, South Australia (35° 05′ S and 139° 06′ E). The region is semi-arid and characterized by a Mediterranean climate; the average temperature is 15.4 °C in winter and 30.5 °C in summer. After transport to the laboratory, soils were air-dried and sieved to <2 mm. The soils had the following EC_e (dS m^{-1}): 1 (non-saline), 11 (low salinity), 31, 41 (medium salinity), 50 (high salinity) (Table 1).

2.2. Experimental set-up

The air-dry soils were pre-incubated for 10 days at 25% of water holding capacity (WHC) to activate the soil microbes after air-dry storage. Ten days incubation was chosen because previous experiments with a range of different soils had shown that microbial respiration becomes stable 7–10 days after rewetting of air-dry soil. Air-drying and rewetting of soils are common in Mediterranean climate, therefore this pre-treatment is not un-natural. After pre-incubation, different amounts of NaCl dissolved in water were added to the soils to increase their electrical conductivity (EC). The water content was adjusted to 50% WHC because Setia et al. (2011) had shown that soil respiration was maximal at this water content in soils of similar texture. Increasing the water content from 25 to 50% of WHC will not induce a flush of respiration as seen after rewetting air-dry soil because wetting induces a flush in respiration only if the soil water content is close to air-dry before rewetting (Chowdhury et al., 2011c).

The EC_e of five different soils was increased to 37 desired EC_e levels, ranging from 3 to 119 dS m^{-1}, to achieve a range from low to very high salinity (Table 2). Pea (*Pisum sativum* L.) straw (ground and sieved to <2 mm) was mixed into the soils at 20 g kg^{-1} to provide a nutrient source, after which 25 g soil were placed into PVC cores (diameter 3.7 cm, height 5 cm) with a nylon mesh base. The soils were packed according to their bulk density in the field. Then the cores were placed individually in 1 L glass jars which were sealed with gas tight lids equipped with septa to allow quantification of the CO_2 concentration in the headspace. The glass jars were kept in the dark at constant temperature (25 °C) for 15 days. The weight of the cores was checked every 1–2 days, reverse osmosis water was added if necessary to maintain the desired weight and thus water content. There were three replicates per EC level.

2.3. Measurements

Soil pH and texture were measured by the standard procedures. The EC was measured in a 1:5 soil: water ratio mixture (5 g soil and 25 ml water) after 1 h end-over-end shaking. The $EC_{1:5}$ was converted to the EC of a saturated paste (EC_e) using the equation: $EC_e = (14.0 - 0.13 \times \text{clay\%}) \times EC_{1:5}$ (Hazelton and Murphy, 2007; Rengasamy, 2006a). Water holding capacity (WHC) was measured by placing thoroughly wetted soils in rings on a sintered glass plate connected to a 100 cm water column, and allowing them to drain for 48 h.

Microbial respiration was measured daily by quantifying headspace CO_2 concentration within each jar using a Servomex1450 infra-red gas analyser (Servomex, UK) as described in Setia et al. (2011). After each measurement, the jars were opened to refresh the headspace and then resealed. The CO_2 evolved from each sample was calculated as the difference between the initial and the CO_2 concentration after each measurement period.

Chloroform-labile C as an indicator of microbial biomass carbon (MBC) was determined by fumigation-extraction (Vance et al., 1987) on days 0, 3, 6, 9, 12 and 15 after residue addition using 10 g soil in 17 salinity treatments (EC_e range from 1 to 50 dS m^{-1}). These EC_e levels were chosen based on previous experiments to cover the range with the greatest impact on cumulative respiration. The soil samples used for chloroform-labile C determination were incubated separately from those used for respiration, but under the same conditions. The C concentration in the filtered extracts was determined by titration (Anderson and Ingram, 1993) after adding 0.0667M $K_2Cr_2O_7$ and sulphuric acid. The remaining dichromate was titrated with 0.033M acidified ferrous ammonium sulphate. Chloroform-labile C was calculated as the difference in C concentration between fumigated and non-fumigated soil.

Table 1
Soil properties.

EC_e dS m^{-1}	pH	Texture	Bulk density g cm^{-3}	Water holding capacity %	Total N g kg^{-1}	Total P g kg^{-1}	Total organic C g kg^{-1}	Available N mg kg^{-1}	Available P mg kg^{-1}	Chloroform-labile C mg kg^{-1}
1	7.8	Loam	1.47	33	0.26	7.9	12.3	17.7	138	47
11	8.6	Sandy clay loam	1.43	37	0.44	7.6	11.0	12.1	130	36
31	8.8	Sandy clay loam	1.42	40	1.04	6.9	9.8	13.1	97	5
41	8.5	Loam	1.42	39	1.11	5.6	5.4	3.7	59	23
50	8.9	Loam	1.43	42	0.90	5.5	6.0	1.9	49	34

Table 2
EC levels of original soils (EC_e 1, 11, 31, 41, 50 dS m^{-1}) and adjusted soils (EC_e: 3–119 dS m^{-1}).

EC_e (dS m^{-1})	Original				
	1	11	31	41	50
Adjusted	3	14	38	49	
	7	18	46	51	
	9	21	48	57	
	12	26	57	69	
	14	31	70	119	
	16	37			
	21	47			
	26	48			
	31	51			
	35	57			
	37	70			
	38				
	46				
	51				
	57				
	70				

2.4. Statistical analysis

Significant differences between different salinity levels in cumulative respiration and microbial biomass C were assessed by 1-way ANOVA with unbalanced design and Tukey test with $P \leq 0.05$ (GenStat® for Windows 11.0,VSN Int. Ltd, UK, 2005). Regression and correlation between salinity and cumulative respiration or microbial biomass were calculated with SPSS (PASW Statistics 18, 2010, Chicago, SPSS Inc.).

3. Results

3.1. Respiration

Cumulative respiration per gram soil on day 15 decreased with increasing EC (Fig. 1). There was a quadratic relationship between EC and cumulative respiration on day 15 ($r = 0.94$, $P < 0.001$). Irrespective of whether the EC was original or adjusted, cumulative respiration decreased by ≥30% at $EC_e \geq 35$ dS m^{-1}.

In general, cumulative respiration expressed in percentage of the soil with EC 1 was similar at a given adjusted EC, irrespective of the original EC (Fig. 2). Therefore in the soil with an originally lower EC (with a higher cumulative respiration), cumulative respiration decreased more strongly than in the soil with an originally higher EC. For example, after increasing soil EC 1 and soil EC 31 to EC 42, their cumulative respiration decreased by 57% and 21%, respectively.

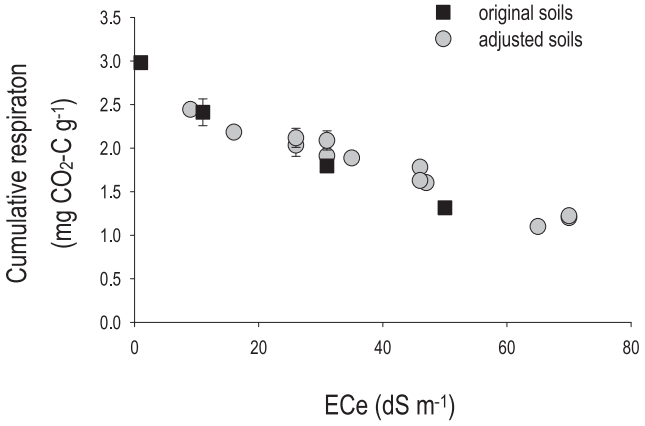

Fig. 1. Cumulative respiration of original (black squares) and adjusted (salt-amended; grey circles) soils as a function of EC_e ($n = 3$, bars indicate standard error, but are often too small to be visible).

3.2. Chloroform-labile C

On day 0 immediately after residue addition, chloroform-labile C (as an indicator for microbial biomass C) ranged from 5 to 226 μg g^{-1} with a negative relationship between chloroform-labile C and EC (Fig. 3A, $r = -0.77$, $P < 0.001$). Similarly, chloroform-labile C on day 15 decreased with increasing EC (Fig. 3B) whereas on day 3 there was no clear relationship between chloroform-labile C and EC (Fig. 3C).

In all soils, chloroform-labile C increased from day 0 (residue addition) to day 3 (Fig. 4). The strong increase in chloroform-labile C from day 0 to day 3 in soils with adjusted high EC was followed by

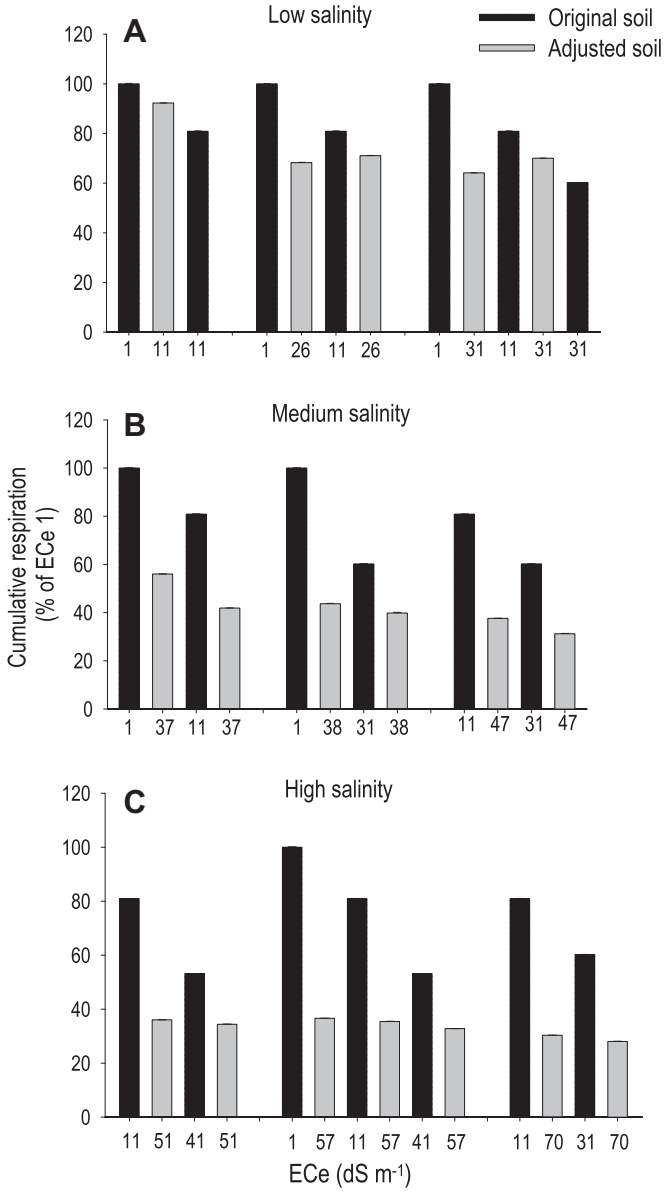

Fig. 2. Relative cumulative respiration (in percentage of the soil with EC_e 1) of original soils (EC_e 1, 11, 31, 41 dS m^{-1}; black bars) and soils in which $_e$EC was adjusted (grey bars) at different ranges of salinity (A) low salinity (1, 11, 26, 31 dS m^{-1}), (B) medium salinity (1, 11, 37, 38, 47 dS m^{-1}) and (C) high salinity (1, 11, 51, 57, 70 dS m^{-1}) ($n = 3$, bars indicate standard error but are often too small to be visible).

a strong decrease to day 9 after which it remained stable. On the other hand in the soils with lower EC, chloroform-labile C decreased more slowly over time. There was a negative relationship between chloroform-labile C on day 0 and the increase of chloroform-labile C on day 3 and day 15 in percentage of chloroform-labile C on day 0 ($r = 0.51$, $P < 0.003$ and $r = 0.66$, $P < 0.004$) (Fig. 5). Thus, the increase in microbial biomass in response to pea straw addition was greater in the more saline soils (which had a lower microbial biomass) than in non-saline soil or soil with low salinity which had a greater microbial biomass on day 0.

4. Discussion

This study showed that the response of microbial activity to increasing salinity depends on the final salinity, not the original salinity. Further, the results indicate that although saline soils may have a low microbial biomass, microbial growth is strongly

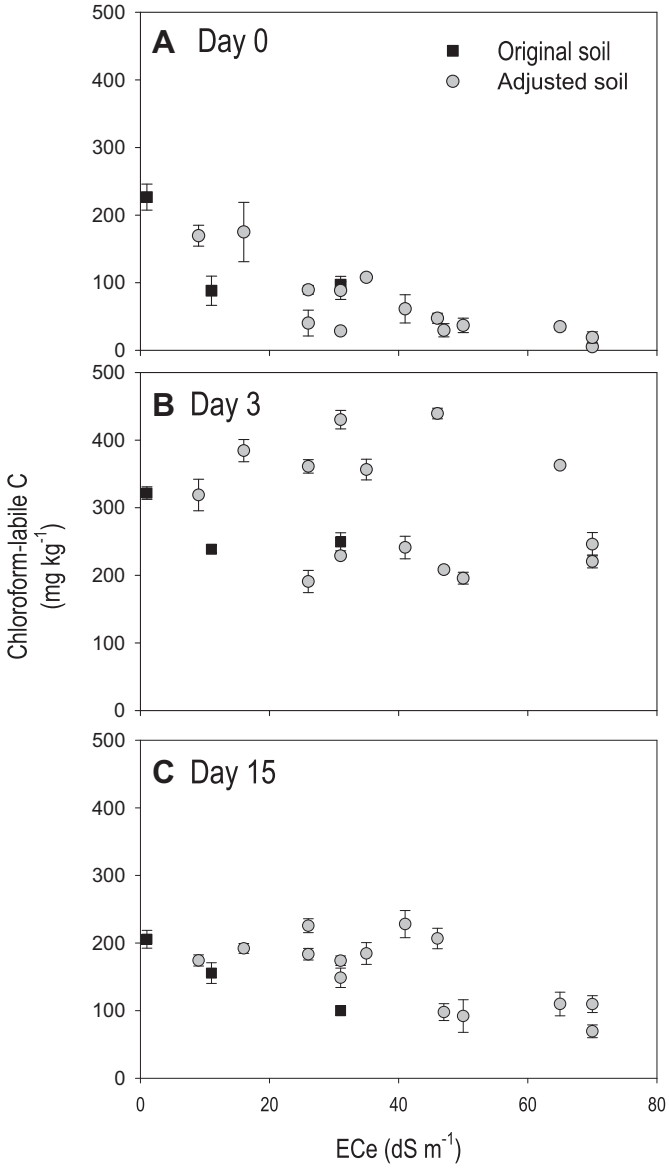

Fig. 3. Chloroform-labile C of original (black squares) and adjusted (salt-amended; grey circles) soils as a function of EC_e on day 0 (A), day 3 (B) and day 15 (C) ($n = 3$, bars indicate standard error).

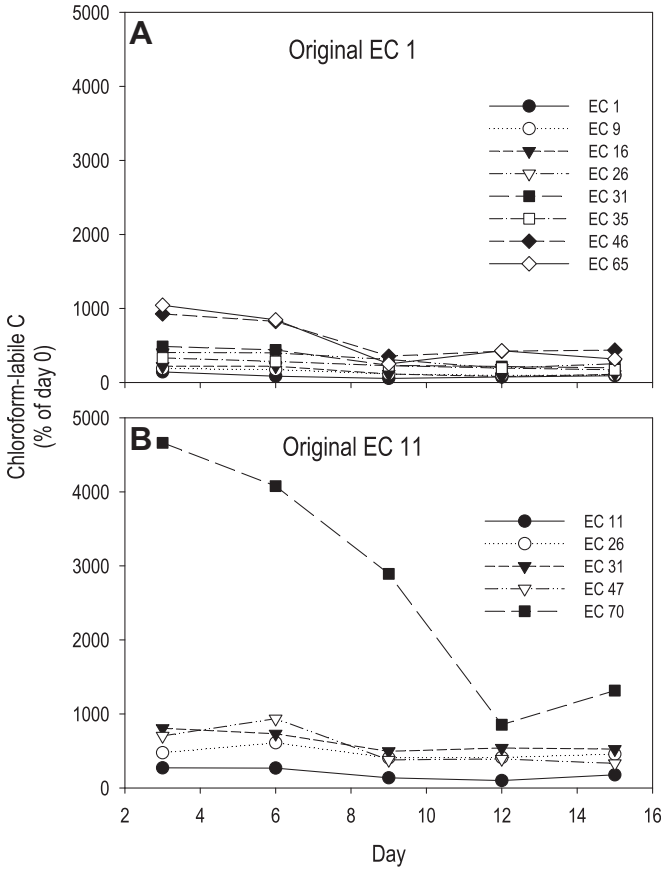

Fig. 4. Chloroform-labile C change percentage of day 0 of soil with original EC_e 1 (A) and EC_e 11 (B) and of soils adjusted to EC_e (from 1 to 70 dS m^{-1}) over time.

increased by addition of easily decomposable substrate. Moreover, the increase in microbial biomass was greater in the more saline soils than in the non-saline soils which had a higher microbial biomass before substrate addition.

4.1. Relationship between EC and microbial activity and biomass

There was a negative relationship between EC and cumulative respiration as well as between EC and microbial biomass C, irrespective of whether the salinity was original or adjusted (Figs. 1 and 3). The decrease in cumulative respiration with increasing EC is in agreement with the previous studies (Adviento-Borbe et al., 2006; Ghollarata and Raiesi, 2007; Setia et al., 2010; Wong et al., 2009; Yuan et al., 2007b). However, we show here that this response to the EC is not influenced by the previous level of salinity. Although the decrease in respiration with increasing adjusted EC relative to the original EC was smaller in the saline soils than in the non-saline soils because the former have lower cumulative respiration, the activity at a given adjusted EC is not affected by the original EC. Similarly, the microbial biomass on days 0 and 15 was a function of the adjusted EC, not the original EC (Fig. 3). Thus our hypothesis has to be rejected because microbes in saline soils are not more tolerant to a given EC than those from previously non-saline soils. This finding is in agreement with previous studies in which microbes extracted from soils of different salinities were exposed to a range of EC levels. Rousk et al. (2011) found that short-term growth rate of bacteria decreased with increasing EC irrespective of the EC of the soil they were extracted from. Likewise, Asghar et al. (2012) found that cumulative respiration at given

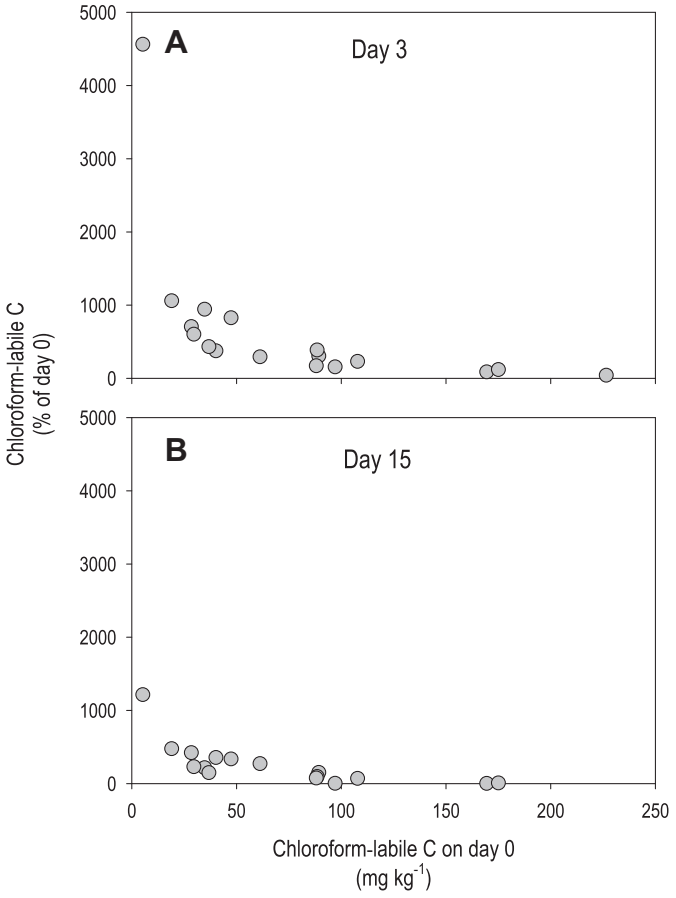

Fig. 5. Increase in chloroform-labile C on day 3 (A) and day 15 (B) in percentage of day 0 in soils with different salinities (EC_e from 1 to 70 dS m^{-1}).

adjusted EC was similar in microbial communities extracted from soils of different salinities. Here we show that this applies to microbial activity and biomass and occurs in situ. These findings suggest that even in previously non-saline soils there is a subset of the microbial community that can quickly acclimate to increases in EC, probably by accumulation of osmolytes which can synthesized within a few hours or days after exposure to salt (Hagemann, 2011). This response may have been stimulated by the addition of the pea residues which provided energy and C for the synthesis of the osmolytes.

4.2. Changes in microbial biomass over time

After addition of the residues on day 0, the increase in microbial biomass (indicated by chloroform-labile C) on day 3 relative to that on day 0 was greatest in the soils with low microbial biomass on day 0 which were also the more saline soils (Figs. 3 and 5). This greater increase can by explained by the greater substrate availability per unit microbial biomass in the saline soils compared to the non-saline or less saline soils. Additionally, the lower cumulative respiration in the more saline soils throughout the experiment resulted in a greater amount of substrate still present on day 15, which explains the higher relative microbial biomass in those soils at the end of the experiment (Fig. 5). On the other hand, at low original or adjusted EC, the greater microbial biomass on day 0 resulted in greater competition for the added C in the first days after addition of the residues, thus a smaller relative increase in microbial biomass (Fig. 5). Further, the higher respiration rates during the experiment would have resulted in a more rapid depletion of the added C, so that by day 15, the microbial biomass was becoming C limited. Therefore the apparently greater increase in microbial biomass in the more saline soils is most likely due to the smaller microbial biomass on day 0 and not to a greater tolerance to the EC per se. This relationship between size of the microbial biomass and relative growth increase in the first few days after residue addition would have to be tested in other, non-saline soils. Other studies in salt-affected soils have shown that microbial biomass is positively correlated with the amounts of labile C (Elgharably and Marschner, 2011; Yuan et al., 2007a; Zahran et al., 1992). However, the results of the present study also show that even at high EC, microbes are able to utilise added substrate for growth. This suggests that the low microbial biomass in saline soils in the field is mainly due to the low amount of substrate as a result of poor plant growth and only secondarily to the high EC.

5. Conclusions

The results of this study show that soil microbial activity and biomass are negatively affected by salinity irrespective of whether the EC is adjusted or already found in the field. Furthermore, microbes in saline soils do not appear to be more tolerant to increases in salinity as respiration and biomass were similar at a given adjusted EC irrespective of the original EC. The microbial biomass in saline soils appears to be limited mainly by substrate availability and only secondarily to salinity. This suggests that there is a subset of microbes in all soils that remains active even in highly saline conditions and that microbial activity and biomass in saline soils can be increased by improving substrate availability, e.g. addition of organic amendments.

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CHAPTER 3

Microbial activity and biomass recover rapidly after leaching of saline soils

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Microbial activity and biomass recover rapidly after leaching of saline soils.

Nan, Y. (Candidate)

Performed experiment, interpreted data, wrote manuscript.

I hereby certify that the statement of contribution is accurate.

Signed: Date: 26/11/2013

Petra, Marschner.

Supervised development of work, data interpretation and manuscript evaluation and correction.

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: 26/11/2013

Yan, N. & Marschner, P. (2012) Microbial activity and biomass recover rapidly after leaching of saline soils.
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CHAPTER 4

Response of soil respiration and microbial biomass to changing EC in saline soils

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STATEMENT OF AUTHORSHIP

Response of soil respiration and microbial biomass to changing EC in saline soils.

Nan, Y. (Candidate)

Performed experiment, interpreted data, wrote manuscript.

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Petra, Marschner.

Supervised development of work, data interpretation and manuscript evaluation and correction.

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: 26/11/2013

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Response of soil respiration and microbial biomass to changing EC in saline soils

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ABSTRACT

The effect of salinity on soil microbes has been studied extensively, but usually with a constant salinity level throughout the experiment. In the field, soil salinity changes over time, but little is known about the effects of these changes on microbial activity and biomass. In this study, two experiments were conducted in which every 5 days, soil cores were dipped into a salt solution to increase or maintain the EC or salinity was decreased by leaching. The solution also contained glucose to ensure that C was not limiting the response of the microbes to salinity. One non-saline soil and two saline soils (ECe 1, 11 and 31 dS m^{-1}) from the field were used. In Experiment 1, soil salinity was increased or reduced between EC 1, 11, and 31 dS m^{-1} repeatedly over six 5-day cycles. In Experiment 2, soil salinity was increased over four 5-day cycles from 1 or 11 to 31 dS m^{-1} either abruptly (within one cycle) or gradually (over at least 2 cycles). Soil respiration was measured daily in both experiments; in Experiment 1, microbial biomass C (MBC) was determined at the start of the experiment and at the end of cycles 1 (day 5) and 2 (day 10) and the last cycle (day 30). In Experiment 2, MBC was measured on day 0 and at the end of experiment (day 20). The results showed that soil microbes can respond quickly to changes in EC with respect to activity and growth when they are supplied with easily available C. A previous exposure to high EC did not limit the ability of the soil microbes to respond to a subsequent decrease in salinity. Compared to the originally saline soils, microbial activity and biomass in the originally non-saline soil were higher, less affected by EC increases and recovered more quickly after the EC was decreased. This suggests that a large biomass can better adapt to changes in EC than a small biomass which was already stressed by salinity in the field. Furthermore, a gradual increase of the EC did not result in greater respiration or microbial biomass compared to an abrupt increase.

1. Introduction

Salinization causes land degradation and is a major threat to soil microbes and plants. Globally 831 M ha of land is affected by salt (Martinez-Beltran and Manzur, 2005). Salinity has a negative effect on plants and soil organisms due to the low osmotic potential of the soil solution and ion toxicity or imbalanced ion uptake. According to the USDA classification system, a soil is considered saline if the electrical conductivity of the saturated paste (ECe) is >4 dS m^{-1}, exchangeable sodium percentage (ESP) is <15 and the pH is <8.5 (US Salinity Laboratory Staff, 1954). Soil salinity is seldom constant over time or uniform in space. In coastal soils, due to the intrusion of seawater in the groundwater, soil salinity fluctuates seasonally (Tripathi et al., 2006). In irrigated soils, salinity varies with the quality of the irrigation water. The salt concentration in the soil solution also varies with soil water content, increasing as the water content decreases because the salt is concentrated in the remaining soil solution.

Microorganisms play a pivotal role in soil nutrient cycling and plant growth. Many studies showed that salinity reduces microbial activity, microbial biomass and changes microbial community structure (e.g. Andronov et al., 2012; Batra and Manna, 1997; Chowdhury et al., 2011; Pathak and Rao, 1998; Rietz and Haynes, 2003; Setia et al., 2011a; Yan and Marschner, 2012b). Salt-tolerant microbes adjust to the low osmotic potential in saline soils mainly by accumulating osmolytes. However, the synthesis of organic osmolytes for example, betaine and malic acid, requires high amounts of energy (Killham, 1994; Oren, 2001).

In a previous study, we showed that there is a subset of microbes in both saline and non-saline soils that can respond to increased substrate availability even when at high salinity (Yan and Marschner, 2012b). Further, when the EC is reduced and substrate

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is added, activity and growth of a proportion of microbial community can rapidly increase (Yan and Marschner, 2012a). But little is known about the response of soil microbes when the salinity changes over time, for example, the EC is increased and decreased alternately or EC is increased gradually or abruptly. To close this knowledge gap, we conducted two experiments with a non-saline soil and two saline soils with similar soil texture from the field. Experiment 1 was designed to determine the response of microbial activity (respiration) and biomass C to changing EC. In Experiment 2 the response of microbes to rapid or gradual increase in salinity was assessed. We hypothesized that (i) an early exposure to high EC would limit the ability of the soil microbes to respond to a subsequent decrease in salinity because the high salinity killed a substantial proportion of the biomass, and (ii) a gradual increase in EC increases the ability to maintain high activity and growth at high EC compared to an abrupt increase to high EC, because they have time to adjust to low osmotic potential.

2. Materials and methods

2.1. Soils

The three soils (one non-saline and two saline soils) were collected (0–10 cm) from Monarto, South Australia (35° 05′ S and 139° 06′ E) under natural perennial vegetation. The region is semi-arid and characterized by a Mediterranean climate; the average temperature is 15 .4 ° C in winter and 30.5° C in summer. Their original electrical conductivity in the saturation paste (EC_e) was 1, 11, 31 dS m^{-1} (hereafter referred to soils A, B and C,Table 1). After collection, the soils were air-dried and sieved to < 2 mm. Before the onset of the experiments, the air-dry soils were pre-incubated for 10 days at 50% of water holding capacity (WHC) at 25 °C to activate the soil microbes and stabilize their activity. This water content was chosen because Setia et al. (2011b) had shown that soil respiration was maximal at this water content in soils of similar texture. Ten days incubation was used because in our previous studies, soil respiration stabilized 7–10 days after rewetting of air-dry soil. After pre-incubation, 25 g soil was placed into PVC cores (diameter 3.7 cm, height 5 cm) with a nylon mesh base. The soils were packed according to their bulk density in the field by adjusting the height of the soils in the cores to achieve the desired volume (approximately 3.3 cm).

2.2. Experimental design

At the start of each cycle, the cores were dipped for 15 min in a solution with 6.25 g glucose C L^{-1} and the appropriate salt (NaCl) concentration to increase the EC to the desired level or to maintain it. We used NaCl because it is the dominant salt in Australian saline soils (Rengasamy, 2010) and in the two saline soils used in this study. To decrease the EC, the soils were leached with the glucose solution until the desired EC was reached. The number of required leaching events was determined in a preliminary experiment in which the EC was measured after each leaching event.

In Experiment 1, the three soils were exposed to six 5-day cycles. The original EC of the soils was either maintained or changed between EC 1 and 11 dS m^{-1} or between EC 1 and 31 dS m^{-1} (for treatments see Table 2). In Experiment 2, there were four 5-day cycles. The EC was maintained throughout the four cycles or the EC of the soils with original EC 1 (soil A) and 11 dS m^{-1} (soil B) was increased to 31 dS m^{-1} either gradually or abruptly (for treatments see Table 3). For the gradual increase, the EC was increased over two or more cycles to 31 dS m^{-1}; for the abrupt increase the EC was increased to 31 dS m^{-1} from one cycle to the next. Therefore, the cycle number at which the soils were exposed to 31 dS m^{-1} varied between cycles one and four.

In both experiments, after the cores were leached or dipped in the glucose solution with the appropriate salt concentration, the soil was dried to 50% WHC by placing the cores in a fan-forced oven at 30 °C for 5–6 h. After 50% WHC was reached, the cores were placed individually into 1 L glass jars with gas tight lids equipped with septa to allow quantification of the CO_2 concentration in the headspace. The glass jars were kept in the dark at constant temperature (25 °C). The weight of the cores was checked every 1–2 days, reverse osmosis water was added if necessary to maintain the desired water content. There were four replicates per treatment.

Soil respiration was measured daily in both experiments. In Experiment 1, microbial biomass C (MBC) concentration was determined before the start of the EC treatments (day 0), on the first day of the first cycle (day 1) and at the end of first (day 5) and the second cycle (day 10) before placing the cores into the solution with salt and glucose and at the end of the 5th cycle (day 30). In Experiment 2, the MBC concentration was measured on day 0 and at the end of the experiment (day 20).

2.3. Measurements

The EC and pH were measured in a 1:5 soil: water ratio after 1 h end-over-end shaking. The $EC_{1:5}$ was converted to the EC of a saturated paste (EC_e) using the equation: EC_e=(14.0–0.13 × clay %) × $EC_{1:5}$ (Hazelton and Murphy, 2007; Rengasamy, 2006). The water holding capacity (WHC) was measured by placing the thoroughly wetted soils in rings in a sintered glass funnel which was connected to a 100 cm water column ($\Psi m = -10$ kPa), and allowed to drain for 48 h. Total P was measured using the phosphovanadomolybdate method (Hanson, 1950), total N by the Kjeldahl method (McKenzie and Wallace, 1954) and total organic C (TOC) by oxidation with potassium dichromate ($K_2Cr_2O_7$) in presence of sulphuric acid followed by titration of the residual $K_2Cr_2O_7$ with acidified $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (Walkley and Black, 1934). Available P was determined by the Cowell P method (Rayment and Higginson, 1992), available N was extracted in a 1:10 soil:2 M KCl ratio with 1-h shaking and measured by the Kjeldahl method (Rayment and Higginson, 1992).

Soil respiration was measured daily by quantifying headspace CO_2 concentration within each jar using a Servomex 1450 infra-red gas analyser (Servomex, UK). After each measurement (t_1), the jars were vented using a fan to achieve ambient CO_2 concentrations, re-sealed and the CO_2 concentration was measured again (t_0). The CO_2 evolved between closing the jars and the next measurement was

Table 1
Properties of sandy clay loam soils.

Soil	ECe (dS m^{-1})	pH	Bulk density (mg m^{-3})	Total N (g kg^{-1})	Total P (g kg^{-1})	Available N (μg g^{-1})	Available P (μg g^{-1})	Total organic C (mg g^{-1})
A	1	7.8	1.47	0.26	7.9	17.7	138	12.3
B	11	8.6	1.43	0.44	7.6	12.1	130	11
C	31	8.8	1.42	1.04	6.9	13.1	97	9.8

Table 2
EC levels (EC_e, dS m^{-1}) in cycles 1–5 for treatments in Experiment 1.

Soil	Treatment	Cycle					
		1	2	3	4	5	6
A	A\1-1	1	1	1	1	1	1
	A\11-1	11	1	11	1	11	1
	A\31-1	31	1	31	1	31	1
B	B\11-11	11	11	11	11	11	11
	B\1-11	1	11	1	11	1	11
	B\31-1	31	1	31	1	31	1
C	C\31-31	31	31	31	31	31	31
	C\1-31	1	31	1	31	1	31

calculated from the difference in CO_2 concentration between t_1 and t_0. Linear regression based on injection of known amounts of CO_2 into jars of similar size was used to define the relationship between CO_2 concentration and detector reading. There were four replicates per treatment with the same core measured throughout the experiment.

The microbial biomass carbon (MBC) concentration was determined in separate destructively sampled cores. Thus, a different core was used at each sampling date. There were four replicates per treatment and sampling time. Soil respiration was measured in the samples for MBC determination at the end of the experiments, but all samples were incubated in similar jars which were opened daily to refresh the air in them. Microbial biomass C (MBC) was determined by fumigation-extraction (Vance et al., 1987). The C concentration in the filtered extracts was determined by titration (Anderson and Ingram, 1993) after adding 0.0667 M $K_2Cr_2O_7$ and sulphuric acid. The remaining dichromate was titrated with 0.033 M acidified $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$. Microbial biomass C was calculated as: MBC = 2.22 × [(organic C extracted from fumigated soil) − (organic C extracted from non-fumigated soil)](Wu et al., 1990).

2.4. Statistical analysis

Data of cumulative respiration was assessed by one-way ANOVA with treatment as main factor, differences between different treatments and in respiration rates on days 1, 2 and 3 of each cycle were assessed by two-way ANOVA (treatments and day as main factors) and microbial biomass C by three-way ANOVA (treatments, soil and day as main factors) (GenStat® for Windows 11.0, VSN Int. UK, 2005). Post-hoc Tukey test ($P \leq 0.05$) was used to determine significant differences.

Table 3
EC levels (EC_e, dS m^{-1}) of original soils and adjusted soils in different cycles for treatments in Experiment 2.

Soil (original ECe (dS m^{-1}))	Treatment	Adjusted ECe (dS m^{-1})			
		Cycle			
		1	2	3	4
		Day 1–5	Day 6–10	Day 11–15	Day 16–20
A (1)	A\1-1-1-1	1	1	1	1
	A\1-1-1-31	1	1	1	31
	A\11-21-31-31	11	21	31	31
	A\11-11-11-31	11	11	11	31
	A\31-31-31-31	31	31	31	31
B (11)	B\11-11-11-11	11	11	11	11
	B\11-11-11-31	11	11	11	31
	B\21-21-31-31	21	21	31	31
	B\31-31-31-31	31	31	31	31
C (31)	C\31-31-31-31	31	31	31	31

3. Results

3.1. Experiment 1 (changing EC)

3.1.1. Soil respiration

In the first three days of each cycle, the respiration rates were high and varied over time whereas the respiration rates during the rest of the cycle were low and constant. Therefore the rates in the first three days were used to compare the treatments. When the EC was maintained constant throughout the cycles (treatments A\1-1, B\11-11, C\31-31), the respiration rates were high in the first three cycles and then decreased (Fig. 1a, d, g); respiration rates were highest in soil A (original EC 1 dS m^{-1}).

In soil A, respiration rates decreased significantly in the first three days only in cycles 1–3 whereas in the later cycles, respiration rates remained unchanged over the three days. In the treatments where the EC was changed between 11 and 1 dS m^{-1} (A\11-1) or between 31 and 1 dS m^{-1} (A\31-1), respiration rates were lower in the cycles with high EC, but recovered in the subsequent cycle when the EC was low (Fig. 1b, c). This recovery was stronger in cycle 2 (first EC decrease) compared to cycles 4 and 6. Previous exposure to low EC did not affect the response of the respiration rate at high EC because respiration rates were similar in cycles 1 and 3. In treatment A\31-1, respiration rates in the first two cycles where highest on day 2 indicating a lag phase when exposed to high EC (31 dS m^{-1} in cycle 1), but also when the EC was subsequently lowered to 1 dS m^{-1} in cycle 2.

In soil B (original EC 11 dS m^{-1}), a low EC (EC 1 dS m^{-1}) in the first cycle (treatment B\1-11) did not increase respiration rates in the first three days of the cycle compared to the controls (B\11-11) where the EC was 11 dS m^{-1} (Fig. 1e). Changing the EC between 31 and 11 dS m^{-1} (treatment B\31-11) resulted in strong fluctuation in respiration rates (Fig. 1f) Respiration rates were up to 5 fold higher at EC 11 dS m^{-1} (in cycles 2, 4 and 6) compared to the previous cycles at EC 31 dS m^{-1} (cycles 1, 3 and 5). In soil C (original EC 31 dS m^{-1}) the respiration rates did not increase when the EC was reduced to 1 dS m^{-1} in cycles 1 and 3. Respiration rates at 1 dS m^{-1} remained lower than in the two other soils.

Cumulative respiration at the end of Experiment 1 was affected by soil and treatment (Table 4). When the EC remained unchanged over the cycles (treatments A\1-1, B\11-11 and C\31-31), cumulative respiration was lowest in soil C and highest in soil B. In soil A, changing the EC increased cumulative respiration compared to treatment A\1-1. In soil B, this was also the case for the strongest changes in EC (B\31-1). But in soil C, changing the EC between 31 and 1 reduced cumulative respiration compared to treatment C\31-31 where the EC was maintained at 31 dS m^{-1} throughout the cycles.

3.1.2. Microbial biomass C

In all soils, the MBC concentrations were higher on day 1 compared to day 0 (after pre-incubation, before glucose addition) (Fig. 2). Except for day 10, the MBC concentrations were higher in soil A than in the other two soils. In soil A, the MBC concentrations were lower on day 5 than on the other sampling dates (Fig. 2 a) even in treatment A\1-1 where the EC was constant over time suggesting that this was not a response to changes in EC. In soil B, the MBC concentration changed over time in treatments B\1-11 and B\31-1being lower on days 5 and 30 than on days 1 and 10. But the higher MBC concentrations were not associated with a lower EC because the low concentrations on day 5 occurred at the end of cycles with EC 1 dS m^{-1} (treatment B\1-11) or EC 31 dS m^{-1} (treatment B\31-1). The MBC concentration changed little over time in soil C.

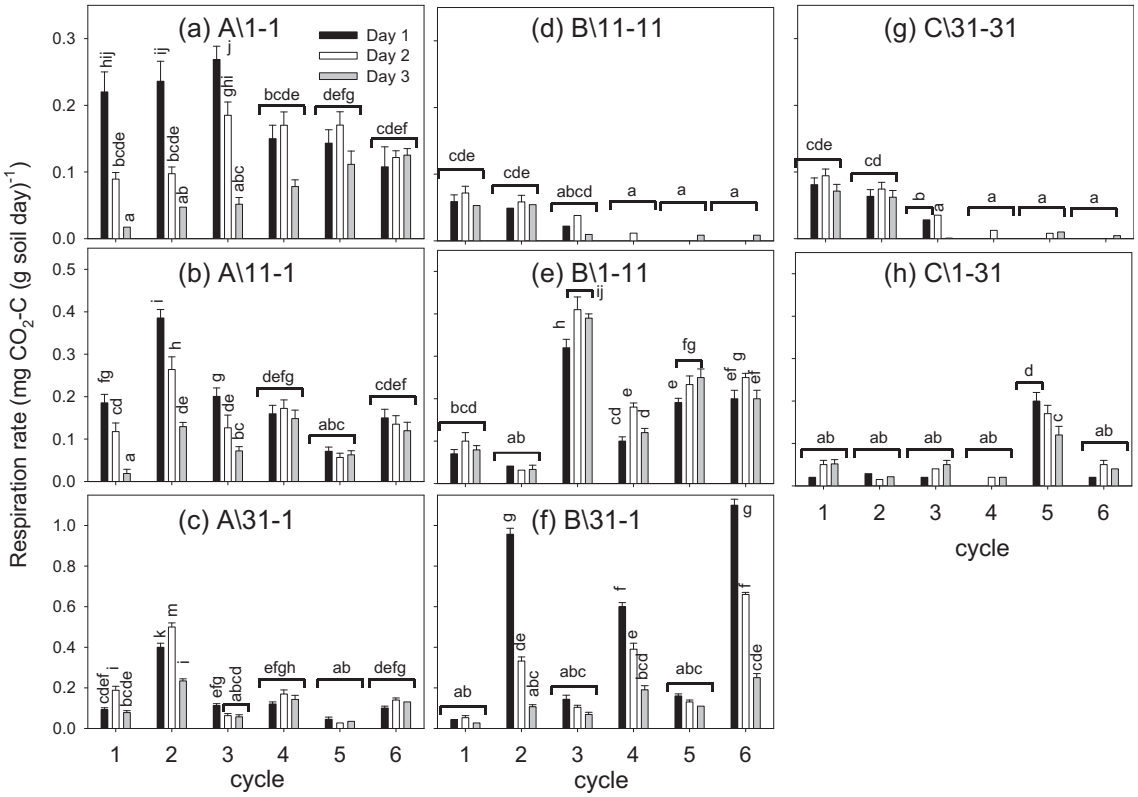

Fig. 1. Respiration rates on day 1 to day 3 of cycles 1–6 in Experiment 1, soil A (a–c), soil B (e–f) and soil C (g–h) ($n = 4$, vertical lines indicate standard error). For a given treatment, bars with different letters are significantly different ($P \leq 0.05$). For treatment abbreviations see Table 2.

3.2. Experiment 2 (gradual or abrupt increase of EC)

3.2.1. Soil respiration

In soil A (original EC 1 dS m^{-1}), respiration rates were highest on day 1 and lowest on day 3 of each cycle except in the first cycle in treatment A\31-31-31-31 where the EC was maintained at 31 dS m^{-1} in all four cycles (Fig. 3a–e). With the exception of latter treatment, respiration rates on the first and second day were highest in the first cycle. Irrespective of whether the EC was changed abruptly or gradually to 31 (treatments A\1-1-1-31, A\11-21-31-31 and A\11-11-11-31), respiration rates were very low at EC 31 dS m^{-1}. However in treatment A\31-31-31-31 where the soil was exposed to EC 31 dS m^{-1} in the first cycle, respiration rates in the first two days of cycles 1–3 were higher than in the other treatments where the high EC was induced in cycles 3 or 4. In the first cycle of treatment A\31-31-31-31, the respiration rate on day 2 was more than twice as high than on days 1 and 3 whereas in the later cycles, respiration rates were highest on day 1. In soil B, respiration rates were lower than in soil A and changed less during the first three days of each cycle or among cycles (Fig. 3f–i). Respiration rates were similar in the first three days of cycle 1 except for treatment B\31-31-31-31. In cycle 2, respiration rates were lower on day 1 than on day 2 irrespective of whether the EC was maintained (B\11-11-11-11) or increased to 21 or 31 (treatments B\21-21-21-31 and B\31-31-31-31). In most treatments, respiration rates were lower in cycles 3 and 4 than in the first two. In soil C, respiration rates were similar in the first 3 days of each cycle and decreased from the first to the third cycle (Fig. 3k).

Cumulative respiration at the end of Experiment 2 differed between soils and treatments (Table 5). When the EC was not changed (treatments A\1-1-1-1), B\11-11-11-11) and C\31-31-31-31), cumulative respiration was highest in soil A and lowest in soil C. Changing the EC in soils A and B reduced cumulative respiration compared to the treatments where the EC was not changed. In soil A, cumulative respiration was similar in treatments where the increase to EC 31 dS m^{-1} was gradually or abrupt (treatments A\1-1-1-31), A\11-21-31-31), A\11-11-11-31) and A\31-31-31-31). In soil B on the other hand, cumulative respiration was lowest when the high EC was imposed in the first cycle (treatment B\31-31-31-31). A gradual increase in EC (treatment B\21-21-31-31) resulted in lower cumulative respiration than an abrupt increase in the last cycle (treatment B\11-11-11-31).

Table 4
Cumulative respiration at the end of Experiment 1 ($n = 4$). Values followed by different letters are significantly different ($P \leq 0.05$). For treatment abbreviations see Table 2.

Soil	Treatment	Cumulative respiration (mg CO_2–C g $soil^{-1}$)
A	A\1-1	2.78 d
	A\11-1	3.37 e
	A\31-1	3.44 e
B	B\11-11	4.33 f
	B\1-11	0.72 a
	B\31-1	6.20 g
C	C\31-31	1.37 c
	C\1-31	0.87 b

3.2.2. Microbial biomass C

On day 0, after pre-incubation and before addition of glucose, the MBC concentration did not differ significantly among the soils (Table 5). In most treatments, the MBC concentration was higher on day 20 than on day 0. In soil A, the MBC concentration was higher when the soil was exposed to EC 31 dS m^{-1} in the third or fourth

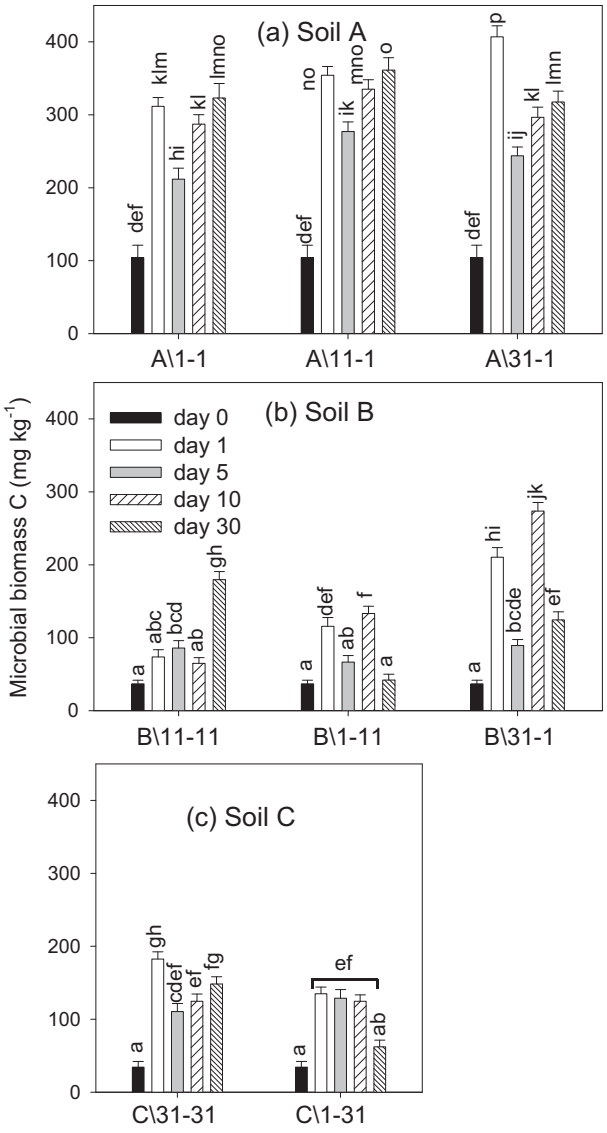

Fig. 2. Microbial biomass C concentrations on days 0, 1, 5, 10 and 30 in Experiment 1 in soils A (a), B (b) and C (c) ($n = 4$, vertical lines at the top of the columns indicate standard error). Bars with different letters are significantly different ($P \leq 0.05$). For treatment abbreviations see Table 2.

cycle (treatments A/1-1-1-31) and A/11-21-31-31) than when EC 31 dS m^{-1} was imposed in the first cycle (treatment A/31-31-31-31) or the control where the EC remained at 1 dS m^{-1} throughout (treatment A\1-1-1-1). But in soil B, the MBC concentration was lower in all treatments where the EC changed compared to the control (B\11-11-11-11). As in soil A, the MBC concentration was lowest when the soil was exposed to EC 31 dS m^{-1} in the first cycle (treatment B\31-31-31-31).

4. Discussion

The results of the two experiments show that soil microbes can respond quickly to changes in EC with respect to activity and growth if they are supplied with easily available C. Although reduced at high EC, activity (respiration) and growth rapidly increased if the EC was subsequently lowered. Further, activity and growth were reduced by high EC irrespective of whether the EC was increased gradually or abruptly.

4.1. Response to increases and decreases in salinity (Experiment 1)

The low respiration rates at high EC are in agreement with other studies (e.g. Rietz and Haynes, 2003; Tripathi et al., 2006; Wichern et al., 2006; Yuan et al., 2007) and can be explained by the low osmotic potential induced by the high salt concentration in the soil solution which reduces water availability to microbes and may draw water out of the cells. In most previous studies, the EC remained stable over longer periods of time (weeks or months), and less is known about the response of microbes to changes in EC. Rousk et al. (2011) and Asghar et al. (2012) showed that the short-term (hours or days) response of bacterial growth or soil respiration to a single change to different EC levels was similar irrespective of the EC of the soil from which the microbes were extracted. The present study further contributes to the understanding of microbial response to short-term changes in salinity because here, microbes were exposed to increases and reductions in salinity in several 5-day cycles.

In the treatments where the EC was adjusted to EC 31 dS m^{-1} in the first cycle (treatments A\31-1 and B\31-1), there was a lag phase indicated by low respiration rates on the first day after which the respiration rates increased to day 2. This lag phase was not observed when the EC was increased to 31 dS m^{-1} in the later cycles. Although the latter could be interpreted as adaptation, this may not be correct because the maximal respiration rate at EC 31 dS m^{-1} was higher when this EC was imposed in the first cycle than when imposed in the later cycles. It seems more likely that the lag phase in the first cycle at EC 31 dS m^{-1} was due to the smaller microbial biomass at the start of the experiment compared to the larger biomass at the end. With a small biomass, the low initial activity of some sensitive microbes in response to the high EC would have a greater impact on respiration rates than with a large biomass where the low activity of some microbes could be compensated by higher activity of less affected microbes.

Although the respiration rates in the first cycle on day 1 were low at EC 31 dS m^{-1}, the strong increase in MBC concentration within one day and the increase in respiration rates on day 2 indicate a rapid adjustment of the microbes to the low osmotic potential in the presence of easily available C. This rapid adjustment may be explained by accumulation of osmolytes which can occur within hours of exposure to low osmotic potential and prevents water loss from the cells (Hagemann, 2011). Further, even though microbial activity was low at EC 31 or 11 dS m^{-1}, a decrease in EC to 1 dS m^{-1} in the following cycle resulted in a strong increase in respiration rate within a day. This shows that microbes can rapidly respond to lower EC in the presence of easily available C. Thus, the first hypothesis that an early exposure to high EC would limit the ability of the soil microbes to respond to a subsequent decrease in salinity because the high salinity had killed a substantial proportion of the biomass has to be rejected. The increase in MBC concentration from day 0 to day 1 in all soils can be explained by the addition of glucose at 6.25 g C l^{-1} at the start of each cycle. The water holding capacity (WHC) of the soils is 400 g kg^{-1}. During pre-incubation and the 5-day cycles, the soils were at 50% WHC, thus they could take up approximately 200 ml kg^{-1} corresponding to 1.25 g glucose C kg^{-1}.

In soil A, the MBC concentration was high in all treatments, changed little over time and was not reduced by high EC although respiration rates varied with EC. This suggests that a large microbial biomass is maintained during changes in EC and responds to such changes mainly by varying the respiration rate.

4.2. Gradual or abrupt increase in EC (Experiment 2)

In the field, salinity develops slowly (e.g. seasonally) or quickly (e.g. changes in salinity of the water used for irrigation),

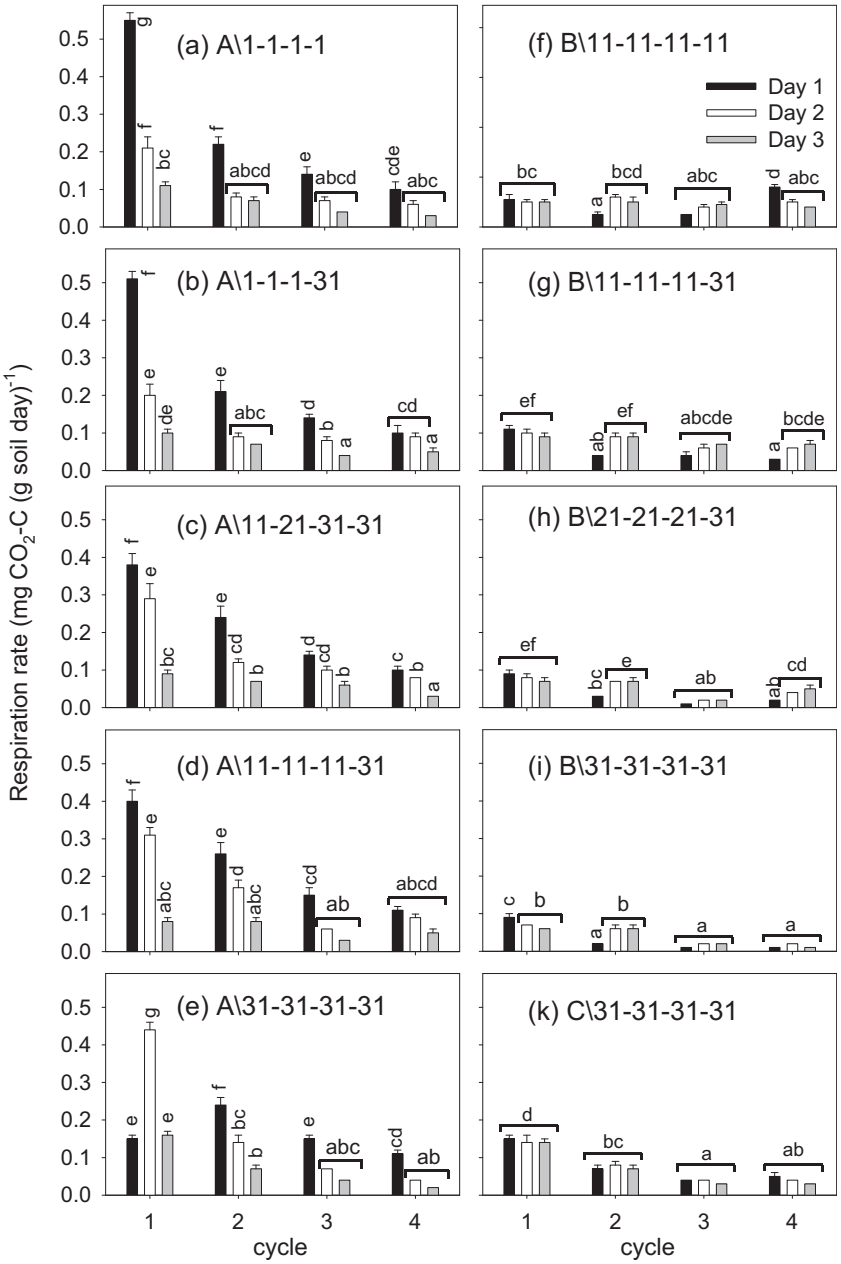

Fig. 3. Respiration rates on days 1–3 of each cycle in Experiment 2 ($n = 4$, vertical lines at the top of the columns indicate standard error). For a given treatment, bars with different letters are significantly different ($P \leq 0.05$). For treatment abbreviations see Table 3.

but most studies with saline soils from the field are carried out without knowledge of the length to time a soil had given EC. In experiments where salt is added to increase salinity, the EC rises abruptly and it could be argued that microbes may do not have sufficient time to adapt to the higher EC. This has raised the question as to whether experiments with salinized soils could adequately represent the effect of salinity on microbes in the field. Modelling by Setia et al. (2011c) suggested that compared to saline soils from the field with the same EC, salinization may lead to a stronger reduction in soil respiration. However in the present study, the gradual increase in EC in soils A and B (over EC 11 and 21 dS m^{-1}) did not increase the respiration at EC 31 dS m^{-1} in the fourth cycle compared to the treatment where the EC was increased abruptly to 31 dS m^{-1} from the third to the fourth cycle. This suggests that microbes can adapt rapidly to increasing EC in presence of an easily available C source, particularly when the initial MBC concentration is high. In soil A, the MBC concentration on day 20 was higher in the treatment with a gradual increase in EC (A\11-21-31-31) compared to an abrupt increase in the fourth cycle (treatment A\11-11-11-31), however this was not observed in soil B where the MBC concentration did no differ between treatments with gradual or abrupt EC increase. Thus our second hypothesis (a gradual increase in EC increases the ability to maintain high activity and growth at high EC compared to an abrupt increase to high EC, because they have time to adjust to a low osmotic potential) has to be rejected. Most detrimental for microbial growth was exposure to EC 31 dS m^{-1} in the first cycle (treatments A\31-31-31-31 and B\31-31-31-31) which suggests that microbes with a low previous supply of C (native organic matter) during the pre-incubation are particularly sensitive to high EC even if the EC increase is accompanied by addition of easily available C.

Table 5
Cumulative respiration on day 20 and microbial biomass C on days 0 and 20 in Experiment 2 ($n = 4$). Values followed by different letters are significantly different ($P \leq 0.05$). For treatment abbreviations see Table 3.

Soil	Treatment	Cumulative respiration (mg CO_2–C g soil^{-1})	Microbial biomass C (mg kg^{-1} soil)	
			Day 0	Day 20
A	A\1-1-1-1	2.05 g	97 abc	412 f
	A\1-1-1-31	2.02 ef		523 g
	A\11-21-31-31	2.01 ef		517 g
	A\11-11-11-31	1.95 ef		384 f
	A\31-31-31-31	1.88 e		301 e
B	B\11-11-11-11	1.69 d	75 ab	284 e
	B\11-11-11-31	1.38 c		161 d
	B\21-21-31-31	0.87 b		134 cd
	B\31-31-31-31	0.71 a		104 bc
C	C\31-31-31-31	1.37 c	61 a	128 cd

4.3. Methodological considerations

The decrease in respiration rates in the first three days of each cycle with increasing number of cycles even in the control soils where the EC was not changed, indicates that microbial activity was negatively affected by the changes in water content from saturation after dipping the cores in the solution containing glucose and salt (or leaching) followed by rapid drying in the oven. However the dipping of the cores was necessary to avoid soil disturbance as it would have occurred when the glucose and salts had been mixed into the soil at the start of each cycle. Leaching may have washed out microorganisms, but the strong increase in respiration and MBC concentration upon the decrease in EC by leaching suggests that this did not compromise the response of the microbes to a lower EC. It should also be noted that some of the added C would have been respired during the drying where CO_2 release was not measured, but this was the case in all treatments.

5. Conclusion

The results show that soil microbes respond rapidly to changes in EC when supplied with an easily available C source, but also suggest that microbial activity and growth at different salinity levels was mainly a function of the original microbial biomass. A large original biomass in an initially non-saline could respond rapidly to changes in EC and take advantage of low EC by rapid growth. The smaller biomass in the originally saline soils on the other hand had a limited ability to respond to changing EC which could be due to the limited genotypic variability in these soils. This study further showed that a gradual EC increase does not increase microbial activity or growth at high EC compared to an abrupt increase.

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CHAPTER 5

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

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STATEMENT OF AUTHORSHIP

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Performed experiment, analysed soil samples, interpreted data and wrote manuscript.

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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 electrical conductivity of the saturated soil extract (ECe) from 2 to 48 dS m^{-1} (EC2, EC9, EC19, EC33 and EC48), were kept at soil water content optimal for respiration 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 decreased with increasing drying time; in the 5D treatment, the water potential ranged between −15 and −30 MPa, with the lowest potentials in soil EC33. In moist and dry conditions, respiration rates per unit soil organic C (SOC) were highest in soil EC19. Respiration rates decreased with increasing time of drying; when expressed relative to constantly moist soil, the decline was similar in all soils. Rewetting of soils only induced a flush of respiration compared to constantly moist soil when the soils were dried for 3 or more days. The flush in respiration was greatest in 5D and smallest in 3D, and greater in EC2 than in the saline soils. Cumulative respiration per unit SOC was highest in soil EC19 and lowest in soil EC2 Cumulative respiration decreased with increasing time of drying, but in a given soil, the relationship between water potential during the dry phase and cumulative respiration at the end of the experiment was weaker than that between respiration rate during drying and water potential. In conclusion, rewetting induced a flush in respiration only if the water potential of the soils was previously decreased at least 3-fold compared to the constantly moist soil. Hence, only marked increases in water potential induce a flush in respiration upon rewetting. 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.

1. Introduction

During summer, top soils may experience drying and rewetting cycles when dry periods are interrupted by occasional rainfall events. 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., 2011a,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). As a result of this and also the death of drought-sensitive microorganisms, soil drying decreases organic matter decomposition and bacterial growth and activity (Iovieno and Bååth, 2008; Yao et al., 2011).

Previous studies using non-saline soils have shown that rewetting of dry soils induces a flush of respiration. 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

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soil (Kieft et al., 1987; Franzluebbers et al., 1994; Mikha et al., 2005; Butterly et al., 2009). The peak in bacterial growth rate occurs several hours after the flush in respiration, suggesting that there is a delay before nutrients released by decomposition are utilised for cell growth (Iovieno and Bååth, 2008).

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). Multiple drying and rewetting cycles have been shown to increase soil organic matter decomposition (Miller et al., 2005; Xiang et al., 2008; Schimel et al., 2011) and N mineralisation (Miller et al., 2005) and microbial biomass (Xiang et al., 2008; Zhao et al., 2010). Drying and rewetting may decrease bacterial growth whereas fungal growth is not affected (Bapiri et al., 2010). In most previous 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). But in a study with forest soils, Fischer (2009) showed that the size of the flush in respiration after rewetting was a function of the water potential during the dry phase, with significant flushes only occurring if the water potential was less than −0.63 MPa.

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., 2011a,b; Chowdhury et al., 2011a,b), bacterial growth rate (Rousk et al., 2011) 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., 2011a). 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. On the other hand, microbes in saline soils may be more tolerant to low water potential and therefore remain more active in dry soil than microbes in non-saline soils. However, Rousk et al. (2011) found no difference in tolerance of growth to low osmotic potential in bacterial communities from soils with differential salinity.

Currently, little is known about the effect of drying and rewetting in saline soils and how this effect is modulated by the extent of drying. Therefore, 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 water potential 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.

2. Materials and methods

2.1. 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). The region is semi-arid and has a Mediterranean climate. After collection from 0 to 10 cm depth, the soils were air-dried and sieved to <2 mm (Table 1). The United States Salinity Laboratory Staff defines a soil as saline when the electrical conductivity of the saturation extract (ECe) 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.

Soil water availability 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.

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):

$$\psi = a\ \theta^{-b}$$

The electrical conductivity of the 1:5 soil:water extract ($EC_{1:5}$) 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 (Richards, 1954):

$$\psi_\pi = -0.036\ EC_{meas}(\theta_{rev}/\theta_{act})$$

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

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.

Table 1
Properties of the soils used in the study.

Soil	Sand (%)	Silt (%)	Clay (%)	$EC_{1:5}$ (dS m^{-1})	ECe (dS m^{-1})	pH 1:5	SOC (g kg^{-1})	MBC (mg g^{-1} SOC)	Water holding capacity (g kg^{-1})
EC2	60.0	21.3	18.8	0.19	2	9.3	16.4	4.1	357
EC9	70.0	15.0	15.0	0.76	9	9.3	10.1	4.5	356
EC19	65.0	16.2	18.8	1.62	19	9.5	2.6	2.4	314
EC33	60.0	21.0	19.0	2.82	33	9.0	4.7	0.7	314
EC48	60.0	21.0	19.0	4.07	48	8.9	5.0	0.8	406

2.2. Experimental set-up

Air-dry soils were wet to 50% WHC and pre-incubated for 10 d at 25 °C before the experiment was 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 (20 g kg^{-1}) to provide a readily-available nutrient source. The soils (30 g) 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 (Fig. 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 (Fig. 1). For the continuously moist treatment (M) maintained at 50% WHC, the experimental period ended on day 22. The desired water content was maintained by weighing the cores and adding reverse osmosis water if required. There were three replicates per moisture treatment and soil.

2.3. Measurements

Respiration was quantified by measuring headspace CO_2 concentrations every 24 h 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 24 h.

Microbial biomass C was determined on day 7 (before onset of the drying) 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.

To account for differences in organic C content among soils, respiration, microbial biomass and K_2SO_4-extractable C are expressed per unit organic C.

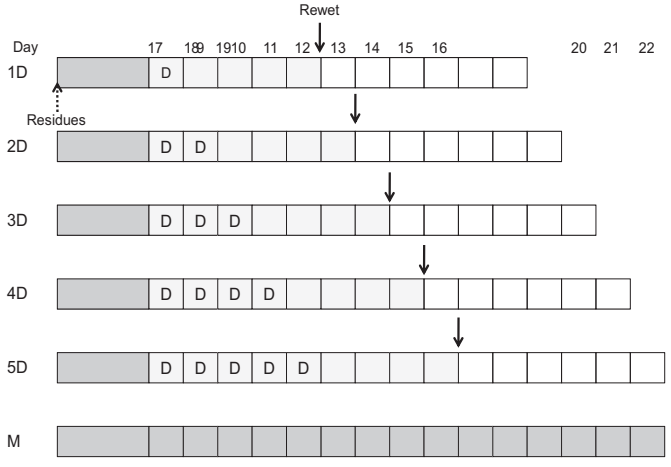

Fig. 1. Experimental design. Grey rectangles indicate moist incubation, white rectangles dry period with D showing days of drying and rectangles without D incubation at the water content reached during the drying phase.

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

Regressions between water potential during the dry phase and respiration parameters or microbial biomass were calculated with SPSS.

3. Results

3.1. 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, osmotic and water potential

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	−10.40
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

decreased exponentially with increasing drying time. In 5D, osmotic and water potential ranged between −15 and −30 MPa, with the lowest potentials in soil EC33. In soil EC2, matric potential was the dominant potential, whereas osmotic potential dominated in the saline soils, contributing between 70 and 91% to water potential.

3.2. Respiration

In moist and dry conditions, respiration rates per unit soil organic C (SOC) were highest in soil EC19 and lowest in soil EC2. Drying for one day reduced average respiration rates in the dry phase compared to the constantly moist soil significantly, but by only 10–20% (Fig. 2). Longer drying periods, which resulted in lower water potentials reduced average respiration rates in the dry phase compared to the constantly moist soil by 46–57% in 2D, 66–77% in 3D, 81–87% in 4D; in 5D the average respiration rate during the dry phase was below the detection limit of the gas analyser. There was a quadratic relationship between water potential and respiration rate during the dry phase (Fig. 3, $r^2 = 0.22$, $P < 0.034$).

In all soils, drying for one day reduced the water potential by only 20–30% compared to the constantly moist soil. In this treatment (1D) rewetting did not increase respiration rates compared to those in the dry phase or the constantly moist soil (Fig. 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 the water potential in the dry phase was approximately 2-fold lower in 2D compared to the constantly moist soil. 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 water potential after rewetting (Fig. 5). There was a linear correlation between the change in water potential and maximal respiration rate after rewetting (in percentage of the constantly moist soil) in soils with ECe ≥9 dS m^{-1} ($r^2 = 0.54$, $P = 0.001$). By day 4 after rewetting, respiration rates were similar

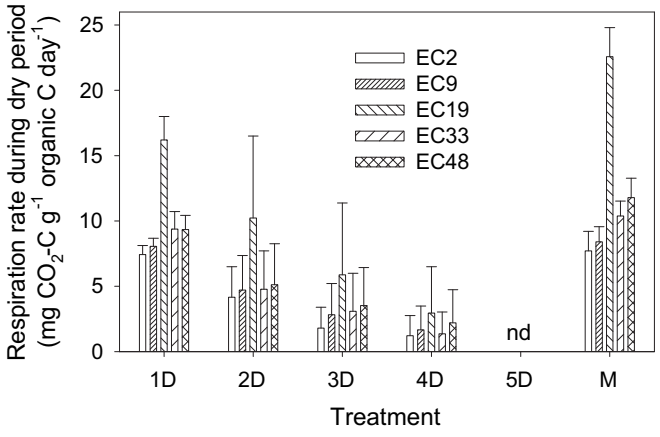

Fig. 2. Respiration rates during the dry phase in soils differing in ECe dried for 1–5 days ($n = 3$, bars indicate standard error), nd indicates that respiration rates were below the detection limit of the infra-red gas analyser.

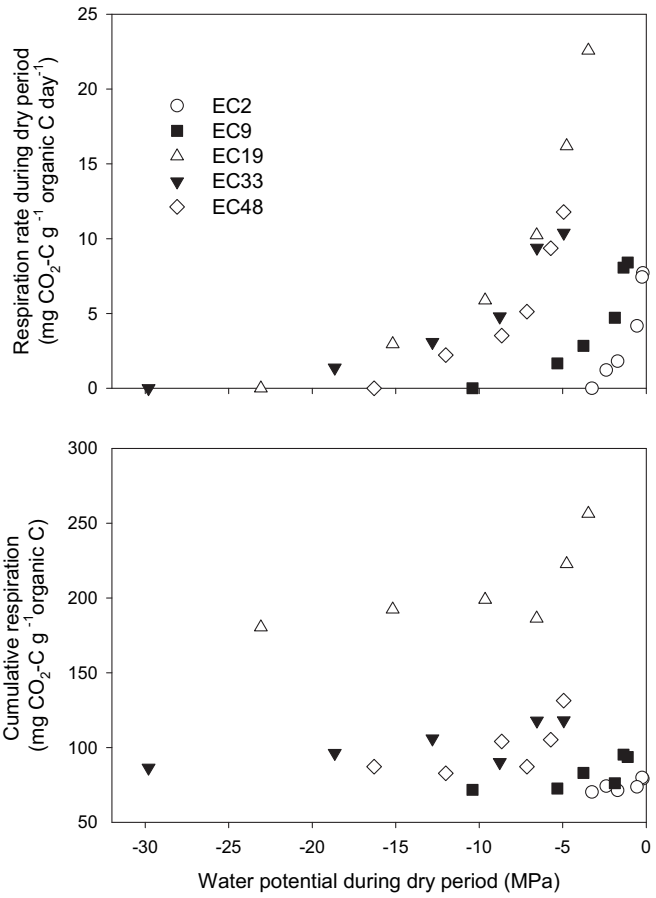

Fig. 3. Relationship between water potential during the dry period in soils differing in ECe dried for 1–5 days and respiration rates during the dry phase or cumulative respiration at the end of the 5-day moist incubation period. Symbols are averages of each treatment for a given soil ($n = 3$).

to those in the constantly moist soil in the saline soils, whereas they remained higher in soil EC2 until day 5 after rewetting.

Cumulative respiration expressed per unit organic C was highest in soil EC19 (Table 3). In soil EC2, cumulative respiration was not significantly affected by the drying treatments; compared to constantly moist soil, cumulative respiration was decreased by only 12% in 5D. Among the saline soils, cumulative respiration was significantly decreased by drying in soils EC9, EC19 and EC48, but not in EC33. In soils EC9, EC19 and EC48, cumulative respiration was 24–34% lower in 5D compared to constantly moist soil. There was no relationship between cumulative respiration and water potential during the dry phase (Fig. 3).

3.3. Microbial biomass carbon

In the unamended soils, microbial biomass C (MBC) was higher in soils EC2 and EC9 with 4.1 and 4.5 mg g^{-1} SOC compared to the soils EC19, EC33 and EC48 where it ranged only between 0.6 and 2.3 mg g^{-1} SOC (Table 1). Seven days after residue addition and before the drying treatments started, MBC ranged from 6 to 18 mg C g^{-1} SOC 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 the constantly moist soils, with the greatest decrease in soil EC48, where MBC was up to 10 fold lower than on day 7. In soils EC2, EC9, and EC33 the drying treatments did not affect MBC at the end of the incubation (Table 3). On the other hand in soils EC19 and EC48, MBC was significantly higher in 5D

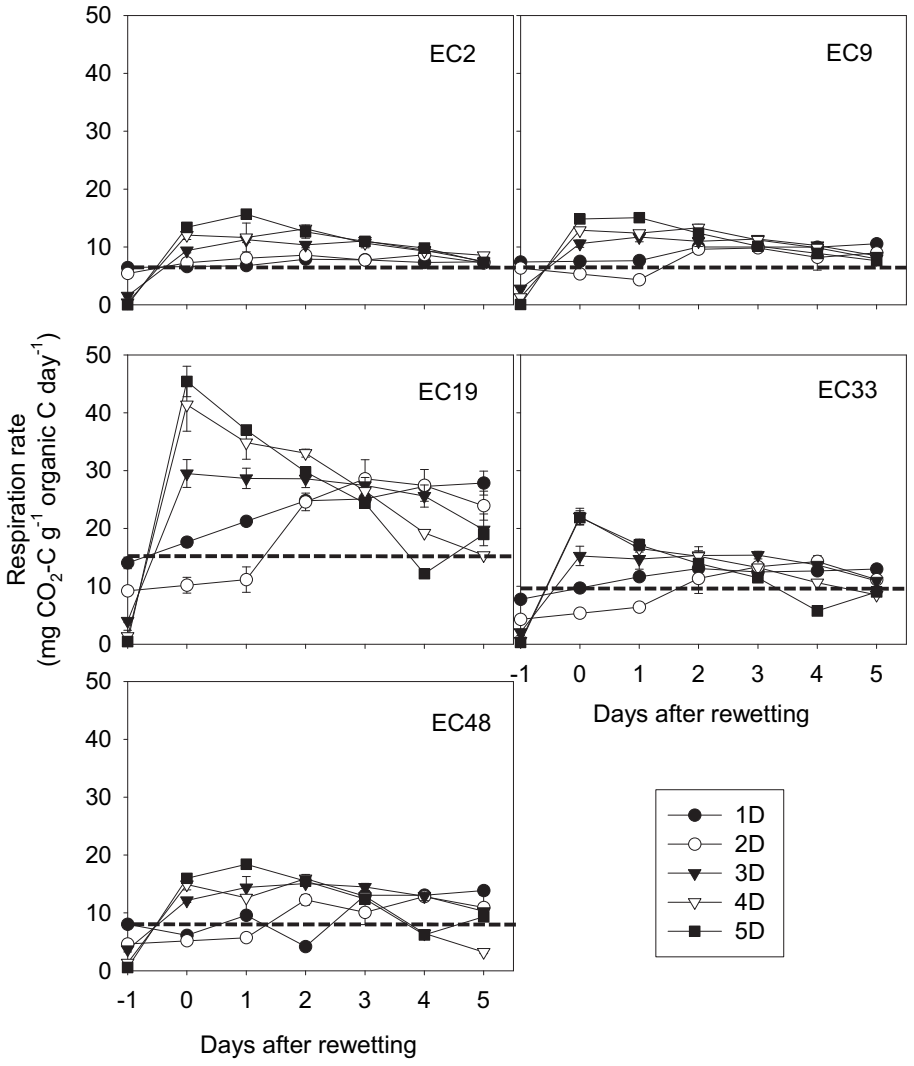

Fig. 4. Respiration rates in soils differing in ECe dried for 1–5 days at the end of the dry phase (day −1), on the day the soils were rewet (day 0) and in the following 5-day moist incubation period ($n = 3$, bars indicate standard error).

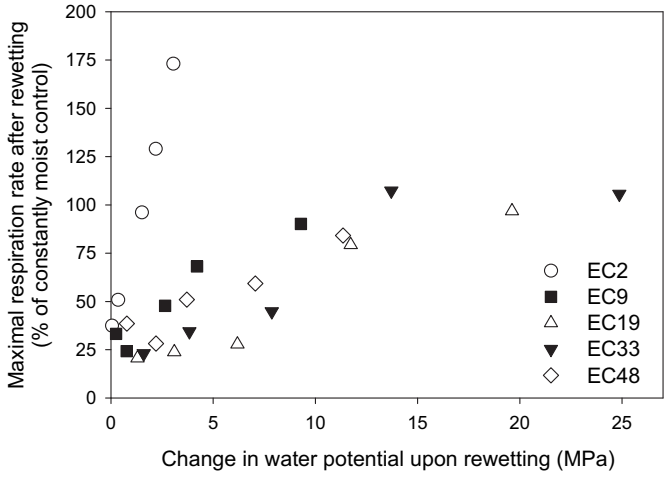

Fig. 5. Relationship between the magnitude of change in water potential at rewetting and maximal respiration rate after rewetting in soils differing in ECe dried for 1–5 days. Symbols are averages of each treatment for a given soil ($n = 3$).

compared to the constantly moist soil. Extractable C per unit organic C was lowest in soil EC2 and highest in soils EC19 and EC48. In soil EC48, extractable C in 4D and 5D was significantly lower than in the constantly moist soil, but there was no effect of drying in the other soils (Table 2).

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

4. Discussion

In agreement with previous studies (Kieft et al., 1987; Franzluebbers et al., 1994; Mikha et al., 2005; Butterly et al., 2009; Bapiri et al., 2010), 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.

4.1. Moist incubation with residues before drying

The similar MBC concentration seven days after residue addition in all soils indicates that microbes in saline soils are capable of rapidly utilising substrates added to the soil. Indeed, the increase in

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} SOC)						
EC2	79	59	63	65	61	70
EC9	91	72	59	72	66	62
EC19	239	159	142	178	147	138
EC33	87	82	69	95	72	66
EC48	104	63	65	90	64	64
			lsd = 12			
Microbial biomass C (mg g^{-1} SOC)						
EC2	9.7	6.2	7.7	10.0	7.2	11.2
EC9	11.4	8.1	10.4	9.8	8.0	15.4
EC19	18.0	30.8	37.1	33.9	49.3	44.7
EC33	11.1	17.8	19.5	17.9	25.5	26.0
EC48	3.2	9.4	6.6	6.6	14.1	18.8
			lsd = 7.7			
K_2SO_4-extractable C (mg g^{-1} SOC)						
EC2	11.1	14.8	16.0	15.9	10.9	10.7
EC9	22.5	27.3	29.1	25.7	23.9	21.8
EC19	76.6	78.9	73.0	75.9	46.9	50.7
EC33	48.8	45.4	48.9	39.7	42.7	30.3
EC48	75.6	83.5	70.5	65.0	60.0	56.6
			lsd = 8.3			

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 (Fig. 4) and in previous experiments with salinized soils (Chowdhury et al., 2011a,b) and saline soils from the field (Setia et al., 2011a). Thus, respiration per unit MBC was lower in saline soils, indicating that substrates were utilised more effectively.

4.2. 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 water potential during the dry phase compared to non-saline soil) has to be rejected. Relative to the constantly moist control, average respiration rates in the dry phase decreased to a similar extent in all soils (Fig. 2), although osmotic and water potential were substantially lower in the saline soils than in soil EC2, particularly in EC19, EC33 and EC48. For example in 3D, water potential was −1.7 MPa in EC2, but ranged from −8.7 to −12.8 MPa in EC19, EC33 and EC48. However, the decrease in water potential in the drying treatments relative to the constantly moist control was quite similar in all soils. This indicates that when comparing different soils, the negative effect of drying on soil respiration is determined by the relative decrease compared to the moist soil and not by the absolute water potential reached during the dry phase. Indeed, in all soils the 2-fold decrease in water potential from the constantly moist soil to 2D decreased respiration in the dry phase by about 50% and the 3-fold decrease in water potential in 3D decreased respiration by about 75%. Nevertheless, the quadratic relationship between respiration rate during the dry phase and water potential (Fig. 3) suggests that respiration rates are very low at water potential <−10 MPa, irrespective of the initial potential of the soils. However, at a given water potential, respiration rates were higher in the saline soils compared to soil EC2 (Fig. 3). This suggests that microbes in saline soils are more tolerant to low water potential than those in non-saline soils. Our finding of the greater tolerance of microbial activity in saline soils to low water potential is in contrast to Rousk et al. (2011), who reported that growth rates of bacteria from saline soils were similarly decreased by high salinity as those of bacteria from non-saline soils.

4.3. Changes 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 the constantly moist control (Fig. 4). In these treatments, rewetting increased water potential by 20–30% in 1D and 2-fold in 2D. Thus, such moderate increases in water potentials 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 not significantly affected by drying (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) and this proportion may differ among the moisture treatments. In forest soils from temperate moist climate, Fischer (2009) found that a substantial flush in respiration after rewetting only occurred if the soils were dried to −0.63 MPa and below. Similarly, there was no flush in respiration in the non-saline soil if the water potential was −0.56 MPa (2D) (Table 2). However in the saline soils, no flush occurred in 2D although water potentials during the dry phase were between −1.88 and −8.77 MPa (Table 2). This suggests that compared to non-saline soils, lower potentials have to be reached in saline soils to induce a flush in respiration upon rewetting. However, since saline soils have a lower water potential than non-saline soils due to the presence of salts, a flush in respiration upon rewetting occurred in all soils after being dried for 3 days or more.

When rewetting increased water potential more than 2-fold (in 3D, 4D and 5D), it induced a strong flush in respiration in all soils, with the relative increase compared to the constantly moist soil greatest in soil EC2 and 5D (Fig. 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 water potential and lowest respiration rates during the dry phase and rewetting induced the greatest increase in water potential (Table 2). Due to the low water potential 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 water potential 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 water potential 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., 2011a,b; Chowdhury et al., 2011a,b). Similarly, growth rates of bacteria decreased with increasing salinity of the soils from which they were extracted (Rousk et al., 2011).

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 (Fig. 2) than for cumulative respiration at the end of the incubation period (Table 3). Moreover, only respiration rates during drying were correlated with the water potential during the dry phase, but not with cumulative respiration at the end of the experiment (Fig. 3). Compared to a water potential of −1 MPa 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.

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 the constantly moist soils (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., 2011a). 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 the constantly moist soils (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 (Fig. 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 the constantly moist soils. Thus, it can be assumed that, compared to the constantly moist soils 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. Other studies have reported that microbial biomass is stimulated by exposure to several drying and rewetting cycles (Zhao et al., 2010, Xiang et al., 2008), Our results suggest that a stimulation may already occur after just one drying and rewetting cycle if the soils are dried to very lower water contents. The difference in MBC between soils EC2 and EC48 was 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.

5. Conclusions

The results of this study showed that at a given water potential during the dry phase, respiration rates per unit soil organic C were higher in saline compared to non-saline soils suggesting that microbes in saline soils are more tolerant to low water potential than those in non-saline soils. The study further showed that rewetting results in a flush in respiration even in highly saline soils. However, the flush in respiration upon rewetting occurred only if the water potential of the soils was decreased at least 3-fold during the dry phase, that is, rewetting increased water potential by a factor of 3 or more. Hence, only marked increases in water potential induce a flush in respiration upon rewetting. The consistent decrease in respiration during the dry phase relative to the constantly moist soil among the soils which differed in original water potential indicates that, if the water potential remains above −10 MPa in the dry phase, respiration is more affected by the relative decrease in water potential than by the absolute water potential reached during drying.

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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CHAPTER 6

Previous water content influences the response of microbes to changes in water content in non-saline and saline soils

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Supervised development of work, data interpretation and manuscript evaluation and correction.

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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Previous water content influences the response of microbes to changes in water content in non-saline and saline soils

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Abstract

Three incubation experiments were carried out with a non-saline soil (electrical conductivity in a saturation paste (ECe) 1 dS m^{-1}) to which NaCl was added to achieve ECe 10 and 30 dS m^{-1}, pea straw was added at 20 g kg^{-1} as a nutrient source. Experiment 1 showed that cumulative respiration was highest in soil EC 1 and lowest in soil EC 30. The optimal water content for respiration was 60-70% of WHC in all soils. There were two periods (days 1-7 and days 8-17) in Experiment 2. In the treatments with the same water content in both periods (O-O and M-M), respiration rates decreased over time and were lower in M-M than in O-O. Cumulative respiration at medium water content did not differ between slow (L-SM) or rapid rewetting (L-RM) from low to medium water content. There were two periods in Experiment 3 with the water content in the first period 50, 40 or 30% of WHC adjusted from 60% during pre-incubation either slowly or rapidly. The water content in the second period was maintained or adjusted slowly to 30-60%. Cumulative respiration differed between water contents but was not consistently different between rapid and slow drying in the first period. We conclude that the response of microbial activity to a certain water content is influenced by the previous water content whereas the speed at which the water content is adjusted had little effect on respiration at target water content.

Keywords: cumulative respiration; osmotic potential, respiration rate; speed of change; target water content

Introduction

Soil water content varies with rainfall and irrigation patterns. Soil water content can be expressed as water potential which describes the tension by which the water is held in the soils. Low water content increases soil water potential (more negative), thus the remaining water is held more tightly in the soil and plants and microbes have to expend more energy to take it up. Further, water films around soil particles become thinner and disconnected which reduces diffusion and thus substrate availability to microbes. Low water content reduces microbial activity and growth (Bottner, 1985; Kieft et al., 1987), C and N mineralization (Pulleman and Tietema, 1999; Sleutel et al., 2008) and induces changes in microbial community structure (Hueso et al., 2012; Sorensen et al., 2013). For example, fungi and gram-positive bacteria may be more tolerant to low matric potential than gram-negative bacteria (Fierer et al., 2003; Schimel et al., 2007). When encountering high water potential (strongly negative), sensitive microbes may die, but others can survive by synthesizing and accumulating osmolytes to lower the osmotic potential inside their cells and thereby retain cell turgor and metabolism (Harris, 1981) which requires high amounts of energy (Oren 2001). In non-saline soils, matric potential is the main component of soil water potential. But in saline soils, soil water potential is also influenced by osmotic potential which is related to the salt concentration in the soil solution.

Soil salinity causes land degradation because salinity reduces plant growth and soil microbial activity. In Australia, about 30% of the land area affected by different types of salinization (Rengasamy, 2006b). Soil salinity is usually determined by electrical conductivity (EC), for example in a 1:5 soil: water extract ($EC_{1:5}$) (Chhabra, 1996). However the salt concentration in the soil solution, that is the osmotic potential, varies with water content. As soils dry, soil matric potential increases but so does the salt concentration in the remaining soil solution and therefore the osmotic potential. Our previous studies showed that when comparing soils with different water content, soil respiration was more closely related to osmotic potential than to EC (Mavi et al., 2012; Setia et al., 2011a).

Soil water content varies with rainfall and irrigation patterns and changes in water content can be rapid (within a few minutes) or slow (over days). The effect of drying and rapid rewetting has been

studied extensively (Chowdhury et al., 2011b; Griffiths et al., 2003; Herron et al., 2009; Ilstedt et al., 2000; Schimel et al., 2007; Xiang et al., 2008). Rapid rewetting of previously dry soil induces a flush in respiration (Chowdhury et al., 2011b; Fierer and Schimel, 2003) This respiration flush is thought to be due to increased substrate availability from cell lysis, release of osmolytes and aggregate breakdown (Borken and Matzner, 2009; Fierer and Schimel, 2002; Kim et al., 2012; Navarro-Garcia et al., 2012). But little is known about the response of soil microbes to less dramatic changes in water content and if the response is influenced by the speed at which the water content is changed. Slow changes may allow microbial community structure to change or accumulation of osmolytes. Therefore microbes may adjust to a low water content (high water potential) better and maintain greater activity if the water content is slowly adjusted compared to rapid adjustment. Further, microbes previously exposed to low water content (high water potential) may be more active at medium (sub-optimal) water content than those at previously optimal water content. This is because water potential decreases in the former whereas it increases in the latter case. The effects of changes in water content could be more pronounced in saline soil than in non-saline soil because they influence not only matric potential but also the salt concentration in the soil solution (osmotic potential).

We conducted three experiments with a non-saline and two saline soils to test the following hypotheses: i) previous exposure to low water content will increase respiration at medium water content compared to soils that maintained at medium water content throughout because the adaptation to low water content will increase tolerance to the lower osmotic potential at medium water content, and ii) slow drying allows microbes to adapt to lower osmotic potential compared to rapid drying which will increase microbial activity at the target water content and result in greater activity during subsequent increases in water content because the microbes were less stressed.

Materials and Methods

Soil

A non-saline sandy loam soil was collected (0-10cm) from Monarto, South Australia (35° 05´ S and 139° 06´ E). The region is semi-arid and characterized by a Mediterranean climate; the average temperature is 15.4 °C in winter and 30.5°C in summer. After collection, the soil was air-dried and sieved to < 2 mm. The soil had the following properties: pH 7.8, electrical conductivity in the saturated extract (EC_e) 1 dS m^{-1}, 16.3% clay, 17.5% silt, 66.2% sand, water holding capacity 32% and 12.3 g kg^{-1} organic carbon content.

Experimental design

Experiment 1. The aim of this experiment was to determine the relationship between osmotic potential and soil respiration in soils with different EC. Soil EC_e was increased to 10 and 30 dS m^{-1} by adding different amounts of NaCl dissolved in water, the control received the same amount of water but no salt. Soil water content was adjusted to 50% of WHC based on previous experiments which showed that this would be close to optimal for microbial activity in soils of this texture. The soils are referred to as EC1 (original soil without salt addition), EC10 and EC30 for soils with EC_e 10 and 30 dS m^{-1}, respectively. After a 15 day pre-incubation at 22-25°C, pea residue (C/N ratio 26, sieved to 0.25-2 mm) was added at 20 g kg^{-1} soil as available nutrient source and the water content was either increased by adding water or decreased by drying in a fan-forced oven at 30°C to achieve osmotic potentials between -0.07.and -9 MPa, then soil respiration was measured daily over 15 days. Three different water contents resulting in high, medium, and low cumulative respiration in the different soils were chosen for following experiments (Table 3).

Experiment 2. The aim of this experiment was to assess the response of soil microbes to rapid or slow changes in osmotic potential. Based on Experiment 1, three different water contents were chosen for each soil for high, medium and low cumulative respiration (% of WHC): 60, 40, 20 for soil EC 1; 60, 30, 20 for soil EC 10 and 70, 40, 20 for soil EC 30. Before adjusting to this water content, the soils were pre-incubated at their optimum water content (60%, 60% and 70% of WHC for EC 1, EC 10 and EC 30,

respectively; determined in Experiment 1) for 22 days. During this time, pea residue (C/N ratio 26, sieved to 0.25-2 mm) was added at 20 g kg^{-1} soil as available nutrient source on day 15. After this 22-day pre-incubation, the experimental period started by adding 25 g soil into cores (diameter 3.7 cm, height 5 cm) with a nylon mesh base which was packed according to the bulk density in the field, there were four replicates in each treatment. The experiment consisted of two periods: days 1-7 and days 8-17. Soil respiration was measured continuously throughout the first and second period. Soil water content was maintained at optimum (O) or were rapidly dried in a 30 °C fan forced oven (within 6 hours) at the beginning of first period to reach medium (M) and low (L) water content. There were five treatments for each soil with similar or different water contents in each period (Table 1). The treatment names indicate the water content in the two periods (days 1-7 and days 8-17): maintained at optimum water content (referred to as O-O); from optimum to medium water content (soils were dried in a fan-forced oven on day 8 and then placed back into the jars for respiration measurement, referred to as O-M); maintained at medium water content (referred to as M-M); slow change from low to medium water content (slow rewetting by adding small amounts of water for a gradual increase in water content in 2-3 days) (referred to as L-SM); rapid change from low to medium water content (rapid rewetting by adding the full amount of required water once) (referred to L-RM). Microbial biomass C was measured on day 1 (start of the first period) and day 17 (end of the second period).

Experiment 3. This experiment was designed to assess the effect of drying (increasing osmotic potential) slowly or rapidly followed by an slow change in water content on soil respiration. Soil EC 30 from Experiment 1 was used in this experiment to represent saline soils. Before the start of the experimental period, the soil was pre-incubated at 60% of WHC for 22 days. During this time, pea residue (C/N ratio 26, sieved to 0.25-2 mm) was added at 20 g kg^{-1} soil on day 15. After the pre-incubation period, the soil was filled into the cores as described for Experiment 2. There were 2 periods: period 1 which lasted from day 1 to day 7 or 12 depending on the mode of drying (slow or rapid) and period 2 which started immediately at the end of period 1 and lasted 10 days. There were 28 treatments separated into four

groups according to the water content in the first period (60%, 50%, 40%, 30% of WHC) (Table 2). In group 60, the water content in the first period was maintained after the pre-incubation. In groups 50, 40 and 30, drying in the first period was achieved by drying the soil either rapidly (30 °C fan forced oven) before placing the cores in the jars for respiration measurement or slowly by placing silica pouches in the jars and replacing them daily. In the second period, soils in each group were either dried or rewetted slowly to 60%, 50%, 40%, 30% WHC. Treatment names indicate the water content in the two periods and the speed by which the water content was adjusted in the second period, e.g. S50-40 refers to slow drying in the first period to 50% of WHC followed by slow drying to 40% of WHC in the second period. In period 1, respiration was measured daily including the days during which the water content was adjusted slowly. It took 1, 2 and 5 days to reach 50, 40 and 30% of WHC slowly. After the target water content was reached, respiration was measured for 7 days. Therefore respiration in the first period was measured for 7 days for treatments with rapid adjustment (day 1 to 7 after adjustment on day 0). In the treatments with slow adjustment of the water content, the number of days during which respiration was measured depended on the target water content: 8 days for target water content of 50% of WHC, 9 days for target water content of 40% of WHC and 12 days for target water content of 30% of WHC. Cumulative respiration data are presented either normalized to 7 days [(cumulative respiration over whole measured period/number of days measured) X 7], or cumulative respiration over the 7 days after the target water content was reached. In period 2, respiration was measured for 10 days only after the target water content was reached.

In all experiment, the soil water content was measured by weight regularly and reverse osmosis water added if necessary.

Measurements

The EC and pH were measured in a 1:5 soil: water ratio after 1 h end-over-end shaking. The $EC_{1:5}$ was converted to the EC of a saturated paste (EC_e) using the equation: $EC_e = (14.0\text{-}0.13 \times \text{clay \%}) \times EC_{1:5}$ (Hazelton and Murphy, 2007; Rengasamy, 2006a). The water holding capacity (WHC) was measured by

placing thoroughly wetted soils in rings in a sintered glass funnel which was connected to a 100 cm water column (Ψm=−10 kPa), and allowed to drain for 48 h. Soil osmotic potential was calculated by the following equation (Richards, 1954):

$O_s = -0.036\ EC_{meas}\theta_{ref}/\theta_{act}$

Where O_s= the soil osmotic potential (MPa) at the actual moisture content (θ_{act}, g g^{-1}) of the soil and EC_{meas}= the measure electrical conductivity (dS m^{-1}) of the extract at the reference water content (θ_{ref}, g g^{-1}) of the 1:5 soil/water mixture.

Soil respiration was measured daily by quantifying headspace CO_2 concentration within each jar using a Servomex 1450 infra-red gas analyser (Servomex, UK). After each measurement (t_1), the jars were vented to achieve ambient CO_2 concentrations, re-sealed and the CO_2 concentration was measured again (t_0). The CO_2 evolved during this period was calculated from the difference in CO_2 concentration between t_1 and t_0. Linear regression based on injection of known amounts of CO_2 into the jars was used to define the relationship between CO_2 concentration and detector reading. For details regarding the calculations see Setia *et al.* (2011b).

In Experiment 2, microbial biomass carbon (MBC) at the start and the end of the experiment was determined by fumigation-extraction (Vance *et al.*, 1987). The soil samples used for microbial biomass C determination were incubated separately from those used for measuring respiration but were also incubated in similar jars which were opened daily to refresh the air in them. There were four replicates per treatment, and sampling time. The C concentration in the filtered extracts was determined by titration (Anderson and Ingram, 1993) after adding 0.0667 M $K_2Cr_2O_7$ and sulfuric acid. The remaining dichromate was titrated with 0.033 M acidified $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$. Microbial biomass C was calculated as: MBC= 2.22 ×[(organic C extracted from fumigated soil)-(organic C extracted from non-fumigated soil)](Wu *et al.*, 1990).

Statistical analysis

Significant differences between different treatments in cumulative respiration and respiration rates were assessed by 1-way ANOVA. In Experiment 2, differences between treatments and sampling times (days 1 and 17) in microbial biomass C were assessed by 2-way ANOVA (GenStat ® for Windows 11.0, VSN Int.Ltd, UK, 2005). The least significant difference values were calculated at 5% probability.

Results

In the description of the results, we will refer mainly to water content, not osmotic potential because readers will be more familiar with water content.

Experiment 1

Between water content 80 to 20% of WHC, the osmotic potential varied between -0.07 and -0.3 MPa in soil EC 1, between -0.7 and -3 MPa in soil EC 10 and between -2.1 and -9 MPa in soil EC 30 (Table 3). Cumulative respiration was highest in soil EC 1 and lowest in soil EC 30. It was influenced by water content in all three soils, particularly in EC 30. Cumulative respiration was highest in soil EC 1 at 50-60% of WHC, in soil EC 10 at 70% WHC) and in EC 30 at 70-80% of WHC.

Based on the results of this experiment, the optimal water content chosen for Experiments 2 and 3 was 60 % of WHC. Although cumulative respiration was highest at 70-80% of WHC in soil EC 30, 60% of WHC was chosen for the following experiments because the soil was rather wet and difficult to mix at 70-80% of WHC.

Experiment 2

Soil respiration

At a given water content, respiration rates were lower in soil EC 30 than in the other two soils EC 1 or 10 (Fig. 1). In the first period (day 1-7), respiration rates decreased with decreasing water content (optimal (O) to medium (M) to low (L)). In the treatment with optimal water content throughout the experiment (O-O), respiration rates decreased over time but were still higher at the end of the

experiment than in the other treatments. Respiration rates also decreased over time in the treatment with medium water content throughout (M-M) and they were lower than with optimal water content. When the soil was dried from optimal to medium water content in the second period (treatment O-M), respiration rates decreased until day 12-13 and then were very low in soils EC 10 and EC 30, but increased again in soil EC 1. After day 12, respiration rates in a given soil were similar in the treatments with medium water content irrespective of whether the water content in the first period was optimal or medium (O-M and M-M). When the water content was increased rapidly from low in the first period to medium in the second period (L-RM), respiration rates sharply increased on day 8, then decreased until day 13 after which they increased again. When the water content was increased from low to medium slowly (L-SM) respiration rates increased gradually but remained stable after day 12. Thus, between days 12 and 15, respiration rates were higher in L-SM than in L-RM.

Cumulative respiration from day 1 to day 17 was lower in soil EC 30 than in the other two soils (EC 1 and 10) (Fig. 2). In all soils, drying from optimal water content in the first period to medium water content in the second period reduced cumulative respiration on day 17 compared to the treatment with optimal water content throughout (O-O). In the treatments with medium water content in the second period (M-M, L-SM, L-RM), cumulative respiration on day 17 did not differ among treatments in soils EC 1 and 10. But in soil EC 30, cumulative respiration was greater when water content was low the first period than if it was medium. When the soil water content was increased from low water content in the first period to medium water content in the second period, cumulative respiration at target water content (medium) was higher in the treatments with rapid rewetting compared to slow rewetting.

Cumulative respiration decreased with decreasing osmotic potential in both periods (Fig.3 A, B). In the first period, the range of osmotic potentials was smallest for soil EC1 and greatest for soil EC 30 (Fig. 3 A). The number of days at the target water content in the second period varied between 7 and 10 days, being shorter when the water content was adjusted slowly. To better compare the effect of slow and rapid wetting on respiration, cumulative respiration in the second period at the target water content

normalised to 10 days for Figure 3 B. The normalised cumulative respiration did not differ between the treatments with rapid or slow rewetting from low to medium water content (L-RM and L-SM).

Microbial biomass C

Before the start of the experiment (day 0, after 22 day pre-incubation), the MBC concentration was higher in soils EC 1 and 10 than in soil EC 30 (data not shown). At the end of the experiment, the MBC concentration differed little among soils and treatments (Table 4). In soil EC 1, treatment had no significant effect on MBC concentration, but in soils EC 10 and EC 30, the MBC concentration was highest in the treatment with optimal water content throughout (O-O). In soil EC 10, the MBC concentration was lowest in the treatment with optimal water content in the first period and medium water content in the second period (O-M) whereas in soil EC 30, it was lowest in the treatment with slow rewetting in the second period (L-SM).

Experiment 3

Respiration rates in the first period decreased over time in all treatments (Fig. 4). When the water content in the first period was 50 and 40% of WHC, respiration rates were higher in the first 3 days in the treatments with slow compared to rapid drying because the water content also decreased more slowly (Fig. 4 A, B). In the treatment with 30% WHC in the first period (Fig. 4 C), respiration rates were higher with slow compared to rapid drying until day 7. In the second period the water content was adjusted slowly in all treatments, respiration was measured when the target water content was reached. Respiration rates were higher in the first 3 days after the target water content was reached with slow compared to rapid drying in the first period when the water content in the first period was 50 or 40% of WHC (Fig. 4 D, E), but not when the water content in the first period was only 30% of WHC (Fig. 4 F). Compared to maintenance of the water content throughout periods 1 and 2 (e.g. 40-40% of WHC), increasing the water content in the second period, e.g. from 40 to 50 or 60% WHC increased respiration

rates for about 5 days after reaching the target water content whereas decreasing the water content in the second period resulted in decreasing respiration rates.

With rapid drying, respiration in the first period was measured for 7 days. In the treatments with slow drying of the water content, the number of days during which respiration was measured depended on the target water content (8, 9 and 12 days for target water content 50, 40 and 30% WHC, respectively).

For Fig. 5, cumulative respiration in the first period was normalised to 7 days in all treatments. The normalised cumulative respiration did not differ between rapid and slow drying in the first period when the target water content was 50 and 40% of WHC (Fig. 5 B, C). But when the target water content was 30% of WHC, normalised cumulative respiration was greater with slow compared to rapid drying (Fig. 5 D). Irrespective of the water content of the first period, cumulative respiration in the second period (8 days) or at the end of the experiment decreased with decreasing water content (Fig. 5). Cumulative respiration at the end of the experiment was not consistently different between rapid and slow drying in the first period, but there were some differences. In the treatments with target water content of 50% WHC in the first period, cumulative respiration at the end of the experiment was higher with slow compared to rapid drying only when the water content in the second period was 60 or 40% WHC (Fig. 5 B). When the target water content of the first period was 40% WHC (Fig. 5 C), cumulative respiration at the end of the experiment was higher with slow compared to rapid drying with 60 or 50% of WHC in the second period (Fig. 5 C). At a target water content of 30% of WHC in the first period, cumulative respiration at the end of the experiment was higher with slow compared to rapid adjustment only when the water content was ≥40% of WHC in the second period.

Cumulative respiration in the first period at the target water content and in the second period decreased with decreasing water content (Fig. 6). Speed of drying in the first period did not influence cumulative respiration in the first period when the water content in the first period was reduced from 60% of WHC during pre-incubation to 50 % of WHC. But when the soils were dried more in the first period (target water content 30 or 40% of WHC), cumulative respiration at the target water content in the first

period was greater with slow compared to rapid drying (Fig. 6 A). Cumulative respiration in the second period was higher with slow compared to rapid drying in the first period when the water content in the first period was 50 or 40% WHC at the highest water content in the second period (60% WHC, osmotic potential -2.5 MPa) (Fig. 6 B, C). But at lower water contents in the second period, there was no consistent difference between rapid and slow drying in the first period. In contrast, when the water content in the first period was 30% WHC (Fig. 6 D), cumulative respiration in the second period was higher with rapid compared to slow drying in the first period at the three higher water contents in the second period (60, 50 and 40% WHC). But there was no difference between rapid and slow drying when at lowest water content in the second period (30% WHC).

Discussion

The first experiment showed that the optimal water content for respiration is higher when the EC is high compared to low EC because the effect of salinity on microbes depends on the salt concentration in the soil solution (the osmotic potential) not the EC measured at a certain soil:water ratio in the laboratory. The salt concentration in the soil solution increases as the water content decreases. Thus, microbial activity will be reduced at higher water contents in soil with high EC than in soil with low EC. This confirms previous studies (Mavi et al., 2012; Setia et al., 2011a) which showed that osmotic potential more closely related to microbial activity than EC when comparing soils with different water contents. The main aim of the present study was to assess if the previous exposure to low or high water content (high or low osmotic potential) and how quickly this water content was reached influenced respiration at a given water content/osmotic potential.

Increasing respiration after rewetting of soil has been shown in many previous studies, however our experiments provide novel information about the response of microbes to rewetting because we used (i) different moisture cycles either from moist to dry or from dry to moist, (ii) soils with different EC which resulted in different osmotic potentials, and (iii) we studied the effect of speed of drying or wetting.

Experiment 2 showed that drying from optimal to medium water content (O-M) reduced respiration rates to similar levels as when the water content was maintained at medium (M-M). Thus previous exposure to high water content did not seem to make microbes more susceptible to lower water content (higher osmotic potential). However cumulative respiration at medium (target) water content was higher when the water content was reduced from optimal to medium (O-M) compared to maintenance of medium water content (M-M). Thus although respiration rates eventually decreased to similar levels in O-M and M-M, respiration rates were higher for a few days at medium water content in O-M resulting in higher cumulative respiration at the end of the experiment compared to M-M. This suggests that previous exposure to optimal water content allows microbes to maintain higher activity at medium water content than microbes which were kept at medium water content throughout. However, this may not be increased tolerance per se but due to non-uniform water content in the soil. The soil dried most likely from the surface, thus when the target medium water content was reached (appropriate soil weight achieved), the soil close to the surface may have been drier whereas the soil at the bottom of the cores may have still had an optimum water content. In contrast the water content would be uniform in the treatment which was kept at medium water content throughout.

Rapid rewetting from low to medium water content (L-RM) in Experiment 2 induced a rapid increase in respiration rates, but respiration rates then declined from day 3 to day 6. This confirms the flush in respiration induced by rapid rewetting in non-saline soils. The rewetting flush is explained by an increase in substrate availability from cell lysis, release of osmolytes and exposure of substrate previously protected in aggregates (Borken and Matzner, 2009; Kim et al., 2012); (Fierer and Schimel, 2002; Navarro-Garcia et al., 2012). The differences between cumulative respiration at medium and low water content were greatest in the most saline soil (EC 30) which can be explained by the high osmotic potential in this soil at low water content (-4.2 MPa) compared to the osmotic potential in the less saline soils at low water content (-0.3 and -2.5 MPa for soils EC1 and EC10). The high osmotic potential in soil EC 30 at low water content will represent a strong stressor to microbes. The osmotic potential at medium water content in soil EC 30 was still quite high (-2.5 MPa) resulting in low overall cumulative

respiration in this soil. The osmotic potential was similar in soil EC 30 at medium water content and in soil EC10 at low water content which explains the similar cumulative respiration in the two treatments. These results emphasise the importance of considering osmotic potential and not just EC measured in a certain soil:water suspension when evaluating the effect of salinity on soil microbes.

With slow rewetting (L-SM) respiration rates increased slowly, but then remained high. Compared to rapid rewetting, respiration rates with slow rewetting were lower from day 1 to 3 after the start of rewetting, but were higher from day 5 to 7. Although the pattern of respiration rates differed between the two treatments, cumulative respiration rates at the end of the experiment were similar in L-RM and L-SM. Speed of rewetting also did not influence cumulative respiration at the target water content in period 2.

However, respiration rates and cumulative respiration at medium (target) water content in the second period were higher when the water content was low in the first period (L-M) compared to optimal or medium water content (O-M and M-M). Thus previous exposure to low water content appears to allow microbes to maintain higher activity at medium water content compared to previous optimal or medium water content. This appears to confirm our first hypothesis (previous exposure to low water content will increase respiration at medium water content compared to soils that maintained at medium water content throughout because the adaptation to low water content will increase tolerance to the lower osmotic potential at medium water content). But this could be not only due to greater tolerance to the water potential at medium water content but also to higher substrate availability because less substrate was decomposed in the previous period when the water content during this period was low compared to optimal or medium water content. Synthesis osmolytes requires large amounts of energy (Oren 2001), therefore greater substrate availability could increase salinity tolerance.

Therefore the results of Experiment 2 suggest that water content in the two experimental periods influenced respiration whereas the speed by which the soils were dried to low water content in the first period had only a small effect.

There was no consistent effect of water treatment on microbial biomass C at the end of Experiment 2. By then, respiration rates were low suggesting substrate limitation which would induce

biomass turnover. Single MBC measurements like in the present study are not suitable for determining treatment effects on microbes. Continuous measurement of respiration on the other hand can provide important insights into microbial response to environmental factors.

Experiment 3 was designed to investigate the effect of changes in water content on respiration in greater detail. The most saline soil was chosen because the microbes were exposed to the highest osmotic at the low water content and because little is known about the effects of changes in water content in saline soils. In Experiment 3, slow drying allowed the microbes to maintain higher activity while the soil dried slowly compared to rapid drying and also increased respiration when the target water content of 40 or 30% WHC was reached. Thus the slow decrease in water content (increase in osmotic potential) apparently gave microbes time to develop tolerance mechanisms. Another reason for the higher activity at the target water content could be changes in microbial community structure during slow drying. Previous studies showed that microbial community structure is influenced by soil water content (e.g. Drenovsky *et al.*, 2004). This confirms our second hypothesis (slow drying allows microbes to adapt to lower osmotic potential compared to rapid drying which will increase microbial activity at the target water content and result in greater activity during subsequent increases in water content because the microbes were less stressed).

Slow drying in the first period to 50 and 40% WHC also resulted in higher respiration for a few days after rewetting the soils in period 2. This increased cumulative respiration at the end of the experiment with slow compared to rapid drying in the first period. The slow drying in the first period caused less stress than rapid drying which allowed the microbes to respond quickly to the more favourable conditions at the higher water content.

But when the water content in the first period was only 30% WHC, respiration after rewetting in period 2 was higher when the soils were rapidly dried to 30% in period 1 compared to slow drying in period 1 which is in contrast to the response of microbial activity to rewetting when the water content in the first period was 50 and 40% WHC and. This contrasting response depending on the water content in

the first period (30 compared to 40 and 50% of WHC) could be due to the greater substrate availability in the second period with 30% of WHC because less C had been respired in the first period with rapid drying. In the treatments with 50 and 40% WHC in period 1 on the other hand, cumulative respiration in the first period differed little between slow and rapid drying, probably because the target water content was still sufficient to maintain a certain level of activity. In the treatment with 30% of WHC in the first period on the other hand, respiration rates were very low once the target water content was reached.

Conclusion

This study showed that in non-saline and saline soils, the response of microbial activity to a certain water content (osmotic potential) is influenced by the previous water content. The previous water content (low, medium or high) had a stronger effect on total cumulative respiration than the speed at which the soils are dried. Respiration at the target water content was higher at medium or high water content when the soils had a low water content before which could indicate increased tolerance to the target water content because of the lower osmotic potential. However, our experiments did not allow distinguishing between increased tolerance due to physiological adaptation or as a result of greater substrate availability because less C was respired previously when the soil was dry. Microbial activity was also influenced by the speed of drying but the effect was less than that of the water content.

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Table 1. Soil water content (%WHC) and osmotic potential (MPa) in different periods in Experiment 2 for soils with ECe 1, 10 and 30 dS m^{-1}.

Soil	Treatment	First period (day 1-7)		Second period (day 8-17)	
		Water content	Osmotic potential	Water content	Osmotic potential
EC1	O-O	60	-0.09	60	-0.09
	O-M	60	-0.09	40	-0.14
	M-M	40	-0.14	40	-0.14
	L-SM	20	-0.3	40	-0.14
	L-RM	20	-0.3	40	-0.14
EC10	O-O	60	-1	60	-1
	O-M	60	-1	30	-1.8
	M-M	30	-1.8	30	-1.8
	L-SM	20	-3	30	-1.8
	L-RM	20	-3	30	-1.8
EC30	O-O	70	-2.5	70	-2.5
	O-M	70	-2.5	40	-4.2
	M-M	40	-4.2	40	-4.2
	L-SM	20	-9	40	-4.2
	L-RM	20	-9	40	-4.2

Table 2. Soil water content (%WHC) and osmotic potential (MPa) in different periods in Experiment 3 for soil ECe 30 dS m^{-1}.

Treatment	First period (7-12 days)		Second period (10 days)	
	Water content	Osmotic potential	Water content	Osmotic potential
60-60	60	-2	60	-2
60-50	60	-2	50	-3
60-40	60	-2	40	-4
60-30	60	-2	30	-5
R50-60	50	-3	60	-2
R50-50	50	-3	50	-3
R50-40	50	-3	40	-4
R50-30	50	-3	30	-5
S50-60	50	-3	60	-2
S50-50	50	-3	50	-3
S50-40	50	-3	40	-4
S50-30	50	-3	30	-5
R40-60	40	-4	60	-2
R40-50	40	-4	50	-3
R40-40	40	-4	40	-4
R40-30	40	-4	30	-5
S40-60	40	-4	60	-2
S40-50	40	-4	50	-3
S40-40	40	-4	40	-4
S40-30	40	-4	30	-5
R30-60	30	-5	60	-2
R30-50	30	-5	50	-3
R30-40	30	-5	40	-4
R30-30	30	-5	30	-5
S30-60	30	-5	60	-2
S30-50	30	-5	50	-3
S30-40	30	-5	40	-4
S30-30	30	-5	30	-5

Table 3. Cumulative respiration after 15 days in Experiment 1 in soils with EC 1, 10 and 30 dS m^{-1} at different water content and the corresponding osmotic potential (n=4). Different letters indicate significant differences in cumulative respiration within one soil ($P \leq 0.05$).

Soil	Water content (%WHC)	Osmotic potential (MPa)	Cumulative respiration	
EC 1	80	-0.07	2.75	c
	70	-0.08	2.74	c
	60	-0.09	2.99	e
	50	-0.11	2.98	e
	40	-0.14	2.81	d
	30	-0.18	2.47	b
	20	-0.3	1.94	a
EC 10	80	-0.7	2.16	c
	70	-0.8	2.28	e
	60	-1	2.20	cd
	50	-1.1	2.24	cd
	40	-1.4	2.18	c
	30	-1.8	1.74	b
	20	-3	0.97	a
EC 30	80	-2.1	2.21	f
	70	-2.5	2.20	f
	60	-2.8	1.85	e
	50	-3.4	1.63	d
	40	-4.2	1.33	c
	30	-5.4	0.81	b
	20	-9	0.50	a

Table 4 Microbial biomass C at the end of Experiment 2 in different moisture treatments in soils with EC 1, 10 and 30 dS m^{-1} (for details about the moisture treatments see Table 2) (n=4).

Soil	Treatment	MBC ($mg\ kg^{-1}$)	
EC1	O-O	188	cde
	O-M	188	cde
	M-M	199	de
	L-SM	160	bcd
	L-RM	202	de
EC10	O-O	209	e
	O-M	114	ab
	M-M	174	cd
	L-SM	165	cd
	L-RM	143	bcd
EC30	O-O	208	e
	O-M	145	bcd
	M-M	127	bc
	L-SM	82	a
	L-RM	157	bcd

Figure 1. Respiration rates in Experiment 2 from days 1 to 17 in soils with EC_e 1 (A), 10 (B) and 30 dS m^{-1} (C) in moisture treatments O-O, O-M, M-M, L-SM and L-RM (n=4, standard error bars are too small to be visible beyond the symbols). Vertical arrows indicate when the target water content was reached in L-RSM. For treatment details see Table 2.

Figure 2. Cumulative respiration in Experiment 2 in the first and second in soils with EC_e 1 (A), 10 (B) and 30 dS m^{-1} (C). Period 2 is subdivided into adjustment and target phases with moisture treatments O-O, O-M, M-M, L-SM and L-RM (n=4). Different letters indicate significant differences between treatments in a given soil and period with lower case letters for the first period and upper case letters for the second period ($P \leq 0.05$). For treatment details see Table 2.

Figure 3 Cumulative respiration at target osmotic potential (water content) in first (A) and second period (B) in Experiment 2 in soils with EC_e 1, 10 and 30 dS m^{-1} (indicated by labels S1, S10 and S30) in moisture treatments O-O, O-M, M-M, L-SM and L-RM (n=4). For treatment abbreviations see Table 2.

Figure 4. Respiration rates in periods 1 and 2 in Experiment 3 at 50 (A, D), 40 (B, E) and 30% (C, F) of WHC in period 1 with rapid or slow drying in period 1 and slow adjustment to 30-60 % WHC in period 2 (n=4, standard error bars are too small to be visible beyond the symbols). Vertical arrows indicate when the target water content was reached. For treatment abbreviations see Table 3.

Figure 5. Cumulative respiration in periods 1 and 2 in Experiment 3 at 60 (A) 50 (B), 40 (C) and 30% (D) of WHC in period 1 with rapid or slow drying in period 1 and slow adjustment to 30-60 % of WHC in period 2. Cumulative respiration in period 1 was normalised to 7 days (n=4). At a given water content in period 1, different letters indicate significant differences between treatments with lower case letters for the first period and upper case letters for the second period ($P \leq 0.05$). For treatment abbreviations see Table 3.

Figure 6 Cumulative respiration at target osmotic potential in different moisture treatments in period 1 (A) at target water content) and in period 2 with rapid or slow drying to 50 (B), 40 (C) and 30% (D) of WHC in period 1 in period 1 and slow adjustment to 30-60 % of WHC in period 2.

Figure 1

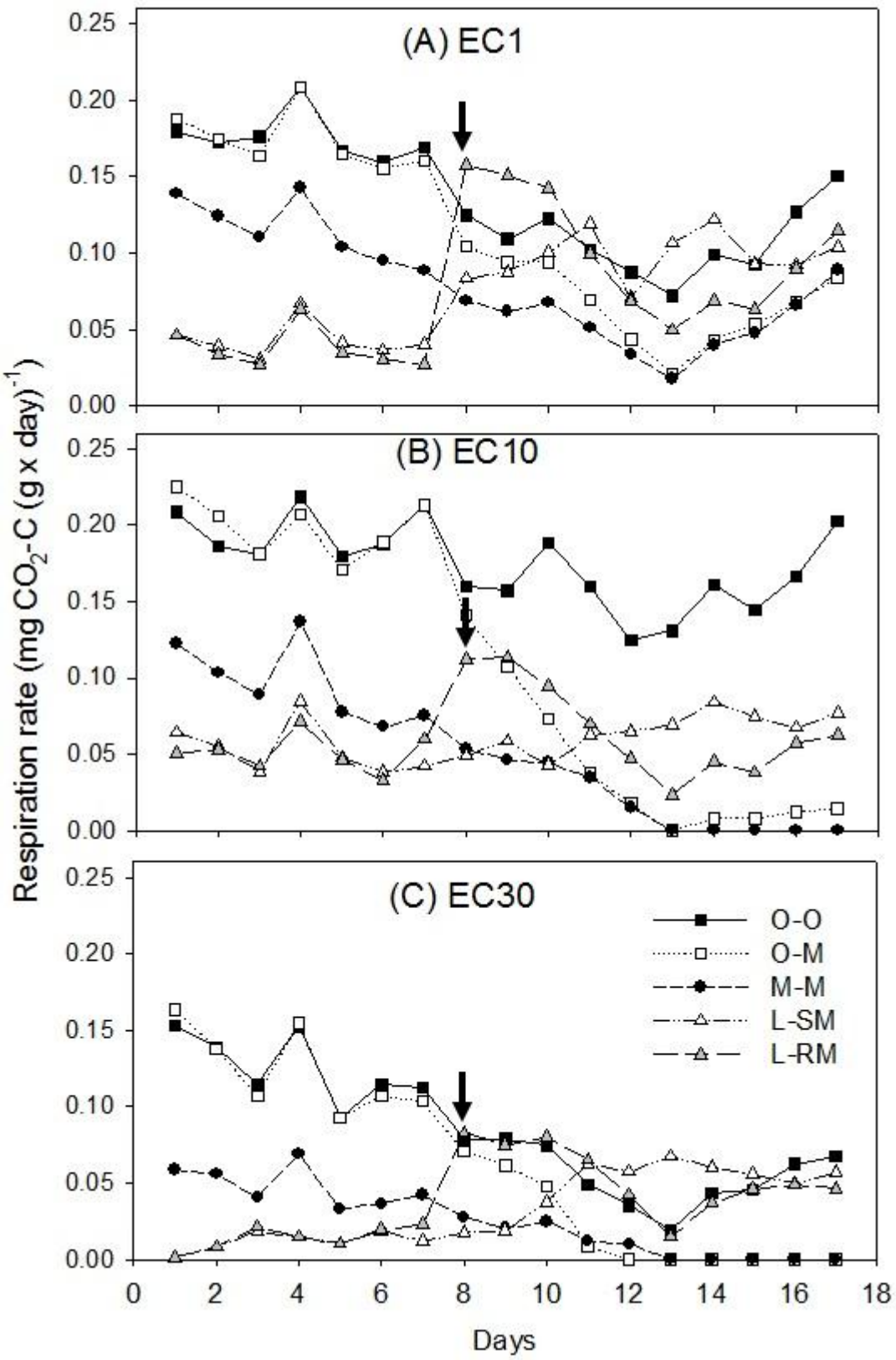

(A) EC1
(B) EC10
(C) EC30
O-O
O-M
M-M
L-SM
L-RM
Respiration rate (mg CO_2-C (g x day)$^{-1}$)
Days
0.25
0.20
0.15
0.10
0.05
0.00
0
2
4
6
8
10
12
14
16
18

Figure 2

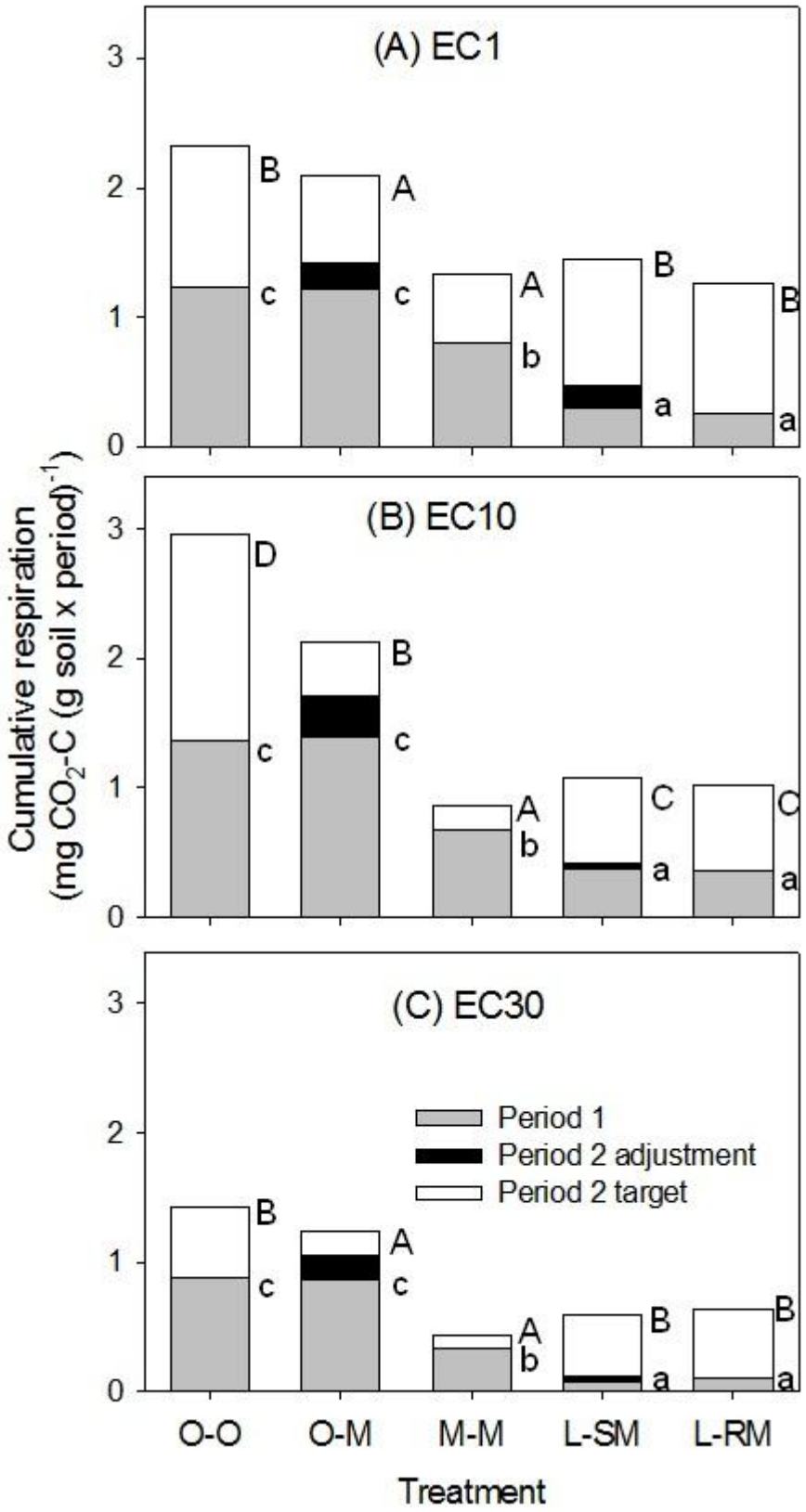

(A) EC1
(B) EC10
(C) EC30
Cumulative respiration
(mg CO_2-C (g soil x period)$^{-1}$)
Period 1
Period 2 adjustment
Period 2 target
O-O
O-M
M-M
L-SM
L-RM
Treatment

Figure 3

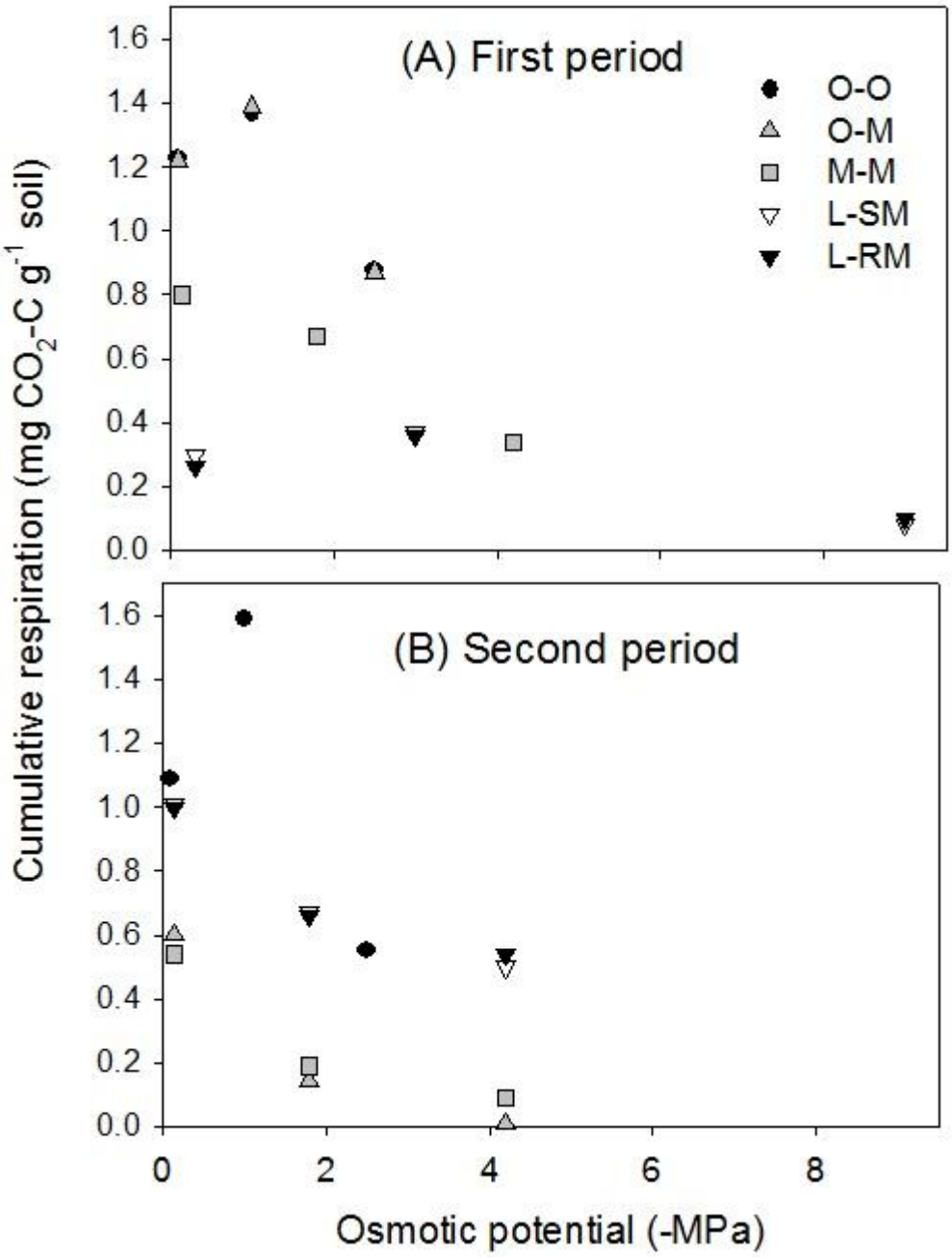

(A) First period
O-O
O-M
M-M
L-SM
L-RM
(B) Second period
Cumulative respiration (mg CO_2-C g^{-1} soil)
Osmotic potential (-MPa)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
2
4
6
8

Figure 4

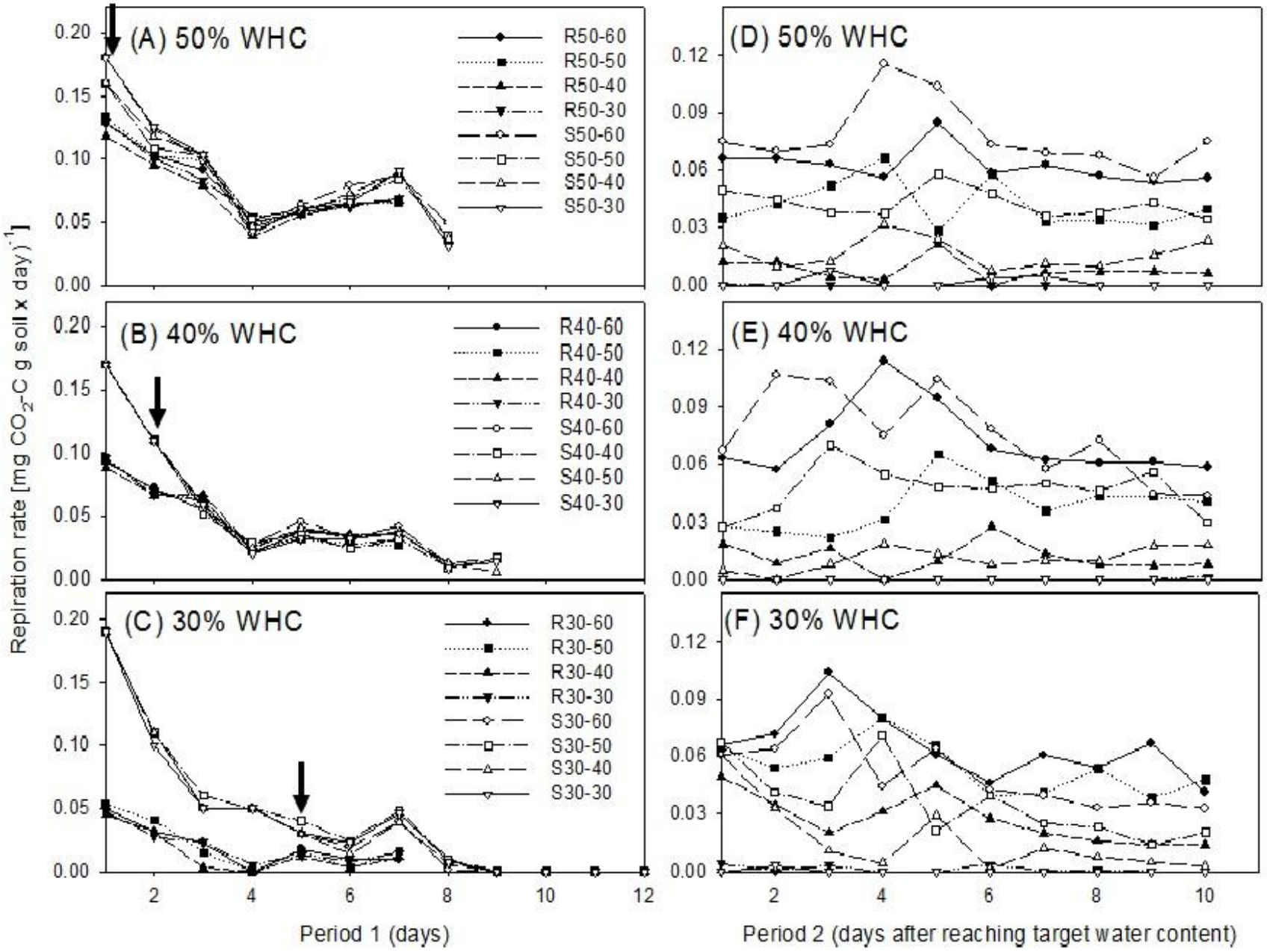

(A) 50% WHC
R50-60
R50-50
R50-40
R50-30
S50-60
S50-50
S50-40
S50-30
(B) 40% WHC
R40-60
R40-50
R40-40
R40-30
S40-60
S40-40
S40-50
S40-30
(C) 30% WHC
R30-60
R30-50
R30-40
R30-30
S30-60
S30-50
S30-40
S30-30
(D) 50% WHC
(E) 40% WHC
(F) 30% WHC
Repiration rate [mg CO_2-C g soil x day)$^{-1}$]
Period 1 (days)
Period 2 (days after reaching target water content)

Figure 5

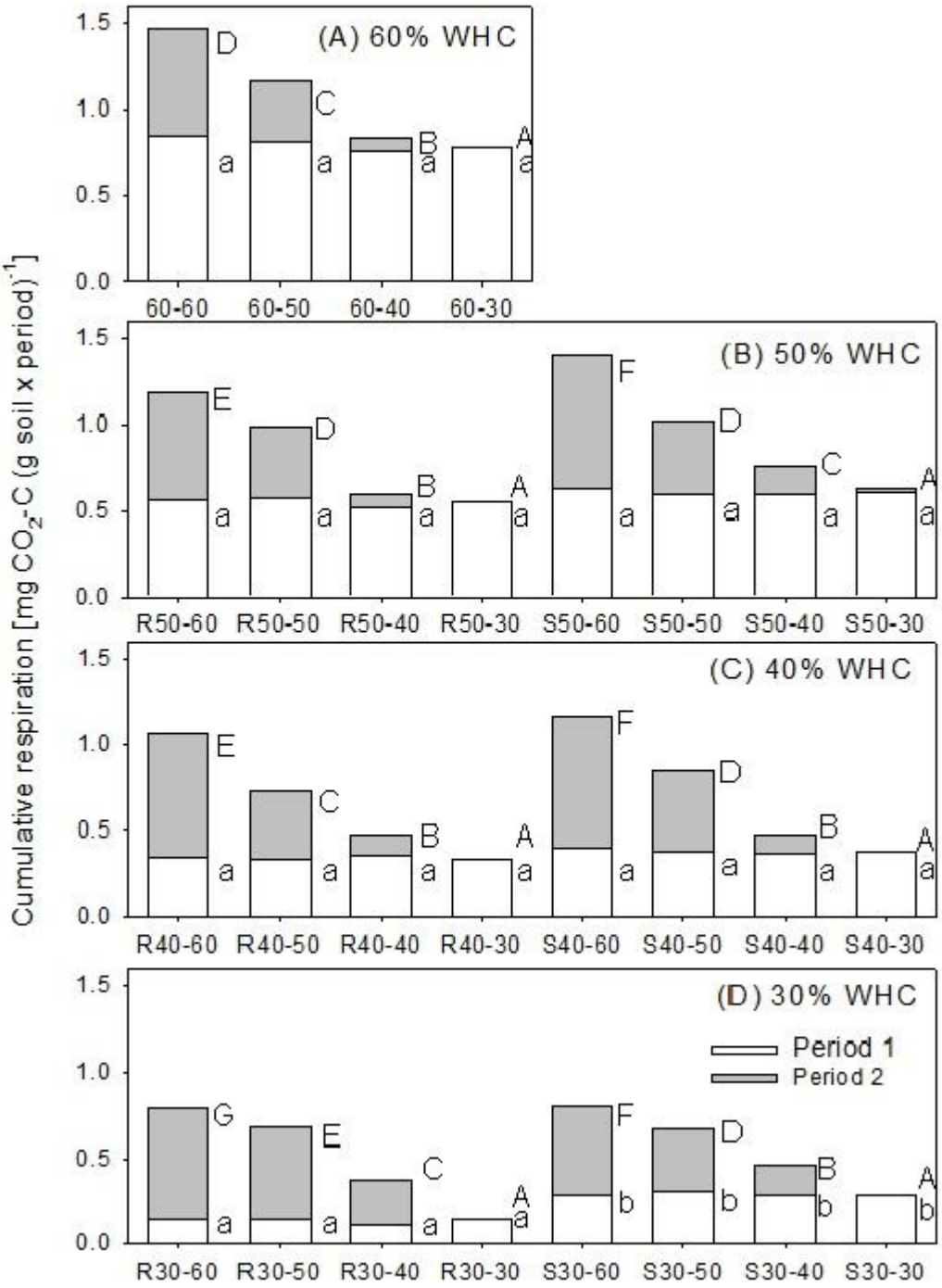
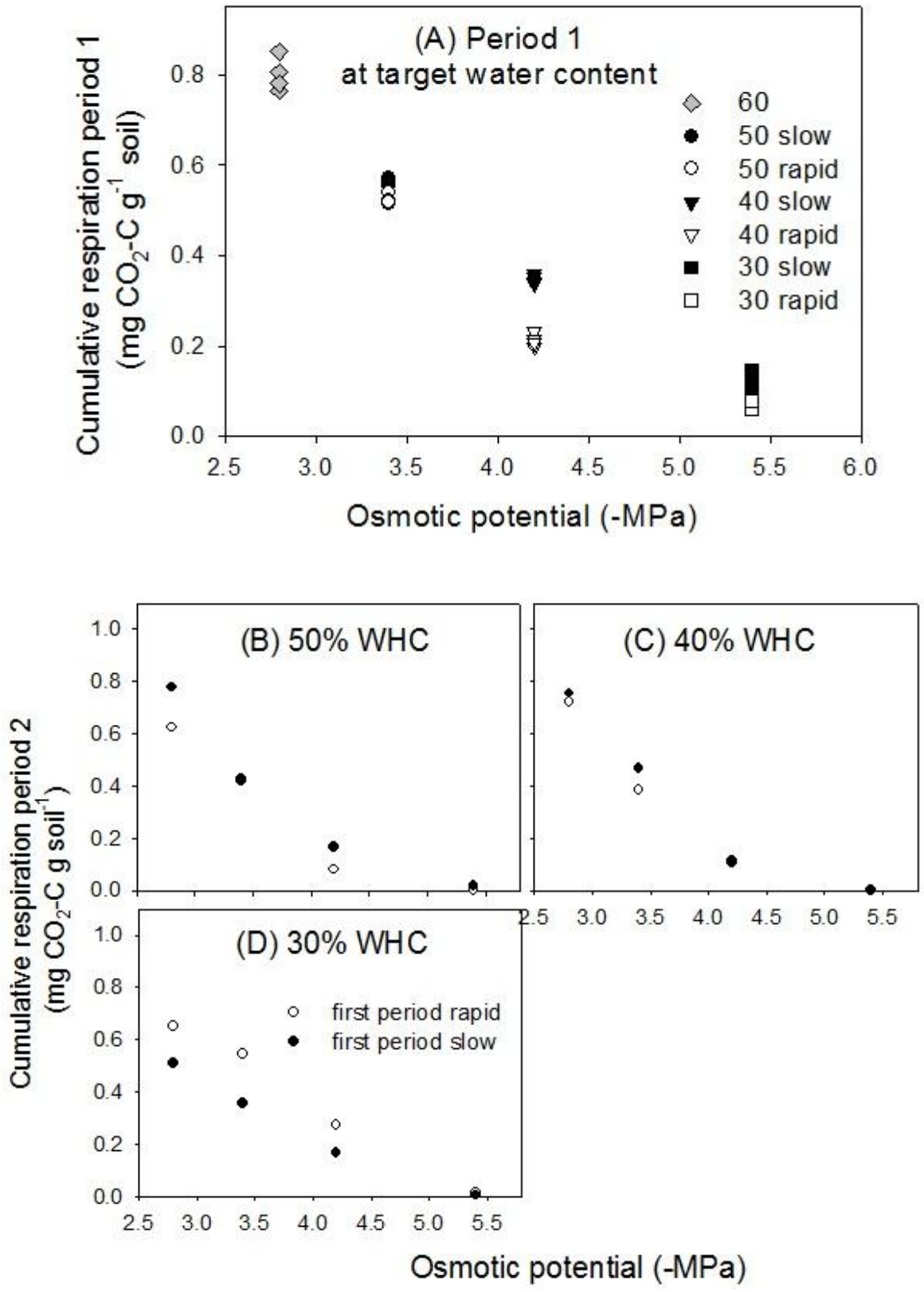

(A) 60% WHC
(B) 50% WHC
(C) 40% WHC
(D) 30% WHC
Period 1
Period 2
Cumulative respiration [mg CO_2-C (g soil x period)$^{-1}$]
60-60 60-50 60-40 60-30
R50-60 R50-50 R50-40 R50-30 S50-60 S50-50 S50-40 S50-30
R40-60 R40-50 R40-40 R40-30 S40-60 S40-50 S40-40 S40-30
R30-60 R30-50 R30-40 R30-30 S30-60 S30-50 S30-40 S30-30

Figure 6


(A) Period 1
at target water content
60
50 slow
50 rapid
40 slow
40 rapid
30 slow
30 rapid
Cumulative respiration period 1
(mg CO2-C g-1 soil)
Osmotic potential (-MPa)
(B) 50% WHC
(C) 40% WHC
(D) 30% WHC
first period rapid
first period slow
Cumulative respiration period 2
(mg CO2-C g soil-1)
Osmotic potential (-MPa)

CHAPTER 7

Conclusion and Future Research

7 Conclusion and Future Research

Soil salinity is a world-wide threat to agricultural production and ecosystems because it reduces plant growth and microbial functioning. The effects of salinity on soil microbes have been studied extensively (Andronov et al., 2012; Batra and Manna, 1997; Pathak and Rao, 1998; Setia et al., 2011a), but usually at constant salinity or without information about changes in salinity prior to sampling. However, in the field salinity is not constant, it varies with the quality of the water used for irrigation. The effect of changes in salinity on soil microbes was investigated in Chapters 2, 3, 4 in this thesis.

Soil water content also changes in the field and its effect on soil microbes has been studied extensively in non-saline soils (Bottner, 1985; Hueso et al., 2012; Kieft et al., 1987; Pulleman and Tietema, 1999; Sorensen et al., 2013). In saline soils, the water content also influences the salt concentration in the soil solution (osmotic potential), but less is known about the interaction between soil water content and salinity on soil microbes. This interaction was studied in Chapters 5, 6 of this thesis.

The main findings of the experiments in this thesis were:

I. Soil microbial activity and biomass are negatively affected by salinity irrespective of whether the EC is adjusted or already found in the field.
II. Microbial activity and biomass are mainly a function of the final EC.
III. Soil microbes respond rapidly to changes in EC when supplied with an easily available C source. A large initial microbial biomass can better adapt to changes in EC and take advantage of low EC by rapid growth than a small microbial biomass.

IV. When studying the interaction between soil water content and salinity, it is important to consider that the salt concentration in the soil solution (the osmotic potential) is a function of water content.

V. Rewetting results in a flush in respiration even in highly saline soils. However, the flush in respiration upon rewetting occurred only if the water potential is decreased at least 3-fold upon rewetting. The respiration flush is lower in saline compared to non-saline soils.

VI. In non-saline and saline soils, the response of microbial activity to a certain water content (osmotic potential) is influenced by the previous water content.

The studies in Chapters 2 and 3 showed that, in the presence of available substrate microbial activity changed rapidly when salinity was increased (Chapter 2) or decreased (Chapter 3). Microbial activity was a function of the final EC irrespective of the original EC. This showed that: (i) microbial activity is not influenced by the extent of EC change (large or small) from original to final EC; (ii) microbes in previously saline soils are not more tolerant to further increases in salinity compared to microbes in previously non-saline soils; and (iii) activity and growth of a proportion of the soil microbes can rapidly increase when the EC is reduced and the substrate is added. The slower recovery of the microbial biomass when the EC was decreased by leaching compared to respiration in the originally highly saline soil suggests that upon an decrease of the osmotic potential, the small initial microbial biomass uses the substrates predominantly for energy generation (respiration) whereas cell division and growth respond more slowly.

In Chapter 4, one non-saline soil and two saline soils were exposed to cycles of different ECs where the EC was changed rapidly or gradually. Glucose was added as an easily available C source at the start of each cycle. The experiments showed that soil microbes can respond quickly to changes in EC with respect to activity and growth when they are supplied with easily available C, which in agreement with the first two experiments. But whereas the experiments described in Chapters 2 and 3 involved only one change in EC, the study in Chapter 4 included several cycles during which the EC was increased or decreased. Nevertheless, the results showed that even upon short-term exposure to different ECs, a previous exposure to high EC did not limit the ability of the soil microbes to respond to a subsequent decrease in salinity because respiration increased within one day when the EC was reduced from EC 31 or 11 dS m^{-1} to 1 dS m^{-1}. Further, a gradual increase of the EC did not result in greater respiration or microbial biomass compared to an abrupt increase. It should be noted that the gradual increase occurred over only a few days which may not give the microbes sufficient time to adapt, e.g. by a change in microbial community structure. However, such rapid changes in EC occur in irrigated fields. The results in Chapter 4 confirm the conclusion that microbial activity is influenced by the current EC.

However, microbial activity and biomass in the originally non-saline soil were less affected by EC increases and recovered more quickly after the EC was decreased than activity and biomass in the originally saline soils. This suggests that the large biomass in the non-saline soil can better adapt to changes in EC than a smaller biomass in the saline soils which was already stressed by salinity in the field.

In the field, soil water content varies in time and space, and these changes can be rapid or slow. Particularly in arid and semi-arid areas, soils often experience dry periods interrupted by occasional rainfall events that induce rapid rewetting. In Chapter 5, the effects of drying extent on the flush of respiration after rewetting was assessed, the results showed that rewetting of dry soils induced a flush of respiration, which in agreement with those reported previously (Fierer and Schimel, 2003; Franzluebbers et al., 2000; Halverson et al., 2000; Kieft et al., 1987). However, the intensity of the flush was influenced by both the extent of drying and soil salinity. The flush in respiration upon rewetting occurred only if the soils were dried for 3-5 days, that is, when the water potential was increased at least 3-fold in the dry period compared to optimal water potential. No flush upon rewetting was observed if the water potential had been increased less than 3-fold. The flush in respiration upon rewetting was lower in saline compared to non-saline soils which suggests that salinity limits the ability of microbes to utilise substrates released upon rewetting.

The effect of changing water content was further investigated in the studies described in Chapter 6. A non-saline and two saline soils were maintained at low, medium and optimum water content during two periods or the water content changed between the periods which changes being either rapid or slow.

The results showed that the response of microbial activity to a certain water content (osmotic potential) is influenced by the previous water content whereas the speed at which the water content was changed had little effect on respiration at target water content. When the previous water content was

low, respiration was higher at medium water content compared to continuous medium water content. This could be due to greater tolerance of the microbes previously exposed to high osmotic potential or because low respiration rates at low water content in the first period resulted in greater substrate availability at medium water content compared to the treatments with medium or optimal water content previously.

The results of this thesis showed that both salinity and soil water content strongly influence microbial activity and nutrient cycling which could in turn influence plant growth. We showed that soil microbes can rapidly respond to improved conditions (reduction of salinity or increased soil water content). Therefore, reducing salinity e.g. by leaching with non-saline water could rapidly increase nutrient mineralisation. However, in the studies presented in this thesis, organic C was added to the soils. Saline soils have a low organic matter content which may limit the ability for rapid recovery suggesting that effectiveness of amelioration strategies such as leaching could be increased by addition of organic matter. The results described in this thesis provide new insights into microbial ecology of saline soils. But they also prompt new questions which could be addressed in future studies.

1) Soil microbial activity and biomass were assessed in this study, but not microbial community structure with respect to functions. It is reported that soil microbial community is influenced by soil salinity and soil moisture content (Sorensen et al., 2013; Thrall et al., 2009; Yu et al., 2012), but little is known about the effect of changes in both salinity and water content on microbial community structure, particularly how this influences microbial functions involved in nutrient

cycling. Presence of functional genes involved in N cycling processes such as ammonification, nitrification, denitrification and N_2 fixation could be investigated using microarrays. The abundance of the genes could be measured by real-time PCR. This could be based on DNA (gene presence) or RNA (gene expression).

2) In the studies described in this thesis, easily decomposable substrates such as pea straw or glucose were used. However, plant residues in saline soils may vary in decomposability. It is possible that salinity has a greater effect on microbial community in the presence of residues that are decomposed by only a small proportion of the microbial community (e.g. lignin-rich material such as wood) because loss of only a few salt-sensitive species could impair decomposition. On the other hand when residues that are decomposed by a large proportion of the microbial community (e.g. legume straw) the death of a few genotypes due to salinity would have a smaller effect. In this thesis, NaCl was used to adjust salinity (EC) because it is the dominant salt in Australian soils. However, salinity may also be due to salts of other cations and anions such as Ca^{2+}, Mg^{+}, K^{+} and SO_4^{2-}, CO_3^{2-} , HCO_3^{-}. Previous studies have shown that NaCl is more toxic to microbes than Na_2SO_4 (McClung and Frankenberger, 1987). Further, the salts differ in solubility which could change the interactions between salinity and water content. Highly soluble salts such as NaCl will remain in solution even at high concentrations (low water content), whereas less soluble salts such as $BaCO_3$ may precipitate and therefore become less toxic.

3) In this study the source of the respired CO_2 or microbial biomass C could not be determined. Therefore, the proportion of utilised substrate could only be estimated assuming no decomposition of native organic C. To differentiate utilisation of added C from that of native organic C, 14C labelled material or differences in 13C natural abundance between soil organic C and substrate C could be used (Jenkinso, 1971), this would lead to a better understanding of the effect of salinity and dry and rewetting on native soil organic matter content and of the importance of native organic matter as energy source for soil microbes.

4) The studies described here were controlled laboratory incubation experiments to allow assessment of mechanisms involved in response of microbes to salinity and soil water content over short periods of time. However field studies measuring CO_2 release from saline soils over longer periods of time in conjunction with measurement of soil water content are needed to better understand the contribution of saline soils to CO_2 release and how it changes over the seasons.

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