MICROBIAL ACTIVITY AND BIOMASS IN SALINE SOILS AS AFFECTED BY CARBON AVAILABILITY

Thesis submitted to The University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

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Dedicated to my parents

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Abstract

Soil salinity is a serious land degradation problem which reduces plant growth and microbial activity due to (1) low osmotic potential which causes plant water stress, and (2) ion toxicity and ion imbalances (nutrient deficiencies) as result of high salt concentrations in the soil solution. Therefore, salinity affects organic matter turnover by influencing the amount of organic matter input in the soil and decomposition rate. Microbial activity and biomass in saline soils have been extensively studied, but a little is known about the effect of organic carbon (OC) addition on adaptation of soil microbes to salinity. The objective of this thesis was to determine the effect of OC availability on adaptation of soil microbial activity and biomass to salinity.

In most experiments described in this thesis, one non-saline and four saline soils from the field with similar texture (sandy clay loam) and electrical conductivities in a 1:5 soil: water extract ($EC_{1:5}$) of 0.1, 1.1, 3.1 and 5.2 dS m^{-1} or electrical conductivity of the saturation extract (EC_e) of 1, 11, 24 and 43 dS m^{-1} were used. In other experiments a non-saline loamy sand was amended with NaCl to achieve a range of EC levels. The optimum water content for respiration was determined by incubating the soils amended with glucose at different water contents and measuring the respiration for 10 days at 25ºC. Glucose, cellulose or pea residue was used as OC sources. Inorganic nitrogen (N) and phosphorus (P) were added in experiments with glucose and cellulose to ensure that N and P availability did not limit microbial growth. Respiration (CO_2 release) was measured throughout the experiments; microbial biomass C (MBC) at selected sampling dates. Available N and P were measured in the first and second experiment. Microbial community structure was measured in the fifth experiment.

The aim of the first experiment was to study the effect of increasing salinity on soil microbial biomass and activity at different addition rates of soluble organic C (glucose). One

non-saline and three saline soils with $EC_{1:5}$ of 0.1, 1.1, 3.1 and 5.2 dS m^{-1} were amended with glucose to achieve five carbon concentrations (0, 0.5, 1, 2.5, 5 g C kg^{-1}). N and P were added to achieve a C/N ratio of 20 and a C/P ratio of 200. Soil respiration was measured continuously over 21 days; MBC and available N and P were determined on days 2, 5, 14 and 21. Cumulative respiration was significantly increased with addition of ≥ 0.5 g C kg^{-1} compared to unamended soils. Cumulative respiration decreased with increasing salinity with smaller relative decrease when C was added than in the soil without C addition. Cumulative respiration decreased with increasing salinity with the strongest decrease in the soils without C addition where, compared to EC 0.1, it was 64% lower at EC1.1 and 80% lower at EC5.2. Addition of glucose reduced the negative impact of salinity; with 5 g C kg^{-1} cumulative respiration decreased by 2% at EC 1.1 and 21% at EC 5.2. MBC concentration was negatively correlated with EC at all C rates and at each sampling date. Addition of C resulted in N and P immobilisation in the first 5 days. Biomass turnover released N and P after day 14, especially in the soils with low EC. It can be concluded that microbes are less affected by increasing EC when they are provided with easily available C.

The second experiment was conducted to determine the response of soil microbes to salinity when supplied with different OC forms. One non-saline and three saline soils were amended with 2.5 and 5 g C kg^{-1} as glucose or cellulose, soluble N and P were added to achieve a C/N=20 and C/P=200. Microbial biomass C and available N and P were determined on days 2, 7, 14 and 21. Cumulative respiration decreased gradually with increasing EC when supplied with glucose, whereas with cellulose it decreased sharply from non-saline to saline soils but differed little among saline soils. Microbial biomass C and available N and P concentrations were highest in the non-saline soil but did not differ among the saline soils. Microbial biomass C concentration was higher and available N was lower with 5 g C kg^{-1} than with 2.5 g C kg^{-1}. With glucose, microbial biomass was highest on day 2 and then

decreased, whereas available N was lowest on day 2 and then increased. With cellulose, microbial biomass C increased gradually over time and available N decreased gradually. It is concluded that salinity decreased the ability of microbes to utilise cellulose more than glucose utilisation.

Two incubation experiments (Experiments 3 and 4) were conducted to investigate the effect of increasing EC on microbial biomass and activity when OC was added once in different proportions of glucose and cellulose or when the carbon form is changed over time. Experiment 3 was carried out using three sandy clay loam soils: a non-saline soil and two saline soils (ECe 11 and 43 dS m^{-1}) amended with 5 g C kg^{-1} as different percentages of glucose and cellulose. The percentages of glucose (G) were 100% and 0-20% and those of cellulose (Ce) were 0-100%. The fourth experiment was conducted with a non-saline loamy sandy soil which was adjusted to ECe 12.5 and 37.4 dS m^{-1} by addition of NaCl. The form of organic C was maintained or changed over time by adding 1.5 g C kg^{-1} every two weeks (on days 0, 15 and 29) as glucose (G) or cellulose (Ce): (Ce+Ce+Ce, G+G+G, Ce+Ce+G, G+Ce+Ce, G+Ce+G, Ce+G+Ce). Experiment 3 showed that compared to 100% cellulose, cumulative respiration was increased by mixing small amounts of glucose with cellulose, but the impact of glucose proportion differed with soil EC. Cumulative respiration increased with increasing glucose proportion in the combined treatments when the proportion of glucose was >2.5%. With 100% G cumulative respiration was greater in the non-saline soil than in the soil EC43, however with 100% Ce and all combined treatments, cumulative respiration was significantly higher in the non-saline than in soils EC11 and EC43. There was no further decrease in cumulative respiration from EC11 to EC43 when amended by 100% Ce but it decreased significantly from EC11 to EC43 in the combined treatments except with 10% G. The MBC concentration was lower in saline soils than in the non- saline soil. In Experiment 4, the impact of salinity on cumulative respiration in the two weeks following OC addition

depended on C form, treatment and period. Regardless of C form added, the effect of salinity was reduced when C was added repeatedly compared to the first addition indicating that high C availability increases microbial tolerance to salinity. Cumulative respiration increased when glucose was added after cellulose addition. Addition of glucose after cellulose alleviated the adverse effect of high salinity on cumulative respiration compared to the previous period with cellulose or when cellulose was added after glucose. It can be concluded that, mixing small amounts of glucose with cellulose increases activity and growth of soil microbes, but may make microbes more susceptible to salinity compared to cellulose alone. The study also indicated that irrespective of C form added, microbial activity and biomass were less influenced by salinity when C was added frequently compared to the first addition showing that high C availability decreases the negative impact of salinity on soil microbes.

To investigate the effect of increasing EC on microbial biomass and activity with repeated addition of plant residues, the fifth experiment was carried out with a non-saline soil (loamy sand, ECe 1 dS m^{-1}) amended with different amounts of NaCl to achieve ECe 12.5, 25 and 50 dS m^{-1}. Two rates of pea residue equivalent to 3.9 and 7.8 g C kg^{-1} (3.9C and 7.8C) were added on days 0, 15 and 29. In the saline soils compared to the first addition, cumulative respiration per g C added was higher after the second and third addition except with 3.9C at EC50. Compared to the first addition, the relative increase in cumulative respiration in the saline soils was greater with 7.8C than with 3.9C. At the end of experiment, the percentage of added C remaining was lowest at non-saline soil and increased with increasing salinity levels. The MBC concentration at the end of experiment was significantly lower than in the non-saline soil at EC25 and EC50 with 3.9C, but only at EC50 with 7.8C. Salinity changed the microbial community composition on day 42 assessed by phospholipid fatty acids, but only in the amended soils. It can be concluded that repeated residue addition reduced the adverse effect of salinity on cumulative respiration which indicates that limiting periods of low

substrate availability can enhance the adaptation of soil microbes to salinity. This positive effect of residue addition was observed although salinity changed microbial community composition, suggesting that OC addition enables the development of a microbial community that can better adapt to salinity.

The aim of the sixth experiment was to assess the response of soil microbes to increasing salinity in rhizosphere compared to non-rhizosphere (bulk) soil using the non-saline soil (ECe 1 dS m^{-1}). The soil was adjusted to ECe 13 and 19 dS m^{-1} by adding NaCl and placed in pots. Barley was planted in half of the pots to obtain rhizosphere soil whereas unplanted pots were used for generation of bulk soil. The pots were placed in a greenhouse and soil moisture was maintained throughout by weight. After 5 weeks the planted and unplanted pots were harvested to collect rhizosphere and bulk soils to be used for the following incubation experiment. The EC levels (EC1, EC13 and EC19) from the pot experiment (referred to as original) were either maintained or adjusted to ECe 13, 19, 31and 44 dS m^{-1} by adding different amounts of NaCl. Cumulative respiration and microbial biomass C in rhizosphere and bulk soil decreased with increasing adjusted EC. Across the whole range of adjusted ECs, the decrease in cumulative respiration with increasing EC did not differ between rhizosphere and bulk soil. However, compared to the treatments where the EC was maintained, the percentage decrease in cumulative respiration when the EC was increased to EC44 was smaller in rhizosphere than in bulk soil. The smaller decrease in microbial activity at the highest EC level in rhizosphere compared to bulk soil suggests that rhizosphere microbes may be less affected by high salinity than bulk soil microbes.

Experiment 7 aimed to determine the response of soil microbial activity and biomass to drying and rewetting of non-saline and saline soils when the salinity levels were maintained or increased upon rewetting. A non- saline loamy sand ($EC_{1:5}$ 0.1 dS m^{-1}) was salinized with NaCl to achieve $EC_{1:5}$ of 1.5 and 3.5 dS m^{-1} (initial EC). The soils were

amended pea straw at 20 g kg^{-1} before the moisture treatments began. The soils were divided into two portions, one portion was dried for four days and the second portion was maintained at 40 % of water holding capacity (WHC). The soils were then wetted to 75% WHC with either water to maintain the EC (EC0.1, EC1.5 or EC3.5) or amended with NaCl to achieve the following EC levels: EC0.1 was increased to 1.5, 2.5 and 3.5 dS m^{-1}. EC1.5 was adjusted to 2.5 and 3.5 dS m^{-1} and EC3.5 was increased to 4.5 dS m^{-1}. A respiration flush upon rewetting only occurred in the initially non-saline soil when the EC was maintained, but not when the EC was increased. At the end of the experiment (day 25), cumulative respiration was higher in the dried and rewet (DRW) treatment compared to the treatment that was maintained moist (CM) only in the initially non-saline soil when the EC was not increased. Cumulative respiration decreased with increasing EC compared to the treatments where the EC was maintained only in treatments with initially EC0.1 where the reduction was greater in DRW compared to CM. The MBC concentration was higher in the treatments in which the EC was maintained compared to the treatments where the EC was increased in both moisture treatments. When the EC was increased, the MBC concentration at the end of the experiment was greater in DRW compared to CM only in soil with initial EC0.1. However, in the saline soils (EC1.5 and EC3.5) when the EC was maintained or increased; the MBC concentration did not differ between moisture treatments. The experiment showed that in the initial non-saline soil, increasing the EC upon rewetting inhibits the ability of microbes to decompose substrates released after rewetting. Drying and rewetting did not consistently increase the sensitivity of soil microbes to salinity compared to constantly moist soil.

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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CHAPER 1

INTRODUCTION AND REVIEW OF LITERATURE

1. Introduction and literature review

1.1 Introduction

Salinity is a major impediment to agricultural production and ecosystem sustainability in arid and semi-arid regions of the world. Globally, it is estimated that 1 billion hectares of lands are salt affected (Singh and Dagar, 2009) and around 100 million hectares (Mha) or 5% of the total arable lands are degraded by salt accumulation (Lambers, 2003). Moreover, about 20 Mha of irrigated land area are affected by salinity (Ghassemi et al., 1995). World-wide, the annual economic loss of agriculture production due to salinity and sodicity is US$12 billion (Ghassemi et al., 1995) and this cost is expected to rise in the future as a consequence of extension of the area affected by salt. In Australia saline soils cover over 17 Mha (NLWRA, 2001). Salinity can be natural, e.g. in the vicinity of the sea or salt lakes, or can be human-induced in rain-fed regions (dry land salinity and transient salinity) and also caused by poor irrigation (Lambers, 2003; Rengasamy, 2002). Most saline soils in Australia are caused by dry land salinity where a raising saline water table leads to salt accumulation in the upper soil layers (Rengasamy, 2006).

Saline soils are characterized by low fertility due to a low organic matter and nutrients and high soluble salt concentrations in the soil solution. Saline soils are defined as having an electrical conductivity of the saturated extract (ECe) > 4 dS m^{-1}, a pH< 8.5 and an exchangeable sodium percentage (ESP) < 15. They are mostly found in arid and semi-arid regions (Siyal et al., 2002). High salt concentrations can have negative impact on soil physical, chemical and biological properties (Rengasamy, 2006). The major effects of salt stress are plant growth inhibition by low osmotic potential of the soil solution and ion toxicity of Na^+, Cl^- and deficiency of K^+ and Ca^{2+} (Marschner, 2012). The lack of plant growth in saline soils leads to increased risk of erosion by water or wind and thus the loss of topsoil fertility. Additionally, the reduction of plant cover will reduce plant residue input into the soil

and therefore, low organic matter (OM) content and soil microbial activity (Rengasamy, 2006; Sumner et al., 1998).

High salt concentration in the soil can stress or kill soil microbes that play a significant role in nutrient cycling (Rietz and Haynes, 2003; Wichern et al., 2006). Tolerant microbes accumulate osmolytes to counteract the low osmotic potential in the soil solution (Oren, 2001; Zahran, 1997). The high energy demand of the tolerance mechanisms (Oren, 1999) leads to reduction in growth and activity of the surviving microbes. Salinity decreases microbial biomass size and activity (Bronicka et al., 2007; Ghollarata and Raiesi, 2007; Tripathi et al., 2006; Yuan et al., 2007b). The decrease in soil microbial activity in saline soils leads to increased plant stress because of the decreased mineralization rate of nutrients such as C, N, P and S and therefore decreases in nutrient availability (Rietz et al., 2001; Rietz and Haynes, 2003). Salinity also alters microbial community structure (Andronov et al., 2012; Gennari et al., 2007; Gros et al., 2003; Llamas et al., 2008) because microbial genotypes differ in tolerance to osmotic stress.

Addition of soluble available C sources like glucose can enhance microbial growth and activity in non-saline soils (De Nobili et al., 2001; Mondini et al., 2006) and could also affect the tolerance of microbes to salinity because it provides the energy required to synthesise osmolytes. Other, more complex carbon forms such as cellulose which require specific enzymes (e.g. cellulase) to break them down before they can be utilised by microorganisms may be less effective in increasing microbial activity, not only due to the increased energy demand for the synthesis of the enzymes but also because only a limited number of microbial species are able to release such enzymes (Killham, 1994). The rhizosphere, which is the zone influenced by roots is characterised by higher concentrations of available C than soil in greater distance from roots because roots release easily decomposable organic compounds as root exudates. This leads to higher microbial density

and activity in the rhizosphere compared to the bulk soil (Marschner, 2012). Therefore, the presence of plant roots (and their exudates) may increase microbial tolerance to salinity. The relationship between carbon availability and microbial activity and biomass in saline soils is not fully understood.

This review begins with an overview of the importance of microbes in nutrient cycling. Then, current knowledge about salt effected soils and their effects on plant growth, soil microbial activity and growth are presented. The review also examines whether the addition of nutrients (e.g. C) increases the tolerance of soil microbes to salinity stress, the importance of C/N and C/P ratios, and the effects of C forms and the rhizosphere on soil microbes.

1.2 Role of soil microorganisms in nutrient cycling

In the soil environment, nutrient cycling is defined as the transformation of nutrients within and among organic matter, soil microbial biomass and plant roots or soil solution and minerals (Van Noordwijk, 1999). Soil microorganisms represent only about 1-5% of soil organic matter (Dalal, 1998; Killham, 1994), but they are the drivers of nutrient cycling (Bloem et al., 1994; Gil-Sotres et al., 2005). It is estimated that 80-90% of soil processes are mediated by microorganisms (Nannipieri et al., 2003). Bacteria, actinomycetes and fungi are dominant soil microbes in terms of number and biomass (Killham, 1994). Therefore, they make a fundamental contribution to ecosystem functioning and soil fertility (Singh and Singh, 1993).

Soil microorganisms play a vital role in nutrient cycling, plant growth and maintaining of soil productivity due to their ability to (i) decompose organic compounds (e.g. plant residues) and release inorganic nutrients (e.g. N, P and S) which can be taken by plant roots (Killham, 1994) and (ii) Influence the availability of nutrients by several processes

including oxidation, reduction, solubilisation and chelation (Marschner and Rengel, 2007). Moreover, the soil microbial biomass is a transient nutrient pool with turnover rates between hours to weeks. Nutrients released after cell death can be used by plants and other microbes (Butler et al., 2004; Marschner and Rengel, 2007). Soil bacteria play a key role in nitrogen fixation by forming symbiotic relationships between rhizobia and legume plants which supply plants with N and free-living diastrophic bacteria such as *Azotobacter sp* and *Azospirilium sp* can also provide N to non-legume plants (Sylvia et al., 2005). There are also other microbial processes that contribute to nutrient uptake by plants such as nitrification, sulfur oxidation and solubilisation and mineralisation of P (Marschner and Rengel, 2007; Sylvia et al., 2005).

1.3.Factors influencing soil microbial activity

Soil microbial activity and community structure are influenced by physical, chemical and biological factors such as moisture (Griffiths et al., 2003; Williams, 2007), temperature (Pettersson and Bååth, 2003; Teklay et al., 2010), pH (Bååth and Anderson, 2003; Rousk et al., 2009), organic matter content (Calbrix et al., 2007; Saison et al., 2006), nutrient availability (Demoling et al., 2007; Lauber et al., 2008), heavy metal concentrations (Åkerblom et al., 2007; Bååth, 1989; Giller et al., 2009) and soil type and texture (Girvan et al., 2003). In arid and semi-arid climate, stressors such as low water content and high salt concentrations can kill sensitive microbes and reduce the activity and growth of surviving microorganisms due to the metabolic burden imposed by the energy required for tolerance mechanisms (Oren, 1999; Schimel et al., 2007). Microbial processes such as nutrient transformation and the degradation of recalcitrant components of plant residues (cellulose and lignin) are reduced by low water content and salinity (Pulleman and Tietema, 1999; Schimel et al., 2007; Wichern et al., 2006; Yuan et al., 2007a). Consequently, salinity and drought influence nutrient cycling and nutrient availability and thus reduce plant growth.

1.4. Salt affected soils

1.4.1 Distribution, causes of salinity and sodicity

Salinity is one of the most important factors limiting growth and productivity of agricultural crops. It has been estimated that 800 Mha are affected by either salinity or sodicity which represent about 6 % of the total world land (FAO, 2008). Globally, more than 45 M ha of irrigated area, which represents about 20% of total irrigated area, has been degraded by salinity and 1.5 M ha are annually taken out of production (Hasanuzzaman et al., 2013; Pitman and Läuchli, 2002). The extent of saline areas is increasing as a result of mismanagement and exploitation of agriculture land to meet the requirements of an increasing world population. Salt affected soils occur in different parts of the world irrespective of climate but are most common in arid and semi-arid regions (Table 1).

Table 1 World distribution of salt-affected soils, in million hectares (Mha**)**

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

Source: FAO Land and Plant Nutrition Management Service (2008)

Salinity can be primary or secondary. Primary salinity occurs naturally from weathering of parent rocks during soil formation which results in accumulation of salts (Pannell and Ewing, 2006; Rengasamy, 2002). In addition, salt can be transported by wind from the salt lakes or ocean to land. Further, intrusion of seawater into soils of coastal lands can cause salt accumulation. In areas with low annual rainfall or poor drainage, salts can also be accumulated over time by rainfall; even though the salt concentration in rain water is low it is concentrated by evaporation. Rising of shallow groundwater also contributes to salinisation (Rengasamy, 2010b).

Secondary (human-induced) salinity includes dry land salinity, transient salinity and irrigation salinity (Pannell and Ewing, 2006; Rengasamy, 2002). Salinity is also caused by poor irrigation practices and drainage as well as expansion of irrigated into areas with high evaporation rates which causes rising of the saline water table (Lambers 2003). In Australia, it is estimated that 5.7 million hectares have the potential for developing dry land salinity and this may increase to 17 million ha by 2050 (NLWRA, 2001).

Dry land salinity is caused by clearing of native vegetation or changes in the water balance which causes rising of saline groundwater in the lower lying areas of the landscape. On the other hand, transient salinity is not related to the groundwater; it is the consequence of accumulation of water over an impermeable subsoil (high content of clay and often sodic) therefore the accumulated water remains in the soil profile (temporary waterlogging) which is widespread in duplex soils. In winter the salts are accumulated above the subsoil as result of leaching out the salt from the top soil. In spring and summer evapotranspiration causes dissolved salts to move upwards into the root zone. Therefore transient salinity changes with seasons and rainfall (Rengasamy, 2002). In Australia, 16% of the cropping area is influenced by dry land salinity while 67% of the area is potentially influenced by transient salinity which annually cost the Australian farming economy about $1.33 billion (Kelly and Rengasamy,

2006; Rengasamy, 2002). Sodic soils can also occur naturally or due to human activities and occur on 23% of Australian arable land (Rengasamy, 2002).

1.4.2. Types and properties of salt affected soils

Salt affected soils are classified as saline, sodic and saline-sodic based on electrical conductivity (EC), sodium absorption ratio (SAR) or exchangeable sodium percentage (ESP) and pH (Table 2) (Brady and Weil, 2008; US salinity laboratory staff, 1954).

Saline soils are characterised by high concentration of soluble cations such as sodium (Na^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}) and anions such as chloride (Cl^{-}), sulphate (SO_4^{-2}), carbonate (CO_3^{-2}), and bicarbonate (HCO_3^{-}) in the soil solution (Rengasamy, 2010b). Saline soils are characterised by electrical conductivity in the saturated soil extract (ECe) >4 dS m^{-1}, SAR<13 or ESP < 15 and pH < 8.5) (US Salinity Laboratory Staff, 1954). Salinity can be caused by Ca salts (Sardinha et al., 2003). However, in Australia, the majority of saline soils are dominated by Na^{+} and Cl^{-} and thus 50-80% of total soluble salt is NaCl (Rengasamy, 2006). Saline soils are flocculated as a result of high ion concentration in soil solution. The high salt concentration causes low osmotic potential, ion toxicity and ion imbalance which have adverse effect on soil biota ant plant growth (Marschner, 2012).

For sodic soils, sodicity is expressed as sodium absorption ratio (SAR) or exchangeable sodium percentage (ESP).

Sodium absorption ratio of the soil water extract is calculated by the following equation:

$$SAR = [Na^{+}]/ [(Ca^{+2}+Mg^{+2})/2]^{1/2}$$

Where, the concentrations of Na^{+}, Ca^{+2} and Mg^{+2} are in mmol L^{-1}

Exchangeable sodium percentage is calculated as:

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

Where Na_{ex} = concentration of exchangeable sodium (cmol kg^{-1}).

CEC = cation exchange capacity (cmol kg^{-1}).

The determination of SAR in the laboratory easier than ESP, therefore SAR is more widely used than ESP to determine sodicity (Brady and Weil 2008). Sodic soils have (ECe < 4, SAR > 13 or ESP >15 and pH > 8.5. In Australia a soil is considered sodic when it has an ESP > 6 (Isbell, 2002), instead of ESP >15 as classified by USDA. The lower ESP in Australian sodic soils is due to low content of soluble salts particularly Ca^{+2} which causes soils to disperse at lower percentages of Na. Furthermore, in Australia, most work has been conducted using soils with fine texture whereas soils with coarse texture were used in US (Qadir and Schubert, 2002; Rengasamy and Olsson, 1991).

Sodic soils are characterised by a high percentage of Na^{+} on the cation exchange sites of soil particles compared to Ca^{+2} and Mg^{+2}, which causes (i) ion toxicity and nutrient imbalance (nutrient deficiency) that reduce the growth of plants and microorganisms and(ii) deterioration of soil structure as result of slaking, swelling and dispersion of clay particles. Moreover, poor drainage and aeration can increase crusting and erosion, as well as reduce plant available water, seeding emergence and root penetration (Oster et al., 1996; Qadir and Schubert, 2002; Rengasamy and Sumner, 1998; Shainberg and Letey, 1984).

Saline -sodic soils have an ECe greater than 4 and SAR greater than 13 or ESP higher than 15. These soils are characterised by high concentration of both neutral and sodium salts and thus, high electrolyte concentrations in the soil solution which leads to flocculation of soil particles (Rengasamy et al., 1984; Shainberg and Letey, 1984). Therefore, these soils have a good structure, aeration and drainage.

Table 2 Classifications of salt affected soils based on electrical conductivity (ECe), sodium absorption ratio (SARe), exchangeable sodium percentage (ESP) and pH measured in saturated paste extract (Brady and Weil 2008; US salinity Laboratory Staff 1954).

Soil	ECe (dSm^{-1})	SARe	ESP	pH
Non-saline	< 4	< 13	< 15	< 8.5
Saline	> 4	< 13	< 15	< 8.5
Sodic	< 4	> 13	> 15	> 8.5
Saline-sodic	> 4	> 13	> 15	> 8.5

1.5 Relationship between salinity and soil water content

The main parameters that have been used to describe soil water status are water content and soil water potential (Hillel, 1998). The water content is expressed as mass water per mass soil or volume water per volume soil. Soil water potential is a measure of the energy (per unit mass or volume) of water relative to the energy of pure free water. Total soil water potential is the sum of forces which influence the energy state in the soil (Papendick and Camprell, 1981), namely matric, osmotic, gravitational, pressure and overburden potentials:

$$\Psi = \Psi_{\pi} + \Psi_{m} + \Psi_{g} + \Psi_{p} + \Psi_{o}$$

Where Ψ = water potential, Ψ_{π}= osmotic potential, Ψ_{m}= matric potential, Ψ_{g}= gravitational potential, Ψ_{p} pressure potential, Ψ_{o} overburden potential.

Plants and soil microbes have to overcome these potentials to take up the water from the soil (Griffin, 1981).

Soil osmotic potential results from the interaction between salts and soil water (Papendick and Campbell, 1981). Therefore osmotic potential of soil solution changes with water and salt content. Reducing soil water content increases the salt concentration of the

remaining soil solution and thus lowers osmotic potential (Chowdhury et al., 2011c) which limits water availability to plant roots and microbes and water be drawn from the cells into the soil (Bray and Weil, 2008). Salinity tolerant plants and microbes respond to salinity by osmotic adjustment to maintain their activity and growth (Killham, 1994, Schimel et al., 2007) by uptake of solutes and/or synthesis of osmoregulatory compounds (Oren, 2001). The accumulation of solutes in the cells of plants and microbes leads to a decrease in osmotic potential in the cells and thus, helps to maintain turgor and metabolic function of cells (Oren, 2001; Wright et al., 1997). The osmotic potential of the soil solution can be calculated by using the equation:

$\Psi\pi = -0.036\ EC_{meas}\ (\theta_{ref}/\theta_{act})$ (Richards, 1954).

Where Ψ_π is the osmotic potential (MPa) at actual moisture content ($\theta_{act,}$ g g^{-1}) of the soil, EC_{meas} is the measured EC (electrical conductivity dS m^{-1}) of the extract at reference water content ($\theta_{ref,}$ g g^{-1}) of the 1:5 (soil: water) mixture.

Matric potential results from adhesion force and capillarity which influences the retention and movement of soil water (Papendick and Campbell, 1981). Low matric potential (dry soil) is another stress that affects plant and microbial growth because low matric potential reduces the thickness of water films around aggregates (Griffiths et al., 2003; Ilstedt et al., 2000).

Water content is a major factor affecting microbial activity and nutrient cycling, because soil water is the transportation medium for nutrients and microbial motility and also is an important for cell metabolism. Changes in soil water content influence matric and osmotic potentials, availability of substrate and nutrients and oxygen diffusion (Chowdhury et al., 2011a; Griffiths et al., 2005; Schimel et al., 2007), therefore, drying and rewetting of

soils are important factors affecting soil microorganisms and plants (Fierer et al., 2003; Schimel et al., 2007).

1.6. Effects of salinity and sodicity on plant growth

Salinity inhibits plant growth due to the low osmotic potential of the soil solution which makes it harder for plants and soil microbes to take up water or retain water in their cells thus causing water deficit and plant wilting. Furthermore, excessive uptake of specific ions (Na^+ and Cl^-) and deficiency of nutrients (Ca^{2+}, K^+, N and P) cause ion toxicity and ion imbalance which inhibit plant growth by reducing seed germination, root growth, photosynthesis, enzyme activity, protein synthesis and evapotranspiration which ultimately leads to reduction in crop yield and/or plant death (Hasanuzzaman et al., 2013; Marschner, 2012; Munns and Tester, 2008; Yadav et al., 2011). Poor structure is the main problem in sodic soils which negatively affect plant physiology, root growth due to poor aeration, lack of pores, micronutrient deficiencies and high concentrations of boron and bicarbonate at high pH (Naidu and Rengasamy, 1993; Qadir and Schubert, 2002).

Several studies have shown a reduction in crop yield as a result of salt stress, for example in wheat (Rengasamy, 2010a), maize (Bajwa et al., 1986), cotton (Meloni et al., 2001), and tomato (Romero-Aranda et al., 2001). The effect of salinity on crop yield depends on water content, plant species and environmental factors. Low water content exacerbates salinity stress because of the lower osmotic potential compared to soils with higher water content. The ability of plant roots to take up sufficient amount of water for growth is reduced as a result of high concentrations of soluble salts in the soil solution (Keren, 2000; Yadav et al., 2011). Therefore, plants growing in saline soils spend a large amount of energy on osmotic adjustment by accumulating organic or inorganic solutes to decrease the osmotic potential inside their cells and thus overcome the low osmotic potential in the soil solution

around the roots (Yadav et al., 2011). The high energy demand for tolerance mechanisms is one factor that affects plant growth. Salt tolerance differs among crop species based on their relative yields. The relative yield often indicates a linear decrease after exceeding the threshold salinity (Fig 1; Maas, 1986; cited in Hasanuzzaman et al., 2013).

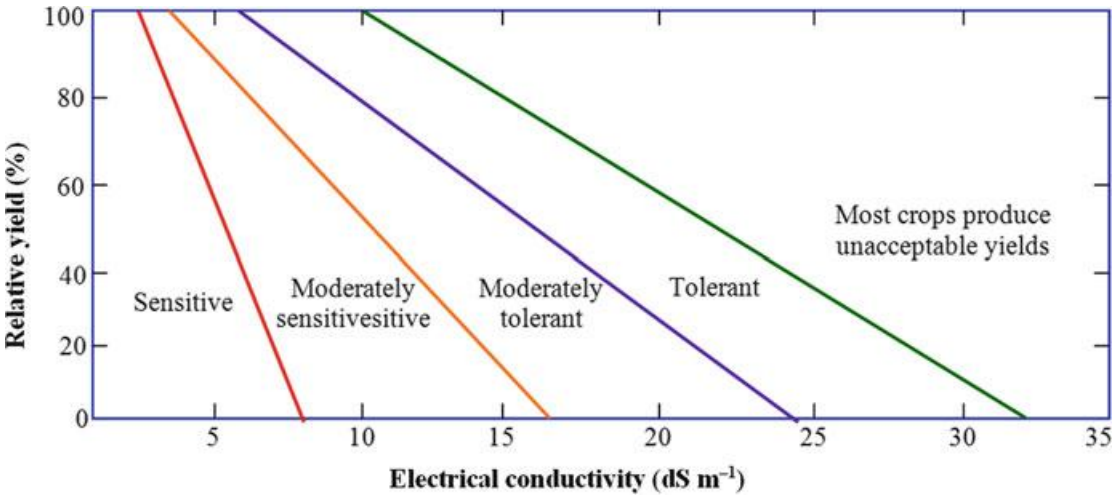

Figure 1 Relative yield in response to different salinity levels and varying degree of salt tolerance (Mass, 1986; cited in Hasanuzzaman et al., 2013).

Crops such barley, cotton and sugar beet whereas sorghum and wheat are considered moderately tolerant and sensitive crops include corn and rice. Sodium and Cl^- are usually the most predominant ions in the saline soils which cause ion toxicity, nutritional and physiological disorders in plants (Munns and Tester, 2008). The reduction in N uptake in the saline soils is due to competition between Na^+ and $NH_4^+$and /or Cl^- and NO_3^- which reduce crop growth and productivity (Fisarakis et al., 2001; Rozeff, 1995). High concentrations of Na^+ reduce K^+, Mg^{+2} and Ca^{+2} uptakes by roots (Hu and Schmidhalter, 2005). Banuls et al. (1991) showed that Cl^- reduces photosynthetic rate through its inhibition of NO_3^- uptake by plant roots. The reduced NO_3^- uptake combined with low osmotic potential can explain the inhibitory effect of salinity on photosynthesis (Yadav et al., 2011). Munns et al. (2006) and Tavakkoli et al. (2010) showed that the ion effect of a salt is prevalent at low salinity levels

whereas the osmotic effect is predominant at high salinity. In arid and semi-arid irrigated areas with pH is greater than 8.5, high concentrations of CO_3^{-2} and HCO_3^- lead to deficiency of Ca^{+2}, Zn and Cu, whereas when pH is greater than 9 in sodic soils B toxicity occurs (Naidu et al., 1992; Qadir and Schubert, 2002; Rengasamy et al., 2003; Rengasamy and Olsson, 1991). The reduction of plant cover in saline soils increases erosion by water and wind which reduces the fertility of the top soil (Lambers, 2003). Additionally, the lack of plant input decreases soil microbial activity.

1.7. Effects of salinity and sodicity on soil microbial activity and biomass and nutrient cycling

Salinity and sodicity adversely affect soil microorganisms and therefore, influence biochemical processes and nutrient cycling due to the changes in soil physical and chemical properties and low organic matter input as result of poor plant growth.

Salinity influences soil microbes mainly by reducing the osmotic potential. Microbial genotypes differ in tolerance to low osmotic potential, less tolerant microbial genotypes die, whereas tolerant genotypes counteract the low osmotic potential by accumulation of osmolytes, therefore salinity changes structure and activity of microorganisms (Pankhurst et al., 2001). There are two major adaptation mechanisms of microorganisms to counteract the osmotic stress, (i) some microorganisms selectively exclude inhibitory ions such as Na^+ and Cl^- from their cells and thus, accumulate ions necessary for metabolism particularly NH_4^+ and K^+ and (ii) Other salt tolerant microbes accumulate osmoregulatory compounds (Killham, 1994; Oren, 2001; Zahran, 1997). These osmolytes are amino acids in bacteria and polyols in fungi (Killham and Firestone, 1984; Schimel et al., 1989). The synthesis of osmolytes requires substantial amounts of energy (Oren, 1999) and therefore imposes a metabolic burden for microbes which can reduce the efficiency of C utilization (less C substrate used

for growth) by microbes under high salt concentrations (Rietz and Haynes, 2003; Wichern et al., 2006).

High salt concentrations in the soil solution reduce microbial biomass (e.g. Egamberdieva et al., 2010; Shah and Shah, 2011; Tripathi et al., 2006; Yuan et al., 2007b). Several studies have shown that in saline soils microbial biomass is negatively correlated with the salt concentrations (Rietz and Haynes, 2003; Sardinha et al., 2003; Shah and Shah, 2011). However, low microbial biomass and activity is not only due to osmotic stress but is also due to low OM content of saline soils (Sparling et al., 1997; Tripathi et al., 2006; Zahran et al., 1992). However, Sarig and Steinberger (1994) found that salinity had no effects on soil microbial biomass. In soils where high salinity was combined with high sodicity, Wong et al. (2008) showed that an increase in soil microbial biomass at a high salinity and sodicity compared to a non-saline soil, which was attributed to an increase C substrate availability as result of increased, solubility and accessibility of soil organic matter due to dispersion of soil aggregates in sodic soils. The effect of salinity on soil microbial biomass C/N ratio is not clear. In several studies, salinity was found to decrease microbial biomass C/N ratio (Muhammad et al., 2006; Wichern et al., 2006) whereas Shah and Shah (2011) found an increase in the ratio. The contradictory results could be due to soil type, salinity levels, water content and microbial community structure.

Salinity changes microbial community structure as consequence of the differences in tolerance of soil microbial genotypes to osmotic stress (Baumann and Marschner, 2013; Chowdhury et al., 2011b; Nelson and Mele, 2007). Fungi have been found to be less tolerant to salinity than bacteria (Badran, 1994; Chowdhury et al., 2011b; Pankhurst et al., 2001; Sardinha et al., 2003). Therefore, salinity decreases the ratio of fungi to bacteria which may influence nutrient cycling because of the inability of most bacteria to decompose the less degradable OM such as cellulose and lignin, whereas fungi release several enzymes

necessary to decompose the complex molecules (Killham 1994). Moreover, salt stress reduces bacteria diversity and species richness (Abed et al., 2007; Ibekwe et al., 2010).

Several studies have reported that salinity decreases microbial activity measured as soil respiration (Adviento-Borbe et al., 2006; Chowdhury et al., 2011c; Elgharably and Marschner, 2011; Laura, 1976; Mavi et al., 2012; Pathak and Rao, 1998; Setia et al., 2010). A few studies found an increase in soil respiration with salinity probably because high salinity occurred in combination with sodicity and therefore increased C availability (Chandra et al., 2002; Wong et al., 2008). Microbial activity in saline soils is not only influenced by the salt concentration in the soil solution but also by the type of salt; sodium chloride is the most toxic salt (Agarwal et al., 1971; Frankenberger and Bingham, 1982). Li et al. (2006) found that cumulative CO_2 evolution declined with NaCl concentration but increased with Na_2SO_4 concentration. The effect of sodicity on soil respiration is unclear. Sodicity can decrease soil respiration (Rietz and Haynes, 2003); increase it (Nelson et al., 1996; Wong et al., 2008). Sodicity can also have no effect on soil respiration (Pathak and Rao, 1998).

Salinity decreases N mineralization (Laura, 1974; Lodhi et al., 2009; McClung and Frankenberger, 1987; Walpola and Arunakumara, 2012), nitrification and ammonification (Rasul et al., 2006; Rietz et al., 2001; Shah and Shah, 2011; Wollenweber and Zechmeister-Boltenstern, 1989). In contrast, an increase in N mineralisation after salt addition to soil was reported by Broadbent and Nakashima (1971). This may be due to microbial biomass turnover as result of osmotic stress (Wichern et al., 2006). Moreover, the activity and growth of N2 fixing bacteria is reduced by salt stress (Sorokin et al., 2008; Zahran, 1999).

High salt concentrations also reduce the activity of enzymes such as β-glycosidase, phosphatase, arysulfatase and protease thereby influencing the cycles of C, N, P and S (Batra and Manna, 1997; Frankenberger and Bingham, 1982; García and Hernández, 1996; Rietz et

al., 2001; Tripathi et al., 2007). Moreover, salinity decreases the uptake of amino acids and protein synthesis (Norbeck and Blomberg, 1998). In addition, the high pH in saline soils may increase the solubility of OM and thus, increase the organic C losses (Pathak and Rao, 1998). The contradictory results of studies mentioned above about the effect of salinity on microbial activity and enzyme activity could also be due to soil type, salinity levels, water content and microbial community structure.

Most of these studies were conducted after adding salt to previously non-saline soils which may not allow microbes to adjust to salinity and could therefore exacerbate the salinity effect (Dendooven et al., 2010; Khan et al., 2008). In saline soils from the field, salinity develops gradually (Askri et al., 2010) allowing microbes to adjust, not only in terms of activity but also with respect to community structure.

1.8. Effects of adding organic matter to saline soils

In saline soils the content of organic matter (OM) is low due to poor plant growth as a result of osmotic stress and ion toxicity. Low input of OM in soils restricts microbial growth by reducing substrate availability (Tripathi et al., 2006). Addition of OM to saline soils can rehabilitate saline soils (Nelson and Sumner, 1998; Tejada et al., 2006) by improving soil structure, decreasing soil bulk density and providing energy and nutrients for soil microorganisms (Tejada and Gonzalez, 2005). It has been reported that the addition of OM to saline soils may increase leaching of Na and reduce EC and ESP, increase aggregate stability and water holding capacity (El- Shakweer M H A, 1998).

1.9. Effects of adding organic carbon on soil microorganisms

1.9.1. Form of carbon (soluble or insoluble)

In all soils, the growth of microbes is mainly limited by OC availability (De Nobili et al., 2001; Demoling et al., 2007; Wardle, 1992). Therefore the addition of OC to soil

stimulates microbial growth and activity. Plant litter contains soluble and insoluble OC forms. Soluble OC form is mainly as sugars (e.g. glucose) whereas insoluble forms include cellulose, hemicelluloses and lignin with cellulose comprising 35-50% of the plant dry matter hemicelluloses 20-35% and lignin 5-30% (Lynd et al., 2002).

The addition of easily soluble C (such as glucose) can enhance decomposition of native soil OM due to the stimulation of soil microbial growth (priming effect) (Kuzyakov et al., 2000) and can change the structure of the microbial community (Blagodatskaya and Kuzyakov, 2008; Hoyle et al., 2008). Due to high solubility of glucose it can be utilised by the majority of soil microorganisms (Anderson and Domsch, 1978; Blagodatsky et al., 2000; Landi et al., 2006) and favours mainly fast-growing microbes (Blagodatskaya et al., 2007; Landi et al., 2006), but slow-growing microbes that are capable of decomposing less available substrate (recalcitrant compounds) may also be stimulated (Fontaine et al., 2003; Landi et al., 2006).

In saline soils C addition may increase the ability of microbes to adapt to low osmotic potential providing the energy needed for tolerance mechanisms. Some studies have shown that increased availability of organic C in saline soils can alleviate the negative effect of salinity on soil microbes (McCormick and Wolf, 1980; Wichern et al., 2006). Addition of soluble C (glucose) induces a rapid, but short-lived increase in respiration (Luna-Guido et al., 2001; Sparling et al., 1981). The rapid decrease in respiration is due to the depletion of glucose (Blagodatsky et al., 2000).

Decomposition of added poorly soluble polysaccharides (e.g. cellulose) which are the major components of plant residues (see Figure 2) is slower because these complex compounds have to be broken down to glucose by producing extracellular enzymes such as cellulases and amylases (Schlegel, 1993; Sylvia et al., 2005). These extracellular enzymes are

released by fewer, mainly slower growing microbes, particularly fungi (de Boer et al., 2005; Killham, 1994; Meidute et al., 2008). Salinity has been shown to reduce the fungi/bacteria ration which may limit the ability of the microbial community to utilise cellulose as C source. There are no published studies comparing the effect of C forms on microbial activity and biomass in saline soils.

Addition of plant residue to soil increases soil respiration for several days followed by a decline the rate of respiration (Franzluebbers et al., 1994). This is indicates that plant residues are decomposed in two distinct stages, the first stage in which easily decomposable materials are utilised followed by a second stage in which more recalcitrant compounds are slowly decomposed (Aneja et al., 2006; Wang et al., 2004). In non-saline soil, repeated addition of small amounts of glucose did not increase cumulative respiration compared to a single addition (Hoyle et al., 2008). However, Duong et al. (2009) showed that when the same total residue amount was added, frequent residue addition of wheat straw increased cumulative respiration compared to a single addition. In saline soils repeated addition of C may provide a more continuous OC supply than a single addition and therefore increase microbial tolerance to salt stress.

1.9.2. Rhizosphere

Rhizosphere is the soil zone that is influenced by plant roots, which is characterised by high availability of soluble C due to the release of root exudates into the rhizosphere whereas in the non- rhizosphere soil the growth of microbes is C-limited (Demoling et al., 2007; Wardle 1992), Therefore soil microbial activity and biomass are higher in rhizosphere than in non-rhizosphere soil (Bodelier et al., 1997) and the two compartments differ in microbial community structure (Kuzyakov et al., 2007; Marschner, 2012). Most root exudates are low molecular weight organic compounds (easily decomposable) such as sugars,

carboxylic acids and amino acids which are released in large quantities (Farrar et al., 2003; Hütsch et al., 2002; Kuzyakov et al., 2007) and induce increased turnover of soil organic C (priming effect; Kuzyakov, 2002). Between 20 and 50% of photosynthetic C is translocated to the root zone (Kuzyakov and Domanski, 2000). Of this C, approximately half remains in the roots whereas about one third is respired by the roots or used for microbial respiration, the remaining portion is integrated into the rhizosphere microbial biomass and soil OM (Dennis et al., 2010; Kuzyakov and Domanski, 2000).

It is not known if microbes in the rhizosphere are better able to cope with salinity than those in non-rhizosphere soil. It can be hypothesised that the sustained supply of easily available C in the rhizosphere leads to greater microbial biomass and activity in saline soils and reduces the impact of salinity on these parameters compared to non-rhizosphere soil.

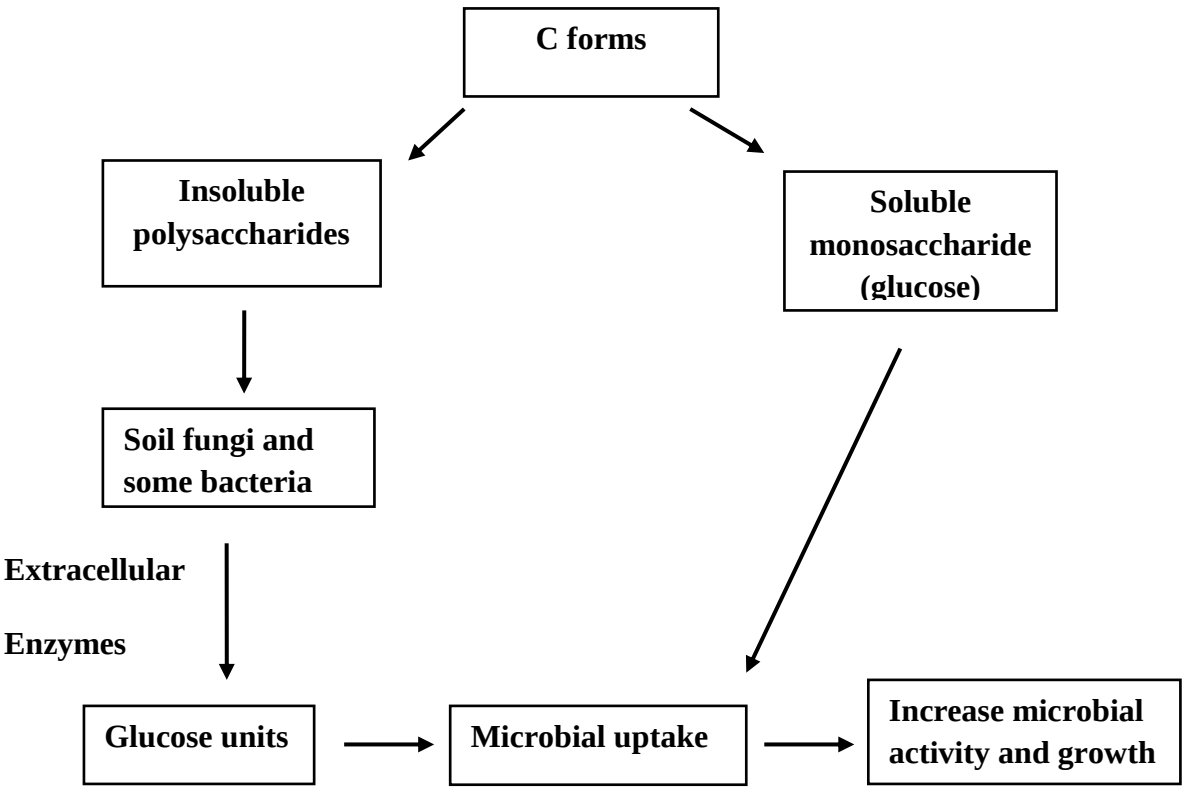

Figure 2 Decomposition of C forms by soil microbes

1.10. Importance of C/N and C/P ratios

Although plant residues provide a large amounts of OC, their C/N and C/P ratio is often higher than that of microbes. Therefore, additional N and P supply may be required for rapid decomposition and utilization of the C and microbial growth (Dilly, 1999). Plants contain mainly polysaccharides which are low in N and P whereas microbes contain predominately proteins and other N and P-rich compounds. Therefore, microbes require relatively large amounts of N and P for growth (Stevenson and Cole, 1999; Sylvia et al., 2005). Nitrogen and P are important because they are necessary for synthesising nucleic acids deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) (Marschner, 2012). Nitrogen is also essential element for synthesis proteins and amino acids. Phosphorus is important for energy production adenosine tri-phosphate (ATP); in addition, phosphate esters such as phosphoglyceraldehyde and glucose-6-phosphate are necessary for transferring energy to a wide range of metabolic processes (Marschner, 2012). Ratios of C/N of 20 and C/P of 200 are optimal for microbial growth (Sylvia et al., 2005) and C/N ratio $\leq$ 25 is considered to be optimal for decomposition of plant residue (Heal et al., 1997). Nutrient demand can be increased under saline conditions. For example, under salt stress the C and N content are increased due to accumulation of amino acids to overcome the osmotic stress (Schimel et al., 1989). Muhammad et al. (2006) reported that microbial biomass C/N and C/P ratios decrease with increasing salt concentration.

The C/N and C/P ratios are considered to be critical factors in terms of whether the nutrients are mineralized or immobilized during the decomposition of soil OM (Curtin et al., 2003; Hadas et al., 1992; Nicolardot et al., 2001; Qiu et al., 2008). Addition of plant residues with low C/N ratio leads to net mineralisation and increases N availability to plants (Hadas et al., 1992). On the other hand, amendment with plant material with a high of C/N can retard plant growth due to reduction of available N (Azam, 2002). Similarly, the C/P ratio is a very

important factor determining the ratio of P immobilisation to mineralisation during degradation of soil OM (Ha et al., 2007). Net P mineralisation can be expected after addition of residues with C/P <200 (Stevenson and Cole, 1999; Sylvia et al., 2005).

1.11. Effects of nutrient availability on microbial adaptation to salinity

Tolerance and adaptation of soil microorganisms to salinity stress requires a high amount of energy (Oren, 1999). Therefore, in saline soils the availability of C, N and P may stimulate microbial growth and activity because it allows the accumulation of osmoregulatory compounds that are required to maintain the osmotic balance between cells and surrounding soil solution (Oren 2001; Zahran 1997). It has been shown that increased substrate availability can help microbes to counteract the detrimental effects of salinity (Wichern et al., 2006). Furthermore, it has been reported that addition of OM as manure or compost can provide microbes with adequate nutrients to become more resistant to salt stress (Tejada et al., 2006). Wada and Toyota (2007) found that repeated addition of manure and chemical fertilizer can increase the resistance and resilience of soil microbial function and stability to soil disinfection. Schmitt et al. (2005) observed that soil microbial communities became more tolerant to stress caused by antibiotics in soils amended with alfalfa and fresh pig slurry. However, the effect of OC availability on microbial activity and biomass in saline soils is poorly understood. To address this knowledge gap, this thesis aims to provide a better understanding of the effect of C availability on adaptation of microorganisms to salinity (see Figure 3).

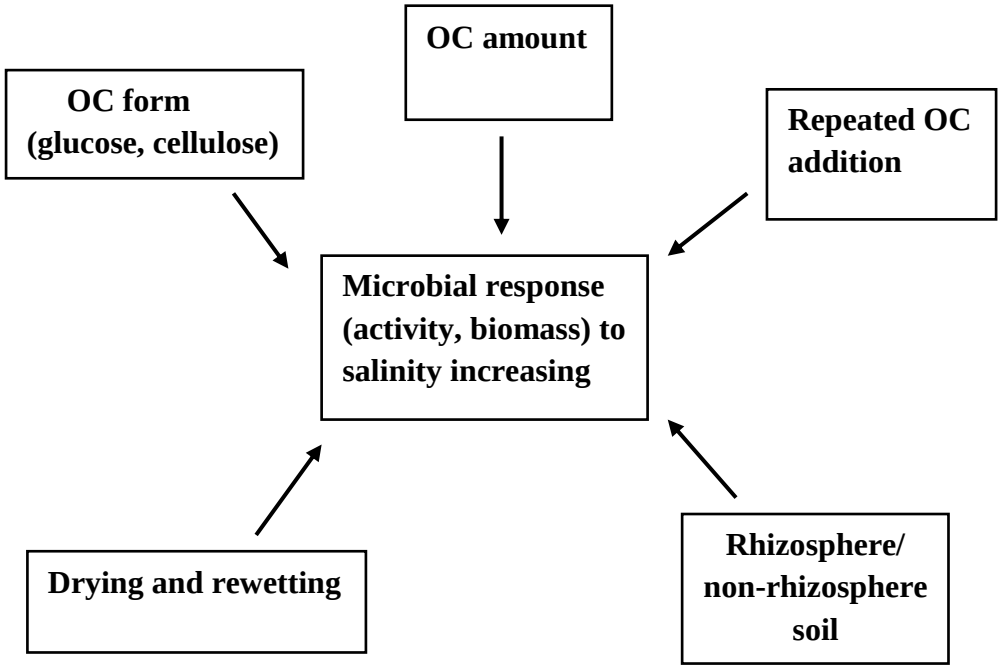

Figure 3 Effects of the carbon availability on microbes in saline soil

1.12. Aims of this study

The present study aims to:

- Assess the effects of increasing salinity on soil microbial biomass and activity at different addition rates of soluble OC (glucose) (Chapter 2).
- Determine the effects of increasing salinity on microbial biomass and activity when supplied with different forms of OC (Chapter 3).
- Investigate the impact of salinity on microbial activity and biomass when OC is supplied as different proportions of glucose and cellulose or when C form is changed over time (Chapter 4).
- Assess the response of soil microbes to increasing salinity with repeated addition of plant residues (Chapter 5).

- Investigate the effect of increasing salinity on microbial activity and biomass in rhizosphere compared to bulk soil (Chapter 6).
- Evaluate the response of soil microbial activity and biomass to drying and rewetting of non-saline and saline soils when the salt concentration is maintained or increased upon rewetting (Chapter 7).

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CHAPTER 2

ADDITION OF GLUCOSE INCREASES THE ACTIVITY OF MICROBES IN SALINE SOILS

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CHAPTER 3

SALINITY REDUCES THE ABILITY OF SOIL MICROBES TO UTILISE CELLULOSE

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CHAPTER 4

RESPONSE OF MICROBIAL ACTIVITY AND BIOMASS TO SOIL SALINITY WHEN SUPPLIED WITH GLUCOSE AND CELLULOSE

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Abstract

In a previous study, we found that the response of soil respiration and microbial biomass to salinity depended on form of organic C (glucose or cellulose) added. Soil microbes were more tolerant to medium salinity when supplied with glucose compared to cellulose. Two incubation experiments were carried out to determine the impact of salinity on microbial activity and biomass when organic carbon is supplied as different proportions of glucose and cellulose or when glucose and cellulose were added every 2 weeks in different order. The first experiment was conducted with three sandy clay loam soils: a non-saline soil and two saline soils (electrical conductivity of the saturated paste ECe of 11 and 43 dS m^{-1}) amended with 5 g C kg^{-1} as different percentages of glucose and cellulose. The percentages of glucose (G) were 100% and 0-20% and those of cellulose (Ce) were 0-100%. The second experiment was conducted using a non-saline loamy sand soil which was salinized to ECe 12.5 and 37.4 dS m^{-1} by adding NaCl. In this experiment the C form was maintained or changed over time by addition of 1.5 g C kg^{-1} every two weeks period (on days 0, 15 and 29) as glucose or cellulose (Ce+Ce+Ce, G+G+G, Ce+Ce+G, G+Ce+Ce, G+Ce+G, Ce+G+Ce). Cumulative respiration was measured continuously for 3 weeks (Experiment1) or 6 weeks (Experiment

2). Microbial biomass C (MBC) was determined on days 2, 7 and 21 (Experiment 1) and on days 14, 28 and 42 (Experiment 2). In Experiment 2, microbial community structure was determined on days 0 and 42. Experiment 1 showed that mixing glucose with cellulose increased cumulative respiration compared to cellulose alone, but the effect of the proportion of glucose differed with soil salinity. Cumulative respiration increased with increasing proportion of glucose in the combined treatments when the glucose proportion was >2.5%. With 100% glucose, cumulative respiration was significantly lower than in the non-saline soil only in EC43 whereas with 100% cellulose and all combined treatments, cumulative respiration was significantly lower than in the non-saline soil in EC11 and EC43. Cumulative respiration did not decrease further from EC11 to EC43 with 100% cellulose, but decreased significantly in the combined treatments. The MBC concentration was higher in the non-saline soil than in the saline soils but did not differ in the saline soils. In Experiment 2, the impact of salinity on cumulative respiration over two weeks following the C addition depended on C form, treatment and period. Cumulative respiration in the first period (0-14) was always higher with glucose than with cellulose; it decreased with increasing EC. Irrespective of C form added, the impact of salinity was lower when C was added repeatedly compared to a single addition showing that high C supply increases tolerance of microbes to salinity. Cumulative respiration increased when glucose was added after cellulose addition. Addition of glucose after cellulose reduced the negative impact of high salinity on cumulative respiration compared to the previous period with cellulose or when cellulose was added after glucose. Microbial community structure was influenced by salinity and C form. In conclusion, mixing small amounts of glucose with cellulose increased microbial activity and growth, but the growth induced by small amounts could make microbes more sensitive to salinity compared to 100% cellulose. The study also showed that irrespective of C form added, the negative impact of salinity on microbial activity and biomass was smaller when C

was added repeatedly compared to a single addition indicating that high C availability reduces the negative impact of salinity on soil microbes.

Keywords: Cellulose; Glucose; Microbial biomass; Respiration; Salinity

1. Introduction

Globally 831 M ha of land is affected by salt (Martinez-Beltran and Manzur, 2005) which is a threat to ecosystems particularly in arid and semi-arid regions. Salt accumulation increases the osmotic potential of the soil solution and thereby reduces water availability to plants and microbes. Further, salinity can affect physiological processes by ion toxicity and ion imbalance (Munns and Tester, 2008). As a result, saline soils are characterized by low organic matter content and reduced organic matter turnover due to poor plant growth and low microbial biomass and activity (e.g. Muhammad *et al.,* 2006; Tripathi *et al.,* 2006). In addition, salinity changes the microbial community composition because microbial genotypes differ in their ability to adapt to salt stress (Nelson and Mele, 2007; Pankhurst *et al.,* 2001). Several studies have shown that bacteria are more tolerant to salinity than fungi (Chowdhury *et al.,* 2011b; Pankhurst *et al.,* 2001), however Wichern *et al*. (2006) found that bacteria are more sensitive to salinity than fungi. In non-saline soil microbial growth is often limited by the availability of organic carbon (C) (De Nobili *et al*., 2001). The organic C demand of microbes in saline soils may be higher than that of microbes in non-saline soils because adaptation to osmotic stress requires a high amount of energy to synthesise organic osmolytes (Hagemann, 2011; Oren, 1999). Addition of organic C to saline soils could allow microbes to synthesise osmolytes and thereby increase their adaptation to salt stress. Previous studies have shown that the response of microbial activity (respiration) to salinity (high osmotic potential) depended on the amount of substrate available with microbial activity being less

sensitive to salinity when C availability is high (Elmajdoub and Marschner, 2013; Wichern *et al.,* 2006).

In a previous study (Elmajdoub and Marschner, 2013) we showed that the response of microbial activity (respiration) to salinity also varied with organic C form. Cumulative respiration decreased gradually with increasing salinity (electrical conductivity in the saturated paste extract (ECe 1, 11 and 43 dS m^{-1}) when C was supplied as glucose, whereas it decreased sharply from non-saline to saline soils when C was added as cellulose. This may be due to the fact that glucose can be utilised rapidly by most microorganisms whereas cellulose is decomposed more slowly by fewer microbes capable of releasing cellulase (Sylvia *et al.,* 2005). Thus, sensitivity of a few genotypes to salinity will have little impact on the utilisation of glucose but can strongly reduce cellulose decomposition. But little is known about the response of soil microbial activity and biomass to salinity when glucose and cellulose were either supplied as different proportions or when the C form is changed over time. To address these knowledge gaps we conducted two experiments. The aim of Experiment 1 was to determine the impact of salinity on microbial activity and biomass when C is supplied as different proportions of glucose and cellulose. Experiment 2 was designed to assess the response of soil microbial activity, biomass and community structure to increasing salinity when the C form was changed repeatedly. We hypothesised that (i) microbial activity and biomass will decrease more rapidly with salinity compared to glucose alone as the proportion of glucose decreases, thus becoming similar to that with cellulose alone (Experiment 1), (ii) compared to a single addition, the reduction of respiration by salinity will become smaller with repeated C addition due to increasing C supply irrespective of the form in which C is supplied (glucose or cellulose) (Experiment 2), and (iii) microbial activity will be little affected by medium salinity when glucose is added whereas it will be reduced with cellulose addition.

2. Materials and Methods

2.1. Experiment 1(different proportions of glucose and cellulose)

Three sandy clay loam soils (one non-saline and two saline) were collected at Monarto (35°05´S and 139°06´ E), South Australia, which is characterised by Mediterranean climate. The soils were air-dried and sieved to < 2 mm (Table 1). Electrical conductivity, sodium absorption ratio (SAR) and pH were determined in a 1:5 soil: water ratio after 1 h end-over-end shaking at 25ºC (Rayment and Higginson, 1992). The $EC_{1:5}$ of the soils was 0.1, 1.1 and 4.2 dS m^{-1} which corresponds to an EC of a saturated paste extract (ECe) of 1, 11, and 43 dS m^{-1} and hereafter referred to as EC1, EC11 and EC43. The $EC_{1:5}$ was converted to ECe using the equation: ECe = (14.0-0.13 × clay %) × $EC_{1:5}$ (Rengasamy, 2006). The soils were saline-sodic, but did not show the dispersive impact of sodicity due to the high salt concentration in the soil solution which causes the soil particles to flocculate (Shainberg and Letey, 1984).

Reverse osmosis (RO) water was added to adjust the water content to 30% of maximum water holding capacity (WHC) after which the soils were pre-incubated at 25ºC for 10 days before the start of the experiment to reactivate and stabilize microbial activity after rewetting of the air-dried soils (Butterly *et al.,* 2010).

To test the range of proportions of glucose and cellulose to be used in the main experiment, a preliminary experiment was conducted using a rate of 5 g C kg^{-1} as five different proportions of glucose (G) and cellulose (Ce): 25%G+75%Ce, 50%G+50%Ce, 75%G+25%Ce, 100%G, 100%Ce and a control without C addition. Soil respiration was measured over 21 days. There were only small differences in cumulative respiration among the different proportions of glucose which all showed higher values than the 100% cellulose treatment. This suggested that even at the lowest glucose proportion (25% of the C added), sufficient C was present as

glucose to satisfy the requirement of the microbes during the experiment. Calculations based on soil respiration indicated that little, if any, cellulose C had been decomposed.

Therefore, in the main experiment (with 5 g C kg^{-1}), smaller proportions of glucose (2.5 to 20%) were used. The treatments were 100%Ce, 2.5%G+97.5%Ce, 5%G+95%Ce, 10%G+90%Ce, 20%G+80%Ce and 100%G. An unamended control was also included. Nitrogen and phosphorus were added as $(NH_4)_2SO_4$ and KH_2PO_4 to achieve a C/N ratio of 20 and a C/P ratio of 200, which are considered to be sufficient for growth of the majority of heterotrophic microbes (Sylvia *et al*., 2005). Glucose, $(NH_4)_2SO_4$ and KH_2PO_4 were added as solutions, cellulose as powder (Sigma cell cellulose, type 20). The unamended soils received reverse osmosis water (RO) water only. The final water content was 50% of WHC which is optimal for microbial activity in soils of this texture (Setia *et al.,* 2011). The nutrient addition did not significantly change the EC (data not shown). After amendment, the soils were mixed immediately to ensure homogeneity of wetting and nutrient distribution. Then 30 g of soil was added to PVC cores with radius 1.85 cm and height 5 cm and a nylon mesh base (0.75µm, Australian Filter Specialist) and packed to a bulk density of 1.4 g cm^{-1}.

The cores were placed individually into 1L glass jars with gas tight lids equipped with septa to allow quantification of the headspace CO_2 concentration. The jars were incubated in the dark at 25ºC for three weeks during which CO_2 emission was measured continuously. Separate cores were destructively sampled on days 2, 7 and 21 to measure microbial biomass C (MBC).

2.2. Experiment 2 (change of C form over time)

In this experiment, a non-saline soil was adjusted to different EC levels by adding different amounts of salt to avoid the differences in organic matter and nutrient concentrations among the soils in Experiment 1.

A non-saline loamy sand was collected from 0-20 cm depth at Monarto, South Australia (sand 83%, clay 12%, silt 5%, $EC_{1:5}$ 0.05 dS m^{-1}, pH 7.5, total organic C 6.2 g kg^{-1}, total N 0.1 g kg^{-1}, bulk density 1.57 g cm^{-3}, water holding capacity (WHC) 170 g kg^{-1}). The soil was air-dried and sieved to < 2 mm.

The soil was adjusted to different salinity levels by adding NaCl (0, 3.05 and 9.25 g kg^{-1}) to achieve $EC_{1:5}$ 0.05, 1 and 3 dS m^{-1} respectively. This corresponds to EC_e 0.6**,** 12.5 and 37.4 dS m^{-1} hereafter referred to as EC0.6, EC12.5 and EC37.4. These EC levels were chosen based on previous experiments to achieve moderate (12**.**5 dS m^{-1}**)** and strong **(**37**.**4 dS m^{-1}**)** reduction of respiration. Nitrogen and P were added as $(NH_4)_2SO_4$ and KH_2PO_4 to achieve a C/N ratio of 20 and a C/P ratio of 200 based on the total amount of C added. NaCl, $(NH_4)_2SO_4$ and KH_2PO_4 were dissolved in RO water and mixed into the soil to achieve a water content of 65% of WHC. As in Experiment 1, the nutrient additions did not significantly influence the EC.

The moist soils were pre-incubated at 25ºC for 18 days before the start of the experiment to revive and stabilise microbial activity. The longer pre-incubation was chosen to allow the microbes to adjust to the different EC levels. A preliminary experiment was conducted to investigate the effect of water content on cumulative respiration in the non-saline soil. The WHC was adjusted between 20% and 80% with RO water in soil amended with 1.5 g C kg^{-1} as glucose and respiration was measured over 10 days. Cumulative respiration was maximal at 40-75% of WHC with no significant differences among these water contents (data not shown). Therefore in this experiment the water content was increased by 5% every two weeks from 65% to 75% of WHC to allow addition of soluble glucose every two weeks. In the unamended control (no C addition) and treatments where cellulose powder was added, the equivalent amount of RO water was added. After the 18-day pre-incubation the soils were amended with 1.5 g C kg^{-1} as glucose or cellulose. Carbon was added again on days 15 and

29, so that the total C addition was 4.5 g C kg^{-1}. There were six C treatments in which the C form was maintained or changed every 2 weeks: Ce+Ce+Ce, G+G+G, Ce+Ce+G, G+Ce+Ce, G+Ce+G, Ce+G+Ce. Glucose was added as solution and cellulose as powder. Unamended soil received only RO water.

On day 0, the soils were mixed with glucose or cellulose and then 30 g of soil was added to PVC cores and adjusted to a bulk density of 1.57 g cm^{-3}. The cores were placed individually in 1L glass jars and incubated in dark at 25ºC for 42 days. Respiration was measured over 42 days. Every two weeks, C was added, mixed thoroughly with the soil and the bulk density re-adjusted. The unamended control received the same amount of water and was mixed and adjusted similarly. During the 14-day incubation between C additions, the soil water content was checked by weight and RO water was added if necessary. Destructive samples for microbial biomass C (MBC) were carried out on days 0, 14, 28 and 42. Microbial community structure was determined on days 0 and 42. The soil samples for community structure were stored at -20ºC before PLFA extraction.

2.3. Measurements

Soil total organic C, N and P were measured by standard methods. Soil respiration was determined by measuring the CO_2 concentration in the headspace of each jar using a Servomex 1450 infra-red gas analyser (Servomex, UK) as described in Setia *et al.* (2011). After each measurement (t1), the jars were vented to refresh the headspace using a fan, and then resealed followed by determination of the CO_2 concentration (t0). The CO_2 evolved during a given interval was calculated as the difference in CO_2 concentration between t1 and t0. Linear regression based on injection of known amounts of CO_2 in the jars was used to define the relationship between CO_2 concentration and detector reading. The respiration rates [in mg CO_2-C (g soil and day)$^{-1}$] were added to calculate cumulative respiration over 21 days

in Experiment 1. In Experiment 2, cumulative respiration was calculated for each 14-day period after C addition separately (days 0-14, 15-28 and 29-42).

Microbial biomass C (MBC) was determined after destructive sampling by fumigation extraction (Vance *et al.,* 1987) as described by Anderson and Ingram (1993) using two subsamples of 5 g. One subsample was fumigated with ethanol-free chloroform for 24 h at 25ºC in sealed desiccators; the non-fumigated subsample was kept at 4ºC during fumigation. After removal of the chloroform, fumigated and non-fumigated subsamples were extracted with 0.5 M K_2SO_4 (1:4 soil to solution ratio). Dissolved organic C in the extracts was determined by titration with 0.033 M acidified $(NH_4)_2Fe\ (SO_4)_2 \cdot 6H_2O$ after dichromate oxidation (Anderson and Ingram 1993). Microbial biomass C was calculated from the difference between the fumigated and non-fumigated samples multiplied by 2.64 (Vance *et al*., 1987).

Microbial community structure was determined by phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from 4 g frozen soil based on Frostegård *et al.*(1993) using a solvent of chloroform, methanol and citrate 1:2:0.8 (v/v/v). The lipid phase was collected and dried under a stream of N_2 at 37ºC. After dissolving the dry sample in 1 ml chloroform, the solution was transferred to silicic acid columns. The columns were washed sequentially with chloroform, acetone and methanol; the methanol fraction which contains the PLFAs was collected. After alkaline methanolysis, the organic phase was collected in dichloromethane, and hexane methylnonadecanoate (C19:0) was added as internal standard to each sample. The PLFAs were separated and analysed in a GC-FID (HP 6890); for more information see Chowdhury *et al.*(2011a). The following signature PLFAs were used as indicators for specific microbial groups: bacteria (14:0, 15:0, 16:0, 17:0, a17:0), Gram-positive bacteria (10 me16:0, i15:0, a15:0, i16:0, i17:0), Gram-negative bacteria (cy17:0, cy19:0, 18:1ω7c, 18:1ω7t) and fungi (18:3ω3c, 18:2ω6, 18:3ω6c) (Pankhurst *al.,* 2001; Zak *et al.,* 2000). The sum of

signature fatty acids for a certain microbial group was used to calculate biomass of that group, the fungi to bacteria (F/B) ratio was calculated by dividing fungal biomass by bacterial biomass.

4.2. Statistical analysis

There were 3 replicates per treatment in both experiments. In Experiment 1, the data of cumulative respiration at the end of the experiment was analysed by two-way ANOVA (analysis of variance) with C treatment (glucose and cellulose mixtures) and EC as fixed factors. Microbial biomass C was assessed by three way ANOVA (C treatment x EC x day). In Experiment 2, the data of cumulative respiration and microbial biomass C were assessed by three-way ANOVA (treatment x time x EC). Bacterial and fungal biomass and the F/B ratio were analysed by two-way ANOVA at given time (day 0 and 42) (treatment x EC). Tukey test was used to determine significant differences (GenStat ® for Windows 14.0, VSN Int.Ltd, UK, 2010). Microbial community composition was analysed by Primer-E software (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK). The PLFA data was transformed using log (x+1) and plotted using non- metric multi- dimensional scaling (MDS) plot. Significant differences in microbial community composition among the treatments were determined by Permanova ($P \leq 0.1$).

3. Results

3.1 Experiment 1 (different proportions of glucose and cellulose)

Cumulative respiration

Addition of 5 g C kg^{-1} significantly increased cumulative respiration compared to the unamended control (Fig. 1). In the amended soils, cumulative respiration was highest with 100% glucose and lowest with 100% cellulose. Mixing glucose with cellulose increased

cumulative respiration compared to 100% cellulose, but the effect of the proportion of glucose varied with soil salinity. In the non-saline soil, cumulative respiration in the treatments with mixes of glucose and cellulose was about 25% higher than with 100% cellulose irrespective of the proportion of glucose. At EC11, cumulative respiration in the combined treatments was about 25% higher than with 100% cellulose when 20% of the C was added as glucose whereas with lower proportions of glucose, respiration did not differ significantly from that of 100% cellulose. At EC43, cumulative respiration increased with increasing proportion of glucose in the mixed treatments when the glucose proportion was > 2.5%. With 100% glucose, cumulative respiration was significantly lower than in the non-saline soil only at EC43. With 100% cellulose and all combined treatments, cumulative respiration was significantly lower than in the non-saline soil at EC11 and EC43. Cumulative respiration did not differ between EC11 and EC43 with 100% cellulose but decreased significantly in the mixed treatments except with 10% glucose. Based on the assumption that only the added C was respired and there was no priming effect (Kuzyakov *et al.,* 2000), it can be calculated that with 100% glucose, all added C was respired by the end of the experiment at all salinity levels. When glucose was combined with cellulose, the proportion of added C respired was 80% in the non-saline soil and 50% in the saline soils. With cellulose alone, 60% of the added C was respired in the non-saline soil, and 35% in the saline soils.

Microbial biomass C

On day 2, the MBC concentration was highest with 100% glucose and similar in the unamended control and soil amended with 100% cellulose or the combined treatment with 2.5% glucose (Fig. 2). Between 5 and 20% glucose, the MBC concentration increased with the proportion of glucose. In the treatments with 100% or 20% glucose, the MBC concentration was lower in the saline soils than in the non-saline soil but salinity had no

effect on the MBC concentration in the other C amended soils or the unamended control. The MBC concentration decreased from day 2 to day 7 in the 100% glucose treatment but increased during this time in all mixed treatments and with 100% cellulose, particularly in the non-saline soil. In the saline soils the MBC concentration in the mixed treatments and with 100% cellulose remained unchanged from day 7 to day 21. With 100% glucose the MBC concentration decreased from day 2 to day 7 and then remained unchanged. Salinity had little effect on the MBC concentration on day 21 except for a lower MBC concentration in the saline soils with 10 or 20% glucose.

3.2. Experiment 2 (change of C form over time)

Cumulative respiration

Cumulative respiration was lower in the unamended than the amended soils (Fig. 3). In the amended soils where the C form changed over time, cumulative respiration over 14 days after C addition was always lower with cellulose than glucose. The effect of salinity on cumulative respiration over 14 days depended on C form, treatment and period (days 0-14, 15-28 or 29-42). Cumulative respiration from day 0 to 14 decreased with increasing EC in all amended treatments. When C was added as cellulose every 2 weeks (treatment Ce+Ce+Ce), cumulative respiration significantly increased over time at all EC levels, but the increase from the period day 0-14 to the period day 29-42 was greatest in the most saline soil: 2-fold in the non-saline soil (EC0.6), 5-fold at EC12.5 and 24-fold at EC37.4 (Fig. 3B). In this treatment, the effect of salinity on cumulative respiration changed over time. Cumulative respiration in the first period (day 0-14) decreased significantly with increasing EC, in the second period (day 15-28) it decreased significantly only from EC12.5 to EC37.4, but was not influenced by EC in the third period (day 29-42). In the treatment where glucose was added every 2 weeks (G+G+G) (Fig. 3C), cumulative respiration was greatest in the first period (day 0-14) in the

non-saline soil whereas it greatest in the third period (day 29-42) in the soil with EC37.4. Increasing salinity significantly reduced cumulative respiration in the first period (day 0-14) but had no significant effect in the later periods. In the treatment where cellulose was added at the start of the first and second period followed by glucose in the start of the third period (Ce+Ce+G) (Fig. 3D), cumulative respiration over 14 days significantly increased with each period. Compared to the first period, cumulative respiration in the third period was increased 2-fold at EC0.6, 13-fold at EC12.5 and 29-fold at EC37.4. Cumulative respiration decreased with increasing EC in the first period, but was reduced only at the highest EC in the second period. Salinity had no effect on cumulative respiration in the third period. When glucose was supplied in the first period and cellulose in the second and third periods (G+Ce+Ce) (Fig. 3E), cumulative respiration at EC0.6 and EC12.5 was similar in the first (day 0-14) and third period (day 29-42), but lower in the second period (day 15-28). At EC37.4, cumulative respiration was lower in the second and third period than in the first period. Compared to the non-saline soil, cumulative respiration in the first and third period was significantly reduced only at EC37.4 whereas in the second period, it was not significantly influenced by salinity. In the treatment where glucose was added at the start of the first and third period and cellulose in the second period (G+Ce+G) (Fig. 3F), cumulative respiration was highest in the third period (day 29-42) and lowest in the second period (day 15-28). In the first period compared to EC0.6, cumulative respiration was significantly lower only at EC37.4. But salinity had no significant effect on cumulative respiration in the second and third period. In the treatment where cellulose was added at the start of the first (day 0-14) and the third period (day 29-42) and glucose was added in the second period (Ce+G+Ce) (Fig. 3G), cumulative respiration was highest in the second period and lowest in the first period. Compared to the non-saline soil, cumulative respiration decreased with increasing EC in the first period. But in

the second period (day15-28) salinity had no effect on cumulative respiration and in the third period it was significantly reduced only at EC37.4.

The amount of C remaining at the end of each period was calculated by subtracting the C respired during the period from the C added at the start of that period (Table 2) assuming no measurable priming effect. At the end of experiment, all added C was respired in the treatment where only glucose was added (G+G+G) at all salinity levels. This was also the case at EC0.6 and 12.5 in the treatments where glucose was added twice (G+Ce+G), but some of the added C remained at EC37.4. In the treatments where C was added as cellulose twice or three times, the amount of C remaining at the end of the experiment increased with increasing EC. In the periods when cellulose was added, the amount of C remaining was greatest when it was added in the first period at all salinity levels. Negative values indicate that more C was respired than added in a given period.

Microbial biomass C

Addition of C increased the MBC concentration at the end of each period (days 14, 28 and 42) compared to the unamended control at all EC levels (Fig. 4). In most C treatments, the MBC concentration on days 14, 28 and 42 was lowest at EC37.4. The exceptions were the treatments where glucose was added once at the start of the first or the third period (Ce+Ce+G; G+Ce+Ce). In these treatments, the MBC concentration on day 42 did not differ between EC0.6 and EC37.4. When cellulose was added in each period (Ce+Ce+Ce), the MBC concentration was highest on day 42 in EC0.6, lowest on day 28 in EC37.4 but did not change over time in EC12.5 (Fig. 4B). At all three sampling dates, the MBC concentration was lowest in EC37.4. When glucose was added in each period (G+G+G) or once at the start of the third period (Ce+Ce+G) (Fig. 4C, 4D), the MBC concentration at all sampling dates was highest with EC12.5 and lowest with EC37.4. This was also true for days 14 and 28 in

the treatment where glucose was added at the start of the first period (G+Ce+Ce) (Fig. 4E). In the treatments where the C form was changed twice (G+Ce+G and Ce+G+Ce) (Fig. 4F, 4G), the MBC concentration was lowest with EC37.4 at all sampling dates. In the treatment G+Ce+G (Fig. 4F), the MBC concentration did not change significantly over time, but in the treatment Ce+G+Ce, the MBC concentration was lowest on day 28 (2 weeks after addition of glucose) in EC0.6 and EC12.5.

On day 42, bacterial and fungal biomass did not differ significantly among EC levels (Table 3), except for a higher bacterial and fungal biomass in the treatment Ce+G+Ce compared to the unamended soil at EC12.5 and EC37.4. The F/B ratio did not differ significantly among EC levels.

Microbial community structure

On day 0 microbial community composition based on PLFA did not differ among EC levels (data not shown). Microbial community structure at the end of the experiment (day 42), was plotted using multi-dimensional scaling (Fig. 5). Permanova showed that microbial community structure differed significantly between EC0.6 and EC37.4 in all C treatments except in G+Ce+G (Table 4). Microbial community structure differed significantly between EC0.6 and EC12.5 only in two C treatments (Ce+Ce+G and Ce+G+Ce). Microbial community structure differed between EC12.5 and EC37.4 in four of the seven treatments but not in treatments where cellulose was added twice or three times (Ce+Ce+Ce, Ce+Ce+G, G+Ce+Ce).

4. Discussion

The results of the two experiments confirmed our earlier study (Elmajdoub and Marschner 2013) that the response of soil microbes to salinity depends on the form in which C is

supplied. However the experiments presented here also show that this response is modulated by the proportion of glucose and cellulose and may change if C is added repeatedly in different C forms.

Addition of glucose induced higher cumulative respiration than addition of cellulose in both experiments. This can be explained by the high availability of glucose compared to cellulose and the fact that glucose can be rapidly utilised by most soil microbes whereas cellulose utilisation requires the synthesis and release of cellulase which can be carried out by fewer microbes and is more energy-demanding than glucose uptake (de Boer *et al.* 2005; Killham 1994). The differences in microbial community structure based on PLFAs between the treatments with only cellulose compared to only glucose addition show that continuous supply of a single C sources changes community structure. However, the lower activity and different community structure with cellulose supply does not seem to limit the ability to utilise glucose when it was added to soils that received cellulose in the previous period in Experiment 2 because cumulative respiration was high at the end of each glucose period where glucose was added even when respiration was low in the previous period with cellulose supply.

The high C availability after glucose addition is also evident in the high MBC concentration on day 2 in the first experiment. However, the rapid decline in MBC concentration from day 2 to day 7 shows that a large proportion of the biomass dies when this easily available C is depleted. Our calculations of the amount of C remaining 2 weeks after C addition in Experiment 2 (Table 2) confirm that all C added as glucose is respired in soils with low or medium salinity. Utilisation of the added C is slower with cellulose, which is evident in the greater amount of added C remaining after 2 weeks in Experiment 2 and the slower build-up of microbial biomass in Experiment 1 where the MBC concentration increased from day 2 to day 7 and then remained stable.

The finding in Experiment 1 that cumulative respiration did not decrease from EC11 to EC43 in the treatment with 100% cellulose but decreased significantly in the glucose-cellulose mixes except with 10% glucose suggests that small amounts of readily available C may make microbes more susceptible to high EC compared to microbes supplied with cellulose only. Mixing glucose and cellulose increased microbial growth particularly from day 2 to day 7 compared to 100% cellulose. Fast growing microbes have been shown to be more sensitive to stress than slow-growing ones (Schimel *et al.,* 2007; Van Gestel *et al.,* 1993). Hence, our first hypothesis (microbial activity and biomass will decrease more rapidly with salinity compared to glucose alone as the proportion of glucose decreases, thus the response to salinity will be similar to that with cellulose alone) has to be declined because the relationship between glucose percentage and adaptation to salinity is more complex.

In agreement with our previous study (Elmajdoub and Marschner, 2013), cumulative respiration with a single addition of only glucose was reduced compared to the non-saline soil at the highest EC but not at the medium EC. With a single addition of cellulose on the other hand, cumulative respiration was already reduced significantly at the medium EC and did not decrease further at the highest EC. This suggests that microbes supplied with an easily available C source (glucose) can maintain a higher activity at medium EC compared to non-saline soils than those supplied with a poorly decomposable C source (cellulose). Synthesis of osmolytes which is an important mechanism to counteract the high osmotic stress in saline soils is very energy-demanding (Oren, 1999). The fast decomposition of glucose is likely to generate the energy for rapid osmolytes synthesis to withstand the medium EC. However, the results of the second experiment show that the effect of C source can change with repeated C addition. The stronger reduction of cumulative respiration compared to non-saline soil at medium EC with cellulose supply than with glucose addition was only found in the first period. When glucose was added after cellulose at the start of the second or third period,

cumulative respiration was not influenced by EC. On the other hand cumulative respiration was lower at the highest EC compared to the non-saline soil when cellulose had been added in the period before or when cellulose was added after glucose. Thus, addition of an easily available C source after cellulose appears to increase microbial tolerance to high salinity. This seems to be in contrast to the first experiment where mixing a small proportion of glucose to cellulose increased sensitivity to salinity compared to 100% cellulose. This apparent contradiction may be explained by the response of the microbial community at the time at which glucose is available. As mentioned above, a small proportion of glucose present at the start is likely to have increased growth of fast-growing microbes which became more susceptible to salinity when C availability was low compared to the more steady but low C supply from cellulose. In the second experiment, the addition of glucose after cellulose may also have induced growth of fast-growing microbes but will also enhance the growth of the more slowly growing cellulose decomposers which were dominating the active community. Further, C depletion after glucose addition is unlikely to have occurred because there was a residual amount of C from the previous cellulose addition.

The importance of high C supply for salinity tolerance was shown in the second experiment in the treatments where only glucose or cellulose was added three times. After three additions of 1.5 g C as glucose or cellulose, cumulative respiration was similar at all EC levels whereas it decreased with increasing EC after the first addition. This increased tolerance after C had been added three times occurred in most amended treatments in Experiment 2 except for those with cellulose added at the start of the third period which confirmed only the first part of our second hypothesis (compared to a single addition, the reduction of respiration by salinity will become smaller with repeated C addition due to increasing C supply) but not the second part (irrespective of the form in which C is supplied glucose or cellulose). Cumulative respiration always increased when the C form was changed from cellulose to glucose and

salinity had no or only a small effect on respiration. This may indicate priming (Kuzyakov *et al.,* 2000), that is glucose enhanced utilisation of cellulose C. However, the switch from glucose to cellulose reduced cumulative respiration compared to glucose supply. Therefore, our third hypothesis that microbial activity will be little affected by medium salinity when glucose is added whereas it will be reduced with cellulose addition is true only for the first addition. In later periods, the response of activity to salinity was not only influenced by the C form added at the start of a period, but also by the C form in the previous period.

The finding that salinity influenced cumulative respiration more strongly than microbial biomass C is in agreement with our previous studies (Elmajdoub and Marschner, 2013; Yan and Marschner, 2012) and can be explained by the fact that cumulative respiration integrates microbial response over a given period whereas MBC concentration is just a snap shot of the biomass at the time of sampling.

Microbial community structure based on PLFA data differed significantly between the non-saline soil and EC37.4 in most C treatments which is agreement with previous studies that also report that salinity alters microbial community structure (Chowdhury *et al.,* 2011a; Pankhurst *et al.,* 2001). This experiment further showed that at a given EC, microbial community structure is also influenced by the form in which C added in the three periods.

5. Conclusion

Mixing small amounts of readily available C (glucose) with poorly available C (cellulose) increased microbial activity and growth. However, the increased growth induced by small amounts of glucose also appears to make microbes more susceptible to negative impact of salinity compared to 100% cellulose. The second experiment showed that irrespective of form of C added, salinity had a smaller negative impact of on respiration when C was added repeatedly compared to a single addition showing that high C supply increases tolerance of

microbes to salinity. The finding that addition of an easily available C source after cellulose supply increased microbial tolerance to high EC deserves further attention because a similar situation could occur when roots grow into patches of largely decomposed residues (only less decomposable compounds left) because root exudates contain easily available C forms.

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Table 1. Physical and chemical properties of the soils used in Experiment 1.

Soil $EC_{1:5}$ dS m^{-1}	pH	SAR	EC_e dS m^{-1}	% sand	% clay	% silt	WHC g g^{-1}	Bulk density g cm^{-3}	TOC g kg^{-1}	Total P g kg^{-1}	Total N g kg^{-1}
	1:5										
0.1	8.4	0.1	1	57.0	25.5	17.5	0.36	1.40	11.8	0.68	0.28
1.1	8.9	12.6	11	60.0	27.5	12.5	0.38	1.40	5.8	0.36	0.20
4.2	8.8	20.4	43	58.5	29.5	12.0	0.40	1.39	7.3	0.37	0.21

Table 2. Amount of remaining C at the end of a given period of C added at the start of the period (days 0, 15, 29) in soils with ECe 0.6, 12.5 and 37.4 dS m^{-1} amended with cellulose (Ce) or glucose (G). Negative values indicate that more C was respired than added in a given period. The sum of remaining C was calculated by adding the amounts remaining in the three periods.

Treatment	EC	Remaining C (g kg^{-1})			
		Period (day)			Sum of remaining C (g kg^{-1})
		0-14	15-28	29-42	
Ce+Ce+Ce	0.6	0.63	0.054	-0.58	0.10
	12.5	1.23	0.29	- 0.07	1.45
	37.4	1.43	1.08	- 0.24	2.27
G+G+G	0.6	0	0	0	0
	12.5	0	0	0	0
	37.4	0.44	-0.21	- 0.23	0
Ce+Ce+G	0.6	0.56	0.14	- 1.02	0
	12.5	1.31	0.18	- 1.08	0.41
	37.4	1.43	1.22	- 0.49	2.17
G+Ce+Ce	0.6	0	0.43	- 0.35	0.08
	12.5	0	0.90	0.26	1.16
	37.4	0.44	1.03	1.03	2.51
G+Ce+G	0.6	0	0.61	- 0.61	0
	12.5	0	0.77	- 0.77	0
	37.4	0.44	1.04	-0.41	1.07
Ce+G+Ce	0.6	0.58	-0.64	0.11	0.06
	12.5	1.23	- 0.41	0.32	1.96
	37.4	1.41	- 0.12	0.94	2.23

Table 3. Biomass of bacteria and fungi and fungi/ bacteria ratios based on PLFAs at the end of the experiment (day 42) in soils with ECe 0.6, 12.5 and 37.4 dS m^{-1} amended with cellulose (Ce) or glucose (G) (n=3), different letters indicate significant differences ($p \leq 0.05$).

Treatments	Bacterial biomass						Fungal biomass						F/ B ratio					
	µg g^{-1}																	
	EC0.6		EC12.5		EC37.4		EC0.6		EC12.5		EC37.4		EC0.6		EC12.5		EC37.4	
unamended	9.89	ab	5.64	a	12.22	abc	5.18	ab	4.51	a	7.2	abc	0. 56	abc	0.81	bc	0.59	abc
Ce+Ce+Ce	32.02	bcd	27.82	abcd	26.65	abcd	19.68	d	18.33	cd	16.48	abcd	0.62	abc	0.70	abc	0.63	abc
G+G+G	15.86	abcd	27.97	abcd	34.81	cd	13.08	abcd	18.21	cd	20.54	d	0.86	c	0.67	abc	0.59	abc
Ce+Ce+G	17.38	abcd	31.24	bcd	22.82	abcd	10.79	abcd	22.95	d	11.75	abcd	0.63	abc	0.74	abc	0.51	abc
G+Ce+Ce	28.28	abcd	23.92	abcd	23.45	abcd	18.06	cd	17.63	bcd	11.04	abcd	0.64	abc	0.79	bc	0.48	ab
G+Ce+G	23.28	abcd	17.97	abcd	34.47	cd	15.66	abcd	13.85	abcd	20.13	d	0.69	abc	0.81	bc	0.59	abc
Ce+G+Ce	29.53	bcd	33	cd	35.44	d	16.71	abcd	22.01	d	14.39	abcd	0.57	abc	0.67	abc	0.41	a

Table 4. Results of Permanova for microbial community structure on day 42 based on PLFA for pair-wise comparison between EC levels in different C treatments.

Pair wise comparison	Treatments						
EC	unamended	Ce+Ce+Ce	G+G+G	Ce+Ce+G	G+Ce+Ce	G+Ce+G	Ce+G+Ce
0.6 and 12.5	ns	ns	ns	*	ns	ns	*
0.6 and 37.4	*	*	*	*	*	ns	*
12.5 and 37.4	*	ns	*	ns	ns	*	*

Asterisks indicates significant differences **(**$P \leq 0.1$) between community structure of a given EC pair, (ns) not significant

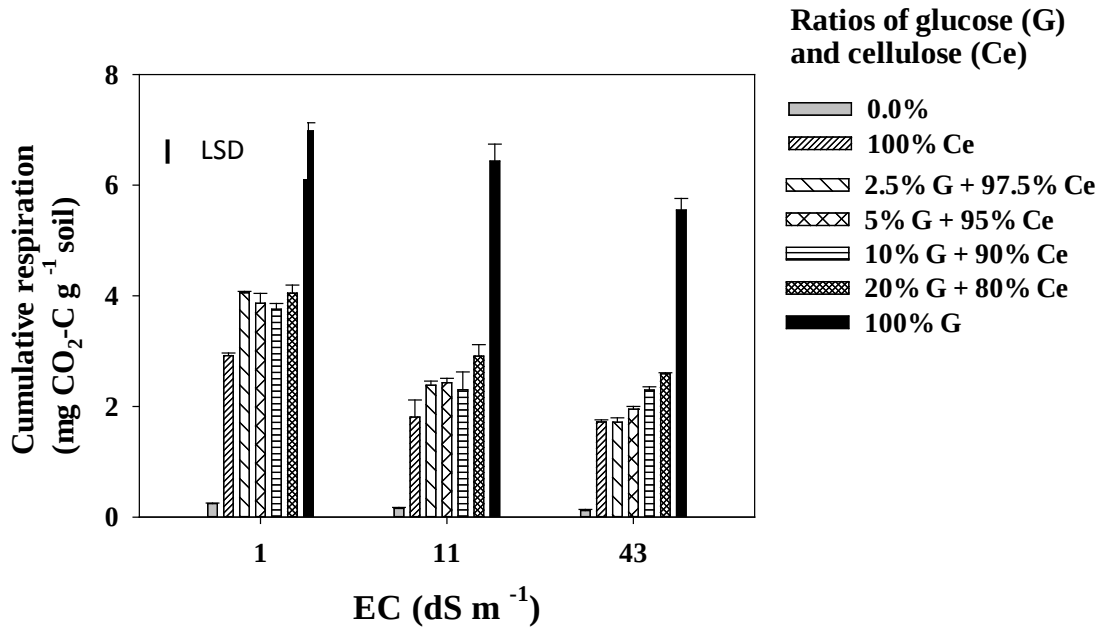

Figure 1. Cumulative respiration after 21 days in soils with EC1, 11 and 43 dS m^{-1} amended with 5 g C kg^{-1} at different ratios of glucose (G) and cellulose (Ce): 0, 100% Ce, 2.5%G+97.5%Ce, 5%G+95%Ce, 10%G+90%Ce, 20%G+80%Ce, 100%G, 100%Ce (n=3, vertical lines indicate standard error). Thick vertical line shows LSD based on the C treatment x salinity interaction.

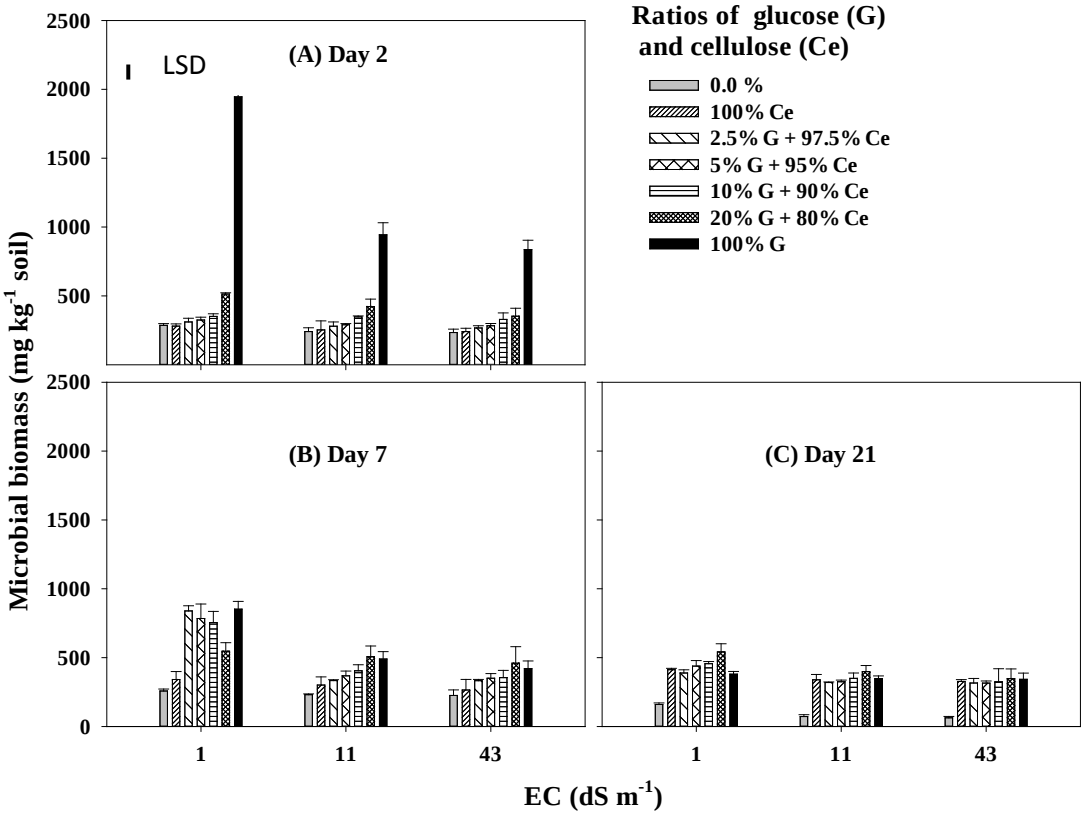

Figure 2. Microbial biomass C concentration on days 2 (A) 7 (B) and 21 (C) in soils with ECe1, 11 and 43 dS m^{-1} amended with 5 g kg^{-1} at different ratios of glucose (G) and cellulose (Ce): 0, 100% Ce, 2.5%G+97.5%Ce, 5%G+95%Ce, 10%G+90%Ce, 20%G+80%Ce, 100% G, 100% Ce (n=3, vertical lines indicate standard error). Thick vertical line in panel (a) shows LSD based on the C treatment x salinity x sampling date interaction.

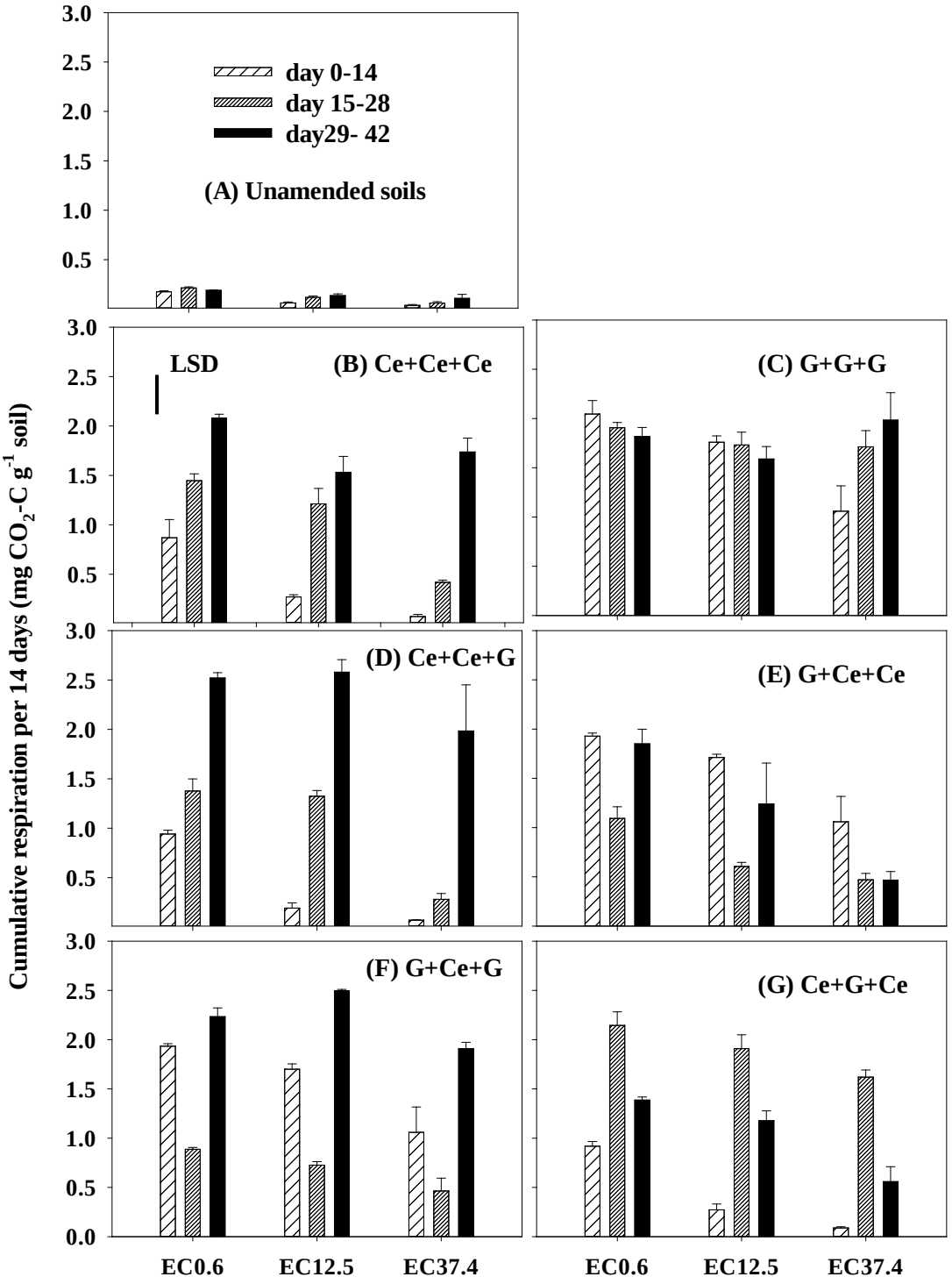

Figure 3. Cumulative respiration in the 2-week periods day 0-14, day15-28 and day 29-42 in soils with ECe 0.6, 12.5 and 37.4 dS m^{-1} in the unamended control or amended at the start of each period with cellulose (Ce) or glucose (G) bars represent standard error (n=3). Thick vertical line in panel (B) shows LSD based on the C treatment x salinity x period interaction.

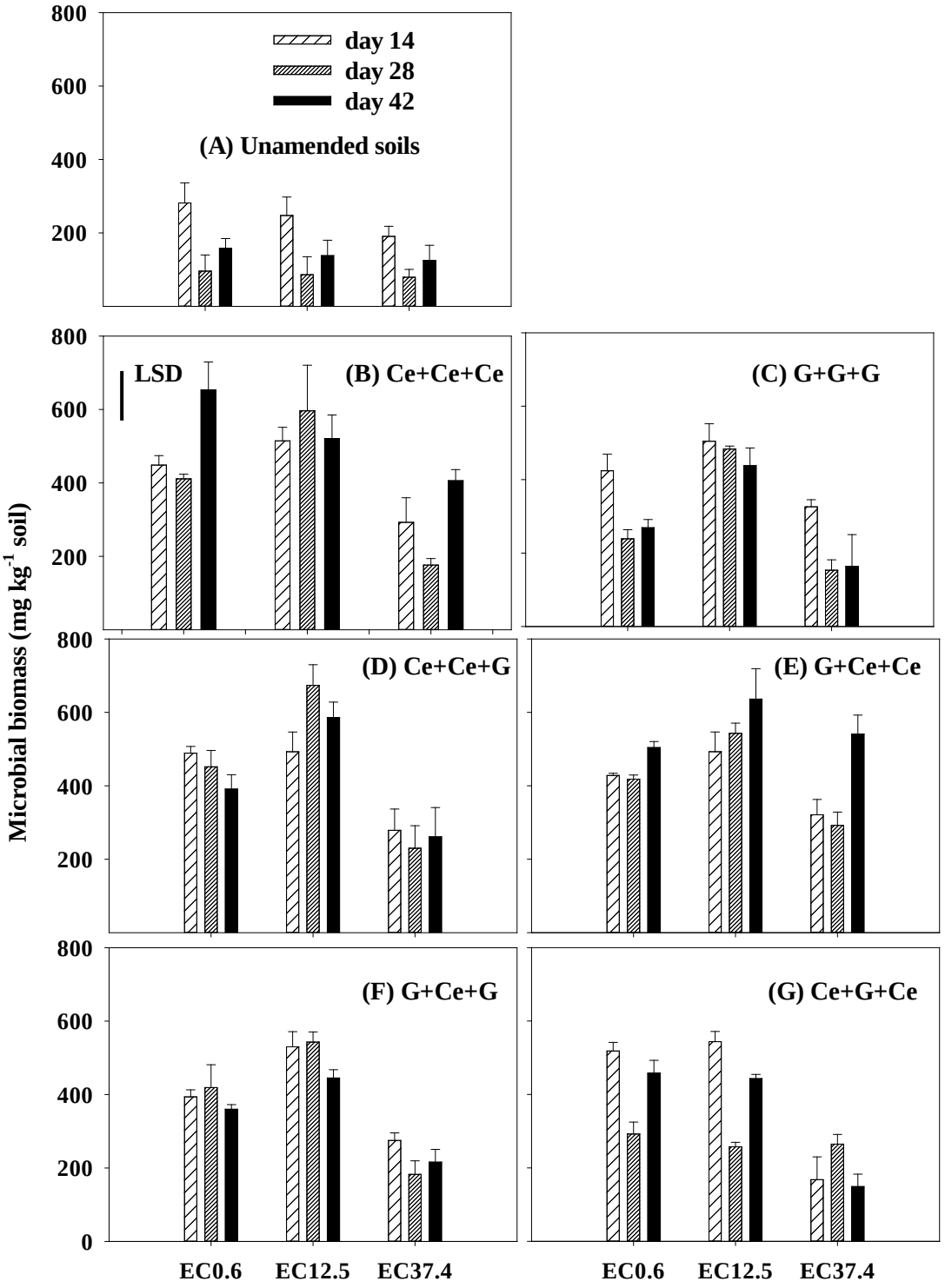

Figure 4. Microbial biomass C on days 14, 28 and 42 in soils with different ECe 0.6, 12.5 and 37.4 dS m^{-1} in the unamended control or amended at the start of each period with cellulose (Ce) or glucose (G) bars represent standard error (n=3). Thick vertical line in panel (B) shows LSD based on the C treatment x salinity x period interaction.

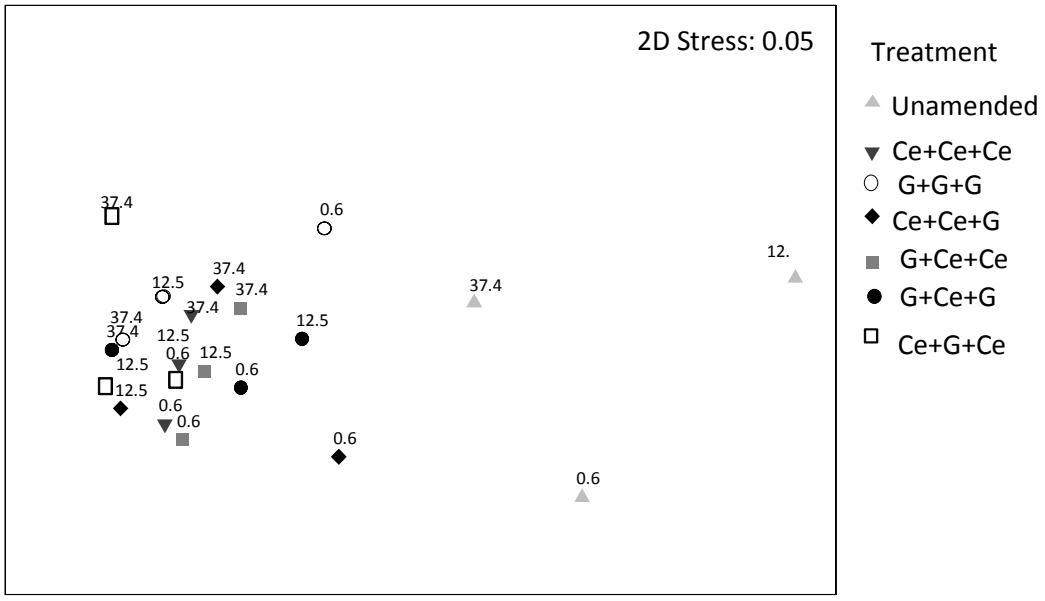

Figure 5. MDS plot of microbial community structure on day 42 based on PLFAs in soils with ECe 0.6, 12.5 and 37.4 dS m^{-1} unamended or amended with different combinations of (glucose (G) and cellulose (Ce). Symbols represent the means of three replicates. The distance among symbols is a measure of similarity of microbial community composition (the greater the distance between symbols the more they differ in microbial community composition).

CHAPTER 5

RESPONSE OF MICROBIAL ACTIVITY AND BIOMASS TO SOIL SALINITY AFTER REPEATED RESIDUE ADDITION

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Response of microbial activity and biomass to soil salinity after repeated residue addition

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Performed experiment, analysis of soil samples, data analysis and interpretation, wrote the manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed Date 15/04/2014

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 manuscript in the thesis

Signed Date 15/04/2014

Response of microbial activity and biomass to soil salinity after repeated residue addition

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Abstract

Previous studies have shown that a single addition of organic carbon or residues in saline soils reduces the negative impact of salinity on soil microbial activity and biomass but the effect may disappear once the available substrate from the added organic compounds is depleted. This depletion could be overcome by repeated residue addition. The aim of this study was to evaluate the response of soil microbes to increasing salinity with repeated residue addition. A non-saline soil was salinized by adding different amounts of NaCl to achieve electrical conductivity in saturated paste extract (ECe) of 1, 12.5, 25 and 50 dS m^{-1}. The soil was amended with two rates of finely ground pea residue equivalent to 3.9 and 7.8 g C kg^{-1}, referred to as 3.9C and 7.8C. The same rates were applied on days 0, 15 and 29. The control received no residues. A soil water content of 75% of water holding capacity (WHC) was maintained throughout the incubation period. Respiration was measured continuously for 6 weeks and expressed as cumulative respiration over two weeks after residue addition. Microbial biomass C (MBC) was determined on day 0, and 14 days after adding each residue addition; microbial community composition was determined on day 0 and 42. Cumulative respiration per g C added was always greater with 3.9C than with 7.8C and higher in the non-saline soil than in the saline soils. In the saline soils cumulative respiration was higher after the second and third addition then after the first addition except with 3.9C at EC50. At the same amount of C added (7.8 g C kg^{-1}), that is after the first addition of 7.8C and the second addition of 3.9C, the decrease in cumulative respiration in the saline soils relative to the non-saline soil was smaller with 3.9C than with 7.8C. After the first residue addition, the MBC concentration was higher in the non-saline soil than in the saline soils only with 3.9C. After the third residue addition, the MBC concentration was significantly lower than in the non-saline soil at EC25 and EC50 with 3.9C, but only at EC50 with 7.8C. At the end of the experiment, the microbial community structure differed significantly between EC1 and EC50

in all C treatments**.** The positive effect of repeated residue addition on adaptation of the soil microbial community to salinity is likely to be due to minimizing fluctuations in substrate supply as they occur with a single residue addition.

Keywords: Microbial biomass; Residue; Respiration; Salinity

1. Introduction

The area affected by salinity is increasing in many areas of the world, especially in arid and semi-arid regions, often induced by poor irrigation and drainage management (*Lambers,* 2003). Salt accumulation negatively influences physical, chemical and biological soil properties (*Rengasamy,* 2006b). Salinity reduces plant growth due to high osmotic potential of the soil solution which inhibits water uptake by plants and due to ion toxicity and ion imbalance which restrict nutrient uptake (*Rengasamy,* 2010). Further, salinity reduces microbial biomass size and activity and decomposition of soil organic matter (*Batra and Manna,* 1997; *Egamberdieva et al.,* 2010; *Elmajdoub and Marschner,* 2013; *Ghollarata and Raiesi,* 2007; *Muhammad et al.,* 2006; *Pathak and Rao,* 1998; *Rietz and Haynes,* 2003; *Setia et al.,* 2011a) and alters soil microbial community structure because microbial genotypes differ in their tolerance to low osmotic potential (*Andronov et al.,* 2012; *Chowdhury et al.,* 2011b; *Pankhurst et al.,* 2001; *Sardinha et al.,* 2003). Salinity decreases the ratio of fungi to bacteria (*Pankhurst et al.,* 2001) indicating that bacteria are more tolerant to salinity than fungi. Salinity tolerant microbes counteract the high osmotic potential by accumulating organic osmolytes to reduce water loss from their cells (*Beales,* 2004; *Oren,* 2001). The synthesis of osmolytes requires a high amount of energy and may therefore reduce microbial growth (*Hagemann,* 2011; *Oren,* 1999).

The addition of organic materials such as farmyard manure, crop straw or green manure can reduce the negative effects of salinity on plants by improving soil physical and chemical

properties (*Yadvinder-Singh et al.,* 2005). This practice could also influence microbial activity because soil microbial activity and biomass are often C limited (*De Nobili et al.,* 2001; *Demoling et al.,* 2007). Organic amendments could be particularly important in saline soils because adaptation to salinity requires large amounts of energy for osmolytes synthesis. Previous studies have shown that a single addition of plant residues increased microbial activity and biomass temporarily after which activity and biomass return to values similar to those in unamended soils (*Elgharably and Marschner,* 2011; *Franzluebbers et al.,* 1994; *Li et al.,* 2012; *Wichern et al.,* 2006). This suggests that adaptation of microbes to salinity requires high amounts of easily available substrates which are depleted rapidly in the first few days after addition of organic material. Decomposition of recalcitrant compounds such as cellulose, hemi-cellulose and lignin is slower and requires release of extracellular enzymes, an ability that is limited to a small number of microbial groups (*de Boer et al.,* 2005; *Meidute et al.,* 2008; *Vargas-Garcia et al.,* 2007). Thus, a single addition of plant residues can only temporarily improve energy supply to the majority of soil microbes. In the field, plant residue supply is more continuous, e.g. through litter fall or root turnover. Previously, *Duong et al.* (2009) showed that when the same total residue amount was added, repeated residue addition of wheat straw increased C mineralisation compared to a single addition. But little is known about the effect of repeated addition of residues on response of microbes to salinity. The aim of this study was to determine the response of soil microbes to increasing salinity with repeated addition of plant residues. We hypothesised that compared to the first addition; residue addition every two weeks will reduce the negative effect of salinity on microbial activity because high C availability is maintained and periods of low C availability are minimized.

2. Materials and methods

2.1 Soil sampling

A non-saline loamy sand was collected under natural vegetation from 0-20 cm depth in Monarto South Australia (35°05′S and 139°06′E). This area has dry Mediterranean climate, the average temperature is 30.1 ºC in summer and 15.9 ºC in winter with annual average rainfall of 352 mm. The soil was air-dried and sieved to < 2mm. In South Australia, top soils often remain dry over summer, therefore air-drying is not an un-realistic treatment. The soil had the following properties: sand 83%, silt 5%; clay 12%, pH 7.5, electrical conductivety in a 1:5 soil water extract ($EC_{1:5}$) 0.05 dS m^{-1}, total organic C 6.2 g kg^{-1}, total N 0.1 g kg^{-1}, total P 0.25 g kg^{-1}, bulk density 1.57 g cm^{-3} and maximal water holding capacity (WHC) 0.17 g g^{-1}.

2.2 Soil amendment and incubation

The soil was adjusted to different EC1:5 levels (control, 1, 2 and 4 dS m^{-1}) by adding different amounts of sodium chloride (NaCl). The salt was dissolved in reverse osmosis (RO) water and added to bring the water content to 75% of WHC. The control soil received only RO water. Then the soils were mixed and the EC measured to ensure that the desired EC was achieved. The $EC_{1:5}$ was converted to electrical conductivity of the saturated paste extract (ECe) by using the following equation:

$ECe = (14.0 - 0.13 \times \text{clay } \%) \times EC_{1:5}$ (*Rengasamy,* 2006a).

The ECe was 0.6, 12.5, 25 and 50 dS m^{-1} hereafter referred to as EC1, EC12.5, EC25 and EC50. These EC levels were chosen based on previous experiments (*Elmajdoub and Marschner,* 2013) to represent non-saline, low, moderate and high salinity with respect to soil respiration. The soils were then pre-incubated at 75% of WHC at 25ºC for 18 days to reactivate and stabilise soil respiration. Soil respiration usually stabilises after 8-10 days after rewetting of air-dry soil (*Butterly et al.,* 2009). The longer pre-incubation was chosen to allow the microbes to adapt to the different EC levels. A preliminary experiment was carried

out to determine the effect of water content on cumulative respiration. The water content was adjusted between 20% and 80% of WHC with RO water in soil amended with 1.5 g C kg^{-1} as glucose and respiration was measured over 10 days. Cumulative respiration was maximal at 40-75% of WHC with no significant differences within this range (data not shown).

The pre-incubated soils were amended with two residue rates (10 or 20 g kg^{-1} soil) as finely ground pea residue (*Pisum sativum* L, particle size between 0.25 and 2 mm; water soluble C 2.7%, total organic C 38.8%, total N 1.5% and C/N 26). This corresponds to C rates of 3.9 or 7.8 g C kg^{-1} soil (referred to as 3.9C and 7.8C). The residues were added at these rates three times: on days 0, 15 and 29. At each addition date, the soil was removed from the PVC cores in which it was incubated and the residues were thoroughly mixed into the soil. A control treatment which received no residues was mixed in the same manner on these days. The addition of residue did not change the EC.

After mixing, 20 g of soil was added to PVC cores with diameter of 3.7 cm and height of 5 cm and nylon mesh base (0.75 µm, Australian filter specialist) and packed to bulk density 1.57 g cm^{-1}; by adjusting the height of the soil in the cores based on the following equation : $Bd = M / \pi r^2 h$, where, Bd = bulk density, M = mass of soil (g), r = radius of PVC core (cm), h = height of the soil in PVC core (cm). The bulk density was adjusted after each residue addition and mixing. The cores were placed in 1 L glass jars together with tubes containing 10 ml of RO water to minimise soil moisture loss during the incubation period. The jars were incubated in the dark at 25ºC. The soil water content was checked by weighing the cores every 3 days after measuring the soil respiration and RO water was added if necessary to maintain the desired water content. There were 3 replicates per C treatment EC level and sampling date (see details of microbial biomass C determination below).

Soil respiration (CO_2 evolution) was measured daily for 42 days. Cumulative respiration was calculated over two weeks after each C addition that is for days 0-14, days 15-28 and days for

29-42 for the first, second and third addition, respectively. For the amended treatments, cumulative respiration was expressed in mg CO_2 per g C added at the start of this period to allow better comparison between the two C addition rates. Destructive sampling for microbial biomass C (MBC) was carried out before addition of residue (day 0) and 14 days after the first (day 14), second (day 28) and the third addition (day 42). The cores to be harvested on day 14 received only one addition of residue whereas the cores harvested on day 28 received residues twice and the cores harvested on day 42 received residues three times. Microbial community structure was determined on days 0 and 42. The soil samples for community structure were stored at -20ºC before phospholipid fatty acid (PLFA) extraction. In this experiment respiration and microbial biomass C are expressed per g organic C added at the start of each period. In the unamended soil they are expressed per g soil. The PLFA data is expressed per g soil.

2.3 Analyses

2.3.1 Soil respiration

Soil respiration was measured by determining the CO_2 concentration in the headspace of each jar using a Servomex 1450 infra-red gas analyser (Servomex, UK) as described in *Setia et al.,* (2011b). After each measurement (t1), the jars were vented to refresh the headspace using a fan, and then resealed followed by determination of the CO_2 concentration (t0). The CO_2 evolved during a given interval was calculated as the difference in CO_2 concentration between t1 and t0. Known amounts of CO_2 were injected into jars of similar size to determine the relationship between CO_2 concentration and detector response. The concentration of CO_2 in the jars with soil samples was calculated from this relationship. The mg CO_2 respired was obtained by multiplying the calculated CO_2 concentration with the gas

volume of the jars (*Setia et al.*, 2011). Cumulative respiration was calculated for each 14-day period after residue addition separately.

The amount of C remaining of added C (g kg^{-1} soil) for each period was calculated by subtracting the amount of C respired from the amount added at the start of that period. Total remaining C was calculated as the sum of C remaining in each period, expressed in g kg^{-1} soil and percentage of total added.

2.3.2 Microbial biomass C

Soil microbial biomass C (MBC) was determined 14 days after residue addition and before the next residue addition, on days (14, 28 and 42) by fumigation extraction (*Vance et al.*, 1987) as modified by *Anderson and Ingram* (1993) using two subsamples of 5 g. One subsample was fumigated with ethanol-free chloroform for 24 h while the non-fumigated subsample was kept at 4ºC during this time. After removal of the chloroform, both fumigated and non-fumigated subsamples were extracted with 0.5 M K_2SO_4 (1:4 ratio). Organic C in the extracts was determined after dichromate digestion and titrated with 0.033 M acidified $(NH_4)_2Fe\ (SO_4)_2 \cdot 6H_2O$ (*Anderson and Ingram,* 1993). Chloroform–labile C was calculated as the difference between the fumigated and non-fumigated samples. Microbial biomass C (mg kg^{-1} soil) was calculated by multiplying the chloroform-labile C by 2.64 (*Vance et al.*, 1987). To allow better comparison between the two C addition rates, the MBC concentration in mg kg^{-1} soil was divided by C rates (3.9 and 7.8 g kg^{-1}) to give MBC mg g^{-1} C added. Respiration was measured on the cores to be harvested at the following sampling date.

2.3.3 PLFA analysis

Microbial community composition was determined by phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from 4 g frozen soil based on *Frostegård et al.* (1993) using a solvent of chloroform, methanol and citrate 1:2:0.8 (v/v/v). The lipid phase was collected

and dried under a stream of N_2 at 37ºC. After dissolving the dry sample in 1 ml chloroform, the solution was transferred to silicic acid columns. The columns were washed sequentially with chloroform, acetone and methanol; the methanol fraction which contains the PLFAs was collected. After alkaline methanolysis, the organic phase was collected in dichloromethane and hexane methylnonadecanoate (C19:0) was added as internal standard to each sample. The PLFAs were separated and analysed in a GC-FID (HP 6890) as described in *Chowdhury et al.*,(2011a). The following signature PLFAs were used as indicators for specific microbial groups: bacteria (14:0, 15:0, 16:0, 17:0, a17:0), Gram-positive bacteria (10 me16:0, i15:0, a15:0, i16:0, i17:0), Gram-negative bacteria (cy17:0, cy19:0, 18:1ω7c, 18:1ω7t) and fungi (18:2ω6,18:3ω3c,18:3ω6c) (*Pankhurst et al.,* 2001; *Zak et al.,* 2000). The sum of signature fatty acids for a certain microbial group was used to calculate biomass of that group. Fungi to bacteria (F/B) ratio were calculated by dividing fungal biomass by bacterial biomass.

2.4 Statistical analysis

The experiment was arranged in a completely randomised design with three replicates per treatment and sampling date. For the amended treatments, the data of cumulative respiration and MBC per g C added were subjected to three-away ANOVA (analysis of variance) with C rate, EC and residue addition times (first, second and third) as fixed factors. For the unamended soil, cumulative respiration and MBC were expressed per g soil and analysed by two-way ANOVA with EC and residue addition times as fixed factors. Biomass of bacteria and fungi as well as F/B ratio were analysed by one-way ANOVA on day 0 (EC) and two-way ANOVA on day 42(C rate x EC). Tukey test was used to determine significant differences (GenStat ® for Windows 14.0, VSN Int.Ltd, UK, 2010). Microbial community composition was analysed by Primer-E software (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK). The data of PLFA was transformed using log (x+1) and plotted

by non-metric multi-dimensional scaling (MDS). Significant differences in the microbial community composition among the treatments were determined by PERMANOVA ($P \leq 0.1$).

3. Results

3.1 Cumulative respiration

In all three periods, cumulative respiration in the unamended soil (per g soil) was significantly higher in the non-saline than in the saline soils and there were no differences between saline soils (Tab.1). Cumulative respiration over 14 days did not differ among periods.

In the amended soils, cumulative respiration per g C added in the 14 days following residue addition was always higher with 3.9 g C kg^{-1} than with 7.8 g C kg^{-1} and higher in the non-saline than in the saline soils (Fig. 1). Among the saline soils it was lowest in EC50. In the non-saline soil, cumulative respiration over 14 days did not differ between first, second and third residue addition. But in the saline soils, cumulative respiration was lower after the first addition than after the second and third addition except for EC50 with 3.9C. In the saline soils, the relative increase after the second and third addition compared to the first addition was greater with 7.8C than with 3.9C and increased with increasing EC. For example, compared to the 14 days after first addition, cumulative respiration after the second addition was increased by 26% at EC12.5, 54% at EC25 and 62% at EC50 with 3.9C and by 59%, 75% and 200% at EC12.5 and EC25 and EC50 with 7.8C. With 3.9C, cumulative respiration in the saline soils was always significantly lower than in the non-saline soil. With 7.8C, cumulative respiration compared to the non-saline soil was significantly lower in EC12.5, EC25 and EC50 after the first addition, but after the second addition it was only significantly lower at EC25 and EC50 and after the third addition only at EC50. When the same amount of C was added (7.8 g kg^{-1}), that is with the first addition in 7.8C and the second addition with

3.9C, the percentage decrease in cumulative respiration in saline soils compared to the non-saline soil was smaller in 3.9C than with 7.8C being. At EC12.5, EC25 and EC50 the percentage decrease in cumulative respiration compared to EC1, was 41%, 50% and 80% with 7.8C but only 19%, 26% and 61% with 3.9C respectively. The amount of C remaining 14 days after residue addition and the total C remaining at the end of the experiment was lowest in the non-saline and increased with increasing EC (Tab. 2).

3.2 Microbial biomass C

On day 0, the MBC concentrations per g soil ranged between 155 and 199 μg g^{-1} and did not differ significantly among EC levels. In the unamended soil at each sampling date, the MBC concentration did not differ between saline and non-saline soil (data not shown). In the amended soils, the MBC concentration per g C added 14 days after residue addition was always higher with 3.9C than with 7.8C except in EC50 after the first addition (Fig. 2). Generally, the MBC concentration varied little between first, second and third residue addition. Two weeks after the first addition the MBC concentration was greater in the non-saline soil than in the saline soils with 3.9C however, there were no differences among EC levels in 7.8C. Two weeks after the second addition, the MBC concentration compared to the non-saline soil was significantly lower only in the most saline soil (EC50). Two weeks after the third addition, the MBC concentration was significantly lower than in the non-saline soil in EC25 and EC50 with 3.9C, but only in EC50 with 7.8C.

3.3 Bacterial and fungal biomass

On day 0, the bacterial biomass concentration was higher at EC50 than with EC1 and EC12.5. But there were no significant differences among EC levels in fungal biomass (Tab. 3). The F/B ratio was significantly lower in EC25 and EC50 than in EC1 and EC12.5. On day 42 compared to the unamended soil, addition of 3.9C significantly increased bacterial

biomass in the non-saline soil and in EC12.5, but not in the more saline soils; it was lower in the saline than in the non-saline soils (Tab. 4). Addition of 7.8C significantly increased bacterial biomass compared to the unamended soil at all EC levels and bacterial biomass was lower at the two higher compared to the two lower salinity levels. Fungal biomass was significantly higher in residue amended treatments compared to the unamended soil at all EC levels except in EC50 with 3.9C. With both residue rates, fungal biomass was significantly lower in the most saline soil compared to the non-saline soil.

3.4 Microbial community structure

Microbial community structure based on PLFA on day 0 did not differ among EC levels (data not shown). At the end of experiment (day 42) microbial community structure significantly differed between EC1 and EC50 in all C treatments (Fig. 3). Compared to the non-saline soil, microbial community structure differed significantly in EC12.5 only in the amended soils (Tab.5). Community structure differed significantly between EC1 and EC25 in the unamended treatment, but not in the amended soils. On the other hand, community structure did not differ significantly between EC1 and EC12.5 in the unamended soil, but was different in the amended soils

4. Discussion

This study showed that with repeated addition of residues, the difference in cumulative respiration per g C added between saline and non-saline soils became smaller, suggesting increased adaption to salinity when high C availability is maintained. This confirms our hypothesis that compared to the first addition; residue addition every two weeks will reduce the negative effect of salinity on microbial activity because high C availability is maintained and periods of low C availability are minimized. This smaller difference in cumulative

respiration per g C added between saline and non-saline soil is due to the fact that repeated residue addition increased respiration in the saline soils, but not in the non-saline soil.

When residues are added to soil, respiration rate sharply increases as easily decomposable compounds are decomposed, but respiration rates decrease after depletion of these compounds because only more recalcitrant material (e.g. cellulose and lignin) is left. Repeated residue addition provided easily available compounds every two weeks, thus minimising the periods of low C availability. Further, the presence of easily available compounds every two weeks may also have increased decomposition of the remaining recalcitrant material (priming effect) (*Kuzyakov et al.,* 2000). This greater amount of energy allowed microbes to synthesise osmolytes to counteract the high osmotic potential in the soil solution. This ameliorating effect can be seen in three comparisons. Firstly, by the smaller difference in cumulative respiration between non-saline and saline soils at a given C rate over the 14 days after the second or third addition compared to the first addition. Secondly, by the finding that cumulative respiration two weeks after the third addition did not differ between the non-saline soil and EC12.5 and EC25 with 7.8C, but was significantly lower with 3.9C. And thirdly, when the same amount of C was added (7.8 g C kg^{-1} soil) in the smaller percentage decrease in cumulative respiration in saline compared to non-saline soils in the two week period after the second addition with 3.9C compared to that after the first addition with 7.8C. Residue addition also changed microbial community structure which may also have contributed to the greater adaptation of the microbial community to salinity. The differences between salinity levels were smaller for MBC concentration than for cumulative respiration. Similar findings were reported before (*Chowdhury et al.,* 2011a; *Elmajdoub and Marschner,* 2013) and can be explained by the fact that cumulative respiration reflects activity over a given period whereas MBC concentration is a snap-shot at the time of sampling. It is likely that two weeks after residue addition, a proportion of the microbial

biomass will have turned over due to the decreased supply of easily available C. In this study, we did not sample for MBC more often because the aim was to measure MBC after the initial burst of easily available C.

Salinity changed microbial community structure because genotypes differ in tolerance to salinity. Further, salinity reduced bacterial and fungal biomass but did not change the F/B ratio which indicates that bacteria and fungi were equally affected by salinity. Previous studies suggested that bacteria are more tolerant to salinity than fungi (*Chowdhury et al.,* 2011a), but the present and other studies (*Wichern et al.,* 2006) did not confirm this. These contradictory findings may be due to differences in soil type and EC levels among the studies.

Both cumulative respiration and MBC concentration per g C added were lower with 7.8C than with 3.9C. Thus, soil microbes utilised more of the added C at the lower addition rate. This is probably due to differences in accessibility of the added C. At the higher addition rate, it is likely that residue particles formed clumps whereas the particles were more uniformly distributed at the lower rate (*Angers and Recous, 1997; Breland, 1994)*. The residue particles within the clumps would be less accessible to microbes than individual particles because the latter can be accessed from all sides and therefore have a greater surface area to volume ratio.

5. Conclusion

Repeated residue addition can minimize periods of low supply of easily available C compounds which increases microbial activity in saline soil. Thus, addition of organic matter to saline soil not only improves chemical and physical soil properties but also microbial activity and growth. This study showed that the negative effect of salinity on cumulative respiration was reduced after two or more additions, suggesting greater adaptation to salinity.

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Table 1: Cumulative respiration in the unamended treatment for three 14 day periods corresponding to the two weeks following the first, second and third residue addition in the amended soils at ECe 1, 12.5, 25 and 50 dS m^{-1} first, second and third (n=3, values with different letters indicate significant differences $P \leq 0.05$).

EC (dS m^{-1})	Cumulative respiration (mg g^{-1} soil) over 14 days after		
	first	second	third
	residue addition		
EC1	0.32 bc	0.38 c	0.38 c
EC12.5	0.13 a	0.18 ab	0.14 ab
EC25	0.10 a	0.14 ab	0.11 a
EC50	0.04 a	0.09 a	0.05 a

Table 2: Amount of remaining C of added C at ECe 1, 12.5, 25 and 50 dS m^{-1} 14 days after the first, second and third residue addition (g kg^{-1} soil) and total remaining C (g kg^{-1} soil and percentage) at the end of the experiment

C treatments (g kg^{-1}soil)	ECe (dS m^{-1})	Amount of remaining C (g kg^{-1} soil)				
		After residue addition C (g kg^{-1} soil)			At the end of the experiment	
		First	Second	Third	Total remaining C (g kg^{-1} soil)	% of added
3.9	EC1	1.14	0.91	0.73	2.78	24
	EC12.5	1.97	1.48	1.37	4.82	41
	EC25	2.46	1.67	1.78	5.91	51
	EC50	3.17	2.72	2.80	8.69	74
7.8	EC1	3.33	2.65	3.10	9.08	39
	EC12.5	5.18	3.64	3.10	11.90	51
	EC25	5.56	3.88	3.55	12.99	55
	EC50	6.92	5.16	5.28	17.37	74

Table 3: Bacterial, fungal biomass and the fungi /bacteria ratio at ECe 1, 12.5, 25 and 50 dS m^{-1} on day 0 (n=3, values with different letters indicate significant differences $P \leq 0.05$).

EC dS m^{-1}	Bacterial biomass		Fungal biomass		F/B ratio	
			µg g^{-1}			
EC1	10.3	a	8.3	a	0.8	a
EC12.5	11.2	a	8.9	a	0.8	a
EC25	17.8	ab	10.4	a	0.6	b
EC50	19.5	b	10.1	a	0.5	b

Table 4: Bacterial, fungal biomass and the fungi /bacteria ratio on day 42 at ECe 1, 12.5, 25 and 50 dS m^{-1} in unamended soil and soil amended three times with 3.9 or 7.8 g C kg^{-1} (n=3, values with different letters indicate significant differences $P \leq 0.05$)

Treatments C rate (g kg^{-1})	EC dS m^{-1}	Bacterial biomass		Fungal biomass		F/B ratio	
		μg g^{-1}					
unamended	EC1	17.5	ab	7.1	a	0.4	a
	EC12.5	12.2	a	7.3	a	0.6	abcd
	EC25	15.3	a	8.2	a	0.5	abc
	EC50	95	a	6.9	a	0.8	cde
3.9	EC1	631	efg	32.4	c	0.5	ab
	EC12.5	39.8	bcd	26.1	bc	0.7	bcd
	EC25	25.2	abc	21.6	bc	0.9	e
	EC50	31.2	abcd	17.0	ab	0.6	abc
7.8	EC1	83.9	g	45.2	de	0.5	abc
	EC12.5	72.3	fg	46.9	e	0.7	bcd
	EC25	42.9	cde	33.7	cd	0.8	de
	EC50	51.9	def	24.6	bc	0.5	ab

Table 5: Pair-wise comparison by Permanova for microbial community composition on day 42 based on PLFA in soils at ECe 1, 12.5, 25 and 50 dS m^{-1} in unamended soil and soils amended three times with 3.9 or 7.8 g C kg^{-1}.

Pair wise comparison*	C rate treatments (g kg^{-1})		
EC	Unamended	3.9	7.8
1 and 12.5	ns	*	*
1 and 25	*	ns	ns
1and 50	*	*	*
12.5 and 25	*	*	ns
12.5 and 50	ns	*	*
25 and 50	*	ns	*

*At a given C rate, pair-wise comparison with asterisks indicates that pairs are significantly different **(*** $P \leq 0.1$); pair-wise comparisons with ns are not significant.

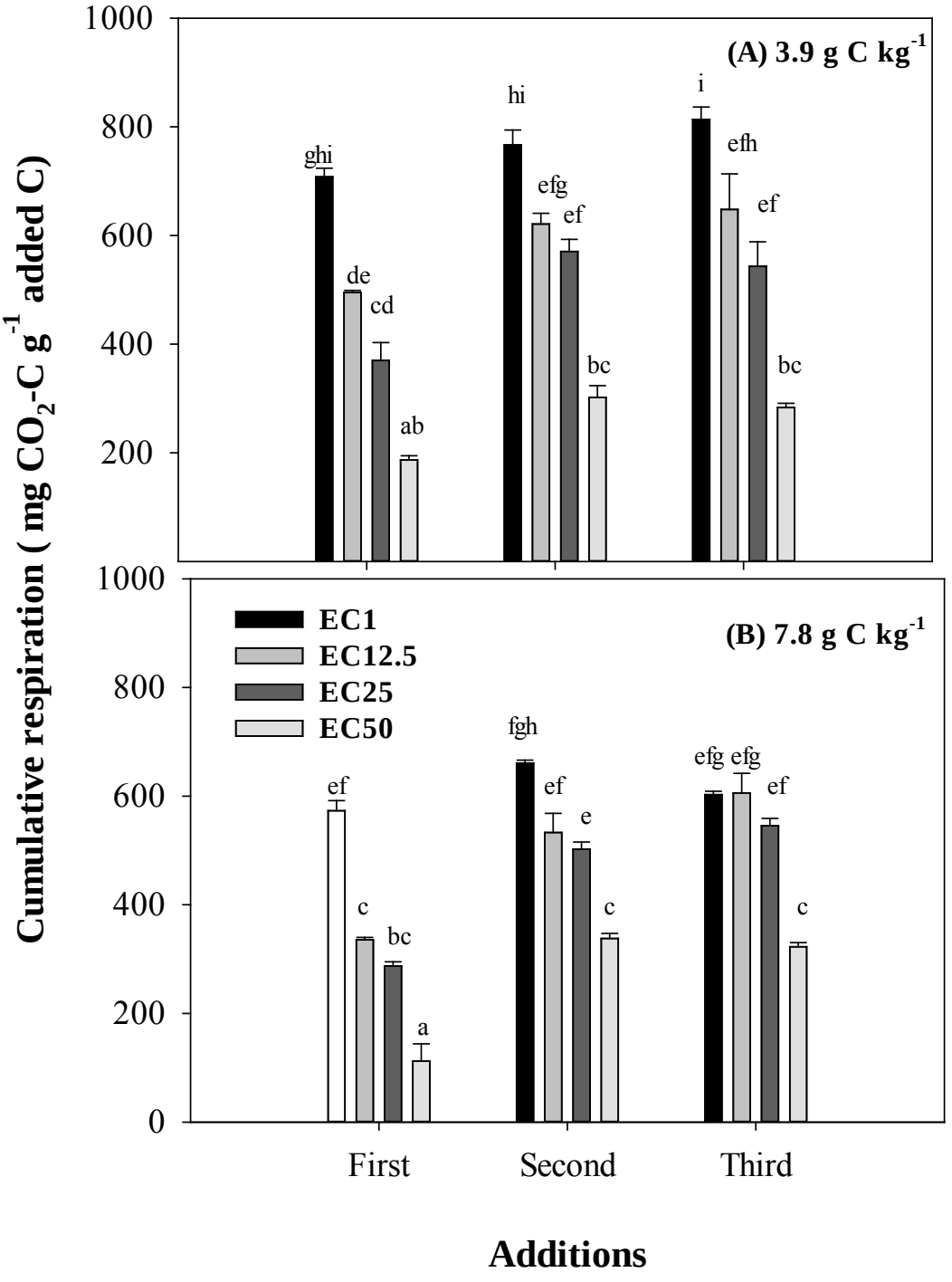

Figure 1: Cumulative respiration per g C added 14 days after the first, second and third addition of residues at (A) 3.9 g C kg^{-1} soil and (B) 7.8 g C kg^{-1} soil), in soils with ECe 1, 12.5, 25 and 50 dS m^{-1},(n=3, vertical bars represent standard error, columns with different letters indicate significant differences $P \leq 0.05$).

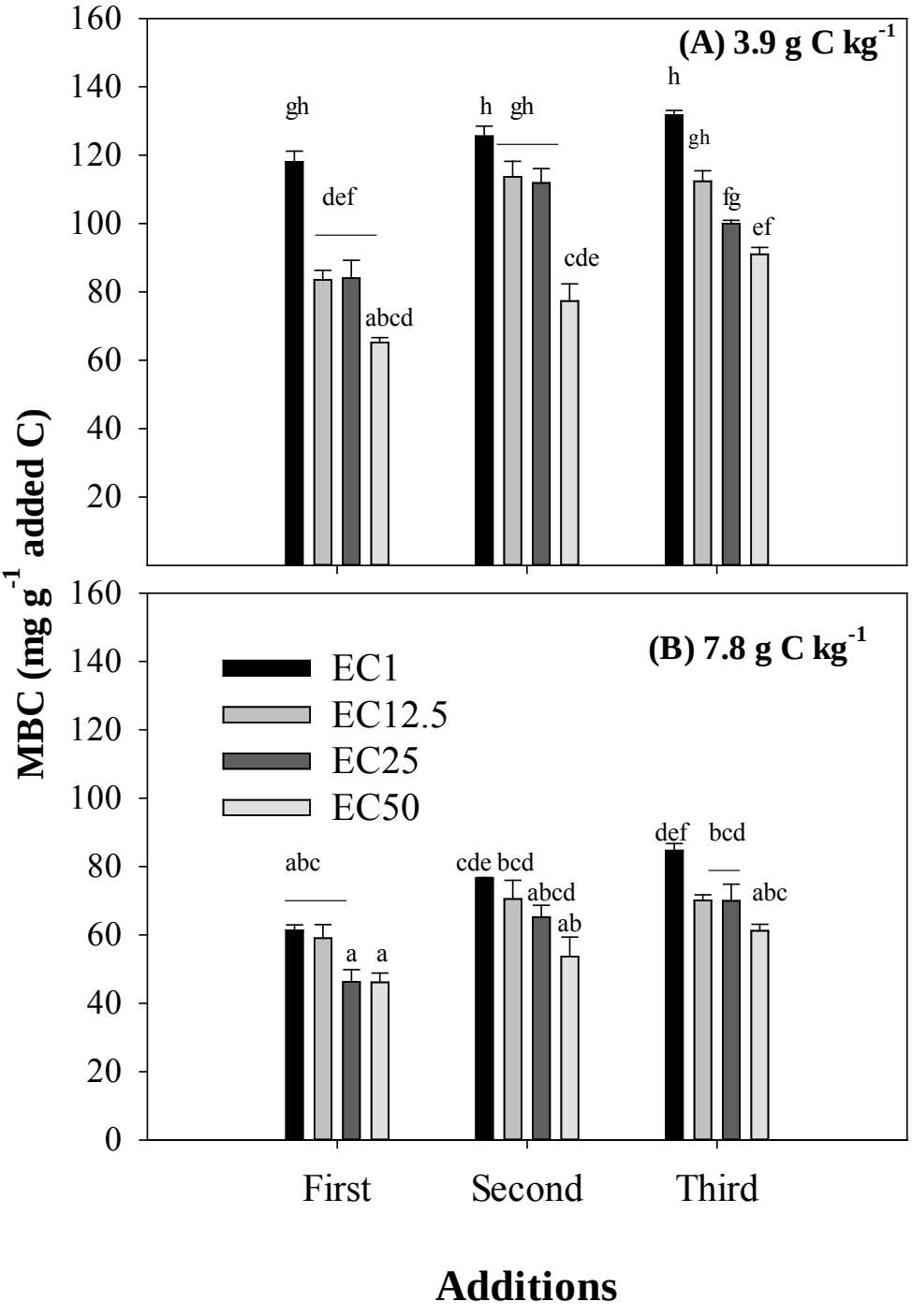

Figure 2: Microbial biomass C 14 days after the first, the second and the third addition in soils with ECe 1, 12.5, 25, 50 dS m^{-1} amended with (A) 3.9 g C kg^{-1} soil and (B) 7.8 g C kg^{-1} soil, (n=3, vertical bars represent standard error, columns with different letters indicate significant differences $P \leq 0.05$).

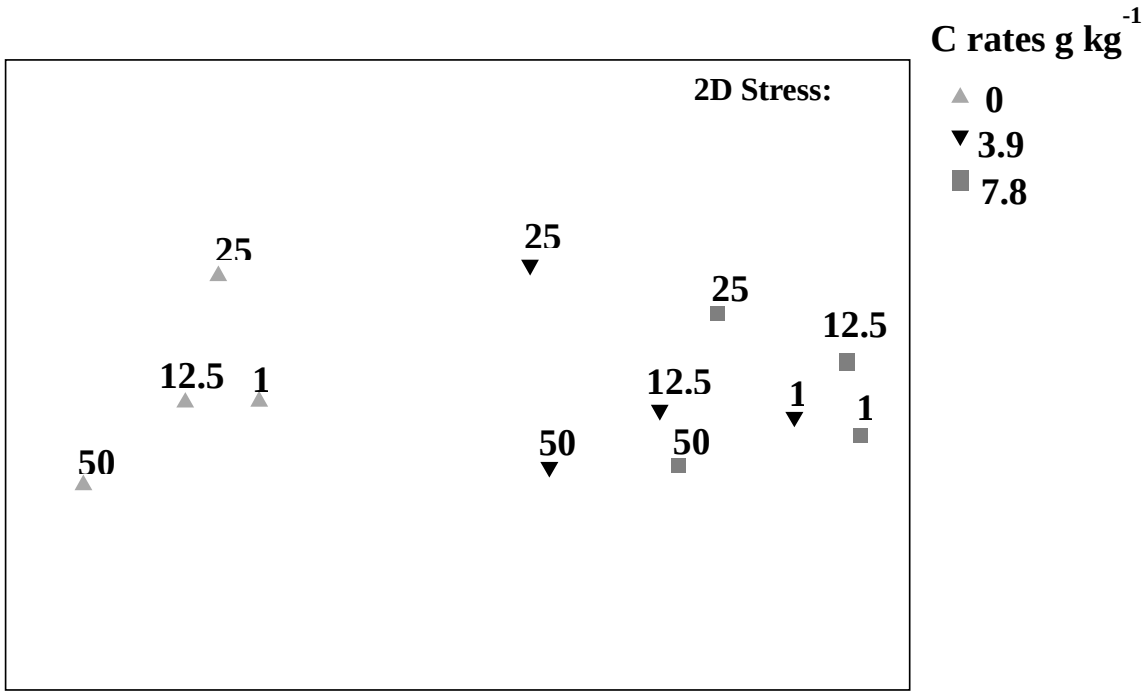

Figure 3: MDS plot of microbial community structure on day 42 based on PLFAs with different C treatments (0, 3.9 and 7.8 g C kg $^{-1}$ soil) at different ECe levels (1, 12.5, 25 and dS m^{-1}), symbols represent means of three replicates (n=3).

CHAPTER 6

RESPONSE OF MICROBIAL ACTIVITY AND BIOMASS IN RHIZOSPHERE AND BULK SOILS TO INCREASING SALINITY

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STATEMENT OF AUTHORSHIP

Response of microbial activity and biomass in rhizosphere and bulk soils to increasing salinity

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Performed experiment, analysis of soil samples, data analysis and interpretation, wrote the manuscript and acted as corresponding author. I hereby certify that the statement of contribution is accurate.

Signed Date 15/04/2014

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Contributed to planning of experiment, manuscript evaluation and supervised development of work.

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

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CHAPTER 7

DOES DRYING AND REWETTING INFLUENCE THE RESPONSE OF SOIL MICROBES TO INCREASING SALINITY?

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Does drying and rewetting influence the response of soil microbes to increasing salinity?

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Abstract

An incubation experiment was conducted to evaluate the response of soil microbial activity and biomass to drying and rewetting of non-saline and saline soils when the salt concentration was maintained or increased upon rewetting. A non- saline loamy sand with electrical conductivity (EC) in a 1:5 soil: water extract ($EC_{1:5}$) of 0.1 dS m^{-1} was amended with sodium chloride (NaCl) to achieve $EC_{1:5}$ of 1.5 and 3.5 dS m^{-1}). All soils were adjusted to 40% of water holding capacity and incubated for 18 days after which the soils were amended with finely ground pea straw and incubated for another 7 days before the start of moisture treatments. The soils were divided into two portions, one portion was dried for four days and the second portion was maintained at 40 % of water holding capacity. On day 12 (after the dried soils were air-dry), the soils were rewet to 75% of water holding capacity with either water to maintain the EC or with solutions with different NaCl concentrations of to achieve the following salinity levels: EC0.1 was adjusted to1.5, 2.5 and 3.5 dS m^{-1}, EC1.5 adjusted to 2.5 and 3.5 dS m^{-1} and EC3.5 adjusted to 4.5 dS m^{-1}. Then the water content was maintained at 75% water holding capacity in all soils until day 25. Respiration was measured from day 0 to day 25. Microbial biomass C was determined on days 0, 7, 11 and 25. A

respiration flush upon rewetting was only observed in the initially non-saline soil when the EC was maintained, but not when salinity was increased. In the initially saline soils, no respiration flush was found even if the EC was not increased. Cumulative respiration at the end of the experiment was higher in drying and rewetting (DRW) treatments compared to the constantly moist (CM) soils only in the initially non-saline soil when the EC was maintained. The reduction in cumulative respiration with increasing EC compared to the treatments where the EC was maintained occurred only in the initially non-saline soil (EC0.1) where the decrease was greater in DRW compared to CM. At the end of the experiment, the microbial biomass C concentration was higher in DRW compared to CM treatments only in the initially non-saline soils when the EC was increased. The study showed that in an initially non-saline soil, increasing the EC upon rewetting limits the ability of microbes to utilise substrates released upon rewetting. Drying and rewetting did not consistently increase the susceptibility of microbial activity or biomass to salinity.

Keywords: Drying and rewetting; Microbial biomass; Respiration; Salinity

1. Introduction

In arid and semi-arid regions, soils are exposed to drying and rewetting cycles during summer when dry periods are interrupted by occasional rainfall or by irrigation. Both drying and rewetting (DRW) are stressors which may change microbial activity, growth and nutrient cycling [1, 2]. Drying of soil reduces water availability (matric potential becomes more negative) and the thickness of water films around the aggregates thereby restricting substrate availability to microbes [6, 7]. Soil drying kills sensitive microbes and reduces microbial growth and activity as well as decomposition of organic matter [3-5]. Particularly in saline soils, drying increases the osmotic potential because of the increasing salt concentration in the remaining soil solution [8]. Some microbes respond to low matric and osmotic potential

by accumulating osmolytes to reduce water loss from the cells [9]. The synthesis of osmolytes requires substantial amounts of energy [9, 10].

Previous studies showed that rewetting of dry soils results in a flush of respiration after which respiration rates decline to levels similar to those in constantly moist soils [11-13]. The flush of respiration after rewetting is due to several mechanisms that result in increased organic matter availability: (i) release of osmolytes accumulated during the dry period, (ii) lysis of microbial cells, and (iii) release of previously protected organic matter as a result of breakdown of aggregates [14-19]. Moreover, DRW cycles can alter microbial community structure due to differences in tolerance to drying and rewetting stresses among microbial genotypes [1, 20-22]. Compared to constantly moist soils, cumulative respiration can be decreased [12,13, 23, 24] or increased by DRW [11, 25, 26].

In arid and semi-arid regions about 5% of arable land is salt affected [27] these soils may experience DRW cycles. Salinity reduces microbial biomass and activity [28-29]. Previous studies have shown that rewetting of saline soils also induces a flush of respiration but the increase in respiration rate immediately after rewetting relative to the constantly moist soil was reduced by high salinity [30-31], indicating that salinity stress may limit the ability of soil microbes to utilise substrates available upon rewetting. When saline water is used for irrigation, rewetting can also be accompanied by an increase in salinity which may affect soil microorganisms. Little is known about the effect of the combined stresses of rewetting and increasing salinity on soil microbes. The aim of this study was to assess the response of microbial activity and biomass to drying and rewetting of non-saline and saline soils when the salinity was maintained or increased upon rewetting. We hypothesised that (i) when rewetting is accompanied by an increase in salinity, the flush in respiration will be smaller than when salinity is not increased, and (ii) drying and rewetting will increase the sensitivity

of microbes to salinity because the microbes are exposed to two stressors: rewetting stress and salinity.

2. Materials and methods

2.1. Soil

A non-saline soil loamy sand soil (sand 83%, clay 12%, silt 5%, electrical conductivity in a 1:5 soil: water extract $EC_{1:5}$ 0.1 dS m^{-1}, pH 7.5, total organic C 6.2 g kg^{-1}, total N 0.1 g kg^{-1}, bulk density 1.57 g cm^{-3}, water holding capacity 170 mg g^{-1}) was collected under natural vegetation from 0-20 cm depth at Monarto, South Australia (35°05′S and 139°06′E), which has a Mediterranean climate. The soil was air-dried and sieved to < 2 mm. Top soils in this region are often air-dry during the long hot and dry summer. Therefore the air-drying is not unusual for this soil.

2.2. Soil amendment and incubation

The soil was adjusted to two salinity levels by adding different amounts of NaCl dissolved in water equivalent to 40% of water holding capacity to achieve an EC in a 1:5 soil water extract ($EC_{1:5}$) of 1.5 and 3.5 dS m^{-1}. The water content of the non-saline soil was adjusted to 40% of water holding capacity by adding reverse osmosis water. The salinity treatments **are** referred to as EC0.1, EC1.5 and EC3.5. Then the soils were pre-incubated at 25ºC for 18 days before the start of the experiment. Previous studies have shown that soil respiration stabilises between 7 and 10 days after rewetting of dry soil [11, 30]. A longer pre-incubation was chosen to allow the microbial community to adapt to the different EC levels. During this pre-incubation, microbial genotypes accumulating osmolytes may become dominant in the community whereas salinity sensitive genotypes die. At the end of pre-incubation, the soils were amended with 20 g kg^{-1} finely ground and sieved pea residue (*Pisum sativum* L, water

soluble C 27 g kg^{-1}, C/N 26, particle size 0.25 -2 mm), as a readily available nutrient source. The soils were mixed with the residue and then 20 g were filled into polyvinyl chloride cores (radius 1.85 cm, height 5 cm) with a nylon mesh base (0.75 µm, Australian filter specialist) and adjusted to a bulk density of 1.57 g cm^{-3}. Hereafter, the number of days refers to days after residue addition. The cores were placed in 1L glass jars with gas-tight lids and incubated in the dark at 25ºC for 7 days before onset of the moisture treatments. On day 8, the cores were divided into two portions (Fig. 1), one portion was maintained at 40% of water holding capacity and the other portion was dried for 4 days by placing small pouches containing self-indicating silica gel (BDH Chemicals) into the glass jars [11]. The silica gel pouches were replaced daily after measuring the CO_2 concentration with a second quantity that was regenerated at 70**°C** for 24 h until the soil was air dry (day 11). On day 12, the soil water content of dried and moist soils was increased to 75% of water holding capacity by adding solutions with different NaCl concentrations; when the EC was to be maintained, only reverse osmosis water was added. A preliminary experiment had shown that respiration was maximal between 40 and 75% of water holding capacity and did not differ significantly within this range (data not shown). Soil with original EC0.1 dS m^{-1} was maintained at 0.1 dS m^{-1} or adjusted to 1.5, 2.5 and 3.5 dS m^{-1}, referred to as EC0.1-0.1, EC0.1-1.5, EC0.1-2.5 and EC0.1-3.5. Soil with original EC1.5 dS m^{-1} was maintained at 1.5 dS m^{-1} or adjusted to 2.5 and 3.5 dS m^{-1} named EC1.5-1.5, EC1.5-2.5 and EC1.5-3.5. The soil with original EC3.5 dS m^{-1} was maintained at 3.5 dS m^{-1} or adjusted to 4.5 dS m^{-1} referred to EC3.5-3.5 and EC3.5-4.5. To ensure uniform salinity and moisture, the soils in the cores were mixed after addition of NaCl solutions or reverse osmosis water and re-packed to a bulk density of 1.57 g cm^{-3}**.** Then the cores were placed back into the glass jars and incubated in the dark at 25°C for 2 weeks (day 12 to day 25). Respiration was measured from day 0 to day 25. Cores were sampled destructively for determination of microbial biomass C on days 0, 7, 11 and 25.

2.3. Analyses

Electrical conductivity and pH were determined in a 1:5 soil: water ratio after 1 h end-over-end shaking at 25°C (Rayment and Higginson 1992). The osmotic potential of the soil solution at 75% of water holding capacity was calculated by using the equation: $\Psi\pi = - 0.036\ EC_{meas}\ (\theta_{ref}/\theta_{act})$, where Ψ_π is the osmotic potential (MPa) at the actual moisture content (θ_{act}, g g^{-1}) of the soil, EC_{meas} is the measured EC (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 [32].

The maximum water holding capacity of the soil was measured by placing the soil in rings in a sintered glass funnel connected to a 1 m water column (Ψ_m= -10 kPa) after which they were thoroughly wetted and allowed to drain for two days. The drained soil was weighed before and after oven-drying at 105°C for 24 hours to determine the water content. The total organic carbon concentration was measured by oxidation with $K_2Cr_2O_7$ and H_2SO_4 and the remaining $K_2Cr_2O_7$ was titrated with $FeSO_4$ [33]. Total N was determined by the Kjehldal method [34].

Soil respiration was determined by measuring the CO_2 concentration in the headspace of each jar using a Servomex 1450 infra-red gas analyser (Servomex, UK) as described in Setia et al. [35]. The jars were opened after each measurement to refresh the headspace by using a fan. Then the jars were closed and the CO_2 concentration was measured (t0). The CO_2 concentration was measured again the following day (t1). The released CO_2 during the measurement period is the difference between the CO_2 concentration t1 and t0. The linear relationship between detector reading and CO_2 concentration was determined daily by injecting known amounts of CO_2 into glass jars similar to those used in the experiment. The mg CO_2 respired was obtained by multiplying the calculated CO_2 concentration with the gas volume of the jars. For further details about the calculations see Setia et al. [35].

Microbial biomass C was determined by fumigation-extraction [36] as described in Anderson and Ingram [37] using two subsamples of 5 g of each soil. One subsample was fumigated with ethanol-free chloroform for 24 h at 25°C in sealed desiccators while the non-fumigated subsample was kept at 4°C during fumigation. After removal of the chloroform, both fumigated and non-fumigated subsamples were extracted with 0.5 M K_2SO_4 (1:4 ratio). Dissolved organic C in the extracts was determined after dichromate digestion and titrated with 0.033 M acidified $(NH_4)_2Fe\ (SO_4)_2 \cdot 6H_2O$ [37]. The microbial biomass C was calculated from the difference between the fumigated and non-fumigated samples multiplied by 2.64 [36].

2.4. Statistical analysis

There were 3 replicates per treatment and sampling time. The data of respiration rate, cumulative respiration and microbial biomass C were assessed by two-way ANOVA (moisture treatments x EC) at a given time. Tukey test was used to determine significant differences between the treatments ($P< 0.05$) (GenStat ® for Windows 14.0, VSN Int.Ltd, UK, 2010).

3. Results

3.1. Respiration

On day 7 (after 7 days at 40% WHC in all treatments), cumulative respiration was highest in the soil without salt addition (EC0.1) and decreased significantly with increasing EC (Table 1). On day 11, after the drying phase in the DRW treatment (days 8 to 11), cumulative respiration in the constantly moist treatments was significantly higher than in the drying treatments (Table 1). Cumulative respiration decreased significantly with increasing EC in both moisture treatments.

In the first 3 days after maintaining or changing the EC and rewetting the dry soil in the DRW treatments (days 12 to 14), respiration rates varied with initial and adjusted EC as well as with moisture treatment (CM or DRW) (Fig. 2 and Table 2). Respiration rates decreased with increasing adjusted EC in all soils and treatments. In the initially non-saline soil (EC0.1), the respiration flush upon rewetting of dry soil compared to the constantly moist soil occurred on day 13 (day 2 after rewetting) only when the EC was maintained at 0.1 dS m^{-1} (EC0.1-0.1). In this treatment, respiration rates decreased from day 12 to day 14 but remained significantly higher in the DRW compared to CM treatment on day 14 (Fig. 2A). Increasing the EC from 0.1 to 1.5, 2.5 or 3.5 dS m^{-1} reduced respiration rates in both moisture treatments. Respiration rates on days 12-14 did not differ between the two moisture treatments when the EC was increased. In the soil with an initial EC 1.5 dS m^{-1} (Fig. 2B), respiration rates on days 12- 14 in the DRW treatment were lower or similar to those in CM irrespective of whether EC was maintained (EC1.5-1.5) or increased to 2.5 and 3.5 dS m^{-1} (EC1.5-2.5, EC1.5-3.5). Respiration rates from day 12 to day 14 were low in the soil with an initial EC of 3.5 dS m^{-1} and did not differ between DRW and CM (Fig. 2C). Cumulative respiration at the end of the experiment (day 25) decreased with increasing initial and adjusted EC (Fig. 3). Only in the non-saline soil treatment where the EC was maintained (EC0.1-0.1), cumulative respiration in the DRW treatment was higher than in CM. When the EC was increased to 1.5, 2.5 or 3.5 dS m^{-1}, cumulative respiration was similar in the two moisture treatments. The decrease in cumulative respiration compared to EC0.1-0.1 with increasing adjusted EC was greater in DRW than in CM. In the soils with initial EC1.5 or 3.5 dS m^{-1} cumulative respiration did not differ between DRW and CM treatments irrespective of whether the EC was maintained or increased. The decrease in cumulative respiration compared to the soils where the EC was maintained (EC1.5-1.5 or EC3.5-3.5) was similar in DRW and CM.

3.2. Microbial biomass C

The microbial biomass C concentrations on day 0 (after 18 days pre-incubation) were 179, 148 and 60 mg kg^{-1} in soils with EC0.1, 1.5 and 3.5 dS m^{-1} respectively. Before drying (day 7) the microbial biomass C concentrations ranged between 438 and 246 mg kg^{-1}) and did not differ between EC levels. After onset of the two moisture treatments, microbial biomass C concentration did not differ significantly between moisture treatments or EC levels. After drying (day11) it ranged from 293 to 435 mg kg^{-1} in CM and from 229 to 398 mg kg^{-1} in the DRW treatment. At the end of the experiment (day 25), in both moisture treatments, the microbial biomass C concentration was higher in the treatments where the EC was maintained compared to those where the EC was increased (Table 3). In the soil with initial EC0.1 dS m^{-1}, the microbial biomass C concentration was higher in DRW compared to CM in all salinity treatments except in EC0.1-3.5. The microbial biomass C concentration was also higher in DRW compared to CM in the soil with initial EC1.5 dS m^{-1} when this EC was maintained (EC1.5-1.5), but did not differ between the two moisture treatments when the EC was increased. In the soil with initial EC3.5 dS m^{-1}, the microbial biomass C concentration did not differ between DRW and CM.

4. Discussion

This study showed that if rewetting of dry soil is accompanied by an increase in EC, the respiration flush usually observed upon rewetting of dry soil does not occur. With respect to cumulative respiration, exposure to DRW increased the sensitivity of the microbes to increasing EC compared to the constantly moist soils only in the originally non-saline soil whereas in the other two soils, increasing the EC reduced cumulative respiration to a similar extent in DRW and CM treatments. The effect of increasing EC on microbial biomass C did not differ between the two moisture treatments in all soils.

In the non-saline soil when the EC was maintained at 0.1 dS m^{-1}, there was a flush in respiration in the DRW treatment compared to the constantly moist soil on days 2 and 3 after rewetting (days 13 and 14) (Fig 2A). This increase in respiration rate upon rewetting has been shown in previous studies with non-saline and saline soils and explained by (i) release of osmolytes accumulated during the dry period (ii) lysis of microbial cells, and (iii) release of previously protected organic matter as a result of breakdown of aggregates [14-19]. The respiration flush after rewetting resulted in higher cumulative respiration in the DRW treatment compared to CM. Increased cumulative respiration in the DRW treatment compared to constantly moist soil has been reported before [11, 30, 31]. However, the respiration flush and increase in cumulative respiration in DRW compared to CM was only found when the EC was maintained (EC0.1-0.1). When the EC was increased to 1.5 and 2.5 dS m^{-1} (EC0.1-1.5, EC0.1-2.5), respiration rates in the first 3 days after rewetting and cumulative respiration at the end of the experiment did not differ between DRW and CM. These results confirm our first hypothesis (when rewetting is accompanied by an increase in salinity, the flush in respiration will be smaller than when salinity is not increased). However, in the soils with initial EC1.5 and 3.5 dS m^{-1}, a respiration flush upon rewetting and increased cumulative respiration in DRW compared to CM were not found even when the EC was maintained. Therefore our first hypothesis is true only for the initially non-saline soil. This suggests that salinity reduces the ability of microbes to utilise substrate released by rewetting of dry soil.

The effect of increasing salinity on soil microbes has been studied before but at constant moisture. Rousk et al. [38] reported that growth rates of bacteria from non-saline soil were similarly reduced by high salinity as those of bacteria from saline soils. Asghar et al. [39] and Yan and Marschner [40] also found that cumulative respiration at a given increased EC did not differ between soils which differed in original EC. On the other hand, Bååth et al.

[41] showed that in a short-term experiment, the growth rate of bacteria extracted from a saline soil was reduced to a smaller extent by addition of increasing NaCl concentrations compared to bacteria from normal agricultural soil. In the present study cumulative respiration at a given adjusted EC did not differ between soils with initially different EC, thus confirming the results of Rousk et al. [38], Asghar et al [39] and Yan and Marschner [40]. But we show for the first time that cumulative respiration at a given EC does not differ between soils with initially different EC when the increase in EC was accompanied by rewetting of dry soil.

Although cumulative respiration did not differ between DRW and CM when the EC was increased in the initially non-saline soil, the microbial biomass C concentration at the end of the experiment was greater in DRW than in CM. Cumulative respiration during dry phase was low (Table 1). Therefore the greater microbial biomass C concentration in the DRW treatment compared to CM is probably due to the lack of decomposition during the dry phase. More of the added pea straw would still be available towards the end of the experiment in the DRW treatment compared to CM where decomposition was not interrupted. The microbial biomass C concentration did not differ between the two moisture treatments in the two originally saline soils; at the end of the experiment they were generally lower than in the initially non saline soil. The lack of difference in microbial biomass C concentration between DRW and CM in these soils suggests that the higher EC limited the ability of the microbes to decompose the substrate remaining after the dry period.

In the initially non-saline soil, the decrease in cumulative respiration with increasing EC relative to the treatment where the EC was maintained (EC0.1-0.1) was greater in DRW compared to CM. However in the originally saline soils, the decrease in cumulative respiration with increasing adjusted EC did not differ between DRW and CM. Further, the reduction in microbial biomass C concentration with increasing EC was similar in the two

moisture treatments. Therefore our second hypothesis (drying and rewetting will increase the sensitivity of microbes to salinity) has to be rejected.

5. Conclusion

This study showed that when rewetting of dry soil was accompanied by an increase in EC a respiration flush did not occur and cumulative respiration was not increased compared to the constantly moist treatment. When the EC was maintained, a rewetting flush and increased cumulative respiration compared to the constantly moist soil was found only in the non-saline soil. This indicates that increasing salinity limits the ability of microbes in non-saline soils to utilise substrates released upon rewetting. This study also showed that drying and rewetting did not consistently increase the sensitivity of the microbes to salinity. This suggests that the impact of drying and rewetting on CO_2 release and soil organic C content observed in non-saline soils may differ from that in irrigated systems where saline water is used.

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Table 1 Cumulative respiration on days 7 [before drying in drying and rewetting (DRW) treatments] and 11 (after drying) in constantly moist or dried soils with initial EC 0.1, 1.5, 3.5 dS m^{-1} (n=3). Values at the same sampling date followed by different letters are significantly different ($P \leq 0.05$), with lower case letters for day 7 and upper case letters for day 11.

	Day 7	Day 11	
	Cumulative respiration (mg CO_2- C g^{-1} soil)		
Soils	Moist (M)	Moist (CM)	Drying (DRW)
EC0.1	2.07 c	1.05 D	0.13 B
EC1.5	1.60 b	0.4 C	0.06 AB
EC3.5	0.57a	0.13 B	0.01 A

Table 2 ANOVA results for respiration rates on days 12, 13 and 14.

Days		12	13	14
Source of variation	df	P	P	P
Soil salinity (EC)	8	***	***	***
Soil moisture (W)	1	**	ns	ns
EC x W	8	*	***	***

Asterisks indicate significant differences (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$), (ns) not significant.

Table 3 Microbial biomass C concentration on day 25 for constantly moist (CM) and drying and rewetting (DRW) treatments when the EC was maintained or increased for soils with initial $EC_{1:5}$ (A) 0.1 (B) 1.5 and (C) 3.5 dS m^{-1} (n=3). The osmotic potential refers to 75% of WHC. Values followed by different letters are significantly different ($P \leq 0.05$).

		Osmotic potential	Microbial biomass C (mg kg^{-1})	
Soil EC	Adjusted $EC_{1:5}$ (dS m^{-1})	(MPa)	CM	DRW
0.1	0.1	- 0.10	309 bcde	472 f
	1.5	-1.58	280 bcd	382 ef
	2.5	-2.64	207 abcd	366 ef
	3.5	-3.70	191 abc	284 bcde
1.5	1.5	-1.58	317 cde	351 cde
	2.5	-2.64	296 bcde	284 bcde
	3.5	- 3.70	279 abcde	233 abcde
3.5	3.5	- 3.70	234 abcde	138 ab
	4.5	- 4.76	182 abc	118 a

Day	1	7	8	11	12	25
CM	40% WHC		40% WHC		+ NaCl solutions or water to 75% WHC	
DRW	40% WHC		Drying		+ NaCl solutions or water to 75% WHC	

Fig. 1. Experimental design for constantly moist (CM) and drying and rewetting (DRW) treatments.Time is expressed as days after addition of pea residues.

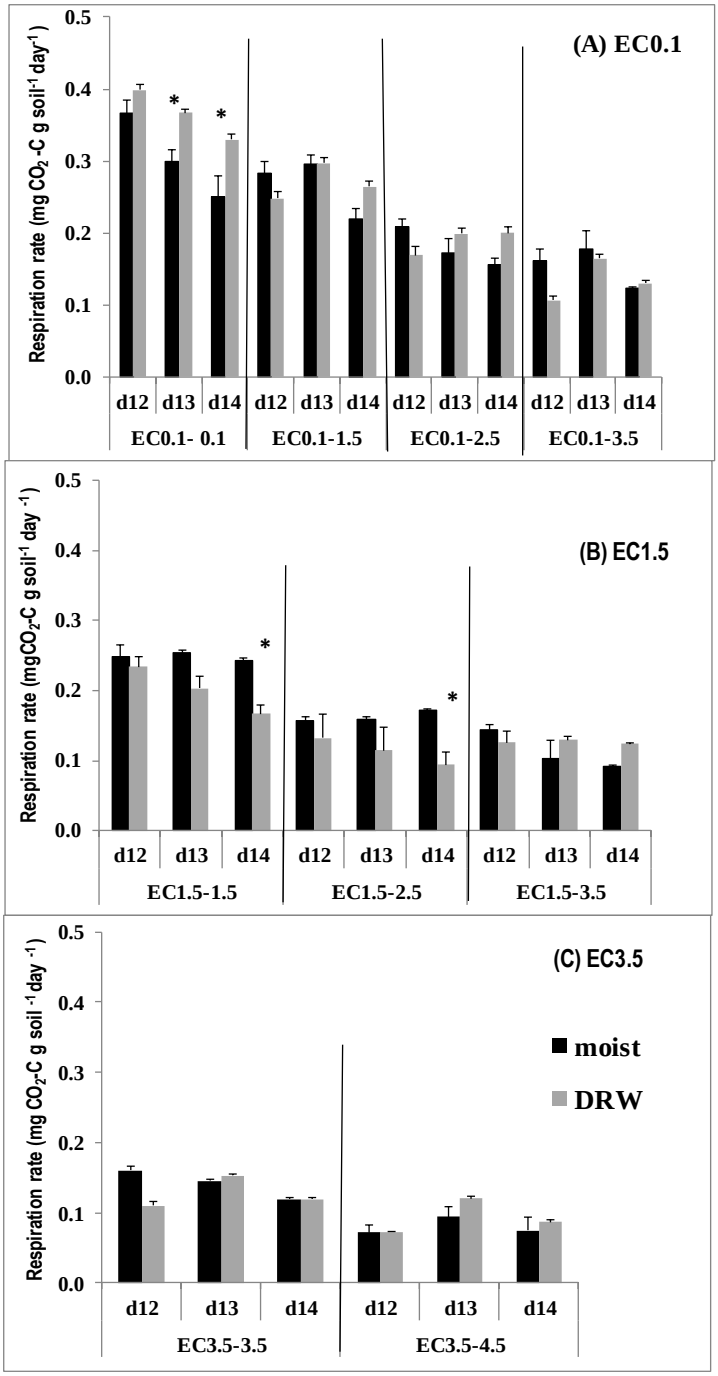

Fig. 2. Respiration rates on days 12, 13 and 14 corresponding to days 1, 2, and 3 after rewetting of dry soils for constantly moist (moist) and drying and rewetting (DRW) treatments when the EC was maintained or increased for soils with initial $EC_{1:5}$ (A) 0.1 (B) 1.5 and (C) 3.5 dS m^{-1}. Asterisks indicate significant differences ($P \leq 0.05$) between moisture treatments (n=3, vertical bars represent standard error).

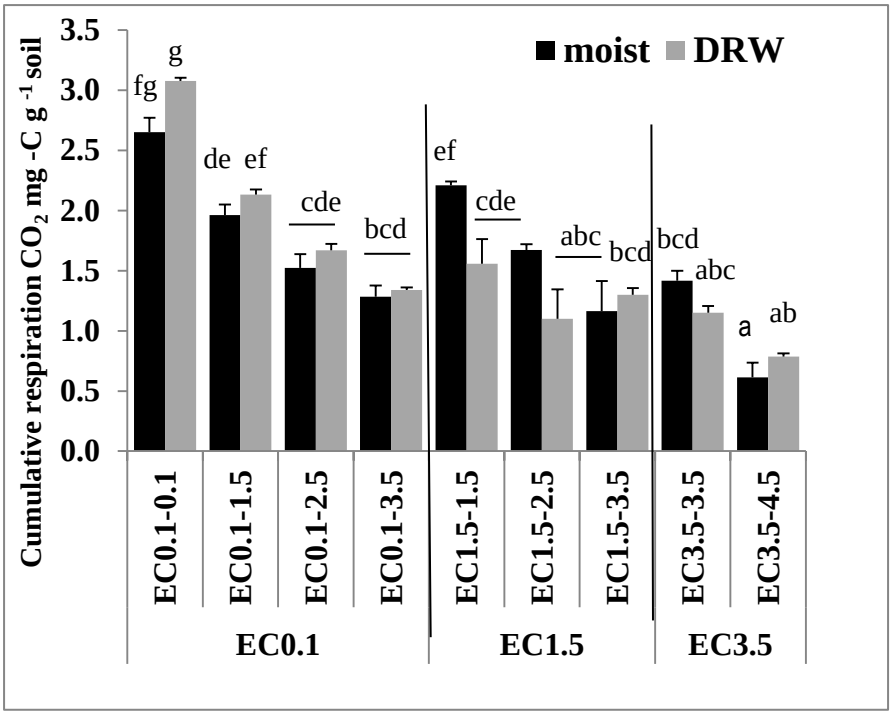

Fig. 3. Cumulative respiration on day 25 (14 days after rewetting and adjusting the EC) for constantly moist (moist) and drying and rewetting (DRW) treatments when the EC was maintained or increased for soils with initial EC (A) 0.1 (B) 1.5 and (C) 3.5 dS m^{-1} (n=3, vertical bars represent standard error, columns with different letters are significantly different $P \leq 0.05$).

CHAPER 8

CONCLUSION AND FUTURE RESEARCH

Conclusion and future research

Salt-affected soils are wide-spread particularly in arid and semi-arid regions where the rates of rainfall are inadequate to leach salt from the root zone. The high salt concentration in the soil solution of saline soils leads to poor crop growth and thus low organic carbon (OC) input. Therefore, amelioration of these soils is important to enhance crop growth and food production to counter the needs of growing population. The amelioration of saline soils could be by (1) supplying optimum rates of nutrients to crops (Lodhi et al., 2009), or (2) addition of OC to enhance soil physical, chemical and biological properties (Liang et al., 2005; Tejada et al., 2006). Several studies have shown that salinity had adverse effects on microbial activity and biomass (Ghollarata and Raiesi, 2007; Setia et al., 2011; Tripathi et al., 2006; Yan and Marschner, 2012; Yuan et al., 2007). Soil microbial activity plays a key role in nutrient turnover in the soil. Microbial adaptation to salinity requires synthesis and accumulation of osmolytes, which is very energy-demanding. Thus, the low OC content of saline soils could limit the ability of microbes to adapt to salinity. It is important to understand the effect of OC supply on microbial activity and microbial biomass in saline soils. A greater salinity tolerance would lead to increased organic matter decomposition and nutrient release which in turn will increase plant growth. The experiments presented in this thesis assessed the effect of addition of different OC sources on microbial activity and biomass in saline soils.

The results presented in Chapters 2, 3 and 4 showed that addition of soluble OC (glucose) increased microbial activity (respiration) and biomass in the first week in saline soils and reduced the negative effect of salinity on respiration. This indicates that addition of glucose reduced the adverse effect of salinity on microbes which could be due to microbes accumulating osmolytes required to maintain cell turgor and thereby metabolic activity (Hagemann, 2011; Oren, 2001). However, glucose is rapidly utilised and therefore depleted

leading to low activity and biomass after 2-3 weeks. In soil, a range of different OC compounds are present, some easily decomposable such as glucose, whereas others are difficult to decompose, such as cellulose. The impact of different C forms (cellulose and glucose) on the effect of salinity on respiration and biomass was investigated in Chapters 3 and 4.

In the experiment described in Chapter 3, glucose and cellulose were added to soil at the same C rate. As expected, respiration and biomass were lower with cellulose than with glucose. But the C form also influenced the effect of salinity on respiration. Whereas there was a gradual decrease in respiration with increasing salinity when glucose was the OC source, respiration decreased strongly from the non-saline to the saline soils with cellulose. This indicates that easily available OC can increase the capability of microbes to mitigate the negative impact of salt stress whereas poorly decomposable OC is less effective.

In soil, glucose and cellulose may be present at the same time, but in different proportions or may occur at different times. This was investigated in the studies described in Chapter 4. The treatments where only glucose or cellulose was present confirmed that microbes are less influenced by moderate salinity when supplied with glucose compared to cellulose. Mixing small amounts of glucose with cellulose increased growth and respiration, but also increased the negative effect of moderate and high salinity on respiration compared to cellulose alone. This indicates that small amounts of easily available C may increase the susceptibility of microbes to salinity. The experiment where C was added as glucose or cellulose every two weeks showed that increasing C supply over time reduces the negative impact of salinity on respiration. It also showed that addition of glucose after cellulose reduced the impact of salinity on microbial activity compared to the previous period with cellulose supply. In the experiments described in Chapters 2, 3 and 4 glucose and cellulose were used as model compounds representing easily and difficult decomposable C forms. But

the main form of C supply for microbes in soil apart from native organic matter are plant residues or root exudates, the effect of which was investigated in Chapters 5 and 6.

In the experiment described in Chapter 5, pea residue was added at two different rates every 2 weeks. Repeated residue addition to saline soils increased the activity and growth of soil microbes and reduced the negative impact of salinity on respiration. This confirmed the results of the experiments with glucose and cellulose showing that high availability of OC in the saline soils ameliorates the negative effect of salinity on microbial activity. Organic C addition has an ameliorative effect on soil microbes probably by providing them with energy needed for synthesising the organic osmolytes to adapt salt stress. Salinity reduced soil respiration to a lesser extent when the same amount of C added in two additions compared to a single addition. This suggests that frequent addition of small residues amounts may be particularly effective in ameliorating saline soils. The positive effect of residue addition was observed although salinity changed microbial community composition in amended soils. This suggests that the increased OC supply with residues enabled the development of a microbial community that was better able to adapt to salinity.

The experiment described in Chapter 6 was conducted to assess the response of soil microbes to increasing salinity in rhizosphere compared to bulk soil. As expected, cumulative respiration in rhizosphere and bulk soil decreased with increasing EC. The decrease in cumulative respiration with increasing EC across whole range of adjusted ECs was similar in both rhizosphere and bulk soil. But the decrease in cumulative respiration at the highest salinity relative to when the EC was not changed was smaller in rhizosphere compared to bulk soil which suggests that microbes in rhizosphere soil have a greater ability to adapt to osmotic stress than microbes in bulk soil due to higher substrate availability from root exudates in the rhizosphere. This study indicates that soluble C from root exudates can supply microbes with the energy needed for tolerance mechanisms.

Saline soils in Mediterranean climate may be exposed to drying and rewetting events through infrequent rainfall events or irrigation. Irrigation can increase salinity if the water quality is poor. The impact of drying and rewetting with saline water was investigated in the experiment described in Chapter 7. Non-saline and salinized soils were exposed to a drying period for 4 days then followed by an increase in EC to different levels at rewetting. The flush of respiration upon rewetting occurred only in the initially non-saline soil when the EC was maintained, but not when salinity was increased. In the initially saline soils, no respiration flush was found even if the EC was not increased. This suggests that the increase in salinity restricted the ability of microbes in non-saline or salinized soils to decompose organic substrates released upon rewetting. In addition, this study indicated that drying and rewetting did not increase the susceptibility of microbial activity and biomass to osmotic stress.

The results presented in this thesis will increase the knowledge of factors influencing microbial activity and biomass in saline soils. But there are some limitations in this research. The experiments in Chapters 2 and 3 used saline soils collected from the field. These soils did not differ only in salinity from non-saline but also had a lower organic matter content and lower N and P concentrations. Therefore it is not clear if the response in microbial biomass and activity with increasing salinity are due to salinity alone. They could also be a consequence of the lower organic matter and nutrient concentrations. Nevertheless the finding that microbial biomass and activity decreased with increasing salinity in the saline soils suggests that this is indeed a salinity effect as the saline soils had similar organic matter and nutrient concentrations. To avoid the differences in OM and nutrient concentrations among salinity levels, different amounts of salt were added to non-saline soil in the studies in Chapters 4, 5, 6 and 7. But addition of salt to a previously non-saline soil may not allow microbial community to adapt because of the rapid increase in salt concentrations and

therefore lead to overestimation the salinity effect (Chowdhury et al., 2011; Khan et al., 2008; Mavi and Marschner, 2012; Wong et al., 2008).

Recommendations for future research

The experiments presented in this research answered a number of questions regarding the effect of OC addition on microbial activity and biomass in saline soils. However, there are several unanswered questions that could be addressed in future research.

1- Most of experiments presented in this thesis were short term experiments (incubation period 3-6 weeks). Further studies could investigate the effect of addition of OC on microbial activity and biomass in saline soils in long term experiments (several months). This could be accompanied by field experiments where soils are irrigated with saline water.

2- In the experiments described in this study, only unlabelled OC forms (glucose and cellulose) were used. Therefore the source of the respired CO_2 and microbial biomass (added or native soil OC) could not be determined. Future experiments could use ^{14}C or ^{13}C labelled C (glucose, cellulose, residues) to differentiate between CO_2 and microbial biomass C from native and from added OC.

3- In most experiments described in this thesis, microbes were only exposed to osmotic stress because the water content (matric potential) was maintained at optimal levels. However, in the field microbes will be exposed to a combination of matric and osmotic potential as the water content of saline soils varies. The combined effect of osmotic and matric potential was studied by Setia and Marschner (2013) using one residue rate. Further studies could investigate the effect of different OC forms as they may influence the sensitivity of microbes to salinity and low soil water content.

4- Future research could investigate the impact of salinity on cellulase activity and abundance of fungi and cellulose utilising- bacteria in soils amended with cellulose by

measuring cellulase activity (Criquet, 2002) or using molecular methods. To determine abundance of cellulase-producing microbes, quantitative PCR targeted to cellulase genes, either based on DNA (presence) or RNA (expression). Community composition of cellulose degraders could be assessed by denaturing gradient gel electrophoresis (DGGE) or terminal restriction length polymorphism (TRFLP).

5- The experiment with rhizosphere and bulk soil indicated that activity of rhizosphere microbes may be less sensitive to high EC than that of microbes in bulk soil. But further studies are required to assess if this is generally the case. Rhizosphere soil from plants growing in patches of soil differing in salinity could be used in a similar manner as in the experiment described in this thesis. Additionally, plants grown in non-saline or saline soil could be irrigated with water differing in salinity and soil respiration measured in-situ (root and microbial respiration) or after removal of the rhizosphere soil. The controls would be unplanted soils irrigated with water of different salinity. Measurements of respiration could be accompanied by determination of microbial community composition using molecular methods to study the impact of salinity on rhizosphere and bulk soil microbial communities.

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