# Alleviating the negative effect of salinity on soil respiration by plant residue addition – effect of residue properties, mixing and amendment frequency

**HASBULLAH**

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**Grateful to God, the Almighty**

Dedicated to my little family

My wife Zatin
And my daughters Syadza and Adella

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# ABSTRACT

Salinity is a major constraint to crop production and also contributes to land degradation, particularly in arid and semiarid regions. Salinity has negative effects on soil microorganisms, reducing soil respiration, microbial biomass and microbial diversity. One of the main reasons for the negative impact of salinity is the low osmotic potential induced by high salt concentrations in the soil solution which reduces water uptake into cells and can cause water loss from cells. Some microorganisms can adapt to salinity by accumulation of osmolytes which is a significant metabolic burden. Rapidly decomposable plant residues contain high concentrations of easily available compounds which can be utilised by many soil microbes. Slowly decomposable residues on the other hand contain complex compounds which can only be utilised by few microbes, those capable of releasing specialised enzymes to break down these compounds. If salinity inhibits or kills some microbes, the decomposition of rapidly decomposable residues may be less affected than that of slowly decomposable residues because the loss of sensitive microbes can be compensated by a larger number of microbes with the former compared to the latter. If this is true, microbial activity after addition of slowly decomposable residues (high in lignin content and C/N ratio and low in water soluble carbon) should decrease more strongly with increasing salinity than after addition of rapidly decomposable residues. However, most previous studies on respiration in saline soils only used one or two types of plant residues (e.g. cereal or legume shoots). A further factor that may influence the impact of salinity on soil respiration is the frequency of residue addition. Frequent residue addition may provide soil microbes with a continuous supply of nutrients and therefore improve

salinity tolerance compared to a single addition where easily available compounds are rapidly depleted. These two assumptions have not been systematically investigated. The aim of this project was to investigate the effect of the chemical composition of added residues, mixing of residues and addition frequency on soil respiration and microbial biomass in soils with different salinity. Three studies were carried out to address the aims in non-saline soil and naturally saline soils with different salinity levels.

The aim of the first study was to investigate the impact of salinity on respiration in soil amended with residues differing in chemical composition (lignin concentration, water soluble organic carbon and C/N ratio). Three incubation experiments were conducted in this study. In the first experiment various residue types (shoots of wheat, canola, saltbush and kikuyu, saw dust, eucalyptus leaves) differing in C/N ratio, lignin and water extractable organic carbon concentration, were applied at 2% w/w to a non-saline soil ($EC_{1:5}$, 0.1 dS $m^{-1}$) and three naturally saline soils with $EC_{1:5}$ 1, 2.5 and 3.3 dS $m^{-1}$. Cumulative respiration decreased with increasing salinity but the negative effect of salinity was different among residues. Compared to non-saline soil, respiration was decreased by 20% at $EC_{1:5}$ 0.3 dS $m^{-1}$ when slowly decomposable residues (saw dust or canola shoots) were added, but at $EC_{1:5}$ 4 dS $m^{-1}$ when amended with a rapidly decomposable residue (saltbush). In the second experiment, the influence of residue C/N ratio and lignin content on soil respiration in saline soils was investigated. Two residues (canola and saw dust) with high C/N ratios but different lignin content were used. The C/N ratio was adjusted to between 20 and 80 by adding ammonium sulfate. Increasing salinity had smaller impact on cumulative respiration after addition of residues with C/N ratio 20 or 40 compared to residues

with higher C/N ratio. In the third experiment, the effect of the concentration of water-soluble organic C (WEOC) of the residues was determined. WEOC was partially removed by leaching from two residues with high WEOC content (eucalypt leaves and saltbush shoots). Partial WEOC removal decreased cumulative respiration in saline soil compared to the original residues, but increased the negative effect of salinity on respiration only with saltbush shoots.

The second study was conducted using the four soils from the first study ($EC_{1:5}$, 0.1, 1, 2.5 and 3.3 dS $m^{-1}$) to compare the impact of single and multiple additions of residues that differ in decomposability on the response of soil respiration to increasing salinity. Two residues with distinct decomposability were used; kikuyu with 19 C/N ratio (rapidly decomposable) and canola with 82 C/N ratio (slowly decomposable). Both residues were added once or 2-4 times (on days 0, 14, 28 and 42) with a total addition of 10 g C $kg^{-1}$ soil and incubated for 56 days. Compared to a single addition, repeated addition of the rapidly decomposable residue reduced the negative effect of salinity on cumulative respiration, but this was not the case with slowly decomposable residues.

The third study was carried out to investigate the effect of the proportion of rapidly and slowly decomposable residues in a mixture on the impact of salinity on soil respiration. This study included two experiments with two residues differing in decomposability (slowly decomposable saw dust and rapidly decomposable kikuyu grass). In the first experiment, both residues were added alone and in mixtures with different ratios into four soils having $EC_{1:5}$ 0.1, 1.0, 2.5 and 3.3 dS $m^{-1}$. The addition of 25% of rapidly decomposable residues in mixture with slowly decomposable residues was sufficient to decrease the negative impact of salinity on cumulative respiration

compared to the slowly decomposable residue alone. In the second experiment, three soils were used ($EC_{1:5}$ 0.1, 1.0 and 2.5 dS $m^{-1}$), residues were added once or 3 times (on days 0, 14 and 28) to achieve a total of 10 g C $kg^{-1}$ soil either with sawdust alone, kikuyu alone or both where final proportion of kikuyu was 25%, but the order in which the residues were applied differed  The negative effect of salinity on cumulative respiration was smaller when the rapidly decomposable residue was added early, that is when the soil contained small amounts of slowly decomposable residues. Salinity reduced soil respiration to a greater extent in treatments where rapidly decomposable residue was added to soil containing larger amounts of slowly decomposable residues.

It is concluded that rapidly decomposable residues can alleviate salinity stress to soil microbes even if they make up only a small proportion of the residues added. By promoting greater microbial activity in saline soils and providing nutrients, rapidly decomposable residues could also improve plant growth through increased nutrient availability.

# DECLARATION

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# CHAPTER 1:

# Introduction and review of literature

# CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

## 1.1 Introduction

Salt-affected soils can be found in all climate zones, but they are most wide-spread in arid and semi-arid regions (Rengasamy, 2006). Due to the low rainfall in these areas, leaching of the salts from the root zone is limited, resulting in the accumulation of soluble salts and affecting soil properties (Farifteh *et al.*, 2006). Salinity affects 5-9% of cultivated land (Pessarakli and Szabolcs, 1999; Lambers, 2003) induced most commonly by sodium salts. However, in some soils relatively high concentrations of other cations can also be found e.g.: calcium and magnesium. Sodium salinity is also a major problem in most saline soils in Australia, which comprise around 30% of the total land. Salt-affected soils can also be sodic, that is, characterised by a high proportion of sodium on exchange sites. Sodicity causes dispersion of soil particles and thus poor soil structure. Furthermore, Australian soils can also be saline-sodic (Rengasamy, 2006). Due to the large extent of salt-affected soils in many regions of the world it is important to better understand nutrient, and particularly organic carbon dynamics in these soils. Salinity affects soil organic matter (SOM) content and turnover in two ways: by reducing plant growth and therefore organic C input and by decreasing microbial activity and thus rates of organic matter decomposition and $CO_2$ release (Setia *et al.*, 2011a). The negative effect of salinity is due to (i) the low osmotic

potential of the soil solution which makes it more difficult for organisms to take up water and maintain cell turgor (Hagemann, 2011), and (ii) ion toxicity (Na and Cl) and imbalance (low K/Na ratio) (Marschner, 2012).

Salinity has been shown to decrease microbial activity (Tripathi *et al.*, 2006; Yuan *et al.*, 2007; Chowdhury *et al.*, 2011a; Setia *et al.*, 2011b), the size of the microbial biomass and may reduce microbial diversity (Rietz and Haynes, 2003). Salinity tolerant microbes may synthesize osmolytes to counteract the low osmotic potential of the soil solution (Hagemann, 2011). However, the synthesis of these osmolytes, e.g. polyols in fungi and amino acids in bacteria, requires large amounts of energy (Oren, 1999; Beales, 2004).

Organic matter addition can increase microbial activity in non-saline and saline soils (Chowdhury *et al.*, 2011a; de Souza Silva and Fay, 2012; Yan and Marschner, 2012) and reduce the negative impact of salinity on soil respiration. Decomposability of organic matter and therefore its availability as energy source for microbes is influenced by organic matter properties such as C/N ratio and lignin content (Abiven *et al.*, 2005; Xu *et al.*, 2006). Rapidly decomposable compounds can be utilised by many different microbial species, whereas slowly decomposable compounds such as cellulose and lignin are decomposed only by a small subset of the community (Berg and McClaugherty, 2008; Sinsabaugh, 2010; Nannipieri *et al.*, 2012). If a certain number of microbial species is killed by salinity, the relative decomposition rate of lignin-rich, high C/N ratio and low WEOC residues may decrease to a greater extent with increasing salinity than that of rapidly decomposable residues. However, most previous studies on decomposition in salt-affected soils used only one type of plant

residue (e.g. clover, cereal or legume shoots) (Nelson *et al.*, 1996; Chowdhury *et al.*, 2011a).

When plant residues are added to soil, the concentration of readily available organic carbon is initially high, but is rapidly depleted through decomposition until only the more recalcitrant compounds remain e.g.: lignin and cellulose (Abiven *et al.*, 2005; Fang *et al.*, 2005). If the supply of readily available organic carbon is more consistent via multiple additions, salinity tolerance might be improved.

Slowly decomposable residues such as sawdust or mature cereal straw are usually available at lower cost and in greater quantity than rapidly decomposable residues (legume shoots or young grass). The utilisation of mixtures of slowly and rapidly decomposable residues in salinity alleviation would be an economical option. The following literature review will discuss (a) the process of salinisation and properties of salt-affected soils, (b) salinity effects on plant growth and soil microbes, (c) soil organic C pools and nutrient turnover in salt affected soils, (d) functions of organic matter in soils, (e) the effect of residue properties on decomposition and nutrient release, and (f) the effect of residue mixing and multiple applications on decomposition.

## 1.2 Salinisation process and properties of salt-affected soils

Although the amount of salt from rainwater is low, over a long period of time large amounts of salt can accumulate in soil in the absence of leaching by fresh water. There are two main types of salinity: primary and secondary salinity (Richards, 1954). Primary salinity occurs when salt accumulates from rock weathering and rainfall. Secondary salinity is due to human activities such as the accumulation of salt caused

by irrigation with saline water or changing the water balance by land clearing (Ghassemi *et al.*, 1995). If the input of salt is greater than the loss through leaching, salt will be accumulated and salt-affected soils are formed (Rengasamy, 2006; Marschner, 2012).

Table 1. Properties of salt affected soils (for explanation of terms, see text below)

| **Soil** | **$EC_e$** (dS $m^{-1}$) | **pH** | **ESP** | **$SAR_e$** |
|---|---|---|---|---|
| **Non Saline** | <4 | <8.5 | <15 | <13 |
| **Saline** | >4 | <8.5 | <15 | <13 |
| **Saline sodic** | >4 | <8.5 | >15 | >13 |
| **Sodic** | <4 | >8.5 | >15 | >13 |

(Brady and Weil, 2008)

Salinity is measured as electrical conductivity (EC) of the soil solution (Marschner, 2012). Saline soils are soils with salt concentrations > 4 dS $m^{-1}$ in the saturated soil paste ($EC_e$), but their Sodium Adsorption Ratio ($SAR_e$) is less than 13 (Brady and Weil, 2008) and the pH of the saturated paste extract is below 8.5 (Table 1). SAR is calculated from soluble $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ concentrations in mmol $L^{-1}$ using the following equation:

$$SAR = \frac{[Na^+]}{\sqrt{([Ca^{2+}] + [Mg^{2+}])}}$$

Plant growth can be reduced at $EC_e$ 4 dS $m^{-1}$ (Ghassemi *et al.*, 1995; Brady and Weil, 2008; Marschner, 2012). However, this threshold depends on other factors, such

as climate, soil-water content and crop genotype (Maas, 1986). For example, the salt concentration in the soil solution of a dry soil is higher than in saturated soil (Rengasamy, 2002).

Salt-affected soils include saline-sodic and sodic soils. Saline-sodic soils have $EC_e$ > 4 dS $m^{-1}$, $SAR_e$ > 13 and pH < 8.5. Sodium salinity is the pre-dominant type of salinity and it can lead to the formation of saline-sodic and sodic soils (Rengasamy, 2006). In general, soils are initially saline and become sodic when sodium replaces calcium and magnesium from the cation exchange sites which causes clay particles to disperse (Rengasamy *et al.*, 1984). However, the high salt concentration in the soil solution of saline-sodic soils ($EC_e$> 4 dS $m^{-1}$ and SAR > 13) can prevent dispersion (Shainberg and Letey, 1984; Sumner and Naidu, 1998). In saline-sodic soils, the excess cations provided by salt move close to the negatively charged colloid surfaces , thereby reducing particle repulsion (Brady and Weil, 2008). When sodium leaches from the soil profile, saline soils can become sodic, that is $EC_e$ < 4 dS $m^{-1}$ but $SAR_e$ > 13 (Rengasamy, 2006). Sodicity can also be expressed in exchangeable sodium percentage (ESP). This value is calculated from the concentrations of exchangeable $Na^+$ in cmol (+) $kg^{-1}$ and cation exchange capacity (CEC) cmol (+) $kg^{-1}$ using the following formula:

$$ESP = \frac{100[\,Na_{ex}^{+}\,]}{CEC}$$

However, the definition of sodicity differs with classification system (Rengasamy, 2006). In Australia, a soil is classified as sodic when the ESP is > 6 (Isbell, 2002), while according to the U.S system sodic soils have ESP > 14 (Soil Survey Staff, 1994). Since it is laborious and time consuming to determine CEC for the calculation of ESP, SAR is

often used to determine sodicity. In 1:5 soil water extracts, the ESP can be calculated using the following equation (Rengasamy *et al.*, 1984):

$$ESP = 1.95 * SAR + 1.8$$

### 1.3 Salinity effects on plant growth and soil microbes

The growth of many plants is negatively affected at $EC_e$ > 4 dS $m^{-1}$ (Mooney, 1999). According to Munns and Tester (2008), there are two main factors for the negative impact of salinity on plants and soil microorganisms: osmotic stress and ion-specific stress. High salt concentration or low osmotic potential inhibit water uptake, nutrient absorption and even draws water out of cells causing dehydration (Tester and Davenport, 2003). At low water content, $EC_e$< 4 dS $m^{-1}$ can reduce plant growth because the concentration of the salts in the soil solution increases and correspondingly the osmotic potential decreases (Rengasamy, 2002; Chowdhury *et al.*, 2011b). Excessive uptake of $Na^+$ and or $Cl^-$ leads to ion toxicity (Munns and Tester, 2008). Ion imbalance can occur for example, when high $Na^+$ uptake reduces $K^+$ concentrations in cells which can reduce enzyme activity (Marschner 2012). These negative effects of salinity can reduce seed germination and density, plant growth and productivity or even cause plant death. It has been shown that salinity stress decreases wheat growth (Rengasamy, 2010) and yield (Richards, 1983; Bajwa *et al.*, 1986), maize and rice yield (Bajwa *et al.*, 1986). The reduced plant growth in saline soils results in low organic matter input into the soil (Rietz and Haynes, 2003). However, the effect of salinity depends not only on the concentration of the salts in the soil, but also other factors such as soil water content, soil texture and type of salt (Marschner, 2012).

High concentration of salts in the soil solution negatively influences not only plant growth, but also affects activity, structure and size of microbial communities and important biochemical processes involved in the turnover of soil organic matter (Pankhurst *et al.*, 2001; Rietz and Haynes, 2003; Chowdhury *et al.*, 2011a). It has been shown that microbial activity (soil respiration) decreases with increasing salinity (Wichern *et al.*, 2006; Chowdhury *et al.*, 2011a; Setia *et al.*, 2011c). Changes in microbial community composition are due to the different ability of microbial genotypes to tolerate salinity and low osmotic potential of the soil solution (Llamas *et al.*, 2008). For example, fungi which are particularly important for decomposition of cellulose and lignin, have been reported to be more sensitive to salinity than bacteria (Pankhurst *et al.*, 2001; Wichern *et al.*, 2006). Some microbes have the ability to tolerate or adapt to salinity, especially those which are regularly exposed to salinity (Sparling *et al.*, 1989). According to Killham (1994), there are two main strategies for microorganisms to adapt to osmotic stress (salinity, drought or freezing). Both strategies are based on the accumulation of cell solutes (so-called osmolytes) to counteract the low osmotic potential in the soil solution (Killham, 1994). The first mechanism is to exclude certain solutes such as $Na^+$ and $Cl^-$, and instead accumulate ions necessary for metabolic processes (e.g. $NH_4^+$). Microbes in very saline environments, such as salt lakes, may also accumulate ions such as $Na^+$ and $Cl^-$. However this requires enzymes tolerant to high concentrations of these ions. The second strategy is producing organic osmolytes. These strategies pose a metabolic burden for microorganisms and reduce energy available for growth. Changes in microbial activity and community composition have important implications for soil fertility and nutrient cycling and therefore plant growth in saline soils.

## 1.4 Soil organic carbon pools and nutrient turnover in salt affected soils

The soil contains the largest active C pool in terrestrial ecosystems. Globally, there is 1580 Pg C stored in soils, which is twice the amount of C in the atmosphere (750 Pg); compared to this there is only 610 Pg C stored in vegetation (Kimble and Stewart, 1995; Janzen, 2004). Hence, changes in soil organic matter (SOM) strongly affect atmospheric $CO_2$ concentrations (Lal *et al.*, 2007). Soils can be a source, but also a sink of $CO_2$ from the atmosphere by formation of stable SOM from plant material (Raich and Potter, 1995).

Soil organic carbon (SOC) can be divided into three main pools. The size of these pools depends on the amount of C input and environmental conditions such as climate and soil type. The active pool has a turnover time of weeks; it consists of readily oxidisable materials, such as microbial biomass and plant residues recently added to soil (Schnürer *et al.*, 1985). The slow pool, also referred to as humus, is the largest pool and contains moderately decomposable materials usually associated with macro and micro-aggregates and has a turnover time of decades (Parton *et al.*, 1987). The third pool is the passive pool, which has a turnover time of millions of years. This pool contains stable C which is resistant to further degradation (Schimel *et al.*, 1994). In Australian soils, this pool is mainly charcoal (Clough and Skjemstad, 2000; Lehmann *et al.*, 2008).

Salinity and sodicity affect SOC and SOM dynamics in two ways: (i) by reducing plant growth and therefore the input of organic matter (Setia *et al.*, 2011a) and (ii) inhibiting microbial activity and therefore organic matter decomposition.

## 1.5 Functions of organic matter in soils

Organic matter improves physical, chemical and biological properties of soils. The addition of organic matter to soils improves porosity (Pagliai *et al.*, 1995), particularly in clay soils. Furthermore, organic matter increases soil aggregate stability (Tisdall and Oades, 1982) and may reduce soil compaction by increasing the resistance to deformation and/or by improving elasticity (Soane, 1990). Organic matter also improves soil structure by increasing fungal and root growth which bind soil particles (Tisdall and Oades, 1982). Organic matter is also a nutrient source by binding cations and through mineralisation of organic nutrients (Nuruzzaman *et al.*, 2005; Hasbullah *et al.*, 2011).

Organic matter is very important for soil microbial activity. According to Swift et al. (1979), there are three major components of organic matter that are significant to decomposer organisms: energy source; nutrients other than C (nitrogen, phosphorus etc.); and modifiers such as polyphenolic compounds and amino acids, which are released in relatively low concentrations during decomposition of organic material and which regulate (stimulate or inhibit) the activity of decomposer organisms. The C in organic material is utilised by decomposer organisms as a source of energy (via respiration) and to build up biomass. This energy is stored in tissues in a variety of compounds, such as lipids, polysaccharides, proteins and aromatic polymers (Swift *et al.*, 1979).

According to Marschner and Rengel (2007), the decomposition of organic matter plays an important role in nutrient cycling. Microorganisms release inorganic nutrients that are available for plants and affect the availability of nutrients via

oxidation, solubilisation, reduction and chelation. They also regulate storage and release of nutrients in or from the microbial biomass and act as a regulator by release of substances that inhibit or stimulate plant growth.

### 1.6 Effect of residue properties on decomposition and nutrient release

The decomposition rate of residues depends on a number of factors, including residue properties and environmental factors. Baldock (2007) summarised that decomposition of organic matter depends on biochemical properties of the organic matter, accessibility and decomposer community composition. This review will focus on residue properties. Residue properties such as particle size and chemical composition including contents of lignin or hemicelluloses, water soluble C and C/N ratio are particularly important (Angers and Recous, 1997; Silver and Miya, 2001; Abiven *et al.*, 2005; Zhang *et al.*, 2008).

Residues with small particle size are decomposed faster than residues with large particle size (Bremer *et al.*, 1991; Angers and Recous, 1997) because they have a greater surface area/volume ratio and therefore greater accessibility to soil microbes (Angers and Recous, 1997).

Simple organic molecules e.g. glucose can be utilised by many microbes, but bacteria have only a limited ability to decompose complex compounds such as lignin because they do not produce the enzymes required for its break-down (Berg and McClaugherty, 2008). Many fungi on the other hand, release hydrolytic enzymes required for lignin breakdown, for example, white-rot fungi have the ability to mineralise lignin into $H_2O$ and $CO_2$ (Berg and McClaugherty, 2008). Residues with a high concentration of water soluble carbon and low lignin content and C/N ratio

decompose rapidly compared to residues with the opposite properties (Swift *et al.*, 1979; Tian *et al.*, 1992; Martens, 2000; Xu *et al.*, 2006). Elmajdoub and Marschner (2013) found that the negative impact of salinity on soil respiration was smaller when organic C was added as glucose compared to cellulose.

The concentration of water soluble C is an important residue property, especially in the early stages of decomposition process (Heal *et al.*, 1997). Previous studies reported that the initial C mineralisation rate was strongly dependent on the amount of initially present soluble C in plant residues (Trinsoutrot *et al.*, 2000; Shi and Marschner, 2014). This is because soil microbes can easily take up and break down soluble compounds such as amino acids and soluble sugars (Marschner and Noble, 2000). After depletion of the soluble compounds, complex compounds such as lignin remain (Abiven *et al.*, 2005) which control the fate of organic C in the medium to long-term (Heal *et al.*, 1997). It has been shown that organic matter with high concentrations of complex compounds (lignin or other polyphenols) decompose slowly (Vanlauwe *et al.*, 1996; Abiven *et al.*, 2005)..

Nitrogen is essential building block for amino acids and proteins and therefore critical for cell metabolism. Thus, plant residue N concentration is an important property not only for microbial activity and growth, but also for N mineralisation (Heal *et al.*, 1997). Plant residues with high N concentration (low C/N ratio) decompose faster than those with low N concentration because they provide microbes with sufficient N (Swift *et al,.* 1979; Tian *et al.*, 1992; Xu *et al.*, 2006 ). However, another study showed that decomposition of added plant residues depended on soluble C, cellulose and lignin content, but not on N concentration (Trinsoutrot *et al.*, 2000).

Many studies have been carried out in non-saline soils, but the relative importance of different residue properties for decomposition is not clear and likely to depend on soil properties (e.g. N availability) and decomposer community composition. However, there is little information about how residue decomposability affects the impact of salinity on soil microbial activity.

### 1.7 The effect of residue mixing and multiple applications on decomposition

In natural and agricultural ecosystems, residues from different plant species may be mixed and decomposed together. Decomposition of mixed residues may be increased, decreased or unchanged compared to that predicted based on the decomposition of single residues (Gartner and Cardon, 2004). Increased decomposition has been attributed to nutrient transfer, for example, N transfer from high N residues to residues with low N concentration (Handayanto *et al.*, 1997; McTiernan *et al.*, 1997; Wardle *et al.*, 1997); or increased diversity of the decomposer community as a result of residue diversity (Schweitzer *et al.*, 2005). A lower than predicted decomposition may be due to release of inhibitory compounds from one of the residue (Gartner and Cardon 2004). There is little information about the impact of salinity on microbial activity in soils amended with mixtures of residues with different properties.

In most of the studies on residue mixing, residues are added at the same time (Bonanomi *et al.*, 2010; Mao and Zeng, 2012; Shi *et al.*, 2012). Multiple additions of residues could be a strategy to manipulate soil respiration and nutrient availability. Recently Marschner *et al.* (2015) found in non-saline soil that nutrient release from low C/N residue following addition of high C/N residue was lower than that of low C/N following low C/N residue. Similarly, Carrillo et al. (2012) reported that available N

after a second addition of residues was influenced by the properties of the previously added residue. These studies were carried out in non-saline soil and less is known about decomposition and nutrient release when residues are added repeatedly to saline soil and how this influences the impact of salinity on microbial activity. Elmajdoub and Marschner (2015) showed that with the same total amount of C added, the effect of salinity on soil respiration was smaller with when a residue was added twice or three times compared to a single addition. In that study, one type of residue was added. It is not clear if this effect of repeated residue addition also applies to other residues or when different types of residues are added repeatedly.

### 1.8 Knowledge gaps and aims

High salt concentration in soil has detrimental impacts not only on crop growth and yield, but also on microbial activity. There are many studies on the impact of salinity on soil microbial activity, however, mostly focusing on soil properties; when amendments were applied only one or two types of organic amendments were used. Further, the effect of residue mixing or repeated residue addition on microbial activity in saline soils has not been considered. For this study, it was hypothesised that salinity has a smaller negative effect on soil respiration with addition of rapidly decomposable residues compared to slowly decomposable residues. And that the effect of residue decomposability on soil respiration in saline soils is modulated by residue addition frequency and mixing. Hence, the present study has the following aims:

a. To investigate the impact of salinity on respiration in soil amended with residues differing in chemical composition (lignin concentration, water soluble organic carbon and C/N ratio).

b. To compare the effect of single and multiple additions of residues differing in decomposability on the response of soil microbial activity to increasing salinity.
c. To determine the impact of the proportion of rapidly and slowly decomposable residues in a mixture on the effect of salinity on soil microbial activity.

## 1.9 Structure of the thesis

Chapter 1 consists of the introductory background and review of literatures on the issue. The structure of the following chapters (from Chapters 2 to 5) is shown in Figure 1.

Previous studies (Chowdhury *et al.*, 2011a; Yan and Marschner, 2012) showed that residue amendments can improve microbial activity in non-saline and saline soils and another study (Setia and Marschner, 2013) suggested that decomposability of residues affects the impact of salinity to soil respiration. However, in most studies only one or two residues were used and therefore provided little information about the influence of residue composition on the impact of salinity to soil respiration. The first study was conducted to investigate the influence of residue chemical properties on the impact of salinity to soil respiration (Chapter 2). In this study, various types of plant residues with different chemical composition were added once to a non-saline soil and soils with different levels of salinity. The study found that properties of plant residues such as lignin and water soluble C concentration and C/N ratio influence the response of microbial activity to increasing salinity.

Another factor affecting plant decomposition and could therefore influence the impact of salinity on soil respiration is repeated additions of residues that differ in

decomposability (Carrillo *et al.*, 2012; Marschner *et al.*, 2015). Hence, the objective of the study in Chapter 3 was to investigate the effect of addition frequency on the impact of salinity to microbial activity. In this study, two residues with distinct properties were chosen from the first study in Chapter 2 and added once, twice or three times to achieve a total addition of 10 g C $kg^{-1}$ soil. The results of this study suggest that repeated addition of rapidly decomposable residues reduced the negative effect of salinity on soil respiration compared to a single addition. Chapter 4 also covers the amelioration potential of multiple additions but with repeated addition of residues with different properties. The study found that 25% of a rapidly decomposable residue mixed with a slowly decomposable residue was sufficient to alleviate the negative effect of salinity compared to a single addition of slowly decomposable residues. The study also suggests that early addition of rapidly decomposable residues, that is when little slowly decomposable residue is present in the soil is more effective than late addition.

Finally, Chapter 5 summarizes the conclusion of the research chapters and gives suggestions for future studies.

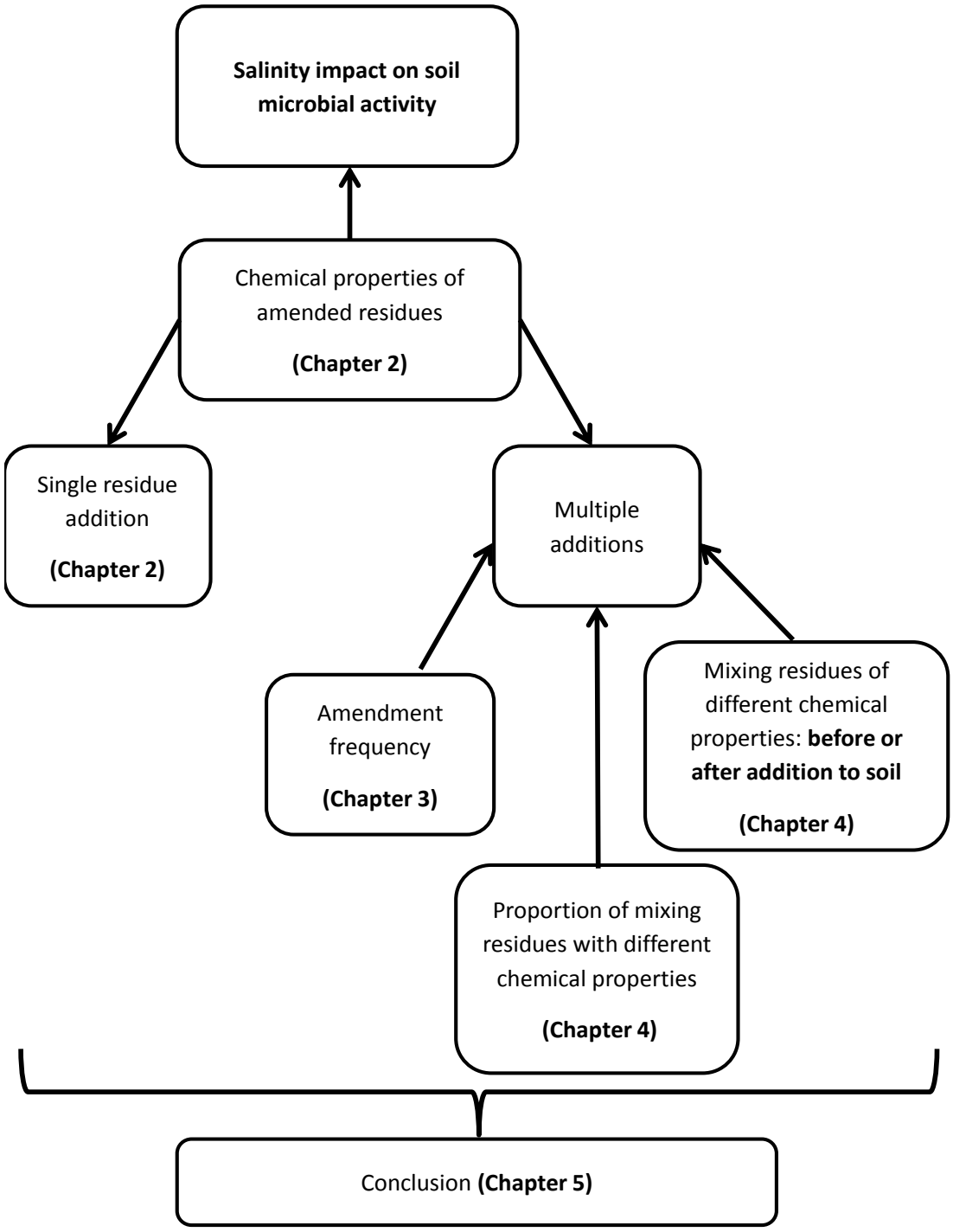


**Figure 1. Thesis structure**

## 1.10 References


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# CHAPTER 2:

# Manuscript 1

# Chapter 2

## Residue properties influence the impact of salinity on soil respiration

Hasbullah and Petra Marschner

School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, Australia.

Biology and Fertility of Soils 2015; **51**(1), 99-111, DOI: 10.1007/s00374-014-0955-2

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## Principal Author

| Name of Principal Author (Candidate) | Hasbullah |
|---|---|
| Contribution to the Paper | Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author. |
| Overall percentage (%) | 85% |
| Signature | Date: 24/07/2015 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| Name of Co-Author | Petra Marschner |
|---|---|
| Contribution to the Paper | Supervised development of work, data interpretation, manuscript evaluation |
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# CHAPTER 3:

# Manuscript 2

# Chapter 3

## Multiple additions of rapidly decomposable residue alleviate the negative impact of salinity on microbial activity

Hasbullah and Petra Marschner

School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, Australia.

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| | |
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| Contribution to the Paper | Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author. |
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## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| | |
|---|---|
| Name of Co-Author | Petra Marschner |
| Contribution to the Paper | Supervised development of work, data interpretation, manuscript evaluation |
| Signature | Date: 24/07/2015 |

# Multiple additions of rapidly decomposable residue alleviate the negative impact of salinity on microbial activity

Hasbullah Hasbullah, Petra Marschner
*School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia*

**Abstract**

Previously we found that the reduction in soil respiration with increasing salinity was smaller in soils amended with rapidly decomposable residues (high concentration of water extractable carbon, low C/N and lignin concentration) compared to slowly decomposable residues (low concentration of water extractable carbon, high C/N and lignin concentration). However, with a single residue addition, available organic carbon will be quickly decomposed until only recalcitrant compounds remain. A more consistent supply of residues may improve the tolerance of microbes to salinity, but this effect may depend on residue decomposability. A 56-day incubation experiment was conducted with four loam soils having $EC_{1:5}$ 0.1, 1, 2.7 and 3.7 dS $m^{-1}$ amended once or 2-4 times with finely ground slowly decomposable canola (C/N 82) and rapidly decomposable kikuyu residues (C/N 19) to achieve a total addition of 10 g C $kg^{-1}$ soil. At all salinity levels and with both residues, cumulative respiration two weeks after addition of 10 g C $kg^{-1}$ was higher with multiple additions compared to a single addition. Compared to a single addition, the reduction of cumulative respiration with increasing salinity was smaller with repeated addition of rapidly decomposable residue, but was this not the case with slowly decomposable residue.

Keywords: crop residues, decomposition, organic C availability, salinity

**Introduction**

Salinity is one of the major constraints to crop production and contributes to land degradation, particularly in arid and semiarid regions (Rengasamy, 2008). In Australia, salinity affects about 3 million hectares and 15 million hectares may become saline in the coming decade (Beresford, 2002). Salinity develops when salt loss by leaching is less than the salt addition from low quality irrigation or rainfall or is the result of rising of saline ground water (Hutson *et al.*, 1990; Pitman and Läuchli, 2002; Rengasamy, 2002). Salinity reduces plant growth by inhibiting water uptake through osmotic effects (Munns, 2002) and induction of ion imbalance or ion toxicity (Munns and Tester, 2008).

Salt accumulation in soil not only adversely affects plant growth and yield, soil physical and chemical properties but also soil microbes (Rietz and Haynes, 2003; Tejada *et al.*, 2006; Tripathi *et al.*, 2006). It can be detrimental for sensitive microorganisms and increases the metabolic load of surviving cells through energy required for tolerance mechanisms, such as accumulation of osmolytes (Oren, 1999; Hagemann, 2011). According to Oren (1999), synthesis of osmolytes requires up to four times more energy than cell wall synthesis. This suggests that supply of energy through addition of organic matter may reduce the negative impact of salinity on microbial activity.

It has been shown that organic matter amendment to saline soil improves chemical, physical and biological properties (El-Shakweer *et al.*, 1998). Soil respiration and microbial biomass are important indicators of soil quality and health (Kennedy and Papendick, 1995) because they are of microbial activity, organic matter

decomposition and nutrient release (Kennedy and Papendick, 1995; Schloter *et al.*, 2003). In a previous study we showed that plant residue addition reduced the negative impact of salinity on respiration, but the ameliorative effect depended on the properties of the organic amendment (Hasbullah and Marschner, 2014). The reduction in respiration with increasing salinity was smaller in soils amended with rapidly decomposable residues compared to slowly decomposable residues. This can be explained by the greater supply of energy for salinity tolerance mechanisms and growth from rapidly decomposing residues compared to slowly decomposing residues. When plant residues are added to soil, the supply of easily available organic C (e.g. sugars, organic acids, amino acids) is high initially, but decreases as they are decomposed until only slowly decomposable compounds such as cellulose and lignin remain (Abiven *et al.*, 2005; Fang *et al.*, 2005). A more consistent supply of easily available organic C through multiple residue additions may improve microbial salinity tolerance.

The aim of this study was to compare the effect of single and multiple additions of residues differing in decomposability on the response of soil respiration to salinity. We hypothesise that the reduction in soil respiration with increasing salinity is smaller with multiple additions of residues compared to a single addition. This hypothesis is based on the assumption that multiple additions provide a more consistent supply of rapidly decomposable organic C.

## Materials and methods

*Soils*

Soils were collected from 0-30 cm depth in Monarto, South Australia (35°05'S and 139°06'E) in areas with salinity patches which were recognisable by poor plant cover (Table 1). The four soils are of similar texture (loam), but differ in electrical conductivity (EC) as a measure of salinity; a non-saline soil $EC_{1:5}$ 0.14 dS $m^{-1}$ and three saline soils with $EC_{1:5}$ 1.3, 2.7 and 3.7 dS $m^{-1}$. This range of salinity was chosen based on previous studies (Chowdhury *et al.*, 2011; Mavi *et al.*, 2012) to induce a moderate to strong decrease in respiration by salinity. The saline soils were also sodic (exchangeable sodium percentage (ESP) >6 for Australian soils) (Isbell, 2002). In 1:5 soil-water extracts ESP can be calculated by following equation (Rengasamy *et al.*, 1984):

$$ESP = 1.95 * SAR + 1.8$$

The soils did not display dispersive behaviour associated with sodicity because the high salt concentration in the soil solution causes flocculation of soil particles (Shainberg and Letey, 1984; Sumner and Naidu, 1998). After collection, the soils were air-dried, sieved to < 2 mm and stored until being used for the experiment.

*Plant residues*

Two residues differing in decomposability were used: slowly decomposable mature canola shoots (*Brassica napus* L.) with high C/N ratio (C/N 82) and lignin content and rapidly decomposable young kikuyu shoots (*Pennisetum clandestinum* L.) with low C/N ratio (C/N 19) and lignin concentration (Table 2). The residues were dried, ground and sieved to particle size 0.25-2 mm. These residues were selected because in the previous study with a single residue addition to the same saline soils (Hasbullah and

Marschner 2014), the negative effect of salinity on soil respiration was smaller when amended with kikuyu compared to canola residues.

*Analyses*

Salinity was measured in a 1:5 soil: reverse osmosis (RO) water (w/w) extract ($EC_{1:5}$) after one hour horizontal shaking at 25°C. The EC of the saturated paste extract ($EC_e$) values were calculated from the $EC_{1:5}$ using the formula of Shaw et al. (1987):

$$EC_e = EC_{1:5}(-2.21 \times \%Clay^{0.5} + 23.78)$$

Soil particle size was assessed by the hydrometer method (Ashworth *et al.*, 2001) and particle size distribution was used for texture classification based on Saxton et al. (1986). Soil maximum water holding capacity (WHC) was measured using a sintered glass funnel connected to a 1 m water column ($\Psi_m$= -10 kPa) (Haines, 1930). The soils were placed in cores in the funnel, thoroughly wetted until saturated and allowed to drain for 48 hours. The drained soil was weighed before and after oven-drying at 105°C for 24 hours to determine the water content. Acid-soluble Al, Fe and K were analysed using ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) after extracting the soils with aqua-regia (0.1% nitric acid to concentrated hydrochloric acid at a 1:3 ratio) as described in Zarcinas et al. (1996). Available P was extracted using the Colwell method and measured colorimetrically at 882 nm (Rayment and Higginson, 1992). Soil inorganic N was extracted in 1 M KCl; ammonium and nitrate concentrations were determined using standard colorimetric methods (Keeney and Nelson, 1982). Soluble $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ concentrations were extracted by one hour horizontal shaking of a suspension with 1:5 soil: water ratio and filtering through Whatman filter No. 42. Element concentrations in the extracts were

measured by ICP-AES. Soluble $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ concentrations in mmol $L^{-1}$ were used to calculate the sodium adsorption ratio ($SAR_{1:5}$) using the following equation:

$$SAR = \frac{[Na^+]}{\sqrt{([Ca^{2+}] + [Mg^{2+}])}}$$

The Walkey and Black's method (1934) was used to measure total organic C concentration in soils and residues according to the following formula which assumes that 1 ml $K_2Cr_2O_7$ oxidizes 3 mg C:

$$\% \text{ organic C} = \frac{3\,(B-T)}{BW} \times \frac{V}{10}$$

Where B: ml ferrous ammonium sulfate (FAS) for blank, T: ml FAS for sample, W: weight of the soil in g and V: volume of $K_2Cr_2O_7$ in ml.

Total N in soils and residues was determined by the Kjeldahl method (Bradstreet, 1965). Water extractable organic carbon (WEOC) of residues was measured by shaking 0.5 g ground residues in 30 ml RO water for 1 hour after which the extract was filtered through a Whatman no. 42 filter. The organic C concentration in the extract was determined as described in Anderson and Ingram (1993) by digesting with 0.0667 M $K_2Cr_2O_7$ and concentrated $H_2SO_4$ and titrating the remaining dichromate with 0.033 M acidified $(NH_4)_2Fe(SO_4)_26H_2O$.

Soil respiration was determined by measuring the $CO_2$ concentration daily in the headspace of the jars using a Servomex 1450 infra-red gas analyser as described in Setia *et al.* (2011a). After each measurement (tm), the jars were vented to refresh the headspace, 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 tm and t0. Linear regression based on injection of known amounts of $CO_2$ into empty jars was used to define the relationship between $CO_2$ concentration and detector reading. Cumulative respiration was calculated as the sum of respiration rates [in mg $CO_2$-C (g soil and day)$^{-1}$] over 14 days following mixing with or without residue addition.

Microbial biomass C (MBC) on day 56 was determined by fumigation extraction (Vance *et al.*, 1987) as modified by Anderson and Ingram (1993). Fumigated and non-fumigated soil was extracted with 0.5 M $K_2SO_4$ at 1: 4 ratio. Organic C in the extracts was determined as described above for WEOC. Microbial biomass C was calculated by subtracting the OC concentration in the fumigated sample from that of the un-fumigated sample and multiplying the difference by 2.64 (Vance et al. 1987).

Nuclear magnetic resonance (NMR) spectroscopy was used to determine the composition of C compounds in the residues. The spectra were obtained on a Varian Unity 200 NMR spectrometer at a $^{13}C$ resonance frequency of 50.3 MHz. The residue samples were packed in a cylindrical zirconia rotor and spun at 5000 ± 100 Hz in a Doty Scientific magic angle-spinning (MAS) probe. The spectra were integrated into eight chemical shift regions for the following C-types: Alkyl (0 - 45 ppm), N-Alkyl/Methoxyl (45 - 60 ppm), O-Alkyl (60 - 95 ppm), Di-O-Alkyl (95 - 110 ppm), Aryl (110 - 145 ppm), O-Aryl (145 - 165 ppm), Amide/Carboxyl (165 - 190 ppm) and Ketone (190 - 215 ppm). For further details about the NMR analysis see Baumann *et al*. (2009). The molecular mixing model of Baldock et al. (2004) was used to calculate the percentage of biomolecules (carbohydrate, protein, lignin lipid and carbonyl) from $^{13}C$ NMR results.

*Experimental design*

The soils were pre-incubated at 55% of maximum water holding (WHC) capacity for 14 days at 19-23 ˚C, to stabilise respiration after the initial respiration flush upon rewetting of the air-dry soil. This water content was chosen because it is optimal for respiration in soils of this texture (Setia *et al.*, 2011b). Reverse osmosis water was used to adjust the water content. After the pre-incubation, the two residues were added between one and four times with a total organic C addition rate of 10 g C $kg^{-1}$ (approximately 20 g residue $kg^{-1}$). At the defined times (see Table 3), residues were mixed into each core; when no residues were added, the soils were mixed in a similar manner. This experimental design allowed comparison of frequency of residue addition. For example, 10 g C had been added in treatments K10-0-0-0 on day zero; in treatment K5-5-0-0 residues were at a rate of 5 g C $kg^{-1}$ on days 0 and 14 therefore a total of 10 g C had been applied on day 14. In K5-2.5-2.5-0 and K2.5-2.5-2.5-2.5a total of 10 g C had been applied on days 28 and 42.

On day 0, soil (equivalent to 25 g moist soil) was mixed with residues and then filled into PVC cores with 1.85 cm radius, 5 cm height 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 1 L Mason glass jars with gas tight lids equipped with septa to allow quantification of headspace $CO_2$ concentration. The jars were kept at room temperature (19-23 ˚C) in the dark. Soil moisture was maintained at 55% of WHC by checking the water content every few days by weight and adding RO water if necessary. Respiration was measured continuously over 56 days and microbial biomass carbon (MBC) was determined at the end of the experiment.

*Statistical analysis*

There were four replicates per treatment. Cumulative respiration and MBC concentration on day 56 were normally distributed and analysed by two-way ANOVA with salinity and residue treatment as fixed factors using Genstat 15$^{th}$ edition (VSN Int. Ltd, UK). Tukey's multiple comparison test at 95% confidence interval was used to determine significant differences among treatments. Regression was used to characterise the relationship between salinity and cumulative respiration in percentage of the non-saline soil for the 14 days after a total of 10 g C kg$^{-1}$ had been added in Excel 2010 (Microsoft, Redmond, WA, United States). This 14-day period was day 0-14 in K10-0-0-0 on day one, day 14-28 in K5-5-0-0, day 28-42 in K5-2.5-2.5-0 and day 42-56 in K2.5-2.5-2.5-2.5. The regression equations were used to calculate percent decrease of cumulative respiration at $EC_{1:5}$ 2 dS m$^{-1}$ compared to the non-saline soil. This salinity level was chosen because in canola amended soils, the greatest decrease in percentage respiration occurred at salinity levels below $EC_{1:5}$ 2 dS m$^{-1}$. At higher salinity, the decrease in respiration with increasing salinity was smaller. Therefore the decrease in percentage respiration at $EC_{1:5}$ 2 dS m$^{-1}$ is a suitable measure for sensitivity to salinity.

**Results**

*Cumulative respiration*

Cumulative respiration on day 56 was higher with kikuyu than with canola at all salinity levels (Figure 1). Among multiple additions, cumulative respiration was highest when residues were added three times (treatment 5-2.5-2.5-0). In canola-amended soils, irrespective of residue addition frequency, cumulative respiration was highest in the

non-saline soil and decreased with increasing salinity until soil EC 2.7, but did not decrease further in soil EC 3.7. With kikuyu, cumulative respiration on day 56 was lower than in the non-saline soil only at in soil EC 3.7 when residues were added once (K10-0-0-0) or twice (K5-5-0-0), but was not influenced by salinity when residues were added three or four times (K5-2.5-2.5-0 and K2.5-2.5-2.5-2.5). Two weeks after the soil was amended with 10 g C $kg^{-1}$ (day 14 28, 42, 56 in treatments K10-0-0-0, K5-5-0-0, K5-2.5-2.5-0 and K2.5-2.5-2.5-2.5, respectively) cumulative respiration was lower with a single addition compared to multiple additions of kikuyu.

In canola amended soils, there was a strong negative logarithmic relationship between salinity and cumulative respiration in percentage of the non-saline soil two weeks after 10 g C $kg^{-1}$. This relationship was linear and weaker in soils with kikuyu (Figure 2). With canola, the strongest reduction of cumulative respiration with increasing salinity was from non-saline soil to soil EC 1.3. The decrease in cumulative respiration with increasing salinity was not affected by canola residue addition frequency. With kikuyu, cumulative respiration in percentage of the non-saline soil decreased more strongly with increasing salinity in the treatment where kikuyu been added only once (10-0-0-0) than when it was added twice (5-5-0-0) and least when it was added three times (5-2.5-2.5-0) (Figure 2).

Using the equations in Table 4, it was calculated that cumulative respiration at $EC_{1:5}$ 2 dS $m^{-1}$ would be reduced by 46-59% compared to non-saline soil in all canola treatments (Table 4). With kikuyu, the decrease in cumulative respiration compared to the non-saline soil was 17% in K10-0-0-0, 6-7% in K5-0-2.5-2.5 and K5-5-0-0, but only 3% in K2.5-2.5-2.5-2.5.

*Microbial biomass carbon*

The MBC concentration in the non-saline soil on day 56 tended to be lower with a single residue addition (10-0-0-0) or residues added twice (5-5-0-0) than with more frequent residue additions (5-2.5-2.5-0 or 2.5-2.5-2.5-2.5) (Table 5). In the non-saline soil, the MBC concentration was higher with kikuyu than with canola in treatments with a single and three times addition. But in the saline soils in a given residue amendment treatment, the MBC concentration was similar with canola and kikuyu. With kikuyu, the MBC concentration was lower in soil EC 3.7 than in non-saline soil.

**Discussion**

This study showed that compared to a single addition, multiple addition of rapidly decomposable residue (kikuyu) reduced the negative effect of salinity on soil respiration, but this was not the case for the slowly decomposable canola (Figure 1). Therefore our hypothesis (the reduction in soil respiration with increasing salinity will be smaller with multiple additions of residues compared to a single addition) can only be confirmed for the rapidly decomposable residue.

The results of this experiment are in agreement with our earlier study Hasbullah and Marschner 2014) as amendment with rapidly decomposable kikuyu reduced the negative effect of salinity on respiration compared to the difficult decomposable canola (Figure 1,). The greater decomposability of kikuyu compared to canola can be explained firstly, by the higher concentration of water-extractable organic C which can be easily taken up by microbes. Decomposition of more complex C compounds requires greater energy for synthesis and release of enzymes (Liang *et al.*, 1996; Uchida *et al.*, 2012). Secondly, the higher N concentration in kikuyu could

improve synthesis of osmolytes many of which are N-rich (Warren, 2013), for example proline and glycine betaine (Singh *et al.*, 1996; Robert *et al.*, 2000; Saum and Müller, 2007).

With both residues and at all salinity levels, cumulative respiration two weeks after addition of 10 g C $kg^{-1}$ was higher with multiple compared to a single addition (Figure 1). This is likely due to the more continuous supply of readily decomposable C with the former. The effect of multiple residue additions on respiration was greater in the non-saline soil and saline soils up to soil EC 2.7 than in the most saline soil. This suggests that at $EC_{1:5}$ 3.7 dS $m^{-1}$ salinity stress limited the ability of microbes to fully take advantage of the more continuous C supply.

With the slowly decomposable canola, multiple residue additions did not change the response of total cumulative respiration to salinity. This low concentration of easily available (water-extractable organic C) in canola (Table 2) which would be depleted within a few days. Kikuyu had a four times higher concentration of water extractable organic C (Table 2) which would provide a longer lasting source of easily available C. With kikuyu, cumulative respiration at the end of the experiment decreased less with increasing salinity with multiple additions compared to a single addition, and among multiple addition treatments the decrease was smaller with three and four additions compared to two (Figure 1). The beneficial effect of multiple additions of kikuyu was also evident when cumulative respiration in the two weeks after addition of 10 g C $kg^{-1}$ was compared (Figure 2). The reduction of cumulative respiration in percentage of non-saline soil was smaller with multiple compared to a single addition.

The MBC concentration at the end of the experiment was less influenced by salinity and residue treatments than cumulative respiration (Table 4, Figure 1). This can be explained by the nature of the measurements. Microbial biomass determination was at a single time point (end of the experiment), whereas cumulative respiration integrates respiration over longer periods of time and includes the higher respiration immediately after residue addition. In general, the MBC concentration tended to be lower with a single or two additions compared to three or four additions (Table 4). This is likely due to the shorter period of time between MBC determination and the last residue addition: 2-4 weeks with three or four additions compared to 6-8 weeks with a single or two additions. The greater amount of remaining residue after the shorter time would be able to support a greater microbial biomass.

**Conclusion**

This study showed that repeated addition of residues can reduce the negative impact of salinity on soil respiration, but only when rapidly decomposable residues are used. In the field, a single residue addition may be more cost effective than repeated additions. Our results indicate that in moderately saline soils, a single addition of rapidly decomposing residues can minimise the negative effect of salinity on microbial activity. However, in more saline soils (in this study $EC_{1:5}$ 3.7 dS $m^{-1}$), multiple additions of rapidly decomposing residues may be required to reduce the negative impact of salinity on microbial activity.

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Table 1. Physical and chemical properties of a non-saline and three saline soils.

| **Parameter**[1] | **$EC_{1:5}$ (dS $m^{-1}$)**[2] | | | |
|---|---|---|---|---|
| | **0.14** | **1.3** | **2.7** | **3.7** |
| $EC_e$ (dS $m^{-1}$) | 2.5±0.02 | 13.9±0.45 | 34.7±1.67 | 44.5±1.08 |
| Sand (%) | 50 | 45 | 47.5 | 47.5 |
| Silt (%) | 35 | 35 | 32.5 | 32.5 |
| Clay (%) | 15 | 20 | 20 | 20 |
| Texture | Loam | Loam | Loam | Loam |
| Water holding capacity (%) | 32 | 37 | 39 | 44 |
| pH | 7.8±0.10 | 9.0±0.06 | 8.7±0.06 | 9.0±0.04 |
| Total organic carbon (%) | 1.2±0.04 | 1.1±0.02 | 0.9±0.03 | 0.7±0.03 |
| Colwell P (mg $kg^{-1}$) | 70±1.13 | 24±0.66 | 25±0.76 | 17±0.19 |
| Total N (g $kg^{-1}$) | 1.5±0.2 | 1.0±0.1 | 1.0±0.1 | 0.9±0.1 |
| **Acid soluble elements** | | | | |
| P (mg $kg^{-1}$) | 848±74 | 423±8 | 318±19 | 279±7 |
| K (g $kg^{-1}$) | 5.7±0.6 | 6.1±0.2 | 6.5±0.3 | 7.0±0.1 |
| Al (g $kg^{-1}$) | 32.4±1.5 | 39.6±0.1 | 40.0±1.2 | 38.3±0.7 |
| Fe (g $kg^{-1}$) | 25.9±1.7 | 31.7±0.2 | 33.0±0.6 | 30.7±1.5 |
| **Available nitrogen** | | | | |
| $NH_4$-N (mg $kg^{-1}$) | 40.1±1.0 | 43.7±0.8 | 41.4±0.9 | 46.7±0.3 |
| $NO_3$-N (mg $kg^{-1}$) | 8.5±0.1 | 8.7±0.1 | 2.7±0.1 | 1.3±0.1 |
| **Soluble cations (1:5 soil: water extract)** | | | | |
| Ca (mmol $L^{-1}$) | 0.51±0.01 | 0.49±0.02 | 0.96±0.03 | 0.44±0.04 |
| Na (mmol $L^{-1}$) | 0.22±0.01 | 9.79±0.31 | 21.31±0.62 | 30.01±0.001 |
| Mg (mmol $L^{-1}$) | 0.22±0.001 | 0.29±0.018 | 0.93±0.03 | 1.01±0.007 |
| $SAR_{1:5}$ | 0.3 | 11.1 | 15.5 | 24.9 |
| $ESP_{1:5}$[3] | 2.4 | 23.4 | 32.0 | 50.4 |

[1] for most variables n=3 ± standard deviation except texture, water holding capacity, SAR and ESP.

[2] EC of 1:5 soil: water extract.

[3] ESP values calculated from SAR values (Rengasamy *et al.*, 1984).

Table 2. Properties of canola and kikuyu shoots (n=3 ± standard deviation)

| **Plant residues** | **Canola shoots** | **Kikuyu shoots** |
|---|---|---|
| C (g $kg^{-1}$) [1] | 388±49.4 | 360±3.46 |
| N (g $kg^{-1}$) | 4.7±0.50 | 19.2±0.71 |
| C/N ratio | 82 | 19 |
| Water extractable C (g $kg^{-1}$) | 10.2±2.5 | 37.9±0.5 |
| Carbohydrate (%)[1] | 66.2 | 63.1 |
| Carbonyl (%)[1] | 2.8 | 2.1 |
| Lipid (%)[1] | 0.7 | 5.6 |
| Protein (%)[1] | 4.4 | 15.8 |
| Lignin (%)[1] | 25.1 | 9.9 |

[1] Concentrations calculated from $^{13}C$ NMR results using a molecular mixing model (Baldock *et al.*, 2004)

Table 3. Organic C addition treatments

| Residues | Treatment | Day | | | |
|---|---|---|---|---|---|
| | | 0 | 14 | 28 | 42 |
| | | C addition rate (g C $kg^{-1}$) | | | |
| Canola | C10-0-0-0 | 10 | 0 | 0 | 0 |
| | C5-5-0-0 | 5 | 5 | 0 | 0 |
| | C5-2.5-2.5-0 | 5 | 2.5 | 2.5 | 0 |
| | C2.5-2.5-2.5-2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Kikuyu | K10-0-0-0 | 10 | 0 | 0 | 0 |
| | K5-5-0-0 | 5 | 5 | 0 | 0 |
| | K5-2.5-2.5-0 | 5 | 2.5 | 2.5 | 0 |
| | K2.5-2.5-2.5-2.5 | 2.5 | 2.5 | 2.5 | 2.5 |

Table 4. Regression equations for the relationship between salinity and cumulative respiration over 14 days after a total of 10 g C $kg^{-1}$ soil had been added in saline soils in percentage of the non-saline soil for soils with single and multiple amendments of canola and kikuyu residues. And calculated percentage decrease in cumulative respiration (y) at $EC_{1:5}$ 2 dS $m^{-1}$.

| | Residue addition treatment[1] | Two week-period after a total addition of 10 g C $kg^{-1}$ soil | Equations | $R^2$ | Percentage decrease in cumulative respiration at $EC_{1:5}$ 2 dS $m^{-1}$ compared to non-saline soil |
|---|---|---|---|---|---|
| Canola | C10-0-0-0 | 0-14 | $y = -22.7\ln(EC_{1:5}\ 2)+57.27$ | 0.98 | 59 |
| | C5-5-0-0 | 15-28 | $y = -18.26\ln(EC_{1:5}\ 2)+66.765$ | 0.91 | 46 |
| | C5-2.5-2.5-0 | 43-56 | $y = -21.69\ln(E\ EC_{1:5}\ 2)+ 57.216$ | 0.94 | 58 |
| | C2.5-2.5-2.5-2.5 | 29-42 | $y = -22.43\ln(EC_{1:5}\ 2)+56.316$ | 0.96 | 59 |
| Kikuyu | K10-0-0-0 | 0-14 | $y = -9.8246\ EC_{1:5}\ 2+103.09$ | 0.78 | 17 |
| | K5-5-0-0 | 15-28 | $y = -5.0428\ EC_{1:5}\ 2+103.03$ | 0.60 | 7 |
| | K5-2.5-2.5-0 | 43-56 | $y = -0.2605\ EC_{1:5}\ 2+94.489$ | 0.0006 | 6 |
| | K2.5-2.5-2.5-2.5 | 29-42 | $y = -1.1868\ EC_{1:5}\ 2+99.167$ | 0.49 | 3 |

[1]For treatment details see Table 3

Table 5. Microbial biomass C on day 56 in non-saline soil ($EC_{1:5}$ 0.1 dS $m^{-1}$) and saline soils ($EC_{1:5}$ 1.3, 2.7 and 3.7 dS $m^{-1}$) with single and multiple amendments of canola and kikuyu residues at 10 g C $kg^{-1}$ soil. Values followed by different letters are significantly different ($p \leq 0.05$)(n=4± standard error)

| EC | Treatment[1] | Microbial biomass C (g $kg^{-1}$) |
|---|---|---|
| Non-saline | C10-0-0-0 | 14.6±3.67 a |
| | C5-5-0-0 | 37.6±13.7 abcd |
| | C.5-2.5-2.5-0 | 71.0±5.4 abcdef |
| | C2.5-2.5-2.5-2.5 | 75.4±2.8 abcdef |
| | K10-0-0-0 | 101.7±4.0 defgh |
| | K5-5-0-0 | 93.9±19.9 cdef |
| | K.5-2.5-2.5-0 | 175.0±30.2 g |
| | K2.5-2.5-2.5-2.5 | 134. 9±13.5 fg |
| EC 1.3 | C10-0-0-0 | 51.1±16.2 abcd |
| | C5-5-0-0 | 16.5±5.1 a |
| | C.5-2.5-2.5-0 | 27.1±11.5 abc |
| | C2.5-2.5-2.5-2.5 | 78.8±10.4 abcdef |
| | K10-0-0-0 | 64.1±7.7 abcdef |
| | K5-5-0-0 | 90.7±13.1 bcdef |
| | K.5-2.5-2.5-0 | 73.9±18.0 abcdef |
| | K2.5-2.5-2.5-2.5 | 130.0±14.5 efg |
| EC 2.7 | C10-0-0-0 | 18.3±3.5 ab |
| | C5-5-0-0 | 39.3±10.5 abcd |
| | C.5-2.5-2.5-0 | 60.9±2.3 abcdef |
| | C2.5-2.5-2.5-2.5 | 47.1±23.0 abcd |
| | K10-0-0-0 | 68.5±6.3 abcdef |
| | K5-5-0-0 | 59.8±2.0 abcde |
| | K.5-2.5-2.5-0 | 67.3±3.9 abcdef |
| | K2.5-2.5-2.5-2.5 | 106.6±24.9 defg |
| EC 3.7 | C10-0-0-0 | 36.1±15.9 abcd |
| | C5-5-0-0 | 69.4±15.7 abcdef |
| | C.5-2.5-2.5-0 | 16.8±7.0 ab |
| | C2.5-2.5-2.5-2.5 | 49.6±8.0 abcd |
| | K10-0-0-0 | 43.9±7.4 abcd |
| | K5-5-0-0 | 36.5±0.3 abcd |
| | K5-2.5-2.5-0 | 42.7±0.2 abcd |
| | K2.5-2.5-2.5-2.5 | 22.7±11.8 abc |

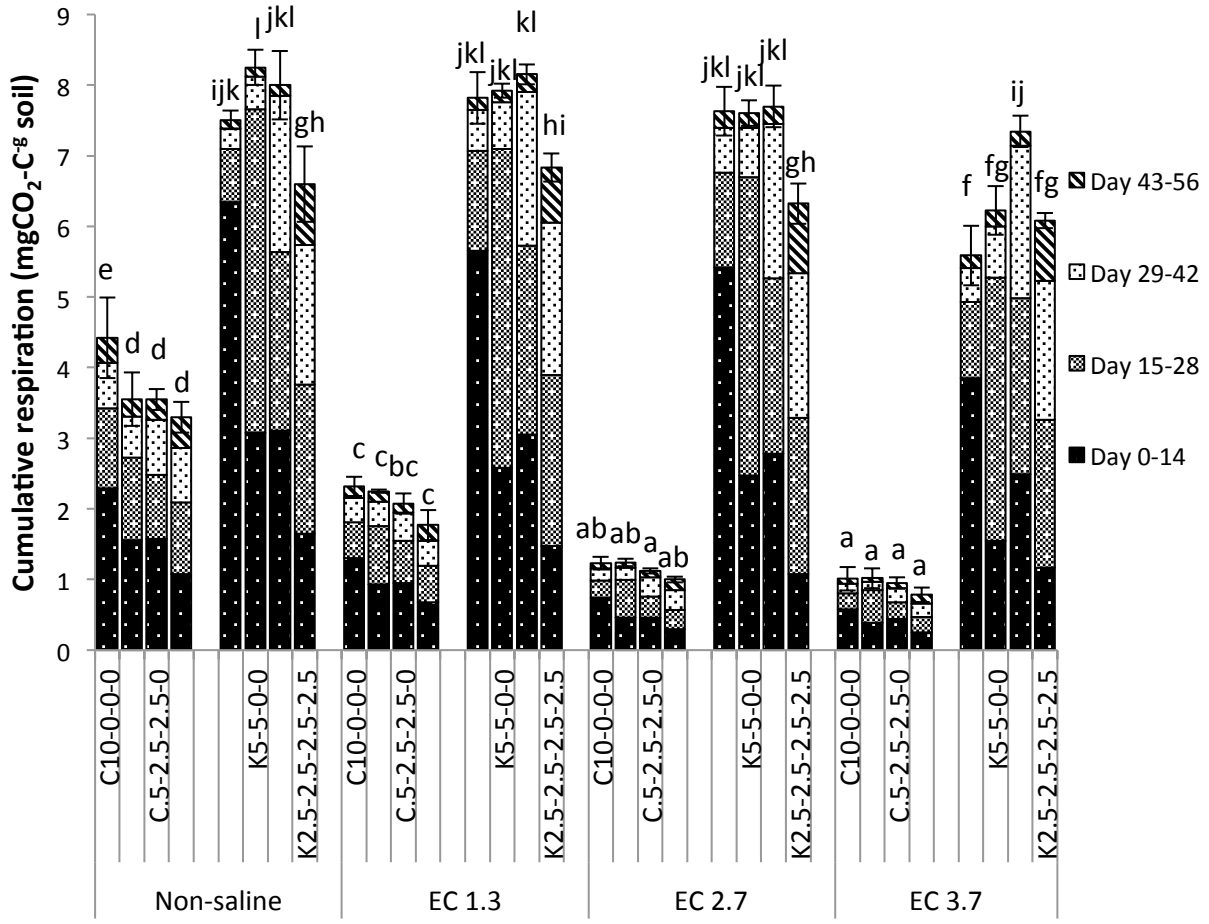


Figure 1. Cumulative respiration over 14 day intervals: from day 0 to 14, day 15-28, day 29-42 and day 43- 56 in non-saline soil ($EC_{1:5}$ 0.1 dS $m^{-1}$) and saline soils (EC 1.3, 2.7 and 3.7 dS $m^{-1}$) with single and multiple amendments of canola and kikuyu residues to achieve a total addition of 10 g C $kg^{-1}$ soil (n=4, vertical lines indicate standard deviation). Different letters indicate significant differences ($P \leq 0.05$). For treatment details see Table 3.

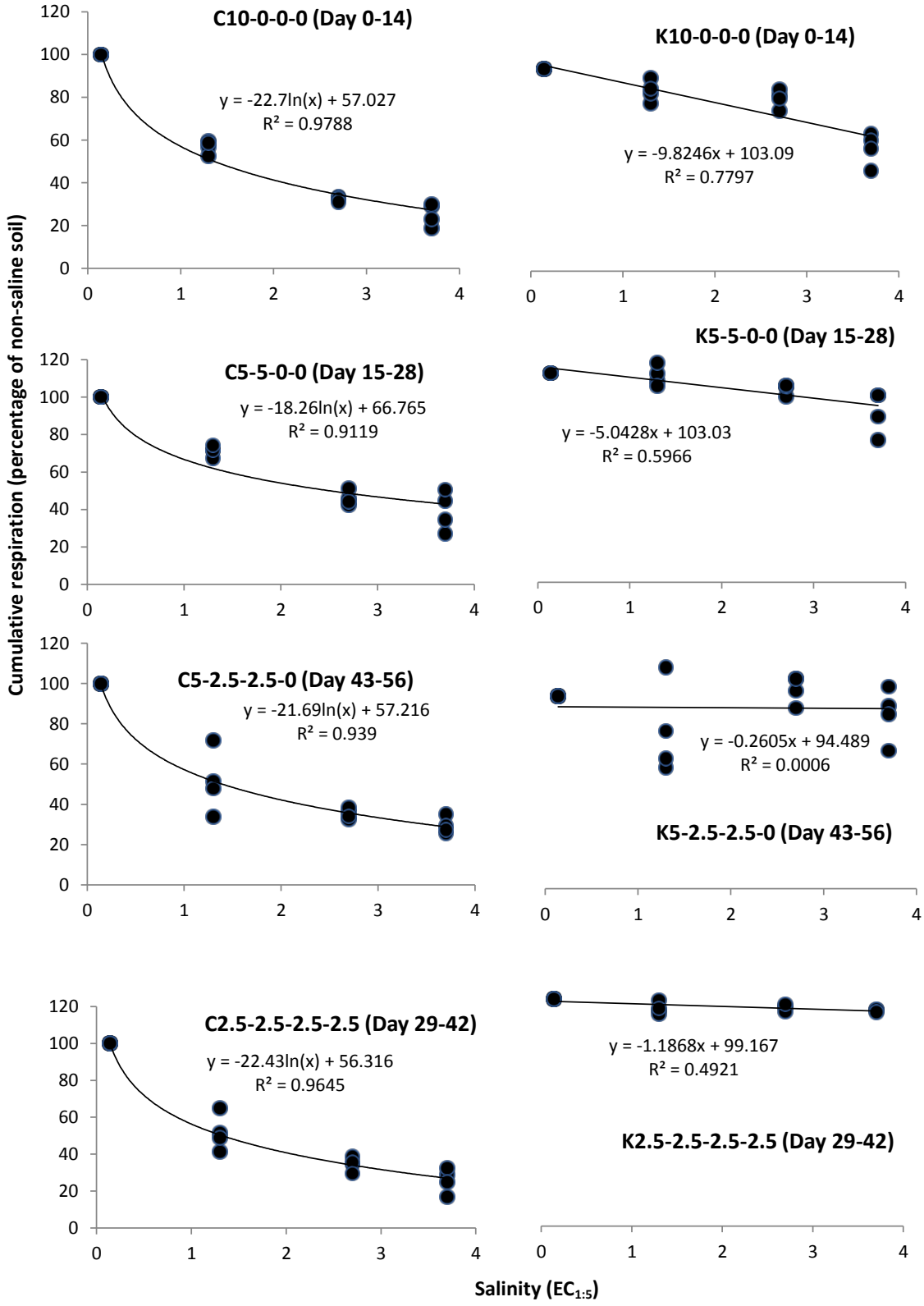


Figure 2. Relationship between cumulative respiration over 14 days after addition of 10 g C $kg^{-1}$ soil and salinity ($EC_{1:5}$ 0.1, 1.3, 2.7 and 3.7 dS $m^{-1}$) in percentage of non-saline soil with single and multiple amendments of canola and kikuyu residues (n=4). Values in brackets indicate two week period after addition of 10 g C $kg^{-1}$ soil.

# CHAPTER 4:

# Manuscript 3

# Chapter 4

## Amending saline soils with residue mixtures - effect of proportion of slowly and rapidly decomposable residue on response of cumulative respiration to salinity

Hasbullah and Petra Marschner

School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, Australia.

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## Principal Author

| | |
|---|---|
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| Contribution to the Paper | Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author. |
| Overall percentage (%) | 85% |
| Signature | Date 24/07/2015 |

## Co-Author Contributions

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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| | |
|---|---|
| Name of Co-Author | Petra Marschner |
| Contribution to the Paper | Supervised development of work, data interpretation, manuscript evaluation |
| Signature | Date 24/07/2015 |

**Amending saline soils with residue mixtures - effect of proportion of slowly and rapidly decomposable residue on response of cumulative respiration to salinity**

Hasbullah Hasbullah*, Petra Marschner
*School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia*

**Corresponding author,*
*E-mail address: hasbullah@adelaide.edu.au*

**Abstract**

Addition of rapidly decomposable residues reduces the negative impact of increasing salinity on soil respiration to a greater extent than slowly decomposable residues. The aim of this study was to investigate the response of soil respiration to salinity when amended with mixtures of rapidly and slowly decomposable residues. Two incubation experiments were carried out with loam soils having $EC_{1:5}$ 0.1, 1.0, 2.5 and 3.3 dS $m^{-1}$. In the first experiment, the soils were amended with 20 g $kg^{-1}$ soil as sawdust (C/N 114, slowly decomposable) or kikuyu (*Pennisetum clandestinum* L., C/N 19, rapidly decomposable) alone (S100/K0 or S0/K100, respectively) or mixed at different ratios. In all mixtures (percentage of kikuyu 25, 50 and 75%), the decrease in cumulative respiration at 1 dS $m^{-1}$ was smaller than with sawdust alone. In the second experiment three soils ($EC_{1:5}$ 0.1, 1.0 and 2.5 dS $m^{-1}$) were amended once or three times (on days 0, 14 and 28) to a total addition rate of 10 g C $kg^{-1}$ soil either with sawdust alone, kikuyu alone or mixtures (final proportion of kikuyu 25%). In treatments with only one residue, the decrease in cumulative respiration with increasing salinity was greater with sawdust than with kikuyu, but did not differ between a single and multiple additions. In the treatments with mixtures of sawdust and kikuyu, the decrease in cumulative respiration from non-saline to EC 1 dS $m^{-1}$ was small in the treatment with three residue additions where the first addition consisted of equal parts of kikuyu and

sawdust [25% sawdust and 25% kikuyu added on day 0, 25% sawdust on days 14 and 28]. We conclude that even a relatively small proportion of rapidly decomposable residue in a mixture is sufficient to alleviate the negative impact of salinity on soil respiration. When different residues are added repeatedly, the ameliorative effect of rapidly decomposable residue will be greater if only small amounts of slowly decomposable residue is present in soil, that is, when it is added early.

Keywords: residue mixes, respiration, salinity

## 1. Introduction

Salinity is one of the major factors limiting agricultural production in many arid and semi-arid areas, including 357 Mio ha land in Australia. This area is predicted to increase by another 15 Mio ha in the next 50 years due to human activities such as poor irrigation and drainage systems, the expansion of irrigation into arid zones and clearing of native vegetation [1, 2].

Excessive amounts of salts have detrimental impact not only on plant growth, but also on biological properties of soils [3, 4]. Salinity has been shown to reduce microbial biomass, activity [5, 6] and change community structure [7]. The adverse effect of salinity on soil microbes can be explained by the low osmotic potential which makes it difficult to take up or retain water [8], and nutrient imbalance because of ion competition [9]. Sensitive microbes die, but some microbes accumulate organic or inorganic osmolytes thereby preventing water loss from the cells [10]. Synthesis of organic osmolytes requires a significant amount of energy [11, 12].

Organic matter decomposition is the main energy source for soil microbes, but organic matter content in saline soils is often low due to poor plant growth [13]. Addition of organic matter has been shown to increase microbial activity in saline soils

[14, 15] and could reduce the negative impact of salinity on microbes by providing energy for osmolyte synthesis. In most studies a single type of organic amendment is used. Setia and Marschner [16] applied mature wheat and pea residues into salinized soils and found that the reduction in cumulative respiration with salinity was greater with pea than with wheat residues. We recently showed that the negative impact of salinity on respiration was greater in soil amended with slowly decomposable residues compared to rapidly decomposable residues [17].

In the field, slowly decomposable residues (e.g. sawdust or mature cereal straw) are often available in greater quantities and at lower cost than rapidly decomposable residues (e.g. young grass or legume shoots). Amelioration of saline soils with residue mixtures of slowly and rapidly decomposable residues may therefore be a cost-effective option. However, it is not clear how much easily decomposable residue has to be in the mix to achieve a similar ameliorative effect as easily decomposable residue alone.

A consistent supply of organic C could be more effective in alleviating the negative effect of salinity on microbial activity than a single addition where decomposable compounds are depleted soon after organic amendment. It is not clear if in soil amended with residue mixtures, proportion or timing of amendment with rapidly decomposable residue influence the impact of salinity on soil respiration or microbial biomass.

Two experiments were conducted with sawdust as slowly decomposable residue and young kikuyu shoots as rapidly decomposable residue. The aim of the first experiment was to investigate the influence of salinity on respiration in soil with a single addition of slowly or rapidly decomposable residues alone or as mixture. The

aim of the second experiment with multiple residue additions was to determine the influence of the proportion of slowly decomposable residue in the soil at the time of addition of rapidly decomposable residue on response of soil respiration to increasing salinity. We hypothesised that (i) with a single addition, the negative effect of salinity on soil respiration will decrease with increasing proportion of rapidly decomposable residue in the mixture, and (ii) with multiple residue additions, the negative impact of salinity on cumulative respiration will increase with proportion of slowly decomposable residue in the soil at the time of addition of rapidly decomposable residue. The second hypothesis is based on the assumption that the likelihood of microbes being in the vicinity of freshly added rapidly decomposable residue will decrease as the proportion of slowly decomposable residue already present in the soil increases.

## 2. Materials and methods

### 2.1 Soils

Four soils were collected in March 2012 from the A horizon (0-30 cm depth) in areas with patches of salinity in Monarto, South Australia (35° 05′ S and 139° 06′ E) classified as Monarto Loam [18] (Table 1) These soils are wide-spread in the region. This area has a Mediterranean climate with hot dry summer (long term average: 29.4°C) and cool wet winter (long term average: 14.7°C) (Meat and Livestock Australia, 2015). The soils were air-dried, sieved to 2 mm and then stored. Salinity was determined as electrical conductivity in a 1:5 (soil: water, w/w) extract ($EC_{1:5}$) and was 0.1 (non-saline), 1.0, 2.5 and 3.3 dS $m^{-1}$. This range was chosen based on previous studies [19, 20] to induce a moderate to strong decrease in respiration. The saline soils

were saline-sodic (Sodium Adsorption Ratio <6 for Australian soils) and therefore do not display dispersive behaviour associated with sodicity because the high salt concentration in the soil solution causes flocculation of soil particles [21, 22].

### 2.2 Plant residues

Two residues with distinct properties were used for both experiments: shoots of kikuyu (*Pennisetum clandestinum* L.) with low C/N ratio and lignin concentration and sawdust (from pine wood (*Pinus* sp.)) with high C/N ratio and lignin concentration (Table 2). Both residues were ground and sieved to particle size 0.25-2 mm. These residues were chosen because in our previous study where we used the same saline soils [17], the decrease in respiration with increasing salinity was greater in soils amended sawdust than in kikuyu-amended soils.

### 2.3 Experimental design

The study included of two experiments, where the second experiment was based on the results of the first. Before the start of the experiments, the soils were wetted to 55% of maximum water holding capacity and incubated for 14 days to stabilise respiration after the initial flush of respiration upon rewetting of air-dry soil. In our previous studies soil respiration in these soils was maximal at this water content.

The objective of Experiment 1 was to investigate the influence of salinity on respiration in soil amended with slowly or rapidly decomposable residues alone or as mixture. After pre-incubation, residues were added into a non-saline soil ($EC_{1:5}$ 0.1 dS $m^{-1}$) and three saline soils (EC 1, 2.5 and 3.3 dS $m^{-1}$) at a rate of 20 g $kg^{-1}$ either as individual residues: sawdust alone (S100/K0) or kikuyu alone (S0/K100) or as mixtures. For the mixture treatments, the residues were thoroughly mixed before addition to

the soils. The following mixtures were prepared where the first number is the weight percentage of sawdust and the second is the percentage of kikuyu: S75/K25, S50/K50 and S25/K75. Soil with residues (equivalent to 20 g moist soil) was filled into PVC cores with 1.85 cm radius, 5 cm height and a nylon mesh base (0.75µm, Australian Filter Specialist) and packed to a bulk density of 1.4 g $cm^{-3}$. The cores were placed individually into 1 L glass jars with gas tight lids equipped with septa to allow quantification of the headspace $CO_2$ concentration (see below). The jars were kept at room temperature (19-23 ˚C) in the dark. Soil moisture was maintained at 55% of maximum water holding capacity (WHC) by checking the water content every few days by weight and adding RO water if necessary. Respiration was measured over 16 days.

The aim of Experiment 2 was to determine the effect of residue addition frequency and order in which residues are added on response of soil respiration to salinity. The two residue were added into a non-saline soil ($EC_{1:5}$ 0.1 dS $m^{-1}$) and two saline soils (EC 1 and 2.5 dS $m^{-1}$) to achieve a total addition of 10 g C $kg^{-1}$. This rate corresponds to approximately 20 g residue $kg^{-1}$ which is the rate used in Experiment 1. In Experiment 1, 25% kikuyu in the mixture was sufficient to alleviate the negative effect of salinity on soil respiration. Therefore, the mixed treatments in Experiment 2 consisted of 75% sawdust + 25% kikuyu. The residues were added once, twice or three times. For the single additions, residues were added on day zero. For the multiple additions, the residues were added every two weeks: on days 0, 14 and 28 (see Table 3 for details). When residue mixtures were applied, the residues were mixed before addition to the soil. The mixed treatments were designed so that the 25% kikuyu was added either on day 0, day 14 or day 28. In treatments where residues were applied only on day 0 (10S, 10K, 7.5S+2.5K where the value indicates the C rate, S and K stand

for sawdust and kikuyu), the soil was mixed on days 14 and 28 in a similar manner as in those treatments with residue addition on these dates. After mixing with residues, soils were treated as described for Experiment 1. Soil respiration was measured daily until day 42 and MBC was measured at the end of experiment.

### 2.4 Soil and residue analyses

Soil EC and pH were measured in a 1:5 soil to reverse osmosis (RO) water suspension after one hour horizontal shaking at 25°C. The formula from Shaw et al. [23] was used to calculate EC in the saturated paste extract ($EC_e$) from $EC_{1:5}$:

$$EC_e = EC1:5(-2.21 \times \%Clay^{0.5} + 23.78).$$

Soil texture was determined by the hydrometer method [24], texture classification was based on Saxton et al. [25]. Maximum water holding capacity (WHC) of the soils was measured by using a sintered glass funnel connected to a 1 m water column ($\Psi_m$= -10 kPa) [26]. The soils were placed in cores in a sintered glass funnel, 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. For total P, Al, Fe and K, the soils were digested with hydrochloric acid as described in Zarcinas et al. [27]. Available P was extracted using the Colwell method and measured colorimetrically at 882 nm [28]. Soil inorganic N was extracted in 1 M KCl; ammonium and nitrate concentrations were determined using standard colorimetric methods [29]. Soluble $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ concentrations were extracted by 1 hour horizontal shaking of a suspension with 1:5 soil: water ratio and filtering through Whatman filter No. 42. Element concentrations in the extracts were measured by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry). Sodium adsorption ratio (SAR) was calculated

from soluble $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ concentrations in mmol $L^{-1}$ using the following equation:

$$SAR = \frac{[Na^+]}{\sqrt{([Ca^{2+}] + [Mg^{2+}])}}$$

Total organic C concentration in soils and residues was measured by the Walkey and Black method [30]. Total N in soils and residues was measured by the Kjeldahl method [31]. Water extractable organic carbon (WEOC) of residues was determined by shaking 0.5 g ground residues in 30 ml RO water for 1 hour after which the extract was filtered through a Whatman no. 42 filter. The organic C concentration in the extract was measured as described in Anderson and Ingram [32] by digesting with 0.0667 M $K_2Cr_2O_7$ and concentrated $H_2SO_4$ and titrating the remaining dichromate with 0.033 M acidified $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$.

The composition of organic C compounds in the residues was determined by nuclear magnetic resonance (NMR) spectroscopy. The spectra were obtained on a Varian Unity 200 NMR spectrometer at a $^{13}C$ resonance frequency of 50.3 MHz. The residue samples were packed in a cylindrical zirconia rotor and spun at 5000 ± 100 Hz in a Doty Scientific magic angle-spinning (MAS) probe. The spectra were integrated into eight chemical shift regions for the following C-types: Alkyl (0 - 45 ppm), N-Alkyl/Methoxyl (45 - 60 ppm), O-Alkyl (60 - 95 ppm), Di-O-Alkyl (95 - 110 ppm), Aryl (110 - 145 ppm), O-Aryl (145 - 165 ppm), Amide/Carboxyl (165 - 190 ppm) and Ketone (190 - 215 ppm). For further details about the NMR analysis see Baumann *et al*. [33]. The percentage of biomolecules (carbohydrate, protein, lignin lipid and carbonyl) was calculated from $^{13}C$ NMR results using the molecular mixing model [34]. Klason lignin was determined as described in Hatfield and Fukushima [35]. Briefly, residues were

washed with 80% ethanol and then digested in 12 M sulfuric acid at 35°C, followed by incubation in 2 M sulfuric acid at 121°C.

Soil microbial biomass carbon (MBC) concentration was determined by the fumigation extraction method [36]. Soil samples were exposed to 48 h chloroform fumigation followed by shaking at 1:5 ratio with 0.5M $K_2SO_4$. The C concentration in the extract of fumigated and non-fumigated soils was determined by adding 0.0667M $K_2Cr_2O_7$ and concentrated $H_2SO_4$. The remaining $K_2Cr_2O_7$ was titrated with 0.033 M acidified $(NH_4)_2Fe(SO_4)_2.6H_2O$ (Anderson and Ingram 1993). The difference in C concentration between fumigated and non-fumigated soil was multiplied by 2.64 to calculate MBC [36].

Soil respiration was determined by measuring the $CO_2$ concentration in the headspace of each jar using a Servomex 1450 infra-red gas analyser as described in Setia et al. [37]. 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. Due to the upper detection limit of the gas analyser (2% $CO_2$) and the decrease in respiration rate over time after residue addition, respiration was measured daily until day 14 and then on day 16 (last day of experiment). 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. Cumulative respiration was calculated as the sum of respiration rates [in mg $CO_2$-C (g soil and day)$^{-1}$].

### 2.5 Statistical analysis

There were three replicates per treatment in Experiment 1 and four replicates in Experiment 2 arranged as randomized block design. Data of cumulative respiration and MBC concentration was normally distributed and analysed by two-way ANOVA with salinity and residue treatment as fixed factors using Genstat 15$^{th}$ edition (VSN Int. Ltd, UK). Tukey's multiple comparison test at 95% confident interval was used to determine significant differences among treatments. Regression was used to characterise the relationship between salinity and cumulative respiration in saline soils as percentage of the non-saline soil in Microsoft Excel 2010. The $EC_{1:5}$ at which respiration is reduced by 20% compared to the non-saline soil was calculated with the regression equations. This percentage decrease was chosen to allow comparison between treatments which had logarithmic or linear responses between cumulative respiration and salinity.

## 3. Results

Compared to sawdust, kikuyu shoots had a lower C/N ratio, concentrations of organic C and water-extractable organic C and lower proportions of carbonyl-C, lipid C and lignin. Total N and protein concentration were higher in kikuyu than in sawdust (Table 2).

### 3.1 Experiment 1

In all treatments, cumulative respiration decreased with increasing EC (Figure 1). At all salinity levels, cumulative respiration was lowest with 100% sawdust and greatest with 100% kikuyu and increased with increasing proportion of kikuyu in the mixes. The decrease in cumulative respiration from the non-saline soil to the soil with EC 1 dS $m^{-1}$

was strongest with 100% sawdust. This decrease was smaller in all residue treatments with kikuyu.

There was a negative relationship between salinity and cumulative respiration in percentage of the non-saline soil in all treatments ($r^2$= 0.8-0.9) (Figure 2). The sharpest decrease in cumulative respiration occurred from non-saline to $EC_{1:5}$ 1 dS $m^{-1}$. The reduction in cumulative respiration with increasing salinity was greater with 100% sawdust than in the other treatments. From the regressions (Table 4), it was calculated that cumulative respiration would be reduced by 20% compared to non-saline soil at $EC_{1:5}$ 0.3 dS $m^{-1}$ in soil with 100% sawdust, at $EC_{1:5}$ 0.5 dS $m^{-1}$ in soil with S75/K25 and at $EC_{1:5}$ 0.6-0.8 dS $m^{-1}$ in soils amended with 100% kikuyu and mixes with ≥ 50% kikuyu.

### 3.2 Experiment 2

Cumulative respiration on day 42 was higher in soils amended only with kikuyu (10K, 5K-2.5K-2.5K) compared to the other treatments at all salinity levels (Figure 3). It was lowest in soils with sawdust only. In treatments where only one residue type was added (either kikuyu or sawdust), cumulative respiration was the same if residues were added once or three times. In treatments with 25% kikuyu [7.5S+2.5K, 5S-2.5S-2.5K, 5S-2.5K-2.5S and (2.5S+2.5K)-2.5S-2.5S)], cumulative respiration was lowest in the treatment where kikuyu was added last (5S-2.5S-2.5K). Cumulative respiration was not significantly influenced by salinity in soils amended with kikuyu only (10K and 5K-2.5K-2.5K), but decreased from the non-saline soil to soil with EC 1 dS $m^{-1}$ in treatments with sawdust alone or mixtures of sawdust and 25% kikuyu. Cumulative respiration did not differ between EC 1 and 2.5 dS $m^{-1}$.

In the two-week period after a total of 10 g C $kg^{-1}$ had been added there was a strong negative logarithmic relationship between salinity and cumulative respiration in

percentage of non-saline soil in soil with a single addition of sawdust (10S). (Figure 4; see Figures S1 and S2 for cumulative respiration after addition of 7.5 and 5 g $kg^{-1}$). The reduction of cumulative respiration with increasing salinity was smaller in soils amended with a single addition of 75% sawdust and 25% kikuyu (7.5S+2.5K) or kikuyu only (10K). In all other treatments, there was a linear relationship between salinity and percentage cumulative respiration with the greatest slope in soil with repeated addition of sawdust only (5S-2.5S-2.5S) and the smallest with repeated addition of kikuyu only (5K-2.5K-2.5K). Among the treatments with multiple additions and 25% kikuyu, the slope was smallest in the treatment with kikuyu added on day 14 (5S-2.5K-2.5S).

The MBC concentration on day 42 was lower in soils with sawdust only compared to those with kikuyu only or mixes of sawdust and kikuyu (Figure 5). In the non-saline soil, the MBC concentration was higher with kikuyu only than in soils with mixtures of kikuyu and sawdust. Salinity influenced the MBC concentration only in some treatments. Compared to the non-saline soil, the MBC concentration was lower in soil with 2.5 dS $m^{-1}$ only in treatments with a single residue addition (10S and 10K).

## 4. Discussion

This study confirmed our earlier study [17] that the reduction in soil respiration with increasing salinity is smaller in soils amended with rapidly decomposable residues compared to slowly decomposable residues. The novel finding of this study is that even a small proportion of rapidly decomposable residues in a mixture with slowly decomposable residues is sufficient to reduce the negative effect of salinity on cumulative respiration compared to the slowly decomposable residue alone.

In Experiment 1, with a single addition, 25% kikuyu in a mixture was sufficient to reduce the negative effect of salinity on cumulative respiration (Figure 2). However, there was little difference in ameliorative effect among mixtures with 25-75% rapidly decomposable residue and also compared to rapidly decomposable residue alone. Therefore our first hypothesis that the negative effect of salinity on soil respiration will decrease with increasing proportion of rapidly decomposable residue in the mixture has to be declined. The alleviation of the negative effect of salinity by rapidly decomposable residues can be explained by several factors. Firstly, a greater amount of energy is available for the synthesis of osmolytes than with slowly decomposable residues because less energy has to be used for the synthesis of enzymes required for the breakdown of more complex compounds such as lignin. Accumulation of osmolytes in cells can counteract the strongly negative osmotic potential in saline soils [10]. However, osmolyte synthesis requires large amounts of energy, 30-110 ATP compared with only 30 ATP for synthesising cell walls [11]. Secondly, the kikuyu residues used here, and rapidly decomposable residues in general [38], have a high N concentration (low C/N ratio) which facilitates osmolyte synthesis many of which are N-rich, for example, glycine betaine, proline and quaternary ammonium compounds [39, 40]. Further potential factors are that (i) rapidly decomposable residues induce a microbial community structure with a greater proportion of salinity-adapted microbes; (ii) the greater amount of energy and N provided may allow synthesis and release of microbial slimes which protect micro-colonies from water stress [41].

In Experiment 2, the effect of salinity on cumulative respiration did not differ greatly between single and multiple additions of either sawdust or kikuyu only (Figure 3). In case of sawdust, multiple additions apparently could not provide energy for

adaptation to salinity, probably because even freshly added sawdust is poorly decomposable due to its high C/N ratio and high lignin concentration [42, 43]. With kikuyu on the other hand, a single addition apparently provided sufficient rapidly decomposable compounds for salinity tolerance mechanisms.

In the treatments with mixtures of sawdust and kikuyu, the decrease in cumulative respiration from non-saline soil to EC 1 dS $m^{-1}$ was greatest with a single addition (7.5S+2.5K) (Figure 4). The smaller decrease in cumulative respiration from non-saline to EC 1 dS $m^{-1}$ in treatment (2.5S+2.5K)-2.5S-2.5S can be explained by the proportion of sawdust and kikuyu in the soil when kikuyu was added. In the two weeks following the first residue addition, 75% of added residues was sawdust In treatment (7.5S+2.5K) whereas it was only 50% in (2.5S+2.5K)-2.5S-2.5S. Therefore a greater proportion of microbes in the soil would have kikuyu in their vicinity and could decompose it in the former. This early provision of rapidly decomposable residue to a greater proportion of soil microbes was sufficient to reduce the salinity effect throughout the 42 days although later only sawdust was added and therefore most soil microbes were in the vicinity of slowly decomposable residues. This confirms the second hypothesis, that with multiple residue additions, the negative impact of salinity on cumulative respiration will increase with proportion of slowly decomposable residue in the soil at the time of addition of rapidly decomposable residue.

The greater decomposability of kikuyu compared to sawdust also lead to a greater MBC concentration in soil amended with kikuyu (Figure 5). However, the differences in MBC concentration between kikuyu and sawdust were smaller than in cumulative respiration. The MBC concentration was about two-fold higher with kikuyu compared to sawdust whereas cumulative respiration was five to six times higher (Figure 4). The

smaller difference in MBC between treatments can be explained by the fact that MBC was determined only once at the end of the experiment whereas cumulative respiration was integrated over the entire experiment. By the end of the experiment a proportion of the microbial biomass may have already died because of depletion of readily available organic C [44]. This can also explain the lack of impact of salinity on MBC concentration.

## 5. Conclusion

The first experiment showed that with a single addition, 25% of rapidly decomposable residue in the mix was sufficient to reduce the negative effect of salinity on respiration compared slowly decomposable residue alone. This could reduce costs of amelioration of saline soils because slowly decomposable residues are often cheaper and available in greater amounts than rapidly decomposable residues. However, when residues are added several times, the ameliorative effect of rapidly decomposable residue will be greater if only a small amount of slowly decomposable residue is present. Thus, rapidly decomposable residue would be more effective if added early than if it is added after amendment with large amounts of slowly decomposable residue. Long-term field studies are required to investigate how often residues or mixtures would have to be applied for a sustained ameliorative effect.

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Table 1. Physical and chemical properties of non-saline and three saline soils (from Hasbullah and Marschner 2014)

| **Parameter**[1] | **$EC_{1:5}$ (dS $m^{-1}$)** | | | |
|---|---|---|---|---|
| | **0.14** | **1.0** | **2.5** | **3.3** |
| $EC_e$ (dS $m^{-1}$) | 2.5±0.02 | 13.9±0.45 | 34.7±1.67 | 44.5±1.08 |
| Sand (%) | 50 | 45 | 47.5 | 47.5 |
| Silt (%) | 35 | 35 | 32.5 | 32.5 |
| Clay (%) | 15 | 20 | 20 | 20 |
| Texture | Loam | Loam | Loam | Loam |
| Water holding capacity (%) | 32 | 37 | 39 | 44 |
| pH | 7.8±0.10 | 9.0±0.06 | 8.7±0.06 | 9.0±0.04 |
| Total organic carbon (%) | 1.2±0.04 | 1.1±0.02 | 0.9±0.03 | 0.7±0.03 |
| **Total elements** | | | | |
| N (g $kg^{-1}$) | 1.48±0.20 | 0.95±0.04 | 0.95±0.08 | 0.88±0.08 |
| P (mg $kg^{-1}$) | 848±73.72 | 423±7.49 | 318±19.12 | 279±6.51 |
| K (g $kg^{-1}$) | 5.7±0.57 | 6.1±0.21 | 6.5±0.30 | 7.0±0.13 |
| Al (g $kg^{-1}$) | 32.4±1.50 | 39.6±0.03 | 40.0±1.18 | 38.3±0.66 |
| Fe (g $kg^{-1}$) | 25.9±1.70 | 31.7±0.23 | 33.0±0.60 | 30.7±1.49 |
| **Available nutrients** | | | | |
| $NH_4$ N (mg $kg^{-1}$) | 40.1±0.97 | 43.7±0.84 | 41.4±0.91 | 46.7±0.32 |
| $NO_3$ N (mg $kg^{-1}$) | 8.5±0.09 | 8.7±0.09 | 2.7±0.09 | 1.3±0.05 |
| Colwell P (mg $kg^{-1}$) | 70±1.13 | 24±0.66 | 25±0.76 | 17±0.19 |
| **Soluble cations (1:5 soil: water extract)** | | | | |
| Ca (mmol $L^{-1}$) | 0.51±0.00 | 0.49±0.02 | 0.96±0.03 | 0.44±0.04 |
| Na (mmol $L^{-1}$) | 0.22±0.00 | 9.79±0.31 | 21.31±0.62 | 30.01±0.00 |
| Mg (mmol $L^{-1}$) | 0.22±0.00 | 0.29±0.02 | 0.93±0.03 | 1.01±0.01 |
| $SAR_{1:5}$ | 0.3 | 11.1 | 15.5 | 24.9 |

[1] n=3 ± standard deviation except for texture, water holding capacity and SAR where n=1.

Table 2. Selected properties of sawdust and kikuyu shoots. n=3 ± standard deviation for organic C, total N, water-extractable organic C and Klason lignin.

| **Plant residues** | **Sawdust** | **Kikuyu shoots** |
|---|---|---|
| Organic C (g $kg^{-1}$) | 437±42.9 | 341±40.2 |
| Total N (g $kg^{-1}$) | 3.8±0.31 | 17.6±0.64 |
| C/N ratio | 114 | 19 |
| Water extractable organic C (g $kg^{-1}$) | 8.4±0.24 | 2.1±0.02 |
| Carbohydrate (%)[1] | 50 | 51 |
| Carbonyl (%)[2] | 5 | 2 |
| Lipid (%)[2] | 20 | 4 |
| Protein (%)[2] | 3 | 19 |
| Lignin (%)[2] | 22 | 15 |
| Klason lignin (%) | 68±2 | 37±1 |

[1]Concentrations calculated from $^{13}C$ NMR results using a molecular mixing model [34]. Values do not necessarily add up to 100% as some C species (e.g. ketones) are excluded from the model.

Table 3. Organic C addition treatments in Experiment 2.

| Treatment | Day | | |
|---|---|---|---|
| | 0 | 14 | 28 |
| | C addition rate (g C/kg) | | |
| 10S | 10S | 0 | 0 |
| 10K | 10K | 0 | 0 |
| 7.5C+25K | 7.5S+2.5K | 0 | 0 |
| 5S-2.5S-2.5S | 5S | 2.5S | 2.5S |
| 5S-2.5K-2.5S | 5S | 2.5K | 2.5S |
| 5S-2.5S-2.5K | 5S | 2.5S | 2.5K |
| (2.5S+2.5K)-2.5S-2.5S | 2.5S+2.5K | 2.5S | 2.5S |
| 5K-2.5K-2.5K | 5K | 2.5K | 2.5K |

Table 4. Regression equations for the relationship between cumulative respiration in saline soils in percentage of the non-saline soil for soils amended with sawdust or kikuyu alone (S100/K0 and S0/K100) or mixed at different ratios (weight percentage saw dust and kikuyu S75/K25, S50/K50, S25/K75) and calculated $EC_{1:5}$ at which cumulative respiration would be decreased by 20% compared to the non-saline soil.

| **Residue mixture % saw dust/kikuyu** | **Equation** | **$R^2$** | **$EC_{1:5}$ at which cumulative respiration is reduced by 20%** |
|---|---|---|---|
| S100/K0 | y = -23.36ln(x) + 50.98 | 0.88 | 0.3 |
| S75/K25 | y = -16.66ln(x) + 68.37 | 0.97 | 0.5 |
| S50/K50 | y = -14.72ln(x) + 71.62 | 0.91 | 0.6 |
| S25/K75 | y = -12.43ln(x) + 76.68 | 0.91 | 0.8 |
| S0/K100 | y = -13.07ln(x) + 73.92 | 0.96 | 0.6 |

**List of Figures:**

Figure 1. Cumulative respiration on day 16 in non-saline soil ($EC_{1:5}$ 0.1 dS $m^{-1}$) and saline soils ($EC_{1:5}$ 1, 2.5 and 3.3 dS $m^{-1}$) amended with 2 % w/w finely ground residues: sawdust or kikuyu alone (S100/K0 and S0/K100) or mixed at different ratios (weight percentage sawdust and kikuyu S75/K25, S50/K50, S25/K75) (n=3, vertical lines indicate standard deviation). Bars with different letters are significantly different (P≤0.05, for the residue treatment x salinity interaction).

Figure 2. Relationship between cumulative respiration on day 15 in saline soils ($EC_{1:5}$ 0.1, 1, 2.5 and 3.3 dS $m^{-1}$) as percentage of non-saline soil amended with 2 % w/w sawdust, kikuyu and their mixtures: (a) S100/K0, (b) S75/K25, (c) S50/K50, (d) S25/K75 (e) S0/K100 (n=3).

Figure 3. Cumulative respiration over 14 day intervals: day 0-14, day 15-28 and day 29-42 in non-saline soil (EC1:5 0.14 dS $m^{-1}$) and saline soils (EC 1.0 and 2.5 dS $m^{-1}$) with mixed, single and multiple amendments of sawdust and kikuyu residues to achieve a total addition of 10 g C $kg^{-1}$ soil (n=4, vertical lines indicate standard deviation). Different letters indicate significant differences (P≤0.05, for the residue treatment x salinity interaction). For treatment structure, see Table 3.

Figure 4. Relationship between cumulative respiration and salinity ($EC_{1:5}$ 0.14 and 1, 2.5 dS $m^{-1}$) as percentage of non-saline over 14 days after addition of a total of 10 g C $kg^{-1}$ as sawdust, kikuyu and their mixtures with single and multiple additions (n=4). Values in brackets indicate the period that was considered. For treatment structure, see Table 3.

Figure 5. Microbial biomass C on day 42 in non-saline soil (EC1: 5 0.1 dS $m^{-1}$) and saline soils ($EC_{1:5}$ 1.0 and 2.5 dS $m^{-1}$) with mixed, single and multiple amendments of sawdust and kikuyu residues at 10 g C $kg^{-1}$ soil. (n=4± vertical lines indicate standard error). Different letters are significantly different (P≤0.05, for the residue treatment x salinity interaction). . For treatment structure, see Table 3.

Figure S1. Relationship between cumulative respiration and saline soils ($EC_{1:5}$ 0.1, 1 and 2.5 dS $m^{-1}$) as percentage of non-saline soil over 14 (from day 15 to 28) after addition of a total of 7.5 g C $kg^{-1}$ as saw dust, kikuyu and their mixtures with single and multiple additions (n=4). Values in brackets indicate the period that was considered.

Figure S2. Relationship between cumulative respiration and saline soils ($EC_{1:5}$ 0.1, 1 and 2.5 dS $m^{-1}$) as percentage of non-saline soil over 14 days (from day 0 to 14) after addition of a total of 5 g C $kg^{-1}$ as saw dust, kikuyu and their mixtures with single and multiple additions (n=4). Values in brackets indicate the period that was considered.

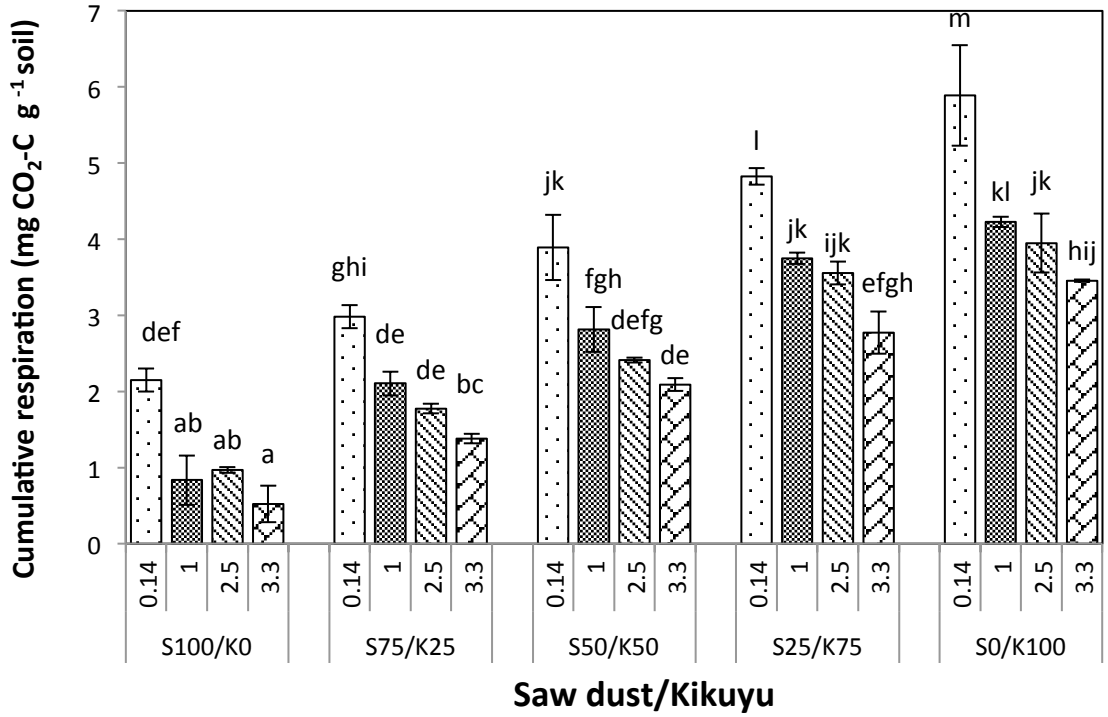
Cumulative respiration (mg $CO_2$-C $g^{-1}$ soil)
7
6
5
4
3
2
1
0
def
ab
ab
a
ghi
de
de
bc
jk
fgh
defg
de
l
jk
ijk
efgh
m
kl
jk
hij
0.14
1
2.5
3.3
S100/K0
S75/K25
S50/K50
S25/K75
S0/K100
Saw dust/Kikuyu

Figure 2.

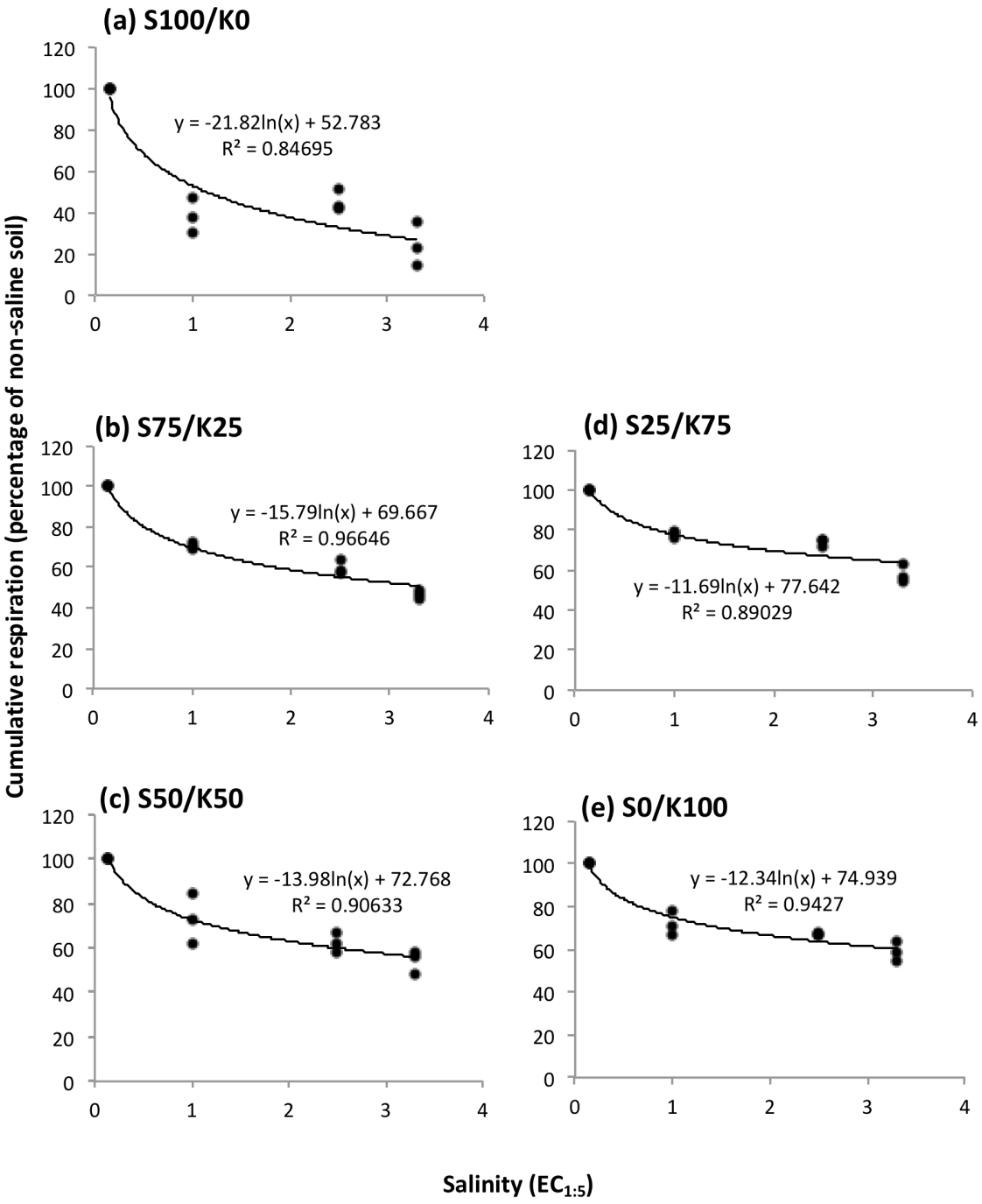
(a) S100/K0
y = -21.82ln(x) + 52.783
R² = 0.84695
(b) S75/K25
y = -15.79ln(x) + 69.667
R² = 0.96646
(d) S25/K75
y = -11.69ln(x) + 77.642
R² = 0.89029
(c) S50/K50
y = -13.98ln(x) + 72.768
R² = 0.90633
(e) S0/K100
y = -12.34ln(x) + 74.939
R² = 0.9427
Cumulative respiration (percentage of non-saline soil)
Salinity (EC1:5)

Figure 2.

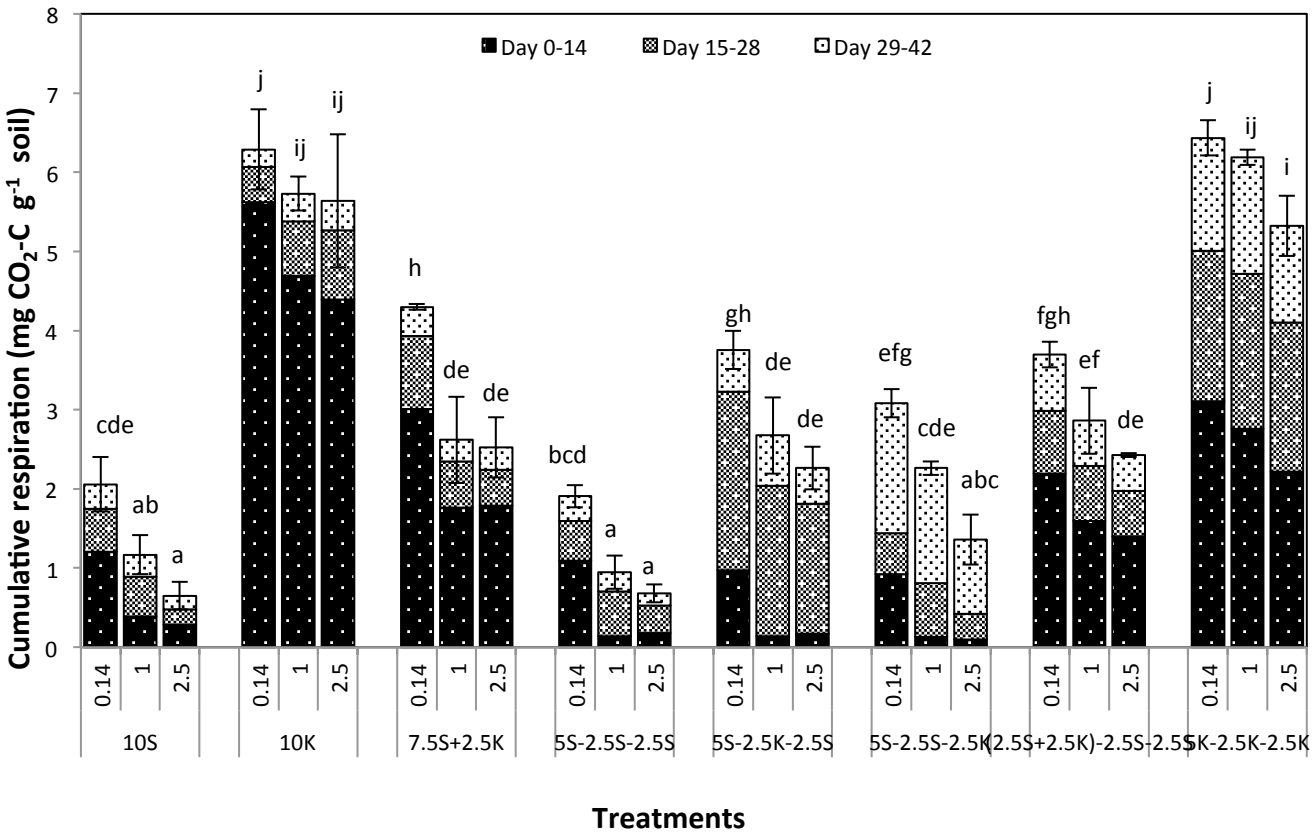
Day 0-14
Day 15-28
Day 29-42
Cumulative respiration (mg $CO_2$-C $g^{-1}$ soil)
0
1
2
3
4
5
6
7
8
cde
ab
a
j
ij
ij
h
de
de
bcd
a
a
gh
de
de
efg
cde
abc
fgh
ef
de
j
ij
i
0.14
1
2.5
10S
10K
7.5S+2.5K
5S-2.5S-2.5S
5S-2.5K-2.5S
5S-2.5S-2.5K
(2.5S+2.5K)-2.5S-2.5S
5K-2.5K-2.5K
Treatments

Figure 3.

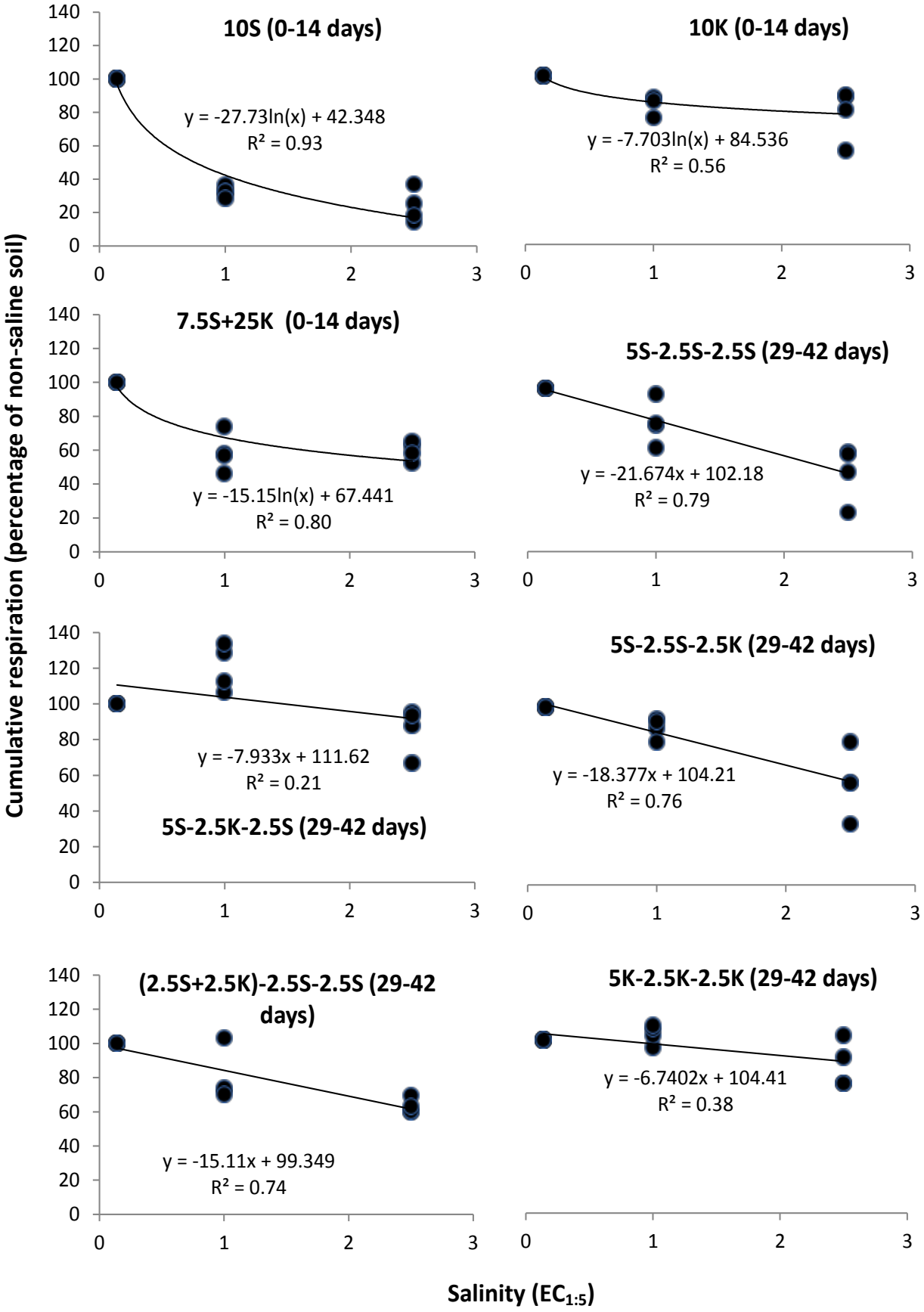
10S (0-14 days)
y = -27.73ln(x) + 42.348
R² = 0.93
10K (0-14 days)
y = -7.703ln(x) + 84.536
R² = 0.56
7.5S+25K (0-14 days)
y = -15.15ln(x) + 67.441
R² = 0.80
5S-2.5S-2.5S (29-42 days)
y = -21.674x + 102.18
R² = 0.79
5S-2.5K-2.5S (29-42 days)
y = -7.933x + 111.62
R² = 0.21
5S-2.5S-2.5K (29-42 days)
y = -18.377x + 104.21
R² = 0.76
(2.5S+2.5K)-2.5S-2.5S (29-42 days)
y = -15.11x + 99.349
R² = 0.74
5K-2.5K-2.5K (29-42 days)
y = -6.7402x + 104.41
R² = 0.38
Cumulative respiration (percentage of non-saline soil)
Salinity (EC1:5)

Figure 4.

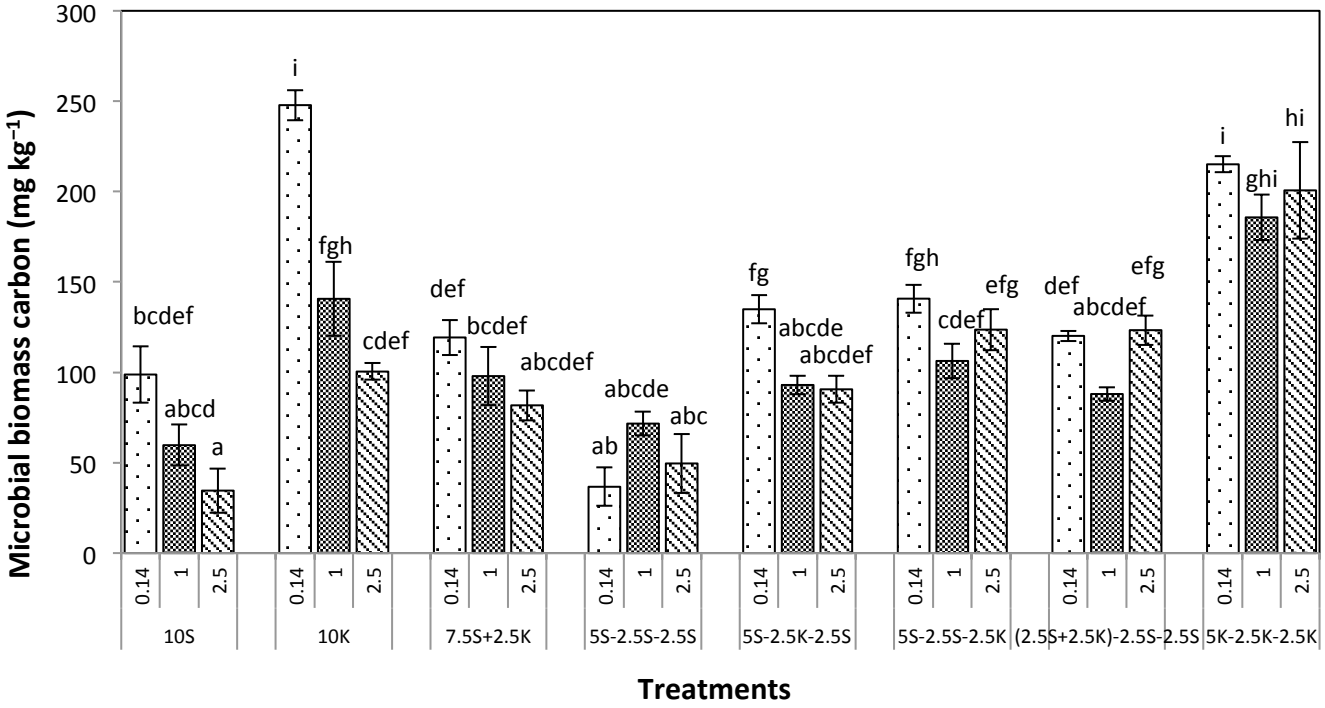
Microbial biomass carbon (mg kg⁻¹)
300
250
200
150
100
50
0
bcdef
abcd
a
i
fgh
cdef
def
bcdef
abcdef
ab
abcde
abc
fg
abcde
abcdef
fgh
cdef
efg
def
abcdef
efg
i
ghi
hi
0.14
1
2.5
10S
10K
7.5S+2.5K
5S-2.5S-2.5S
5S-2.5K-2.5S
5S-2.5S-2.5K
(2.5S+2.5K)-2.5S-2.5S
5K-2.5K-2.5K
Treatments

Figure 5.

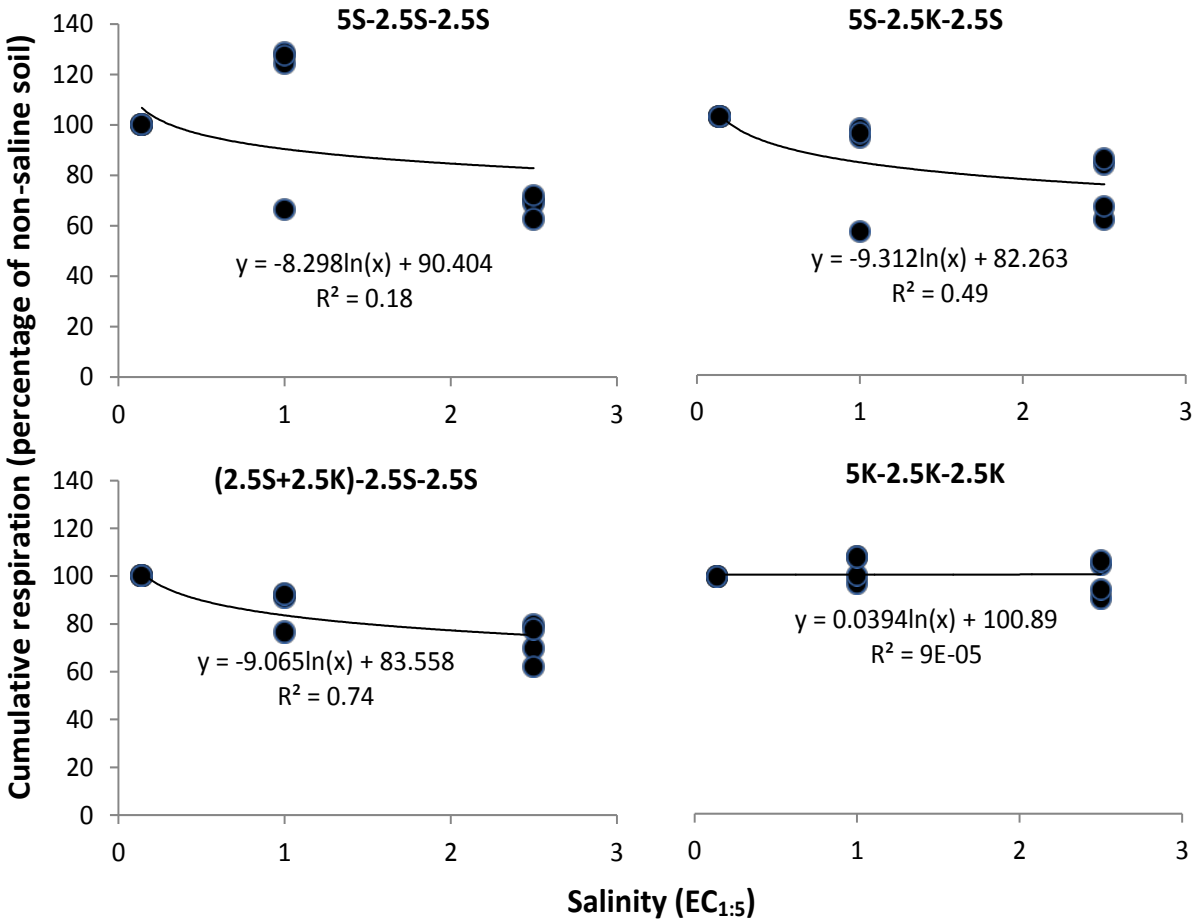
5S-2.5S-2.5S
y = -8.298ln(x) + 90.404
R² = 0.18
5S-2.5K-2.5S
y = -9.312ln(x) + 82.263
R² = 0.49
(2.5S+2.5K)-2.5S-2.5S
y = -9.065ln(x) + 83.558
R² = 0.74
5K-2.5K-2.5K
y = 0.0394ln(x) + 100.89
R² = 9E-05
Cumulative respiration (percentage of non-saline soil)
Salinity (EC1:5)

Figure S1.

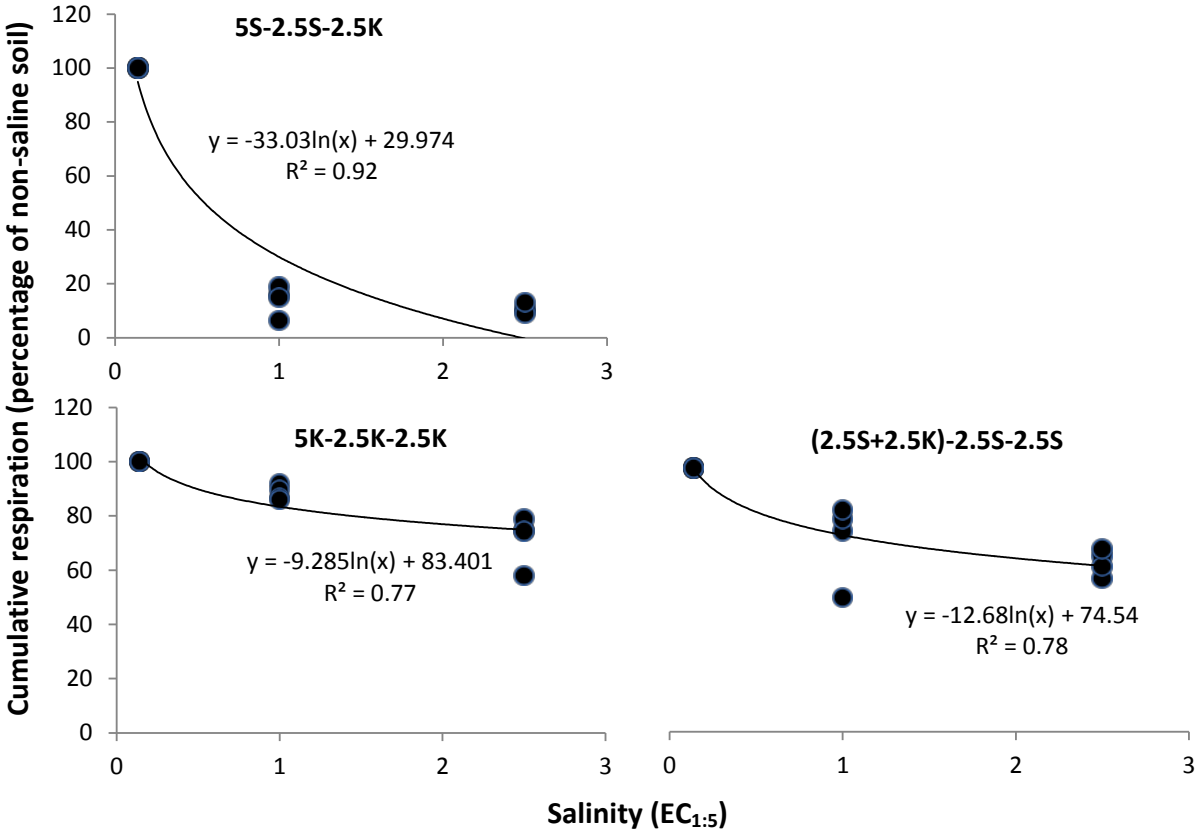
5S-2.5S-2.5K
y = -33.03ln(x) + 29.974
R² = 0.92
5K-2.5K-2.5K
y = -9.285ln(x) + 83.401
R² = 0.77
(2.5S+2.5K)-2.5S-2.5S
y = -12.68ln(x) + 74.54
R² = 0.78
Cumulative respiration (percentage of non-saline soil)
Salinity (EC1:5)

Figure S2.

# CHAPTER 5:

# General conclusions and future studies

# Chapter 5

# GENERAL CONCLUSIONS AND FUTURE STUDIES

## 1. General conclusions

Salinity is one of the major threats to agricultural production and contributes to land degradation, adversely affecting 5% of arable land or 100 million hectares worldwide (Lambers, 2003). Salt accumulates in soil particularly in arid and semi-arid areas where precipitation is lower than evapotranspiration (Flowers *et al.*, 1977; Rengasamy, 2008). In Australia, 30% of total land is affected by salinity and this area is predicted to increase due to human activities such as poor irrigation water quality and drainage systems, irrigation expansion into arid areas and increased clearing of native vegetation for agricultural use (Lambers, 2003; Rengasamy, 2006).

Salinity adversely affects crop growth and yield as well as soil biological properties through lowering the osmotic potential and induction of imbalanced ion uptake (Tejada *et al.*, 2006; Chowdhury *et al.*, 2011). With regard to soil biological properties, salinity can reduce microbial biomass and activity, resulting in reduced soil respiration (Wichern *et al.*, 2006; Setia *et al.*, 2010; Elgharably and Marschner, 2011) and altered community structure (Rietz and Haynes, 2003). The latter effect is due to differences in ability among microbial genotypes to adapt to salinity (Llamas *et al.*, 2008). An important adaptation mechanism is accumulation of osmolytes (Hagemann, 2011) which requires large amounts of energy (Oren, 1999).

Organic matter decomposition is the main energy source for soil microbes. However, saline soils generally have low organic matter content due to a poor plant growth (Setia *et al.*, 2011). It has been shown that plant residue amendment increases microbial activity in saline soil (Chowdhury *et al.*, 2011; Yan and Marschner, 2012) and has been used for saline soil remediation (Tejada *et al.*, 2006). However, the effect of residue amendment on microbial activity in saline soils may depend on the chemical composition of the residues as well as how they are applied.

Residue chemical properties (lignin content, C/N ratio and water extractable organic carbon) are important factor influencing residue decomposability (Abiven *et al.*, 2005; Xu *et al.*, 2006). Previous studies with saline soils used only one or two types of organic matter. Therefore little is known about how residue composition influences the impact of salinity on microbial activity.

When plant residues are added to soils, the supply of rapidly decomposable organic C is initially high, but decreases rapidly, leaving only slowly decomposable compounds e.g. cellulose and lignin (Abiven *et al.*, 2005; Fang *et al.*, 2005). A consistent supply of rapidly decomposable organic C through repeated residue addition may be more effective in alleviating the negative effect of salinity to soil microbial activity than a single amendment. However, little is known about the effect of residue addition frequency on microbial activity in saline soils. If plant residues that have a strong ameliorative effect on microbial activity in saline soils are difficult to obtain or more expensive than those with little effect, mixing of residues could be an economic option to maximise the beneficial effect of residue addition. To our knowledge, there are no published reports on the effect of mixed residues on response of microbial activity to increasing salinity.

The aim of the studies in this thesis was to investigate if the impact of salinity on microbial activity in residue amended soil is influenced by residue chemical properties, mixing of residues and addition frequency. The first study (Chapter 2) showed that residue chemical properties play an important role in amelioration of saline soil with residues. The addition of rapidly decomposable residues with low C/N ratio and lignin content, but high concentration of water extractable organic C reduced the negative effect of salinity on soil respiration compared to slowly decomposable residues. The importance of C/N ratio of the residues, that is N supply to the decomposing microbes, for reducing the negative effect of salinity on soil respiration was shown in the experiment where the C/N ratio of high C/N residue was decreased by addition of inorganic N. On the other hand, removal of water-extractable organic C from residues did not reduce the ameliorative effect of residues. Hence, C/N ratio was shown to be more important in alleviating the negative effect of salinity on soil respiration than water extractable C, possibly by stimulating the synthesis of N-containing osmolytes.

In Chapter 3, the influence of residue addition frequency on microbial activity and growth with increasing salinity was investigated. Two residues with distinct decomposability were selected from the previous study: mature canola with high lignin concentration and C/N ratio (C/N 82) as a slowly decomposable residue; and young kikuyu shoots with low lignin concentration and C/N ratio (C/N 19) as a rapidly decomposable residue. The residues were added once or two to four times, achieving the same total organic C addition (10 g C $kg^{-1}$). Repeated addition of kikuyu reduced the negative effect of salinity on soil respiration compared to a single addition. However, this was not the case for slowly decomposable canola residue where salinity

reduced soil respiration strongly when added once or multiple times. Thus the slowly decomposable residue did not become more effective when added repeatedly compared to a single addition.

The effect of mixing on the amelioration potential of residues was investigated in the experiments described in Chapter 4. A rapidly decomposable residue (young kikuyu, C/N 19) and a slowly decomposable residue (saw dust with 114 C/N ratio) were used. In the first part of the study, mixed residues were added once with different proportions of rapidly and slowly decomposable residues. A proportion of only 25% rapidly decomposable residue was sufficient to reduce the negative impact of salinity on soil respiration compared to a single amendment of slowly decomposable residues. In the second experiment of Chapter 4, residues were added once or three times (on days 0, 14 and 28) with a final proportion of 25% kikuyu and 75% sawdust. The impact of salinity on soil respiration was smallest when kikuyu addition was early in the incubation period, that is, if it was added to soil with no or little slowly decomposable residue present. This may be due to the proportion of soil microbes in proximity of the rapidly decomposable residue which will be greater if little slowly decomposable residue is present in the soil when the former is mixed into the soil.

**2. Suggestions for future studies**

The experiments described in this thesis provided new insights about how residue amendments could be used to reduce the negative effect of salinity on soil microbial activity. However, several areas could be investigated in future studies.

A possible reason for the reduction of the negative impact of salinity on soil respiration by amendment with low C/N residues is the greater ability to synthesise

osmolytes many of which are N-rich (Ashraf and Foolad, 2007; Warren, 2013). In a future study, residues with different C/N residues could be produced by growing a cereal at different N supply. The residues with different C/N ratio could then be applied to soils differing in salinity. Over a three week period, soil respiration would be measured continuously and compared with soil osmolyte concentration that could be determined weekly as described in Warren (2014).

To investigate which residue is decomposed if mixtures of residues are applied, a cereal could again be grown with different N supply, but half of the plants are supplied with $^{13}CO_2$ (Baumann *et al.*, 2011). The residues from these plants would then be added to soil in mixtures of low and high C/N residues, one of which is $^{13}C$ labelled. The $^{13}C$ could be tracked in both released $CO_2$ and microbial biomass C as well as in the soil at the start and the end of the experiment.

Using molecular biology methods, it could be determined to what extent residue addition alters the microbial community composition and its metabolic potential. Soils differing in salinity without and with residues of different C/N ratio could be sampled at different times for extraction of DNA and RNA combined with continuous measurement of soil respiration. Methods such as pyrosequencing or metatransciptome sequencing using an Illumina MiSeq or HiSeq platforms could be used for detailed information about microbial community composition. Quantitative PCR could be used to quantify abundance of specific groups such as bacteria or fungi (Rousk *et al.*, 2010; Stoeck *et al.*, 2010; Damon *et al.*, 2011; Caporaso *et al.*, 2012).

In the experiments described in this thesis, the ameliorative effect of residues was investigated with respect to soil respiration and microbial biomass. However, the main goal of most soil amelioration methods is to improve plant growth, particularly

on agricultural land. Future experiments could determine the effect of addition of residues with different C/N ratio (single or mixed) on plant growth, nutrient uptake and yield. Such experiments could initially be carried out in pots in a glass house and later in the field. The experiments could be conducted over several growing seasons to investigate the longer term effect of residue amendment.

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