

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

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the degree of Doctor of Philosophy

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Dedicated to my parents

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Abstract

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

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

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

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

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

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

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

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

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

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

The aim of the sixth experiment was to assess the response of soil microbes to increasing salinity in rhizosphere compared to non-rhizosphere (bulk) soil using the non-saline soil ($EC_e 1 \text{ dS m}^{-1}$). The soil was adjusted to $EC_e 13$ and 19 dS m^{-1} by adding NaCl and placed in pots. Barley was planted in half of the pots to obtain rhizosphere soil whereas unplanted pots were used for generation of bulk soil. The pots were placed in a greenhouse and soil moisture was maintained throughout by weight. After 5 weeks the planted and unplanted pots were harvested to collect rhizosphere and bulk soils to be used for the following incubation experiment. The EC levels (EC_1 , EC_{13} and EC_{19}) from the pot experiment (referred to as original) were either maintained or adjusted to $EC_e 13$, 19 , 31 and 44 dS m^{-1} by adding different amounts of NaCl. Cumulative respiration and microbial biomass C in rhizosphere and bulk soil decreased with increasing adjusted EC. Across the whole range of adjusted ECs, the decrease in cumulative respiration with increasing EC did not differ between rhizosphere and bulk soil. However, compared to the treatments where the EC was maintained, the percentage decrease in cumulative respiration when the EC was increased to EC_{44} was smaller in rhizosphere than in bulk soil. The smaller decrease in microbial activity at the highest EC level in rhizosphere compared to bulk soil suggests that rhizosphere microbes may be less affected by high salinity than bulk soil microbes.

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

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

Declaration

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

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