DOCTOR OF PHILOSOPHY

Effects of salinity on soils of the Gefara Plain, Libya

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Award date:
2011

Awarding institution:
Bangor University

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PRIFYSGOL
BANGOR
UNIVERSITY

A thesis submitted to Bangor University in candidature for the degree of Doctor of Philosophy in Soil and Environment

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September 2011

Abstract

In arid and semi-arid ecosystems, salinization is a major threat to the productivity of agricultural land. The Gefara Plain located in the northwest of Libya; contain about 80% of the total agricultural activity. The high water requirements for the populations and agriculture are depleting the groundwater aquifer, resulting in intrusion of seawater in the first few kilometres along the coast. Due to increasing salinity in the groundwater used for irrigation, the soils of the Gefara Plain are becoming increasingly saline. The objective of this study is to investigate the effects of salinity on soil respiration in the Gefara Plain. The rate of CO_2 evolution from soil is principally a function of soil microbial activity. This study investigated the sensitivity of these soils to increased salinity using soil respiration as an integrating measure of soil function. Soil was collected from four sites located in the Gefara Plain, Almaya, Janzur, Gargaresh and Tajura. The soils were shown to differ in salinity and the amount of organic matter in the soil. Soil collected from Tajura had the highest background salinity, and Janzur had the highest organic matter content. All of the soils had relatively low organic matter content, ranging between 0.49-1.25%. *In vitro*, addition of NaCl decreased soil CO_2 efflux in all soils, with the greatest decrease shown in Tajura. The smallest decrease was shown in Janzur the soil with the highest organic matter content. Similar results were also shown for substrate induced respiration using glucose and glutamate. In a field investigation, *in situ* addition of NaCl showed the same sensitivity of the soils as in the laboratory investigation. In further laboratory investigations a greater decrease in soil CO_2 efflux was shown with NaCl compared to similar concentrations of Na_2SO_4 and KCl respectively. The cumulative rate of $^{14}CO_2$ of added ^{14}C-labelled *Lolium perenne* and ^{14}C-labelled glucose to soils was decreased under effects of water containing different concentrations of NaCl at 20, 50, 70, 90, 150 and 200 mM compared to the control at any time of incubation in Tajura, Gargaresh, Almaya and Janzur respectively. To determine the effects of additional organic matter on the influence of salinity, and the decomposition of soil organic matter under salinity, investigations using coconut husk compost and ^{14}C-labelled *Lolium perenne* were carried out. The coconut husk compost, addition to the soils increased pH, electrical conductivity and K^+ concentrations in the soil solution. Addition of coconut husk compost both sterilized and unsterilized increased the rate of soil CO_2 efflux, but did not change the relative sensitivity of the different soils to addition of NaCl. Similarly after addition of ^{14}C-labelled *Lolium perenne*, again a similar sensitivity of the soils to NaCl was shown.

In further investigations attempts were made to separate mycorrhizal hyphal respiration from soil microbial heterotrophic respiration. Using cultures of wheat (*Triticum aestivum L. var*) grown with separate plant, mycorrhizal hyphal and soil microbial, as well as soil microbial only compartments, the effect of NaCl addition was investigated. Growth of external hyphae, and CO_2 evolution rate in the presence external hyphae were reduced by increased concentrations of NaCl to the soil compared to the control. In the mycorrhizal compartment the decrease in soil CO_2 efflux was proportional to the decrease in hyphal length.The tolerance to salt of extracted soil bacterial communities was investigated using an assay based on the leucine incorpation method to measure bacterial growth. Clear concentration-response relationships between microbial growth and soil salinity could be established, providing estimates of the salt tolerance. However, there was no relationship between *in situ* soil salinity and the salt tolerance of the soil bacterial communities, suggesting that other factors were more influential for the actively growing decomposer community in these soils.

Dedication

"I dedicate this thesis to my wife Aziza , whose patience and understanding long ago passed my understanding"

Acknowledgements

I am most thankful to Almighty Allah for giving me the patience and making it possible for me to produce this work. I would like to acknowledge my supervisors Professor Douglas Gold bold, Professor Davey Jones for their continual support and guidance throughout my research and the encouragement, they have been an excellent mentors.

I shall always remember all the phrases and words learnt throughout the meetings. It has been an enjoyable education experience.

I would like to thank Dr. Johannes Rousk, and my many colleagues in the SNERG group for advice and support throughout the period of the research. I would like to thank to General Authority for Water-Tripoli and Dr. Saleh Asadg the main provider of data and information about the study area.

I am grateful to many people for help, both direct and indirect with the research.

The Libyan High Education, Faculty of Engineering are acknowledged for providing me with a grant to carry out this research.

I am grateful to my wife Aziza, my sons Mohammed and Khalifa, my daughters Mariam, Mowada and may for their encouragement, understanding, and tolerance during of the study.

List of abbreviations

ANOVA-Analysis of variance

$BaCl_2$- Barium chloride

C- Carbon

^{14}C- Carbon 14 isotope

Ca^{2+}- Calcium

CEC- Cation exchange capacity

CO_2- Carbon dioxide

Cu- Copper

d- Day

EC- Electrical conductivity

IRGA- Infra-red gas analyser

g- Gram

h- Hour

ha- Hectares

H^+- Hydrogen

HCO_3^- - Bicarbonate

K^+- Potassium

KCl- Potassium chloride

(Log(EC50))- Logarithm of effect concentration at 50% inhibition of the bacterial growth

M- Molar

MBS- Microbial biomass

mg- Milligram

Mg^{2+}- Magnesium

Mh- Million of hectors

mM- Millimolar

N- Nitrogen

Na^+- Sodium

NaCl- Sodium chloride

NaOH- Sodium hydroxide

Na_2SO_4- Sodium Sulfate

NH_4^+- Ammonium

NO_3^-- Nitrate

O_2- Oxygen

OH^-- Hydroxide

OM- Organic matter

P- Phosphorus

S- Sulfur

SAR- Sodium adsorption ratio

SE- Standard error

SIR- Substrate induced respiration

SO_4^{2-}- Sulfate

SOC- Soil organic carbon

SOM- Soil organic matter

WHC- Water holding capacity

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Chapter 1

Overview of thesis

1.1 Introduction

Soil salinity has become a major environmental issue and excessive salinity in the soil has been considered the main limiting factor for plant growth (McKersie and Leshem, 1994). The arid and semi-arid regions of the Mediterranean combine a low rate of rainfall and a high rate of evapotranspiration, and irrigation in these areas is often increasing soil salinity (Heatwole et al. 1992). The Gefara Plain located in the northwest of Libya, contains about 80% of the total agricultural activity. Population growth, continuous development and agriculture are depleting the groundwater aquifer, resulting in intrusion of seawater in the first few kilometres along the coast (Sadag and Karahanolu, 2001). Sea water intrusion has been accelerated by the pumping of ground water in the Gefara Plain, and this progressive seawater intrusion in most of the water wells in the Gefara Plain, especially in shallow aquifers, and the use of water of poor quality for irrigation has led to the deterioration of the soil's physical and chemical properties and a decreasing infiltration rate (Suarez and Gonzalez Rubio, 2010).

1.2 The objective of this study

In the work presented here, soil was collected from four sites located in the Gefara Plain, Almaya, Janzur, Gargaresh and Tajura. The objective of this study is to investigate the effects of salinity on soil fertility in the Gefara Plain. The study investigated the sensitivity of these soils to increased salinity using soil respiration as an integrating measure of soil function. The rate of CO_2 evolution from soil is principally a function of soil microbial activity, and measurements of CO_2 efflux reflect the stress existing in a saline soil.

In addition, microbial activity also contributes to improved aggregation stability in sandy soils, which in turn leads to increased infiltration, reduced erosion and runoff in soil. This attempts were also made to investigate the effects of salinity on the total microbial biomass, mycorrhizal hyphae as well as on bacterial growth rates.

1.3 The plan of thesis

The thesis is divided into eight chapters as detailed below:

Chapter 2 gives the background and a literature review, detailing (1) geological and climate of Gefara Plain (2) seawater intrusion in the coastal region of the Gefara Plain (3) soil salinity.

Chapter 3 assesses soil quality, and includes an investigation of soil properties and processes as they relate to the ability of a soil to function effectively as a component of a healthy ecosystem. Soil properties assessed include water holding capacity, moisture content, organic matter, water infiltration, soil pH, soil EC and soil exchangable cations.

Chapter 4 aims to identify effects of salinity on soil respiration at the four sites. In this work, in a laboratory investigation, substrate induced respiration was determined. To do this both the effects of adding glucose and glutamate, in both dry from and as a solution was investigated. To estimate the effects of salt addition, solutions of NaCl, Na_2SO_4 and KCl were used. Soil respiration rate was also measured *in situ* four fields located in Almaya, Janzur, Gargaresh and Tajura during the summer season 2009. At the sites, each field was subdivided into 16 sites: 4 sites were untreated, 4 sites were treated with distilled water as the control, 4 sites were treated with NaCl at a concentration of 20 mM, and 4 sites with 50 mM.

Chapter 5 investigates salt effects on mycorrhizal hyphal growth. A laboratory experiment was designed to examine CO_2 efflux from soil with and without external hyphae growing from wheat plants. A rhizobox was used comprising of a plant tube and hyphal tube separated by 35 μm nylon mesh to allow the external hyphae to pass. A solid tube totally prevented the ingrowth of roots and fungal hyphae. The plants were watered with distilled water as the control, and treated with concentrations of NaCl added at 20 and 50mM, every two days. Hyphal length was determined by extraction of hyphae from both the hyphal and soil tubes, and measured using the gridline intersection method. Reported are the findings of the analysis of the relationship between the length of external hyphae and CO_2 evolution under the effects of NaCl.

Chapter 6 to determines both the effects of salinity on decomposition of soil organic matter, and the effects of soil organic matter addition on the influence of salinity on soil microbial activity.

This was carried out by amending the soils with ^{14}C-labelled *Lolium perenne*, ^{14}C-labelled glucose or 1% and 2% coconut husk compost. Soils were treated with NaCl at 20, 50, 70, 90, 150 and 200 mM, or distilled water as the control.

Chapter 7 to assesses the tolerance of the bacterial communities from these soils to salt (NaCl), by extracting bacterial communities and subsequently determining the concentration-response relationship between bacterial growth and NaCl exposure. The Gefara Plain soils used are low in organic matter. Bacterial growth was investigated after a one month incubation with 0, 1 or 2% added coconut husk compost additions to stimulate microbial growth levels, and under treatment with eight different concentrations of NaCl (0, 10, 20, 70, 90, 150, 200 and 400 mM).

Chapter 8 provides a general discussion of the results from all the experimental chapters, highlights the key conclusions and identifies areas for further work.

Chapter 2

Background and literature review

2.1 Introduction

In arid and semi-arid areas, soil salinity has become a major environmental issue and excessive salinity in the soil has been considered the main limiting factor for plant growth - and, consequently, for sustainable crop production. Salinity is common in topographical lowlands near the sea, where intrusion of seawater to the aquifer occurs (McKersie and Leshem, 1994). The salt composition of these soils is the same as that of the seawater (Kelley, 1951). Increases in settlement and population in these areas, together with increases in agricultural and industrial activities, place pressure on water resources. The greatest effect of this pressure is the change in quality (salinity) of underground water sources (Jones et al., 1999). This is the result of various factors. The most important of these – considering proximity to the coast - is seawater intrusion (Richter and Kreitler, 1993).

Libya is a southern Mediterranean country with a shoreline extent of about 1,900 km. Most of the population lives in the coastal Gefara Plain. The total area of the Gefara Plain is 17000 km^2. It runs from the Tunisian border in the west and extends east to Al Khams, to the Mediterrean in the north, and southward to Gebal Nafusah where the elevation reaches 400 to 700 m above sea level. The Gefara Plain area forms fertile lands that contain more than 80% of the country's total agricultural activity. Figure 1.1 shows the location of the Gefara Plain, and also the study areas – these include the zones Almaya, Janzur, Gargaresh and Tajura and Figure 2.2 shows sample collection from Janzur site. Sadeg and Karahanoolu (2001) have shown that aquifer water was partially replaced by intruding sea water in the first few kilometers along the coast. Sea water intrusion has been accelerated by the pumping of ground water, especially in the Gefara Plain.

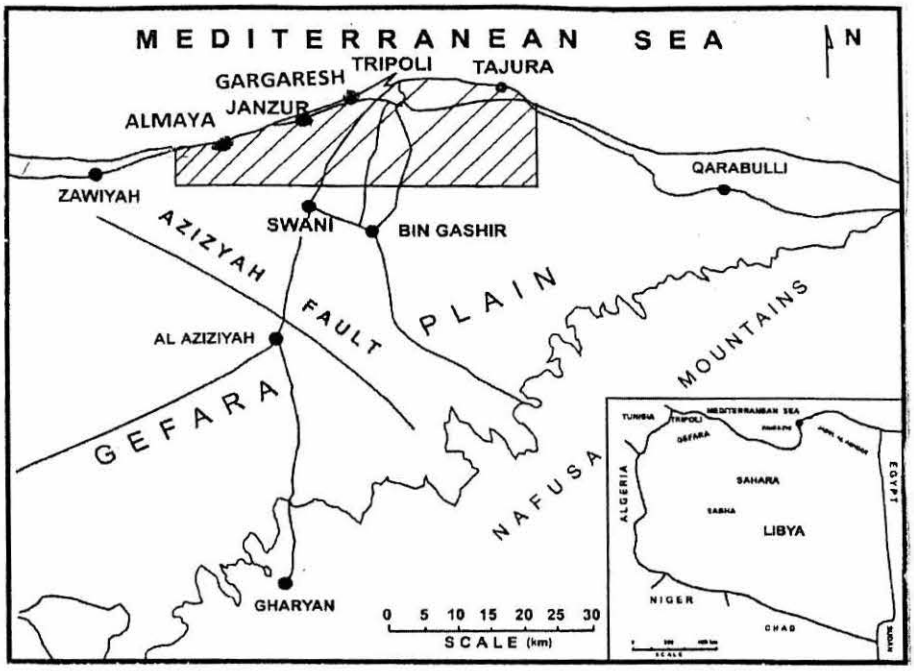

Figure 2.1 Location map of the study area

Figure 2.2 Shows sample collection from Janzur site.

2.2 Geological formations of Gefara Plain

Geological and morphological studies of the Gefara Plain indicate that the land used at present – along with that which is currently under reclamation and development - developed from transported sands or material deposited in the middle and late Quaternary. In the sublayers of the soil profile there is an accumulation of limestone in varying formations of old pedological origin. The overall study area is located in the northern coastal part of the Gefara Plain, and forms an almost rectangular area of 763 km^2 between the Mediterranean Sea and the cities of Swani and Bin Gashir in the south. The Gefara Plain, including the study area, has been the subject of numerous geological studies (GEFLI, 1972; IRC 1975; Kruseman, 1977; Krummenacher 1982; NCB and MM, 1993). A review is given below that summarizes the geological outline of the Gefara Plain as a whole.

Detailed information about the geology of the area can be obtained from the cited literature. The plain can be divided into three different parts: the coastal strip, the central part and the foot-hill strip. The plain is covered by Quaternary deposits with occasional outcrops of limestone hills belonging to the Azizia Formation. Calcarenites, covered by coastal sandstones and brown silts, form the coastal strip. The central part is covered mainly by poorly consolidated, A eolian deposits mixed with brownish silts. The southern border of the central part interlocks with the foot-hill strip, which is made up of fluvial and proluvial coarser sediments. The topographic elevation rises gradually toward the south and reaches 200 m above mean sea level at the southern end of the plain. Seasonal streams flow over the central part drain the plateau and fan out before reaching the sea. Other streams have longer courses and deepen their channels over the plain surface to form wadis. The Gefara Plain consists of Late Tertiary to Quaternary strata resting on a faulted and a tilted Mesozoic basement.

The Azizia formation is present throughout the Gefara Plain, especially in the western and north central parts. The Aziza formation consists of dolomitic limestone and limestone. North of Azizia, the fault dips to a great depth and reaches 900 m depth in the coastal area. The Abu Shaybah formation overlies the Azizia formation in the northern area, and contains light colored, cross-bedded, friable quartz sandstone and conglomerates with brown, yellow and red silt and clay intercalations. The Ain Tobi formation (Upper Cretaceous) consists of limestone and forms most of the Nafusa escarpment at the southern border of the Gefara plain. An Upper Tertiary- Quaterary series, consisting of argillaceous sandstone with clay lenses, reaches its greatest thickness in the Tripoli area. The Miocene-Pliocene- Pleistocene complex forms the topmost series in the northern part of the Gefara plain. This unit consists of fossiliferous detrital limestone and marl formations alternating with yellow clay and sand lenses. The total thickness varies from 40 to 150 m. The Quaternary deposits cover the major part of the Gefara Plain as well as the plateau surface. The Gefara formation consist mainly of fine materials: mostly silt and sand with occasional gravel and caliche bands. In the study area, the Gargaresh formation forms steep cliffs along the shore of the Mediterranean Sea. This formation is made up of calcarenite, including shell fragments and minor sand grains, interbedded with silt lenses. The sand dunes and sheets that cover large areas in this region represent eolian deposits in the Gefara Plain (IRC, 1975; Krummenacher, 1982).

2.3 The Gefara Plain soils.

The soils of Gefara Plain, especially in Gargaresh, Almaya, Janzur and Tajura areas, usually contain 1% to 10% calcium carbonate. Organic matter content is <1% (Ben Mhamod, 1995). With huge areas of calcrete, the Gefara Plain outcrops developed during the Pleistocene epoch.

Saline soils are usually of the solontchak type in the low-lying areas in the north western parts of the Gefara area. Sandy arid and semi-arid soils prevail in the eastern parts of the Gefara area.

The types of soils of the Gefara Plain are either entisols or aridisols that include psamments and orthents (Bin Mahmoud et al., 2000). Cation exchange capacity (CEC) for these soils is low due to the low clay percentage, and ranges between 4.3 to 8.2 meq/100 gm soils. The percentage total nitrogen ranges between 0.023 to 0.007% and the exchangeable sodium percentage (ESP) ranges from 0.6 to 14.8% (Soil–Ecological expedition Selkhozprom Export company, 1980; Bin Mahmoud et al., 1995). Table 2.1 shows the particle size distribution of the Gefara soils.

Table 2.1 Particle size distribution for the Gefara Plain soils (Gargaresh, ALmaya, Janzur and Tajura areas) (Soil–Ecological expedition Selkhozprom Export company, 1980; Ben Mahmoud, 1995).

Fraction	Percentage
Sand	88.6-94.1
Silt	3.3-6.8
Clay	2.6-4.6
Type of soil	Entisols
Soil texture	Sandy

2.4 Climate of the Gefara Plain

The dominant climate is Mediterranean, but to the south it is semi-desert. The annual rainfall at Tripoli is between 300 to 380 mm, and decreases towards the south of the Gefara Plain where it reaches 50 mm. In the south, it may be rainless for several years. The average temperature on the coast in the Gefara plain area is 10°C to 12°C in the winter season, while average temperature in the summer season is from 26 °C to 29 °C. Figure 2.4 and Figure 2.5 show the climate classification and the temperature distribution (National Atlas, 1977).

The climate of the Gefara area is classified as arid in the west, semi-arid in the middle, and dry sub-humid in the east (Figure 2.3). These climate types are characterized by a high level of incident radiation, high seasonal temperature variations, low humidity and strong winds causing dust storms.

Precipitation is intense and sporadic. Throughout the Gefara area, precipitation does not exceed 400 mm, but more than 90% of the Gefara Plain receives more than 150 mm rain per year. Furthermore, precipitation occurs irregularly: Figure 2.6 shows a rain fall distribution map (mm/year) as an average of 38 years (Sadag and Karahanolu, 2001). Higher temperatures could result in a reduction of soil fertility due to higher rates of decomposition and losses of organic matter, and could affect nutrient cycling (Dubief, 1971).Evaporation rates are in the Gefara are high, ranging from 1,700 mm in the north to 6,000 mm in the south, (IMB, 1980). A high rate of potential evapotranspiration can further reduce yields. Most precipitated water evaporates without any benefit, whereas a small percentage infiltrates to the groundwater.

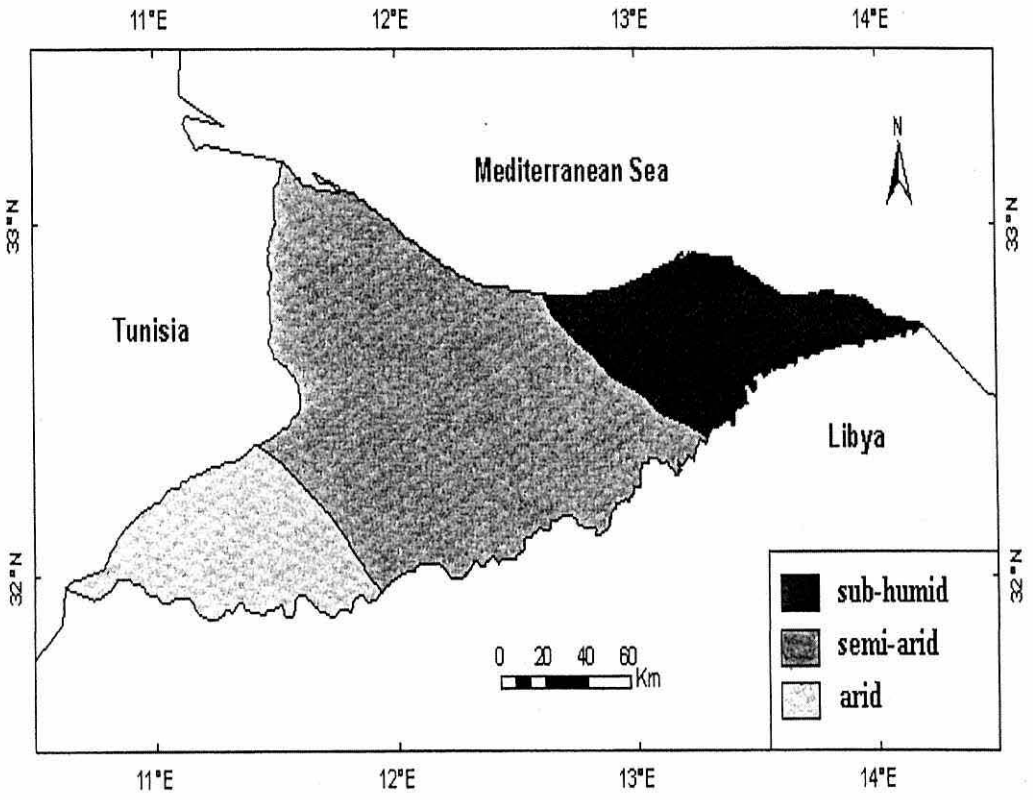

Figure 2.3 Climate regions in the Gefara Plain after the De Martonne climate Classification (Source: Libyan Meteorological Department, Tripoli, 1978).

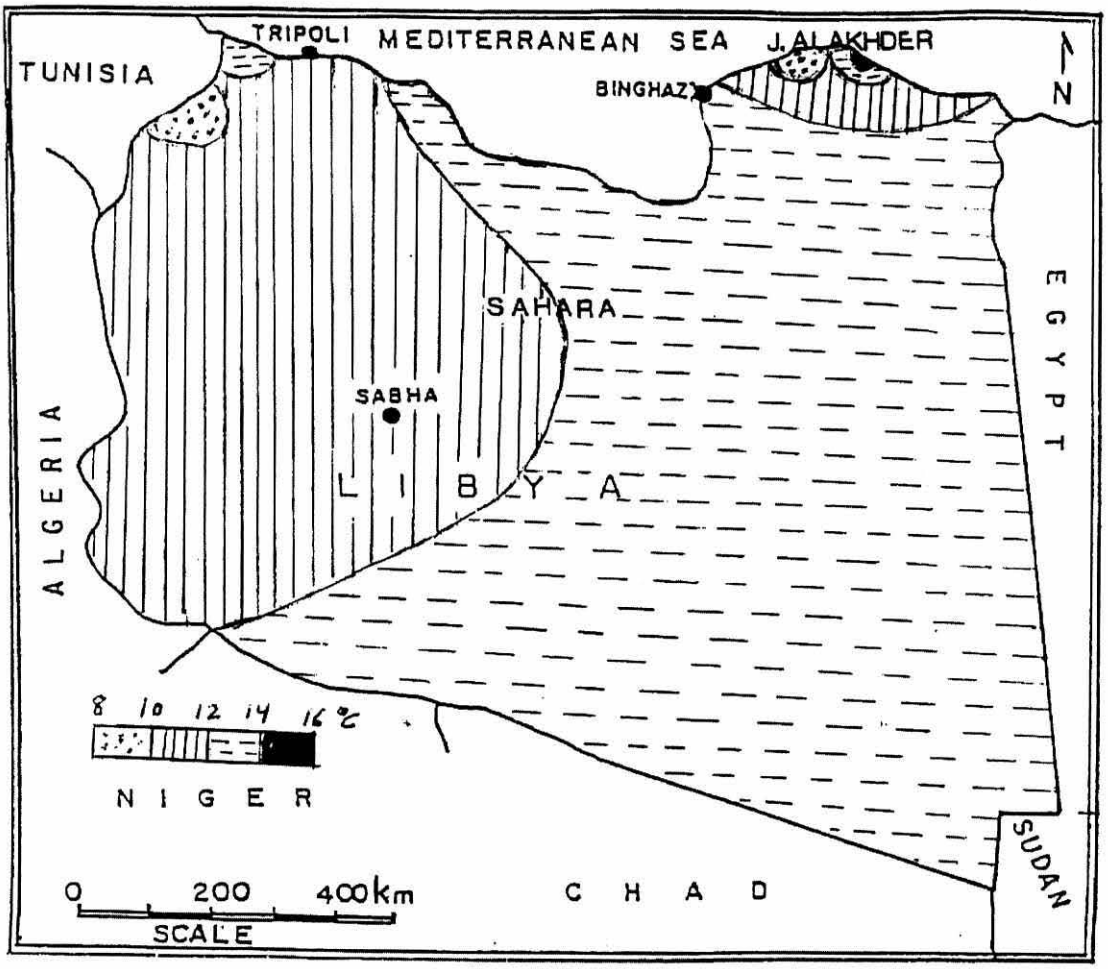

Figure 2.4 Temperature distribution in the winter season (National Atlas, 1977).

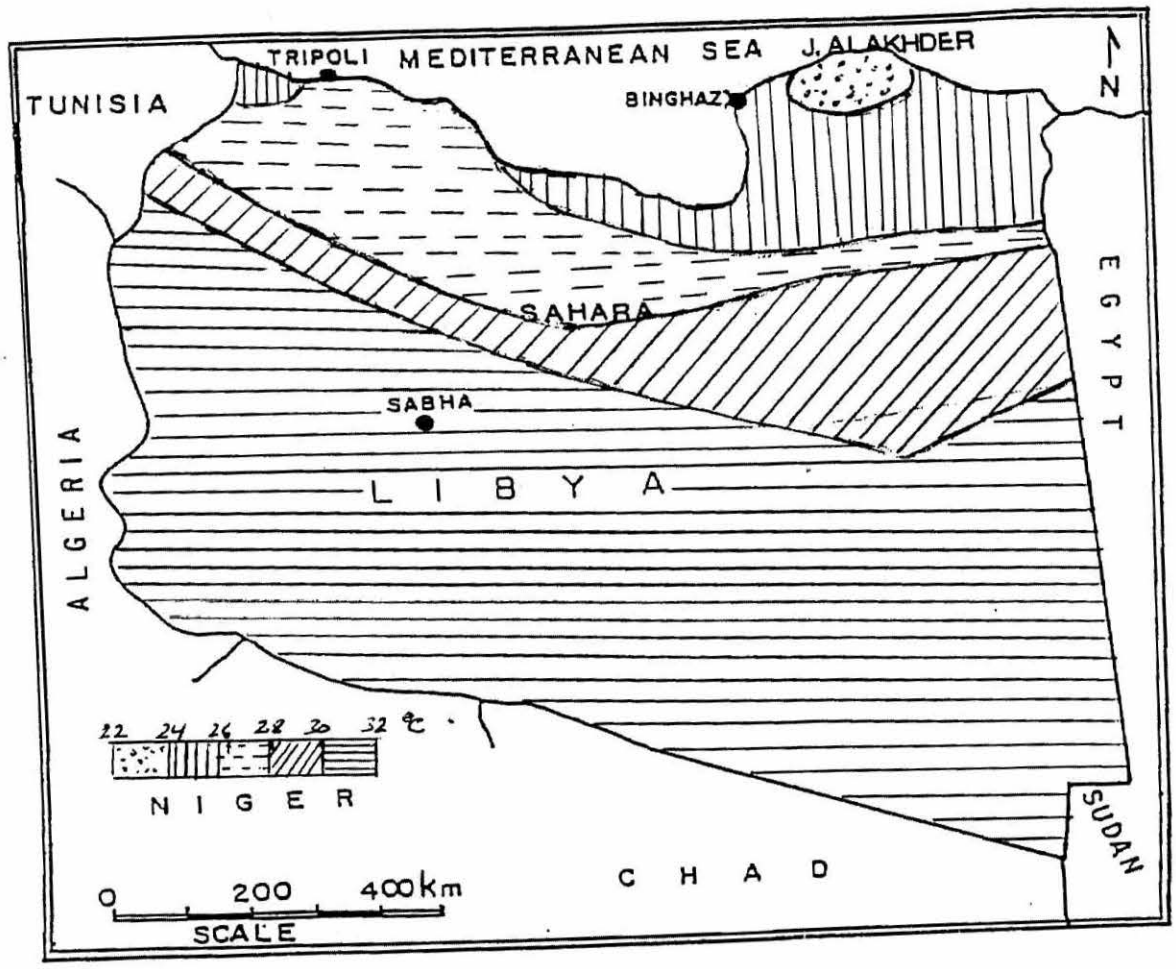

Figure 2.5 Temperature distribution in the summer season (National Atlas, 1977).

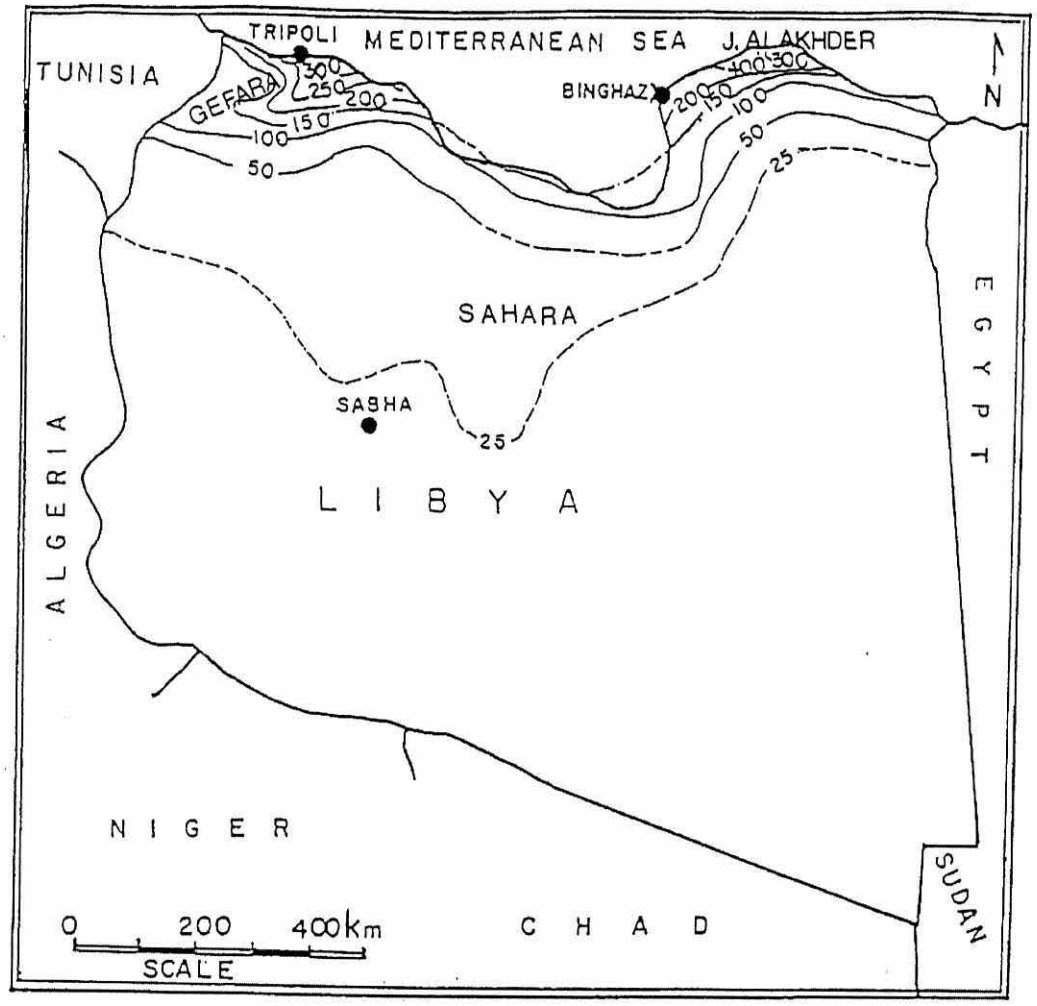

Figure 2.6 Rainfall distribution in Libya (mm/year) (Sadag, 2001).

2.5 Population growth in the Gefara Plain

The rapidly increasing population of Libya is a great problem. The population currently increases 4% annually, which is considered one of the highest rates of increase worldwide. This results in overexploitation of water and land resources through over-cultivation and poor irrigation practices. Increasing water demand for social, agricultural, and industrial developments has accelerated groundwater deterioration (Salem, 2005). The increase in population of the Gefara area has led to settlement expansion on agricultural lands. There is high emigration from the rural areas to the urban areas, especially to Tripoli. Modern societies may also threaten the dry lands in many ways; they need roads and highways, which results in the construction of pipelines and canals, and also need land for the establishment of factories and buildings. The assessment and prediction of the population over the time period 1950-2015 shows a sharp increase in growth from 1960 (Salem, 2005). This increasing population needs an increasing food supply, resulting in intensification of agriculture, which further leads to heavy and concentrated use of fertilizers and pesticides. The destruction of vegetation has always proceeded from regions under human influence in response to the need for agricultural areas, roads, water sources and so on.

2.6 Seawater intrusion in the coastal region of the Gefara Plain

2.6.1 Introduction

Throughout the world, areas with arid and semi-arid climates are suffering from a water shortage problem. Population growth and continuous development require larger quantities of water in coastal regions. However, groundwater is vital to many nations. Overexploitation of groundwater has become a common issue along coasts where good quality groundwater is available. Consequently, many coastal regions in the world have extensive saltwater intrusion in aquifers, resulting in severe deterioration of the quality of groundwater resources. Saltwater intrusion problems in coastal aquifers are not new. Limited seawater intrusion is a natural process that occurs in most coastal aquifers, but when it causes ground water salinization it becomes a concern. The deleterious effects of salinization resulting from seawater intrusion have caused significant losses in potable water supplies and of agricultural production (FAO, 1997). The arid and semi-arid regions of the Mediterranean combine a low rate of rainfall and a high rate of evapotranspiration, and are subject to extreme recurrent droughts. Long-term aquifer overexploitation near the sea shore creates an ever-increasing gap between available supply and demand for water, which leads to deterioration in the quality of this resource (US EPA, 1990; Ford et al. 1992; Heatwole et al. 1992; Hrkal, 1992). However, intrusion of seawater is now affecting many coastal areas in the world. In Italy, for example, the Ministry of the Environment in 1997 classified 15 coastal aquifers as at risk of salinization (Grassi et al., 2007). However, Libya is considered as one of those countries which suffer most from limited water resource availability because most parts of the country are either semi-arid or arid, and situated in one of the driest regions of the world.

2.6.2 Water resources in Libya

2.6.2.1 Groundwater: Libya has limited renewable water resources because most parts of the country are either semi-arid or arid. Groundwater is the main source of water supply, providing 88% of the country's water needs. It is found in five basins, three of them in northern Libya - The Gefara Plain, El Jebal El-Akhdar, El-Hamada El-Hamra - and two in southern Libya: Murzuq and El-Kufra-Serir. The basins in the north suffer from severe deterioration; their recharges have not been precisely determined, but it is estimated to be about only 500 million m^3 per year from precipitation (General Water Authority, 1985). The basins in the south have a large storage of water, and some of this water is transferred to the northern areas by the Great Man-Made River Project (El Tantawi, 1998a). Based on a water balance of the groundwater basins in Libya, a severe deficit in water supply occurs in the Gefara Plain basin and moderate deficit in Jebal El-Akhdar basin, caused by the concentration of the population in north western and north eastern Libya, while there is no deficit water in the southern basins (El-Kufra-Sarir and Murzuq). UN research groups estimate the amount of groundwater in the El-Kufra basin to be 200 billion m^3 and in Sarir 15 billion m^3 (Schliephake, 2004).

2.6.2.2 Surface water: Surface water is controlled by precipitation. Surface water supplies about 3 % of Libya's total water consumption. The country has established 16 dams on wadis with a total storage capacity of 385 million m^3 and an average annual capacity of 60.6 million m^3 per year; this water appears in winter season only (General Environmental Authority, 2002). These dams serve both as water reservoirs and for flood and erosion control. The wadis are heavily settled because the soil in their bottoms is often suitable for agriculture.

In addition, an often a high water table in the wadis makes them logical locations for well digging (McMorris, 1979).

Many springs are found in mountainous areas. A large number of reservoirs in the coastal regions are of Roman origin; these reservoirs are considered to be one of the country's earliest storage systems for runoff water. Additional dams are planned, with the aim of achieving a total storage capacity of 686 million m^3/year of precipitation water (El-Tantawi, 1998a).

2.7 The Man-Made River Project

The Man-Made River Project is considered the biggest project to combat desertification in Libya. The pumping of large amounts of groundwater in the Gefara area has resulted in deterioration of groundwater quality and quantity. The project will alleviate the pressure on water resources in northern parts of Libya, including the Gefara area. It is a massive project with four-meter-diameter pipes and a length of about 4000 km, aiming to divert part of the groundwater from the southern basins to the coastal areas where about 90% of Libya's population has settled. The project consists of three phases (The Great Man-Made River Water Utilization Authority, General Water Authority of Libya, Unep et al., 1996, Tarbush, 1988, and EL-Tantawi, 1998a). In the first and largest phase, water was sourced from two well fields (Serir and Tazirbu) in south eastern Libya to carry 2 million m^3/day to the coastal areas, extending from Benghazi to Sirt. This phase was completed in September 1991. The second phase delivers 1 million m^3/day of water to the fertile Gefara Plain in north western Libya from more than 500 wells distributed in several fields in the Murzuq Basin in south western Libya. This phase was completed in September 1996. The third phase consists of three parts: the first adds1.68 million m^3/day of water to the first phase from an additional well field within El-Kufra-Serir Basin. The second and the third phases do not involve any additional water production. Instead, conveyance lines from the first phase (Agedabia reservoir) will be extended further to the east to reach Tobruq in the east

of coastal area, and further to the west to link (Sirt Reservoir) with the second phase pipelines.

The execution of the third phase will be dependent upon financial possibilities and water needs (Schliephake, 2004). The Man-Made River Project will carry 5.68 million m^3 of water a day from the southern basins to the heavily populated areas in the north: 3.68 million m^3 in the eastern conveyance system and 2 million m^3 in the western system, with 80% of the water being used for irrigation. It is noted that in Libya the amount of water withdrawal is over eight times the country's renewable water resources. The gap is filled largely by the pumping of fossil groundwater (FAO, 2001).

2.8 Seawater intrusion in the coastal region of the Gefara Plain.

The phenomenon of seawater intrusion in coastal aquifers can be explained by the hydraulic gradients that occur towards the sea. An essentially stable interface between discharging groundwater and seawater exists under natural conditions. Persistent disturbances in the hydraulic quasi-equilibrium of coastal aquifers, as caused by excessive groundwater abstraction, induce encroachment of saline waters. Seawater intrusion may occur through a number of different processes, including lateral seawater intrusion, inter-aquifer exchange (Figure 2.8). However, Libya has experienced seawater intrusion in the coastal aquifers since the 1930s because of the ever-increasing demands the country has placed on underground water resources. The seawater intrusion covers an area of 250 km^2, and the contamination front has already progressed 10 km inland in the Tripoli region (Sadeg and Karahanoolu, 2001). The Tripoli region has been exposed to problems of seawater intrusion since the construction of the Salt Canal in the 1930s. To investigate seawater intrusion, water samples have been collected from water wells located along selected sea to land profiles Figure 2.7 shows a network water wells in the Gefara plain area.

Table 2.2 shows the chemical analysis of the water samples collected during 2008. Irrigation water containing large amounts of Na is of special concern due to the effects of Na on the soil.

The sodium hazard is usually expressed in terms of the sodium adsorption ratio (SAR). Table2. 2 shows that water irrigation of the Tajura site carries a higher danger than at other sites

Table2.2 The chemical analysis of the water samples collected during 2008 in the Gefara area.

Sites	pH	Electrical conductivity (EC) ($mScm^{-1}$)	sodium adsorption ratio (SAR)
Almaya	7.5– 7.9	0.86- 3.9	6.40 – 28.3
Janzur	7.2- 7.7	0.57- 2.1	6.25 – 15.5
Gargaresh	8.0- 8.2	0.90- 3.5	7.36 – 38.8
Tajura	7.9- 8.3	2.7- 4.4	23.7 – 44.7

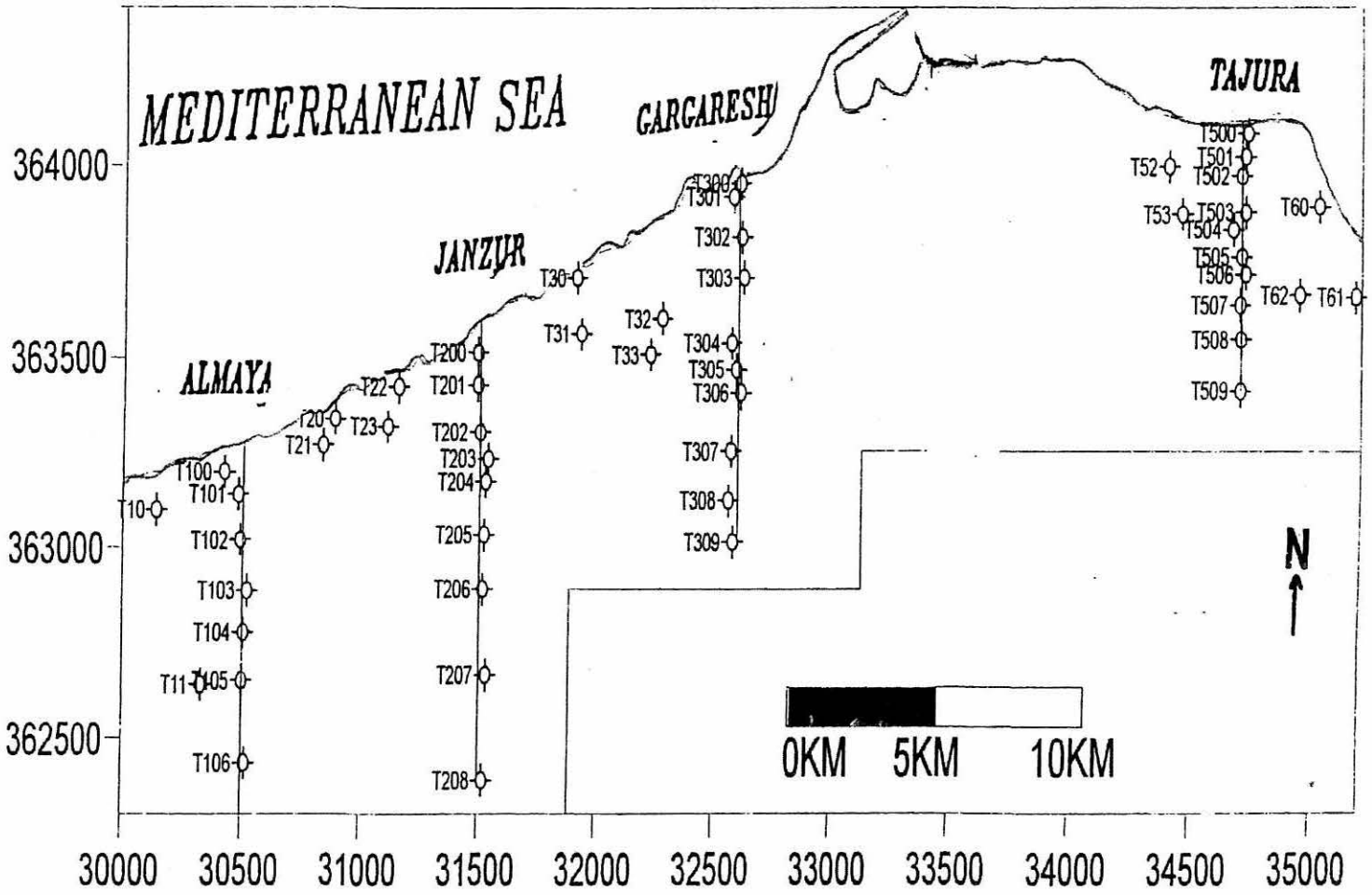

Figure 2.7 A network water wells in the Gefara plain area

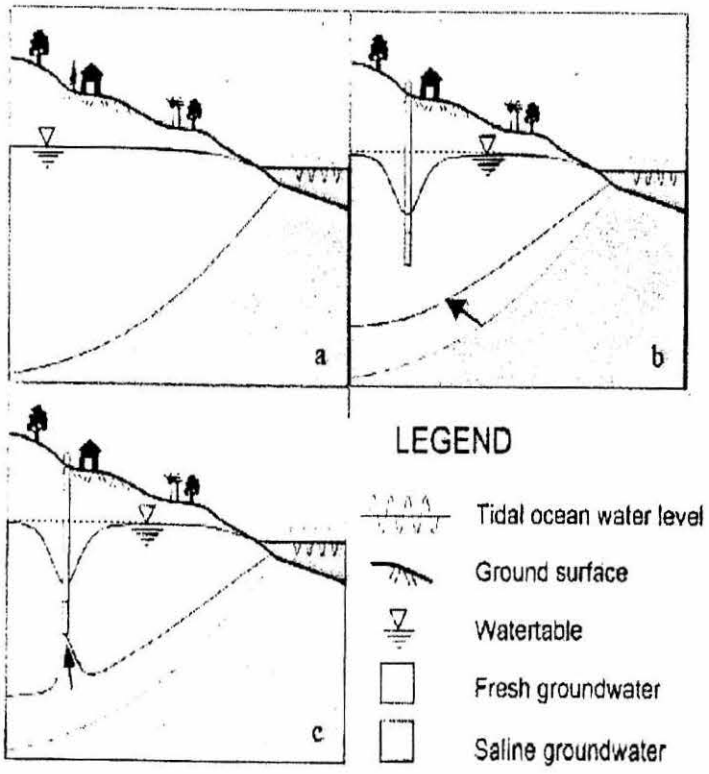

Figure 2.8 A schematic example of the progression of pumping-induced seawater intrusion: (a) An initial state of equilibrium, (b) lateral seawater intrusion, and (c) up-coning. (Werner, et al. http://www.internationalsalinityforum.org).

2.9 Definition of soil salinization

The excessive use of low-quality ground water for irrigation may lead to an increase in soil salinization (Poustini and Siosemardeh, 2004). Salt-affected soils have an accumulation of soluble salts cations such as sodium, calcium, and magnesium, as well anions such as carbonate, chloride and sulphate (Manguet, 1991; Aydin et al., 2004). Salt-affected soils are catagorised as saline or saline-sodic (Abrol and Yadav, 1988; Szabolcs, 1989). The concentration of salt in land and water resources can increase through physical and chemical processes, or through human activities and processes, which are referred to as primary and secondary salinization respectively (Ghagemi et al., 1995). Soil salinity that occurs naturally is classified as primary salinization. Soil salinity that results from human activities is called secondary salinization (Minhas and Sharma, 2003).

Primary salinization is the accumulation of salts in soils as a result of processes such as weathering of soil parent materials and the deposition of marine salts carried in wind and rain (Isbell et al., 1983). Secondary salinization is the gradual build-up of salt in previously salt-free topsoil that occurs in irrigated areas as salt is introduced into the soil with every round of irrigation. Szabolcs (1989) stated that primary and secondary salinization have a wide distribution in many countries. Saline regions are often found in poorly drained areas within semi-arid and arid climates in which large quantities of salts have leached from regions of higher elevation. These leached salts accumulate in the slow-flowing ground water and are brought to the soil surface in these low-lying areas through high evaportranspiration rates (Goudie, 1990). Goudie (1990) also pointed to the effect of irrigation in the raising of the water table in such areas, resulting in them becoming waterlogged and salinized.

In addition, salts can come from vegetation growing on salt-affected soils. Russell (1989) pointed out that up to a quarter of plant dry weight is ash and the large proportion of the

ash may be soluble salts. Vegetation brings about 200 kg ha^{-1} of salts annually to the soil surface. Although small compared to amounts of salts added by irrigation, plants can contribute to the accumulation of salts in the top soil over long periods of time.

2.10 Importance of salinization

Soluble salts are a natural feature of the landscape, being present usually in small amounts in all waters, soils and rocks (Yaalon, 1967). Soil salinization is an *in situ* form of soil degradation that arises due to the buildup of soluble salts to deleterious levels at or near the surface of the soil. Salinity of soils and ground water is a serious soil degradation problem, which is growing steadily in many areas of the world. Food production in many areas of the world, particularly in arid and semi-arid areas is being severely affected due to a decrease in area under cultivation, an increase in the land area affected by salinization, and a decrease in overall productivity of fertile soils as a result of improper irrigation and water management practices (IAEA, 1995; van Antwerpen and Meyer, 1996; Haynes and Hamilton, 1999).

Salt-affected soils are more extensive in arid and semi-arid regions compared to humid regions (Szaboles, 1979; Abrol and Yadav, 1988; Summer, 1995). However, soil salinity is becoming an increasing problem, especially in arid and semi-arid regions, as the consequence of a progressive decrease of water resources driven by the use of saline water for irrigation. The use of this water increases the risk of salt accumulation in the rooting zone and consequent damage to soil fertility (Maas, 1986 and Lauchli and Epstein, 1990). Ashraf and Rehman (1999) reported that soil salinity is characterized by high concentrations of soluble salts, and a low nitrogen and organic matter content. Salt-affected soils can be found in the tropical regions of Africa and Latin America, and in all continents and under all climatic conditions. The accumulation process can also be caused by other factors (Chhabra, 1996), such as capillary rise from shallow groundwater, or from seawater

intrusion in coastal areas. Salinity is expressed in terms of soil electrical conductivity and is reported in units of dSm^{-1}.Soils with an EC > 4 dS m^{-1} are considered saline (Richards, 1954; Brady and Weil, 2002). Irrigation water with an EC > 0.7 dSm^{-1} may restrict growth of several crops (Ayers and Westcot, 1985). Ions that contribute to soil salinity include Cl^-, SO_4^{2-}, HCO_3^-, Na^+, Ca^{2+}, Mg^{2+}, and rarely NO_3^- or K^+. The salts of these ions occur in highly variable concentrations (Leon, 1975). However, salt addition to soil alters its physical and chemical properties, including soil structure and hydraulic conductivity. Excessive exchangeable sodium and high pH decrease the soil permeability and infiltration capacity through swelling and dispersion of clays (Lauchli and Epstein, 1990). This also results in potential flooding, erosion, and soil degradation. Salt-affected soils contain sufficient concentrations of soluble salts to reduce the growth of most plant species.

Ghassemi et al. (1995), Pessarakli and Szabolics (1999) and Li et al. (2005) reported that soil salinity occurs on nearly 10% of the earth's total land surface, or that 954 Mha are covered with salt-affected soils, and up to 100 Mha of saline soils are the result of irrigation. Around 10 to 20 Mha of irrigated land deteriorates to zero productivity each year due to salinity (Hamdy, 1996; Choukr-Allah, 1996). About 20–30 million ha of irrigated land is seriously damaged by the build-up of salts, and 0.25–0.5 million ha are estimated to be lost from production every year as a result of salt build-up (FAO, 2002). Salt-affects about 91.6 Mha of the land area of north and central Asia (Table 2.3). Sodic-affected lands in Australasia cover an area of 340 Mha. Saline soils cover about 53.5 Mha of Africa, while sodic soils cover about 27 Mha. Salinization affects between 1 and 3 million ha of the area of the European Union and Candidate Countries (European Commission, 2003). About 3% of the 3.5 million hectares of irrigated land in Spain is severely affected by salts and another 15% is at serious risk (European Commission, 2002). Salt-affected lands in the Indus basin of Pakistan cover an area of 4.22 Mha, which

is 26% of the total irrigated area (Ghassemi et al., 1995). Qureshi et al. (2007) also indicated that about 6.3 million ha are affected by different levels and types of salinity in Pakistan. Nlwra (2001) stated increasing soil salinity in Australia is a serious land degradation issue, with the area affected by dry land salinity was estimated to be approximately 4 million ha in 2000, and is predicted to increase to 20 million ha by 2020. At least 2.5 Mha are affected by dry land salinity and approximately 5.7 Mh of Australian farm land has a high salinity risk. This may rise to 12 Mha by 2050 (Coram et al., 2001). Soil salinity affects 1.7% (1.518.746 ha) of Turkey and 3.8% (837.405 ha) of agricultural land in Turkey (Topraksu, 1978; Kurucu et al., 2002). Hamdy (1996) stated that saline soils cover about 80 million hectares in the Mediterranean basin. Mtimet (2001) stated salinity is also one of the major factors that affect plant growth in Tunisia. FAO (1986) stated that the decline of productivity in Egypt is attributed to the increase in primary and secondary salinization. In China there are more than 30 Mha of saline soil and 9 Mha of secondary salinized soil (Wang, 1993). About 4.5% of Brazil's land is salt- affected (Dias et al., 2003). In India, about 30 million ha of coastal land remains barren because of salinity (Singh and Surendra, 1994). Anon (2000b) indicated to an estimated 5.7 Mha of agricultural land is currently at risk of developing dry land salinity, with the area predicted to expand to 17 Mha by 2050. Szabolcs (1989) described the global distribution of saline and sodic soils in different continents (Table 2.3).

Table 2.3 Global distribution of salt-affected soils (Szabolcs, 1989). Values show area in millions of hectares (Mh).

Continent	Saline	Sodic	Total
North America	6.2	9.6	15.8
Central America	2.0	--	2.0
South America	69.4	59.6	129.0
Africa	53.5	27.0	80.5
South Asia	83.3	1.8	85.1
North &Central Asia	91.6	120.1	211.7
Southeast Asia	20.0	--	20.0
Australasia	17.4	340.0	357.4
Europe	7.8	22.9	30.7
TOTAL	351.5	581.0	932.2

2.11 Classification of salt-affected soils

Salt-affected soils are classified into different groups based on the pH of water-saturated soil paste (pH), the total soluble salt or electrical conductivity of the water saturated soil paste extract, and the exchangeable sodium percentage (ESP). Salt–affected soils are classified as saline and sodic soils.

This classification is on the basis of determinations made on soil samples and the effects of neutral and alkaline salts on the soil properties and plant growth (Abrol and Bhumbla,

1978; Bhmbla and Abrol, 1979; Szabolcs, 1989).

The pH of the saturation paste of a saline soil is less than 8.5, while that of a sodic soil is greater than 8.5. Pearson and Bauder (2003) and Rengasamy et al. (2003) found that high ESP and high pH in the soil may reduce soil porosity and permeability. In the past several efforts were made to characterize and classify such soil into different categories (e.g. US Salinity Laboratory Staff, 1954). US Salinity Laboratory Staff (1954) suggested numerical criteria for categorizing saline-sodic soils (Table 2.4) which have been commonly used.

Table 2.4 Classification of salt-affected soils (Bohn et al., 1985; Rengasamy and Olsson, 1991).

Classification	Saline	Sodic	Saline-Sodic
USSL, 1954	EC>4 dSm^{-1}	EC <4 dSm^{-1}	EC >4dS m^{-1}
	ESP <15	ESP>15	ESP>15
	SAR<13	SAR>13	SAR at least 13
	pH<8.5	pH>8.5	pH>8.5

2.12 Effects of salinity on soil physical and chemical properties

Even moderate amounts of salts have adverse effects on soil physical and chemical properties (Pathak and Rao, 1998). In sodic soils, carbonate and bicarbonate are the principal anions, and Na^+ is the dominant cation. Sodicity is based on Na^+ concentration relative to other cations (i.e. Ca^{2+}, Mg^{2+}, K^+, NH_4^+) adsorbed to soil particles on the exchange complex (Russell, 1961). Accumulation of excess Na^+ in soil causes changes such as in soil pH, exchangeable and soil solution ions, destabilization of soil structure, impairment of soil hydraulic properties, increased susceptibility to crusting and specific ion effects on plants (Mustafa et al., 1966; Shainberg and Levy, 1992; Barzegar et al., 1997; Qadir and Schubert, 2002).

Buckland et al. (2002) showed aggregate stability in sodic soil is reduced due to the increase in exchangeable sodium percentage (ESP). Soil clay particles have negative electrical charge, attracting large number of cations. The zone surrounding the cations at the negative charges on soil particles is termed the diffuse double layer. The concentration of cations is high within the diffuse double layer close to the surface of the clay particles and decreases with distance. Clay particles are subject to forces that can move them together (aggregation) or apart (dispersion). The accumulation of salts in non-sodic soils can affect soil physical properties by causing fine particles to bind together into aggregates. This process is known as flocculation (Pearson and Bauder, 2003). When dissolved in water, Na^+ ions are larger in size than K^+ and Mg^{2+} is larger than Ca^{2+}, pushing clay particles apart (Shainberg, 1992). On other hand, the concentration of Ca^{2+} prevents the disruption of aggregates and the occlusion of pores by dispersed clay particles (Greene et al., 1988). Flocculation is beneficial in terms of infiltration, soil aeration, root penetration, and root growth. However, Keren and Shainberg (1984) pointed out flocculation occurs as the soluble salt concentration increases. Calcium and magnesium will generally keep soil flocculated because they compete for the same spaces as sodium to bind to clay particles. Thus the amount of sodium-induced dispersion is decreased due to increased amounts of calcium and magnesium. However, sodium builds up in the soil and interferes with soil structure. The amount of sodium and salt left determines whether the soil is non–sodic (very little sodium) or sodic (a lot of sodium or saline) (Pichu and Leigh, 1994). Under arid and semi-arid conditions, irrigation water quality (SAR and EC), soil properties (texture, structure, mineralogy and ESP) and even irrigation methods are interrelated factors affecting soil permeability. Quirk and Schofield (1955) showed that the permeability of soil to water depends on its exchangeable sodium percentage (ESP).

Porosity has been used extensively to characterize the soil structure, as the size, shape and continuity of the pores affect many important soil processes (Lawrance, 1977). In addition, Utomo and Dexter (1981) reported that wetting and drying of the soil can increased soil porosity as a result of crack development. The primary physical processes associated with high sodium concentrations are soil dispersion, and clay platelet and aggregate swelling. The forces that bind clay particles together are disrupted when too many large sodium ions come between them. When this separation occurs, the clay particles expand, causing swelling and soil dispersion. Several investigations have shown that the structure of the soil is degraded due to a high degree of clay dispersion (e.g. Gupta and Verma, 1983; Rengasamy et al., 2003). However, in sodium- affected soils, water infiltration rates and hydraulic conductivities are low due to swelling and dispersion of clays (Shainberg, 1990). Also, Acharya and Abrol, (1991, 1998) found a negative effect of salinity on soil aeration and water conductivity. Salinity leads to change in the soil surface, and saline soils tend to have a white crusty surface, while sodic soils tend to have a dark surface due to the dispersion of clay and organic particles (Lax et al., 1994).

2.13 Soil aggregates

Soil aggregates are an important component of soil structure and are important for maintaining soil porosity and aeration favourable for plant and microbial growth, infiltration of water, and stability against erosion (Tisdall and Oades, 1982; Oades, 1984; Dexter, 1988), as well as physical protection of soil organic matter (Tisdall and Oades, 1982). Borie et al. (2008) refer to aggregation as essential to maintain soil physical properties. Aggregate stability is used as an indicator of soil structure (Six et al., 2000). Horn et al. (1989) and Becher (1992) mention that the size of aggregates may vary greatly from crumbs (diameter < 2 mm), to sub angular blocks (0.005-0.02 m).

Smaller soil aggregates are divided into two general groups based on aggregate size (diameter), macroaggregates (> 250 μm) and microaggregates (less than 250 μm).

Aggregation results from the rearrangement of particles, flocculation and cementation (Duiker et al., 2003). Aggregates of different sizes are joined and held together by different organic and inorganic materials (Figures 2.9 and 2.10). Thus, aggregate formation includes the processes of formation and stabilization. Nevertheless, some authors report that the formation of soil aggregates occurs as a result of physical forces, while the stabilization of soil aggregates is produced by a number of factors, in particular the quantity and quality of inorganic and organic stabilizing agents (Lynch and Bragg, 1985; Oades, 1993; Dalal and Bridge, 1996). However, aggregate formation and aggregate strength depends on swelling and shrinkage processes, and on increased organic matter levels and microbial activity (Diaz et al., 1994). In soils containing more than 15% clay (particle size < 2 μm), the mineral particles (sand, silt and clay) form structured aggregates. This process occurs due to biological activities, and also when soils dry and swell (Hillel, 1980; Wolters, 1991). Singer et al. (1992) showed that climate affects soil aggregation through alterations in temperature and moisture regimes. Soil moisture and wet-dry cycles have a variable effect on aggregation. Wet-dry cycles can disrupt aggregation in swelling clays.

Some soils of arid regions have higher levels of aggregation and stable microaggregates than those in humid regions. In the Mediterranean region. Boix-Fayos et al. (1998) found that a lower soil organic carbon (SOC) and clay content could decrease aggregate stability. These authors also found that increased infiltration and reduced erosion may due to increased vegetation cover and aggregate stability. Barthes and Roose (2002) found reduced run-off and erosion due to increased aggregate stability as well.

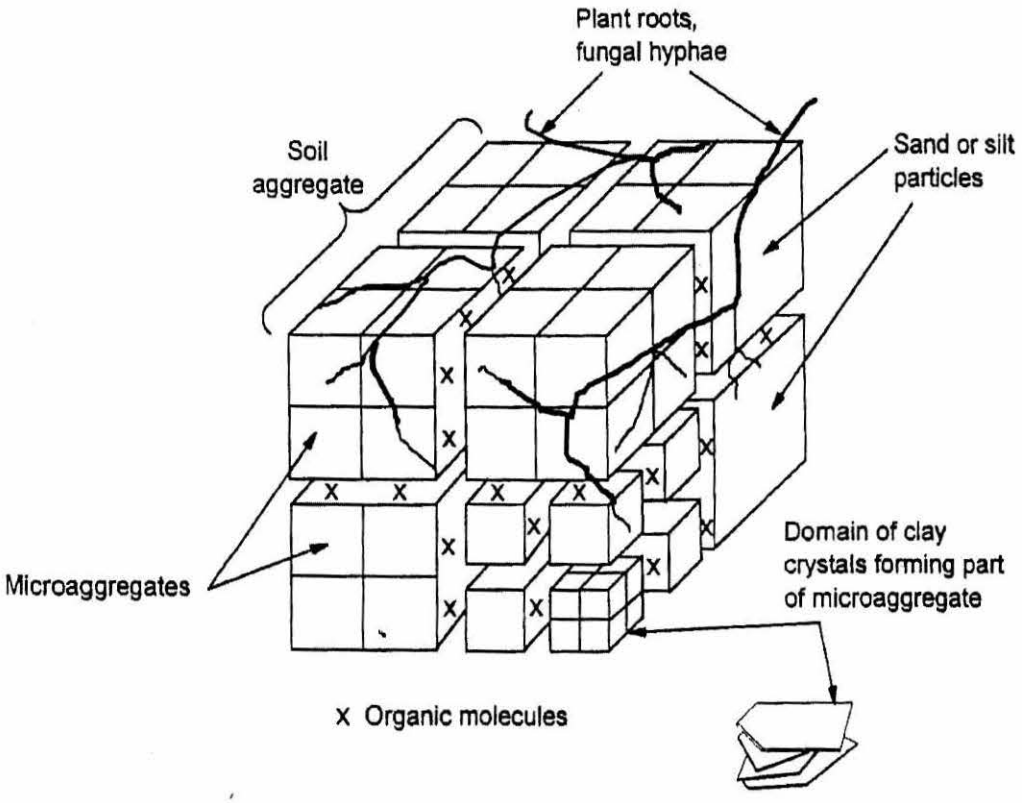

Figure 2.9 Structure of aggregates (Adapted from Greenland, 1979).

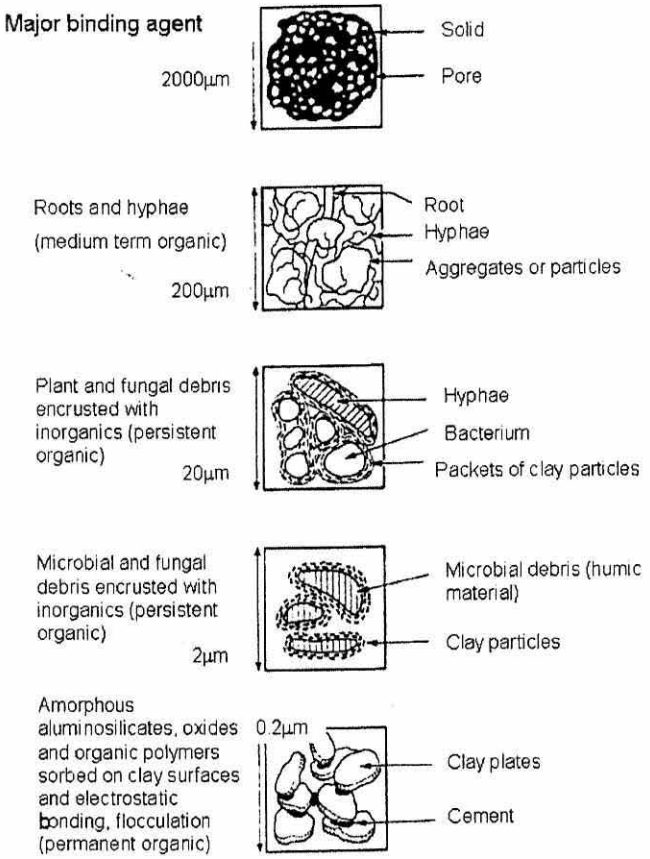

Figure 2.10 Schematic diagram of aggregate formation (Adapted from Tisdale and Oades, 1982).

2.14 Contribution of microbial activity to aggregate formation

Soil microbial activity plays a vital role in ecosystem processes, maintaining soil structure (Skinner, 1979; Haynes and Swift, 1990; Helal, 1991; Oades, 1993; Kennedy and Gewin, 1997; Ryan and Graham, 2002), contribuing to stabilization of soil aggregates (Smith et al., 1990; Diaz et al., 1994; Kennedy and Gewin, 1997; Fliebach et al., 2000), reducing erosion (Kennedy and Gewin, 1997; Oades, 1993). Live/dead bacteria and hyphae are considered temporary binding agents (Tisdall and Oades, 1982). In addition, mucilages or gums are considered temporary binding agents (Tisdall and Oades, 1982) because they are decomposed rapidly by microorganisms. Skinner (1979) and Edgerton et al. (1995) reported that microbial activity is lower in compacted soils with poor aggregation. Bacteria play an important role in maintaining soil structure (Gray and Williams, 1971, Lynch, 1976, Tisdall and Oades, 1982). Bacterial cells themselves can hold soil particles together even after the bacteria die, as their necromass continue to bind the particles (Lynch, 1976; Tisdall and Oades, 1982). Thus the breakdown products of plant residues resulting from the actions of bacteria and fungi are also important in contributing to the formation of soil aggregates (Elliott and Papendick, 1986; Beare et al., 1994b; Cambardella and Elliott, 1994). In addition, most microbial activity takes place on the surfaces of aggregates where the microbial substrates are most available (Hattori, 1988; Nietfeld et al., 1992).

2.15 Role of fungal hyphae in improving aggregation of sandy soils.

The aggregation process in sandy soils depends on biological activity (Espindola et al., 2007). Low proportions of clay and silt limit aggregation in sandy soils (Oades, 1993). An alternate way to improve aggregation in these soils may be to increase the amounts of external mycorrhizal hyphae (Degens et al., 1996). Nasim (2005) found external hyphae can extend more than 9 cm beyond the roots. Coarse, thick-walled hyphae have diameters >20 μm and fine, thin-walled hyphae have diameters in the range 2-10 μm (Mosse, 1959; Nicolson, 1959). A number of authors have found aggregates appeared to be stabilised in sandy soils by a network of hyphae cross-linking the sand grains (Bond and Harris, 1964; Clough and Sutton, 1978; Degens, 1997).

There are also many previous studies showing the role of hyphae in sandy soil aggregation (e.g. Goodchild and Myers, 1987; Degens et al., 1996 and Othman et al., 2004). Soil binding by external hyphae involves cross-linking and sand particle enmeshment binding the soil particles together to create water–stable aggregates, which in turn creates pore spaces in the soil that enhances water drainage (Lyn and Daniel, 2003). Ternan et al. (1996), Haynes and Beare (1997) and Bronick and Lal (2005) reported that hyphae improve aggregate stability. Degens et al. (1996) and Miller and Lodge (1997) showed that hyphae appear to be important in the stabilisation of macro-aggregates in sandy and loam soils, and also that aggregation increased with hyphal density (Ternan et al., 1996; Haynes and Beare, 1997). Moreno-Espindola et al. (2007) reported that the larger the sand particles, the greater the length of hyphae that is required to stabilize a set of sand particles into macro-aggregates. Examination of the maximum and minimum lengths of hyphae associated with individual grains of sand in the sandy soil showed that the average length of hyphae per grain of sand sharply declined with decreasing sand grain size (Degens et al., 1996).

Hyphae were able to contribute to the stability of the sand aggregates, as most of the soil particles were > 250 μm, which favoured cross-linking of the sand grains by short lengths of hyphae (Degens et al., 1996). The influence of hyphae on aggregation stability also depends on the tensile strengths of the hyphal cross-linkages. In addition, the size of sand particles adhered to by external hyphae was affected by plant species (Moreno-Espindola et al., 2007). However, there are many studies which show that mycorrhizal hyphae were visually identified as the dominant hyphae stabilising aggregates in sand, and formed a network separate from the roots (e.g. Koske et al., 1975; Sutton and Sheppard, 1976; Clough and Sutton, 1978; Forster and Nicolson, 1981). The binding effect of mycorrhizal hyphae also persisted after plant death, and the decomposition of the roots in the sand (Clough and Sutton, 1978; Forster, 1979).

2.16 Effects of salinity on soil aggregates

The saturation level of sodium and the saline concentration in a soil solution determine the dispersion of clay particles. This may cause clogging of soil pores and, in the channels where the water flows, lead to a reduction in soil permeability and soil porosity (Frenkel et al., 1978; Shainberg and Levy, 1992; Amezketa, 1999). Varallyay (1977a, b) showed that a high sodium saturation level may affect aggregate stability in water by destroying soil aggregates (Abusharar et al., 1987; Rietz and Haynes, 2003). Sodium is a highly dispersive agent that causes the breakup of aggregates, and this can lead to decreased plant productivity (Bronick and Lal, 2005). However, saline irrigation water may also reduce production of organic matter by microorganisms and thus aggregate stability (Sarig et al., 1992). For example, in Pakistan, the use of irrigation water with high sodium causes a degradation in the structure of fine-textured soils, and poor infiltration of water results in salinization through evaporation of irrigation water at the soil surface and waterlogging (Qureshi and Barrett-Lennard, 1998).

In contrast, Lax et al. (1994) found that enhancing soil structure stability may lead to accelerated leaching of sodium, and decrease sodicity (Qadir et al., 2001).

2.17 Soil respiration

Soil respiration is the biological oxidation of organic matter (OM) to CO_2 by microorganisms; it is positively correlated with soil organic carbon (SOC) content and microbial biomass (MBS) (Ivarson, 1977; Parton et al., 1987; Illmer and Schinner, 1991; Alef, 1995). Microbial activity is often determined in terms of CO_2 evolution from the soil. As soil respiration is a good indicator of the overall biological activity of soil, it is often used in studies of the soil carbon cycle (Feiziene, 2008), and also used as an index of soil fertility for agricultural production (Russell and Appleyard, 1915). The efflux of CO_2 from the soil surface is usually used to measure all the mineralization processes occurring in the soil profile (Hanson et al., 2000; Kuzyakov, 2006). Hogberg et al. (2001) suggest that measurements of total soil respiration provides vital evidence towards understanding soil responses to environmental perturbation. However, total soil respiration is combination of several processes, such as root respiration, microbial respiration (Hanson et al., 2000; Hoogberg et al., 2001; Rastogim et al., 2002), litter decomposition (Singh and Gupta, 1977; Kuzyakov and Cheng, 2001; Nguyen, 2003). Cheng (1996); Dilly et al. (2000) and Kutsch et al. (2001b) defined microbial respiration using carbon from both live roots (rhizomicrobial respiration) and microbial respiration using soil carbon. Edwards and Sollins (1973) estimated that total soil respiration from a forest soil was 35% from roots, 48% from litter and 17% from soil. Keith et al. (1997) found that microbial respiration is related to the quantity and quality of the substrate. Vanessa et al. (2008) indicated that carbon dioxide efflux as a result of microbial activity depends on the C pool defined as soil organic carbon (SOC).

Moyano et al. (2007) indicated that higher soil respiration is indicative of high biological activity and is a good sign of organic residue decomposition to make recycled nutrients more available to plants. Root- Soil respiration is divided into hetertrophic (microbe) and autrotrophic (plant root) respiration (Sotta, et al., 2004). In root-free soil, soil respiration is a measure of microbial respiration (Ross et al., 1995; Insam et al., 1999). However, there is not a clear division between hetertrophic and autrotrophic respiration, as root and hyphal exudates also drive respiration of root associated organisms (Moyano et al., 2007). Autrotrophic respiration is normally that due to root respiration. Luo et al. (1996), Rouhier et al. (1996), Hungate et al. (1997) and Pregitzer et al. (2000) found an increase in soil respiration rates was due to an increase of root productivity, while Bouma et al. (2001) and Fahey and Yavilt (2005) found young fine roots have much higher respiration rates than older roots.

2.18 Factors affecting CO_2 efflux from soil

There are many reports of factors affecting CO_2 efflux rates from soil, such as temperature and moisture, nutrient content, root respiration, microbial processes, organic matter, soil aeration, soil porosity, soil water, vegetation type (Lundegardth, 1927; Reiners, 1968; Rixon, 1968, Edwards, 1974; Weber, 1990; Johnson, 1993; Mielnick and Dugas, 1999, Maier and Kress, 2000; Schlesinger and Andrew, 2000), and increased salinity (Agarwal et al., 1971; Johnson and Guenzi, 1963; Singh et al., 1969; Rietz and Haynes, 2003; Jannike et al., 2006; Sirulink et al., 2007).

2.18.1 Temperature

Many studies have shown the influence of environmental factors such as temperature on rates of soil respiration (e.g. Witkamp, 1969; Singh and Gupta, 1977; Schleser, 1982; Schlenter and Van Cleve, 1985; Peterjohn et al., 1993, 1994; Kirschbaum, 1995; Winkler et al., 1996; Rustad and Fernandez, 1998).

Soil respiration rates strongly vary with soil temperature and increase as soil temperature rises (Kucera and Kirkham, 1971; Medina and Zelwer 1972; Singh and Gupta, 1977; Schlenter and Van Cleve, 1985; Carlyle and Ba Than, 1988; Lloyd and Taylor, 1994; Rustad et al., 2000). The increase in reaction rate per 10°C increase in temperature is known as the Q_{10}. The exponential function Q_{10} is commonly used to express the relationship between soil biological activity and temperature. Global soil respiration is very sensitive to the selected Q_{10} value for various biomes (Holland et al., 1995). Howard and Howard (1993), Lloyd and Taylor (1994), and Raich and Potter (1995) explained that the Q_{10} value is frequently observed to change with temperature, with higher values typically found in colder climates. Edward (1975) found a strong relationship between CO_2 evolution and mean daily temperature. Wiant (1967) observed no CO_2 efflux at 10°C followed by logarithmic increase in CO_2 evolution between 20 and 40°C, above 50°C, CO_2 evolution declined rapidly.

Evanylo (2000) found occurrences of microbial respiration inhibition at higher temperatures, but Bunt and Rovira (1954) found that, in a temperature range from 10 to 70°C, CO_2 efflux increased with temperature until 50°C, after which there was a decline. In general, Jenkinson et al. (1991) and Lal et al. (1995) indicated the rate of release of soil C increases with annual temperature increase. Brito et al. (2005) found that high soil temperatures accelerate soil respiration and thus increase CO_2 efflux. Fernando et al. (2007) noted that root respiration is more sensitive to changes in temperature. However, bacterial and fungal activity had optimum temperature ranges of about 25-30°C, while at higher temperatures activity tends to be lower (Pettersson, 2004). In this regard, Persson et al. (1999) and Agren (2000) also reported that fungi are more active at low temperatures than bacteria.

2.18.2 Soil texture

Soil texture is characterized on the basis of the percentages of sand, silt, and clay (Eswaran 2003). Soil texture is related to porosity, which in turn determines water movement and gas diffusion in the soil (Luo and Zhou, 2006). Generally, many researchers suggest that the effect of texture on microbial activity is through the ability of the soil to hold water in a microbially available state (e.g. Scott et al., 1996; Franzluebbers, 1999; Strong et al., 1999). Fine–textured soils have a larger proportion of small pores than coarse-textured soils. Foster (1988) suggested that the most favourable environment for bacteria is in aggregates with small diameter pores. Soil texture affects the growth of bacteria and fungi through the supply of moisture and air and thus affects formation of CO_2. Van Veen et al. (1985) found that in coarse-textured soils turnover of microbial biomass C and N is faster than in fine-textured soils. The rate of soil respiration is higher in coarse textured soils than in fine textured soils (Franzluebber et al., 1996). Changes in the pore size distribution to a greater proportion of large pores, such as progressing from a clay to sand, are followed by higher rates of organic carbon mineralisation at equivalent values of air-filled porosity (Franzluebber 1999). Also, soil texture has effects on water infiltration and gas diffusion rates and thereby CO_2 formation and efflux. Kowalenko et al. (1978) observed that soil respiration rate was greater in clay loam soil than in sandy soil.

2.18.3 Salinity

Excess amounts of salt have adverse effects on physical, chemical and microbiological processes in soil including carbon and nitrogen mineralization and enzyme activities, which are crucial for decomposition of OM (Rastogim et al., 2002). Sodicity had a slight negative effect on residue decomposition, and may decrease OM decomposition directly by inhibiting microbial growth and activity, reported by Nelson et al. (1997).

In addition, the negative effect of salinity have been shown on carbon mineralization, reported by Frankenberger and Bingham (1982), Nelson et al. (1996) and Nelson and Mele (2007), and negative effects on nitrogen mineralization reported by Pathak and Rao (1996). There are many investigations that have indicated that CO_2 efflux is significantly decreased with increased salinity (e.g. Agarwal et al., 1971; Johnson and Guenzi, 1963; Singh et al., 1969; McCormick and Wolf, 1980; Sarig et al., 1996; Zahran, 1997; Pathak and Rao, 1998; Rietz and Haynes, 2003; Jannike at al., 2006; Sirulink et al., 2007). Salinisation has been suggested to be one of the most stressing environmental conditions for soil microorganisms, especially for fungi (Sardinha et al., 2003).

2.19 Measurements methods for soil respiration

Many methods have been developed to measure soil respiration (Jenkinson and Powlson, 1976; Anderson and Domsch, 1978; Knapp et al., 1983). Original measurement techniques are methods such as trapping CO_2 in sodium hydroxide, with a subsequent titration (Isermeyer, 1952). As discussed, the CO_2 efflux from soils is the result of the respiration of different groups of organisms, a fact which has lead to the development of methods to partition and measure these fluxes separately. Several different methods for measuring soil respiration have been employed, each having its own strengths and weaknesses. Furthermore, there is no standard or reference for determining the accuracy of any one of these methods (Nakayama, 1990). The following methods allowing partitioning of root-derived CO_2 into root respiration and rhizomicrobial respiration.

A-non-isotopic methods

1- The component integration method is the first method designed to separate root respiration and rhizomicrobial respiration (Edwards and Sollins, 1973; Singh and Shekhar, 1986). The principle of this method is manual separation of sources and measuring CO_2 from each source by incubation.

Samples are taken from the field are analyzed in the laboratory. The advantages of this method are that it is cheap, and works for various ecosystems. The disadvantages of this method are the possibility of strong CO_2 efflux after disturbance.

2- Respiration by excised roots method. This method is based on measurement of specific respiration rates of excised roots separated from soil. Samples taken from field are analyzed in the laboratory. The advantages of this method are it is cheap, and useful for roots of various plants. The disadvantages include roots may be damaged and that the results depend on the duration of CO_2 trapping.

3-Substrate induced respiration method (SIR). The principle of this method is based on the different responses of microbial respiration and root respiration to the addition of glucose, and it can be applied to estimate microbial respiration and root respiration (Panikov et al., 1991). The addition of glucose to soil leads to a strong increase in microbial respiration, which was limited before glucose addition by easily available substrate (Anderson and Domsch, 1978). This method can be applied both in field and the laboratory. The advantages of this method are that it is cheap, it works in various ecosystems, and it is more exact than other methods.

An advanced development of the SIR method involves applying C_4 sugar instead of glucose to soil developed under C_3 vegetation and measuring total CO_2 evolution (Ekblad and Hogberg, 2000).

The disadvantages of this method are that it involves many treatments and many measurements are necessary.

B- Isotopic methods:

1- Comparison of root -derived $^{14}CO_2$ with rhizomicrobial $^{14}CO_2$ method. The principle of this method is comparison of root-derived $^{14}CO_2$ during continuous labeling with rhizomicrobial $^{14}CO_2$ by decomposition of uniformly ^{14}C labeled rhizodeposits from the

same plants. This method was suggested by Johansson (1992), and is applied in the laboratory. The advantages of this method are that it allows estimation of additional parameters as total C input by plants into the soil and separation of SOM-derived and root-derived CO_2. The disadvantages are that very long incubation and many treatments are necessary; furthermore, there are uncertainties because of estimation of the stabilization factor. It is only useful for grasses and crops.

2-The isotope dilution method. This method is based on the addition of a solution of unlabeled glucose to the soil with growing plants that were pulse-labeled in $^{14}CO_2$ atmosphere. The added unlabeled glucose dilutes the ^{14}C-labeled rhizodeposits (Cheng et al., 1993). This method is applied in the laboratory. The advantages of this method are that roots have no effect on glucose addition, and it gives reasonable separation results. The disadvantages are that assumed constant ratio between root respiration and rhizomicrobial CO_2 after $^{14}CO_2$ labeling are not checked, and it needs many treatments.

3-Model rhizodeposition technique method is based on adding artificial ^{14} C- labelled rhizodeposits to the soil (Swinnen, 1994). The $^{14}CO_2$ evolution from this soil is then compared with the $^{14}CO_2$ evolution from plants labeled previously in a $^{14}CO_2$ atmosphere. This method is applied in the laboratory. The advantage of this method is that it is comparatively simple; the disadvantages are that it results in strong underestimation of rhizomicrobial CO_2 and overestimation of root respiration.

4- Dynamics of $^{14}CO_2$ evolution method. The basis of this method is the modeling of $^{14}CO_2$ dynamics evolution from soil after pulse labelling (Kuzyakov et al., 1999, 2001). Kuzyakov (2002) found that the $^{14}CO_2$ effluxes from root respiration earlier than from microbial respiration. This method is applied in the laboratory. The advantages of this method are that it is based on dynamics of processes and has reasonable separation results.

The disadvantages are that the assumption of delayed rhizomicrobial $^{14}CO_2$ is not checked, many measurements are necessary, model parameterization is necessary, and results may be biased by diurnal CO_2 dynamics.

5- Exudate elution method. This method is based on the elution of root exudates labeled with ^{14}C before microorganisms decompose them, and the simultaneous trapping of $^{14}CO_2$ from root respiration (Kuzyakov and Siniakina, 2001). This method is applied in the laboratory. The advantage of this method is that it is the only one that allows physical separation of CO_2 and substances exuded by roots. The disadvantages are that it results in strong underestimation of rhizodeposition, and overestimation of root respiration.

6- $\delta^{13}C$ values of CO_2 and microbial biomass method. This method is based on the natural abundance of ^{13}C by growing C_4 plants on a C_3 soil (Kuzyakov, 2004) and calculation by $\delta^{13}C$ values of CO_2, microbial biomass, soil and roots. This method can be applied both in the field and the laboratory.

The advantages of this method are the absence of strong disturbance, the fact that many measurements in one canopy are possible, and it allows estimation of C sources for microbial biomass. The disadvantages of this method are that it is not tested; unconsidered isotopic effects may strongly shift the partitioning results, and inactive microbial biomass may dilute $\delta^{13}C$ of active microbial biomass.

2.19.1 Dynamic chambers- infrared gas analysis (IRGA)

Soil respiration can be measured with an infrared gas analyzer IRGA for CO_2 detection (Haney et al., 2008). This method is based on the automatic estimation of CO_2 by an IRGA system developed by Heinemeyer et al (1989). Ewel et al. (1987) pointed out that IRGA system-based techniques are considered more accurate than results derived from other means of analysis. The infrared gas analyzer it was first developed in the 1950s and used for the measurement of soil respiration.

The first uses of IRGA included the method for calibrating alkali absorption method by Haber (1958). Portable infrared gas analyzers have been widely used for the measurements of soil surface CO_2 fluxes since the early 1990s (Norman et al., 1992). The IRGA method requires relatively less operator training, but provides more accurate measurements of soil respiration than the traditional alkali or soda lime absorption methods. Below is a classification of chamber methods for measuring soil respiration with dynamic chambers. It is divided into open system and closed system approaches (Luo and Zhou, 2006).

2.19.1.1 The open dynamic chamber method

This method uses a differential mode to estimate CO_2 evolution. In contrast to the closed dynamic system, which uses changes in CO_2 concentration over a period of time, in this method ambient air flows from an inlet through a chamber to an outlet (Iritz et al., 1997; Fang and Moncrieff, 1998).

The air leaving the chamber is enriched in CO_2 concentration relative to the air entering the chamber, as a result of the CO_2 evolution from respiration at the soil surface. The CO_2 evolution is obtained from the difference in the amounts of CO_2 in, respectively, the inlet air and the outlet air of the chamber (Rayment and Jarvis, 1997). This method has three principle advantages: (1) high accuracy, if artifacts are removed; (2) steady-state measurement; (3) the opportunity to take continuous measurements and at high temporal resolution. There are four disadvantages: (1) the method is sensitive to pressure differences inside and outside the chamber; (2) it takes time to reach a steady state in the chamber; (3) it needs a power supply; (4) a differential gas analyzer and a mass flow controller are also required (Luo and Zhou, 2006).

2.19.1.2 The closed static chamber method

In this method, an area of soil surface is covered with a chamber which contains a chemical absorbent inside to capture CO_2 molecules.

Chemical absorbents for CO_2 trapping include alkali (NaOH or KOH) solution and soda lime (a mixture of NaOH and $Ca(OH)_2$). The alkali solution method is the oldest method of soil respiration measurement (Lundegardh, 1927). Since Monteith et al. (1964), the soda-lime technique has been used to measure CO_2 evolutions from soil under field conditions for more than 40 years. It is a static technique that is easy and inexpensive to use (Monteith et al., 1964; Edwards, 1982; Jensen et al., 1996; Grogan 1998). However, the amount of CO_2 absorbed by soda lime in a chamber on the soil surface is determined by the gain in soda-lime dry weight during the sampling period. The increase in weight is related to the absorption of CO_2, with a correction factor. However, the operating principle for this method is based on a temporal gradient by building up CO_2 in the chamber. This method has three advantages: (1) it is commercially available and easy to use; (2) IRGA calibration is less important due to the non-steady state approach; (3) it uses a short measurement time and is flexible for spatial sampling with a portable system. There are two principle disadvantages: (1) it promotes builds-up of CO_2 concentration in the chamber that distorts the gradient for diffusion; (2) it is labour-intensive, using a portable system to sample temporal variation (Luo and Zhou, 2006).

Chapter 3

Chemical and physical properties of the Gefara Plain soils

3.1 Introduction

The Soil quality includes an assessment of soil properties and processes as they relate to the ability of a soil to function effectively as a component of a healthy ecosystem (Schoenholtza et al., 2000). Physical properties such as the water holding capacity (WHC) of soil are very important; soils that hold generous amounts of water are less subject to leaching losses of nutrients. As most plants extract water directly from the soil, the physical characteristics of the soil influence the quantity and availability of water to plants. Microbiological measurements are often made after adjusting the water content to a constant value for all soils, as availability of water, is crucial for their growth and metabolic activity (Forster and Zech, 1993). The water holding capacity of soils is controlled primarily by (I) the number of pores and pore-size distribution of soils and (II) the specific surface area of soils - because with increased aggregation total pore space is increased (Kladivko and Nelson, 1979; Tiarks et al., 1974; Volk and Ullery, 1973; Williams and Cooke, 1961). To determine water holding capacity an excess amount of water is percolated through a known amount of field-moist soil; the volume of the percolate is determined and the volume of water stored in the soil is calculated to determining maximum water holding capacity WHC (Forster, 1995). The moisture content can be defined as the amount of water present in a quantity of soil in terms of its dry weight and is expressed as a percentage. The moisture content plays an immense role in influencing microbial activity (as soil respiration) (Adviento-Borbe, 2006).

This has been identified in much research: the first to document a consistent relationship between soil water content and microbial activity were Greaves and Carter (1920).

Infiltration is the process of water entering the soil. Some water that infiltrates will remain in the soil layer, where it will gradually move vertically and horizontally through the soil and subsurface material. Lowery et al. (1996) reported that the rate at which water enters the soil, the infiltration rate, is dependent on the soil type, soil structure, or amount of aggregation and the soil water content. Radke and Berry (1993) indicated that infiltration characteristics are closely related to soil structure and may be a good indicator of changes in soil physical and biological properties. There are several factors involved, such as the slope of the landscape, soil texture and structure, vegetation cover, and management systems in place; antecedent water content and soil organic matter also have an effect on infiltration (Radke and Berry, 1993). Compacted soils will have less pore space, resulting in lower infiltration rates. Soils that tend to form surface crusts which seal the soil surface can have severely reduced infiltration rates. Good water infiltration rates depend on aggregate stability (Kemper and Rosenau, 1986; Emerson et al., 1986). Boix-Fayos et al. (1998) reported that the increased aggregate stability increased infiltration. Ayers and Westcot (1985) pointed out that the amount of Na^+ and the total amount of soluble salts in a water source influence infiltration rate. Sumner (1993) and Agassi et al. (1981) found decreasing infiltration rates caused by increasing Na^+ in water irrigation, which also leads to dispersion of the soil particles. Le Bissonnais and Arrouays (1997) indicated that soil aggregate breakdown due to dispersion results in pore collapse which reduced infiltration rate, leading to runoff and erosion and, eventually, soil degradation. Chemical properties such as the soil electrical conductivity (EC) of a soil-water mixture is an indication of the amount of ions of dissolved salts present in the soil solution.

Increased salt content affects plant growth and soil water balance (Fitter and Hay, 1987). However, all soils contain some salts, which are essential for plant growth.

The EC measurement detects the amount of cations or anions (salts) in solution. The ions generally associated with salinity are Na^+, K^+,Ca^{2+}, Mg^{2+}, H^+ (cations), or Cl^-, NO_3^-, SO_4^-, HCO_3^-, OH^- (anions), soil electrical conductivity measurement is a means of easily quantifying and monitoring soil salinity in irrigated agricultural areas of arid-zone soils (Rhoades, 1993, Smith and Doran, 1996).. Salinity is quantified in terms of the total concentration of the soluble salts in units of $mScm^{-1}$ (U.S. Salinity Lab. Staff, 1954). Most soils are considered slightly saline if the soil salinity (EC) exceeds 2 m S cm^{-1} (Smith and Doran, 1996). Soil pH is a measure for estimating hydrogen ion activity in the soil solution i.e. the acidity or alkalinity. Soil pH affects the solubility of soil minerals, the availability of plant nutrients, and activity of microorganisms. Acidity is associated with leached soils, whereas alkalinity occurs in drier regions. However, agricultural practices such as liming or the addition of ammonium fertilizers can alter the soil pH. In general pH values between 6.0 and 7.5 are optimal for crop growth. There are several authors who have shown the influence of soil pH on the microbial activity of a soil (Beyer et al., 1992). The soil respiration rate usually increases with pH that is less than 7 and decreases with pH beyond 7 (Kowalenko and Ivarson 1978). Sitaula et al. (1995) observed lower CO_2 efflux in soils at pH 3.0 than in soils at pH 4.0; this is attributed to adverse effect of low pH on soil microbial activity, which contributes to lower respiration rate and consequently lower CO_2 efflux. A soil pH above 7.0 adversely affects CO_2 efflux (Rao and Pathak, 1996). In addition, Stevenson and Verburg (2006) indicated that high soil pH and calcium carbonates content are likely to favour the contribution of inorganic C to CO_2 evolution, while Muhammad et al. (2006) found that at high pH 8.9 with increasing salinity to above soil EC 5.7 $mScm^{-1}$ in soil amended with 1% maize led to a decrease in microbial activity. The purpose of this study has been to determine the similarities or differences of the different soil sites by physical, chemical methods.

3.2 Materials and methods

3.2.1 Collection of soil samples

Soil samples were collected from 4 farms located in the Gefara Plain (30° 00 NW, 35° 00 NE). A total of four sites were included in this study. The sites are named Almaya, Janzur, Gargaresh and Tajura. Figure 1.1 shows the map locations of each site. The soil samples were taken in July 2008, 2009, 2010 with a mean annual temperature of 26 to 29°C (National Atlas, 1977).

Agriculture in these locations in the summer season depends on irrigation water from subterranean sources. Soil was collected from each site from three replicate locations using a stainless steel corer (0-18 cm depth). After removal of the litter from the surface, and the soils were then sieved to pass a 2 mm screen, with any discernible roots and stones being removed. Soils were subsequently stored at field moisture in polyethylene bags at 4°C until analysis.

3.2.2 Determination of water holding capacity (WHC)

The principle of this method is that an excess amount of water is percolated through a known amount of field moist soil. The volume of water stored in the soil is calculated as described by Alef and Nannipieri (1995). Duplicate 20 g field moist soil samples (triplicate for each site (n=12) were placed in funnels and filter paper mounted 100 g distilled water was added and allow to stand overnight. The funnels were covered with aluminum foil to prevent evaporation. The funnel was tapped at the neck of the glass to move adhering water drops into the flask, then weighted water was collected in the flask.

Determine the % water holding capacity from this formula:

$$[(100-W_p)+W_i] /dwt \times 100$$

Where W_p is the weight of the percolated water in grams, W_i is the initial amount of water in grams contained in the samples and dwt is the soil dry weight in grams.

3.2.3 Determination of soil moisture content and organic matter (OM)

To determine the water content, a clean dry porcelain crucible (W_1) was weighed, and approximately 10 g of soil was placed in the crucible (triplicate for each site (n=12)) and weighed (W_2), and then dried in an oven overnight at 105°C and reweighed (W_3) after cooling is desiccators for 30 minutes.

The moisture content percentage was calculated using formula (1). After recording the moisture loss, the samples were then heated to 450°C overnight, to measure loss on ignition (total organic matter). After ignition the crucibles were again cooled in a desiccators and reweighed (W_4). The organic matter in percentage loss on ignition was calculated using formula (2).

Soil moisture (g/g) = $[W_2- W_3] / [W_3-W_1] X 100$.....1

Loss on ignition (g/g) = $[W_3$ pre 450°C$]-[W_4] / [W_3$ pre 450°C$–W_1] X100$.....2

3.2.4 Infiltration rate in the Gefara Plain soils

The infiltration rate was measured in situ at the four sites using a double-ring infiltrometer (Turf-Tec International, Coral Springs, Florida, U.S.A.). Measurements were made in November 2007. The infiltration rate readings were recorded after 1, 5, 15, 30 and 60 minutes (n=12 in total). The double-ring infiltrometer test directly measures soil infiltration rates (Bouwer, 1986). The double-ring is constructed from thin walled steel pipe with the inner and outer cylinder diameters being 20 and 30 cm respectively (Bouwer, 1986). The infiltrometer was installed with as little disturbance to the soil as possible.

During use the cylinders penetrated about 5 cm into the soil, both rings are filled up with water but only the inner ring is measured. The reason for this is that you will notice that during operation, the outer ring will infiltrate much faster than the inner one because there will be lateral movement of water around the cutter blade.

The soil was checked to ensure that there was no separation of the soil from the cylinder edge. If this occurred soil was pushed back against the cylinder wall. Equal water levels were maintained in the inner and outer ring. Differences in water level would have resulted in flow from one cylinder to the other and in an erroneous infiltration reading.

The water level was kept constant within the two cylinders by manually adding small quantities of water. The infiltration rate was then calculated from the rate of fall of the water level in the reservoir. The measurements were continued until the infiltration rate became constant.

3.2.5 Soil electrical conductivity (EC)

Soil solution was extracted by adapting the centrifugal drainage procedure described by Giesler and Lundstrom (1993). 50 g of each soil was placed in each centrifuge tube and treatment soil samples with 12.5 ml distilled water as the control or treated with solutions of NaCl, Na_2SO_4 or KCl at 20 and 50 mM. In addition, soils were treated with 12.5 ml of NaCl 70, 90, 150 and 200 mM. All soil samples were treated with 12.5 ml at 50% WHC. Triplicate soil samples were used for each site (n=144 in total). Soil solutions were removed by centrifugation (Giesler and Lundstrom, 1993), and the collected solutions passed through a Whatman 42 filter paper prior to storage at 4°C to await chemical analysis. Soil EC was measured using a conductivity meter (CDM 210) at 25°C, after calibration with a 0.01 M KCl standard solution. This solution has an electrical conductivity of 1413 μScm^{-1}.

3.2.6 Soil pH

Soil pH was measured after measured soil EC for all the samples. Soil pH was determined a using pH electrode (ANNA, Model 410A), after calibration.

3.2.7 Soil exchanged cations (Ca^{2+}, Na^{+} and K^{+})after addition of salt.

Briefly, 50 g of each soil were placed in each centrifuge tube and treatment soil samples with 12.5 ml distilled water as the control or treated with a concentration of NaCl added (20 and 50mM at 50% WHC). Soil samples were carried out in triplicate for each site (n=84 in total). Soil solutions were removed by centrifugation (Giesler and Lundstrom, 1993), and the collected solutions passed through a Whatman 42 filter paper prior to storage at 4°C to await chemical analysis. The cations exchanged after addition of distilled water as the control or salinity were determined by flame photometer (Sherwood 410).

3.3 Statistical and data analysis

The experiments were conducted with three replicates in the laboratory, and means and standard errors were calculated in Microsoft Excel. The data on organic matter percentage, moisture content percentage, water holding capacity, soil electrical conductivity (ECe), soil pH and cations exchanged after addition of salt in four soils were subjected to ANOVA analysis of variance using the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) statistical analysis program to test for significant differences between soils of four sites. Means were compared for experimental variables by Tukey test, where significant differences were accepted at $(P<0.05)$ or non-significant probability values $(P>0.05)$. Graphs were performed using Sigma Plot 8.0 for Windows using means and standard errors.

3.4 Results

The physical properties of the soils are shown in Table 3.1. The water holding capacity was similar for all the soils, at about 25%. Statistically there was no significant difference *(P>0.05)* between the sites. Soil moisture content percentage ranged from a high of value 8.9% at Janzur to the lowest of 6.6% at Tajura (Table 3.1). The moisture content was significantly higher *(P<0.05)* at Janzur and Almaya compared to Tajura and Gargaresh. There was no significant difference between Tajura and Gargaresh, and neither between Janzur and Almaya. Organic matter percentage was lower at Tajura at 0.49% compared to the other sites, while in Janzur the highest value was at 1.25%.

There were significant differences *(P<0.05)* between Janzur and Tajura (Table 3.1), but no significant difference between the other sites. Figure 3.1 shows the infiltration rate determined at the sites on the Gefara plain.

The highest rate of initial infiltration was shown for Janzur, with an intermediate value determined at Almaya, and the lowest similar values determined at Gargaresh and Tajura. For all sites, the rates of infiltration decreased rapidly after 5 minutes. Only Tajura showed a lower infiltration rate compared to the other sites. Figure 3.2 Shows during measurement infiltration rate in Almaya site by using a double-ring infiltrometer.

Table 3.1 Physical properties of four soils samples of the Gefara area. Shown are means of triplicate samples with the standard error in parentheses. Different letters indicate a significant differences between sites. Tukey HSD comparison were performed at the $P<0.05$ level.

Sites	Water holding capacity %	Soil moisture content %	Organic matter %
Gargaresh	24.3 (±0.11)a	7.0 (±0.33)a	0.62 (±0.19)a
Almaya	24.1 (±0.47)a	8.5 (±0.36)b	0.87 (±0.08)ab
Janzur	25.1 (±0.34)a	8.9 (±0.14)b	1.25 (± 0.05)b
Tajura	24.5 (±0.26)a	6.6 (±0.09)a	0.49 (±0.03)a

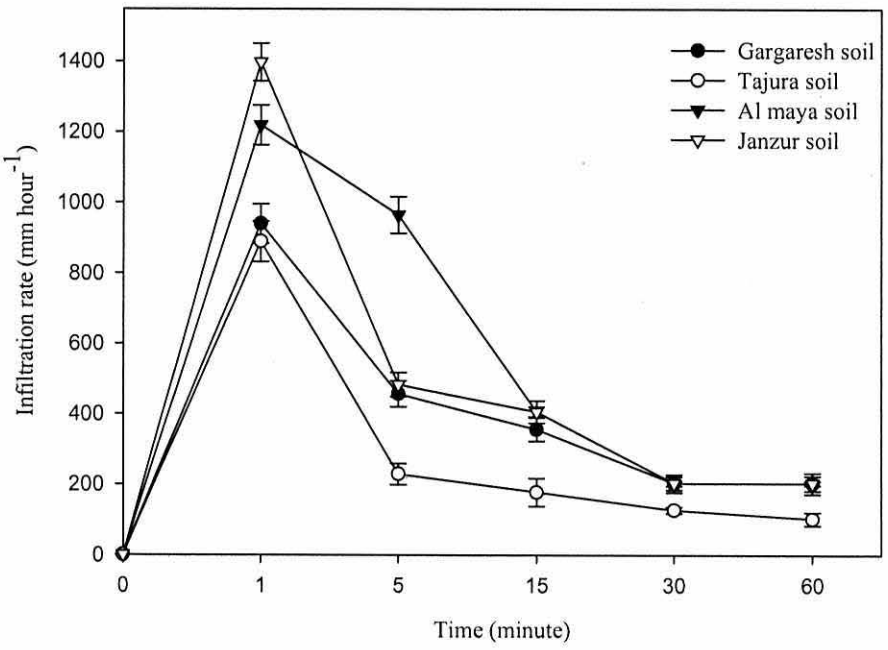

Figure 3.1 Showing infiltration rate in four soils of the Gefara Plain area. Measured in July 2007. Bars show means (±S.E.) (n = 3).

Figure 3.2 Shows measurement infiltration rate in Almaya site by using a double-ring infiltrometer.

The soil EC was significantly lower (*P<0.001)* in soil samples from Janzur (0.73 $mScm^{-1}$) than soil samples from Tajura, Gargaresh and Almaya: 3.34 $mScm^{-1}$, 2.14 $mScm^{-1}$ and 1.19 $mScm^{-1}$ respectively (Table 2.2). While in soil samples from Tajura it was significantly higher (*P<0.001)* than at Janzur and Almaya, there was also was a statistically significant difference (*P<0.001)* between Tajura and Gargaresh. Table 2.2 shows the soil electrical conductivity measured after addition of salt solutions. The greatest increase in soil electrical conductivity is found NaCl > Na_2SO_4> KCl for each concentration. Soil electrical conductivity was significantly different (*P<0.01)* after the addition of NaCl at 20 and 50 mM in four soils compared to the control. After the addition of Na_2SO_4 at concentration 20 mM, there was no significant difference *(P>0.05)* in four soils compared to the control, but when the concentration was increased to 50mM, there was significant difference *(P<0.05)* in Tajura, Gargaresh and Almaya, while in Janzur there was no significant difference *(P>0.05)* compared to the control. In the case of KCl, soil electrical conductivity was not significantly different *(P>0.05)* at four sites at concentrations of 20 and 50 mM. There are no significant difference *(P>0.05)* between Janzur and Almaya when NaCl was added at concentrations of 20 and 50 mM. In the case of Na_2SO_4, there was no significant difference *(P>0.05)* between Janzur and Almaya, nor between Tajura and Gargaresh. Soil electrical conductivity was only significantly different *(P<0.01)* between Tajura, Janzur and Almaya when NaCl was added at concentrations of 20 and 50 mM. When treated with concentration of NaCl at 20, 50, 70, 90 and 150 mM, there was no significant difference *(P>0.05)* between Janzur and Almaya. There was significant increase in Tajura compared to Janzur *(P<0.001)* and other sites *(P<0.01),* but when the soils were treated with a concentration of NaCl 200 mM, there was significant difference *(P<0.05)* between four sites (Figure 3.3).

Table 3.2 Soil electrical conductivity (ECe in mS cm^{-1}) of four soils samples as the control and under the effects of the addition of 12.5 ml of a concentration of NaCl, Na_2SO_4 and KCl added at 20 and 50 mM. Shown are means of triplicate samples with the standard error in parentheses. Different letters indicate a significant difference between sites. Tukey HSD comparisons were performed at the $P<0.05$ level.

Sites	Control	NaCl (20 mM)	NaCl (50 mM)	Na_2SO_4 (20 mM)	Na_2SO_4 (50 mM)	KCl (20 mM)	KCl (50 mM)
Gargaresh	2.14 (±0.06)b	3.34 (±0.26) b	4.27 (±0.14)b	2.73 (±0.11)b	3.82 (±0.26)b	2.40(±0.07)b	2.92(±0.34)a
Almaya	1.12 (±0.06)a	2.12 (±0.20)a b	3.02 (±0.04)a	1.74 (±0.46)ab	2.50 (±0.07)a	1.22(±0.33)a	1.89(±0.24)a
Janzur	0.74 (±0.02)a	1.64 (±0.19)a	2.62 (±0.16)a	1.29 (±0.06)a	2.11 (±0.15)a	1.12(±0.17)a	1.62(±0.07)a
Tajura	4.12(±0.26)c	5.19 (±0.38)c	6.15 (±0.26)c	4.78 (±0.23)c	5.49 (±0.18)c	4.22(±0.30)c	4.71(±0.41)b

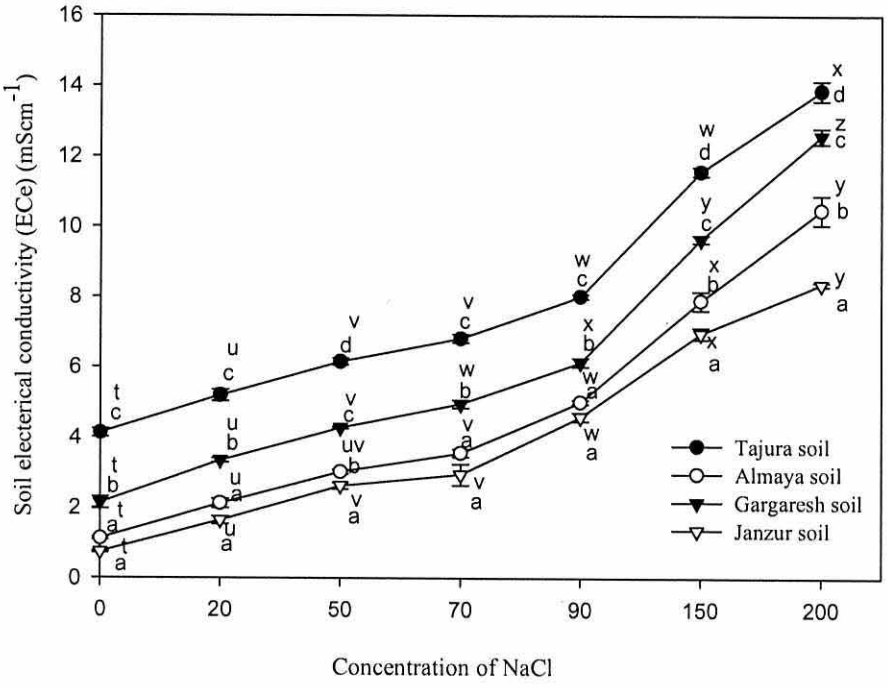

Figure 3.3 Soil electrical conductivity (EC in $mScm^{-1}$) of soils from the four sites after addition of 12.5 ml 0, 20, 50, 70, 90, 150 and 200 mM NaCl. Mean of triplicate samples with the standard error. Between sites bars not marked with the same indices (a-d) are significantly different. Between treatments bars not marked with the same indices (t-z) are significantly different. Tukey HSD comparisons were performed at the $P<0.05$ level.

Table 3.3 shows the soil pH values were similar for all four sites. Statistically there was no significant difference *(P>0.05)* between the sites and the control. Soil pH value ranged between 7 and 8 in the untreated soils indicating the soils are slightly alkaline. pH values in Tajura were slightly lower than Janzur, Almaya and Gargaresh respectively. When soil samples in four sites were amended with concentration of NaCl, Na_2SO_4 and KCl added at 20 and 50mM, soil pH was not affected *(P>0.05)* with increasing concentration added in each site (Table 3.3). Increased concentrations of NaCl added at 20, 50, 70, 90, 150 and 200 mM led to slightly decreased pH (Table 3.4).

In the untreated soils, the concentration of Na^+ ion was significantly higher in Tajura compared to other sites. This may be that the reason that the soil electrical conductivity (EC) in native soil is also higher in Tajura (Figure 3.4). Concentration of K^+ ions was only significant different *(P<0.05)* between Tajura and Janzur. Concentration of Ca^{2+} was not significantly different *(P>0.05)* between all sites. When soil samples were treated with different concentrations of NaCl at 20 and 50mM, the concentration of Na^+ ion was increased in four soils. Statistically it was significantly increased in Tajura (compared to Janzur) *(P<0.001)*, Almay *(P<0.01)* and Gargaresh *(P<0.05)* respectively (Figure 3.4). The concentration of K^+ was not significantly different *(P>0.05)* between sites on treatment with concentration of NaCl added at 20 and 50mM. The concentration of Ca^{2+} also was not significantly different *(P>0.05)* between Tajura, Almaya and Gargaresh (Figure 3.4).

Table 3.3 Soil pH of four soils samples as the control and under effects of addition of 12.5 ml of a concentration of NaCl, Na_2SO_4 and KCl 20 and 50 mM. Shown are means of triplicate samples with the standard error in parentheses. Different letters indicate a significant difference between sites. Tukey HSD comparisons were performed at the $P<0.05$ level.

Sites	Control	Na_2SO_4 (20mM)	Na_2SO_4 (50mM)	KCl (20mM)	KCl (50mM)
Gargaresh	7.54 (±0.05)a	7.48 (±0.04)a	7.38 (±0.05)a	7.50 (±0.06)a	7.47 (±0.06)a
Almaya	7.60 (±0.11)a	7.56 (±0.07)a	7.47 (±0.05)a	7.57 (±0.12)a	7.50 (±0.06)a
Janzur	7.69 (±0.03)a	7.52 (±0.05)a	7.49 (±0.09)a	7.60 (±0.07)a	7.54 (±0.06)a
Tajura	7.38 (±0.09)a	7.33 (±0.05)a	7.30 (±0.05)a	7.36 (±0.04)a	7.34 (±0.11)a

Table 3.4 Soil pH in of four soils samples as the control and under effects of addition of 12.5 ml of a concentration of NaCl 20, 50, 70, 90, 150 and 200 mM. Shown are means of triplicate samples with the standard error in parentheses. Different letters indicate a significant difference between sites. Tukey HSD comparisons were performed at the $P<0.05$ level.

Sites	Control	20mM	50mM	70mM	90mM	150mM	200mM
Tajura	7.38 (±0.05)a	7.33 (±0.03)a	7.29 (±0.12)a	7.25 (± 0.10)a	7.22 (± 0.08)a	7.18 (± 0.07)a	7.13 (± 0.11)a
Almaya	7.60 (±0.02)a	7.53 (±0.17)a	7.41 (±0.10)a	7.32 (± 0.07)a	7.28 (± 0.04)a	7.25 (± 0.04)a	7.23 (± 0.10)a
Gargaresh	7.54 (±0.09)a	7.48 (±0.09)a	7.43 (±0.09)a	7.37 (± 0.09)a	7.31 (± 0.07)a	7.26 (± 0.09)a	7.09 (±0.19)a
Janzur	7.65 (±0.03)a	7.61 (±0.21)a	7.58 (±0.02)a	7.52 (± 0.05)a	7.48 (± 0.03)a	7.41 (± 0.06)a	7.37 (± 0.18)a

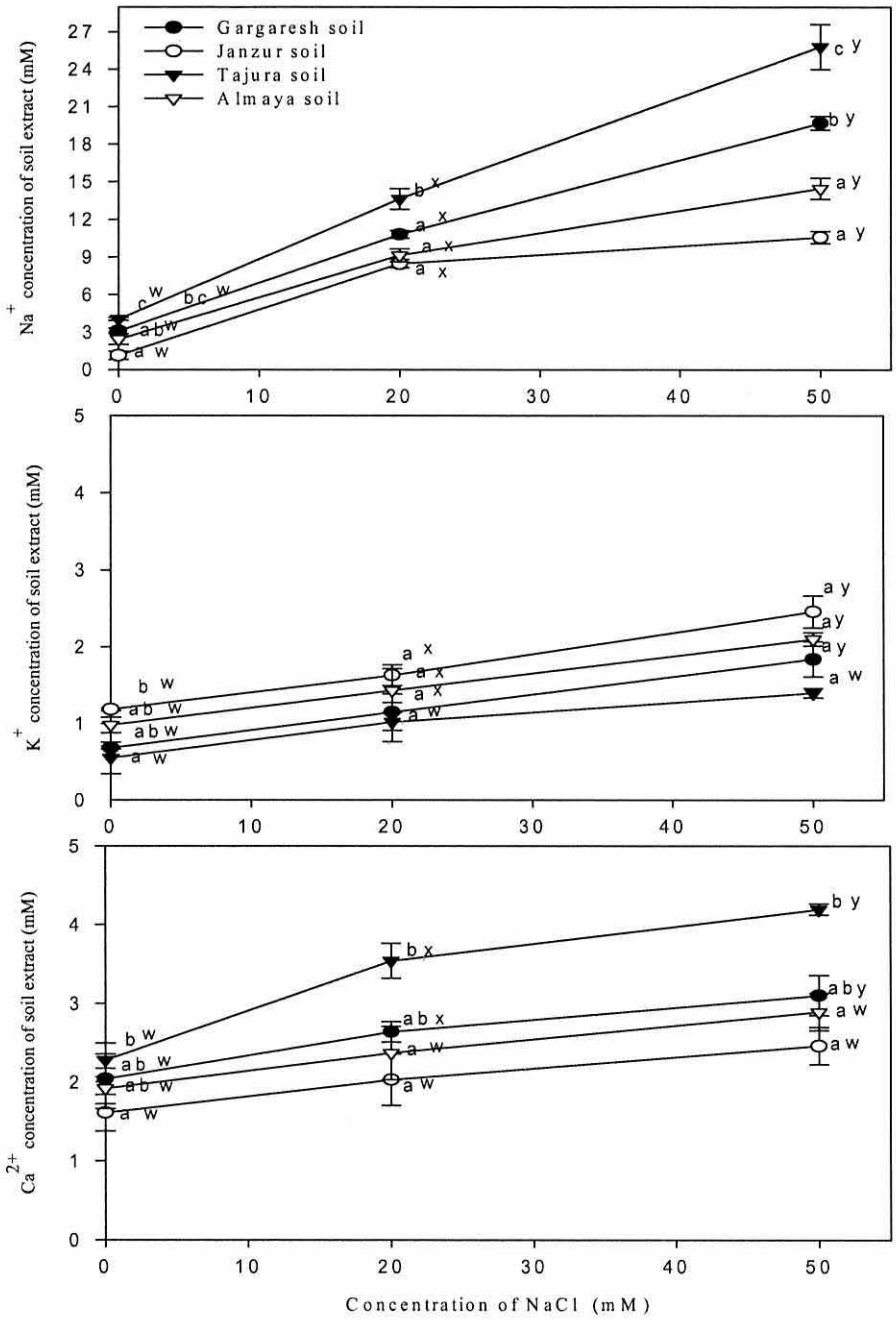

Figure 3.4 Concentration of Na^+, K^+ and Ca^{2+} of four soils samples as the control and under effects of concentration of NaCl added 20 and 50 mM. Mean of triplicates with standard error. Between sites bars not marked with the same indices (a-b) are significantly different. Between treatments bars not marked with the same indices (w-y) are significantly different. Tukey HSD comparisons were performed at the $P<0.05$ level.

3.5 Discussion

Treatment with NaCl at 20 and 50 mM led to an increase in the total soluble salts in the soil. However, NaCl added at concentrations of 20, 50, 70, 90, 150 and 200 mM, (ECe) will usually be greater than the salinity of the irrigation water used (Blaylock, 1994). Soil EC values increased with increasing salinity levels of water irrigated (El-Boraie, 1997; Ragab et al., 2008). This is more obvious in calcareous soils. It may be due to the greater surface area of fine particles which absorb soluble and exchangeable cations of saline solution. This finding is in agreement with Abd El-Nour (1989) and Ragab et al. (2008). Treatment of the soil samples with concentrations of Na_2SO_4 and KCl at 20 and 50 mM led to an increase in ECe (Table 3.2). Increased soil salinity led to an effect on pH (Alden et al., 2001). Soil pH was slightly decreased under less alkaline conditions with an increase in salinity (Lai and Stewart, 1990, Busaidi and Cookson, 2003) (Table 3.3). This may be due to the increased soil salinity increasing the removal of hydrogen from the cation exchange surfaces (Dielman, 1963, Al Zbidi, 1989). Tajura also had a higher concentration of Ca in the soil solution than Janzur. After addition of NaCl this difference was greatly increased (Figure 3.4), suggesting that addition of Na removes Ca from the cation exchange sites. This result may be of biological significance as the effect of Na on plants has been shown to be mediated by Ca (Ehret et al., 1990; Tuna et al., 2007). This was also a clear relationship between the initial infiltration rate, and the organic matter level in the soils. The size of aggregates was not determined in this investigation, but a change in aggregates due to higher organic matter contents (Boix-Fayos et al., 1998) may be a possible reason for the higher infiltration rates.

Chapter 4

Effects of soil moisture and salinity on soil respiration

4.1 Introduction

4.1.1 Soil moisture

Biological activity requires air and moisture. There have been many studies on the effects of drying and rewetting soils on soil respiration rate (e.g. Wardle, 1998; Johnson et al., 1994; Meenakashi et al., 2000). Feiziene (2008) showed that rates of soil respiration are highly dependent upon soil moisture content. Differences in the rate of degradability of soil carbon have been shown to be strongly linked to soil water holding capacity (Schonning et al., 1999; Thomsen et al., 1999). Linn and Doran (1984) found optimal microbial activity occurs at or near field capacity.

In general, increasing soil moisture increases carbon dioxide efflux rapidly to an optimum level (Gaarder, 1957), above which it reduces CO_2 efflux. Ino and Monsi (1969); Bowden (1993); Bowden et al.(1998); Liu et al.(2002a) and Xu et al.(2004) have all demonstrated that soil moisture below 40% or above 80% of the field holding capacity (WHC) decreases microbial respiration. Periodic drying and wetting of soil has a strong influence on soil respiration. When the soil is rewetted the activity of the microbes, which were dormant in the dry soil, increases - accompanied by release of air trapped in the soil pores. This contributes to an increase in CO_2 efflux (Orchard and Cook, 1983). Most studies have demonstrated an increased CO_2 efflux after rewetting compared to soil kept moist (e.g. Lloyd and Taylor, 1994; Franzluebber et al., 2000; Rustadet al., 2000).

The effect of soil moisture on soil respiration rate is important not only ecologically, but also in methods such as substrate induced respiration when substrates are added in solution.

4.1.2 Substrate-Induced Respiration (SIR)

The substrate induced respiration (SIR) method is a physiological method for measurement of the soil microbial biomass. The principle of this method is the measurement of the maximum initial respiratory response of the soil sample after the addition of glucose (Heinemeyer et al., 1989). When an easily degradable substrate (e.g. glucose) is added to a soil, an immediate increase in the respiration rate is obtained, the size of which is assumed to be proportional to the size of the microbial biomass (Anderson and Domsch, 1978). Glucose is commonly used as a substrate because most soil microorganisms can readily utilize it as a carbon source (Stotzky and Norman, 1961). Most microorganisms in the soil are dormant (Jenkinson and Ladd, 1981), so their rate of respiration is low. However, with substrate-induced respiration the microbes in soil are activated by addition of readily decomposable respiratory substrate (e.g. glucose) and leads to respiration unlimited by C (Tomomi and Sayo, 2004). Respiration may rapidly increase to a maximum and remain at a constant rate for more than 4 hours (Drobnik, 1960). Long-term incubations also lead to decreases in the amount of available carbon (Robertson et al., 1988; Bonde et al., 1988; Ross, 1991).

4.1.3 Effects of salinity on soil respiration rate

Microbial biomass size and activity is concentrated in the top few centimetres of the soil (Murphy et al., 1998). In agricultural fields with low rainfall, irrigation is reliant on underground water, which generally has higher soluble salt content than rainwater (Okur, 2002). Also, greater salinity exists in lowlands near the sea where intrusion of seawater to the aquifer occurs. Such saline waters are used on the agricultural soils of the Gefara Plain close

to the sea, which are exposed to high temperatures and low rainfall in summer months. The salts affect the soil's chemical, physical and biological properties. Irrigation waters that are saline lead to an unbalanced nutrient supply, and the osmotic potential decreases in the soil solution – a factor that will consequently affect crop yield (Curtin et al., 1993) and microbial activity (Okur, 2002). A soil's osmotic pressure is a critical factor for microbial growth and activity, and osmotic potential is closely related to salt concentration (Harris, 1981; Garcia and Hernandez, 1996; Okur, 2002). There are several comprehensive reviews on the effects of salinity and sodicity on chemical and physical properties of soil, however the effects soil microbial activity are less investigated (Sumner and Naidu, 1997; Keren, 1999; Levy, 1999). Of the studies which have been carried out on biological processes, several show contradictory results (e.g., Laura 1973, 1976; Sarig et al., 1993; Chander et al., 1994; Nelson et al., 1996; Okur, 2002; Rietz and Haynes, 2003). Several of the studies have shown inhibition of microbial activity in soils due to the influence of the irrigation with high salinity water (e.g. Adviento-Borbe et al., 2006; Nelson and Mele, 2007; Sirulink et al., 2007), for example the rate of CO_2 efflux was depressed by salinity in a sandy soil (McCormick and Wolf, 1980).There are some studies that have reported that microbial activity is not related to soil salinity or high soil pH (Beltran- Hernandez et al., 1999; Luna-Guido et al., 2000). Thus, there are several studies that report salinity's negative or positive effects of soil salinity on C mineralization in soils (Xiaogang et al., 2006). With increased salinity, osmotic stress limits, microbial growth and activity, and it has been suggested that microbes dehydrate (Oren, 1999). In addition, Zahran (1997); Juniper and Abbott (1993) and Okur et al. (2002) found dissolved salts may directly affect microbial growth and activity due to the specific toxicity of high concentration of ions such as Na^+ or Cl^-. The type of salinity has also been shown to be

important, Garcia and Hernandez (1996) found microbial respiration negatively correlated with increased salinity caused by NaCl and Na_2SO_4. They also found that this effect was more noticeable with NaCl than with Na_2SO_4.

In the work presented here, the effect salt added as NaCl; Na_2SO_4, or KCl on the microbial respiration has been investigated.

4.2 Materials and methods

Soil samples were collected as described in Chapter 3 section 3.2.1.

4.2.1 Soil moisture

Three samples of each soil, each equivalent to 50 g soil, dry wet were placed in centrifuge tubes, with triplicate samples for each site (n=12 in total), and rewetted with distilled water to 50% WHC (12.5 ml). Another set of samples were kept field moist at 25% WHC, also using triplicate samples for each site (n=12 in total). CO_2 efflux rates from each sample were measured with a PP systems SRI soil respirometer (PP-system, Hitchin, UK) at ±20°C for 24 hours.

4.2.2 Substrate-Induced Respiration (SIR)

To carry out the SIR, substrates were add both as a dry powder and as an aqueous solution. For the dry addition, the soil was amended with a powder mixture of 1mg glucose, 0.1 mg glutamate and 10 mg quartz flour (to avoid clumping of the substrate) per gram of soil. In the solution treatment the powder was first dissolved in 12.5 ml of distilled water, then mixed. Soil samples treated with distilled water (basal respiration) were used as the control. All experiments were carried out using sieved soil (< 2 mm) and three replicates of each treatment (n=36 in total). Rates of CO_2 efflux from each sample were measured with a PP systems SRI soil respirometer (PP-system, Hitchin, UK) at ±20°C for 24 hours.

4.2.3 Effects of salinity on soil respiration rate

50 g of soil was placed in a centrifuge tube, and adjusted to a water holding capacity (WHC) of 50% with 12.5 ml of solution. To estimate the effects of salt addition the 12.5 ml of solution was either distilled water as the control, or taken from 20 and 50 mM solutions of NaCl, Na_2SO_4 and KCl.

The soils were allowed to equilibrate for 7 days (Carcia and Hernandez, 1996), and all the samples were carried out in triplicate for each site (n=108 in total).

Soil respiration rate was also measured *in situ* four fields located in Almaya, Janzur, Gargaresh and Tajura during the summer season 2009. Figure 4.1 shows the study locations in the field. At the sites, each field was subdivided into 16 sites: 4 sites were untreated, 4 sites were treated with distilled water as the control, 4 sites were treated with NaCl at a concentration of 20 mM, and 4 sites with 50 mM. In each sub-plot a tube constructed from thin-walled polyvinyl chloride (PVC) (diameter 15 cm, volume 3535 cm^3) was inserted into the soil, leaving a small collar All addition treatments used 1.15 L of solution, which was based on the soil volume in the tube and the amount of water needed to increased the soil moisture to 50% of WHC. The litter layer was removed from the soil surface before treatment (Bowden et al. 1993). Soil respiration was measured for 8 hours at 27°C using an EGM-4 portable system (PP Systems, Hitchin, U.K.), consisting of a portable infrared gas analyzer (IRGA), and a measuring chamber (diameter 95 mm, volume 991 cm^3). To carry out the measurements the chamber end was inserted into the soil collars.

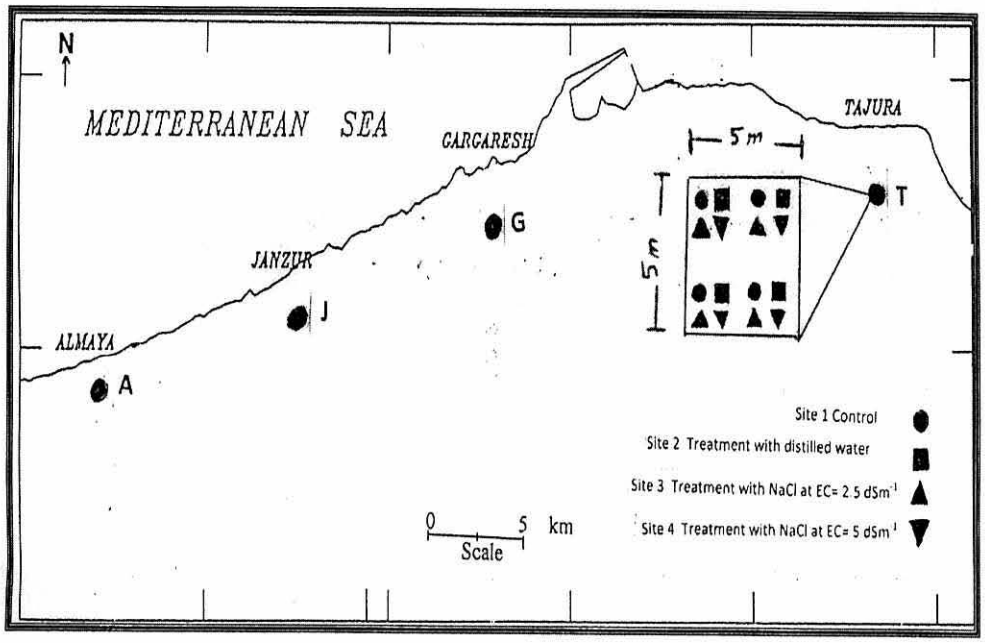

Figure 4.1 The study locations in the field.

4.3 Statistical and data analysis

The experiments were conducted with three replicates in the laboratory, and means and standard errors were calculated in Microsoft Excel. The sets of data (soil respiration rates in four soils between the samples kept at field moist as the control and after rewetting with distilled water at 50% WHC (12.5 ml), the data of soil respiration rates in four soils between the samples as the control and after treatments with glucose dry and glucose wet) and the data of soil respiration rates in four soils between the samples as the control or treatments with salinity in laboratory and field were subjected to an independent samples t-test and one way-ANOVA analysis of variance were compared for experimental variables by Tukey test using the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) with significant differences accepted at *(P<0.05)* or non-significant probability values *(P>0.05).*Graphs were created with Sigma Plot 8.0 for Windows using means and standard errors.

4.4 Results

The respiration rate of soil samples kept at field moisture (see Table 3.1) was significantly higher in soils from Janzur than Tajura *(P<0.01)* and also Gargaresh *(P<0.05)*. After wetting of the soil samples to 50% WHC, the rate of CO_2 evolution was significantly higher *(P<0.001)* in soil samples from Janzur compared to soils from Tajura and Gargaresh, there was also a significant difference *(P<0.01)* between Almaya and Tajura. The results indicate that there is a statistically significant difference *(P<0.05)* between soil samples kept at field and soil samples after wetting in all soils (Figure 4.2). In all soils after wetting, the rate of CO_2 efflux increased to 138-140% compared to soil samples kept at field moisture. With added dry glucose and glutamate, rates of substrate-induced respiration (SIR) was not significantly different *(P>0.05)* between Tajura and Gargaresh, nor between Janzur and Almaya while the SIR rate was significantly higher *(P<0.01)* at Janzur than at Tajura and Gargaresh (Figure 4.2). In soil samples amended with glucose and glutamate as a solution, were adjusted to 50% WHC by with 12.5 ml, the SIR rate was significantly higher at Janzur than at Tajura *(P<0.001)*, Gargaresh *(P<0.01)* and Almaya *(P<0.01)*. Generally, all four soil samples had the greatest rates of SIR when glucose was added in solution, reaching values of 400%, 370%, 340% and 265% *(P<0.001)* at Janzur, Gargaresh, Almaya and Tajura above the control (basal respiration) respectively (Figure 4.2). In the investigation of the effects of salts on soil respiration (Figure 4.3A and B), in the control, the rate of CO_2 evolution was significantly higher in soil taken from Janzur than that taken from either Tajura *(P<0.01)* or Gargaresh *(P<0.05)* (Figure 4.4A). In soils from Tajura at 20 mM NaCl, the rate of CO_2 efflux decreased significantly more than those from Janzur *(P<0.001)* and Almaya *(P<0.01)* (Figure 4.3A).

The rate of CO_2 evolution in Tajura was decreased by nearly 57% *(P<0.01)* at 20 mM NaCl and decreased by 27% *(P<0.001)* at 50 mM NaCl, compared to the control (Figure 4.3B).

In contrast, in the soil samples from Janzur, the rate of respiration was not significantly decreased *(P> 0.05)* after treatment with 20 mM NaCl compared to the control (Figure 4.3A). The rate of CO_2 evolution was decreased to 89% *(P> 0.05)* at 20 mM and to 76% *(P< 0.05)* at 50 mM NaCl compared to the control (Figure 4.3B). In soil samples from Gargaresh the rate of CO_2 evolution was significantly inhibited *(P<0.01)* by adding 20 mM NaCl, at *(P<0.001)* and treating with 50 mM NaCl compared to the control (Figure 4.3A). The rate of soil respiration was decreased in soil samples of Gargaresh from 75% *(P<0.05)* to 56% *(P<0.01)* by concentrations of NaCl added at 20 and 50 mM respectively, compared to the control. In Almaya, it was decreased 84% *(P>0.05)* by concentrations of NaCl added at 20 mM and 62% *(P<0.05)* at 50 mM compared to the control (Figure 4.3B). The decreased rate of CO_2 evolution was higher in soil samples from the Tajura site than in other sites at 20 and 50 mM NaCl. In the case of Na_2SO_4, when the Tajura samples were treated with 20 mM, the CO_2 rate was decreased 83% *(P>0.05)* compared to the control, and when the concentration of Na_2SO_4 was increased to 50 mM the decreased rate reached to 47% *(P<0.01)* compared to the control (Figure 4.4 A and B). There was a significant difference *(P<0.01)*, *(P<0.001)* in soil samples of Tajura at amended with 20 and 50 mM Na_2SO_4 respectively, compared to Janzur. The rate of decreased in soil samples from Gargaresh was 84% *(P>0.05)* compared to the control at amended by 20 mM Na_2SO_4. In soil samples amended by 50 mM Na_2SO_4 the decreased rate reached 70% *(P<0.05)* compared to the control (Figure 4.4 A and B). Between Gargaresh and Janzur there was a significant difference at *(P<0.05)*, *(P<0.01)* when samples were amended with 20 and 50 mM Na_2SO_4 respectively. The effects of Na_2SO_4 on soil samples from Almaya

were similar to Gargaresh. In samples from Janzur the rate of CO_2 evolution was not significantly different following treatment by 20 and 50 mM (89 and 81%, *(P>0.05)*) respectively compared to the control.

In the case of KCl, the soil respiration rate was not significantly decreased *(P>0.05)* by a 20 mM of concentration of KCl added to soil samples of from Janzur (99%), Almaya (96%) and Gargaresh (97%). There was also a similar tendency with 50 mM KCl the rate of soil respiration was not significantly decreased *(P>0.05)* by 95%, 93% and 88% in soil samples from Janzur, Almaya and Gargaresh respectively (Figure 4.5 A and B). But, the rate of CO_2 evolution was decreased by 56% *(P<0.01)* when soil samples from Tajura were treated with 50 mM KCl. In the field study, the rate of CO_2 evolution from the control (no treatment) was only not sigifcantly different *(P>0.05)* between Gargaresh and Almaya. In Janzur it was significantly higher *(P<0.001)* than Tajura, Gargaresh and Almaya respectively (Figure 4.6 A and B). When the four soils were amended with distilled water, there was no significant difference in the rates of soil respiration *(P>0.05)* between Almaya and Janzur. With treatment with concentrations of NaCl at 20 mM, the rate of CO_2 was decreased by (68%; *P>0.001* and 81%; *P>0.01*) in Tajura and Gargaresh respectively, compared to the soils treated with distilled water (Figure 4.6 A and B). The rate of CO_2 was decreased by (94% and 90%; *P<0.05)* in Janzur and Almaya respectively. When the concentration of NaCl was increased to 50 mM, the soil respiration rate was decreased by (54% and 67%; *P<0.001*) in Tajura and Gargaresh respectively compared to soils treated with distilled water. The rate of CO_2 efflux in Alymaya was decreased by 80% *(P<0.001)*, and in Janzur was decreased by 89% *(P<0.001)* (Figure 4.6 A and B). The CO_2 efflux rate was higher *(P<0.001)* at Janzur than Tajura and Gargaresh in soils amended with distilled water.

In a comparsion between results obtained from Tajura in laboratory and *in situ* in the field, the inhibition of the rate of CO_2 efflux was significantly different between the laboratory and field investigations after the treatment with 20 and 50 mM.

The effects at 20 and 50 mM were less *in situ* (57 and 68% respectively *($P>0.05$)*) than in the laboratory (27 and 54% respectively, *($P<0.001$)*).

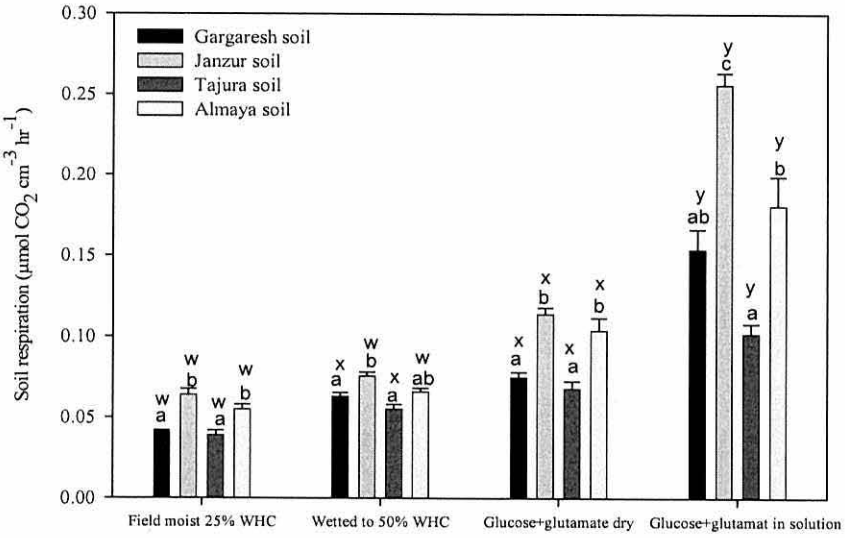

Figure 4.2 Effects soil moisture content, glucose and glutamate added dry and glucose and glutamate add in solution at 50% WHC on soil respiration in four soils samples compared to the control. Bars show mean (±S.E.) of triplicates. Within a treatment bars not marked with the same indices (a-c) are significantly different for each soil. Between treatments bar not marked with the same indices (w-y) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

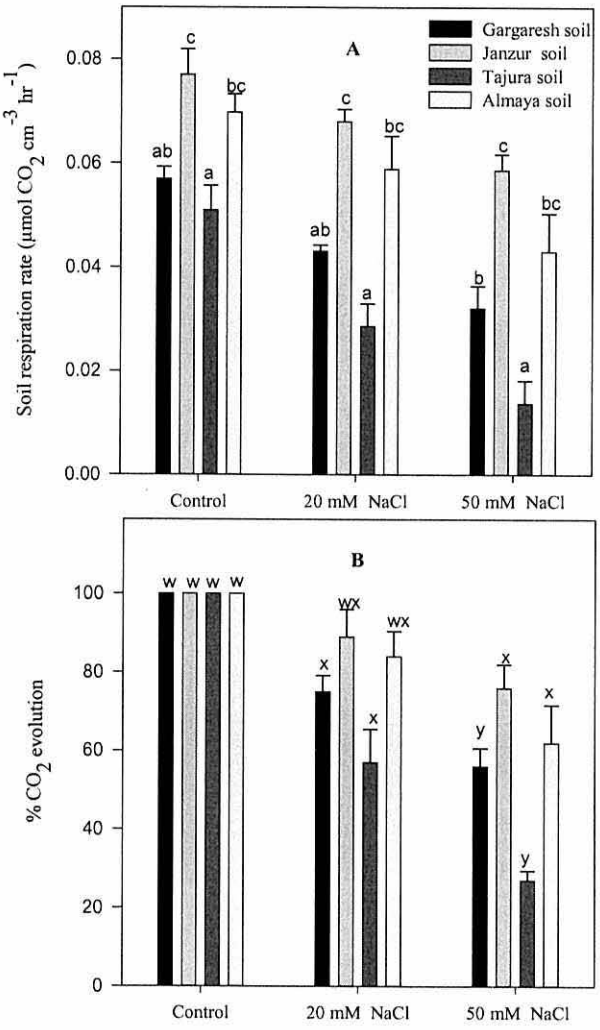

Figure 4.3 (**A**) Shows the effects of addition of 12.5 ml of a concentration of NaCl 20 and 50 mM or distilled water as the control, for 24 hr on soil respiration of four soils. Bars show means (±S.E.) (n = 3). (**B**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-c) are significantly different for each soil. Between treatment bars not marked with the same indices (w-y) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

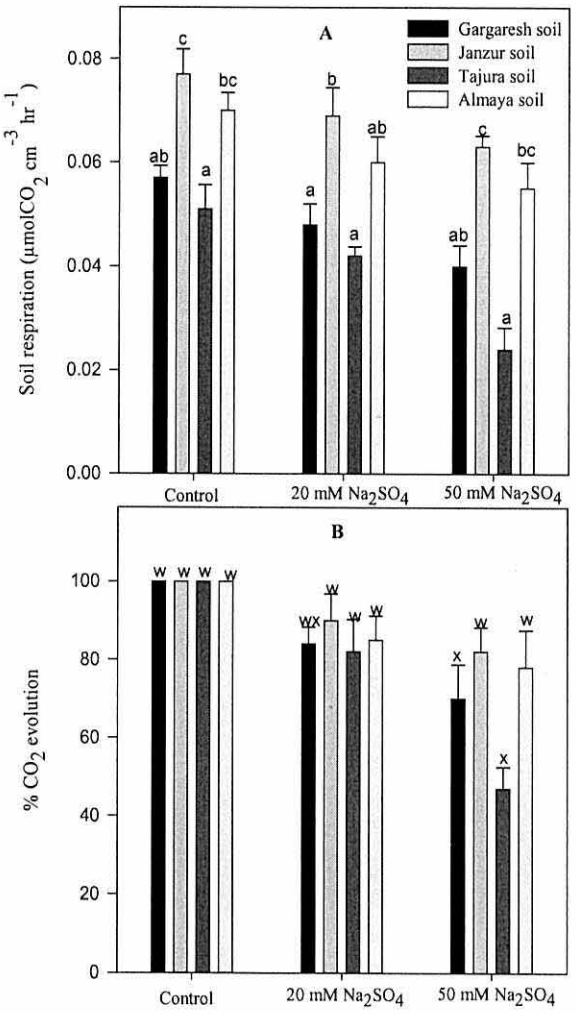

Figure 4.4 (**A**) Shows the effects of the addition of 12.5 ml of a concentration of Na_2SO_4 20 and 50 mM or distilled water as the control, for 24 hr on soil respiration of four soils. Bars show means (±S.E.) (n = 3). (**B**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-b) are significantly different for each soil. Between treatment bars not marked with the same indices (w-x) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

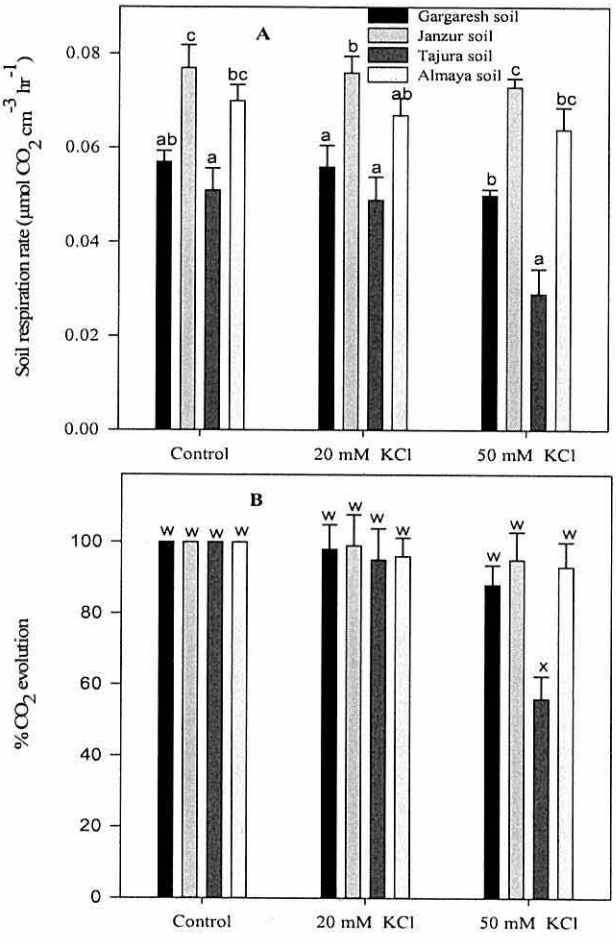

Figure 4.5 (**A**) Shows the effects of addition of 12.5 ml of a concentration of KCl 20 and 50 mM or distilled water as the control, for 24 hr on soil respiration of four soils. Bars show means (±S.E.) (n = 3). (**B**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-c) are significantly different for each soil. Between treatment bars not marked with the same indices (w-x) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

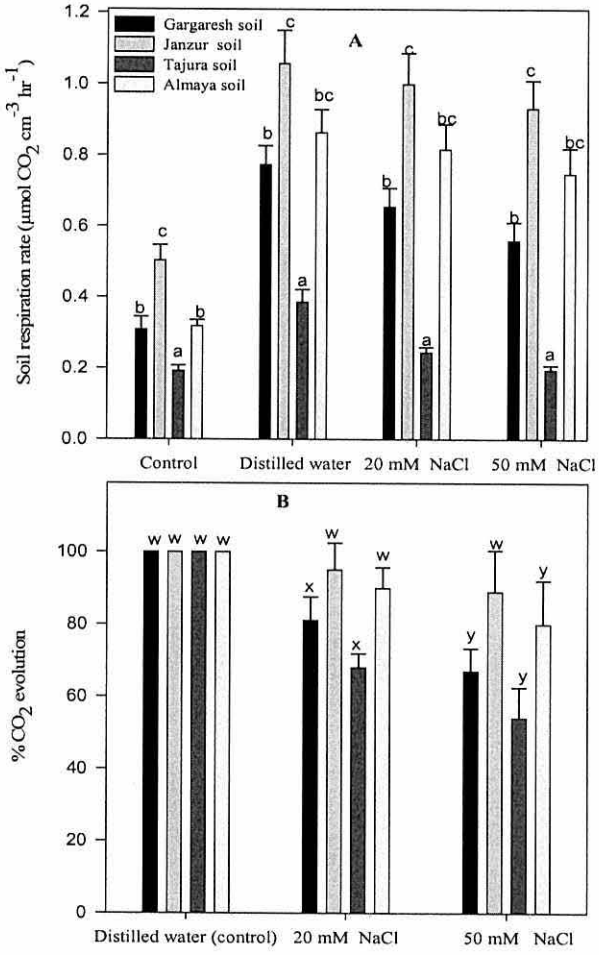

Figure 4.6 (**A**) The effects of addition of 1.15 ml of a concentration of NaCl 20 and 50 mM or distilled water for 8hr on soil respiration of four soils in the field. Bars show means (±S.E.) (n = 4) (**B**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-d) are significantly different for each soil. Between treatment bars not marked with the same indices (w-z) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

4.5 Discussion

The rate of soil respiration increased doubled after wetting soil samples, to 50% WHC, compared to soil kept at field moisture as the control. Rewetting is known to make organic matter more readily mineralizable (Sorensen, 1974). In addition, field capacity has been shown to be the optimal soil moisture level for soil microbial activity (Doran et al., 1991; Orchard and Cook, 1983; Borken et al., 1999). The rate of soil respiration rose to 48% in soil samples amended to 50% WHC compared to the field moist soil, similar to results shown by Ross (1989) and Ilstedt et al. (2000). These authors also found that CO_2 efflux rate increased when soil samples were wetted to 50% WHC. In soils, Jenkinson and Ladd, (1981) suggested most microorganisms are dormant, and thus have a low rate of respiration. This is shown as the rate of respiration in the control (basal respiration) (Figure 4.2), and may be due to the lack of organic material, which does not exceed 1% in the Gefara Plain soils (Ben Mhamod, 1995). Their respiration can be stimulated by adding easily decomposable substrate. Glucose and glutamate were used as a substrate as most soil microorganisms can readily utilize glucose as a carbon source (Stotzky and Norman, 1961) and the addition of glutamate ensure the micro-organisms are not N limited. C-mineralization increased rapidly when the soil samples were treated with glucose (Drobnik, 1960; Johansson et al., 1998). When glucose and glutamate were added in solution to the soil samples, the substrate-induced respiration (SIR) rate increased, about 3 times compared to the control basal respiration (Figure 4.2). Part of this effect is due to addition of water at 50% WHC, but part may also be due to the solution giving a better distribution of glucose and glutamate in soil (West and Sparling, 1986); another reason for this may be because the osmotic potential of the soil solution is changed (Ilstedt et

al., 2000). The greatest increase in soil respiration to the solution of glucose and glutamate was seen in the soil from Janzur, the lowest in the soil from Tajura.

This suggests that there may be differences in microbial biomass between the soils, possibly due to the differences in organic matter in these soils. However, difference in the rate of substrate-induced respiration (SIR) may also be due to differences in soil EC (Vuelvas-Solorzano et al., 2009) in all four soils of the Gefara Plain area. In particular, soil samples from the Tajura site have high EC compared to samples from the other sites. This may have caused a lack of response in the rate of substrate-induced respiration (SIR) (Vuelvas-Solorzano et al., 2009). The increase in CO_2 evolution in soil samples amended with distilled water may be due to increased contact between the native SOM and enzymes produced by a more active microbial population (Conde at al., 2005). However, when an equal molar concentration of NaCl, Na_2SO_4 and KCl was added to the soil, NaCl was produced the greatest increase in ECe (Table 2.2). This can be explained by the higher activity of Cl^- ions than SO_4^{2-} ions. Soil ECe reached 3.02, 4.27, 6.15 and 2.62 $mScm^{-1}$ at a maximum concentration of NaCl 50 mM. At the same concentration of Na_2SO_4, EC reached 2.50, 3.82, 4.68 and 2.11 $mScm^{-1}$, while at the same concentration of KCl it reached 1.89, 2.92, 3.91 and 1.63 $mScm^{-1}$, in Almaya, Gargaresh, Tajura and Janzur respectively. The rate of soil respiration decreased on treatment with NaCl > Na_2SO_4 > KCl respectively. An increase in EC has been shown to have negative effects on microbial activity in the soil (Okur, 2002), which in turn led to a decreased rate of CO_2 evolution (Okur, 2002; Rietz and Haynes, 2003; Sirulink et al., 2007). In part the decrease seen in this work may be due to changes in EC, however the effect of salt addition on soil respiration rate differed according to whether NaCl, Na_2SO_4 or KCl were added, suggesting specific ion effects.

The CO_2 evolution rate was clearly affected by the addition of saline solutions (Laura 1974, 1976; Sarig et al., 1993; Nelson et al., 1996; Pathak and Rao, 1998; Luna-Guido et al., 2001), even at low concentrations - especially when NaCl was added.

The rate of CO_2 efflux was further inhibited at a 50 mM concentration of NaCl compared to the control in Tajura, Gargaresh, Almaya and Janzur respectively. Similar findings are reported by Sarig et al. (1993). Chloride and sulphate salts often dominate in saline soils (Bresler et al., 1982; Wang et al., 1991). As a result, chloride and sulphate have different activities in the soil solution, thus chloride salts are more effective than sulphate salts (Heilman, 1975). Sindhu and Cornfield (1967) also pointed out sulphate salts have a lower toxicity than chlorides due to lower uptake by microbial cells. In the case of KCl no effect on CO_2 efflux was observed, even though it decreased the EC. This suggests that Cl has little effect directly or that K may ameliorate the potential effects of Cl or the background Na in the soils. An increase in KCl increased organic C mineralization in soil and enhanced the microbial activity (Chandra et al. 2002). But they found that influence of K_2SO_4 was significantly higher than KCl in stimulating C mineralization in soil.

In the *in situ* field exposures to NaCl, the rate of soil respiration was suppressed by concentrations of NaCl at 20 and 50 mM. The decrease was significantly greater than those obtained in the laboratory by addition of the same amounts of NaCl relative to the soil volume for some of the soils. For example, no significant difference was shown in the effects concentrations of 20 mM NaCl on the Janzur samples in laboratory and in the field. However, the rate of CO_2 evolution was more affected in the Tajura soil following application of a concentration of NaCl 20 and 50 mM in both the laboratory and the field. This difference in sensitivity appears to be due mainly to increasing salinity and low organic matter percentages

in Tajura samples when compared to Gargaresh, Almaya and Janzur. The variation of soil respiration rate in four soils without addition of NaCl may be due to the heterogeneity of vegetation coverage, root distribution (Xu, Qi, 2001; Maestre and Cortina, 2003; Epron et al., 2004; Tang, Baldocchi, 2005).

The four areas where soils were sampled were differ in the amount and heterogeneity of vegetation coverage, there was more vegetation coverage in Janzur and Almaya than in Gargaresh and Tajura.

In general, the results obtained suggest that salinity has negative effects on soil respiration rate, especially when NaCl is responsible for the salinity. The inhibition of CO_2 evolution at high salt concentrations could have been due to decreases in water availability and accumulation of Na^+ and Cl^- ions to toxic levels in microbial tissues, which impedes the use of the other essential anions and cations by the microbes (Laura, 1974). High concentrations of salts can also affect decomposition of organic materials (Broadbent and Nakashima 1971; Kaur et al. 1998). It is concluded that soil microbial activity represent sensitive and useful indicators for determining the effects of salinity on soil quality. The microbial activity of soil and cycling of C is harmed by continuous and gradual salinization of soils, and alkalinity will likely decrease soil organic matter content (Xiao-Gang and Zed, 2007), which in turn leads to decreased CO_2 evolution.

Chapter 5

Salt effects on mycorrhizal hyphal growth and respiration

5.1 Introduction

Soil salinity may lead to a reduction in the growth and yield of many crops. The degree of reduction is dependent upon plant species, salinity level and the ionic composition of the salts that contribute to salinity (Kent and Lauchli, 1985). In addition, deterioration of the soil's physical properties decreases soil aeration, creating a poor environment for root penetration, and waterlogging due to poor drainage. Soil crusting and compaction of the top and subsoil also reduces seedling emergence, and increases runoff and erosion. Salinity increases the osmotic potential of the soil solution (Bernstein et al., 1974; Maas and Hoffman, 1977), resulting in reduced availability of water to plant roots (Al-Karaki, 1997). Greenway and Munns (1980) indicated that ion toxicity and imbalanced nutrition in saline conditions are the main constraints on plant growth. Rietz and Haynes (2003) noted that in salt-affected soils, reduction in the mineralization of C, N, S and P, reduction in the rate of SOM decomposition and reduced nutrient availability are all additional growth-limiting factors. Many investigations have reported retardation of germination and seedling growth at high salinity, while at later stages of growth, plants become more tolerant of salinity stress (Ramoliya and Pandey, 2003). Other investigations indicate that salt accumulation in the soil can also negatively affect plant growth by reducing nutrient availability in the soil and decreasing nutrient uptake (e.g. Pessarakli, 1991; Grattan and Grieve, 1999) leading to nutritional imbalance in plants (Grattan and Grieve, 1999). High salt concentrations in soil have a number of effects that can decrease plant growth. These include the specific ion effects of Na and Cl, but also negative effects on water uptake (Munns et al., 2006).

Salinity has also been shown to suppress shoot growth more than root growth (e.g. Bernstein and Pearson, 1954; Ayers and Eberhard, 1960; Meiri and Poljakoff, 1970).

5.2 Effects of the mycorrhizal on plant growth in soil salinity

Mycorrhizs are mutualistic symbiose between a plant's roots and a fungus (Allen, 1991). Mycorrhizs increase the surface area associated with the plant roots, which improves the acquisition of nutrients and water from the soil (Smith and Read, 1997; Aerts, 2002; Kodie, 1991; Miller and Jastrow, 2000; Smith et al., 2001; Saito, 2000; George, 2000). Mycorrhizal fungi source their carbohydrates from the plant root they are living in/on and are usually beneficial to the plants by transferring P and N from the soil into the root (Smith and Read, 1997). Mycorrhizal fungi also contribute to the sustainable maintenance of plant health (Jeffries et al., 2003). An increasing number of studies indicate the importance of mycorrhizs for the survival of plants in places with problem soils, such as soil affected by drought (Auge, 2001; Davies et al., 2002a; Estrada-Luna and Davies, 2003; Estrada-Luna et al., 2000), and salinity (Al-Karaki and Clark, 1998; Al-Karaki and Hammad, 2001; Cabello, 2001; Mohammad et al., 2003). Sengupta and Chaudhuri (1990) showed also mycorrhizs occur naturally in saline areas, although salinity may affect the formation and function of mycorrhizas as observed by Juniper and Abbott (1993) and McMillen et al. (1998). There are two types of mycorrhizas as endomycorrhizae (penetrating the cell wall) and ectomycorrhizae (no penetrating the cell wall) (Allen, 1991).

Arbuscular mycorrhizal fungi, these fungi infect a wide variety of plants, such as grasses, herbs, agricultural crops and some legumes.

5.3 Effects of salinity on length and respiration of external hyphae.

Fungal hyphae play an essential role in soil aggregation processes (Miller and Jastrow, 1990) and nutrient uptake to the plant (Jakobsen et al., 1992) from immobile or fixed elements (P, Zn, Cu) in acid or alkaline soils (Allen et al., 1995; Mosse, 1973). There have only been a few investigations of the rates of respiration of hyphae (Rillig and Allen, 1999 and Heinemeyer et al., 2006). However, there are many studies that have shown that fungal hyphae occur naturally in saline soils (e.g. Pond et al., 1984; Van Duin et al., 1989; Cooke and Lefor, 1990; Sengupta and Chaudhuri, 1990; Bohrer et al., 2003), and that the hyphae support plant growth in saline soils (Pfeiffer and Bloss, 1988). Levy et al. (1983) and Hartmond et al. (1987) reported that external hypha are not reduced by salinity. There are some studies reporting that external hyphae growth is inhibited by increasing concentrations of NaCl in soil solution (e.g Hirrel, 1981; Estaun, 1989; Juniper and Abbott, 1993). This may be due to the direct effects of ion toxicity or the indirect effects of osmotic stress (McMillen et al., 1998). In this chapter the effect of NaCl on the growth and respiration of external hyphae is investigated.

5.4 Materials and methods

5.4.1 Soil samples and plant culture

Soils were collected as described in Chapter 3, section 3.2.1. A laboratory experiment was designed to examine CO_2 efflux from soil with and without external hyphae growing from wheat plants. The wheat cultivar *Triticum aestivum L. var.* was used in the experiments. Six seeds were placed in the plant tube of a rhizobox (Figure 5.1), at 1 cm depth into the soil. Each tube contained 50 g of soil. The rhizoboxes were placed in a growth cabinets (Sanyo Gallenkamp Fitotron®) at day/night temperatures of 24/15°C, a light intensity 1.250 μmol m^{-2} sec^{-1}, humidity 70% and a day length of 16h.

The plants were watered with distilled water as the control, and treated with concentrations of NaCl were added at 20 and 50mM (12.5 ml at 50% WHC), every two days. The rhizobox comprised a plant tube and hyphal tube separated by 35 μm nylon mesh to allow the external hyphae to pass the size of the window cut into the tubes were with length 6 cm and width 2cm. The other tube, made up of a complete tube, containing only soil acted as the control. The soil respiration rate was measured 6 weeks from planting, by which time the shoot reached approximately 15cm; all the samples were carried out in triplicate (n=36 in total). Soil respiration rate was measured by using PP Systems SRI soil respirometer (PP-Systems, Hitchin, UK) at ±20°C for 24 hours.

5.4.2 Determination of hyphal length

The hyphal length was determined by extraction of hyphae from both the hyphal and soil tubes from a soil sample (5 g), using the method of Jakobsen et al. (1992). (n=36 in total). This was modified by rinsing the soil through a 53 μm sieve to remove fine clay and organic matter particles. The samples were stirred in 250 ml of distilled water for 10 s and allowed to settle for 10 s. The water was then poured off through a 53 μm sieve. Larger debris remained in the beaker. This was repeated three times to ensure most of the hyphae were obtained. The material on the 53 μm sieve was rinsed into a blender using 250 ml distilled water, then blended, extracted and filtered onto cellulose membrane filters (Whatman 47 mm diameter, 0.45 mm pore size), and placed on a Buchner flask with a perforated support for the membrane. Water was removed from the filter under vacuum. The membrane filters, containing the extracted hyphae, were then stained with trypan blue solution, left for 15 minutes, then rinsed with distilled water and again placed under vacuum. The filter was allowed to air dry. A filter paper was cut to a size that fitted a microscope slide, then moistened with acidic glycerol and

overlaid with a cover-slip. A $1cm^2$, 10 x 10 square gridded eyepiece, formed by 11 horizontal and 11 vertical lines intercrossed perpendicularly, was incorporated into the lens (x10) of a compound microscope (Axioscope, Zeiss, Germany).

The hyphae on the membrane filters were viewed at x100 magnification counting the intersections between the hyphae and the grid-lines.

Then the external hyphal lengths were quantified using the formula devised by Marsh (1971)

$$R = 11/14\ N \times G$$

Where R is hyphal length; N is the number of intersections; G is length of the grid unit.

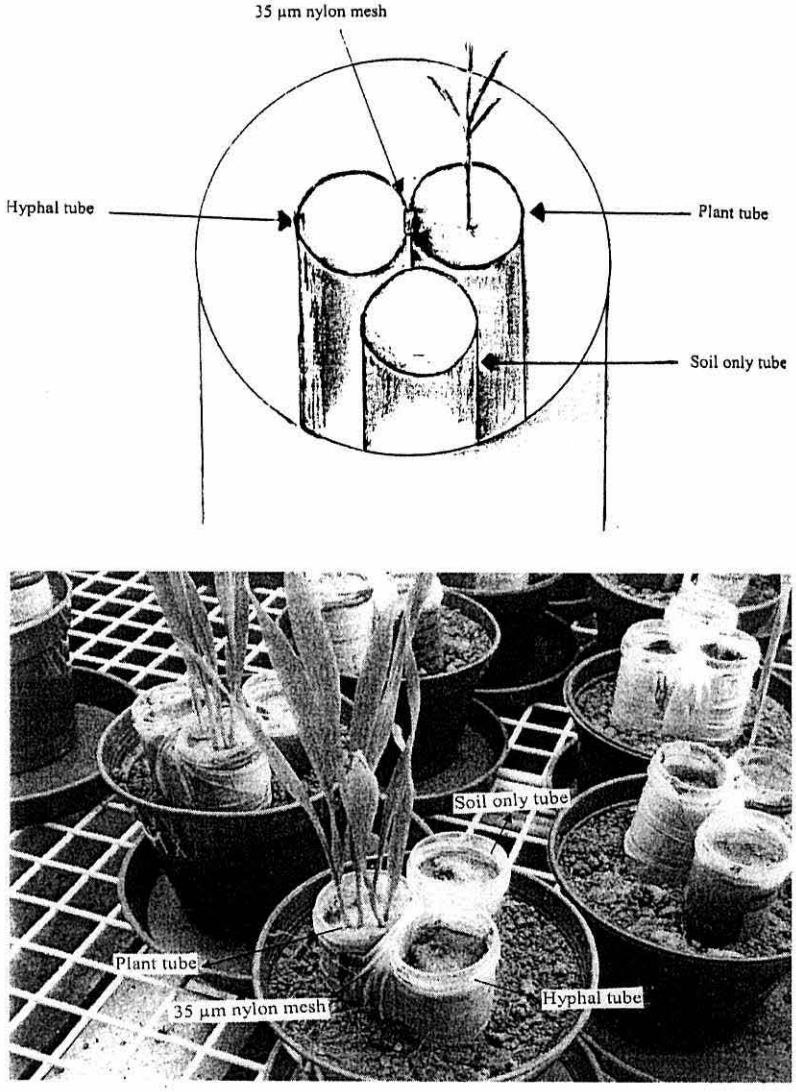

Figure 5.1 Diagram showing the design of the rhizobox, consisting of a plant tube, a hyphal tube and a soil-only tube. The plant and hyphal tubes are separated by 35 µm nylon mesh pore size.

5.5 Statistical and data analysis

The experiments were conducted with three replicates in the laboratory, and means and standard errors calculated in Microsoft Excel. The data of CO_2 evolution rate and length of external hyphae after addition of distilled water and salt in four soils were subjected to ANOVA using the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) statistical analysis program to test for significant differences between soils from the four sites. Means were compared for experimental variables by Tukey test, where significant differences were accepted at *($P<0.05$)* or non-significant probability values *($P>0.05$)*. Graphs were created using Sigma Plot 8.0 for Windows using means and standard errors.

5.6 Results

The soil without the presence of external hyphae, and without addition of NaCl, exhibited an CO_2 efflux rate that was considerably higher *($P<0.001$)* in samples from Janzur compared to Tajura (Figure 5.2A). Addition of increasing concentrations of NaCl from 20 to 50mM led to a decrease in the rate of CO_2 efflux (Figure 5.2A). Considering soil from all four sites, the decrease in CO_2 efflux was greater *($P<0.01$)* in samples from Tajura than samples from Janzur. Also, there was a relatively smaller decrease *($P<0.05$)* in the rate of soil respiration in soil samples from Gargaresh than from Janzur. The rate of soil respiration in the presence of external hyphae was significantly increased *($P<0.001$)* compared to the soil without external hyphae. A higher rate of CO_2 efflux was seen in both the unamended treatment but also in soils amended with concentrations of NaCl at 20 and 50 mM. There was a significant difference *($P<0.05$)* in CO_2 efflux from the hyphal compartment between Janzur and Gargaresh compared to Tajura and Almaya (Figure 5.2B).

But CO_2 the efflux rate from the hyphal compartment was not significantly different *(P>0.05)* between Tajura and Gargaresh, or between Janzur and Almaya. The addition of NaCl to the hyphal compartment decreased the rate of soil CO_2 efflux in all soils. At 50 mM NaCl the decrease was 60%, *(P<0.001)* for Tajura, 74%, *(P<0.05)* for Gargaresh, 88%, *(P>0.05)* for Almaya and 92%, *(P>0.05)* for Janzur compared to the control (Figure 5.3B). In comparison, in the tubes without hyphae the decrease was 56%, *(P<0.05)* for Tajura, 72%, *(P<0.05)* for Gargaresh, 86%, *(P>0.05)* for Almaya and 90%, *(P>0.05)* for Janzur (Figure 5.3A). By subtracting the values from the soil compartment from those of the hyphal compartment, the soil CO_2 efflux from only the hyphae was calculated (Figure 5.2C). Addition of NaCl decreased the rate of CO_2 efflux, with the greatest effect seen in the Tajura soils. Hyphal length (Figure 5.4A) was determined in both the hyphal and the soil only compartments. No hyphae were observed in the soil only compartment. The external hyphal length without addition of NaCl was significant higher *(P<0.01)* in Janzur compared to Tajura. There was no significant difference *(P>0.05)* in the length of external hyphae between Tajura and Gargaresh, nor between Janzur and Almaya. Addition of NaCl to the hyphal tube led to decrease in the length of external hyphae. At the highest NaCl concentration the decrease was 26%, *(P<0.001)* for Tajura, 47%, *(P<0.01)* for Gargaresh, 63%, *(P<0.05)* for Almaya and 85%, *(P>0.05)* for Janzur and this was the largest effect seen (Figure 5.4 B). Figure 5.5A shows soil CO_2 efflux expressed in terms of hyphal length. There was no significant different *(P>0.05)* between the four soils amended with either water or 20 mM NaCl. When 50 mM NaCl was added the decrease was 73%, *(P<0.001)* for Tajura, 80%, *(P<0.05)* for Gargaresh, 91%, *(P>0.05)* for Almaya and 95%, *(P>0.05)* for Janzur compared to the control (Figure 5.5B).

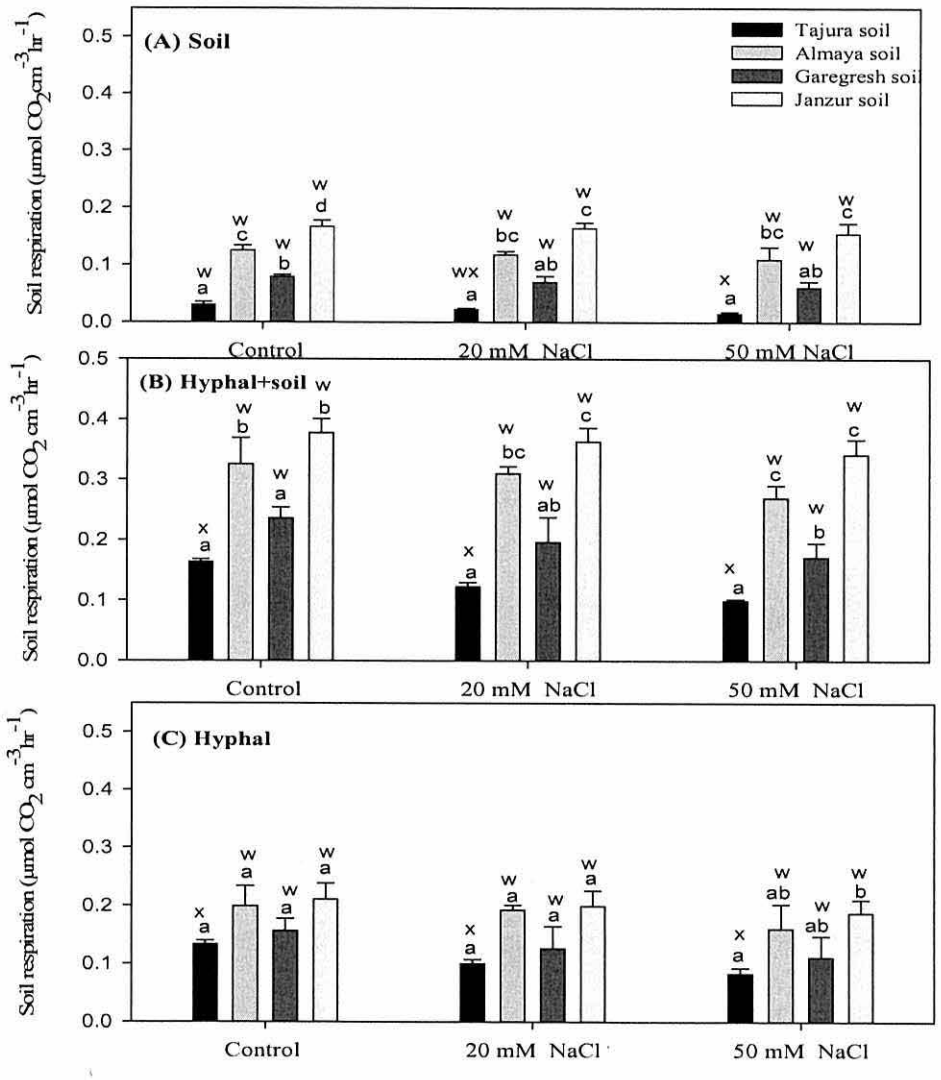

Figure 5.2 Effects of addition NaCl at 20 and 50 mM on CO_2 efflux from the soil only compartment (**A**) and the hyphal compartment (**B**). Panel C shows the calculated hyphal respiration ((hyphal+soil)-soil). Bars show means (±S.E.) (n = 3). Within a treatment, bars not marked with the same indices (a-d) are significantly different for each soil. Between treatments bar not marked with the same indices (w-x) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

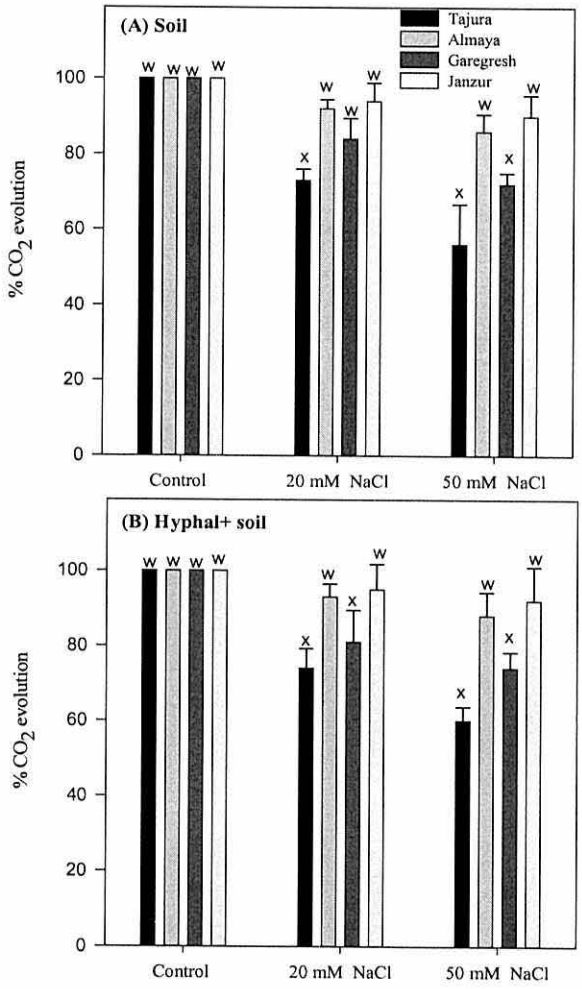

Figure 5.3 Shows % CO_2 evolution under effects of addition of 12.5 ml of a concentration of NaCl 20 and 50 mM or distilled water as the control, for 24 hr on soil respiration of four soils (**A**) and hyphal+soil (**B**). Bars show means (±S.E.) (n = 3). Between treatment bars not marked with the same indices (w-x) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

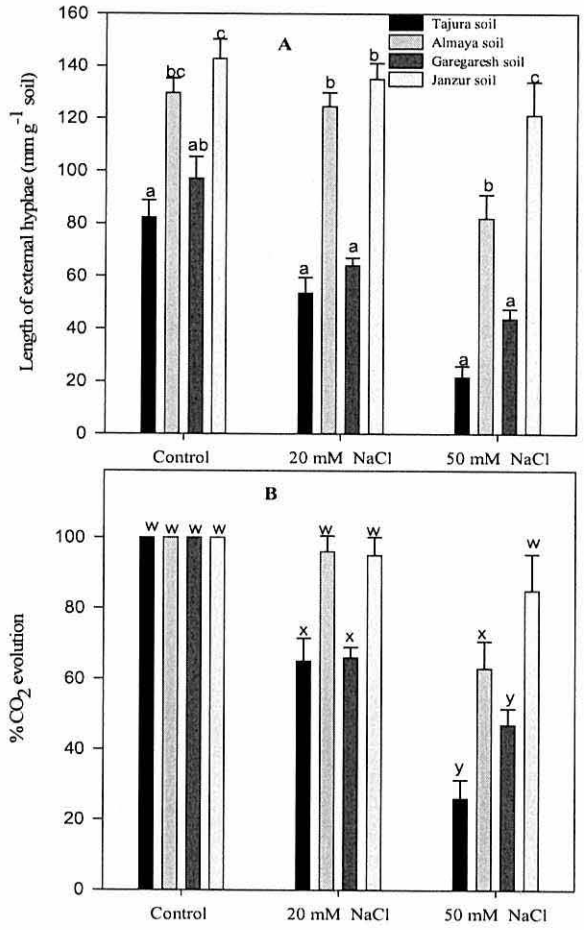

Figure 5.4 (**A**) Effects of addition of 12.5 ml of concentrations of NaCl at 20 and 50 mM or distilled water as the control on length of external hyphae. Bars show means (±S.E.) (n = 3). (**B**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-c) are significantly different for each soil. Between treatment bars not marked with the same indices (w-y) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

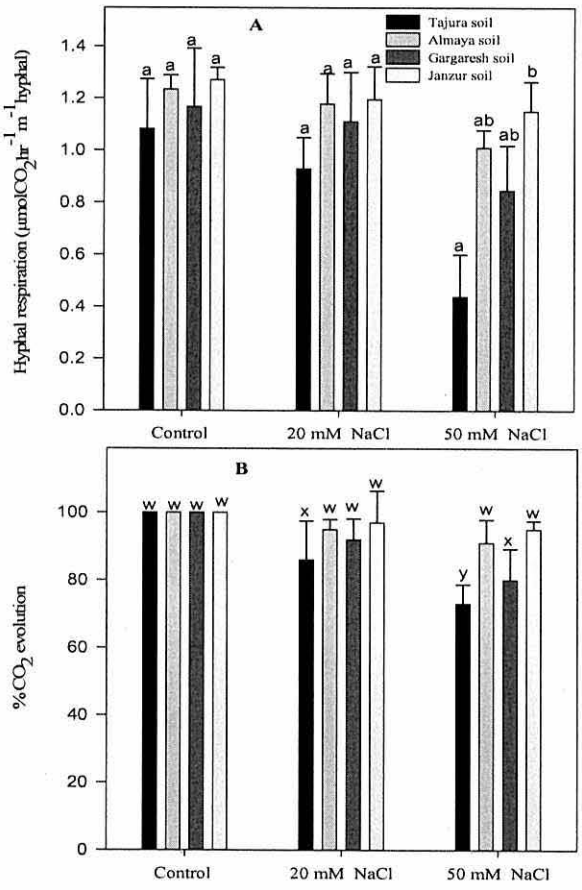

Figure 5.5 (**A**) Effect of NaCl on soil CO_2 efflux expressed in terms of hyphal length. Bars show means (±S.E.) (n = 3). (**B**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-b) are significantly different for each soil. Between treatment bars not marked with the same indices (w-y) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

5.7 Discussion

In the tubes only containing soil, clear differences between soils were shown in the rates of CO_2 efflux (Figure 5.2A). These tubes measure only the heterotrophic respiration in the soil and the difference in CO_2 evolution rate between four soils may be due to differences in the proportions of organic material between the four soils. As shown in table 2.3 the soil organic matter content increased in the order Tajura < Gargaresh < Almaya < Janzur. A large part of the CO_2 evolution from these soils is the result of decomposition of organic matter (Toland and Zak, 1994). A comparison of the rates of CO_2 efflux from the soil only compartments (Figure 5.2A) and that calculated to be from mycorrhizal hyphae (Figure 5.2C), shows that there are large difference between the contributions from heterotrophic and apparent hyphal respiration. Much research has indicated that rates of respiration tend to be much higher when external hyphae are present (Bouma et al., 2001 and Fahey and Yavilt, 2005). Addition of NaCl reduced the rates of soil CO_2 efflux in both the soil only and the hyphal compartments. As shown in the investigations in other chapters (chapters 4 and 6), Tajura was shown to be the soil most sensitive to NaCl addition, and was shown for Tajura in both the soil and the hyphal compartments. The reduced rate of CO_2 evolution from the soil with external hyphae after addition of NaCl at 50 mM, may be related to reduced lengths or density of external hyphae. Hyphal length also showed the greatest decrease in the Tajura soil, and hyphal biomass is known to be negatively affected by increasing salinity (Mexal and Reid, 1973; Griffin and, Luard, 1979; Wilson and Griffin, 1979; Dixon et al., 1993; Hogberg and Hogberg, 2002; Juniper and Abbott, 2006) (Figure 5.4). There was also a positive relationship between decreased length of external hyphae and decreased rate of CO_2 evolution such that there were no significant differences between the soils or when NaCl was added at 20 mM.

Only after addition of 50 mM NaCl was a depression of soil CO_2 efflux greater than that accountable from the decrease in hyphal length shown. This was again shown only in the Tajura soil, the soil with the highest background salinity. Soil electrical conductivity in Tajura was 3.34 $mScm^{-1}$, this appeared to be the main reason for the decrease of external hyphal growth when increasing concentrations of NaCl were added. There are many studies that show the effects of salinity on hyphal growth (Hirrel, 1981; Estaun, 1989; Juniper and Abbott, 1993; Omar et al., 1994). Inhibition of growth may be due to effects of ion toxicity or osmotic stress (Juniper and Abbott, 1993). Other soil factors such as P may to increase hyphal growth, with subsequent dilution of toxic ion effects (Juniper and Abbott, 1993).

Potentially increases root exudation leading to stimulation of microbial respiration in the rhizospher (Luo and Zhou, 2006), thus increased soil respiration (Cheng, 1999).

Chapter 6

Impacts of salinity on microbial carbon turnover on the Gefara soils

6.1 Introduction

Soil organic matter is known to affect soil biological activity (Tian et al., 1993). Organic matter and soil microbial activity are concentrated in the top few centimetres of soil (Murphy et al., 1998) and soil ecosystem function can be restored if amendments are made with OM (Crecchio et al., 2004). Organic matter can be considered a critical component of soil because of its role in physical processes such as (1) binding soil particles together in stable aggregates and (2) influencing water holding and aeration; and its role in chemical processes such as acting as a source of: (1) cation exchange capacity and (2) pH buffering. Furthermore, it is involved in soil biological processes as: (1) a food source for microbes and (2) a major reservoir of plant nutrients. The greater part of a soil's carbon is usually found in OM (Imamul Huq, 2005). Decomposition of OM is a biological process (Brussaard, 1994) in which different products are released: CO_2, energy, water and plant nutrients. Bacteria break down the OM, and any excess nutrients such as N, P and S are released into the soil in forms that plants can use. Higher microbial populations are often found in soil due to an accumulation of OM, thus improving decomposition, and resulted in high CO_2 evolution (Iovieno et al., 2009). Organic matter is added to soil by incorporating plant material, animal residues, manure, sewage sludge or municipal waste (Crecchio et al., 2004). Eriksen (2005) and Randhawa et al. (2005) reported that amending soil with OM stimulates soil microbial growth and activity. However, conventional agricultural practices and climatic conditions in Mediterranean agricultural soils have led to a decrease in OM content and hence to a decrease in soil quality (Jones et al., 2004).

Organic matter amendment of Mediterranean soils has led to increases in microbial activity (Perucci, 1992; Pascual, et al., 1999; Garcia-Gil et al., 2000; Ros et al., 2003; Crecchio et al., 2004; Bastida et al., 2008). Soils with organic matter contents are higher in clay and silt than sandy soils (FAO, 2005). However, activity decreases due to the presence of some contaminants (Garcia-Gil et al., 2000; Marcote et al., 2001; Ros et al., 2003; Crecchio et al., 2004). There are many studies that indicate decreases in the decomposition of OM in saline soil (Laura, 1973, Laura, 1974; El-Shakweer et al., 1976; Malik and Haider, 1976). Primavesi (1984) showed that in highly alkaline or strongly acid soils, the growing conditions for microbial life are poor, resulting in low levels of biological oxidation of organic matter. On other hand, OM overcomes any inhibitory effects of high metal concentrations (Lai Wong, 2007). Using isotopically labeled plant material provides a sensitive method to determine decomposition (Meharg, 1994; Cheng, 1996; Lin et al., 1999; Trumbore, 2000). There are many studies that have estimated rates of decomposition of ^{14}C-labelled plant materials under laboratory and field conditions (e.g., Jenkinson, 1977; Broadbent and Nakashima, 1974; Voroney et al., 1989; Aita et al., 1997), and also under a different soil types and climatic conditions and a variety of plant tissues (Nyhan, 1975; Jenkinson and Ayanaba, 1977; Sauerbeck and Gonzales, 1977). The methodology consisted of incorporating previously ^{14}C-labelled plant material into the soil and determining the amount of plant material-derived C remaining or evolution in/from the soil after a given period. This technique provides accurate estimates of decomposition rates (Cheng, 1996).

Respiration rates provide quantitative information on the short-term dynamics of the decomposition processes.

The rate of substrate degradation via microbial mineralization is measured by trapping any evolved $^{14}CO_2$ in a strong alkali trap (1 M NaOH) causing the formation of stable $NaH^{14}CO_3$, which can be assayed by liquid scintillation counting (Jones et al., 1996).

Merckx et al. (1985) reported the concentrations of organic-^{14}C in soil consisting of residual ^{14}C-labelled plant material and derived microbial cells and products decreases rapidly initially, but rates of decomposition tend to slow down considerably within a few months. The rate of mineralization is measured as the maximum slope of $^{14}CO_2$ release; it is usually determined that the maximum extent of mineralization is the maximum cumulative $^{14}CO_2$ release after the period of maximum rate of mineralization. Anderson, (1978a) showed that it is possible to use ^{14}C-labelled plant material to obtain better information on the effects of pesticides on soil microbial activities. Thomsen et al. (2001) reported that soil moisture was better than soil texture for explaining differences in the turnover of ^{14}C-plant material. However, the effects of salinity on organic matter decomposition in soil are poorly understood (Xiao-gang et al., 2006). A contradiction has been reported in the effects of salinisation on the decomposition of added plant material, which in some cases increases (Nelson et al., 1996; Conde et al., 2004) and decreases in others (Pathak and Rao, 1998). These contradictory findings may be due to differences in quality of the added organic amendments or related to the soil properties, especially the levels of salinity and soil pH (Muhammad et al., 2006). Addition of labeled materials measures the turnover of both easily decomposable and less available organic substances. The turnover of easily decomposable substrates can also be measured using ^{14}C-labeled glucose (Anderson and Domsch, 1978). Vuelvas-Solorzano et al. (2009) concluded that increases in soil salinity can reduce also mineralization of the easily decomposable C- substrate.

The objective of this study was to determine both the effects of salinity on decomposition of soil organic matter, and the effects of soil organic matter addition on the influence of salinity on soil microbial activity.

6.2 Materials and methods

6.2.1 Determining the turnover of ^{14}C-labelled Pant material in soil

Soil samples were collected from four sites located in the Gefara Plain as described in Chapter 3 section 3.2.1. To determine the effects of NaCl on decomposition of plant material 5 g of field-moist soil was placed in centrifuge tubes (n=84 in total), then treated with 12.5 ml of NaCl at 20, 50, 70, 90, 150 and 200 mM, or distilled water as the control (n=3). Soil samples were left at room temperature for 7 days after saline treatment, a period considered sufficiently long for the soil solution to reach equilibrium. 100mg of ^{14}C-labelled *Lolium perenne* (specific activity, 12.3 kBq g^{-1}) was added to the soil. The ^{14}C-enrichment of *Lolium perenne* plant material was performed by pluse labeling with $^{14}CO_2$ at a constant specific activity according to Hill et al. (2008).

A $^{14}CO_2$ trap consisting of 1 ml of 1 M NaOH in a polypropylene vial was placed above the soil. $^{14}CO_2$ evolution over time was measured by replacing the NaOH trap after 1, 2, 4, 7, 14, 21 and 28 days. $^{14}CO_2$ was trapped in the 1M NaOH which was mixed with 4 ml scintillation fluid (Perkin Elmer, Waltham, MA), and counted using a Wallac 1404 liquid scintillation counter (Perkin Elmer, Waltham, MA).

6.2.2 Determining the ^{14}C-labelled microbial biomass in soil

5g of field-moist soil was placed in centrifuge tubes (n=84 in total). The soil samples were treated with water containing different concentrations of NaCl (20, 50, 70, 90, 150 and 200

mM) or distilled water as the control. Soil samples were maintained at 50% WHC by adding 12.5 ml, and the samples were carried in three replicates.

Soil samples were left at room temperature for 7 days after saline treatment, a period considered sufficiently long for the soil solution to reach equilibrium, and then amended with 0.5 ml of a solution containing 10 mM ^{14}C-labelled glucose (specific activity, 37 kBq ml^{-1}). $^{14}CO_2$ efflux over time was measured by replacing the NaOH trap after 2 hours, 5 hours, 1, 2, 4, 7, 14, 21 and 28 days. $^{14}CO_2$ was trapped in 1 ml of 1 M NaOH which was mixed with 4 ml scintillation fluid (Perkin Elmer, Waltham, MA), and counted using a Wallac 1404 liquid scintillation counter (Perkin Elmer, Waltham, MA).

6.2.3 Determining the effects of addition of coconut husk compost on soil microbial activity under saline conditions

Briefly, 50g soil samples from Tajura and Janzur soil were amended with 0% (control), 1% and 2% coconut husk compost (n=108 in total), then were adjusted to 50% WHC by with 12.5 ml of either distilled water or a solution of NaCl at concentrations of 20 and 50 mM soils were incubated for one month. The soil was placed in centrifuge tubes, and all the treatments were carried out in triplicate. The soil solutions were separated by centrifugation at 4000 rpm for 30 min at 20°C to obtain the soil solution. The collected solutions were passed through a Whatman 42 filter paper and kept at 4°C until analysis.

To characterize the coconut husk material, 5g ground coconut husk compost (n=54 in total) was placed in centrifuge tubes and extracted in water (1:5 husk:water) by shaking for 30 minutes, after which the solution were passed through a Whatman 42 filter paper, and kept at 4°C until analysis (again, all the samples were carried out in triplicate. Soil respiration rate was measured with a PP systems SRI soil respirometer (PP-Systems, Hitchin, UK) at ±20°C

for 24 hours before and after sterilized. Coconut husk compost was sterilized by autoclave at 121°C for 30 minutes, to inactivate all bacteria.

Cations such as Ca^{2+}, Na^{+} and K^{+} were measured by flame photometer (Sherwood 410). NO_3^- concentrations was determined in a microtiter plate format by addition N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDD), sulfanilamide (SULF), vanadium (III) chloride (VCl_3) this method called spectrophotometric method procedure described by Miranda et al.(2001) and total carbon and nitrogen were determined with a CHN-2000 analyzer (Leco Corp., St Joseph, MI) of coconut husk compost.

6.3 Statistical and data analysis

The experiments were conducted with three replicates in the laboratory, and means and standard errors were calculated in Microsoft Excel. The data on (1) the soil respiration rate as amended with ^{14}C-labelled plant material and ^{14}C-labelled glucose; (2) soil respiration rate before and after amendation with sterilized coconut husk compost in Janzur and Tajura soils; (3) ECe; (4) pH; (5) NO_3^- and (6) concentration of cations in soil extracts from four soils were subjected to ANOVA analysis of variance using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) statistical analysis program to test for significant differences between soils from all four sites. Means were compared for experimental variables by Tukey test, where significant differences were accepted at *($P<0.05$)* or non-significant probability values *($P>0.05$).*Graphs were created with Sigma Plot 8.0 for Windows using means and standard errors.

6.4 Results

The effects of NaCl on the decomposition of ^{14}C-labelled *Lolium perenne* in the soil samples depended on the length of the incubation period. The data obtained showed the percentage $^{14}CO_2$ evolution was increased significantly *($p<0.001$)* with increasing incubation periods in

all four soils. In soil samples from Janzur (with distilled water amendation as the control) the cumulative of $^{14}CO_2$ evolved was significantly lower *($p<0.01$)* after 2 days than after 28 days (Figure 6.1).

The percentage decreased significantly (60%; *$p<0.01$*) after 28 days when soil samples were amended with concentrations of NaCl at 200 mM compared to the control (Figure 6.1). When ^{14}C-labelled *Lolium perenne* was added to soil samples from Tajura, the mineralization of ^{14}C-labelled *Lolium perenne* was decreased with increasing salinity levels (Figure 6.1). 78% of ^{14}C-labelled *Lolium perenne* was mineralized after treatment with 20 mM solution of NaCl over 28 days compared to the control *($p<0.05$)* (Figure 6.1). Also, when samples were amended with different concentrations of NaCl (50, 70, 90, 150 and 200 mM), mineralization decreased to 70%, 58%, 53%, 52% and 48% respectively; (*$p<0.001$*) after 28 days incubation compared with the control (Figure 6.1). In soil samples from Almaya the mineralization of ^{14}C-labelled *Lolium perenne* was significantly decreased only when amended with 90, 150 and 200 mM, which reached to 71%, 70%; *$p<0.05$*; and 63%; *$p<0.01$* respectively after 28 days incubation compared with the control (Figure 6.1). The effects on mineralization rate under treatment by different concentrations of NaCl were tested on soil samples from Gargaresh. When amended with 20 mM mineralization it was not significantly decreased *($p<0.05$)* with any amount of incubation (Figure 6.1). While at treatment by 50, 70 mM decomposition decreased to 72% and 70% *($p<0.05$)* respectively within 28 days compared to the control. The production of $^{14}CO_2$ tended to decrease with increasing concentrations of salinity levels (90, 150 and 200 mM, to 63%; *($p<0.01$)*; 57% and 50%; *($p<0.001$)* respectively after 28 days compared to the control). The production of $^{14}CO_2$ was lower in samples amended with high concentrations of NaCl after 28 days' incubation.

The production of ^{14}C-labelled *Lolium perenne* was decreased in samples from Tajura, Garegarsh, Janzur and Almaya amended with 200 mM NaCl after 28 days' incubation (by 48%, 51%; $p<0.001$; 60% and 61%; $p<0.01$ respectively) compared to the control (amended with distilled water) (Figures 6.1).

The production of $^{14}CO_2$ in Tajura was greatly decreased compared to Janzur and Almaya. The production of $^{14}CO_2$ was similar in all treatments between Janzur and Almaya, and also between Tajura and Gargaresh within the first days of the incubation.

The cumulative production of $^{14}CO_2$ showed a lag of 2 hours in four soil samples after amendation with^{14}C–labelled glucose. $^{14}CO_2$ increased with increasing incubation periods after treatment with distilled water as the control (Figures 6.2). In soil samples amended with water containing different concentrations of NaCl (20, 50, 70, 90, 150 and 200 mM), the cumulative production of $^{14}CO_2$ showed a decrease with increasing salinity levels at any time of incubation. For example, after 28 days, the rate of production of $^{14}CO_2$ from Janzur reached to 97%, 94%, 90%, 88% and 82% respectively compared to the control (Figure 6.2). While in samples amended with 200 mM, NaCl was significantly decreased (73%, $p<0.05$) compared to the control. In soil samples from Tajura, rates of mineralized tended decrease when soil samples were amended with increasing salinity levels (Figure 6.2). The production of $^{14}CO_2$ following treatment by 20 mM NaCl was not significantly decreased $(p>0.05)$, but when the samples were amended with the 50 and 70 mM NaCl solution, the production of $^{14}CO_2$ was significantly decreased $(p<0.05)$, reaching 69% and 64% of the control respectively after 28 days. Also, when NaCl solution at 90 and 150 mM was added the rate of $^{14}CO_2$ significantly decreased (58% and 43%, $p<0.01$ respectively) after 28 days compared to the control.

The amount of ^{14}C-labelled glucose mineralized was 36%, $p<0.001$ within 28 days after treatment with a 200 mM concentration (Figure 6.2).

The observed effected of salinity levels at 20, 50, 70, and 90 mM on the amount of ^{14}C-labelled glucose mineralized in soil samples of Almaya was not significantly decreased $(p>0.05)$ during the whole period of incubation.

In soil samples with 150 and 200 mM of solution NaCl, the cumulative production of $^{14}CO_2$ was significantly decreased after 28 days (67% and 63%, $p<0.05$ respectively) (Figure 6.2). The $^{14}CO_2$ production rate was significantly lower $(p<0.05)$ following treatment with 70 mM (65%) and 90 mM (61%) after 28 days compared to the control. This affect increased with increasing salinity, at 150 and 200 mM, the cumulative production of $^{14}CO_2$ significantly decreased $(p<0.001)$ to 59% and 50% respectively after 28 days' incubation compared to the control. When amended with 200 mM NaCl after 28 days incubation, the production of $^{14}CO_2$ was decreased by 37%, 50%; $p<.001$; 63% and 73%; $p<0.05$, in samples from Tajura, Gargaresh, Almaya and Janzur respectively (Figures 6.2).The production of $^{14}CO_2$ was decreased more in Tajura compared to Janzur, Almaya and Gargaresh.

The characteristics of the coconut husk compost are shown in Table 6.1 The pH of the coconut husk compost is much lower than that of the Libyan soils (Chapter 3 section 3.12), and cations a moderate amount of NO_3^-. However, the OM-incubation of the soils did not affect soil pH or EC noticeably compared to the control. Soil EC was significantly increased $(P<0.05)$ compared to the control in Tajura after the addition of 2% coconut husk, amended with 20 and 50 mM of NaCl. At Janzur it was significantly increased $(P<0.05)$ compared to the control after the addition of 2% coconut husk, amended with50 mM of NaCl (Figure 6.3).

Soil pH was also significantly increased *(P<0.01)* compared to the control after the addition of 2% coconut husk, amended with 20 and 50 mM of NaCl (Table 6.2).

Table 6.3 shows that nitrate (NO_3^-) levels in the two soils was significantly increased *(P<0.05)* when 2% coconut husk compost was added, amended either with distilled water or with 20 and 50 mM of NaCl.

But it was significantly increased *(P<0.05)* in the Tajura soil after addition of 50 mM of NaCl. Figure 6.4 shows that Na^+ did not significantly change with the coconut husk compost treatments. Only in samples from Tajura was it significantly increased *(P<0.01)* with 2% coconut husk added compared to the control. K^+ was significantly increased *(P<0.001)* in samples amended with 1% and 2% coconut husk compared to the control in two soils. Coconut husk led also to increased *(P<0.01)* Ca^{2+} of only 2% in samples from Tajura and Gargaresh compared to the control.

Where 1% non-sterilized coconut husk, compost was added to soil samples from Janzur and Tajura. The rate of CO_2 efflux decreased to 88% and 74% *(P>0.05)* after treatment with 20mM NaCl, after addition of 50 mM NaCl, it decreased to 87% *(P>0.05)* and 63% *(P<0.05)* (Figure 6.5 B and D). When non-sterilized 2% coconut husk compost was added to soil amended with 20 and 50 mM of NaCl, soil respiration decreased to 93% and 86% *(P>0.05),* and decreased to 90% *(P>0.05)* and 67 % *(P<0.05)* in Janzur and Tajura respectively compared to the control. The rate of CO_2 evolution following the addition of 1% sterilized coconut husk to soil samples from Janzur was decreased to 82% *(P>0.05)* and 47% *(P<0.05),* while in samples from Tajura it was decreased to 60% and 40% *(P<0.05)* when amended with 20 and 50 mM of NaCl respectively compared to the control (Figure 6.6 B and D). The soil respiration rate in soil samples from Janzur decreased to 89% and 53 % *(P>0.05)* when 2%

sterilized coconut husk compost was added was, while in Tajura it decreased to 62% *($P<0.05$)* and 45% *($P<0.01$)* when amended with 20 and 50 mM of NaCl respectively compared to the control (Figure 6.6 B and D). Comparison (Figure 6.5 A and C and 6.6 A and C) of the non-sterilized and sterilized coconut husk compost treatments shows that without addition of NaCl, the rate of CO_2 efflux in the non-sterilized are slightly higher, but the difference was not statistically significant. However, after addition of NaCl a greater inhibition of CO_2 efflux was shown in the sterilized compared to the non-sterilized treatment at 50 mM of NaCl.

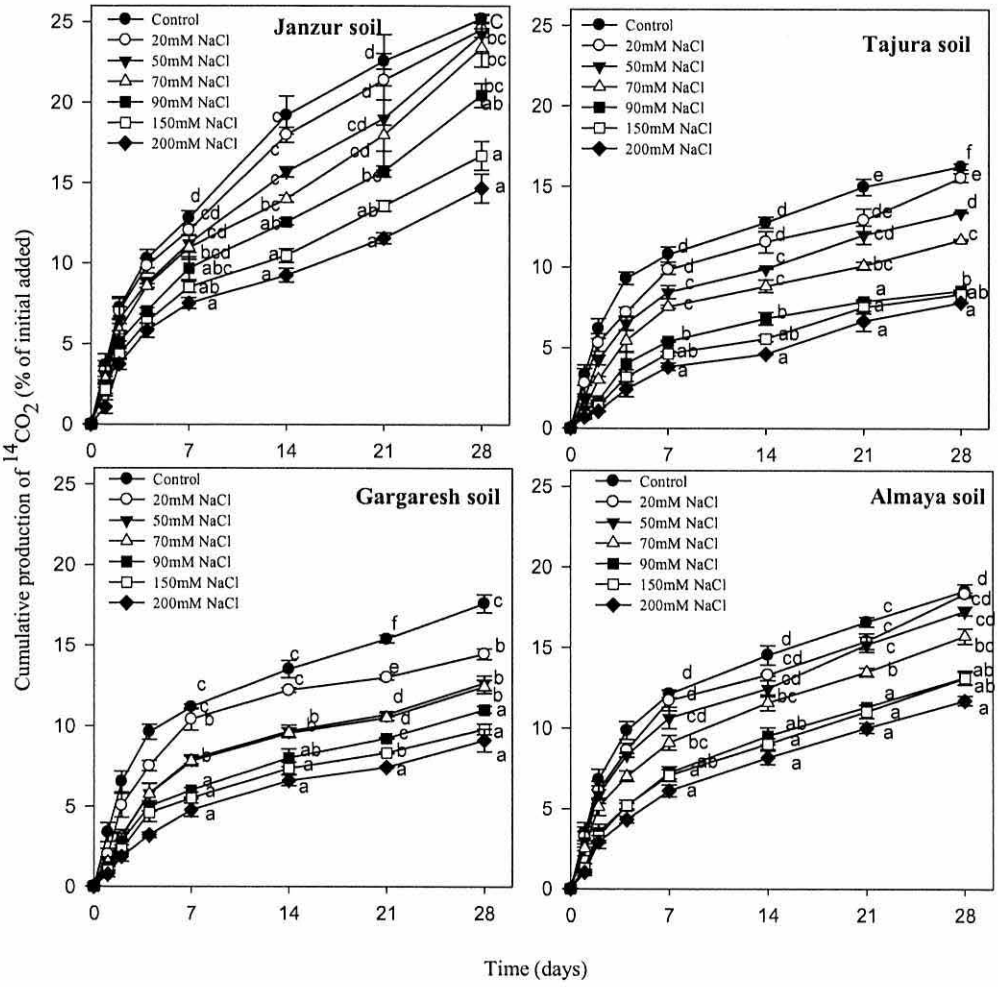

Figure 6.1 The production of $^{14}CO_2$ from soils after amended with 100 mg ^{14}C-labelled *Lolium perenne* under effects of addition of 12.5 ml of concentrations of NaCl 20, 50, 70, 90, 150 and 200 mM or distilled water as the control. Bars show means (±S.E.) (n = 3). Between treatments at a single time point, data points not marked with the same indices (a-f) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

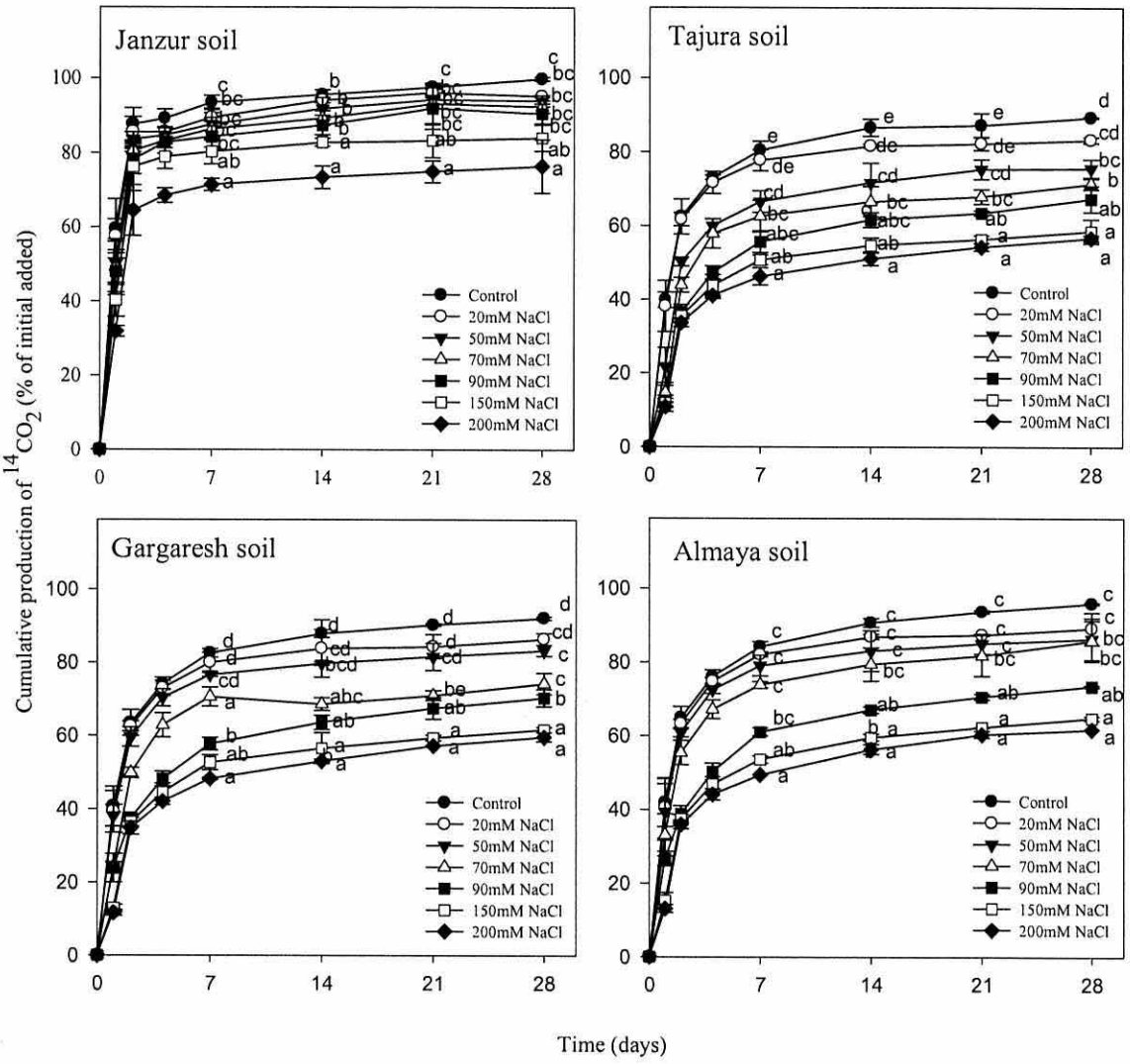

Figure 6.2 The production of $^{14}CO_2$ from soils, after amended with 0.5 ml ^{4}C-labelled glucose under effects of addition of 12.5 ml of concentrations of NaCl 20, 50, 70, 90, 150 and 200 mM or distilled water as the control. Bars show means (±S.E.) (n = 3). Between treatments at a single time point, data points not marked with the same indices (a-f) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

Table 6.1 Characteristics of organic matter, coconut husk compost. Mean of triplicates with standard error in parentheses.

pH	4.3 (±0.07)
EC ($mScm^{-1}$)	2.1 (±0.22)
Moisture content %	676 (±6.93)
C %	41.6 (±0.11)
N %	0.48 (±0.02)
NO_3^- (mM)	0.84 (±0.93)
Na^+ (mM)	3.1 (±0.13)
K^+ (mM)	9.3 (±0.37)
Ca^{2+} (mM)	6.1 (±0.28)

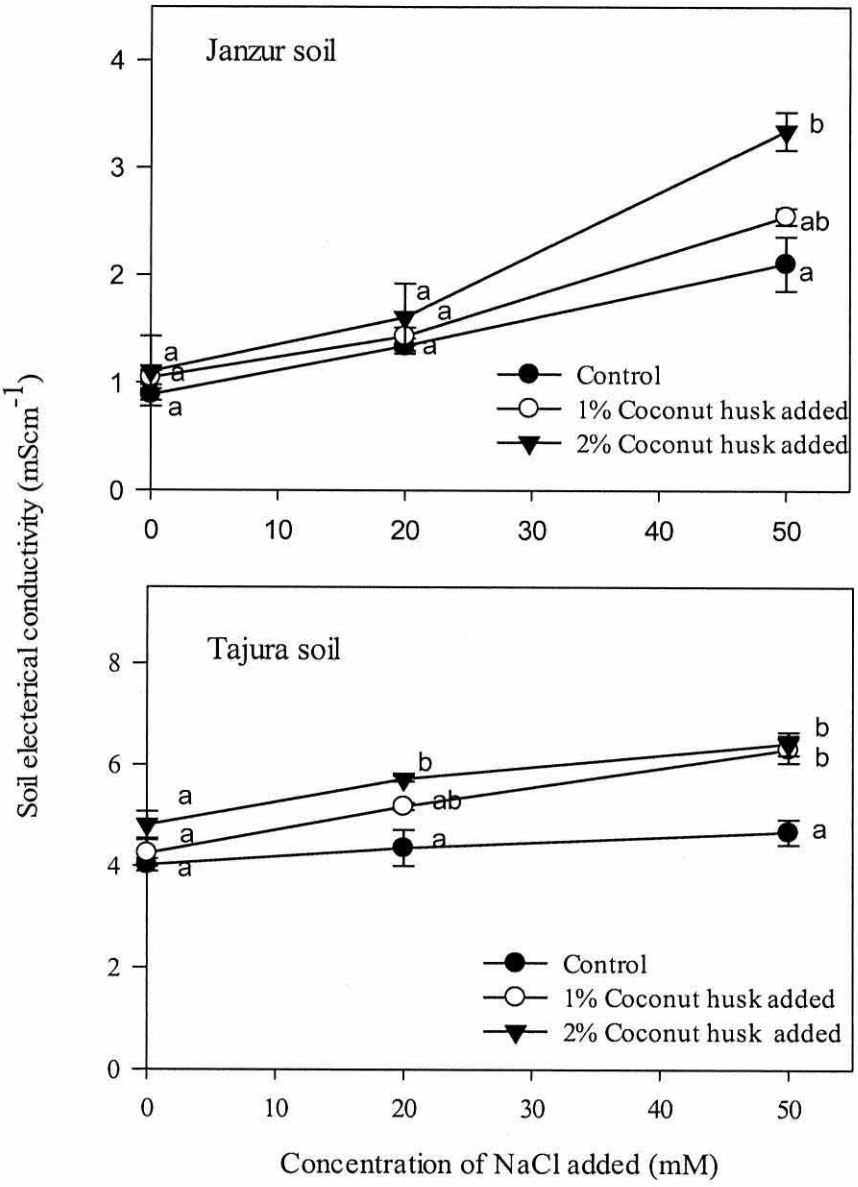

Figure 6.3 Soil electrical conductivity (EC in $mScm^{-1}$) of Janzur and Tajura soils after addition of 12.5 ml of a concentration of NaCl 20 and 50 mM or distilled water as the control, amended with 1% and 2% of coconut husk compost. Bars show means (±S.E.) (n = 3). Within a treatment and between the different amendments with coconut husk compost points not marked with the same indices (a-b) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

Table 6.2 Soil pH of Janzur and Tajura soils after addition of 12.5 ml of a concentration of NaCl 20 and 50 mM or distilled water as the control, amended by 1% and 2% of coconut husk compost. Mean of triplicates with standard error in parentheses. Different letters indicate statistically significantly differences between salinity treatments. Tukey HSD comparison were performed at the $P<0.05$ level.

Soil	Control	20 mM NaCl	50 mM NaCl
Janzur			
No addition	7.46 (±0.04)a	7.38 (±0.05)a	7.36 (±0.03)a
1% Coconut husk	7.61 (±0.05)a	7.47 (±0.03)a	7.53 (±0.04)a
2% Coconut husk	7.67 (±0.06)a	7.58 (±0.04)a	7.74 (±0.06)a
Tajura			
No addition	7.40 (±0.03)a	7.27 (±0.03)a	7.24 (±0.01)a
1% Coconut husk	7.44 (±0.02)a	7.39 (±0.05)a	7.34 (±0.01)a
2% Coconut husk	7.53 (±0.04)a	7.50 (±0.04)a	7.48 (±0.05)a

Table 6.3 NO_3^- (in mg l^{-1}) of Janzur and Tajura soils after addition of 12.5 ml of a concentration of NaCl 20 and 50 mM or distilled water as the control, amended by 1% and 2% of coconut husk compost. Mean of triplicates with standard error in parentheses. Different letters indicate statistically significantly differences between salinity treatments. Tukey HSD comparison were performed at the $P<0.05$ level.

Soil	Control	20 mM NaCl	50 mM NaCl
Janzur			
No addition	40.1 (±0.01)a	33.0 (±0.04)ab	29.4 (±0.04)b
1% Coconut husk	42.1 (±0.04)a	35.6 (±0.05)ab	32.3 (±0.01)b
2% Coconut husk	44.2 (±0.02)a	38.1 (±0.03)a	38.2 (±0.03)a
Tajura			
No addition	31.2 (±0.01)a	28.2 (±0.03)ab	23.4 (±0.01)b
1% Coconut husk	32.4 (±0.02)a	30.0 (±0.01)ab	27.8 (±0.01)b
2% Coconut husk	35.2 (±0.03)a	33.0 (±0.02)a	30.1 (±0.03)a

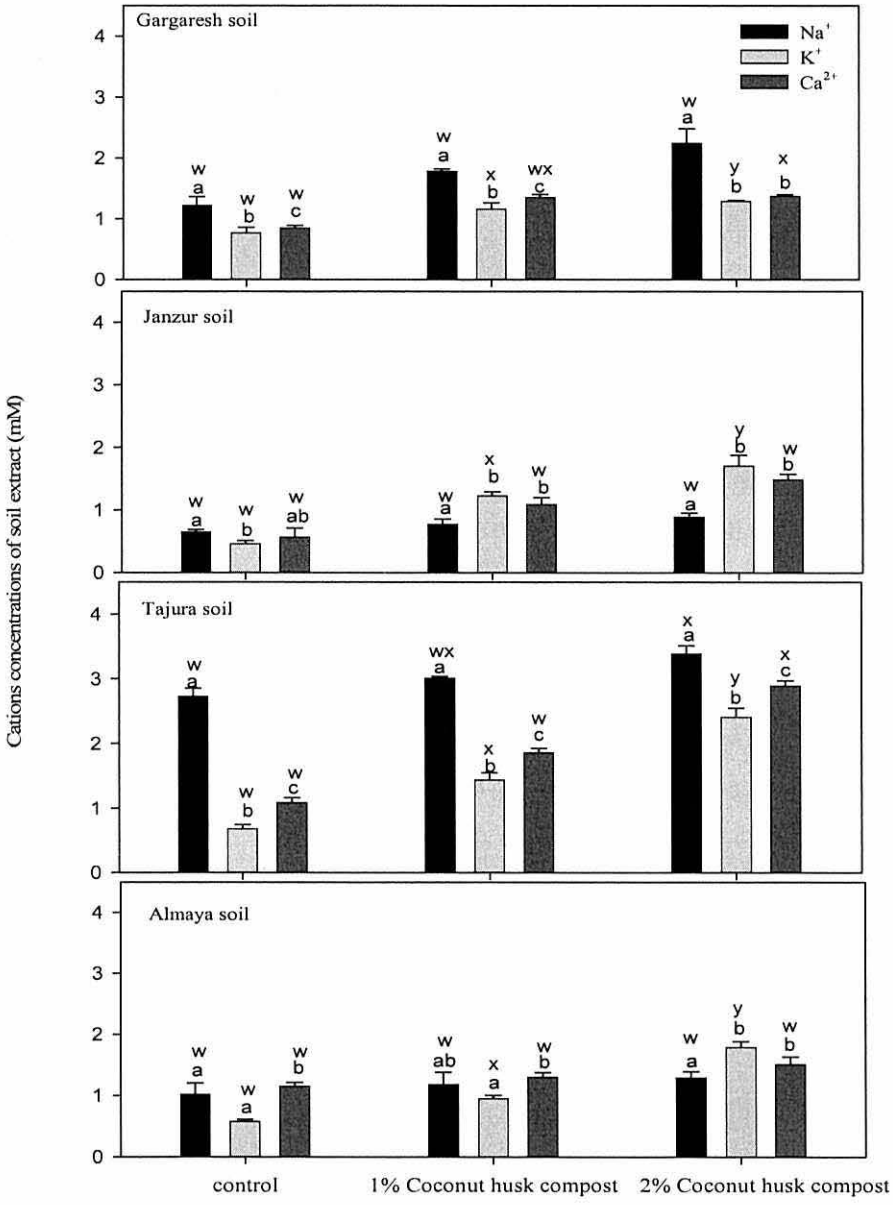

Figure 6.4 Concentration of Na^+, K^+ and Ca^{2+} in four soil samples as the control or under effects of the addition of 1% and 2% coconut husk compost. Bars show means (±S.E.) (n = 3). Within a treatment bars not marked with the same indices (a-b) are significantly different for each soil. Between treatments bars not marked with the same indices (w-y) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level

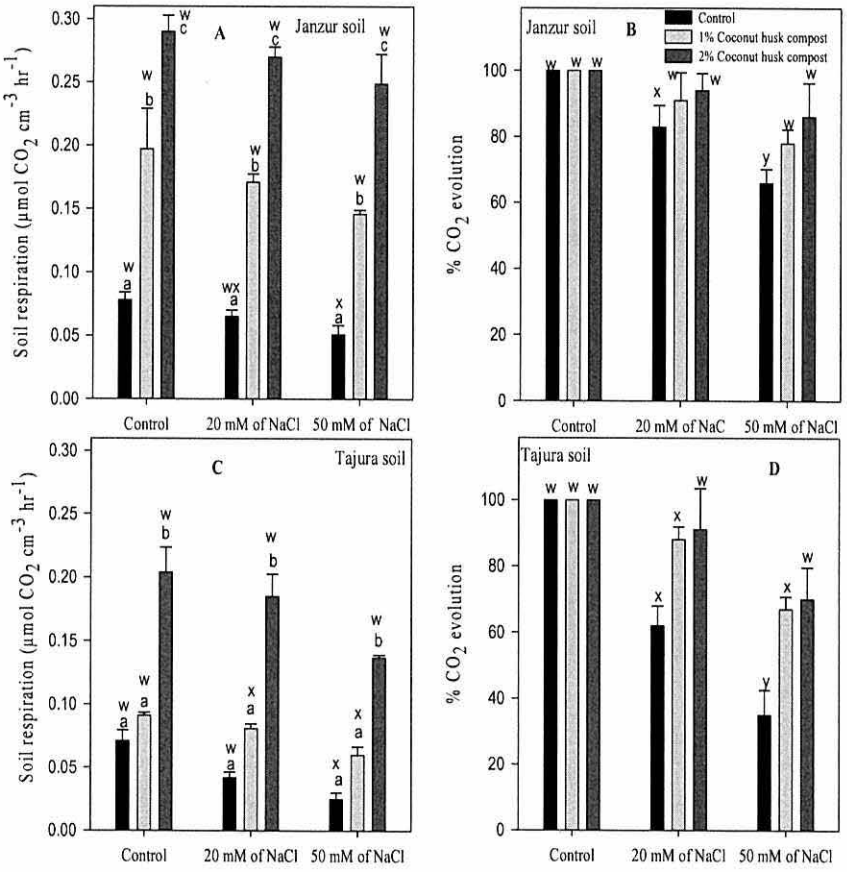

Figure 6.5 (**A** and **C**) The effects of addition of 1% and 2% of non-sterlized coconut husk, and 12.5 ml of concentrations of NaCl 20 and 50 mM or distilled water as the control on soil respiration of Janzur and Tajura soils measured over 24 hr. Bars show means (±S.E.) (n = 3). (**B** and **D**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-c) are significantly different for each soil. Between treatments bars not marked with the same indices (w-y) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level

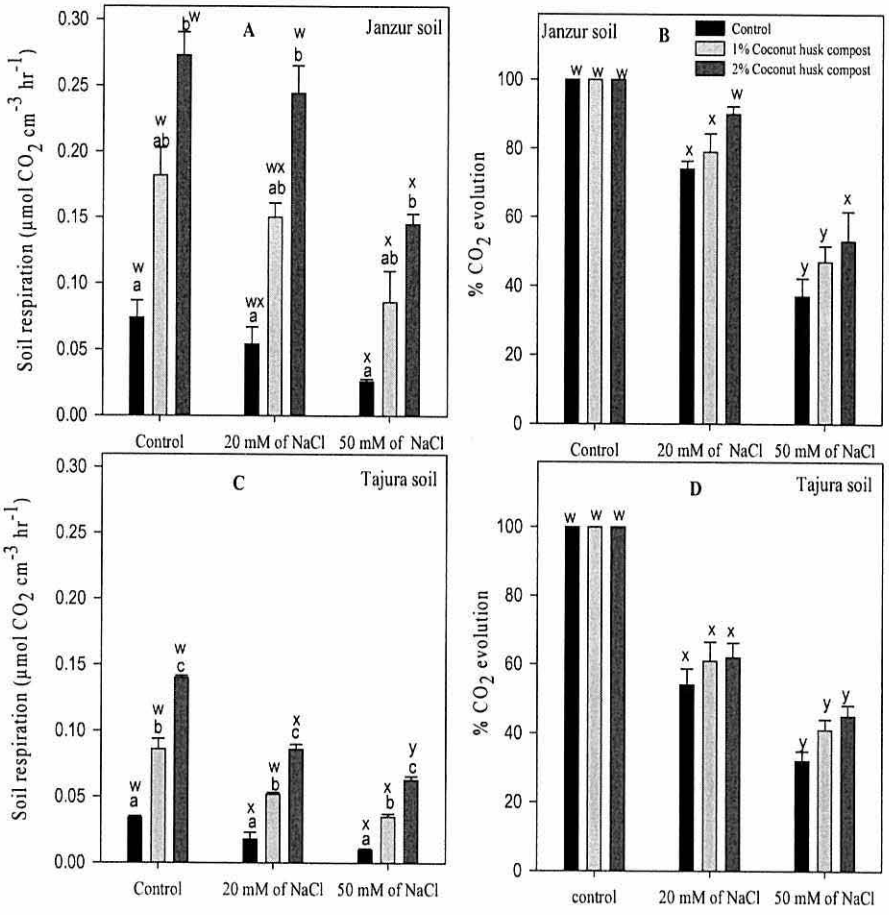

Figure 6.6 (**A** and **C**) The effects of addition 1% and 2% of sterilized coconut husk compost and addition of 12.5 ml of a concentrations of NaCl 20 and 50 mM or distilled water as the control on soil respiration of Janzur and Tajura soils for 24 hr. Bars show means (±S.E.) (n = 3). (**B** and **D**) Shows % CO_2 evolution. Within a treatment bars not marked with the same indices (a-c) are significantly different for each soil. Between treatments bars not marked with the same indices (w-y) are significantly different. Tukey HSD comparison were performed at the $P<0.05$ level.

6.5 Discussion

The soil microbial community respiration is usually limited by the availability of C substrates (Raich and Tufekcioglu, 2000). As shown in Chapter 4, addition of glucose greatly increases the rates of soil respiration. Both the addition of glucose and the addition of plant material can act as substrates for the microbial biomass. This may lead to enhanced microbial biomass in soils after amendment (Anderson, 1984). The amount of $^{14}CO_2$ evolved from glucose additions greatly exceeded those from the plant material, this may be due to an easily degradable substrate (e.g. glucose) was added to a soil (Anderson and Domsch, 1978).

The amount of ^{14}C evolved from the ^{14}C-labelled *Lolium perenne* and ^{14}C-labelled glucose was greatly decreased in soil samples treated with salinity. However, the amount of ^{14}C-labelled *Lolium perenne* and ^{14}C-labelled glucose was less mineralized in soil samples from Tajura compared to soil samples from Janzur, Almaya and Gargaresh respectively at any time of incubation in the control, and also at any concentrations of NaCl added (Figures 6.1 and 6.2). It appears that increasing soil EC due to NaCl addition affected the decomposition of ^{14}C-labelled *Lolium perenne* and ^{14}C-labelled glucose (Rietz and Haynes, 2003; Conde at el., 2005). However, Pathak and Rao (1998) indicated that additional organic material in the short term provides additional substrates for the microbial population and also relieves osmotic and pH stress on the microorganisms. But the clay content and pH values are similar in the Gefara soils (Tables 2.1 and 3.3). Many studies revealed the adverse effects of salinity on the microbial biomass (Sarig et al., 1996; Batra and Manna, 1997; Rietz and Haynes, 2003). In contrast, the effect of salinisation on C and N mineralization or added plant material is uncertain, i.e. they both increase (Nelson et al., 1996) and decrease (Pathak and Rao, 1998). Application of salinity levels led to decreased decomposition of ^{14}C-labelled plant material

and ^{14}C-labelled glucose in all four soils. This is in agreement with Pathak and Rao (1998) and Vuelvas-Solorzano et al. (2009), and may depend on the increasing soil EC (Luna-Guido et al., 2001; Ramirez-Fuentes et al., 2002).Nelson et al. (1996) pointed to a decomposition rate that was significantly reduced at moderate salinity. In the last half of the incubation period, the production of $^{14}CO_2$ showed a sharp increase with all treatments in all four soils. The percentage of mineralization was lowest with increasing concentration of salinity levels in soils; this indicates that salts decreased microbial activity (Vuelvas-Solorzano, 2009). In addition, Pathak and Rao (1998) found that microorganisms in the soils do not tolerate high salt content, and this may be have strongly inhibited their activity.

Decreased rates of $^{14}CO_2$ evolved after the addition of ^{14}C-labelled glucose to soil samples at different concentrations of NaCl. This confirmed by Ramirez-Fuentes et al. (2002) and Vuelvas-Solorzano (2009), and may be due to microorganism experiencing stress caused by the saline and alkaline conditions, leading to low substrate use efficiencies (Muhammad, 2005). In general, the reduced CO_2 efflux with an increase in salinity in our incubation experiment revealed a decrease in C-mineralisation (Pankhurst et al., 2001; Pathak and Rao, 1998). Considering the above, it appears that effect of salinity could be attributed to the direct influence of osmotic potential on microbial activity (Johnston and Guenzi, 1963, Singh et al., 1969). However, the differences in percentages between ^{14}C-lablled glucose and ^{14}C-labelled *Lolium perenne* mineralized may be due to the effect of large salt concentrations on metabolic processes (Saggar et al., 1999). The coconut husk compost incubation of the soils of about one month did not affect the soil pH noticeably in soils from Tajura and Janzur (Table 6.2). On other hand, increasing the amount of coconut husk compost in soils led to increased soil electrical conductivity (Figure 6.3).

Organic matter also leads to increased nitrate accumulations in soil (Zhou et al., 2010). Nitrate production in soil is a significant part of the nitrogen cycle. This process is called nitrification. Nitrification was reduced in all soils by chloride salts (Heilman, 1975), and the nitrification process was most sensitive to NaCl salt (Viro, 1962; Johnson and Guenzi, 1963). Treatments with concentrations of NaCl at 20 mM had little effect on NO_3^- levels in soil, while NO_3^- decreased with increasing concentrations of NaCl, especially in soil samples from Tajura (Table 6.3). Organic matter is an important source of nutrients for plants, derived from the mineralization of SOM (Wolf and Snyder, 2003). Increases in cation exchange capacity (CEC) in soils after the application of compost or pig slurry have been found previously (Lax, 1991; Bernal et al., 1992a; Walker and Bernal, 2004). The addition of organic matter may have reduce the effects of salinity on nitrification possibly by adsorbing Cl^-, However, the addition of OM leads to increased microbial activity (Crecchio et al., 2004; Bastida et al., 2008). The rate of CO_2 evolution from soil samples amended with non-sterilized coconut husk compost was increased compared to soil samples amended with sterilized coconut husk compost and was maintained after the addition of 20 and 50 mM of NaCl (Figure 6.5 and 6.6). This may be due to sterilization removing the microbial community (Salonius et al., 1967, Jeakison and Powlson, 1976). It may also accelerate the decomposition of organic matter (Jenkimson, 1966). Figure 6.5 and 6.6 showed increased CO_2 efflux rate from soil samples relative to increasing organic matter (Hassink, 1994), compared to the control.

Rates of CO_2 were increased in soil samples with 1% and 2% coconut husk compost added and amended with 20 and 50 mM of NaCl, compared to the control.

This has been ascribed to organic matter overcoming any inhibitory effects. Lai Wong (2007) found that organic matter overcomes any inhibitory effects of high metal concentrations.

However, salinity effects were evident as different responses at different levels of NaCl. It was found that the application of organic matter to all four soils led to alleviation of the effects of salinity on CO_2 efflux, and nitrification, and may be a means to ameliorate salt-affected soils.

Chapter 7: Article

Bacterial salt-tolerance is unrelated to soil salinity across an arid agroecosystem salinity gradient

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This paper has been accepted for publication in Soil Biology & Biochemistry.

Abstract

In arid and semi-arid ecosystems, salinization is a major threat to the productivity of agricultural land. While the influence of other physical and chemical environmental factors on decomposer microorganisms have been intensively studied in soil, the influence of salinity has been less exhaustively assessed. We investigated the influence of soil salinity on soil bacterial communities in soils covering a range of salt levels. We assessed tolerance of the bacterial communities from Libyan agricultural soils forming a salinity gradient to salt (NaCl), by extracting bacterial communities and instantaneously monitoring the concentration-response to added NaCl with the Leucine incorporation technique for bacterial growth. To maximise our ability to detect differences in bacterial salt tolerance between the soils, we also repeated the assessment of bacterial growth tolerance after one month's incubation with 1 or 2 % added organic matter additions to stimulate microbial growth levels. We could establish clear concentration-response relationships between bacterial growth and soil salinity, demonstrating an accurate assessment of bacterial tolerance. The *in situ* soil salinity in the studied soils ranged between 0.64 to 2.73 mM Na (electrical conductivities of 0.74 to 4.12 mS cm^{-1}; cation exchange capacities of 20 to 37 mmolc kg^{-1}) and the bacterial tolerance indicated by the concentration inhibiting 50% of the bacterial growth (EC_{50}) varied between 30 and 100 mM Na or between electrical conductivities of 3.0 and 10.7 mS cm^{-1}. There was no relationship between *in situ* soil salinity and the salt tolerance of the soil bacterial communities. Our results suggest that soil salinity was not a decisive factor for bacterial growth, and thus for structuring the decomposer community, in the studied soils.

Keywords: Soil salinity, decomposition, arid soils, salt, tolerance, ecotoxicology, selective pressure, Leucine incorporation, bacterial growth, microbial community composition, biomass.

7.1 Introduction

In arid and semi-arid ecosystems, salinization is a major threat to the productivity of agricultural land (Sumner, 1995). Irrigation with saline water combined with high evapotranspiration continually intensifies the salinization problem in arid soils. While the influence of other physical and chemical environmental factors on decomposer microorganisms have been intensively studied in soil, including e.g. tilling (Van Groenigen et al., 2010), soil pH (Baath and Anderson, 2003; Fierer and Jackson, 2006; Rousk et al., 2009b; 2010a, b, c; 2011) and heavy metal pollution (Brookes and McGrath, 1984; Rajapaksha et al., 2004; Baath et al., 2005), the influence of salinity has been less exhaustively assessed. Over recent decades, studies on the impact of salinity on the concentration of soil microbial biomass (Muhammad et al., 2006; 2008; Egamberdieva et al., 2010) and composition (Muhammad at al., 2006; Wichern et al., 2006) have started to emerge, together with studies of the influence of soil salinity of the decomposition on plant materials (Li et al., 2006; Muhammad et al., 2006) and microbial activity (Rietz and Haynes, 2003; Ghollarata and Raiesi, 2007). Most of these assessments of the soil microbial community and its functioning have used different soils with a range of in situ salt levels. Often, salinity is correlated to other chemical aspects of the soils, typically soil sodicity, which confounds the analysis of the direct salt impact. An alternative approach is to use a detection phase, where soil samples are exposed to a range of salt additions, to investigate the sensitivity of the in situ microbial community, or processes mediated by it, to salinity (e.g. Wichern et al., 2006). This direct investigation of the tolerance of the microbial community to salt has the advantage of being more specific to salt, and less confounded by other correlated factors, although addition of salt to soil can also result in secondary effect on e.g. chemical and physical factors.

To date, the influence of salt on soil microorganisms has mostly been assessed using biomass measurements (e.g. Muhammad et al., 2008). However, microbial biomass is relatively slow to react to disturbances, especially detrimental ones, as it takes time for an inhibitory effect on the microbial community to result in a biomass decrease, due to the lag between inactivity, death, and turnover of the microorganisms. Alternatively, soil microorganisms have been assessed using its respiratory response to salt exposure. However, the relationship between the microbial respiration and the status of the microbial community is ambiguous; exposure to salt is often regarded as a stressor for the microbial community, and respiration per microbial biomass (qCO_2) has been proposed as an index for the level of stress the soil microbial community is exposed to (Anderson and Domsch, 1993; Ghollorata and Raiesi, 2007), although its use has also been contended (Wardle and Ghani, 1995). Subsequently, it is unclear what a detrimental salt effect on the soil microbial community should result in using respiration as a response variable, and both increases and decreases in respiration with higher salt levels have been reported (Singh et al., 1969; Laura, 1974; Nelson et al., 1996; Pathak and Rao, 1998; Muhammad et al., 2008; Wong et al., 2009). A sensitive response variable to assess the soil bacterial community's response to various factors has been the determination of growth using leucine (Leu) incorporation (Baath, 1994; Baath et al., 2001; Rousk and Baath, 2011). Using this method, the direct influence of factors and pollutants on the soil bacterial have been assessed, including heavy metal toxicity (Baath et al., 2005), antibiotics (Alden Demoling and Baath, 2008a; Alden Demoling et al., 2009; Brandt et al., 2009; Milenkovski et al., 2010), and phenols (Alden Demoling and Baath, 2008b).

The influence of toxic substances on the microbial community is not random. Species and strains that can tolerate toxins better will be favoured, and their ecological role will thus

increase as they grow more abundantly represented within the community. This results in a directional change of the makeup of the soil biota (e.g. population of a single strain or integral measurement of a whole community) toward one harbouring a composition with a higher tolerance to the presence of the toxin. Consequently, the active and growing organisms found in a certain habitat must be able to tolerate the prevalent conditions there. This means that assessing and comparing the tolerance of a community to a certain toxicant will be informative of this toxicant's "historical" influence on the microbial community in this system until the time of study. Furthermore, the tolerance measurement will only respond to ecologically relevant impacts, i.e. to factors that significantly influence the ability of the organisms' ability to actively participate in various processes such as decomposition. These ecological principles are also the basis for the Pollution Induced Community Tolerance (PICT) concept (Blanck, 2002), and while this mostly has been employed to screen for environmental toxins (e.g. Alden Demoling and Baath 2008a, b), it is relevant for any factor that can affect the survival, activity and growth of the biotic community, including e.g. salt. Importantly, assessing the tolerance of e.g. soil bacterial communities in soils differently exposed to a toxicant can determine if it has been an important selective agent on the studied biological indicator (i.e. an ecologically relevant factor), but the mechanism that led to the tolerance (e.g. species sorting, or adaptation of already present species, etc.) is arbitrary, and additional assessments would be needed to determine this. In the present study we investigated the influence of soil salinity on the bacterial communities in soils covering a range of salt levels.

To do this, we used agricultural soils from the Gefara Plain in Libya representing a range of soil salinities. We assessed tolerance of the bacterial communities from these soils to salt (NaCl), by extracting bacterial communities and subsequently determining the concentration-

response relationship between bacterial growth and NaCl exposure. The Libyan soils used were low in organic matter content and to maximise our ability to detect differences in bacterial salt tolerance between the soils the concentration response relationships can be determined with higher accuracy the further we are from the detection limit–we also repeated the assessment of bacterial growth tolerance after one month incubation with 0, 1 or 2% added organic matter additions to stimulate microbial growth levels. We hypothesized that bacterial communities from higher salinity soils should be more salt tolerant.

7.2 Materials and methods

7.2.1 Soils and organic matter amendments

Soil samples were collected from farms located in the Gefara Plain in Libya (30° 00 NW, 35° 00 NE). Agriculture productivity in the summer season depends on irrigation water from subterranean water supplies. The soil in this region was previously described in detail (Ben-Mahmoud, 1995), and is characterized as a sandy (sand: 88.6-94.1%; silt: 3.3-6.8%; clay: 2.6-4.6%) Aridisol (USDA, 1992). In each area, one farm was sampled. The four sampled areas, in order of increasing soil salinity level, were Janzur (continuous cropping with wheat, minimum tilling, grazing animals between harvest and sowing; Henceforth "Soil A"), Almaya (continuous cropping with wheat, minimum tilling, grazing animals between harvest and sowing; Henceforth "Soil B"), Gargaresh (cropping with barley, left fallow part of the year, frequent tilling, and low intensive grazing between harvest and sowing; Henceforth "Soil C") and Tajura (cropping with barley, left fallow part of the year, frequent tilling, and no grazing; Henceforth "Soil D"). The soil samples were collected in July 2009, with a mean annual temperature of 26 to 29°C. Soil monoliths were taken to a depth of 18 cm. After removal of the surface litter, the soils were then sieved (< 2 mm), and any discernible roots and stones

were removed. Each soil sample was composed from four randomly sampled subsamples from each soil, and homogenized. Soils were subsequently stored at field moisture in polyethylene bags at 4°C until preparation for analysis. The soils were adjusted to 50% of water holding capacity (WHC; the gravimetric difference between water saturated-and-drained samples and oven-dried samples), and left at 20°C for 1 week before the bacterial growth tolerance assays or organic matter incubation treatments. These soils were used to determine in situ bacterial growth tolerance to salinity. A subset of soil from each of Soil A, B, and D (Soil C was excluded due to logistic reasons that resulted in a limited supply of this soil) were then incubated with 0%, 1% or 2% added organic material (ground coconut husk compost, (Table 7.2) for one month at 20°C, maintained at 50% WHC. Each soil organic matter incubation was performed in three independent replicates (3 soils with 3 treatments and 3 replicates,) totaling 27 soil samples, with equivalent to 100 g dw in each. The bacterial growth tolerance to salinity was then also investigated in these organic matter treated soil samples.

7.2.2 Physiochemical measurements

Soil pH and soil electrical conductivity (EC) were determined in 1:1 (w/v) soil:H_2O extracts and total C and total N were determined with a CHN-2000 analyzer (Leco Corp., St Joseph, MI). Soil solution was obtained using centrifugation-drainage (Giesler and Lundström, 1993) and filtered with Whatman 42 filters prior analysis. Exchangeable cations were determined using a 1:5 (w:v) extraction of soil samples with 1.0 M $BaCl_2$.Concentrations of K, Na and Ca were analysed using a Sherwood 410 flame photometer (Sherwood Scientific, Cambridge, UK), and Mg was determined using a Varian 220FS atomic absorption spectrometer (Varian, Palo Alto, CA, USA). The Cation Exchange Capacity (CEC) was determined as the total concentration of Na, K, Ca and Mg, each multiplied by its ionic charge.

NO_3^- was determined colourimetrically using the Cu-Zn hydrazine reduction method of Downes (1978). Loss on ignition (400°C overnight) was used to determine the organic matter content of the soils. Soil basal respiration was measured on 50 g fresh samples for 24 h at 22 °C using an automated multichannel infrared gas analyser (PP-Systems Ltd, Hitchin, UK). Substrate induced respiration (Anderson and Domsch, 1978) was measured using the same system as the average respiration rate (0.5-6 h after glucose addition, at 22°C) following a 6 mg glucose g^{-1} soil addition, and the conversion factor to microbial biomass used was 1 mg CO_2 corresponded to 20 mg biomass-C (recalculated from Anderson and Domsch, 1978).

7.2.3 Bacterial growth tolerance

Soil bacterial growth rates were estimated using the leucine incorporation technique (Baath, 1994; Baath et al., 2001). Briefly, 2 g of wet weight (ww) of soil was mixed with 20 ml of distilled water. The soil suspensions were vortexed for 3 min and then centrifuged for 10 min at 1000 *g* to obtain a bacterial suspension in the supernatant. From the soil bacterial suspension, 1.35 ml was used for the leucine incorporation measurements in 2 ml microcentrifuge tubes. Subsequently, 0.15 ml of increasing concentrations of salt (NaCl) was added to the 1.35 ml of the bacterial suspension in the microcentrifuge tubes.

Eight different concentrations of Na were used, 0, 10, 20, 70, 90, 150, 200 and 400 mM NaCl, to determine the in situ tolerance, and 0, 10, 20, 40, 80, 160, 320, and 640 mM in the tolerance determination after the organic matter incubation, as final concentrations in the bacterial suspensions in the microcentrifuge tubes.

Distilled water was added to the control with no added salt. Leucine (2 µL) was then added as 7.8 nM L-[4,5-^{3}H]leucine (171 Ci $mmol^{-1}$, 1.0 mCi ml^{-1}, Amersham) with nonradioactive L-leucine to reach a final concentration of 260 nM.

The samples were then incubated at approximately 20 °C for 2 h. The incorporation of leucine was terminated by the addition of 75μL of 100% trichloroacetic acid. Non-incorporated leucine was then removed by centrifugation, and ^{3}H-leucine incorporation was measured in a scintillation counter.

7.2.4 Statistics and calculations

Tolerance values were expressed as the logarithm of the concentration resulting in 50% inhibition (log (EC_{50})) of the bacterial growth in the soil suspensions. A more tolerant community is inhibited at higher concentrations and, therefore, has a higher value of log EC_{50} than a less tolerant community. The log (EC_{50}) values of the bacterial communities were calculated using a logistic model, $Y = c/[1 + eb(X-a)]$, where Y is the leucine incorporation rate, X is the logarithm of the salt concentration, a is the value of log (EC_{50}), c is the leucine incorporation rate in the control, and b is a parameter (the slope) indicating the inhibition rate. Kaleidagraph 4.0 for Mac (Synergy software) was used to fit a curve to the data using the equation. Relationships between variables of the different soils were investigated using regression analyses or using one way analysis of variance (ANOVA) with Tukeys HSD posthoc pair-wise comparisons using JMP 7.0 for Mac (SAS Institute, Cary, NC, USA).

7.3 Results

7.3.1 Soils

Soil salinity increased from 0.64 mM Na (an electrical conductivity of 0.74 mS cm^{-1}) in Soil A to 2.73 mM Na (an electrical conductivity of 4.12 mS cm^{-1}) in Soil D, with Soils B and C intermediary (Table 7.1), confirming the salinity gradient. While Na concentration was closely related to the shift in electrical conductivity (R^2=0.96, P<0.05), the soil solution concentration of K and Ca ions were not significantly different along the salinity gradient. The cation exchange capacity changed along the gradient (P<0.01, Table 7.1), increasing progressively from about 20 mmolc kg^{-1} in soil A to about 40 mmolc kg^{-1} in soil D. This increase was predominantly related to the exchangeable Na concentration, increasing three-fold (P<0.001), but also coincided with increases in exchangeable K (50% increase toward higher salinity, P<0.01) and Mg (two-fold increase with higher salinity, P<0.001), while the concentration of exchangeable Ca did not change along the salinity gradient (Table 7.1). Except for the salinity of the four different soils, they were similar in most respects except for the organic matter concentration (Table 7.1). There was a non-significant trend between higher salinity and lower organic matter concentrations. There were no large differences in soil pH, which varied between 7.4 and 7.7 (Table 7.1). Soil microbial biomass (P<0.05; R^2=0.94) and soil respiration (P<0.01; R^2=0.98) varied systematically with the concentration of soil organic matter, but was only significantly distinguishable between soils for biomass. The bacterial growth was higher in Soils A and D, at around 80 pmol Leu g^{-1} h^{-1}, and lower in Soils B and C, at around 40-50 pmol Leu g^{-1} h^{-1}, with no clear relationship with the measured factors (Table 7.1).

7.3.2 Organic matter addition incubations

The organic matter added to the soils at 0, 1 or 2% was coconut husk compost (Table 7.2). The treatment with organic matter did not noticeably affect soil pH or electrical conductivity (data not shown). The bacterial growth in the organic matter treated soil samples with 0% OM addition were reduced between 40-60% compared with the fresh soil, the growth in the 1% addition were similar to the initial values, while the 2% additions resulted in a 20-40% increase in bacterial growth following an incubation for one month.

7.3.3 Direct salt toxicity on soil bacteria

We were able to demonstrate clear concentration-response relationships between bacterial growth and salt for the four Libyan soils (Figure 7.1), with lower bacterial growth following exposure to higher concentrations of salt. The relationship between bacteria growth and salt exposure could be modeled well using a logistic model (R^2 = 0.964, 0.999, 0.996 and 0.996 for soils A, B, C and D, respectively). Higher concentrations of salt inhibited bacterial growth by more than 90% at the highest salt concentration used (400 mM NaCl) in all the four soils. The log (EC_{50}) values estimated from the logistic model ranged between 1.43 in soil D to 1.82 in soil B, corresponding to EC_{50} values of 27-66 mM NaCl (electrical conductivities of 3.0-5.8 $mScm^{-1}$). The clear concentration-response relationships between bacterial growth and increasing levels of salt exposure were reproduced in all levels of organic matter addition in the incubated soils (Figure 7.3). Higher salt concentrations reduced bacterial growth by more than 90% at the highest concentration (640 mM NaCl). Again, the logistic model consistently described the relationships well (R^2 values ranged between 0.953 and 0.999 with an average of 0.983).

Indices for the tolerance of the bacterial community to salt, log (EC_{50}) values, were estimated from the logistic equation and ranged between 1.65 (in 0 and 1% organic matter additions to soil B) and 2.04 (1% organic matter addition to Soil D), corresponding to EC_{50} values of 45 – 110 mM NaCl, or electrical conductivities of 3.9 – 10.7 mS cm^{-1}. The log (EC_{50}) values were used as indices for the bacterial salt tolerance, and were related to the *in situ* salt level (Figure 7.3). There was no clear relationship between the log (EC_{50}) and the level of salt in the fresh soils, although there was a non-significant trend for lower log (EC_{50}) in soils with higher levels of salt. There was a positive relationship between soil salt level and bacterial growth tolerance to salt in the organic matter treated soils (Figure 7.3), that was significant when the EC was used to reflect soil salt level (P=0.038; R^2=0.48), and bordering on being significant when the Na concentration was used to indicate soil salt level (P=0.052; R^2=0.44). However, when all soils were included in the analysis, there was no discernible relationship between bacterial salt tolerance (log (EC_{50})) and soil salinity (Na) (R^2=0.04; P=0.51; Figure 7.3).

Table 7.1: Soil characteristics

	Soil A (Janzur)	Soil B (Almay)	Soil C (Gargaresh)	Soil D (Tajura)
Water holding capacity (WHC,%[1])[NS]	25.1 (0.34)	24.1 (0.47)	24.3 (0.11)	24.5 (0.26)
Organic matter (% of dry wight)***	1.25 (0.05) a	0.87 (0.08) b	0.62 (0.19) c	0.49 (0.03) b
NO_3^- (mgl^{-1})	43.4 (1.67) a	38.0 (1.42) ab	28.5 (2.27) c	31.2 (1.11) bc
Basal respiration ($\mu g\ CO_2\ g^{-1}\ h^{-1}$)	1.4 (0.24)	1.2 (0.49)	1.0 (0.17)	1.0 (0.17)
Microbial biomass C ($\mu g\ C\ g^{-1}$)[3]**	141 (8.2) a	120 (8.8) a	74 (16.1) b	64 (2.3) b
Bacterial growth (pmol Leu $g^{-1}\ h^{-1}$)[2]***	82.6 (5.44) a	43.6 (1.49) b	56.8 (1.68) c	80.7 (5.16) a
Soil solution				
Na^+ (mM)***	0.65 (0.03) a	1.02 (0.33) a	1.22 (0.12) a	2.73 (0.21) b
K^+ (mM)[NS]	0.46 (0.33)	0.58 (0.13)	0.77 (0.43)	0.68 (0.13)
Ca^{2+} (mM)[NS]	0.57 (0.14)	1.15 (0.23)	0.85 (0.30)	1.09 (0.16)
EC ($mS\ cm^{-1}$)***	0.74 (0.03) a	1.12 (0.03) a	2.14 (0.11) b	4.12 (0.17) c
pH (H_2O) [NS]	7.7 (0.03)	7.6 (0.11)	7.5 (0.05)	7.4 (0.09)
1 M $BaCl_2$ extract				
Na^+ ($mmol_c\ kg^{-1}$) ***	3.0 (0.07) a	4.8 (0.37) b	5.4 (0.21) b	10.3 (0.67) c
K^+ ($mmol_c\ kg^{-1}$)**	2.1 (0.04) a	2.4 (0.23) ab	3.4 (0.17) c	3.0 (0.12) bc
Ca^{2+} ($mmol_c\ kg^{-1}$)[NS]	7.1 (1.80)	10.1 (3.16)	7.7 (0.69)	9.7 (2.11)
Mg^{2+} ($mmol_c\ kg^{-1}$)***	7.4 (0.09) a	11.6 (0.04) b	13.7 (0.54) c	14.1 (0.66) c
Cation exchange capacity (CEC, $mmol_c\ kg^{-1}$)**	19.6 (0.92) a	28.9 (3.34) ab	30.1 (0.53) b	37.0 (2.45) b

Values are mean with 1 SE within brackets (n=3). Nutrient and ion concentrations in soil solution are determined on soil solutions using centrifugation, exchangeable cations are determined as the concentrations multiplied by the charge of each cation in a 1:5 (W:V) 1.0 $BaCl_2$ extract, and the cation exchange capacity (CEC) is the sum of these (see materials and methods). [1] Water holding capacity (WHC, %):% of fresh weight of soil. [2] Electrical conductivity. [3] Substrate-induced biomass (SIR-biomass). Different lower case letters indicate significantly different treatments P<0.05; Tukeys pairwise comparisons), stars designate significance level of ANOVA, NS not significant, * P<0.05; ** P< 0.01; *** P<0.0001.

Table 7.2: Characteristics of the organic matter amendment, Coconut husk compost, added to soils at 0, 1 or 2%.

pH	4.3 (0.07)
Electrical Conductivity ($mScm^{-1}$)	2.1 (0.22)
Organic C ($mg\ g^{-1}$)	416 (1.0)
Total N ($mg\ g^{-1}$)	5.0 (0.2)
NO_3^- ($mg\ g^{-1}$)	52.1 (0.93)

Values are mean with 1 SE within brackets (n=3)

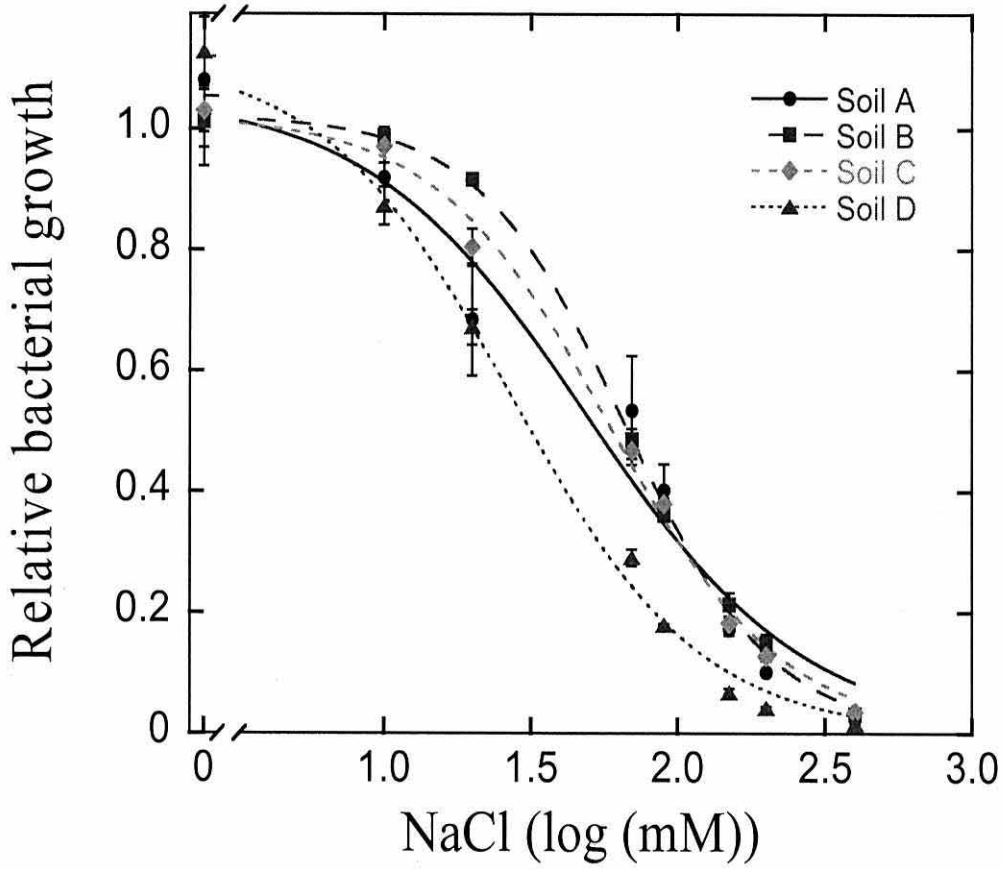

Figure 7.1 Concentration-response relationships of salt (NaCl) exposure on the relative bacterial growth in the four studied soils, A (Janzur), B (Almaya), C (Gargaresh) and D (Tajura), with increasing levels of soil salinity, and salt level. A logistic model was used to fit the curves to the data points.

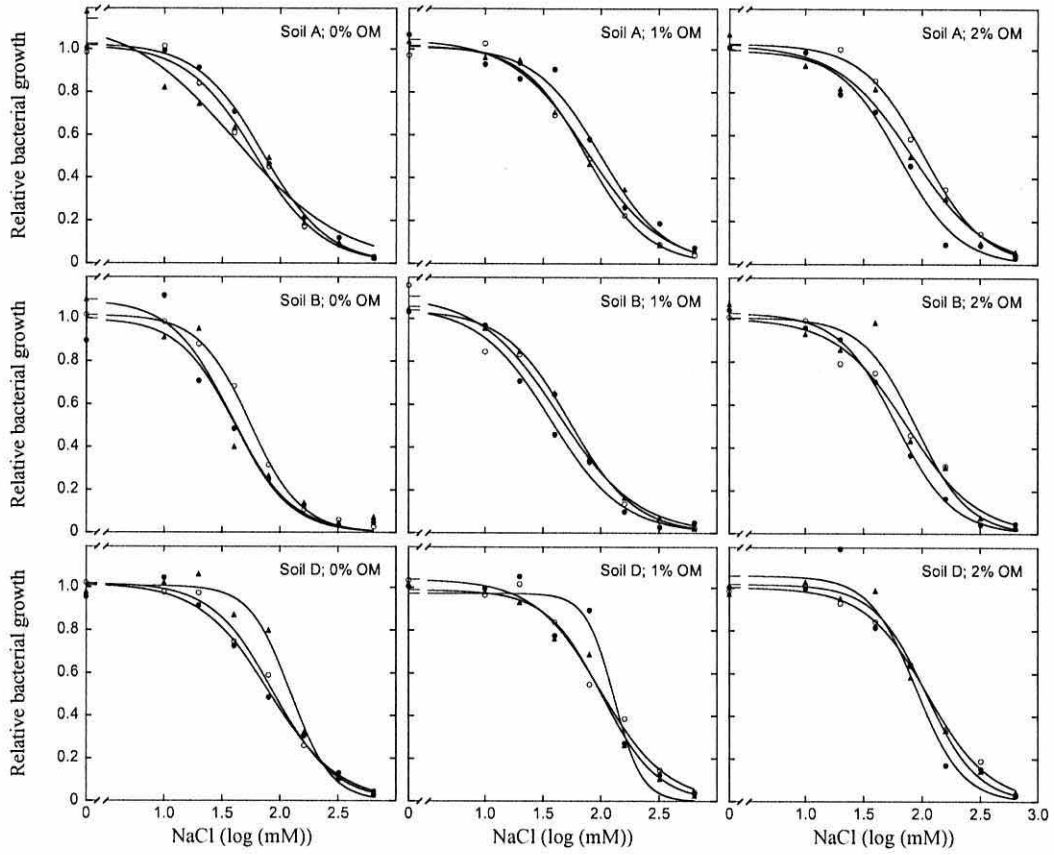

Figure 7.2 Concentration-response relationships of salt (NaCl) exposure on the relative bacterial growth in soils A (Janzur), B (Almaya) and D (Tajura) following a one-month incubation with 0, 1 or 2% organic matter (see Table 7.2). The three lines represent single independent replicate incubations.

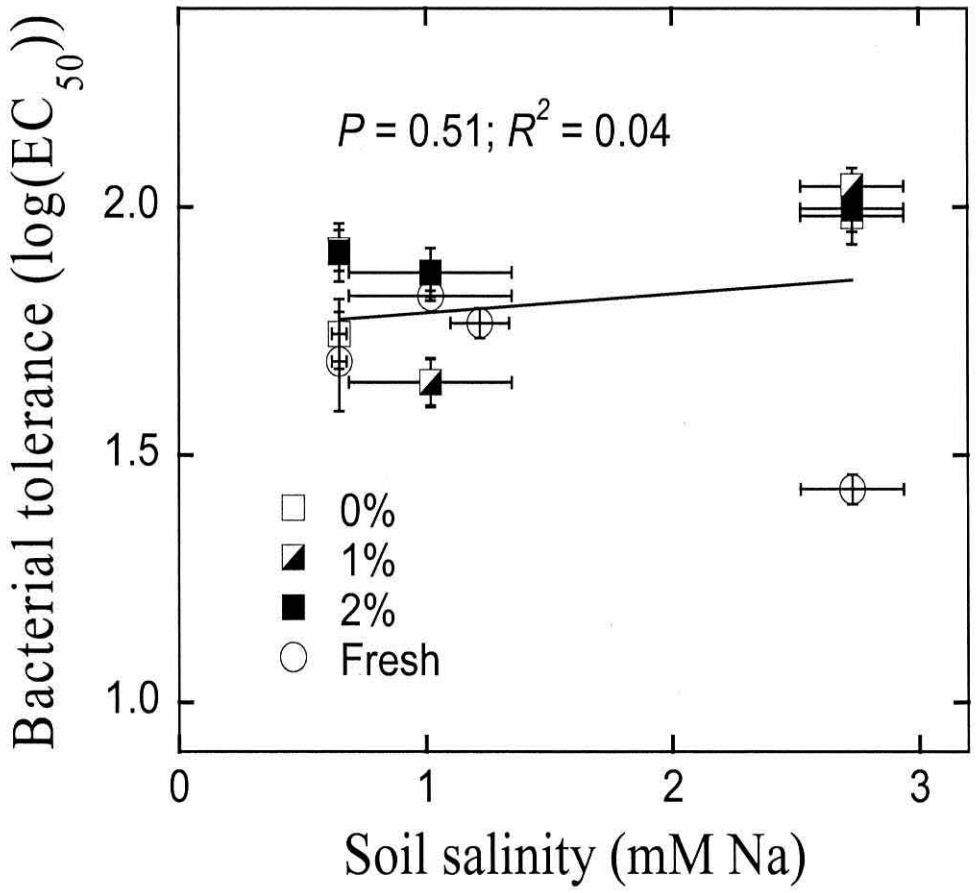

Figure 7.3 Tolerance (log (EC_{50})) of the bacterial communities regressed against the soil salinity (Na) in the four fresh soils (circles) and the tolerance (log (EC_{50})) of the bacterial communities in the three soils A (Janzur), B (Almaya) and D (Tajura) incubated for one month with 0 (open squares), 1 % (half-closed squares) or 2% organic matter (closed squares). A linear regression is used to test the relationship between bacterial salt tolerance (log (EC_{50})) against soil solution salinity (Na).

7.4 Discussion

7.4.1 Using leucine incorporation to determine concentration-response relationships to salt for bacterial growth

The use of bacterial growth to determine concentration-response relationships and thus screen for bacterial tolerance to salinity proved to be highly sensitive. The concentration-response relationships for all the different soils were very clear, and the effective inhibition of the bacterial growth, by more than 90% for all soils at the highest salt exposures, generated very clear inhibition curves that could be used to estimate bacterial tolerance (log (EC_{50})) with high precision. Wichern et al. (2006) investigated the response of respiration (with and without organic matter additions) to the application of salt. Immediately following salt application, the respiration was reduced by up to 20- 50 % in the absence of fresh substrate, and more reliably by up to 50-70% in the presence of plant material amendments. Over the course of a 48-day incubation, the concentration response relationship of respiration to salt application grew clearer (Wichern et al., 2006). Similar results of limited inhibition, and clearer effects with increasing time of exposure have also been reported for e.g. heavy metal toxicity (Akerblom et al., 2007). It should be noted that a problem with long detection phases in estimating the tolerance of microbial communities, including culture-based methods such as BIOLOG (Alden, Demoling et al., 2009), the use of biomass responses (Sardinha et al., 2005; Wichern et al., 2006), relying on the lack of biomass accumulation in toxin treated samples to detect effects, and respiration measurements (Wichern et al., 2006; Akerblom et al., 2007) is that it can provide ample time for community adaptation (i.e. selective growth of tolerant microorganisms) to the toxicant studied. After several days of incubation, the microbial communities can shift sufficiently to bias the result, and the resulting estimate of microbial

tolerance is then removed from the microbial community in the soil studied, compromising the estimate's relevance. In the presently used bacterial growth assays, the concentration-response relationships are measured within a few hours (on average about 3 h) of exposure, eliminating potential shifts in the microbial community during the detection phase, and thus better reflecting the tolerance of the *in situ* bacterial communities compared to previous approaches with longer detection phases. The high sensitivity and precision (as seen by the inhibition curves, reaching levels above 90% inhibition, with very low error terms) of the bacterial growth measurement (Leu) and the immediate response makes it a more sensitive method to determine the tolerance of the bacterial community than previously used biomass-based, culture-based and respiration-based measurements. This is consistent with studies of tolerance of other toxins, including antibiotics, where the Leu incorporation method has been found to be more sensitive than alternative methods including BIOLOG (Alden-Demoling et al., 2009), potential denitrifictation (Milenkovski et al., 2009), single-cell esterase probing by flow-cytometry (Brandt et al., 2009), and compared with both short term potential and basal respiration (Rousk et al., 2009a).

7.4.2 Bacterial salt tolerance across the salinity gradient

We predicted that increasing levels of salt in soil would result in higher bacterial tolerance to salt of the extracted community, showing the direct influence by salt on the microbial community. There was no statistically significant relationship between the bacterial tolerance to salt and *in situ* soil concentration of salt across the four studied soils (Figure 7.2), suggesting that other factors must have been more important as selective forces acting on and shaping the actively growing bacterial community in these soils.

The four studied soils were low in organic matter content, and it is possible that lack of substrate could have obscured the pattern for important growth factors for the bacterial communities. To maximise the resolution of the influence of salt on the bacterial communities in the soils, we also incubated them with a range of concentrations of organic matter. Following one-month incubation with the organic matter additions, we again tested the bacterial tolerance to salt (Figure 7.3). There was support for an overall increase in bacterial salt tolerance with higher levels of salt, but it was only marginally significant, and the effect was small. In short, the bacterial tolerance to salt was only weakly related to the in situ soil salt level, again emphasizing that other growth factors were likely to have been more influential in structuring the bacterial communities. Previous direct assessments of the influence by soil salinity on the microbial community are few, and are to date not very comprehensive. Wichern et al. (2006) compared two soils with different salinity and found some results suggesting that respiration and decomposition of plant material were less detrimentally affected by exposure to salinity in the soil with higher in situ salt concentration. Baath et al. (2001) investigated the bacterial tolerance to salt additions in two soils with very different (albeit not quantified) in situ salt levels using growth measurements. While the bacterial growth was found to be sensitive to added salt in the low salinity soil, the bacterial community of the high salinity soils appeared unaffected by corresponding levels of salt exposure (although a positive control, where the bacterial growth could be demonstrably inhibited by salt, was not included in the salt range of the exposure phase). These suggestions are consistent with the weakly positive connection between bacterial salt tolerance and soil salinity, however, they are not comprehensive enough (2 soils compared in each study, differing in many factors) to aid our ability to generalize from our results.

Characteristic for the studied Libyan soils are high levels of salt as well as alkaline arid conditions (Ben-Mahmoud, 1995; Ben-Mahmoud et al., 2000). Soil pH has previously been shown to be a very powerful influence on the structure of soil bacterial communities (Baath and Anderson, 2003; Fierer and Jackson, 2006; Rousk et al., 2010a, b, c; 2011). However, soil pH did not differ significantly amongst the presently studied soils, and in addition, the soil pHs were within an interval (around pH 7) where differences in soil pH have been found to coincide with relatively minor shifts in bacterial community composition (Rousk et al., 2010c). It is consequently unlikely that soil pH differences between the studied soils obscured the influence of salt. Soil moisture has been found to be highly influential for shaping bacterial communities in soil (Iovieno and Baath, 2008). In addition, arid soils also suffer more intensive drying rewetting events, which are perturbations that can greatly affect the growth and structure of bacterial communities (Fierer et al., 2003; Williams, 2007; Bapiri et al., 2010; Chowdhury et al., 2011). We note that the concentrations at which 50% of the bacterial growth was terminated (EC_{50}) are consistently higher by about an order of magnitude than the average *in situ* salinity determined in the soils. Using the 50% level of inhibition as an index for salt tolerance may not appropriately reflect the level of salt relevant for the bacteria to be competitively viable in. It is likely that bacteria whose growth is inhibited by 10% or even less rapidly will be outcompeted by more tolerant strains in a natural environment. Even so, the significantly higher salt tolerance of the bacterial community compared to the *in situ* soil level is unexpected and surprising. As a possible explanation, the average salinity determined using a soil solution may be an under-representation of levels experienced by microorganisms in the microenvironments of the soil, with occasional very high concentrations of salt caused by e.g.

fluctuating moisture conditions due to high evapotranspiration (DeAngelis et al., 2010), especially relevant on the soil crust.

It is thus possible, that the bacterial communities in the studied soils were all similarly tolerant to these transient higher micro-scale salinity levels and that these, unlike average salinity, were similar between the soils. Our results do not substantiate that the influence of average soil salinity was a strong influence shaping the actively growing soil bacterial community. Rather, our results suggest that another factor likely was more influential. Previous assessments have indicated that the moisture regime, including dry rewet perturbations, is the prime candidate for such a factor, and we propose that future studies should be focused on partitioning between the influence of soil salinity and moisture (and related dry rewet cycles) on arid and saline soils, adding to the work initiated by e.g. Chowdhury et al. (2011). It should be noted, however, that the bacterial growth assay used to determine salt-toxicity does not take into account bacterial strategies for tolerance that involve e.g. the induction of resting stages (with no contribution to the growth measurement here employed) with higher salt levels, and the influence of survival strategies also need to be investigated further.

Acknowledgements

J.R. was supported by a Swedish research council postdoctoral grant (Project No. 623- 2009-7343). F.K.E. was supported by The Higher Education, Libya.

Chapter 8

General discussion

8.1 General discussion

Microbial communities play vital role in soil processes such as the formation and stabilization of soil aggregate and the decomposition of plant residues, and soil fertility. However, they are affected by adverse environmental conditions in Mediterranean areas - such as salinity induced by seawater intrusion into shallow aquifers in the Gefara Plain area. Saline waters are in relatively frequent use on the agricultural soils of the Gefara Plain, which is proximal to the sea and experiences high temperature and low rainfall in the summer months. Irrigation-induced salinity through sea water intrusion not only has an effect on plant growth but also effects soil microbial activity (Haynes and Chidoma, 2001). There are many researchers who have reported the effects of salinity on soil physical and chemical properties, but their effects on soil microbial activity are poorly investigated (Sumner, 1995; Keren, 2000; Levy, 2000).

The objective of the present study was to investigate how the microbial activity of agricultural soils from the Gefara Plain area is affected by increased NaCl induced salinity. We predicted that increasing levels of NaCl in soil would result in decreased microbial activity. Addition of NaCl changed some of the chemical properties of the soils. Soil electrical conductivity values increased with increasing concentrations of NaCl (El-Boraie, 1997; Ragab et al., 2008) in all soils (Figure 3.2, chapter 3). Increased soil salinity also decreased soil pH (Alden et al., 2001, Lai and Stewart, 1990) (Table 3.4, Chapter 3). This may be due to removal of hydrogen ions from cation exchange (Dielman, 1963, El Zbidi, 1989).

Soil respiration was used as a measure of microbial activity. The rate of soil respiration increased in all of the soil when the soil moisture was increased to 50% of water holding capacity (Figure 4.1, Chapter 4). Rewetting a dry soil will result in a flush of respiration (Liu et al., 2002a; Xu et al., 2004; Miller et al., 2005), that often increases within an hour (Rey et al., 2005) to levels more than soil kept at field moisture (Iovieno and Baath, 2008). Much of this may result from the metabolism of organic matter released by microbial death during drought and rewetting (Bottner, 1985; Van Gestel et al., 1991). However, Illstedt et al. (2000) reported that the maximal rate of CO_2 evolution occurs at 50% WHC. This may be also due to microbes being dormant in the dry soil, with their activity increasing when the soil undergone rewetting (Saetre and Stark, 2005), accompanied by the release of air trapped in the soil pores which contributes to an increase in the rate of soil respiration (Orchard and Cook, 1983). Rewetting is known to make organic matter more readily mineralizable (Sorensen, 1974). In general, the organic matter supply is the main rate limiting processes for microbial activity in dry soil, whilst O_2 diffusion controls the activity in wet soil.

In Chapter 4, the substrate–induced respiration (SIR) method was used to determine microbial biomass (Anderson and Domsch, 1978; Jenkison and Powlson, 1976). This method is a sensitive and fast CO_2 monitoring system (Heinemeyer et al., 1989). SIR was directly increased when glucose was added (Johasson et al., 1998) compared to a control without added glucose. This could be due to the fact that when glucose is used as a substrate, most soil microorganisms can utilize it as a C source (Stotzky and Norman, 1961). When of the microorganisms are dormant, (i.e., temporarily inactive) the switch from a dormant (Morita, 1988) to active state a triggered by release of easily available C to the soil solution (De Nobili et al., 2001; Stenstrom et al., 2001).

When glucose was added in solution, the rate of SIR was greatly increased compared to when dry glucose was added., This may be due to the solution form offering better distribution of glucose in the soil (West and Sperling 1986) and the fact that it changes the soil's osmotic potential (Ilstedt et al., 2000) (Figure 4.2, Chapter 4).

Although the rate of SIR was different between the four soils, it was lower in soil samples from Tajura compared to samples from Janzur, Almaya and Gargresh. This may be due to increased salinity and a lack of OM in the Tajura soils (Figure 3.2, chapter 3). The rate of SIR usually increases even further 6 to 10 hours after glucose addition, until the maximum rate of CO_2 is reached. However, glucose addition leads to increased respiration rate, indicating that microbial growth has occurred (Alden et al., 2001; Vuelvas-Solorzano et al., 2009) compared to unamended soil. The initial CO_2 efflux rate upon glucose addition does not necessarily indicate growth, but merely that microorganisms are using the carbon source for energy production (Alden et al., 2001).

Chapter 4 examined which type of salts (NaCl, Na_2SO_4 and KCl) at concentrations of 10 and 50 mM had the greatest negative influence on the Gefara Plain soils' microbial respiration. It was found the rate of CO_2 efflux was more inhibited by NaCl than by Na_2SO_4 or KCl in all four sites (Figure 4.4, 4.5 and 4.6). This may be due to chloride and sulphate salts decreasing the rate of decomposition (El-Shakweer et al., 1977). Cl^- salts have been shown to be more toxic than SO_4^- salts (Agarwal et al., 1971; Heilman, 1975; Sindhu and Cornfield, 1967). Cl^- ions to toxic levels in microbial tissues, which impedes the use of the other essential of anions and cations by the microbes (Laura, 1974).

Sindhu and Cornfield (1967) also pointed out sulphate salts have a lower toxicity than chlorides due to lower uptake by microbial cells.

In the field study we investigated the effects of distilled water or NaCl at 20 and 50 mM on soil respiration in four soils. The soils were amended with 1.5 L distilled water or concentrations of NaCl at 50% WHC. From the results obtained from this study were similar to the results obtained in the laboratory study.

The rate of CO_2 evolution was decreased also by increasing concentrations of NaCl levels in four soils, especially in samples from Tajura, which showed a rate of evolution significantly decreased compared to Janzur, Almaya and Gargaresh (Figure 4.7, Chapter 4). This negative effect of salinity on CO_2 efflux rate is in agreement with previous studies (e.g. Laure, 1974; Rietz and Haynes, 2003).

In general, ionic strength of the soil solution in addition to the directly toxic effect Na and Cl has on soil respiration (Lai Wong, 2007).

In Chapter 5 roots fungal hyphae in soil were separated by used of a 35 μm nylon mesh, which allow to the external hyphae to pass through without roots. When soil samples with external hyphae were amended with 12.5 ml distillated water at 50% WHC was rate of CO_2 evaluation increased compared to the control without external hyphae. We examined the effects of NaCl levels on soil respiration in the presence of external hyphae.

The results obtained in this study provide evidence that the rate of CO_2 evolution with external hyphae decreased with increasing concentrations of NaCl (McMillen et al., 1998), especially in soil samples from Tajura compared to Janzur, Almaya and Gargaresh (Figure 5.2).

This may be due to different soil electrical conductivity between the four soils. In the same regard, we measured the length of external hyphae after measuring soil respiration rates, and found the length of external hyphae decreased with increasing concentrations of NaCl (Juniper and Abbott, 1993) (Figure 5.3).

This may be due to the direct effects of ion toxicity or the indirect effects of osmotic stress (McMillen et al., 1998). However, Tresner and Hayes (1971) found external hyphae are intolerant of salt stress in the laboratory.

However, the studies have found that salt stress in the field. Levy et al. (1983) and Hartmond et al. (1987) found that external hypha are not reduced by salinity. In general, external hyphae growth is inhibited by increasing salinity; this is also reflected by decreased rates of CO_2 efflux in the presence of external hyphae under the effects of NaCl (Figure 5.4).

In Chapter 6 we investigated the effects of distilled water or water containing different concentrations of NaCl (10, 50, 70, 90, 150 and 200 mM) on the decomposition of ^{14}C-labelled *Lolium perenne* litter and ^{14}C-labelled glucose. The use of ^{14}C-radiollabeled substrates allowed very sensitive and specific measurements of $^{14}CO_2$ efflux. Addition of NaCl decreased the mineralization of ^{14}C-labelled *Lolium perenne* and ^{14}C-labelled glucose.

The cumulative production of $^{14}CO_2$ following the addition of ^{14}C-labelled *Lolium perenne* litter was particularly altered between samples from Tajura and Janzur. In samples from Tajura it decreased to 47% after 28 days when amended with a high concentration of NaCl (200 mM) (Figure 6.1), while in Janzur it reached 61% at the same concentration after 28 days' incubation compared to the control (Figure 6.1).

This may be due to organic matter content in native soil being lower at Tajura compared to Janzur, and also soil electrical conductivity being higher in native soil from Tajura compared to Janzur.

However, dissolution of readily decomposable plant material and microbial metabolites (plant-derived C) by Na^+ early in the incubation enhanced decomposition of plant residues, while later in the incubation the amount of these materials was insignificant (Nelson et al., 1996).

The cumulative production of $^{14}CO_2$ on addition of ^{14}C-labelled glucose to soil samples of Tajura was decreased to 37% within 28 days when amended by 200 mM used NaCl (Figure 6.2), while it decreased to 74% in Janzur compared to the control (Figure 6.2). The cumulative production of $^{14}CO_2$ increased with time reflecting the initial metabolism of substrate, which leads to increase in the number of metabolizing cells (Maier et al., 2009). In general, increased soil electrical conductivity led to decreased rates of decomposition (Luna-Guido et al., 2001; Vuelvas-Solorzano et al., 2009). This was clearer in Tajura compared to Gargaresh, Almaya and Janzur respectively.

On the other hand, incorporated ^{14}C-labelled substrates acts as a carbon and nutrient source, maintaining increased microbial biomass activity relative to the control (Witter and Lopez Real, 1998; Pascual et al., 1999). This is reflected by increased $^{14}CO_2$ evolution rates, and was clearer at Janzur than other sites (Figures 6.1 and 6.2, chapter 6).

Although soil pH affects on soil respiration (Kewalenko and Ivarson, 1978), it appears that soil EC was the factor that explained most of the reduction in mineralization when glucose and the plant material was added (Vuelvas-Solorzano et al., 2009).

In Chapter 6 we investigated the effects of the addition of 1% and 2% coconut husk as organic matter to soil on rate of CO_2 efflux when amended by 12.5 ml distilled water or with concentrations of NaCl at 50% WHC.

This was performed on samples from two sites only, Tajura and Janzur, because Tajura had the highest EC and lowest organic matter content and Janzur has lowest EC and highest OM content relative to the other sites (Tables 3.3 and 3.4, Chapter 3). There are positive correlations between organic matter and size of microbial community (Zahran et al., 1992).

This was clear from the increased rate of CO_2 efflux following the addition of organic matter (Leifeld et al., 2002; Dioamaeva et al., 2003). Several other studies have showed the effect of the amount of added substrate on its microbial mineralization to CO_2 (Bremer and van Kessel, 1990; Mary et al., 1993; Bremer and Kuikman, 1994) and found positive correlations (Wu et al., 1993; Bremer and Kuikman, 1994; Marstop and Witter, 1999).

The organic matter incubation of the soils did not affect soil pH or electrical conductivity significantly compared to the control (Table 6.2 and Figure 6.3; Chapter 6). 2% of coconut husk led to increased ECe in samples from Tajura and Janzur respectively, after the addition of 2% of coconut husk amended with 12.5 ml of concentrations of NaCl at 10 and 50 mM. Nitrate (NO_3^-) also played a role in increased soil electrical conductivity, because soil EC is tightly linked to nitrate concentration in the soil (Patriquin et al., 1993). The addition of coconut husk to soil led to decreased nitrate accumulation (Table 6.3).

Concentrations of NaCl added to soils had little effect on NO_3^- levels at low concentrations, while it decreased with increasing salinity.

This can be attributed to an inhibition of microbial activity (Pathak and Rao, 1998; Irshad et al., 2005). However, organic matter incorporated into soils led to increased soil pH (Pocknee and Sumner, 1997), although this was only was significant following the addition of 2% coconut husk in samples amended with 10 and 50 mM NaCl compared to the control (Table 6.2, Chapter 6).

On the other hand, until now the detailed processes responsible for a change in soil pH after incorporation of OM were not completely understood (Yan et al., 1996; Butterly et al., 2010).

The addition of coconut husk had an effect on cations (such as Na^+, K^+ and Ca^{2+}) in four soils.

The coconut husk at 1% and 2% change significantly the K^+ compared to the control; this may be due to K^+ supplied by the amendments (Figure 6.4). There were increases in cations released during decomposition (Noble and Randall, 1999; Pocknee and Sumner, 1997). Cations Ca^{2+} and Na^+ had a similar effect on CO_2 efflux (Setia et al., 2010).

The rate of CO_2 evolution also increased when soil samples were amended with 10 and 50 mM of NaCl compared to the control (without amendation by coconut husk) in Janzur and Tajura respectively. Organic matter seems to reduce the effects of salinity in soil (EL-Abyad et al., 1979; Zahran et al., 1992).

The addition of OM to saline soils leads to changes in the size of microbial biomass, which would clarify some of these variable patterns in microbial respiration at different soil salinity levels (Li et al., 2006). This has been ascribed to OM decreasing Na toxicity (Bhojvaid and Timmer, 1998).

In Chapter 7 the tolerance of the bacterial communities from the Gefara agricultural soils was a stressed, by extracting bacterial communities and subsequently monitoring their growth response (Leucine incorporation) to NaCl exposure.

We also repeated the assessment of bacterial growth tolerance after one month's incubation with 1% or 2% added organic matter added to stimulate microbial growth levels. Eight different concentrations of Na were used (0, 10, 20, 70, 90, 150, 200 and 400 mM NaCl). The concentration response relationships for all the different soils were very clear, and the effective inhibition of the bacterial growth, by more than 90%, for all soils at the highest salt exposures generated very clear inhibition curves that could be used to estimate bacterial tolerance (log (EC_{50})) with high precision.

There was a non-significant relationship between the bacterial tolerance to salt and *in situ* soil concentrations of salt in all four soils (Figure 7.2).

There was support for an overall increase in bacterial salt tolerance with higher levels of salt, but it was only marginally significant, and the effect was small. The bacterial tolerance to salt was only weakly related to the *in situ* soil salt level. This suggests that other growth factors must have been more influential in structuring the bacterial communities. While bacterial growth was found to be sensitive to salt in the low salinity soil, the bacterial community of the high salinity soils appeared unaffected by corresponding levels of salt exposure. The Gefara soils, once low organic matter conditions experimentally are compensated for, show characteristically high levels of salt, alkaline conditions and arid conditions (Ben-Mahmoud, 1995; Ben-Mahmoud et al., 2000). Soil pH has previously been shown to be a very powerful influence on the structure of soil bacterial communities (Baath and Anderson, 2003; Fierer and Jackson, 2006; Rousk et al., 2010a, b, c; 2011). However, the differences in soil pH in these particular soils were marginal, and differences were within an interval in which relatively minor shifts in bacterial community composition have been observed (Rousk et al., 2010c).

Throughout this investigation clear differences could be shown in the response of the soils from Tajura and Janzur to addition of NaCl. The strongest effects were seen throughout in Tajura the soil with lowest level of organic matter and the highest level of background Na. Particularly noticeable was the effect of NaCl addition on hyphal biomass in the Tajura soil.

8.2 General conclusions

The water holding capacity was similar for all the soils, at about 25%. Organic matter percentage was lower at Tajura at 0.49% compared to the other sites, while in Janzur the highest value was found at 1.25%.

There was a clear relationship between the initial infiltration rate, and the organic matter level in the soils, the infiltration rate in Janzur was higher than at the other sites this due to higher organic matter content.

Soil EC values were more increased with increasing salinity levels when added as NaCl than when added as Na_2SO_4 or KCl in Tajura, Gargresh, Almaya and Janzur respectively. Tajura also had a higher concentration of Ca in the soil solution than Janzur. Soil pH was slightly decreased to a less alkaline condition with an increase in salinity.

The rate of soil respiration doubled after the soil was wetted to 50% of WHC, compared to soil kept at field moisture (25% WHC) in Janzur, Almaya, Gargresh and Tajura respectively. The substrate-induced respiration (SIR) rate increased 3 times after addition of glucose and glutamate in solution to the soil samples compared to the basal respiration (soil kept at field moisture), and was greater in Janzur than at the other sites. The rate of soil respiration decreased on treatment with NaCl > Na_2SO_4 > KCl respectively. An increase in EC has been shown to have negative effects on microbial activity in the soil, which in turn led to a decreased rate of CO_2 evolution in Tajura, Gargresh, Almaya and Janzur respectively.

In the field study, the rate of CO_2 evolution was decreased by increasing concentrations of NaCl levels in four soils, especially in samples from Tajura, where the rate of CO_2 evolution was significantly decreased compared to Janzur, Almaya and Gargaresh.

The rate of CO_2 evolution with external hyphae decreased with increasing concentrations of NaCl, in soil samples from Tajura compared to Janzur, Almaya and Gargaresh. The length of external hyphae was decreased with increasing concentrations of NaCl. External hyphae growth was inhibited by increasing salinity, this was also reflected by decreased rates of CO_2 efflux in the presence of external hyphae under the effects of NaCl.

The decomposition of ^{14}C-labelled *Lolium perenne* litter and ^{14}C-labelled glucose were most affected at high concentrations of NaCl. Increased soil electrical conductivity led to decreased rates of decomposition, this was clearer in Tajura compared to Gargaresh, Almaya and Janzur respectively.

There was a clear increase in the rate of CO_2 efflux following the addition of coconut husk compost to soil in Janzur, Almaya, Gargaresh and Tajura respectively. Addition of coconut husk compost to the soils increased pH, electrical conductivity and K^+ concentrations in the soil solution, and lowered the sensitivity of the soils to salinity. This work suggests that soils with high salinity in the Gefara Plain can be rapidly affected by further salt addition, but these can be in part be alleviated by addition of organic matter.

The assessment of bacterial growth tolerance after one month's incubation with 1% or 2% added coconut husk compost added to stimulate microbial growth levels, and under with eight different concentrations of NaCl (0, 10, 20, 70, 90, 150, 200 and 400 mM), showed clear concentration response relationships for all the different soils, and the effective inhibition of the bacterial growth, by more than 90%, for all soils at the highest salt exposures. There was support for an overall increase in bacterial salt tolerance with higher levels of salt, but it was only marginally significant, and the effect was small. There was a non-significant relationship between the bacterial tolerance to salt and *in situ* soil concentrations of salt in all four soils. Results suggest that another factor likely was more influential. Previous assessments have indicated that the moisture regime, including dry rewet perturbations, is the prime candidate for such a factor.

8.3 Further work

Considerations for future work have been stated in the experimental chapters and here a summary is provided:

1. Further investigation may need to consider finer resolution of effects of NaCl, Na_2SO_4 and KCl on soil respiration in the field. This would allow for a better comparison with results obtained *in vitro*.
2. Further investigation about the effect of NaCl on the growth and respiration of external hyphae in field are also needed. This work showed that the external hyphae of mycorrhizas are sensitive to salinity, and it is suggested that the decrease in hyphae may play an important role in plant response to salinity.
3. The results suggest that another factor likely was more influential on bacterial growth than salinity alone. Previous assessments have indicated that the moisture regime, is the prime candidate for such a factor and it is proposed that future studies should be focused on partitioning between the influence of soil salinity and moisture. In addition, the methods used here investigated to the response of the total bacterial community, a determination of the structure of the community and any changes under salinity would greatly enhance understanding of the effects of salinity on soil microbiology.

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