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Understanding the impacts of changing soil temperature, water irrigation source and fertiliser types on C and N cycling in arid soils

Almulla, Abdulaziz

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Understanding the impacts of changing soil temperature, water irrigation source and fertiliser types on C and N cycling in arid soils

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Abdulaziz Abdulrahman A AlMulla

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Supervisors: Dr. P. Roberts and Prof. D.L. Jones

School of Environment, Natural Resources and Geography

Bangor University, Bangor, Gwynedd

LL57 2UW

United Kingdom

Abstract

Nitrogen (N) is a key regulator of ecosystem development, and the cycling and competition for N resources in ecosystems remains poorly understood. Arid ecosystems are primarily found in North Africa, Middle East and Australia, and cover about one-third of the total global area. Soil organic N (SON) and C cycles are linked; both immobilization and mineralization of N pathways are linked by heterotrophic microorganisms that require C from organic material for production of energy and growth. Therefore, studies into N and C cycles are key to understanding biogeochemical cycles in these areas. Typical agricultural practices in Saudi Arabia (KSA) focus on the production of dates. This study focussed on Al-Hassa oasis - the largest oasis and date supplier in Eastern KSA. The aim of this thesis was to investigate the effect of changing soil temperature, water irrigation sources and fertiliser types on soil C and N cycles within the oasis. To increase our understanding of the effect of aridity on N and C cycle we used ^{14}C techniques to investigate the rate of DON or DOC mineralization by soil microorganisms in response to different temperatures, moisture content and different water types. Initial experiments showed that soil properties decreased dissolved organic nitrogen (DON) and carbon (DOC) mineralization rate whereas, temperature increased mineralization rate in the soil by altering carbon dioxide (CO_2) emissions and C partitioning between catabolic and anabolic processes within the microbial biomass. Therefore, we would recommend the farmers and government managers to reduce fertiliser on hot summer that can reduce environmental pollution and cost. Higher C mineralization rates were observed in the soil with the lowest contents of silt, clay, and salinity. The rate of $^{14}CO_2$ evolution from DON is greater than from DOC compounds with shorter half-life for DON substrates because they are likely to be processed by different metabolic pathways inside the cell. Following on from this, further experiments showed that changing the irrigation water source significantly increased the mineralization of C contained in insoluble plant residues in comparison to that present in the soluble component. The rate of insoluble plant material mineralization was slower than for the soluble component leading to lower rates of $^{14}CO_2$ loss and a ca. 11-fold longer half-life compared to soluble fractions. Results from the final experiments indicated that applying organic fertilisers would reduce nitrate (NO_3^-) leaching more than inorganic fertilisers. The information contained in this thesis has improved our

fundamental understanding of C and N cycling in arid soil systems. We would recommend the farmers and government managers to reduce inorganic fertiliser and use more organic N fertiliser that can reduce environmental pollution, cost, save more water, and increased yield. Further studies are, however, still needed to investigate the long-term effects of changing soil temperature, water irrigation source and fertiliser types on soil microbial processes in arid soil for developing better water and fertiliser management and reducing N or C gaseous emissions.

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Abbreviations

°C	The degree of Celsius
^{14}C	14-Carbon isotope
AMES	Ammonium molybdate-sulphuric acid
C	Carbon
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
cm	Centimetre
CN ratio	Carbon to nitrogen ratio
CO_2	Carbon dioxide
d	Day
DIN	Dissolved inorganic nitrogen
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
EC	Electrical conductivity
EEA	Extracellular enzyme assay
EON	Extractable organic nitrogen
g	Grams
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit
GM	Green manure
GW	Groundwater
h	Hour
ha	Hectares
HIDA	Al-Hassa Irrigation and Drainage Authority
HMW	High molecular weight
K_2SO_4	Potassium sulphate
kBq	Kilobecquerel
KCl	Potassium chloride
kg	Kilogram
KSA	Kingdom of Saudi Arabia
l	Litre

LMW	Low molecular weight
M	Molar
MAW	Ministry of Agriculture and Water
MC	Moisture content
mg	Milligrams
Mic_{eff}	Microbial biomass carbon use efficiency
ml	Millilitre
µl	Microliter
mm	Millimetre
µM	Micromole
MMRA	Ministry of Municipal and Rural Affairs
MOA	Saudi Ministry of Agriculture
mS	Milisiemens
MW	Mixed water
MWE	Ministry of Water and Electricity
N	Nitrogen
N_2	Dinitrogen
N_2O	Nitrous oxide
NaOH	Sodium hydroxide
NEDD	N-(1-Naphthyl) ethylenediamine dihydrochloride
NH_3	Ammonia
NH_4^+	Ammonium
NH_4NO_3	Ammonium nitrate
NO	Nitric oxide
NO_2	Nitrogen dioxide
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Oxygen
OH	Hydroxide
OM	Organic matter
P	Phosphorous
pH	Power of hydrogen
PLFA	Phospholipid fatty acids

rcf	Relative centrifugal force
rev min^{-1}	Revolutions per minute
RNA	Ribonucleic acid
S	Sulphur
SEM	Standard error of the mean
SOM	Soil organic matter
SON	Soluble organic nitrogen
SPSS	Statistical package for social sciences
SR	Basel soil respiration
TC	Total carbon
TDN	Total dissolved nitrogen
TFAA	Total free amino acids
TN	Total nitrogen
TOC	Total organic carbon
TON	Total organic nitrogen
TTWW	Tertiary treated wastewater
UK	United Kingdom
USA	United States of America
w/v	Weight to volume
WD cycles	Wetting and drying cycles
WHC	Water holding capacity

Chapter 1. Introduction

Climate change is expected to raise temperatures in Saudi Arabia (KSA) by 1.8 to 4.1°C by 2050 (Chowdhury and Al-Zahrani, 2013). The subsequent increase in soil temperature may influence water availability and decrease soil moisture (43.80 - 237.25 mm $year^{-1}$), reduce relative humidity (0.8 - 2.3%), and increase evapotranspiration (76.1 - 195.6 mm $year^{-1}$) across the kingdom (Chowdhury and Al-Zahrani, 2013). The changing of soil temperature is expected to affect soil nutrient status (e.g., increasing mineralization process) through increasing maintenance cost of microorganisms this may cause an accumulation of nitrate, organic nitrogen, and salts over time (Ewing et al., 2008; Baumann and Marschner, 2013). High soil salt contents in solution can negatively affect the soil's microbiological, chemical, and physical properties (Shah and Shah, 2011). The Al-Hassa oasis is the largest oasis in KSA, and is located in the eastern region. This oasis is one of the largest date suppliers for the KSA. As a result of increasing demand on limited groundwater supply for agricultural, industrial and domestic uses, the Al-Hassa Irrigation and Drainage Authority (HIDA) has been utilizing alternative sources of irrigation water, such as tertiary treated wastewater and mixtures of drainage water and groundwater. The changing of water sources is expected to affect soil nutrient status and the environment. Typically, TTWW may supply mineral nutrients and organic matter advantageous to crop production, or it may increase heavy metal loading and expose crops to toxic substances (Lucho-Constantino et al., 2005). Pescod (1992) shows that the effluents from selected TTWW in California supplied different forms of N at different range (e.g. 0.4 - 21.3 mg NO_3^--N l^{-1}, 0.1 - 16.6 mg NH_4^+-N l^{-1} and 0.2 - 2.6 mg organic-N l^{-1}). The addition of organic substrate stimulate the mineralization rate with greater CO_2 release resulting in priming effect (Rukshana et al., 2013) depending on many factors (e.g., soil texture, microbial community, soil temperature, moisture content, …etc). According to Cai et al. (2016), there is a lower mineralization of soil organic C and N in the silt+clay fraction than in the sand fraction in both non-cultivated and cultivated soil. These differences could be due to large surface area and ion exchange reactions that involves adsorption and binding of organic matter, increased micro-aggregation, and decreased microbial decomposition in the clay fraction (Cai et al., 2016). In addition, since 2005, the Ministry of Agriculture in Saudi Arabia (MOA) has begun supporting and developing

organic agriculture; 33710 ha is currently under organic management and around 2890 ha was in development in 2014 (Hartmann et al., 2012). We believe that changes the fertiliser types to organic N may reduce N losses to groundwater due to high microbial N demand from higher available C in organic N fertiliser. This could be due to higher available C will increase the immobilization process (Robertson and Groffman, 2007) therefore, reducing leachate and gaseous losses (Tan, 2009). Conversely, the excessive use of inorganic N fertilisers can cause adverse environmental effects, including groundwater pollution from increasing NO_3^- leaching, air pollution from increased emission of greenhouse gasses such as nitrous oxide and ammonia, acidification of soils, decreasing aggregate stability, and promoting soil erosion (Fließbach et al., 2000; Inselsbacher et al., 2009; Chirinda et al., 2010; López-López et al., 2012; Zhang et al., 2012; Iqbal et al., 2014). Climate change and management changes (i.e., changing water sources and soil fertiliser types) such as these are expected to negatively affect crop production (Chirinda et al., 2010), physical and chemical properties of soil (Chirinda et al., 2010; Almadini, 2011; Brar et al., 2015), nutrient use efficiency (Chirinda et al., 2010), and soil N and C turnover (Glanville et al., 2012; Li et al., 2014). This is likely to lead to increased greenhouse gas emissions and losses of nutrients to groundwater. N is a key regulator of ecosystem development, and the cycling and competition for N resources in all ecosystems remains poorly understood (Jones et al., 2004; Boddy et al., 2008; Hill et al., 2008; Jan et al., 2009; Dungait et al., 2012). Soil organic N (SON) and C cycles are linked; both immobilization and mineralization of N pathways are linked by heterotrophic microorganisms that require C from organic material for production of energy and growth (Fisk et al., 2015). We know that temperature, soil moisture content, soil management, vegetation litter, microbial community and soil organic matter will increase or decrease the rate of C and N turnover in soil (Simfukwe et al., 2011; Glanville et al., 2012; Qiu-Hui et al., 2012; Li et al., 2014) depending in their situation (e.g., increasing temperature will increase the rate of N or C turnover, decrease moisture content to drought will decrease the rate). Nitrate and organic N accumulate with time as a result of extreme aridity (high temperature and low moisture content) but low accumulation of organic C from atmosphere deposition due to slow C cycling that sufficient to limit the accumulation of organic C (Ewing et al., 2008). To our knowledge, there are no studies investigating the

dynamics of dissolved organic nitrogen (DON) and carbon (DOC) in Al-Hassa oasis sites, and it is important to increase our understanding of the controlling factors that influence the microbial mineralization rate of DON and DOC in arid systems, if we are to fully understand soil management implications on a global scale and develop more sustainable agricultural systems and reduce environmental pollution (e.g., reduce CO_2 emission, NO_3^- leachating).

1.1 Aims and objectives

The overall aim of this project is to investigate the DON and DOC dynamics of different soil types from the Al-Hassa eastern oasis. The specific objectives are:

1) To determine the C mineralization of DON and DOC in response to temperature from non-agricultural or agricultural arid soils (Chapter 3, 4).
2) To study the effect of using alternative sources of water irrigation on soil chemical and physical properties in the oasis farms (Chapter 5).
3) To determine the C mineralization of soluble and insoluble fractions of plant components in response to different water irrigation source or moisture contents from agricultural arid soils (Chapter 5).
4) To investigate the influence of different fertiliser types on the diffusion of N compounds in arid soils (Chapter 6).

1.2 Hypothesis

1) Increasing silt, clay, and salinity will reduce the rate of utilization of DON and DOC compounds due to the reduction of microbial activity.
2) The mineralization rate of DON is higher than for DOC in arid soils because they are likely to be processed by different metabolic pathways inside the cell.
3) The mineralization rate of DON and DOC increases with increasing temperature due to temperature tends to increase maintenance cost of microorganisms.
4) Increasing labile C concentration in irrigation water source will increase the decomposition of the insoluble fractions of plant residues due to increase microbial activity.

5) N from inorganic N fertiliser will diffuse faster than N contained in an organic fertiliser due to the rapid increase of inorganic N compound from inorganic fertiliser.
6) Organic fertiliser can reduce the amount of NO_3^- produced over time that could be due to high microbial N demand from higher available C in organic N fertiliser.

1.3 Thesis plan

This thesis plan attempts to answer the aforementioned objectives by undertaking different experimental studies. These different experimental chapters are presented in the form of scientific papers with the aim that they may be submitted to international scientific journals. This thesis is divided into an abstract, introduction, literature review, four experimental chapters, and general discussion. The thesis structure is detailed below (Figure 1.1):

Chapter 1: Introduction

General introduction to the subject area, aim, objectives, and hypothesis of the study.

Chapter 2: Literature review

A critical review of the N cycle in arid soil, a background of the KSA and the Al-Hassa eastern oasis, and the different factors affecting the concentrations of N in soil.

Chapter 3: Understanding the impacts of temperature on DON and DOC mineralization in arid non-agricultural soils

Using ^{14}C techniques to determine DON and DOC mineralization rates in response to temperature in non-agricultural arid soils. In addition, determined various physical and chemical characteristics and microbial community structure of the soils.

Chapter 4: Substrate influences temperature sensitivity of dissolved organic carbon (DOC) and nitrogen (DON) mineralization in arid agriculture soils

The aim of this experimental chapter was to investigate the rate of DON or DOC mineralization by soil microorganisms in response to different temperatures. Using ^{14}C-labelled glucose, amino acids, and trialanine in laboratory incubation

experiments at different temperatures, measured different physical and chemical characteristics, and the microbial community structure of the soil.

Chapter 5: Do soil moisture regimes and irrigation water source affect DOC and DON mineralization in arid agricultural soil?

This chapter used ^{14}C techniques to measure DON and DOC mineralization rates in response to irrigation water source and moisture content applied to arid agricultural soils by using soluble and insoluble ^{14}C plant materials obtained from ^{14}C-labelled *Lolium perenne* L. shoots. In addition, measured the changes of various physical and chemical characteristics of the soil with water irrigation source and moisture content.

Chapter 6: Diffusion of soluble nitrogen away from organic and inorganic fertilisers in an arid soil

This chapter used a microtome to investigate the effects, at mm scale, of different fertiliser types on the vertical diffusion of N compounds available to plants in arid soil. Furthermore, the influence of applying different fertilisers (green manure, protein, ammonium nitrate, and control) on soil pH, electrical conductivity (EC), carbon to nitrogen (C:N) ratio, and gaseous emission (ammonia (NH_3) and CO_2) was determined.

Chapter 7: General discussion

The aim of this chapter was to highlight and discuss the results presented in this thesis, identify future research work, and recommendations.

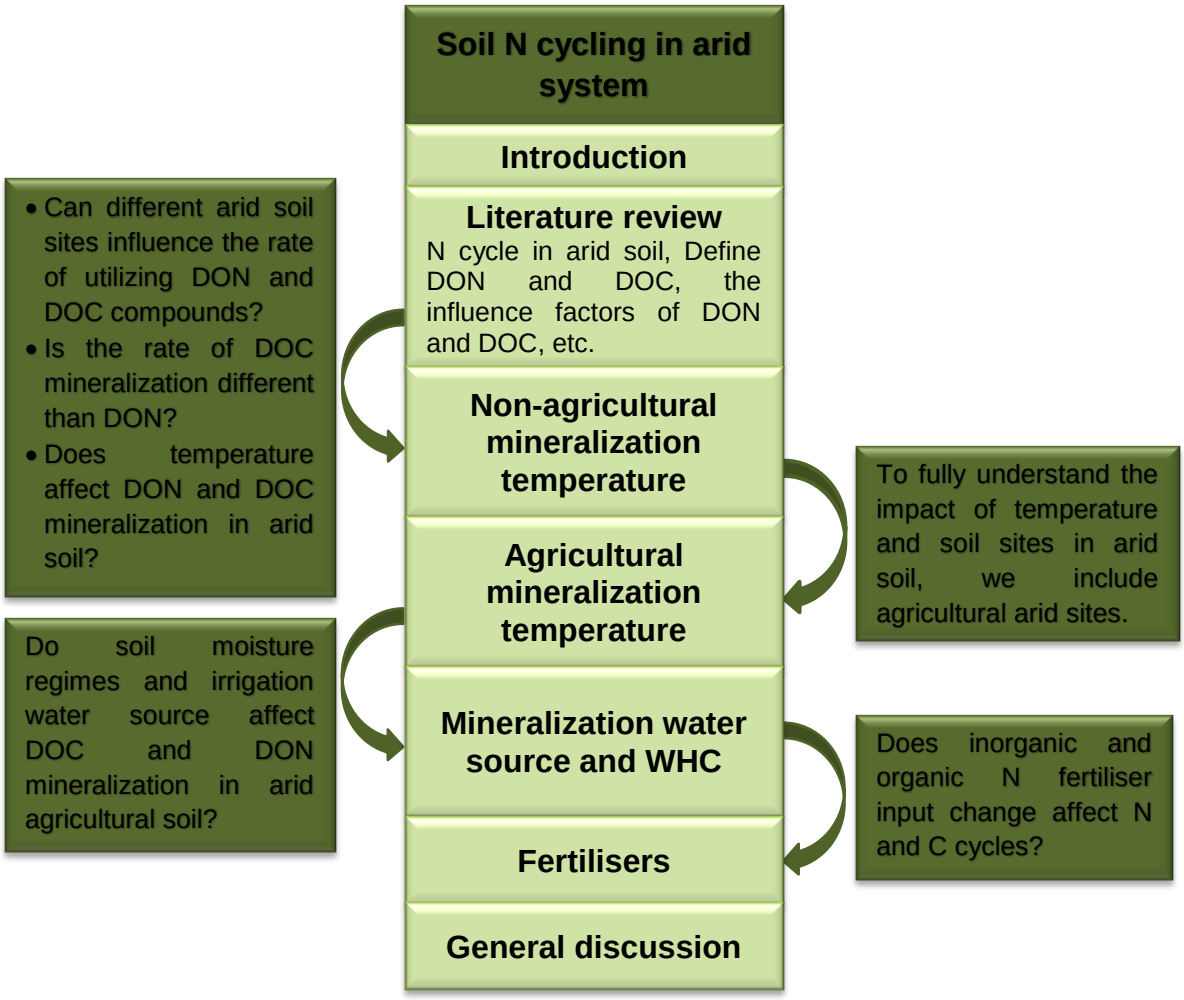


Figure 1.1 Schematic representation of thesis chapters.

Chapter 2. Literature review

2.1 Introduction

Nitrogen (N) is a primary nutrient regulator of plant productivity in terrestrial desert ecosystem. N is a key regulator of ecosystem development, and the cycling and competition for N resources in all ecosystems remain poorly understood. Since 1990s, there has been a shift in the N paradigm (the traditional view; that plant can uptake only nitrate (NO_3^-) and ammonium (NH_4^+) from soil), from a sole focus on inorganic N uptake pathways to one that includes dissolved organic N (DON) (Farrell et al., 2011; Hill et al., 2011a). This arose primarily due to the discovery that plants can compete with microorganisms for amino acids in soil by passing the need for organic N to be mineralized to NO_3^- and NH_4^+ (i.e., short-circuiting the N cycle; Boddy et al., 2007; Hill et al., 2011a). Soil organic N (SON) and C cycles are linked; both immobilization and mineralization of N pathways are linked by heterotrophic microorganisms that require C from organic material for production of energy and growth (Fisk et al., 2015). In addition, changes to more sustainable irrigation systems using alternative sources of water to irrigate oasis farms are expected to affect soil nutrient status. Here, we challenge the current paradigm, as we believe that competition between plants and microbes primarily occurs at a higher point in the N cycle, and that alternative sources of water for irrigation may create a faster short circuit of the N and C cycle in arid ecosystems due to increasing labile compounds. Typically, TTWW may supply mineral nutrients and organic matter advantageous to crop production, or it may increase heavy metal loading and expose crops to toxic substances (Lucho-Constantino et al., 2005). Pescod (1992) shows that the effluents from selected TTWW in California supplied different forms of N at different range (e.g. 0.4 - 21.3 mg NO_3^--N l^{-1}, 0.1 - 16.6 mg NH_4^+-N l^{-1} and 0.2 - 2.6 mg organic-N l^{-1}). The addition of organic substrate stimulate the mineralization rate with greater CO_2 release resulting in priming effect (Rukshana et al., 2013). In addition, we believe that changes the fertiliser types to organic N may reduce N losses to groundwater due to high microbial N demand from higher available C in organic N fertiliser. This could be due to higher available C will increase the immobilization process (Robertson and Groffman, 2007) therefore, reducing leachate and gaseous losses (Tan, 2009). To understand DON and DOC dynamics in soils from the Al-

Hassa eastern oasis, background knowledge of the Kingdom of Saudi Arabia (KSA), the Al-Hassa eastern oasis, and the N and C cycle are required.

2.1.1 Background of the Kingdom of Saudi Arabia (KSA)

Climate of KSA

The climate of Saudi Arabia is known as a desert climate, which is characterized by high heat in the daytime, and decreased temperature at night, with slight and irregular rainfall (Safar, 2011). The average annual temperature is 26 - 27°C, but the maximum daily temperature is 32 - 33°C, while the highest temperature recorded is 46 - 47°C. The mean annual rainfall is about 36 mm in the west coast and 89 mm in the east coast (Safar, 2011). During periods of rainfall, precipitation is very intense. The highest recorded rain in the west coast is 48 mm day^{-1} and in the east coast is 98 mm day^{-1} (Safar, 2011). Global warming is predicted to raise temperatures in KSA by between 1.8 - 4.1°C by 2050 (Chowdhury and Al-Zahrani, 2013). The subsequent increase in soil temperature may influence water availability and decrease soil moisture (43.80 - 237.25 mm $year^{-1}$), reduce relative humidity (0.8 - 2.3 %), and increase evapotranspiration (76.1 - 195.6 mm $year^{-1}$) across the kingdom (Chowdhury and Al-Zahrani, 2013). These changes are expected to affect crop production (Chirinda et al., 2010), soil physical and chemical properties (AlMadini, 2001; Chirinda et al., 2010; Brar et al., 2015), nutrient use efficiency (Chirinda et al., 2010), and soil N and C turnover (Glanville et al., 2012; Li et al., 2014), thereby increasing greenhouse gas emissions and nutrient use efficiency. Therefore, it is important to increase our understanding of the impact of the potential increases of temperature and climate change in arid soil to determine future effects of climate change, develop more sustainable agriculture systems, and reduce N or C pollution.

General types of soil in KSA

According to Hussain et al. (2010), the Kingdom's lands are classified into three extensive areas of sand dunes, which are shale, siltstone and sandstone and thoroughly mixed by wind, or deposited in a layer of different soil texture (e.g., deposited from sandstorms). Soil contains loamy sand or sandy loam textural classes, with predominantly coarse soil. They also contain calcareous soil, which, in some cases, contains gypsum. The development of different soil types is due to the

difference in soil forming processes such as high temperature, wind erosion and aridity. There is a slow rate of chemical weathering due to low rainfall and high levels of soluble salt contents in some areas (Hussain et al., 2010). Typically, organic matter is low (less than 1%), and amounts of available nitrogen, phosphorous and potassium are adequate for plant growth (Hussain et al., 2010).

2.1.2 Background of the Al-Hassa eastern oasis

Climate of the Al-Hassa eastern oasis

The largest oasis of KSA is the Al-Hassa oasis, which is located in the eastern region, between 25°37′12″ and 25°20′24″ N latitude and 49°32′24″ and 49°46′48″ E longitude, and covers an area of around 20,000 hectares (ha) (Aldakheel, 2011) (Figure 2.1). The aridity of Al-Hassa climate is characterized by extended, rainless, hot summers with northerly winds, often carrying sandstorms. The rain falls mostly in December and January with annual values differing from 45 - 150 mm. The mean annual rainfall is about 70 mm. The annual temperature ranges is 28 - 47.5°C, with an average of 34.1°C, where the highest temperature recorded is 50°C. The air humidity is remarkably high, and could reach 90% in summer (Al-Fredan, 2011; Aldakheel, 2011).

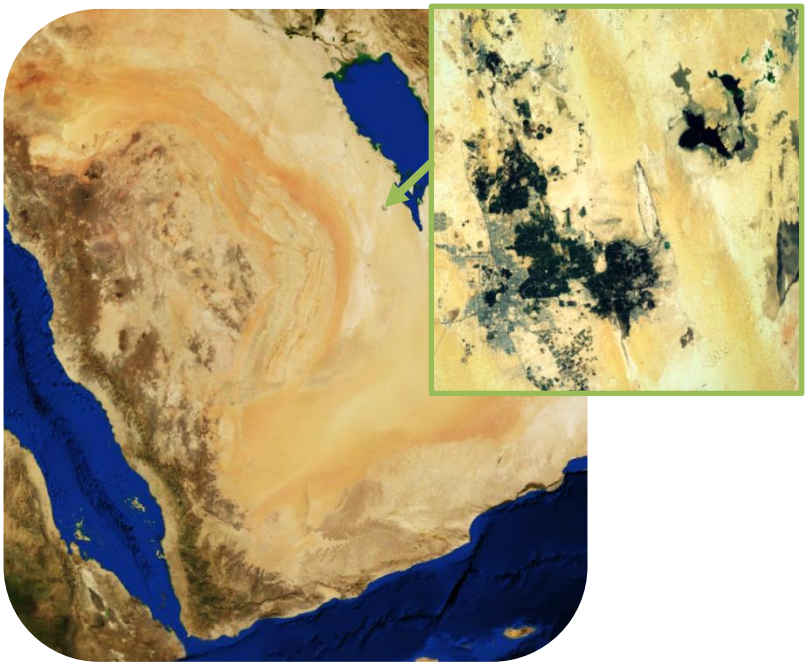


Figure 2.1 Map of Saudi Arabia showing sampling sites at Al-Hassa eastern oasis (Produced by the author).

Water resources for irrigation in the Al-Hassa eastern oasis

Since 1980, Al-Hassa Irrigation and Drainage Authority (HIDA) have been utilizing alternative sources of water to irrigate the oasis farms due to increasing demand on limited groundwater supply for agriculture, industrial and domestic uses in arid areas (Figure 2.2) (Al-Kuwaiti, 2010; Aldakheel, 2011). The major water source for irrigating the cultivated area in the oasis is groundwater (GW) from 32 springs; alternatively, water used to support agricultural production was sourced from tertiary treated wastewater (TTWW), and a mixture of tertiary treated wastewater, drainage water and groundwater (MW) (Al-Kuwaiti, 2010; Aldakheel, 2011). Utilizing TTWW and MW for irrigation can decrease the pressure on GW supplies significantly. Currently, the Irrigation and Drainage Authority in Al-Hassa Oasis, KSA, sources more than 64% of its total irrigation water from alternative sources; 24% comes from TTWW and 40% from MW (Al-Kuwaiti, 2010). The changing of water sources is expected to affect soil nutrient status and the environment. Typically, TTWW may supply mineral nutrients and organic matter advantageous to crop production, or it may increase heavy metal loading and expose crops to toxic substances (Lucho-Constantino et al., 2005). Pescod (1992) shows that the effluents from selected TTWW in California supplied different forms of N at different range (e.g. 0.4 - 21.3 mg NO_3^--N l^{-1}, 0.1 - 16.6 mg NH_4^+-N l^{-1} and 0.2 - 2.6 mg organic-N l^{-1}). The addition of organic substrate stimulate the mineralization rate with greater CO_2 release resulting in priming effect (Rukshana et al., 2013). Al-Kuwaiti (2010), at the Al-Hassa agriculture research department (1974 - 1976), showed that it was possible to use MW to grow salinity-resistant crops such as tomato (*Solanum lycopersicum* L.), clover (*Trifolium* spp.) and okra (*Abelmoschus esculentus*).

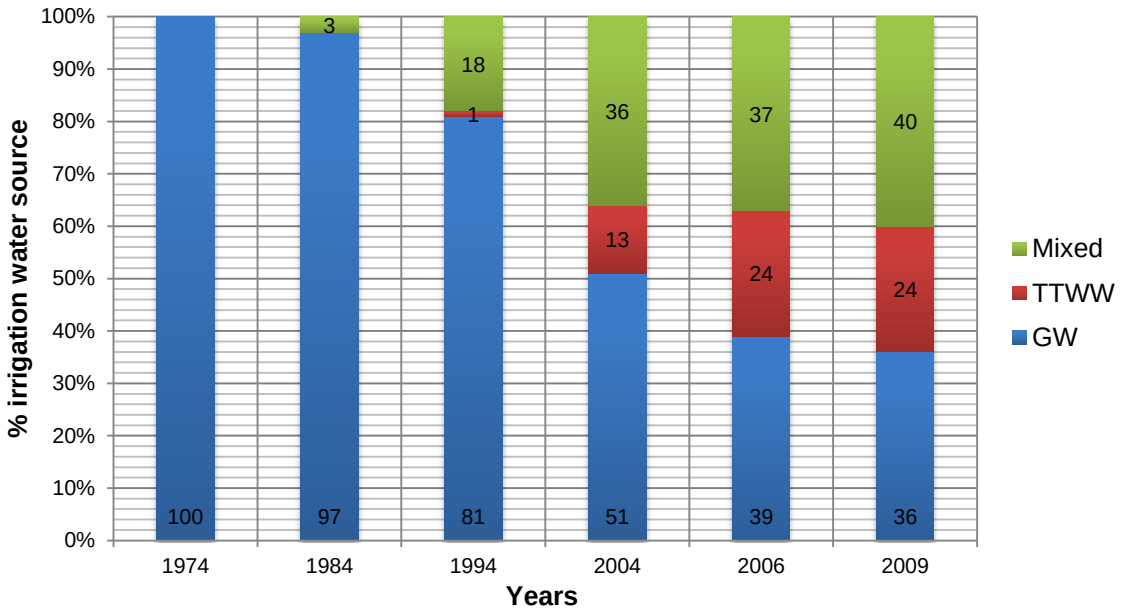


Figure 2.2 Development of water resources in Al-Hassa eastern oasis from 1974 to 2009 (Modified from Al-Kuwaiti, 2010).

In KSA, there are several standards for reusing TTWW in the landscape, and the Ministry of Agriculture and Water (MAW) issued agriculture irrigation guidance with restricted (the water cannot be used if any parameter is does not meet the standard) and unrestricted standards (can use the water if single parameter is similar or lower than the standard) in 1986 (Al-Jasser, 2011). New standards were issued in 2003 by the Ministry of Municipal and Rural Affairs (MMRA), which was replaced by the Ministry of Water and Electricity (MWE) in 2006 (Al-Jasser, 2011) (Table 2.1) with set chemical and biological parameters over water that can be used for agricultural irrigation.

Table 2.1 Restricted and unrestricted irrigation standards in tertiary treated wastewater by 2003-MMRA and 2006-MWE (Reproduced from Al-Jasser, 2011).

Parameters	Unit	Unrestricted irrigation		Restricted irrigation	
		2003-MMRA	2006-MWE	2003-MMRA	2006-MWE
Physical parameters					
Floatable materials		Absent	Absent		Absent
Total suspended solids (TSS)	mg l^{-1}	10	10	40	40
pH		6 - 8.4	6 - 8.4		6 - 8.4
Turbidity	NTU		5		5
Chemical parameters					
Organic chemicals parameters					
Biochemical oxygen demand (BOD_5)	mg l^{-1}	10	10	40	40
Chemical oxygen demand (COD)	mg l^{-1}	50			
Total organic carbon (TOC)	mg l^{-1}	40			
Oil and grease	mg l^{-1}	Absent	Absent		Absent
Phenol	mg l^{-1}	0.002	0.002		0.002
Inorganic chemicals parameters					
Heavy metals					
Arsenic (As)	mg l^{-1}	0.1	0.1		0.1
Cadmium (Cd)	mg l^{-1}	0.01	0.01		0.01
Chromium (Cr)	mg l^{-1}	0.01	0.1		0.1
Copper (Cu)	mg l^{-1}	0.2	0.4		0.4
Lead (Pb)	mg l^{-1}	5	0.1		0.1
Mercury (Hg)	mg l^{-1}	0.001	0.001		0.001
Nickel (Ni)	mg l^{-1}	0.02	0.2		0.2
Zinc (Zn)	mg l^{-1}	2	4		4
Aluminium (Al)	mg l^{-1}	5	5		5
Barium (Ba)	mg l^{-1}	1			
Manganese (Mn)	mg l^{-1}	0.2	0.2		0.2
Silver (Ag)	mg l^{-1}	0.5			
Selenium (Se)	mg l^{-1}	0.02	0.02		0.02
Molybdenum (Mo	mg l^{-1}	0.01	0.01		0.01
Boron (B)	mg l^{-1}	0.75	0.75		0.75
Vanadium (V)	mg l^{-1}	0.1	0.1		0.1
Lithium (Li)	mg l^{-1}	2.5	2.5		2.5
Beryllium (Be)	mg l^{-1}	0.1	0.1		0.01
Iron (Fe)	mg l^{-1}	5	5		5
Cobalt (Co)	mg l^{-1}	0.05	0.05		0.05
Chemical compounds					
Total dissolved solids (TDS)	mg l^{-1}	2000	2500	2000	2500
Chloride (Cl_2)	mg l^{-1}	100			
Sulphate (SO_4)	mg l^{-1}	600			
Ammonia (NH_3-N)	mg l^{-1}	5	5		5
Nitrate (NO_3-N)	mg l^{-1}	10	10		10
Free residual chlorine	mg l^{-1}	0.2	0.5		0.5
Fluoride (F)	mg l^{-1}		1		1
Cyanide (Cn)	mg l^{-1}	0.05			
Biological parameters					
Faecal coliforms per 100 ml		2.2	2.2	1000	1000
Intestinal nematodes per litre	No. l^{-1}	1	1		1

Vegetation of the Al-Hassa eastern oasis

Al-Hassa eastern oasis is one of the largest date suppliers and most influential agricultural areas in the KSA (Al-Jabr, 2002). There are approximately 7000 ha of cultivated land within the oasis, which are divided into 8091 farms (AlMadini, 2001, Aldakheel, 2011). The most common crop grown in the oasis is date palm trees (*Phoenix dactylifera*), with a total of 1.6 million trees covering nearly 92% of the total cultivated area. Each date palm tree is planted at distance of 4 m x 6 m from the other, and usually interplanted with other crops including (AlMadini, 2001; Al-Jabr, 2002):

1) Vegetables, e.g., onions (*Allium cepa* L.), eggplants (*Solanum melongena*), okra (*Abelmoschus esculentus*), parsley (*Petroselinum crispum*), carrots (*Daucus carota*), lettuce (*Lactuca sativa*), and cucumbers (*Cucumis sativus*).
2) Fruits, e.g., watermelon (*Citrullus lanatus*), tomato (*Solanum lycopersicum* L.), rough lemons (*Citrus jambhiri* L.), figs (*Ficus carica* L.), lemons (*Citrus Limon* L.), peaches (*Prunus persica*), and pomegranates (*Punica granatum*).
3) Cereal, e.g., wheat (*Triticum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and Hassawi rice (*Oryza sativa* L.).
4) Hassawi alfalfa (*Medicago sativa* L.) and clover (*Trifolium* L.) crops.

Fertiliser use in the Al-Hassa eastern oasis

Fertilisers are usually used every two or three years, where the most commonly applied fertiliser for date palm trees is cattle farmyard manure (AlMadini, 2001). A few kilograms (kg) of N fertiliser per hectare (ha) such as urea with the manure is a common practice amongst farmers, and depends on the farmer's financial status (AlMadini, 2001). For the other crops, different types of fertilisers are used, such as phosphorus (P) fertilisers (e.g., mono and triple super phosphate), 46% N as urea (AlMadini, 2001) and ammonium nitrate (NH_4NO_3) (AlMadini, 2011). Saudi Arabia started to support and develop organic agriculture in 2005, owing to the Saudi Ministry of Agriculture (MOA) and Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) (Hartmann et al., 2012). In 2012, 16400 ha became designated as an organic area (78 farms) in Saudi Arabia and around 2200 ha are currently under development to become organic areas (Hartmann et al., 2012). These changes in agriculture management practices may influence crop production (Chirinda et al., 2010), soil physical and chemical properties (Chirinda et al., 2010;

AlMadini, 2011; Brar et al., 2015), nutrient use efficiency (Chirinda et al., 2010), and soil N transformations (Zhang et al., 2012).

2.1.3 Essential nutrients for plant nutrition in arid soils

To achieve good produce yield and normal growth, plants require light, temperature, water, and essential nutrients in appropriate quantities (FAO, 2006). There are sixteen essential nutrients for crop growth. Three of them are taken up from the atmosphere in the form of water and carbon dioxide (i.e., H, O_2, and C), and the other thirteen are taken up from the soil, which are classified into two groups (macronutrients and micronutrients) according to the plant, where an appropriate amount is needed in a balanced ratio for each plant (FAO, 2006). Macronutrients required for plant growth include nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), sulphur (S), and magnesium (Mg). Micronutrients required by the plant include iron (Fe), zinc (Zn), manganese (Mn), chlorine (Cl), copper (Cu), boron (B), and molybdenum (Mo). In the soil of arid regions, soil properties are generally characterized by low organic matter (OM), neutral to slightly high calcium carbonate ($CaCO_3$) and pH content, as well as some expected nutrient concentrations (Table 2.2) (FAO, 2006).

Table 2.2 Some nutrient concentrations' range in arid regions' soil (Reproduce from FAO, 2006).

	Very low	Low	Medium	High	Very high
	(PPM or mg kg^{-1})				
NO_3^-	0 - 10	10 - 20	20 - 40	40 - 60	> 60
K	0 - 85	85 - 170	170 - 300	300 - 500	> 500
P	0 - 7	7 - 15	15 - 30	30 - 50	> 50
Ca	0 - 500	500 - 1200	1200 - 2500	2500 - 3500	> 3500
S	0 - 10	10 - 20	20 - 35	35 - 50	> 50
Mg	0 - 85	85 - 200	200 - 300	300 - 500	> 500
Fe	0 - 2	2 - 4	4 - 6	6 - 10	> 10
Zn	0 - 0.5	0.5 - 1.5	1.5 - 4	4 - 6	> 6
Mn	0 - 0.5	0.5 - 2	2 - 5	5 - 10	> 10
Cu	0 - 0.1	0.1 - 0.3	0.3 - 0.8	0.8 - 3	> 3
B	0 - 0.5	0.5 - 1	1 - 2	2 - 4	> 4
Mo	-	0 - 0.1	0.1 - 2	2 - 5	5 - 10
Na	-	-	0 - 300	> 300	-
$CaCO_3$	-	0 - 5	5 - 15	15 - 25	> 25

* Molybdenum levels > 10 ppm are toxic to plants.

* Soil salinity (EC of soil saturated extract, in mS cm^{-1}): 0 - 4 no hazard; 4 - 6 low hazard; 6 - 8 medium hazard; 8 - 10 high hazard; > 10 very high hazard.

2.2 Nitrogen cycling

N is a primary nutrient regulator of plant productivity in most terrestrial ecosystems. Desert ecosystems have different input sources and forms of organic and inorganic N; the latter may exist in any of eight different oxidation states (Table 2.3) (Robertson and Groffman, 2007). The earth's atmosphere is the source of all N in the soil and contains 79% (by volume) in the form of dinitrogen gas (N_2) (Schulten and Schnitzer, 1997). Most living organisms need combined N for carrying out their biological functions, while only some microorganisms have the capability to directly use N_2 and convert this into a form that is accessible to other plants and microorganisms (Schulten and Schnitzer, 1997). The conversion of N_2 into forms of N that have any biological function is described in Figure 2.3.

Table 2.3 Nine oxidation states of N in soil (Reproduce from Robertson and Groffman, 2007).

Name	Formula	Oxidation States
Organic N	$R\text{-}NH_2$	-3
Ammonium, Ammonia	NH_4^+, NH_3	-3
Dinitrogen	N_2	0
Nitrous oxide	N_2O	+1
Nitric oxide	NO	+2
Nitrite	NO_2^-	+3
Nitrogen dioxide	NO_2	+4
Nitrate	NO_3^-	+5

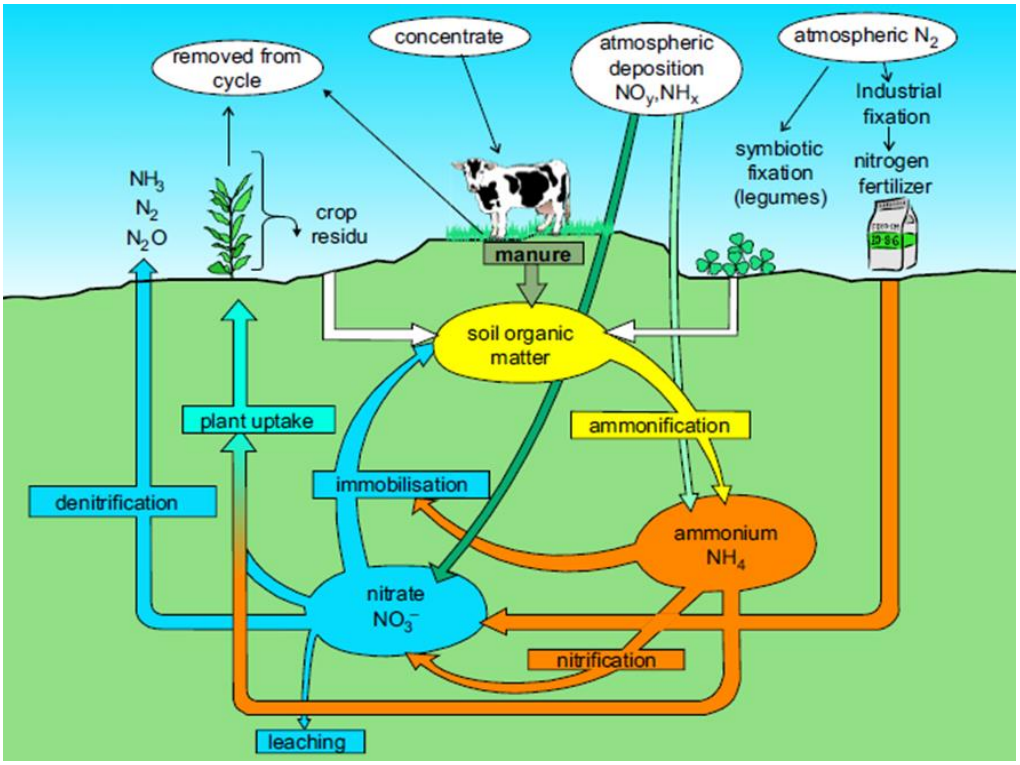


Figure 2.3 Soil N cycle (Erisman et al., 2007).

2.2.1 Nitrogen fixation

Nitrogen fixation is the reduction of N_2 gas into -3 oxidation states in NH_4^+ and NH_3 or +5 oxidation states NO_3^-. This can occur by some specialized bacteria that live in root nodules on plant roots (e.g., *Rhizobium*), cyanobacteria that symbiotic relationship with plant (symbiotic N fixation) or free-living bacteria (i.e., cyanobacteria) in the soil (non-symbiotic N fixation), or by combining hydrogen and N_2 to produce NH_3 (industrial fixation) (Schulten and Schnitzer, 1997; Tan, 2009; AlMulla, 2012). A supply of NH_4^+ or NO_3^- is essential for most plants because plants will take up N from the soil, and then use it to produce amino acids and other organic N compounds.

2.2.2 Nitrification

The nitrification process produces NO_3^- from NH_4^+ and occurs when the dissolved oxygen level in the soil is 1.0 mg l^{-1} or more. NH_4^+ is oxidized and converted to NO_2^- by *Nitrosomonas* bacteria. NO_2^- is subsequently oxidized and converted to NO_3^- ions by *Nitrobacter.* This rapid conversion into NO_3^- ions is beneficial as a result of the health effect of NO_2^-, which is toxic to plants and living organisms (Robertson and Groffman, 2007; AlMulla, 2012). Low molecular weight compounds (LMW) in the DON pool may affect the rate of nitrification and ammonification, because it will act as an important substrate for N transformation pathways such as C substrate. Once soil has sufficient C substrate, heterotrophic microbial growth (NH_4^+ immobilization) will be greater than autotrophic growth (NH_4^+ oxidation), where unavailable C substrate in the soil can stop microbial heterotrophs from immobilizing NH_4^+ but the nitrifier bacteria can still produce NO_3^- from oxidizing NH_4^+ (Jones et al., 2004).

2.2.3 Denitrification

Denitrification involves producing NO, N_2O, and N_2 from NO_3^-; this occurs when the dissolved oxygen levels are less than 0.5 mg l^{-1}, and ideally less than 0.2 mg l^{-1}. Anaerobic microorganisms will use NO_3^- as an alternative respiratory electron acceptor to gain O_2, which will reduce it into NO_2^-. Then, NO gas could produced from the reduction of NO_2^- by microorganisms. After that, N_2O gas could produced from the reduction of NO and finally to N_2, which returns to the atmosphere (Egboka, 1984; Robertson and Groffman, 2007; Cole, 2008). Anaerobic microorganisms release N in the forms of NO, N_2O, or N_2 as described earlier. Chemical

denitrification can occur in aerobic conditions without help from microbial enzymes and release NO or NO_2 as a final product (Tan, 2009). These products (i.e., NO or NO_2) will remain in the soil and form nitric and nitrous acid due to rapidly dissolving in soil water (Tan, 2009).

2.2.4 Ammonification (mineralization)

Ammonification, which is also known as mineralization, is a critical process that produces inorganic forms (i.e., NH_4^+ and NO_3^-) from organic nitrogen compounds such as urea-based fertilisers, crop residues, sludge, manures and soil organic matter (Robertson and Groffman, 2007; AlMulla, 2012). This can occur by microorganisms that use C from organic nitrogen compounds as a source of energy, fulfilling needs for nutrients (e.g., N) and supporting growth (Robertson and Groffman, 2007) or by soil enzymes (Jones et al., 2008; Tan, 2009). Many soil enzymes are involved in breaking down organic residues, large proteins, amino sugars, and amino acids into simple inorganic N. For example, proteases produce peptides by breaking down proteins, oxidases and dehydrogenases will produce amino group by breaking down amino acids, and deaminases will produce NH_4^+ by breaking down LMW DON (Jones et al., 2008; Tan, 2009). Net mineralization will occur when the C:N ratio of the substrates in $<$ 25:1 (Jones et al., 2004; Robertson and Groffman, 2007).

2.2.5 Immobilization

Immobilization is the production of organic nitrogen from inorganic nitrogen NO_3^- and NH_4^+ by microorganisms or plants (Robertson and Groffman, 2007; Tan, 2009). Microorganisms or plants will take inorganic nitrogen NO_3^- and NH_4^+ up as food and convert them into organic N compounds (Robertson and Groffman, 2007; Tan, 2009). The immobilization process is changing the chemical form of N without affecting N contents in the soil and reducing leachate and gaseous losses (Tan, 2009). The immobilization will occur when the substrate C:N ratio exceeds 25:1 (Robertson and Groffman, 2007).

2.2.6 Plant uptake

Plants can take up N as organic N or inorganic forms (Figure 2.4) (Hill et al., 2011b). There are many forms of organic N that plants can take up, such as amino sugars, amino acids, or peptides (Robertson and Groffman, 2007). Most plants can

take up NO_3^- and NH_4^+ by the roots. NO_3^- is highly soluble in water, and can flow to root surfaces by both mass flow and diffusion. NH_4^+ is attracted to soil particles and typically only a small amount is present in soil water (Robertson and Groffman, 2007).

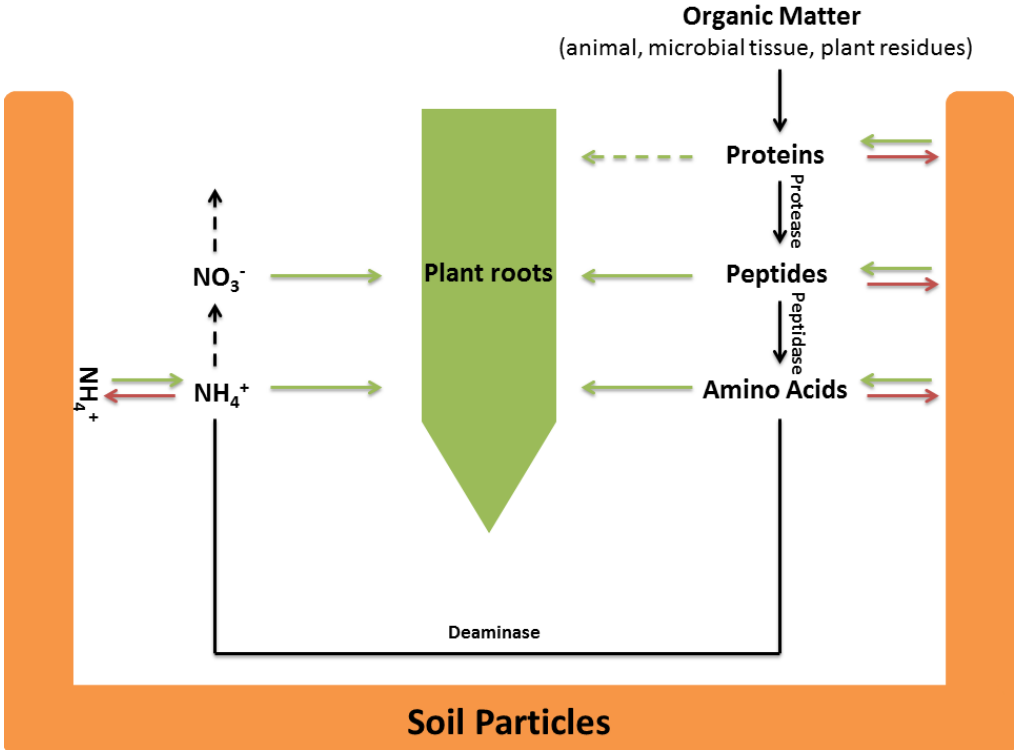


Figure 2.4 Plant roots' uptake of different soil N forms (Modified from Hill et al., 2011b).

2.3 Soil organic nitrogen and carbon

Soil dissolved organic N (DON) and C (DOC) can be defined as the soluble organic N and C present in soil solution (Murphy et al., 2000; Ros et al., 2009; van Kessel et al., 2009). It can also be defined as the soil organic N and C fraction, which is collected in-situ using suction cups, Rhizon, soil moisture samplers, or lysimeter, where no extraction is used. DON and DOC plays an important role in mineralization, N leaching, and plant uptake processes (Ros et al., 2009; van Kessel et al., 2009). On the other hand, Extractable Organic N (EON) or Soluble Organic N (SON) and C (SOC) can be defined as N or C extracted from soil by shaking field-moist or dried soils with a salt solution. Many salt solutions are used, such as potassium chloride (KCl), calcium chloride ($CaCl_2$), potassium sulphate (K_2SO_4), or water for a short duration, followed by filtering or centrifugation to isolate the solid phase from the solution phase (Ros et al., 2009; van Kessel et al., 2009). However, DOC and DON

consists of many individual components, ranging from high molecular weight compounds (HMW) such as cellulose, hemicellulose, lignin, chlorophyll, DNA, RNA and proteins, to low molecular weight compounds (LMW) such as, sugars (e.g., glucose), amino sugars, amino acids, short peptides, purines and urea (Christou et al., 2006; Hill et al., 2008; Ge et al., 2010).

2.3.1 Sources of DON

There are many sources of DON inputs into soil (Table 2.4). Each source depends on the nature of the natural or the agro ecosystem, its spatial input pattern (topsoil versus subsoil, rhizosphere versus bulk soil), and its temporal dynamics (e.g., time of day and seasonality) (Jones et al., 2008). DON may also come from wet and dry deposition (e.g., rainfall, irrigation water), plant canopy throughfall, residues of roots and organisms, microbial exudation, organic fertiliser additions, and urine and faeces of livestock (Christou et al., 2006; Jones et al., 2008; Ge et al., 2010). The concentrations of DON is varied from low (e.g., rainfall, irrigation water, plant canopy throughfall, residues of roots and organisms, and microbial exudation) to high (e.g., urine and faeces of livestock, green manure, industrial waste, and biosoilds) depending on the source (see Table 2.4; Jones et al., 2008).

Table 2.4 The main inputs of DON into soil (Reproduce from Jones et al., 2008).

Sources	Major Characteristics
Rainfall	Typically low concentrations of DON (< 1 mg N l^{-1}; DON comprises ca. 10 - 30% of the total N)
Irrigation Water	Typically low concentrations of DON (0.1 - 5 mg N l^{-1})
Canopy throughfall	Typically low concentrations of DON (0.1 - 10 mg N l^{-1}; concentrations higher from trees than crops)
Rhizodeposition	Extremely variable release rates depending upon species and environmental conditions
Microbial exudates	Known to occur but rarely quantified; ecological significance largely unknown in soil environments
Livestock urine	Very high concentrations of DON (100 - 5000 mg N l^{-1}; Dominated by urea)
Livestock faeces	High concentrations of DON (10 - 500 mg N l^{-1} but dependent upon age and type of faeces
Human-made fertilisers	Extremely high concentrations of DON but localized
Green manures and composts	High concentrations of DON but highly dependent upon source
Industrial waste	Dependent upon the source it can be high (abattoir waste) or low (paper waste)
Biosolids	High concentrations of DON but lesser than inorganic N concentrations

2.4 Carbon cycling

Carbon can enter the soil in the form of animal excreta and remains, plant litter, dead root all are consumed by soil organisms (e.g., heterogeneous population)

which is the important part of global C cycle (White, 2009). The decomposition of SOM can increase the CO_2 emissions to the atmosphere leading to enhanced global warming (Boddy et al., 2007; White, 2009). The annual average emission of CO_2 increased 5.08% from 2005 to 2015 and it represent 1.90% of global CO_2 emission (624.5 million tonnes in 2015; BP, 2016). Soil organic N (SON) and C cycles are linked; both immobilization (C:N ratio > 25 increase net immobilization and microbial biomass) and mineralization (C:N ratio < 25 increase net mineralization) of N pathways are linked by heterotrophic microorganisms that require C from organic material for production of energy and growth (Robertson and Groffman, 2007; White, 2009; Fisk et al., 2015). Plant and microbial residues are the major inputs of organic matter into soil and most losses are from microbial decomposition (by bacteria and fungi; Hill et al., 2008) of organic C that return CO_2 to the atmosphere (Christou et al., 2006; Jones et al., 2008; Simfukwe et al., 2011). Plant residues can be separated into two fractions (Simfukwe et al., 2011). The soluble fraction generally consists of a mixture of common plant exudate compounds (e.g., organic acids, sugars, and amino acids) that easily turn over in soil within hours. The second fraction, the insoluble fraction, are generally compounds with high molecular weight that contain complex plant polymers (e.g., some proteins, lignin, cellulose, and hemicellulose) which typically turnover over days or months (Simfukwe et al., 2011; Glanville et al., 2012). The soluble fraction is regularly released to soil from root exudation, microbial and plant cells lysis, and throughfall (Simfukwe et al., 2011), whereas the insoluble fraction tends to be the less easily degraded components of plant residue. Dissolved organic C (DOC) is continuously changing pool of C, soil organic material, litter, root may increase the C pool whereas, mineralization, microbial adsorption, mineral adsorption, leachate may reduce the pool.

Microbial species can use extracellular enzymes to breakdown the polymers (i.e., insoluble plant material) into low molecular weight compounds (LMW) (i.e., soluble plant material, peptides, amino acids, glucose, etc) that can be taken up by microbes and used for both anabolic and catabolic pathways (Hill et al., 2008). In general, there are many factors affecting microbial species diversity that can influence the rate of SOM decomposition (e.g., arid climate, extreme temperature see Chapter 3 and 4, low soil pH, soil poor drainage, waterlogging see Chapter 5, or excess fertilizer see Chapter 6 all will reduce soil biomass and decrease species

diversity; see Figure 2.5; White, 2009) and release plant-available N. Therefore, we need to increase our understanding of the influence of moisture regimes and irrigation water source on mineralization of soluble and insoluble plant materials in arid agricultural systems such as those found in KSA (see Chapter 5).

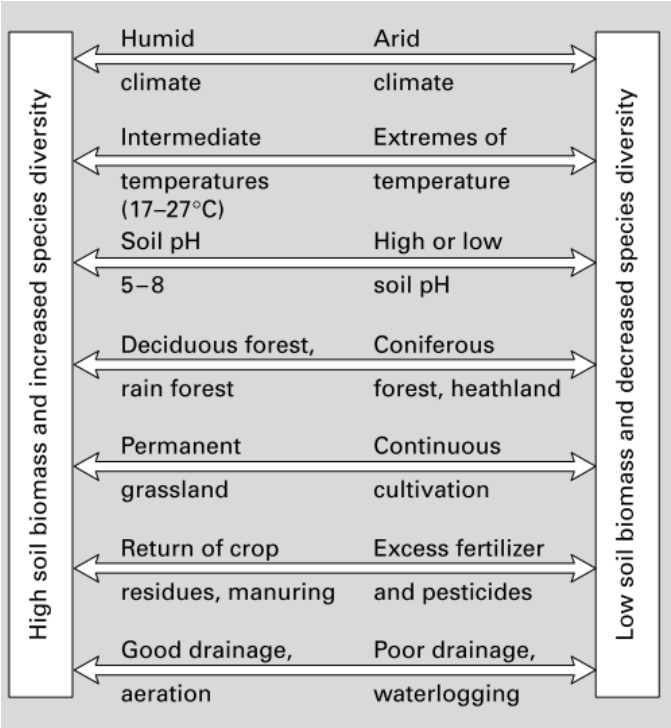


Figure 2.5 General factors affecting species diversity (White, 2009).

2.5 Factors affecting concentrations of N and C in soil

There are many factors that affect the concentrations of N and C in soil, such as, soil texture (Sollins et al., 1996; Zhang and Wienhold, 2002; van Kessel et al., 2009; Filep and Rékási, 2011; Cai et al., 2016), soil depth (Barber, 1995; Deenik, 2006; Ros et al., 2009), soil pH (Barber, 1995; Aciego and Brookes, 2008; Filep and Rékási, 2011), electrical conductivity (EC) (Zhang and Wienhold, 2002; Christou et al., 2006; Filep and Rékási, 2011; Shah and Shah, 2011; Yan and Marschner, 2013; Gao et al., 2014), soil carbon to nitrogen (C:N) ratio (Robertson and Groffman, 2007; Batlle-Aguilar et al., 2011), calcium carbonate ($CaCO_3$) content (Christou et al., 2006; Bashour and Sayegh, 2007; Ros et al., 2009; Filep and Rékási, 2011), temperature (Barber, 1995; Jones et al., 2004; Deenik, 2006; Cookson et al., 2007; Ghani et al., 2007; AlMulla, 2012; Butler et al., 2012), and moisture content (Barber, 1995; Deenik, 2006; Robertson and Groffman, 2007; Ros et al., 2009; van Kessel et al., 2009). These factors will discussed in the following sections.

2.5.1 Soil texture

Soil texture can affect the N and C cycle from the percentage of sand, silt, and clay contents. In general, there is a positive correlation between clay content with organic matter content or moisture content as discussed before organic matter content is low in arid ecosystem. The content of DON and DOC compound will be higher (e.g., after fertiliser applications, microbial exudation, ...etc) in soil with clay texture compared to sand texture this could be due to the ion concentration of clay and accumulation of organic compounds through an increase in the negative charge of the molecules (Zhang and Wienhold, 2002; Filep and Rékási, 2011). There is also a positive correlation between silt+clay with organic matter, which can be associated with the presence of organo-mineral complex as a result of sorption of organic anion by anion exchange (Sollins et al., 1996; Filep and Rékási, 2011). Turnover times of soil organic matter ranged 100 - 300 years with silt+clay fraction, and about 15 - 50 years with sand fraction (Cai et al., 2016). These differences could be due to large surface area and ion exchange reactions that involves adsorption and binding of organic matter, increased micro-aggregation, and decreased microbial decomposition in the clay fraction (Cai et al., 2016). According to Cai et al. (2016), there is a lower mineralization of soil organic C and N in the silt+clay fraction than in the sand fraction in both non-cultivated and cultivated soil. DON leaching is also expected to decrease with increasing clay content, as a result of DON binding to clay minerals (Figure 2.6) (van Kessel et al., 2009).

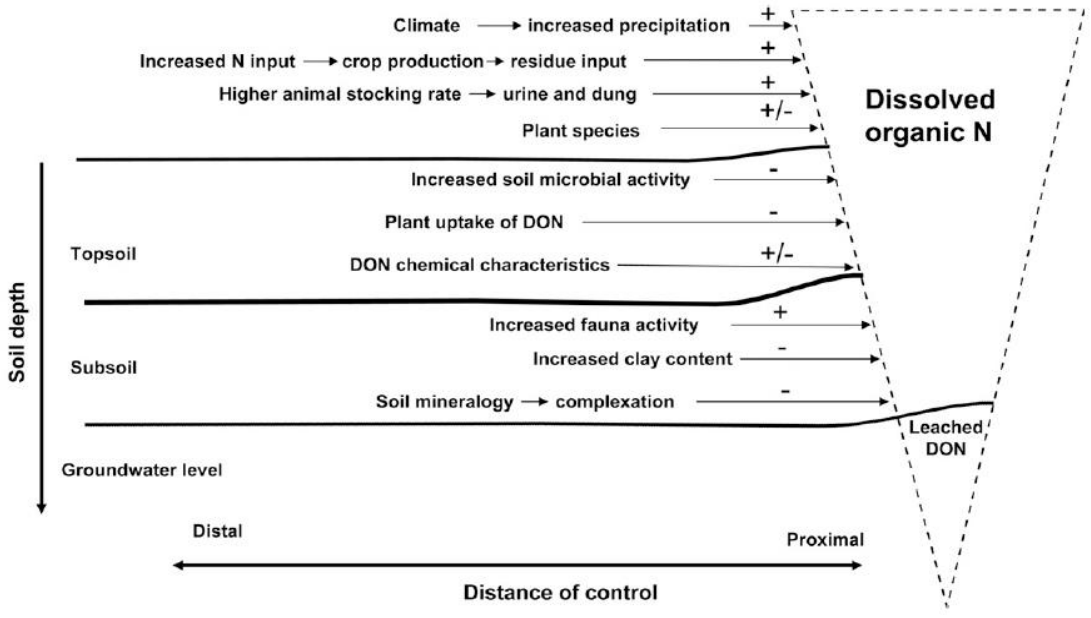


Figure 2.6 Factors influencing dissolver organic N leaching (van Kessel et al., 2009).

2.5.2 Soil depth

Soil depth affect nitrification, denitrification and mineralization processes. The denitrification process will increase, whereas nitrification and mineralization processes decrease with increasing soil depth because of increasing moisture content, decreasing organic matter (e.g., DOC and DON content), microbial activity and oxygen level in the soil due to higher rate of irrigation water (high rate application in arid soil). The latter, in particular, results in microorganisms converting to using NO_3^- as an electron acceptor in place of oxygen, which will reduce NO_3^- to NO_2^-. NO_2^- is then further reduced by microorganisms to NH_4^+ due to microorganisms will use NO_2^- as an electron acceptor in place of oxygen (Barber, 1995; Deenik, 2006; Ros et al., 2009).

2.5.3 Soil pH

The uptake of NO_3^- and NH_4^+, nitrification, denitrification, and DOM (that contain DOC and DON) decomposition are affected by soil pH. The uptake of NH_4^+ by plants increases and NO_3^- decreases with increasing pH (Barber, 1995). Barber (1995) found that increasing the pH from 5.0 to 7.8 would decrease one-third of NO_3^- influx. The nitrification and denitrification processes will increase with increasing soil pH. NH_4^+ concentrations will decrease and NO_3^- will increase with increasing pH from 5.0 up to approximately 8.0. While in low pH, the nitrification process and DOM decomposition are reduced, albeit not stopped, it will increase with increasing soil pH (Barber, 1995; Aciego and Brookes, 2008; White, 2009). However, there is a negative correlation between DON and pH: DON will decrease with increasing soil pH from 6.0 to 8.0. This could be due to increased microbial activity that can consume the high concentrations of H^+ ions and decompose the organic matter in soil, which will increase the availability of soluble molecules and reduce DON and DOC concentrations (Aciego and Brookes, 2008; Filep and Rékási, 2011). In addition, it could be due to higher soil microbial biomass and higher species diversity occur at soil pH between 5 - 8 pH unit (White, 2009) that increase the DOC and DON mineralization, leading to increase CO_2 emissions, and reduce the DOC and DON content from soil.

2.5.4 Electrical conductivity (EC)

EC is affected by many factors such as soil temperature, soil type, ion concentrations, and moisture content (Zhang and Wienhold, 2002). The mineralization, immobilization, and plant uptake processes would be affected by soil EC. The mineralization process will increase; immobilization and plant uptake processes will decrease with increasing EC under aerobic conditions as a result of increases in both cation concentration, ion strength, and accumulation of organic compounds through an increase in the negative charge of molecules (Zhang and Wienhold, 2002; Filep and Rékási, 2011). Other studies have shown that high salinity can reduce C and N mineralization by supressing extracellular enzyme activity, reducing microbial biomass, and inhibiting microbial growth due to osmotic stress, ion toxicity, sodic condition, and reduction of essential nutrient absorption (Shah and Shah, 2011; Yan and Marschner, 2013; Gao et al., 2014).

2.5.5 Carbon to nitrogen (C:N) ratio

An important factor affecting overall soil organic matter turnover rates is the carbon to nitrogen (C:N) ratio (Robertson and Groffman, 2007; Batlle-Aguilar et al., 2011). Once soil residues have a high C:N ratio $>$ 25:1 and the soil does not provide enough N to the microbes, immobilization will occur, which in turn decreases inorganic N and increases organic N in the soil (Robertson and Groffman, 2007; Batlle-Aguilar et al., 2011). When the C:N ratio is low, i.e. $<$ 25:1, the soil has enough N to sustain microbial processes, and thus, mineralization occurs, which increases inorganic N and decreases organic N in the soil. Microbial N uptake is affected by organism growth efficiency; for example, fungi can grow more efficiently on low N substrate than bacteria as a result of having wider C:N ratio in their tissues (Robertson and Groffman, 2007).

2.5.6 Calcium carbonate content ($CaCO_3$)

$CaCO_3$ has a negative correlation with DON and DOC concentrations. DON and DOC will decrease with increasing $CaCO_3$ as a result of high Ca^{2+} concentrations from DOM flocculation or adsorption by cation bridges and microbial consumption of DOM (Ros et al., 2009; Filep and Rékási, 2011). Increasing concentrations of $CaCO_3$ will affect soil chemical and physical characteristics, and its distribution down the soil profile affects soil-water relationships. Soil water movement and N leaching will decrease with increasing $CaCO_3$ (Bashour and Sayegh, 2007).

The soil pH will increase as a result of the presence of $CaCO_3$ (Christou et al., 2006). Soil pH and $CaCO_3$ have a significant effect on microbial productivity, therefore providing a link between soluble organic matter and insoluble organic compounds (Filep and Rékási, 2011).

2.5.7 Soil temperature

According to Barber (1995), nitrification, mineralization and plant uptake processes will increase with increasing temperature. NO_3^- uptake increased when temperature increased between 5 and 30°C, while the maximum rate of NO_3^- uptake will occur in the range between 20 and 25°C. At lower temperatures (8°C) the uptake of NH_4^+ by plants is greater than NO_3^-; nitrification and mineralization (organic C and N) processes will reduce as a result of the reduction of microbial activity at low soil temperature (Barber, 1995; Deenik, 2006; White, 2009; AlMulla, 2012). The higher soil microbial biomass and higher species diversity occur at soil temperature between 17 - 27°C depending on microbial species (White, 2009) that increase the DOC and DON mineralization leading to increase CO_2 emissions. On the other hand, leaching of DON will increase as a result of the reduction of microbial activity at low soil temperature (Ghani et al., 2007), whereas at high temperatures, at 23°C, the NO_3^- uptake/absorption becomes greater than NH_4^+. Nitrification and mineralization processes will increase when temperatures are in the range of 30 and 35°C as a result of increasing microbial activity in nitrifying bacteria and decreasing microbial immobilization of N, which leads to increasing N availability for plants (Barber, 1995; Deenik, 2006; AlMulla, 2012; Butler et al., 2012). In addition, at high temperature, the LMW DON and DOC mineralization and N immobilization rate in the soil will increase as a result of decreasing C availability from the soil by soil respiration and microbial biomass, which constrains microbial heterotrophs from immobilizing NH_4^+, which causes decreasing DOC availability. Therefore, N mineralization rate is greater than N immobilization rate (Jones et al., 2004; Cookson et al., 2007; Paul, 2007). Soil temperature enhance SOM or plant residue decomposition by stimulating the diffusion of substrate in soil or induce a shift in microbial community composition (Paul, 2007). According to Paul (2007) increasing temperature from 15 to 25°C tends to increase the rate of C and N mineralization up to threefold due to increase in biological activity from the shift in microbial community structure. Whereas, at cold weather the decomposition is reduced and C tends to accumulate (Paul, 2007).

However, the loss of gaseous N and C will decrease as a result of increasing available mineralized N for microbial and plant uptake (Butler et al., 2012).

2.5.8 Moisture content

Moisture content is varied between different soil texture and environment. For example, arid ecosystem is characterized by high temperature with slight and irregular rainfall therefore low moisture content. Regular irrigation system into arid site will increase the moisture content and could generate waterlogging in poor drainage soil (with excessive irrigation in poor soil drainage). That leads to decrease soil biomass and species diversity (White, 2009) that decrease the DOC and DON mineralization In general, moisture content can affect nitrification, denitrification and mineralization (Barber, 1995; Deenik, 2006; Robertson and Groffman, 2007; Ros et al., 2009). NO_3^- content decreases and the level of NH_4^+ will increase with increasing moisture content as a result of increasing soil water content (i.e., after regular application of water irrigation at high rate), reducing the level of oxygen diffusion, which decreases the nitrification process and increases the denitrification process (Barber, 1995; Deenik, 2006; Robertson and Groffman, 2007; Ros et al., 2009). According to Paul (2007) the decomposition of SOM is inhibited at low oxygen level and C tends to accumulate. The mineralization process will decrease with increasing moisture content as a result of limited microbial activity at high soil water content and low oxygen level (Barber, 1995). According to Li et al. (2014), the lowest mineralization rate was at 20%, 80%, and 100% WHC. At low WHC, cellular desiccation of microbes occurred, whereas at high WHC, the activity of aerobic microbes decreased due to inhibition of air exchange and increased denitrification. At 10% WHC the microbial activity is limited due to limiting the diffusion of available nutrients and reducing water potential inside the microorganism to prevent dehydration, whereas at 90%, reduced oxygen diffusion due to high water content and therefore oxygen availability can limit microbial activity, thus reducing enzyme production, which is key to substrate mineralization (Agehara and Warncke, 2005; Glanville et al., 2012). According to Glanville et al. (2012) increasing soil water content tends to increase nutrient flow and changing in microbial community structure. NO_3^- and some DON will leach from the soil with increasing water content as a result of their solubility in soil water (Figure 2.7) (van Kessel et al., 2009).

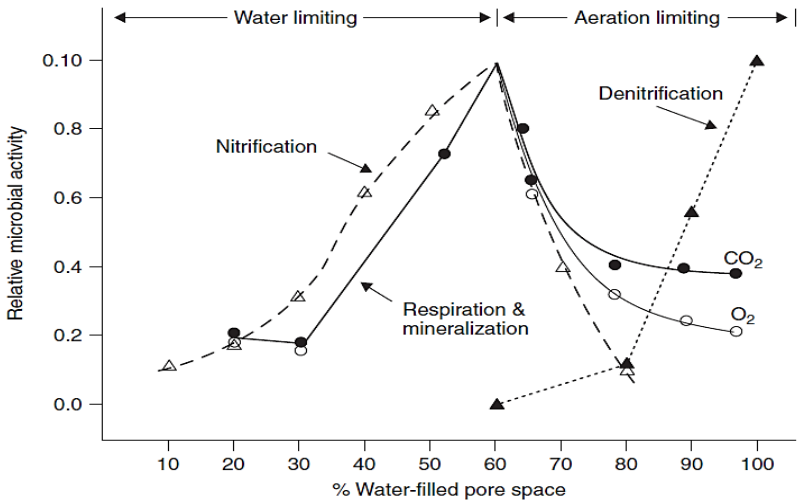


Figure 2.7 Moister content affects the microbial activity of nitrification and denitrification processes (Robertson and Groffman, 2007).

2.6 Conclusions

Arid and semiarid ecosystems are important to study because they cover one-third of the world's land area (Cable et al., 2008) and relatively little is known about DON and DOC cycling in these ecosystems, particularly in the Middle East. One of the largest date suppliers and most influential agriculture area in the KSA is the Al-Hassa eastern oasis (Al-Jabr, 2002). Currently, this oasis obtains more than 64% of its total irrigation water from alternative sources, with 24% coming from TTWW and 40% from MW (Al-Kuwaiti, 2010). In addition, some of its farms have or are converting to organic agriculture (Hartmann et al., 2012). These changes in agriculture management practices may influence soil physical and chemical properties (Chirinda et al., 2010; AlMadini, 2011; Brar et al., 2015), nutrient use efficiency (Chirinda et al., 2010), and soil N transformations (Zhang et al., 2012). We know that mineralization is a critical process within the C and N cycle (Robertson and Groffman, 2007) in arid ecosystems and it is controlled by many factors such as temperature, soil moisture content, soil management, vegetation litter, microbial community and soil organic matter (Simfukwe et al., 2011; Glanville et al., 2012; Qiu-Hui et al., 2012; Li et al., 2014). Therefore, the aim of this research is to expand our fundamental understanding on the impacts of changing soil temperature (Chapter 3 and 4), water irrigation source (Chapter 5) and fertiliser types (Chapter 6) on soil C and N cycles within arid ecosystems to develop more sustainable agriculture systems in terms of both nutrient and water use efficiency and reduce C and N emissions.

Chapter 3. Understanding the impacts of temperature on DON and DOC mineralization in arid non-agricultural soils

A.Z. AlMulla, D.L. Jones, P. Roberts

School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd LL57 2UW, UK

Abstract

Saudi Arabia (KSA) is currently susceptible to global warming, with a predicted rise in temperature from 1.8 - 4.1°C by 2050. An increase in soil temperature may influence the rate of low molecular weight (LMW) DON and DOC compound utilization by soil microbial communities, thereby increasing losses of soil organic matter and greenhouse gases. It is important to increase our understanding of the main factors controlling the rate of C and N turnover in arid soils to develop better management regimes and reduce N or C gaseous emissions. Little is known about DON and DOC cycling in arid soils such as those found in KSA. The aim of this research is to further our understanding to enable prediction of the effects of temperature rise on fundamental soil processes, and to further investigate soil N and C substrate cycling in arid systems by: 1) studying the effect of aridity on DOC and DON mineralization in three contrasting typical arid sites (mountain 1, mountain 2, and desert soil; that different in soil texture and salinity); and 2) determining the rate of DOC and DON mineralization by soil microorganisms at different incubation temperatures (10, 20, 30, 40, and 50°C). We show that the production of $^{14}CO_2$ is affected by soil type and follows a pattern of mountain 1 = desert > mountain 2. Our results from these soils showed a significant effect of soil type on microbial use efficiency (Mic_{eff}) and followed the pattern of mountain 2 > desert = mountain 1. In addition, there was an increase in the $^{14}CO_2$ evolution with increasing temperature resulting in a positive non-linear correlation. Temperature tended to increase the pool size of rapidly mineralized substrate (a_1) and decrease substrate half-life ($t_{1/2}$) in most cases. The rate of LMW DON and DOC utilization in the different soils was related to differences in their physical and chemical properties, and microbial community structure. Our findings have increased our understanding of the effect of future soil temperature rises in arid systems on the microbial mineralization rate of DON and DOC, and greenhouse gas emissions. Therefore, it is important to focus the attention of HIDA and KSA farmers about the effect of future soil temperature rises in arid systems and to develop appropriate soil management practices that reduce N or C gaseous emissions.

Keywords: microbial mineralization rate, Al-Hassa eastern oasis, mountain, desert

3.1 Introduction

Global warming is predicted to raise average temperatures in Saudi Arabia (KSA) between 1.8 - 4.1°C by 2050 (Chowdhury and Al-Zahrani, 2013). In addition, it is predicted that more extreme temperatures will occur (≥ 55°C). An increase in soil temperature may reduce soil moisture and increase evapotranspiration across the kingdom (Chowdhury and Al-Zahrani, 2013). The changing of soil temperature is expected to affect soil nutrient status (e.g., increasing C and N mineralization process) through increasing maintenance cost of microorganisms this may cause an accumulation of nitrate, organic nitrogen, and salts over time (Ewing et al., 2008; Baumann and Marschner, 2013). Increasing organic substrate tends to stimulate the mineralization rate with greater CO_2 release resulting in priming effect (Rukshana et al., 2013) depending on many factors (e.g., soil texture, microbial community, soil temperature, moisture content, …etc). According to Cai et al. (2016), there is a lower mineralization of soil organic C and N in the silt+clay fraction than in the sand fraction in both non-cultivated and cultivated soil. These differences could be due to large surface area and ion exchange reactions that involves adsorption and binding of organic matter, increased micro-aggregation, and decreased microbial decomposition in the clay fraction (Cai et al., 2016). However, high soil salt contents in solution can negatively affect the soil's microbiological, chemical, and physical properties (Shah and Shah, 2011). High soil salt contents in solution can negatively affect the soil's microbiological, chemical, and physical properties (Shah and Shah, 2011). Increasing soil salinity can cause osmotic stress, which reduces the activity and size of the microbial community and decreases soil respiration rates (Shah and Shah, 2011; Baumann and Marschner, 2013; Yan and Marschner, 2013).

The following changes can be expected when salinity increases: 1) an exponential decline of extracellular enzyme activity involved in C mineralization; 2) inhibition of microbial growth due to ion toxicity (e.g., Na^+ and Cl^-); 3) reduction in microbial biomass may cause a major decline in N mineralization; 4) reduction in fungal population leads to a reduction in the decomposition of complex organic materials; and 5) decrease in ammonification, nitrification, immobilization and mineralization of N (Shah and Shah, 2011).

Little is known about DON and DOC cycling in arid soils such as those found in KSA. Therefore, it is important to increase our understanding of the impact of

potential increases in temperature and climate change in KSA on the rate of DON or DOC mineralization by soil microorganisms in typical arid sites (i.e., non-agricultural arid soils). This will enable better management of these soils and may help reduce N or C gaseous emissions. Soil microorganisms are require C from DON and DOC for production of energy and growth in both N pathway (i.e., immobilization and mineralization) (Robertson and Groffman, 2007; White, 2009; Fisk et al., 2015). We therefore hypothesize that: 1) increasing silt, clay and salinity will reduce the rate of utilization of DON and DOC compounds due to the reduction of microbial activity; 2) the mineralization rate of DON and DOC increases with increasing temperature due to temperature tends to increase maintenance cost of microorganisms; and 3) the mineralization rate of DON is higher than for DOC in arid soils because they are likely to be processed by different metabolic pathways inside the cell. In order to examine these hypotheses, we used ^{14}C labelling techniques to determine DON and DOC mineralization rates in response to different temperature regimes in non-agricultural arid soils. We further determined various soil physical and chemical characteristics, and microbial community structure of the different soils to help explain the observed differences in C and N turnover.

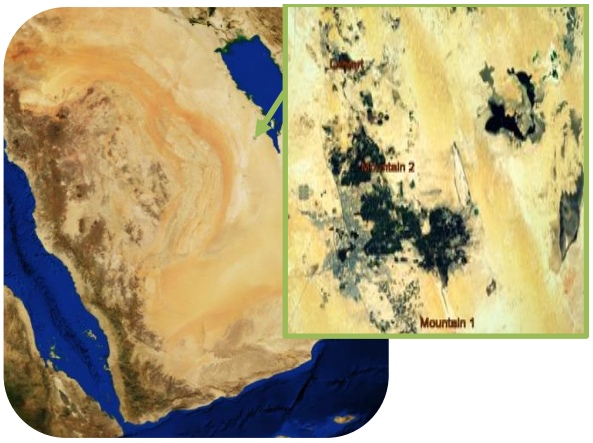
3.2 Material and methods

3.2.1 Soil sampling

The samples were collected from three sites. Four independent replicates were collected from three blocks (0 - 30 cm depth) from three non-agricultural sites (independent replicates in each blocks were pooled into one replicate due to no significant differences was observed between each independent replicates) located in the Al-Hassa eastern oasis, KSA. Sites 1 and 2 were different mountain sites located at 25°17.362`N 049°43.049`E and 25°28.212`N 049°38.170`E respectively; site 3 was a desert located at 25°35.347`N 049°34.786`E, as shown in Figure 3.1. All samples were collected during the fourth week of April 2013. We chose these sites to investigate the typical arid environment without any effect from irrigation and fertiliser and improve our fundamental understanding of N and C cycle process. Soil was collected from the topsoil 0 - 30 cm using an auger in multiple samplings across a *ca.* 6 m x 6 m block. Each individual sample was stored in a clean, labelled,

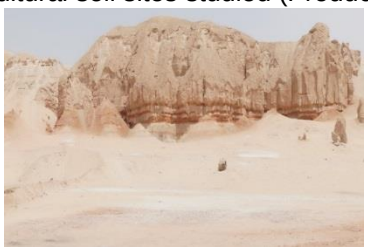
oxygen-permeable plastic bag. All soils were cooled (4°C) then immediately shipped to the UK for analysis.

For each of these sites, the annual mean rainfall is about 70 mm. The average annual temperature is 28 - 47.5°C, with an average of 34.1°C, where the highest temperature recorded is 50°C. The air humidity is remarkably high for arid systems, and could reaching 90% in summer (Al-Fredan, 2011; Aldakheel, 2011). The average daily soil temperature at the non-agricultural arid sites typically varies from 16.3 to 35.5°C at 20 cm depth, 13.4 to 31.5°C at 50 cm depth, and 12.1 to 35.1°C at 100 cm depth, as shown in Figure 3.2 (AlKhouly et al., 2004).



Map of non-agricultural soil sites studied (Produced by the author)

Mountain 1



Mountain 2

Desert

Mountain 1 soil profile

Mountain 2 soil profile

Desert soil profile

Figure 3.1 Photographs of the three non-agricultural soil sites studied.

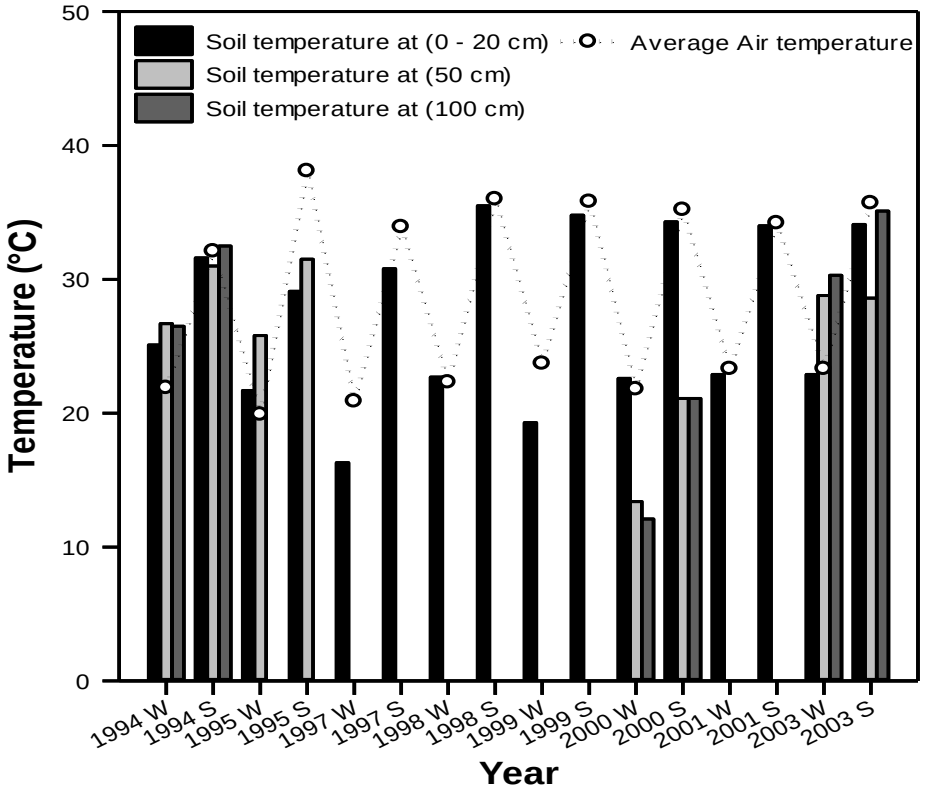


Figure 3.2 Air temperature, soil temperature at 20, 50, and 100 cm depth in non-agricultural arid soil from Al-Hassa from 1994 - 2003 (AlKhouly et al., 2004). W indicate winter and S indicate summer.

3.2.2 Physical and chemical soil properties

Soil texture was measured using the Bouyoucos method according to Bashour and Sayegh (2007). pH and EC were determined by standard electrodes (1:2.5 w/v soil:distilled water extraction). The moisture content (MC) was determined gravimetrically by drying the samples in an oven at 105°C overnight, and organic matter (OM) content was determined by ashing the samples in an oven at 550°C overnight. $CaCO_3$ was measured by the titration method (Bashour and Sayegh, 2007). Total elements were analysed by digesting the biological materials from dried soils with 70% HNO_3 (1:4 w/v) for 1 h at 100°C and filtered through a Whatman filter paper No. 541 according to US-EPA method 3051A (2007). Na was measured using a Model 410 flame photometer (Sherwood Scientific, Cambridge, UK) (Bashour and Sayegh, 2007; Burden et al., 2013) and Mg using a Spectr*AA* 220FS atomic absorption spectrometry (Varian Inc., Palo Alto, CA). The remaining elements (e.g., Al, Ca, ..etc) were measured using a S2-Picofox TXRF (Burker Inc., Billerica, MA). Soil C:N ratio was determined using a CHN2000 analyser (Leco Corp., St Joseph, MI). Fresh soils were extracted with 0.5 M K_2SO_4 (1:5 w/v) to determine available P,

phenolics, NO_3^-, NH_4^+, protein and amino acids. Available P, phenolics, NO_3^-, NH_4^+, and protein were measured colourimetrically using a Powerwave XS spectrophotometer (Biotek Instruments Inc., Winooski, VT). Available P was measured using the ammonium molybdate-sulphuric acid (AMES)/ascorbic acid assay (Murphy and Riley, 1962). Phenolics was measured using the Folin reagent and sodium carbonate (Swain and Hillis, 1959); NO_3^- by vanadium (III) chloride reduction and N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD) (Miranda et al., 2001). NH_4^+ was determined using the sodium salicylate-sodium nitroprusside with hypochlorite (Mulvaney, 1996), and the Bradford method was used to determine protein (Roberts and Jones, 2008). Amino acids were determined using a fluorescence spectrophotometer according to Jones et al. (2002). Soil solutions were analysed for total dissolved N (TDN) and DOC using a Multi-N/C 2100 S TOC/TN analyser (Analytik-Jena AJ, Jena, Germany). DON concentrations = TDN concentrations - (NO_3^- + NH_4^+ concentrations). Microbial biomass N and C was determined by measuring them before and after 7 days of chloroform fumigation-0.5 M K_2SO_4 (1:5 w/v) extraction (K_{EN} = 0.5; K_{EC} = 0.35) according to Hill et al. (2013), and soil respiration (SR) was measured using an automated CIRAS-SC soil respirometer at 20°C (PP Systems Ltd, Hitchin, UK).

3.2.3 Microbial community structure

Microbial community structure was measured by phospholipid fatty acid (PLFA) analysis following the method of Buyer and Sasser (2012). Samples (2 g) were then freeze-dried with Bligh-Dyer extractant (4.0 ml) containing added internal standard. Tubes were sonicated in an ultrasonic cleaning bath for 10 min at room temperature before rotating end-over-end for 2 h. After centrifuging (10 min), the liquid phase was transferred to clean 13 mm × 100 mm screw-cap test tubes and 1.0 ml each of chloroform and water added. The upper phase was removed by aspiration and discarded, while the lower phase, containing the extracted lipids, was evaporated at 30°C. Lipid classes were separated by solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well (Phenomenex, Torrance, CA, USA). Phospholipids were eluted with 0.5 ml of 5:5:1 methanol:chloroform:H_2O (Findlay, 2004) into glass vials, and the solution evaporated (70°C, 30 min). Trans esterification reagent (0.2 ml) was added to each vial, and the vials sealed and incubated (37°C, 15 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were

added. The chloroform was evaporated just to dryness and the samples dissolved in hexane. An Agilent (Agilent Technologies, Wilmington, DE, USA) 6890 gas chromatograph (GC) equipped with auto sampler, split-split less inlet, and flame ionization detector was used. FAMEs were separated on an Agilent Ultra 2 column, 25 m long × 0.2 mm internal diameter × 0.33 µm film thickness. FAMEs were identified using the MIDI PLFAD1 calibration mix and naming table.

3.2.4 Determining the depletion of ^{14}C-substrate

10 g of soil from each site was placed in a 50 ml labelled centrifuge tube and 0.5 ml (10 µM; 3.7 kBq ml^{-1}) of uniformly radiolabelled ^{14}C glucose, an equimolar mixture of 15 ^{14}C amino acids (L-alanine, L-arginine, L-aspartic acid, L-leucine, L-tyrosine, L-valine, L-phenylalanine, glutamic acid, threonine, proline, L-lysine, L-histidine, glycine, L-isoleucine, L-serine), or $^{1\text{-}14}$C trialanine (that labelled the carboxyl group), injected into the soil surface. The addition of substrate in liquid form will increase the moisture content into arid soil (i.e. the average of water holding capacity of soil sites studied in this experiment increased from 9% to 21% after the addition of substrate) that will help microbial population to uptake the substrate faster. These substrate were chosen due to they represent the major component of soil organic matter (SOM) decomposition, root exudation, and organic N cycle (Jones et al., 2005a; Hill et al., 2008; Jan et al., 2009). Triplicate samples were incubated after addition of labelled ^{14}C at different temperatures (10, 20, 30, 40, and 50°C; total n = 135). The production of $^{14}CO_2$ over time was measured by replacing a polypropylene vial containing 1 ml of 1 M NaOH trap at fixed time intervals of 0.5, 1, 3, 6, 24, 48, 168, 336, 504, and 672 h. $^{14}CO_2$ was determined by using a Wallac 1409 liquid scintillation counter (PerkinElmer Inc., Waltham, MA) with 4 ml of OptiPhase Hi-safe 3 scintillation cocktail (PerkinElmer Inc.). After the removal of the NaOH trap at 672 h, the soils were shaken for 20 min at 200 rev min^{-1} with 25 ml of 0.5 M K_2SO_4 to extract any remaining unmetabolized ^{14}C label from the soil (Farrell et al., 2011; Roberts and Jones, 2012). The extracts were measured by liquid scintillation counting as described earlier. The same experiments were conducted on sterilized soil (autoclaved at 121°C, 1 h and cooled for 10 min in room temperature) to confirm that the release of $^{14}CO_2$ is a biological process and not due to abiotic mineralization of the substrate (Boddy et al., 2008; Hill et al., 2008).

The ^{14}C-labelled substrate mineralization from the soil was described by double first-order exponential decay model:

$$f = [a_1 \times \exp^{(-k_1 t)}] + [a_2 \times \exp^{(-k_2 t)}]$$

Where: f is the amount of ^{14}C label remaining in the soil. a_1 describes the pool size of rapidly mineralized substrate, k_1 is the exponential coefficient describing depletion by the soil biomass in the first rapid phase of $^{14}CO_2$ production from catabolic processes (respiration). a_2 describes the pool size of the slower second phase of $^{14}CO_2$ production, k_2 is the exponential coefficient describing depletion by second slower phase of $^{14}CO_2$ production from immobilized or taken up labelled ^{14}C within microbial community, and t is time.

The half-life ($t_{1/2}$) of first pool a_1 was defined as:

$$t_{1/2} = \ln(2) / k_1 \text{ (Boddy et al., 2008; Hill et al., 2008)}$$

The total microbial uptake ($^{14}C_{uptake}$) can be defined as:

$$^{14}C_{uptake} = {}^{14}C_{Tot} - {}^{14}C_{K_2SO_4} \text{ (Roberts and Jones, 2012)}$$

Where: $^{14}C_{Tot}$ is the total amount of labelled ^{14}C added to the soil and $^{14}C_{K_2SO_4}$ is the labelled ^{14}C recovered in K_2SO_4 extraction.

The amount of labelled ^{14}C incorporated into the microbial biomass ($^{14}C_{mic}$) was quantified as:

$$^{14}C_{mic} = {}^{14}C_{Tot} - {}^{14}CO_2 - {}^{14}C_{K_2SO_4} \text{ (Roberts and Jones, 2012)}$$

Where: $^{14}CO_2$ is the total amount of labelled ^{14}C recovered in the NaOH traps.

The C use efficiency of microbial biomass (Mic_{eff}) was determined as:

$$Mic_{eff} = {}^{14}C_{mic} / ({}^{14}C_{mic} + {}^{14}CO_2) \text{ (Roberts and Jones, 2012)}$$

3.2.5 The effects of temperature on $^{14}CO_2$ respiration rate from substrate

The influence of temperature on $^{14}CO_2$ respiration rate from each substrate was determined by measuring added labelled ^{14}C at different incubation temperatures (10, 20, 30, 40, and 50°C). A square root of the linear-relationship between $^{14}CO_2$ production and temperature modified from Rousk et al. (2012) and Birgander et al. (2013) was used to describe the relationship between $^{14}CO_2$ and temperature. The duration of the incubation period was 0 - 168 h for 10°C and for the other temperatures it was 0 - 48 h for 20°C, 0 - 24 h for 30°C, 0 - 6 h for 40°C, and 0 - 3 h for 50°C. These times reflected the linear part of the $^{14}CO_2$ evolution plots.

3.2.6 Statistical analysis

Statistical analyses (ANOVA with Tukey's pairwise comparison) were performed using SPSS 20 (SPSS Inc., Chicago, IL) or Minitab 16 (Minitab Inc., State College, PA, USA) with significance differences set at $P \leq 0.05$ unless otherwise stated. The double first-order exponential decay equation was fitted to experimental results using Sigmaplot 12.3 (SPSS Inc., Chicago, IL, USA). Linear regression models describing ^{14}C mineralization rate and temperature were made using Sigmaplot 12.3. We calculated √ rate of ^{14}C substrate mineralization by the following equation:

√ rate of ^{14}C substrate mineralization = √ (C atom in substrate (e.g., C atom in glucose = 6) X C molecular weight (i.e., C MW = 12) X $^{14}CO_2$ evolution plots at specific time (see section 3.2.6) / 100) / specific time (see section 3.2.6).

3.3 Results

3.3.1 General physical and chemical soil properties

Physical and chemical characteristics of the soils tested in this study are presented in Table 3.1. Soils were classified as being sand or sandy loam textured (Certini and Scalenghe 2006) with very low to medium OM contents according to Boulding (1994), and low to very high NO_3^- concentrations according to Bashour and Sayegh (2007). There were significant differences between all physical and chemical characteristics of soils except DON, DOC, microbial N, microbial C, and available P. Mountain 1 showed the lowest content of silt, EC, OM, $CaCO_3$, Na, Al, S, Cl, K, Ca, Mg, Cr, Mn, Fe, total C (TC), total N (TN), CN ratio, NO_3^-, NH_4^+, amino acids, and soil respiration but the highest sand, pH, protein, and phenol of the three soil sites. Mountain 2 showed the highest value of silt, clay, EC, OM, $CaCO_3$, Na, K, Mg, Cr, Mn, Fe, TN, NO_3^-, NH_4^+, and soil respiration, but the lowest sand, pH, protein and DOC/DON ratio. The desert soil showed the highest Al, S, Cl, Ca, TC, CN ratio, amino acids, and DOC/DON ratio, but had the lowest phenol content of the three sites.

The highest pH value occurred in mountain 1, with about 0.10 pH unit higher than the desert and 0.56 pH unit higher than mountain 2 ($P \leq 0.05$). Mountain 2 showed the highest EC content between all three sites and about 7-fold more than desert and 42-fold more than mountain 1 ($P \leq 0.05$). The highest OM content was

seen in mountain 2 with about 2-fold or 8-fold more content than mountain 1 and the desert respectively. Mountain 2 showed the highest $CaCO_3$ contents for all soils and contained 29% more than mountain 1 and 113% more than the desert soil ($P \leq 0.05$). The highest Na was observed in mountain 2 across all sites, with about 38 and 486-fold more than desert and mountain 1 respectively. Desert soil showed the highest TC content across all sites, with about 7% and 152% more than mountains 2 and 1 respectively. The highest TN content was observed in mountain 2, with about 2-fold more content than desert and mountain 1. The desert soil had the highest C:N ratio, with about two times more content than mountain 1 and 2. NO_3^- concentrations were highest in mountain 2 with about 6 and 17-fold more than desert and mountain 1 respectively this could be due to livestock faeces and urine that accumulate with time in mountain 2. Mountain 2 showed the highest NH_4^+ concentrations, with about 11 and 18-fold more than desert and mountain 1. The desert soil had the highest amino acids concentrations, with about 2-fold more content than mountains 1 and 2. Mountain 1 showed 2-fold and 3-fold protein concentration compared to desert and mountain 2 respectively.

3.3.2 Microbial community structure

Soil microbial community structure for the three sites are presented in Figure 3.3. Significant differences exist between soil sites; the amount of Gram-negative bacteria followed a pattern of mountain 1 > desert > mountain 2, while Gram-positive bacteria was only detected in desert soil. Actinomycetes was the only soil microbial group present in mountain 2. The highest amount of actinomycetes was observed in mountain 2 about 3 and 4-fold more than mountain 1 and desert. There were significant differences between total microbial biomass measured by PLFA and followed desert soil > mountain 2 = mountain 1 with the concentrations of 3.79, 1.83, 1.38 nmol g^{-1} respectively.

Table 3.1 Physical, biological and chemical characteristics of the soils used in the experiments. Values represent mean ± SEM (n = 4). Different letters identify significant differences between sites ($P \leq 0.05$), and no letters shows there are no significant differences.

Soil	Mountain 1	Mountain 2	Desert
Sand (%)	94.00 ± 0.00^{a}	55.00 ± 1.33^{c}	70.00 ± 0.01^{b}
Silt (%)	4.00 ± 0.00^{c}	39.00 ± 1.78^{a}	27.00 ± 0.67^{b}
Clay (%)	2.00 ± 0.00^{b}	6.00 ± 1.15^{a}	3.00 ± 0.67^{ab}
Soil Texture	Sand	Sandy Loam	Sandy Loam
pH	8.28 ± 0.01^{a}	7.72 ± 0.11^{c}	8.18 ± 0.02^{b}
EC (mS cm^{-1})	0.49 ± 0.04^{c}	20.26 ± 3.89^{a}	2.76 ± 0.34^{b}
OM (%)	0.34 ± 0.02^{c}	2.69 ± 0.32^{a}	1.09 ± 0.13^{b}
$CaCO_3$ (%)	13.75 ± 0.14^{c}	29.25 ± 1.96^{a}	22.75 ± 2.31^{b}
Na (g kg^{-1})	0.02 ± 0.02^{b}	11.25 ± 2.14^{a}	0.29 ± 0.12^{b}
Al (g kg^{-1})	0.37 ± 0.11^{b}	0.50 ± 0.06^{b}	1.27 ± 0.14^{a}
S (g kg^{-1})	0.03 ± 0.01^{b}	0.21 ± 0.05^{b}	9.40 ± 0.82^{a}
Cl (g kg^{-1})	0.10 ± 0.01^{b}	0.14 ± 0.002^{b}	0.32 ± 0.03^{a}
K (g kg^{-1})	0.35 ± 0.02^{b}	1.01 ± 0.02^{a}	1.01 ± 0.11^{a}
Ca (g kg^{-1})	16.82 ± 1.07^{b}	23.34 ± 1.29^{b}	70.02 ± 5.50^{a}
Mg (g kg^{-1})	2.19 ± 0.23^{b}	5.66 ± 1.19^{a}	3.98 ± 0.18^{ab}
Cr (g kg^{-1})	0.01 ± 0.0003^{c}	0.03 ± 0.002^{a}	0.02 ± 0.002^{b}
Mn (g kg^{-1})	0.03 ± 0.002^{b}	0.12 ± 0.02^{a}	0.08 ± 0.01^{a}
Fe (g kg^{-1})	1.11 ± 0.09^{b}	4.93 ± 0.58^{a}	2.45 ± 0.26^{b}
Total C (g C kg^{-1} soil)	5.85 ± 0.26^{b}	13.74 ± 2.60^{a}	14.74 ± 1.32^{a}
Total N (g N kg^{-1} soil)	0.25 ± 0.03^{b}	0.48 ± 0.05^{a}	0.29 ± 0.05^{b}
CN Ratio	25.40 ± 4.46^{b}	28.50 ± 3.71^{b}	53.49 ± 4.97^{a}
NO_3^- (mg N kg^{-1} soil)	6.73 ± 0.77^{b}	113.29 ± 34.10^{a}	17.49 ± 5.21^{b}
NH_4^+ (mg N kg^{-1} soil)	0.22 ± 0.14^{b}	4.04 ± 1.27^{a}	0.38 ± 0.25^{b}
Amino acids (mg N kg^{-1} soil)	0.05 ± 0.01^{b}	0.06 ± 0.01^{b}	0.10 ± 0.01^{a}
Protein (mg N kg^{-1} soil)	2.92 ± 0.04^{a}	0.85 ± 0.22^{c}	1.75 ± 0.31^{b}
DON (mg N kg^{-1} soil)	4.95 ± 2.99	12.04 ± 6.16	4.46 ± 1.50
DOC (mg C kg^{-1} soil)	9.48 ± 5.99	14.25 ± 11.10	16.78 ± 2.63
Microbial N (mg N kg^{-1} soil)	5.17 ± 2.64	13.62 ± 2.80	15.34 ± 5.87
Microbial C (mg C kg^{-1} soil)	31.85 ± 5.27	24.48 ± 5.19	45.14 ± 11.70
DOC/DON ratio	1.84 ± 0.19^{ab}	0.96 ± 0.34^{b}	4.50 ± 1.29^{a}
Phenolics (mg kg^{-1} soil)	5.72 ± 1.59^{a}	5.36 ± 1.59^{ab}	1.23 ± 0.02^{b}
Available P	1.55 ± 0.57	0.55 ± 0.49	2.09 ± 0.55
Soil respiration (μgCO_2 g^{-1} h^{-1})	0.03 ± 0.002^{b}	0.05 ± 0.01^{a}	0.04 ± 0.004^{a}

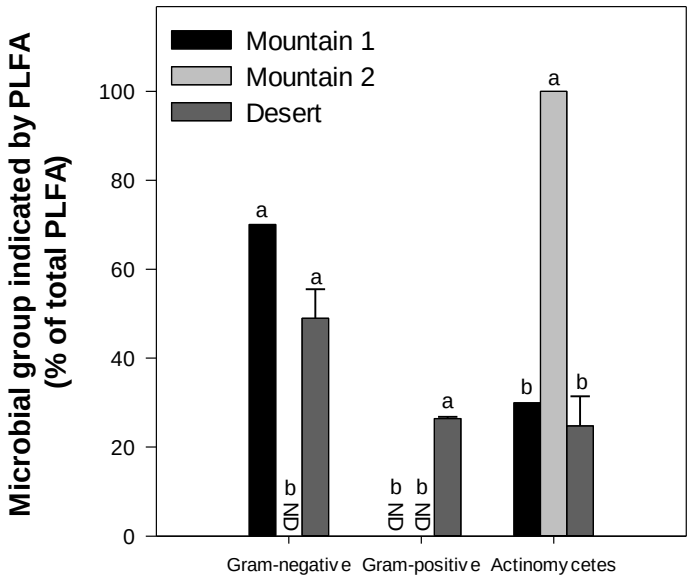


Figure 3.3 Abundance of soil microbial community indicator PLFAs in different non-agricultural soil sites. Values represent means ± SEM (n = 3). ND indicates not detected. Different letters identify significant differences between sites ($P \leq 0.05$), and no letters shows there are no significant differences.

3.3.3 DON and DOC compounds mineralization and half-life

The effect of temperature on different ^{14}C-substrate mineralization in non-agricultural arid soils are shown in Figure 3.4, while the kinetic parameters from double first-order exponential decay equation of ^{14}C-labelled substrate during the 672 h incubation period in different soils are presented in Table 3.2.

In almost all soils, significant effects were observed between soil sites and the production of $^{14}CO_2$ ($P \leq 0.05$). Mountain 2 showed the lowest production of $^{14}CO_2$ compared with soil sites with about 2-fold lower $^{14}CO_2$ production than desert and mountain 1 ($P \leq 0.05$). The production of $^{14}CO_2$ increased with increasing temperature in almost all cases ($P \leq 0.05$) as shown in Figure 3.4, except after the addition of ^{14}C glucose and ^{14}C trialanine in desert soils or after the addition of ^{14}C trialanine in mountain 1 ($P > 0.05$; Figure 3.4.C1, C3, and A3 respectively). Where temperature has an effect on mineralization, this is particularly between 20 and 30°C. Mountain 2 was the most affected soil site after increasing temperatures from 20 and 30°C with $^{14}CO_2$ production across all sites, and about 2-fold more after the addition of ^{14}C glucose and ^{14}C amino acids, where, after the addition of ^{14}C trialanine, it increased 4-fold compared to 20°C ($P \leq 0.05$). In addition, the most affected soil site on increase of $^{14}CO_2$ production after increasing temperature from

40 to 50°C was mountain 2, with about 11%, 12%, and 27% more $^{14}CO_2$ production after the addition of ^{14}C trialanine, ^{14}C glucose, and ^{14}C amino acids compared to 40°C.

The model pool size a_1 describes the amount of ^{14}C-substrates immediately mineralized by microbial community in catabolic processes. In all cases, there were significant differences between three soil sites with respect to the size of the a_1 pool ($P \leq 0.05$; Table 3.2), particularly after the addition of ^{14}C glucose at 10, 20, or 50°C ($P \leq 0.05$), which followed a pattern of mountain 1 = desert > mountain 2. a_1 pool size increased with increasing temperature between 20 and 30°C after the addition of ^{14}C glucose and trialanine in mountain 1. Temperature tended to increase pool a_1 size in mountain 2 after the addition of ^{14}C glucose between 10 and 20°C and between 20 and 30°C after the addition of ^{14}C trialanine, whereas in the desert soil, there is an increase of a_1 pool size between 20 and 30°C after the addition of ^{14}C glucose.

The shortest $t_{1/2}$ of glucose was observed in the desert soil (9.80 ± 0.87 h; Table 3.2). Amino acids $t_{1/2}$ was shortest in mountain 1 (2.74 ± 0.44 h). The desert soil showed the shortest $t_{1/2}$ of trialanine (4.42 ± 0.88 h). Some statistical differences were observed between $t_{1/2}$ of the three ^{14}C-substrate in the soils ($P \leq 0.05$; Table 3.2). In most cases, the $t_{1/2}$ of DON was shorter than for DOC. There were some significant differences in $t_{1/2}$ between soil types, following a pattern of mountain 2 > desert = mountain 1 in most cases ($P \leq 0.05$; Table 3.2). Only a small amount of unmetabolized ^{14}C label was recovered from the soil by 0.5 M K_2SO_4 at the end of 672 h incubation period (< 7% of the total ^{14}C applied in most cases; data not presented).

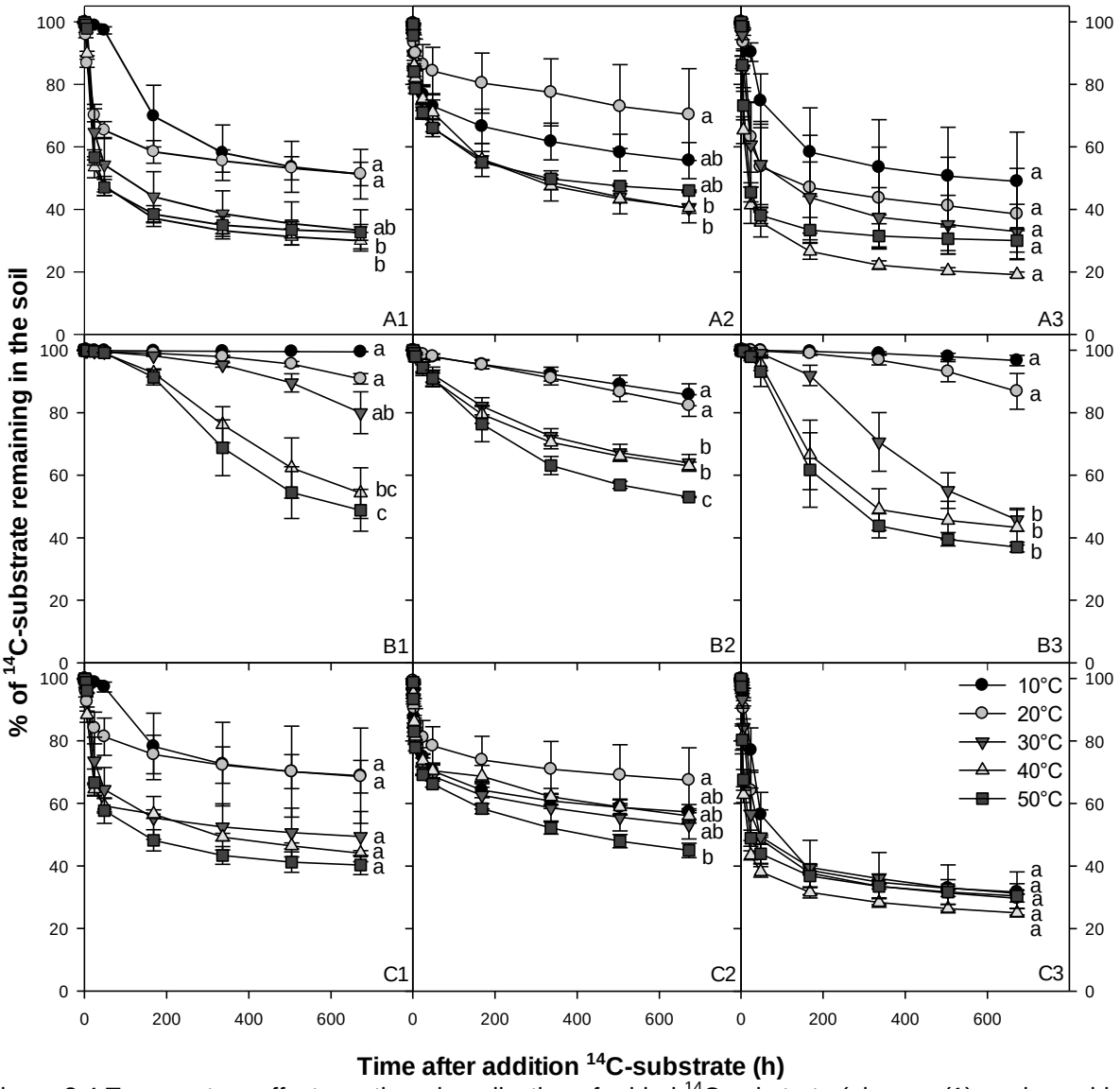


Figure 3.4 Temperature effects on the mineralization of added ^{14}C-substrate (glucose (1), amino acids (2), and trialanine (3)) in different non-agricultural soils (mountain 1 (A), mountain 2 (B), and desert (C)) during the periods of incubation 28 days. Points represent means ± SEM (n = 3). Letters indicate significant differences between temperature within the same substrate and site ($P \leq 0.05$).

Table 3.2 Influence of soil temperature on the modelled kinetic parameters describing the turnover of ^{14}C-labelled glucose, amino acids, and trialanine in different non-agriculture sites. Values represent mean ± SEM (n = 3). Significant differences between sites are indicated by different numbers; different lower case letters indicate different substrates ($P \leq 0.05$), different capital letters indicate significant differences between temperatures ($P \leq 0.05$), and no letters shows there are no significant differences.

Temperature (°C)	a_1		
	Glucose (%)	Amino acids (%)	Trialanine (%)
Mountain 1			
10	$^{AB}57.96 \pm 4.68^{1}$	$^{AB}26.11 \pm 4.22$	44.52 ± 16.77
20	$^{B}38.86 \pm 3.75^{1,2}$	$^{B}15.28 \pm 7.34$	54.83 ± 16.66
30	$^{AB}58.01 \pm 9.80$	$^{A}33.35 \pm 3.18$	53.84 ± 12.60
40	$^{A}64.20 \pm 3.21^{a}$	$^{AB}29.85 \pm 3.59^{b}$	70.13 ± 6.35^{a}
50	$^{A}66.50 \pm 3.40^{a1}$	$^{A}35.68 \pm 1.03^{b}$	67.23 ± 2.82^{a}
Mountain 2			
10	$^{B}7.88 \pm 3.72^{b2}$	34.29 ± 16.08^{ab}	$^{B}46.40 \pm 0.78^{a}$
20	$^{A}48.08 \pm 0.45^{1}$	32.38 ± 15.36	$^{B}48.09 \pm 0.75$
30	$^{A}48.75 \pm 0.48$	31.08 ± 7.56	$^{AB}65.41 \pm 15.11$
40	$^{A}49.03 \pm 0.45^{b}$	29.73 ± 7.63^{a}	$^{AB}67.61 \pm 0.48^{c}$
50	$^{A}57.15 \pm 6.90^{2}$	54.57 ± 14.80	$^{A}76.91 \pm 7.81$
Desert			
10	$^{AB}33.57 \pm 16.55^{ab1,2}$	28.05 ± 2.65^{b}	66.23 ± 2.21^{a}
20	$^{B}21.71 \pm 7.00^{b2}$	21.27 ± 5.61^{b}	63.18 ± 1.53^{a}
30	$^{AB}46.68 \pm 7.06$	32.05 ± 3.12	58.49 ± 10.62
40	$^{AB}44.58 \pm 2.70^{b}$	28.20 ± 2.54^{c}	67.67 ± 1.91^{a}
50	$^{A}53.60 \pm 4.16^{a2}$	32.89 ± 0.18^{b}	61.08 ± 3.59^{a}
Temperature (°C)	$t_{1/2}$		
	Glucose (h)	Amino acids (h)	Trialanine (h)
Mountain 1			
10	$^{A}211.07 \pm 87.05^{a2}$	5.21 ± 1.59^{b}	$^{A}44.75 \pm 6.42^{ab}$
20	$^{B}9.86 \pm 1.80^{a2}$	2.74 ± 0.44^{b}	$^{B}13.58 \pm 1.69^{a}$
30	$^{B}19.06 \pm 3.18^{ab2}$	3.55 ± 0.23^{a2}	$^{B}16.32 \pm 6.43^{b2}$
40	$^{B}13.34 \pm 0.97^{2}$	10.54 ± 7.38	$^{B}5.80 \pm 0.84a2$
50	$^{B}15.80 \pm 0.34^{2}$	8.47 ± 5.39	$^{B}7.85 \pm 2.09^{2}$
Mountain 2			
10	$^{A}6832.83 \pm 3426.84^{1}$	2773.23 ± 2116.34	50975.60 ± 39850.60
20	$^{AB}5775.00 \pm 1155.00^{1}$	1760.33 ± 976.26	9383.11 ± 6504.16
30	$^{AB}4042.50 \pm 1527.92^{a1}$	160.43 ± 68.48^{c1}	623.55 ± 110.22^{b1}
40	$^{B}883.67 \pm 263.41^{a1}$	126.09 ± 70.75^{b}	193.83 ± 76.35^{b1}
50	$^{B}589.00 \pm 204.94^{1}$	247.65 ± 144.81	191.53 ± 87.65^{1}
Desert			
10	$^{A}86.25 \pm 41.25^{2}$	6.95 ± 3.07	$^{A}35.31 \pm 12.49$
20	$^{B}15.50 \pm 6.90^{2}$	3.78 ± 0.61	$^{AB}18.65 \pm 4.64$
30	$^{B}21.34 \pm 4.80^{a2}$	3.05 ± 0.91^{b2}	$^{B}11.98 \pm 3.09^{ab2}$
40	$^{B}9.80 \pm 0.87^{a2}$	3.10 ± 0.36^{b}	$^{B}4.42 \pm 0.88^{b2}$
50	$^{B}17.74 \pm 2.00^{a2}$	3.04 ± 0.23^{b}	$^{B}4.62 \pm 1.42^{b2}$

3.3.4 Microbial biomass C use efficiency

Microbial C use efficiency gives an indication of relative partitioning of ^{14}C between the immobilized pool and the respired pool (immobilization to mineralization ratio). Microbial C use efficiency ranged between 0.19 ± 0.01 and 0.99 ± 0.00 (Figure 3.5). There was a significant effect of soil type on Mic_{eff}, generally following a pattern of mountain 2 > desert = mountain 1, except between soil sites after the addition of ^{14}C amino acids at 20°C or after the addition of ^{14}C trialanine at 30 or 50°C ($P > 0.05$). Temperature tended to decrease Mic_{eff}, particularly between 20 and 30°C in most cases ($P \leq 0.05$; Figure 3.5).

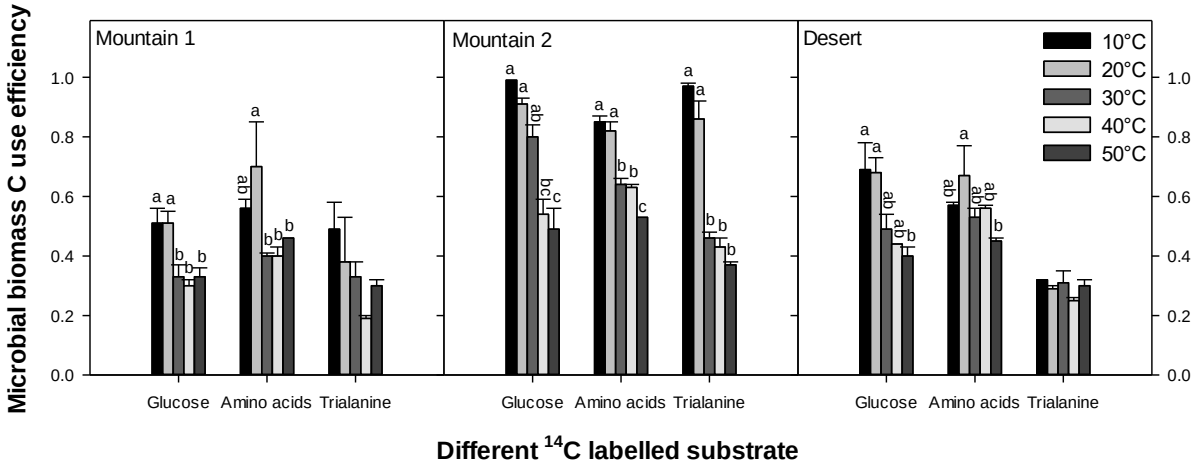


Figure 3.5 Effect of soil temperature on the microbial carbon use efficiency for different substrates in non-agricultural soils. Values represent means ± SEM (n = 3). Letters indicate significant differences between temperature in the same substrate and site ($P \leq 0.05$).

3.3.5 Temperature dependency of $^{14}CO_2$ respiration rate

For estimating the effect of temperature on instantaneous respiration rate of labelled substrates added to soil sites, we plotted the square root of linear-relationships between temperature and $^{14}CO_2$ respiration rate (Rousk et al., 2012; Birgander et al., 2013) (Figure 3.6). In most cases, temperature tends to increase the square root value of $^{14}CO_2$ respiration rate ($P \leq 0.05$). The only exceptions that decreased the square root value of $^{14}CO_2$ respiration rate were observed in mountain 1 and desert soil after the addition of ^{14}C glucose between 40 and 50°C ($P \leq 0.05$: Figure 3.6. A1 and C1). The square root relationships fit well with R^2 ranged from 0.85 to 0.99 in all cases (Figure 3.6). In almost all cases, mountain 2 showed the lowest square root value of $^{14}CO_2$ respiration rate across all sites, about 6-fold lower than desert soil and mountain 1.

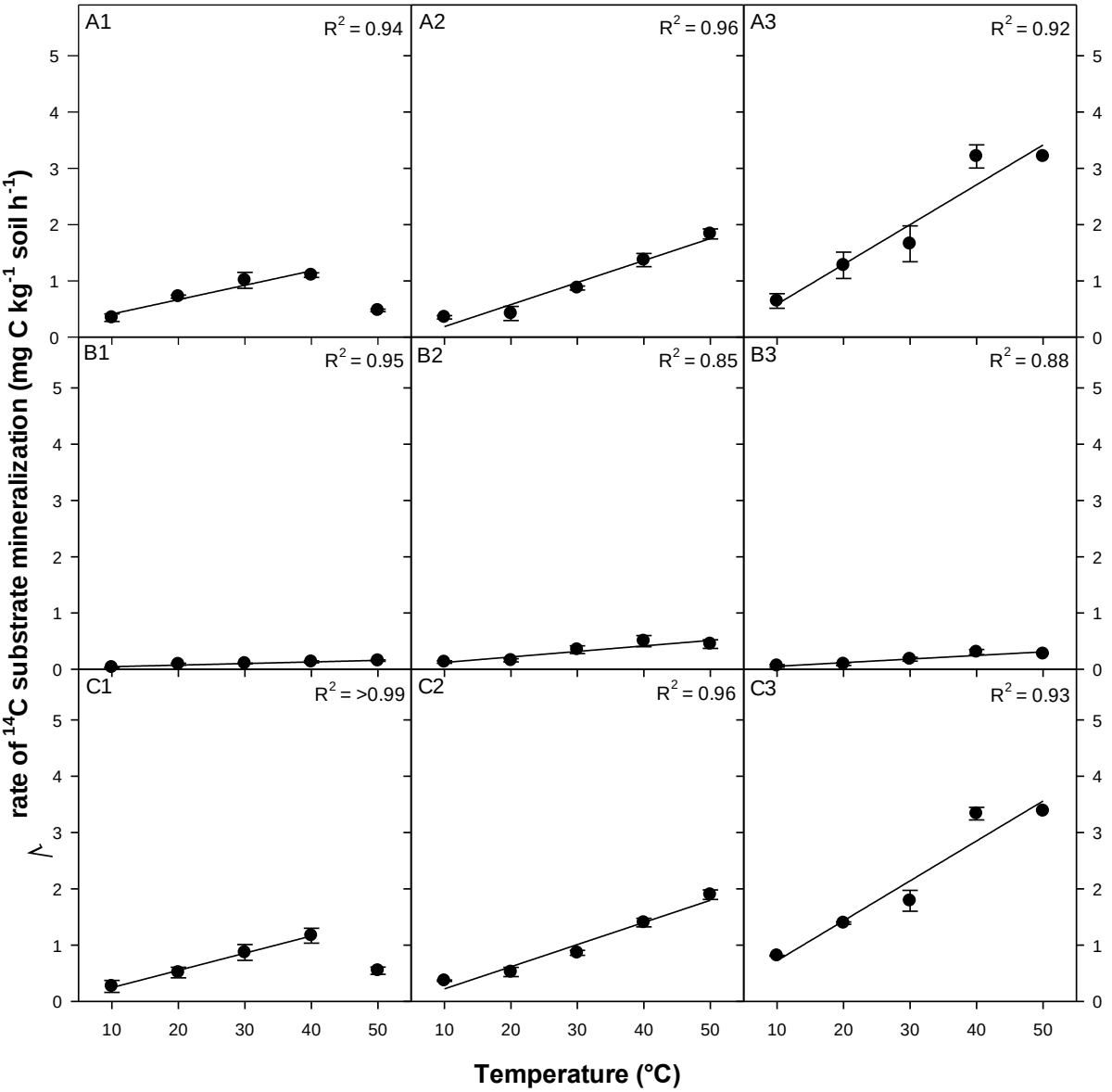


Figure 3.6 Relationship between soil temperature and the square root of mineralization rate for three different ^{14}C-substrates (glucose (1), amino acids (2), and trialanine (3)) in different non-agricultural soils (mountain 1 (A), mountain 2 (B), and desert (C)). Points represent means ± SEM (*n* = 3).

3.4 Discussion

3.4.1 Mineralization and half-life of DON compounds

Temperature, soil moisture content, soil management, vegetation litter, microbial community and soil organic matter - all influence the rate of microbial mineralization of DON and DOC in soil (Simfukwe et al., 2011; Glanville et al., 2012; Qiu-Hui et al., 2012; Li et al., 2014). This study focused only on the short-term effects of temperature changes on DOC and DON mineralization in three non-agricultural sites from the Al-Hassa eastern oasis in KSA. The range of temperature

in this study (10 - 50°C) was designed to reflect the actual temperature and predicted future increased temperatures resulting from global warming at the sites. To our knowledge, an evaluation of DON and DOC mineralization responses to temperature in non-agricultural oasis soils has not previously been examined. We examined the temperature factor on mineralization rate of LMW DON and DOC compounds to extend our understanding of C and N cycling in arid systems to develop better management options and reducing N or C gaseous emissions. We know that the depletion of ^{14}C glucose, ^{14}C amino acids and ^{14}C trialanine in soil can only happen as a result of either abiotic mineralization or microbial uptake (Hill et al., 2008; Jan et al., 2009). Here, we assume that the production of $^{14}CO_2$ after the addition of radiolabelled substrate was from microbial uptake, since no evolution of $^{14}CO_2$ was observed from the sterile treatments (data not presented).

We hypothesized that different arid soil sites would influence the rate that soil microbial communities utilize DON and DOC compounds due to differences in their soil properties (e.g., soil texture, chemistry and microbial community). Our results showed that the lowest $^{14}CO_2$ evolution across all non-agricultural soil sites at different temperature were observed in mountain 2. This could be due to the highest contents of silt, clay, salinity (EC, Na, K, and Mg), and actinomycetes observed in mountain 2 ($P \leq 0.05$; Table 3.1 and Figure 3.3). Higher silt and clay contents in mountain 2 could increase N immobilization, which could be due to clay minerals having a large surface area and ion exchange reaction that adsorbs immobilizing enzymes, some DOC, DON, and NH_4^+ to the surface of clay minerals (An et al., 2015) which may reduce the microbial activity. This is consistent with Cai et al. (2016), who found that there is a lower mineralization of soil organic C and N in the silt+clay fraction than in the sand fraction in both non-cultivated and cultivated soil. These differences could be due to large surface area and ion exchange reactions that involves adsorption and binding of organic matter, increased micro-aggregation, and decreased microbial decomposition in the clay fraction (Cai et al., 2016). Higher salinity in mountain 2 can decline N and C mineralization by reducing microbial biomass, decline extracellular enzymes activity, and inhibit microbial growth due to osmotic stress, ion toxicity, sodic condition, and reduction of essential nutrient absorption (Shah and Shah, 2011; Yan and Marschner, 2013). This is consistent with Gao et al. (2014), who found that microbial mineralization of N decreased at

higher salinities through the inhibition of microbial activity. Further studies are needed to investigate how increasing salinity in one control soil type influence microbial community composition, microbial biomass, and soil enzymes in the short-term in arid soil for developing better management and reducing N or C gaseous emissions. Actinomycetes was the only soil microbial group present in mountain 2; this could reduce $^{14}CO_2$ evolution at low temperatures, since most actinomycetes are mesophilic (i.e., growth temperature ranged from 20 to 42°C) or thermophilic (i.e., growth temperature ranged from 45 to 60°C) (Kurapova et al., 2012).

In most cases, the square root rate value of $^{14}CO_2$ respiration from DON was higher than the DOC rate value (Figure 3.6); this could be due to higher N and C contents that accumulate more C and N for a rapidly cycling pool rather than the soil microbial community structure defined by PLFA. This result is consistent with Farrell et al. (2013), who showed that microbial uptake rate of trialanine > dialanine > L-alanine and could be due to higher N and C contents in trialanine compared to amino acids pool. In addition, it could be due to methodical reason (i.e., we used $^{1\text{-}14}C$ trialanine that labelled the carboxyl group which can breakdown quickly by microbial population). It is logical to conclude, therefore, that the amino acids have a higher square root value of $^{14}CO_2$ respiration rate than glucose due to higher N and C contents and shorter $t_{1/2}$ of amino acids (Figure 3.6 and Table 3.2). In addition, it could be due to the production of organic acid skeletons from the rapid internal transamination and deamination of amino acids in the cell, which can be used directly in the respiration pathway (Jones et al., 2005a; Boddy et al., 2008). This result supports our hypothesis that the mineralization rate of DON is higher than for DOC in arid soils because they are likely to be processed by different metabolic pathways inside the cell.

3.4.2 Temperature dependency of ^{14}C respiration rate

We hypothesized that the mineralization rate of LMW DON and DOC compounds by soil microbial communities would be influenced by soil temperature. Our results showed that temperature tends to increase the pool size of rapidly mineralized substrate (a_1) and decrease $t_{1/2}$ in most cases. This result is consistent theoretically with Boddy et al. (2008), who showed that temperature tends to increase the partitioned ^{14}C into a_1 pool. Our results show that, in most cases,

respiration rate after the addition of DON and DOC substrate is positively correlated with temperature increases; this could be due to:

1) Increases in soil permittivity due to water movement in soil column with higher temperature, thus making the compound more mobile and less protected with in the soil matrix (Baumhardt et al., 2000).
2) Microbial communities favouring high temperature for uptake, turnover and metabolism of the added substrate. Agehara and Warncke (2005) show an increase in microbial respiration with increasing temperatures from 5 to 25°C due to shift in microbial communities' composition and activity with increasing temperature that increases the ability for metabolizing more substrate at a higher temperature than lower temperature. In this study, the results of $^{14}CO_2$ respiration continually increased even after 25°C suggesting that it could be due to higher size and activity of soil microbial community in arid soil at higher temperature that increases $^{14}CO_2$ evolution
3) Temperature tends to increase maintenance cost of microorganisms, which means that microbes will used more ^{14}C for respiration processes rather than for growth and storage (Boddy et al., 2008).

3.4.3 Microbial biomass C use efficiency

The results from these soil sites also show a significant effect of soil types on microbial use efficiency (Mic_{eff}), following a pattern of mountain 2 > desert = mountain 1 ($P \leq 0.05$; Figure 3.5). This supports our hypothesis that increasing silt, clay, and salinity will reduce the rate of utilization of DON and DOC compounds due to the reduction of microbial activity. Temperature tended to decrease Mic_{eff} after the addition of DON and DOC substrate into the arid non-agricultural soil studied in most of the cases. We suggest that temperature could affect the relative balance of microbial C gain through different metabolic pathways. This result is consistent with Schindlbacher et al. (2015), who showed that the microbial substrate (mixture of amino acids, amino sugars, sugars, and organic acids) use efficiency tends to decrease with increasing temperature, because changing temperature may affect different energy demanding processes such as increasing C respiration and microbial turnover rate. The highest affected value of Mic_{eff} with increasing temperature was found in mountain 2 (Figure 3.5); this could be due to soil microbial community structure in this site (only actinomycetes were detected; Figure 3.3).

According to Kurapova et al. (2012), most actinomycetes are mesophilic or thermophilic species with optimum growth temperature from 20 to 42°C for mesophilic or 45 to 60°C for thermophilic species, and high temperature activates the growth of the mesophilic or thermophilic actinomycetes species. This suggested higher size and activity of mesophilic or thermophilic actinomycetes in mountain 2 with increasing temperature that increases $^{14}CO_2$ evolution (Figure 3.4 B1 - B3) and decreases Mic_{eff} value (the higher Mic_{eff} value was found at 10°C; Figure 3.5). These results support our hypothesis that the mineralization rate of DON and DOC increases with increasing temperature due to temperature tends to increase maintenance cost of microorganisms.

3.5 Conclusions

The three non-agricultural soil sites used in this study differ in their soil chemical properties and ^{14}C-substrate mineralization patterns. Our results showed significant effect of soil types on ^{14}C respiration rate and this follows a pattern of mountain 1 = desert > mountain 2. The results from the soil sites also showed a significant effect of soil types on microbial use efficiency (Mic_{eff}) and followed a pattern of mountain 2 > desert = mountain 1, thus proving our hypothesis that increasing silt, clay, and salinity contents will reduce the rate of utilization of DON and DOC compounds due to the reduction of microbial activity. Our data showed that temperature tended to increase the pool size of rapidly mineralized substrate (a_1) and decrease $t_{1/2}$ in most cases. The results from the soil sites also showed a positive correlation between $^{14}CO_2$ respiration rates and increasing temperature after the addition of LMW DON or DOC into soil, thus proving our second hypothesis that the mineralization rate of DON and DOC increases with increasing temperature due to temperature tends to increase maintenance cost of microorganisms. These findings can be valuable to extend our understanding of the effects of temperature on the microbial mineralization rate of DON and DOC in arid non-agricultural systems for developing better management and reducing N or C gaseous emissions. Further studies are needed to investigate the effect of using different arid-agricultural soil sites on soil function, in particular the response of various temperature, moisture content, and N fertiliser on soil chemical characteristics, and DOC and DON mineralization rate.

Chapter 4. Substrate influences temperature sensitivity of dissolved organic carbon (DOC) and nitrogen (DON) mineralization in arid agriculture soils

A.Z. AlMulla[a,b], D.L. Jones[b], P. Roberts[b,*]

[a]*Department of Environmental Health, College of Applied Medical Sciences, University of Dammam, Dammam, KSA*

[b]School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd LL57 2UW, UK

[*]Corresponding author. Tel.: +44 1248 382976; +44 1248 354997. *E-mail address:* p.roberts@bangor.ac.uk (P. Roberts).

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Abstract

The bioavailability of nitrogen (N) in soil relies to a great extent on the progressive breakdown of plant- and microbially-derived necromass protein to peptide and amino acid components and their subsequent conversion to inorganic forms of N. While we have a good understanding of the fluxes and pathways of the N cycle downstream from amino acids, our understanding of the factors controlling peptide and amino acids mineralization, particularly in arid soils is lacking. To address this knowledge gap, we investigated the influence of temperature on the rate of dissolved organic carbon (DOC) and nitrogen (DON) cycling in agricultural soils from the Al-Hassa eastern oasis, Saudi Arabia. Soils were incubated in the laboratory with ^{14}C-labelled glucose, amino acids or trialanine at different temperatures (10, 20, 30 and 40°C) and $^{14}CO_2$ production evaluated over 28 d. Although the physical and chemical properties of the three arid soils differed markedly, PLFA analysis revealed that they had very similar topsoil and subsoil microbial communities. In addition, the soils behaved very similarly in terms of the rate of substrate use, microbial C use efficiency and response to temperature. Overall, substrate mineralization rate increased with temperature with significantly more C being allocated to microbial catabolic rather than anabolic processes. In conclusion, our results show that climate change is likely to lead to changes in soil organic matter turnover and shifts in C allocation pattern within the soil microbial community. This is expected to reduce soil quality and exacerbate nutrient losses (NO_3^- leaching and N gaseous emissions). Management strategies are therefore required to promote the retention of organic matter in these soils (e.g., more research on the soil quality is required to reduce environmental pollution).

Keywords: Carbon cycling; Groundwater; irrigation; Microbial uptake kinetics; Substrate-induced respiration; Wastewater.

4.1 Introduction

The Kingdom of Saudi Arabia (KSA) is potentially highly susceptible to global warming with temperatures predicted to increase by 1.8 - 4.1°C by 2050 (Chowdhury and Al-Zahrani, 2013). This increase in temperature is likely to impact upon both the availability and quality of water used for irrigating agricultural fields and the intrinsic amount of water present in the soil. For example, climate change is predicted to lead to an increased loss of soil water (43.80 - 237.25 mm $year^{-1}$), reduced relative humidity (0.8 - 2.3%), and increased evapotranspiration (76.1 - 195.6 mm $year^{-1}$) across Saudi Arabia (Chowdhury and Al-Zahrani, 2013). In combination, the changing climate together with an increased demand on the use of limited groundwater supplies for agriculture, has led to some irrigation and drainage authorities promoting the use of alternative sources of water for crop irrigation (Al-Rawahi et al., 2014). In addition to groundwater (GW), tertiary treated wastewater (TTWW), and a mixture of tertiary treated wastewater, farm drainage water and groundwater (MW) are currently used to support agricultural production (Hussain and Al-Saati, 1999; Al-Kuwaiti, 2010; Aldakheel, 2011). For example, the Irrigation and Drainage Authority in the Al-Hassa Oasis, KSA (the largest oasis in the world), currently sources more than 64% of its total irrigation water from alternative sources, 24% comes from TTWW and 40% from MW (Al-Kuwaiti, 2010).

In oasis-based agroecosystems, N represents the key nutrient regulating primary productivity, however, the cycling and competition for N resources in these ecosystems remains poorly understood (Buerkert et al., 2005; Yang et al., 2015). Recently, it has been discovered that plants can directly compete with microorganisms for both organic (e.g. oligopeptides, amino acids) and inorganic (e.g. NH_4^+ and NO_3^-) forms of N present in soil (Hill et al., 2011b, 2013; Farrell et al., 2013). This challenges the traditional paradigm of nutrient cycling in oasis soils which suggests that plants will only acquire N in an inorganic form. It also highlights the need to better understand how solid soil organic N (SON) and dissolved organic N (DON) cycles within these soils.

DON and DOC can enter arid soils from many sources with the quantitative importance of each source highly dependent on management regime, its spatial input pattern (topsoil *vs* subsoil, rhizosphere *vs* bulk soil), and its temporal dynamics (e.g. time of day and seasonality) (Jones et al., 2008). In oasis ecosystems, a small amount of DON and DOC may enter the soil surface from both wet and dry

deposition (Javid et al., 2015), with inputs also occurring from canopy throughfall especially if spray irrigators are used, from leaf litter (Li et al., 2013) or directly in irrigation water or organic residues (Buerkert et al., 2005). In addition, inputs can also occur below-ground from both root and microbial exudation and from root and microbial death (Jones et al., 2009; Ferguson and Nowak, 2011). The DOC and DON pool are continuously changing pool of C and N; e.g., soil organic material, litter, root exudation tends to increase the C and N pool whereas, mineralization, microbial adsorption, mineral adsorption, leachate tends to reduce the pool.

DOC and DON consists of many individual components, ranging from high molecular weight compounds (HMW) such as cellulose, hemicellulose, lignin, DNA, RNA and proteins, to low molecular weight compounds (LMW) such as, sugars (e.g., glucose), amino sugars, amino acids, short peptides and urea (Christou et al., 2006; Hill et al., 2008; Ge et al., 2010; Glanville et al., 2012). The subsequent breakdown of HMW DON compounds by microbial exoenzymes is a key bottleneck in the supply of N to plants and microorganisms (Jan et al., 2009; Roberts and Jones, 2012) and understanding agricultural practices that affect or change exoenzymes activity in oases are key to their better management (Rui et al., 2015).

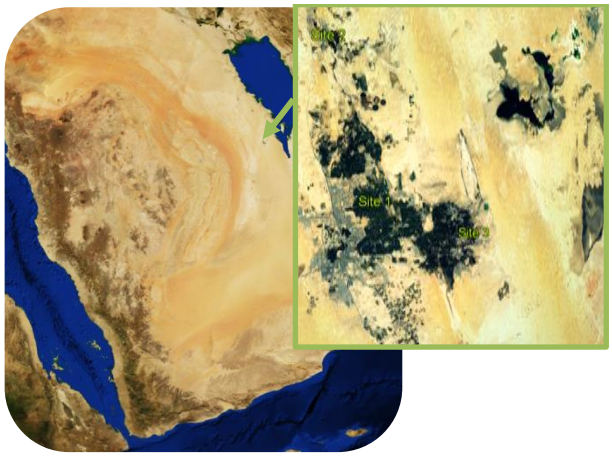
Due to ongoing climate change in KSA, we need to better understand the factors regulating C and N dynamics in soil with the aim of developing more sustainable agriculture systems in terms of both nutrient and water use efficiency (Alkolibi, 2002; Chowdhury and Al-Zahrani, 2013). To date, relatively little is known about DON and DOC cycling in arid soils, particularly in the Middle East. Consequently, the aim of the research is to expand our fundamental understanding of the effects of high soil temperature on N and C cycling within oasis-based agroecosystems.

4.2 Material and methods

4.2.1 Soil and water sampling

Soil and water samples were collected from three agricultural farm sites with contrasting irrigation regimes within the Al-Hassa eastern oasis, Saudi Arabia. Site 1 (25°25.430’N, 49°37.460’E) irrigated its crops with groundwater (GW), Site 2 (25°35.839’N, 49°34.144’E) irrigated with tertiary treated wastewater (TTWW) and Site 3 (25°23.461’N, 49°44.553’E) irrigated with mixture of groundwater, treated

wastewater and farm drainage water (MW), as shown in Figure 4.1. A summary of the main chemical properties of the irrigation water used at each site is presented in Table 4.1 (will discuss later in section 5.3.1). The dominant crop grown at the three sites was date palms (*Phoenix dactylifera* L.). Each date palm tree is planted at a distance of 4 m × 6 m and interplanted with vegetables, fruit, and Hassawi alfalfa (*Medicago sativa* L.) crops except at Site 2 where they are interplanted with Hassawi alfalfa only. At each site, four independent replicates of topsoil (0 - 30 cm) and subsoil (30 - 60 cm) were collected from within a 6 m × 6 m block using an auger (independent replicates in each blocks were pooled into one replicate due to no significant differences was observed between each independent replicates). Each individual field-moist soil sample was placed in an O_2-permeable plastic bag. Additionally, replicate samples of irrigation water (n = 3) were collected in clean polypropylene bottles from the pump outlet at each experimental site. All soil and water samples were cooled (4°C) then immediately shipped to the UK for analysis.



Map of three agricultural soil sites studied (Produced by the author)

Site 1 irrigated with GW

Site 2 irrigated with TTWW

Site 3 irrigated with MW

Figure 4.1 Photographs of the three agricultural soil sites studied.

Table 4.1 Physical and chemical characteristics of irrigation water source. Values represent means ± SEM (n = 3). Different letters indicate significant difference between irrigation water source ($P \leq 0.05$).

Water/ Irrigation source Parameters	Ground water	Treated wastewater	Mixed
pH	7.27 ± 0.03 [a]	7.22 ± 0.05 [a]	6.83 ± 0.04 [b]
EC (mS cm^{-1})	4.92 ± 0.16 [b]	2.21 ± 0.03 [c]	7.04 ± 0.09 [a]
Na (mg l^{-1})	624.65 ± 35.78 [b]	175.42 ± 2.22 [c]	724.62 ± 7.78 [a]
S (mg l^{-1})	187.79 ± 8.27 [b]	98.99 ± 6.70 [c]	306.20 ± 21.92 [a]
Cl (mg l^{-1})	1103.63 ± 52.75 [b]	406.35 ± 24.31 [c]	1755.29 ± 85.42 [a]
K (mg l^{-1})	17.94 ± 0.89 [b]	15.03 ± 1.25 [b]	47.97 ± 6.86 [a]
Ca (mg l^{-1})	185.20 ± 12.65 [b]	99.56 ± 4.28 [c]	270.72 ± 19.73 [a]
Mg (mg l^{-1})	130.29 ± 6.91 [b]	68.16 ± 0.50 [c]	206.84 ± 3.98 [a]
Cr (mg l^{-1})	0.09 ± 0.01	0.02 ± 0.01	0.05 ± 0.02
Fe (mg l^{-1})	0.18 ± 0.11	0.07 ± 0.03	0.21 ± 0.06
Zn (mg l^{-1})	0.24 ± 0.04 [a]	0.03 ± 0.01 [b]	0.05 ± 0.03 [b]
Available P (mg l^{-1})	0.04 ± 0.001 [c]	4.99 ± 0.46 [a]	2.50 ± 0.11 [b]
Phenolics (mg l^{-1})	1.74 ± 0.58	1.33 ± 0.45	3.10 ± 0.54
TN (%)	0.09 ± 0.02	0.14 ± 0.01	0.14 ± 0.01
TC (%)	0.008 ± 0.001	0.005 ± 0.002	0.005 ± 0.0001
NO_3^- (mg N l^{-1})	7.02 ± 0.05 [b]	7.11 ± 0.22 [b]	9.68 ± 0.41 [a]
NH_4^+ (mg N l^{-1})	0.02 ± 0.01	0.02 ± 0.004	0.08 ± 0.04
DON (mg N l^{-1})	0.41 ± 0.01 [b]	0.61 ± 0.09 [a]	0.43 ± 0.18 [b]
DOC (mg C l^{-1})	1.03 ± 0.30 [c]	4.69 ± 0.36 [b]	6.44 ± 0.18 [a]
DOC/DON ratio	2.49 ± 0.70	8.13 ± 0.87	23.84 ± 11.65
Amino acids (mg N l^{-1})	0.01 ± 0.001 [b]	0.05 ± 0.02 [a]	0.03 ± 0.001 [ab]

For all three experimental sites, the annual mean rainfall is about 70 mm. The average annual temperature is 28 - 47.5°C, with an average of 34.1°C, where the highest temperature recorded is 50°C. The relative humidity of the air is generally high for arid systems, and could reaching 90% in summer (Al-Fredan, 2011; Aldakheel, 2011). The average daily soil temperature in the Al-Hassa eastern oasis typically ranges from 16.4 to 37.9°C at 20 cm depth and 17.1 to 37.6°C at 30 cm (Figure 4.2).

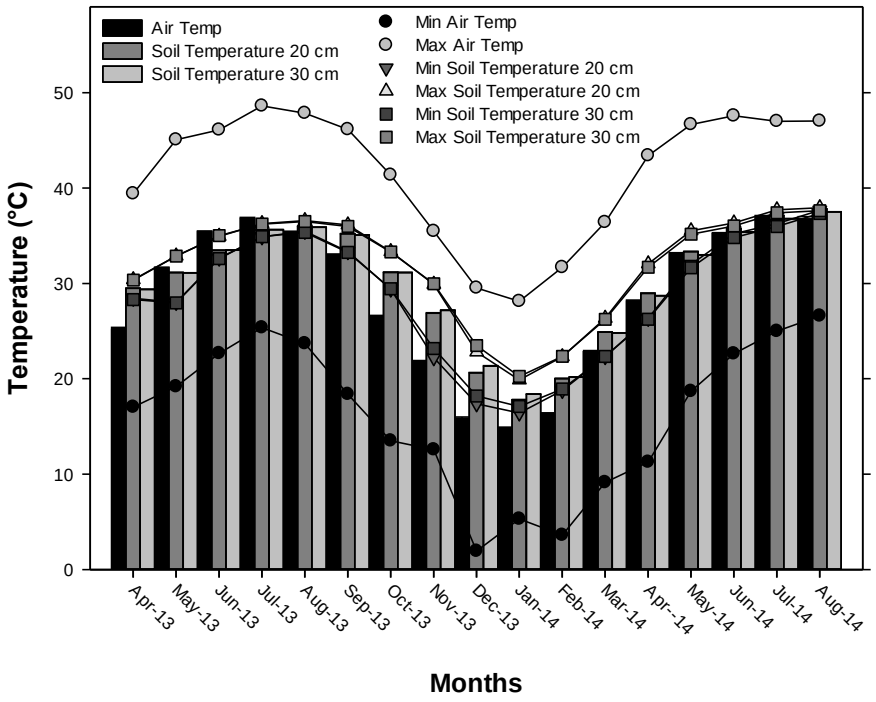


Figure 4.2 Air temperature, soil temperature at 20 cm and at 30 cm depth in Al-Hassa from April 2013 to August 2014 (data from Agriculture & Veterinary Research Station and Training, King Faisal University, Al-Hassa, KSA).

4.2.2 General soil and water characterization

pH and EC were determined by standard electrodes either directly for irrigation water or in 1:2.5 w/v soil:distilled water extracts for soil. Soil moisture content was determined gravimetrically by oven-drying (105°C, 16 h) while organic matter content was determined by loss-on-ignition (550°C, 16 h). Soil $CaCO_3$ was measured by the titration method of Bashour and Sayegh (2007) while soil texture was measured by sedimentation using the Bouyoucos method according to Bashour and Sayegh (2007). Total elemental concentrations were determined by digesting the air-dry soil with 70% HNO_3 (1:4 w/v) for 1 h at 100°C and filtering through a Whatman filter paper No. 541 according to method 3051A (US-EPA, 2007). Na was measured using a Model 410 flame photometer (Sherwood Scientific, Cambridge, UK) (Bashour and Sayegh, 2007; Burden et al., 2013) while Mg was measured using a Spectr*AA* 220FS atomic absorption spectrometer (Varian Inc., Palo Alto, CA). The remaining elements were measured using a S2-Picofox TXRF (Bruker Inc., Billerica, MA). Soil total C, total N and C:N ratio were determined using a CHN2000 analyser (Leco Corp., St Joseph, MI). Fresh soils were extracted with 0.5 M K_2SO_4 (1:5 w/v) for determining plant-available P, total extractable phenolics, NO_3^-, NH_4^+, protein and free amino acids. Available P, phenol, NO_3^-, NH_4^+, protein were measured

colorimetrically using a Powerwave XS spectrophotometer (Biotek Instruments Inc., Winooski, VT). Available P was measured using the ammonium molybdate-H_2SO_4-ascorbic acid method of Murphy and Riley (1962). Phenolics were measured using the Folin-Ciocalteu reagent in the presence of sodium carbonate (Swain and Hillis, 1959), NO_3^- by reaction with VCl_3 and N-(1-naphthyl)ethylenediamine dihydrochloride (Miranda et al., 2001). NH_4^+ was determined using the sodium salicylate-sodium nitroprusside-hypochlorite method of Mulvaney (1996). The Bradford method was used to estimate protein content (Roberts and Jones, 2008). Amino acids was determined by fluorescence according to Jones et al. (2002) while total dissolved N (TDN) and DOC were determined using a Multi-N/C 2100 S TOC/TN analyser (Analytik-Jena AJ, Jena, Germany). DON concentrations = TDN concentrations - (NO_3^- + NH_4^+ concentrations). Microbial biomass C and N were determined by the 7 d chloroform fumigation-0.5 M K_2SO_4 extraction procedure of Hill et al. (2013) (K_{EN} = 0.5; K_{EC} = 0.35). Basal soil respiration (SR) was measured using an automated CIRAS-SC soil respirometer at 20°C (PP Systems Ltd, Hitchin, UK).

4.2.3 Microbial community structure

Microbial community structure was measured by phospholipid fatty acid (PLFA) analysis following the method of Buyer and Sasser (2012). Briefly, replicate samples of soil from each site were sieved to pass 5 mm and stored at -20°C prior to analysis. Samples (2 g) were then freeze-dried and Bligh-Dyer extractant (4.0 ml) containing an internal standard added. Tubes were sonicated in an ultrasonic cleaning bath for 10 min at room temperature before rotating end-over-end for 2 h. After centrifuging (10 min), the liquid phase was transferred to clean 13 mm × 100 mm screw-cap test tubes and 1.0 ml each of chloroform and water added. The upper phase was removed by aspiration and discarded while the lower phase, containing the extracted lipids, was evaporated at 30°C. Lipid classes were separated by solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well (Phenomenex, Torrance, CA). Phospholipids were eluted with 0.5 ml of 5:5:1 methanol:chloroform:H_2O (Findlay, 2004) into glass vials, the solution evaporated (70°C, 30 min). Trans esterification reagent (0.2 ml) was added to each vial, the vials sealed and incubated (37°C, 15 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were then added. The chloroform was evaporated just to dryness and the

samples dissolved in hexane. An Agilent 6890 gas chromatograph (GC) (Agilent Technologies, Wilmington, DE) equipped with auto sampler, split-split less inlet, and flame ionization detector was used. FAMEs were separated on an Agilent Ultra 2 column, 25 m long × 0.2 mm internal diameter × 0.33 µm film thickness. FAMEs were identified using the MIDI PLFAD1 calibration mix (Microbial ID, Inc., Newark, DE) and classified according to Frostegård et al. (1993).

4.2.4 Rate of ^{14}C-labelled substrate mineralization in soil

Replicate samples of soil from each site (10 g) were placed in individual 50 ml sterile polypropylene tubes and 0.5 ml of a ^{14}C-uniformly labelled substrate (10 µM; 3.7 kBq ml^{-1}) added to the soil surface. The ^{14}C-labelled substrate added to individual tubes included: (i) glucose, (ii) an equimolar mixture of 15 different L-amino acids (alanine, arginine, aspartic acid, leucine, tyrosine, valine, phenylalanine, glutamic acid, threonine, proline, lysine, histidine, glycine, isoleucine, serine), or (iii) or $^{1\text{-}14}$C oligopeptide L-trialanine (that labelled the carboxyl group). After addition of the ^{14}C-labelled substrate, a 1 M NaOH trap (1 ml) was placed inside each tube to catch respired $^{14}CO_2$ and the tubes sealed and incubated at either 10, 20, 30 or 40°C. Production of $^{14}CO_2$ over time was measured by periodically replacing the NaOH trap after 0.5, 1, 3, 6, 24, 48, 168, 336, 504, and 672 h. $^{14}CO_2$ was determined by liquid scintillation counting using a Wallac 1409 liquid scintillation counter (PerkinElmer Inc., Waltham, MA) and OptiPhase Hi-safe 3 scintillation cocktail (PerkinElmer Inc.). After the removal of the final NaOH trap at 28 d, the soils were shaken for 20 min at 200 rev min^{-1} with 25 ml of 0.5 M K_2SO_4 to extract any ^{14}C remaining in the soil (Roberts and Jones, 2012). The extracts were measured by liquid scintillation counting as described earlier. The same experiments were conducted on sterilized soil (autoclaved at 121°C, 1 h and cooled for 10 min prior to use) to confirm that the release of $^{14}CO_2$ was biologically mediated and not produced from abiotic processes. The rate of ^{14}C-substrate mineralization in the soil was described by a double first-order exponential decay model:

$$f = [a_1 \times \exp^{(-k1\ t)}] + [a_2 \times \exp^{(-k2\ t)}] \quad \text{(Eqn. 1)}$$

where f is the percentage of ^{14}C-substrate remaining in the soil at time t (hours). Parameters a_1 and a_2 represent the size (% of total ^{14}C added) of the fast and slow ^{14}C turnover pools respectively (Boddy et al., 2007; Glanville et al., 2016). k_1 is the rate constant describing the turnover rate of C pool a_1 which is ascribed to the

immediate use of substrate-derived ^{14}C in catabolic processes (i.e. respiration). k_2 is the rate constant describing the turnover of C pool a_2 and is attributed to the second slower phase of $^{14}CO_2$ production associated with turnover of substrate-derived ^{14}C immobilised in the microbial biomass (Glanville et al., 2016). The half-life ($t_{1/2}$) of pool a_1 is defined as

$$t_{1/2} = \ln(2) / k_1 \text{ (Eqn. 2)}$$

Total microbial uptake ($^{14}C_{uptake}$) can be defined as

$$^{14}C_{uptake} = {}^{14}C_{Tot} - {}^{14}C_{K_2SO_4} \text{ (Eqn. 3)}$$

where $^{14}C_{Tot}$ is the total amount of ^{14}C label added to the soil and $^{14}C_{K_2SO_4}$ is the amount of ^{14}C label recovered in the K_2SO_4 extract (Roberts and Jones, 2012). The amount of labelled ^{14}C remaining in the microbial biomass ($^{14}C_{mic}$) after 28 d was calculated as follows:

$$^{14}C_{mic} = {}^{14}C_{Tot} - {}^{14}CO_2 - {}^{14}C_{K_2SO_4} \text{ (Eqn. 4)}$$

where $^{14}CO_2$ is the total amount of label recovered in the NaOH traps (Roberts and Jones, 2012). Lastly, microbial C use efficiency (Mic_{eff}) for each substrate can be defined as

$$Mic_{eff} = {}^{14}C_{mic} / ({}^{14}C_{mic} + {}^{14}CO_2) \text{ (Eqn. 5)}$$

4.2.5 Effects of temperature on ^{14}C mineralization rate

The effect of temperature (T) on ^{14}C mineralization rate (MR) was described by applying a square root transformation model to the data according to Rousk et al. (2012) and Birgander et al. (2013) using the equation

$$MR^{½} = \alpha (T - T_{min}) \text{ (Eqn. 6)}$$

where MR is the mineralization rate (defined as the initial linear rate of $^{14}CO_2$ production at each temperature), α is a slope parameter linked to the absolute rates, and T_{min} is the temperature at which mineralization ceases. The linear phase of $^{14}CO_2$ evolution was defined as 0 - 48 h at 10°C, 0 - 24 h at 20°C, 0 - 6 h at 30°C, and 0 - 3 h at 40°C. We calculated √ rate of ^{14}C substrate mineralization by the following equation:

√ rate of ^{14}C substrate mineralization = √ (C atom in substrate (e.g., C atom in glucose = 6) X C molecular weight (i.e., C MW = 12) X $^{14}CO_2$ evolution plots at specific time / 100) / specific time.

4.2.6 Statistical analysis

Statistical analyses (ANOVA with Tukey's pairwise comparison) were performed using SPSS 20 (SPSS Inc., Chicago, IL) or Minitab 16 (Minitab Inc., State College, PA) with significance differences set at $P \leq 0.05$ unless otherwise stated. The double first-order exponential decay equation was fitted to experimental results using Sigmaplot 12.3 (SPSS Inc., Chicago, IL). Linear regression models describing ^{14}C mineralization rate and temperature were made using Sigmaplot 12.3.

4.3 Results

4.3.1 General physical and chemical soil properties

Physical and chemical characteristics of the soils tested in this study are presented in Table 4.2. Soils were classified as sand or sandy loam (Certini and Scalenghe, 2006); the average pH and EC values for all soils were 8.55 and 0.72 mS cm^{-1} respectively. No differences in soil pH and EC were observed between sites or sampling depths. The soils tested in this study can be classified as having low to medium OM contents, according to Boulding (1994); the average OM and $CaCO_3$ contents for all soils were 1.7% and 23.6% respectively. Significant differences in OM and $CaCO_3$ were observed between sites ($P \leq 0.05$), but no significant differences were observed between sampling depths, except in OM contents of site 1. In topsoils, the highest total C (TC) content was observed in site 3, in the subsoils; the highest content was shown in site 2 (23.6 ± 2.8 g C kg^{-1} soil, and 34.5 ± 3.6 g C kg^{-1} soil in topsoils and subsoils respectively); the lowest TC content was observed in site 1 in both topsoils and subsoils (13.3 ± 1.3 g C kg^{-1} soil, and 10.5 ± 1.4 g C kg^{-1} soil in topsoils and subsoils respectively) with significant differences between sites ($P \leq 0.05$), but no significant differences between sampling depths ($P > 0.05$). The average total N (TN) contents and C:N ratio for all soils were 0.94 g N kg^{-1} soil and 22.0 respectively. No differences were observed between sites or sampling depths, but there were significant differences between sites in the subsoils in C:N ratio. All soils contain very low NO_3^- content, according to FAO (2006). The highest NO_3^- concentration was shown in site 2 (7.7 ± 0.6 mg NO_3^--N kg^{-1} soil, and 3.2 ± 0.6 mg NO_3^--N kg^{-1} soil in topsoils and subsoils respectively) and the lowest NO_3^- concentration in site 1 (4.6 ± 0.6 mg NO_3^--N kg^{-1} soil, and 0.7 ± 0.1 mg NO_3^--N kg^{-1} soil in topsoils and subsoils respectively), with significant differences between sites

($P \leq 0.05$), but no significant differences between sampling depth, except in site 1. Site 1 shows the highest NH_4^+ concentration in top and subsoils (1.05 ± 0.13 mg NH_4^+-N kg^{-1} soil and 1.3 ± 0.2 mg NH_4^+-N kg^{-1} soil respectively), and the lowest NH_4^+ concentration in the topsoils was observed in site 2; in the subsoils, the lowest content was observed in site 3 (0.05 ± 0.02 mg NH_4^+-N kg^{-1} soil, and 0.33 ± 0.18 mg NH_4^+-N kg^{-1} soil respectively) with significant differences between sites ($P \leq 0.05$), but no significant differences between sampling depths ($P > 0.05$). Total free amino acid (TFAA) concentrations show similar patterns to NH_4^+ but no significant differences exist between sites or sampling depths ($P > 0.05$), except between the depths in site 1 (0.19 ± 0.01 mg TFAA-N kg^{-1} soil, and 0.13 ± 0.01 mg TFAA-N kg^{-1} soil in topsoils and subsoils respectively). Amino acid concentration is lower than DON concentration in all sites and follows a pattern of site 2 > site 3 ≥ site 1, with significant differences between them ($P \leq 0.05$). The average protein, DON, DOC, microbial N and microbial C content for all soil sites were 4.17 mg N kg^{-1} soil, 6.73 mg N kg^{-1} soil, 41.0 mg C kg^{-1} soil, 10.7 mg N kg^{-1} soil, and 105.2 mg C kg^{-1} soil respectively. No differences were observed between sites or sampling depths, except between sites in the subsoils and DOC concentrations, and follows a pattern of site 3 > site 2 ≥ site 1 between sites in the topsoils and microbial N, and follows a pattern of site 2 > site 3 > site 1 between sampling depths and microbial N or C in site 2 ($P \leq 0.05$). Amino acid concentrations represent 2.44% of DON concentrations in topsoils and 2.03% in subsoils, following a pattern of site 1 > site 2 > site 3. DOC/DON ratio shows that there were significant differences between sites in the subsoils, following a pattern of site 3 ≥ site 1 > site 2 (5.70 ± 0.65, 5.33 ± 0.29, and 3.25 ± 0.21 respectively) ($P \leq 0.05$), but there were no significant differences between sites in the topsoils or sampling depths ($P > 0.05$), except between the sampling depths in site 2 ($P \leq 0.05$).

4.3.2 Soil microbial community structure

Soil microbial community structure is presented in Figure 4.3. In most soils, the presence of Gram-negative bacteria > Gram-positive bacteria > actinomycetes > AM fungi ≥ fungi ≥ anaerobic bacteria ≥ eukaryote. No major differences were observed between sites or sampling depths, except for Gram-positive, anaerobic bacteria, or actinomycetes in subsoils and sampling depths, with few exceptions between sampling depths and Gram-negative in site 2 or anaerobic bacteria in site 3. There

were no significant differences observed between total microbial biomass measured by PLFA in different soil or depths except, between subsoil and followed site 3 > site 2 = site 3 with the concentrations of 26.36, 21.30, and 10.04 nmol PLFA biomass g^{-1} soil respectively.

Table 4.2 Physical, biological and chemical characteristics of the soils used in the experiments. Values represent mean ± SEM (n = 4). Significant differences between depths are indicated by * = $P \leq 0.05$, different lower case letters identify significant differences between sites ($P \leq 0.05$) in topsoil and different capital letters identify significant differences between sites ($P \leq 0.05$) in subsoil.

Soil/ Irrigation source	Site 1		Site 2		Site 3	
Parameter	(0 - 30 cm)	(30 - 60 cm)	(0 - 30 cm)	(30 - 60 cm)	(0 - 30 cm)	(30 - 60 cm)
pH	8.51 ± 0.03	8.51 ± 0.06	8.66 ± 0.02	8.51 ± 0.01	8.56 ± 0.07	8.55 ± 0.07
EC (mS cm^{-1})	0.91 ± 0.07	0.95 ± 0.14	0.40 ± 0.02	0.59 ± 0.03	0.67 ± 0.15	0.81 ± 0.15
MC (%)	12.78 ± 0.25^{b}	13.43 ± 0.39^{C}	*12.66 ± 0.69^{b}	27.10 ± 1.13^{A}	18.16 ± 0.60^{a}	20.31 ± 0.49^{B}
OM (%)	*1.26 ± 0.05^{b}	0.89 ± 0.04^{C}	1.56 ± 0.06^{ab}	1.80 ± 0.05^{B}	2.65 ± 0.23^{a}	2.05 ± 0.23^{A}
$CaCO_3$ (%)	23.54 ± 0.78^{b}	25.58 ± 1.65^{AB}	27.92 ± 2.07^{a}	35.50 ± 3.13^{A}	14.96 ± 2.38^{c}	14.08 ± 2.38^{B}
Soil Texture	Sand	Sand	Sand	Sandy Loam	Sandy Loam	Sandy Loam
Na (g kg^{-1})	0.56 ± 0.15	0.42 ± 0.17	0.32 ± 0.06	0.51 ± 0.06	0.49 ± 0.11	0.53 ± 0.06
Al (g kg^{-1})	*1.47 ± 0.04^{a}	0.68 ± 0.09	0.60 ± 0.08^{b}	0.41 ± 0.04	1.32 ± 0.31^{ab}	0.71 ± 0.20
S (g kg^{-1})	0.20 ± 0.02	0.04 ± 0.04	0.04 ± 0.02	0.03 ± 0.01	0.09 ± 0.04	0.08 ± 0.02
Cl (g kg^{-1})	*0.25 ± 0.02	0.14 ± 0.01^{B}	0.21 ± 0.02	0.18 ± 0.002^{AB}	0.25 ± 0.07	0.21 ± 0.01^{A}
K (g kg^{-1})	*0.70 ± 0.01^{ab}	0.49 ± 0.03^{B}	0.58 ± 0.04^{b}	0.50 ± 0.02^{B}	0.93 ± 0.13^{a}	0.98 ± 0.08^{A}
Ca (g kg^{-1})	*33.00 ± 0.74	21.61 ± 1.03^{B}	37.75 ± 2.35	38.50 ± 2.54^{A}	37.55 ± 4.34	37.09 ± 2.41^{A}
Mg (g kg^{-1})	3.05 ± 0.10^{b}	2.58 ± 0.15^{C}	3.46 ± 0.34^{b}	4.32 ± 0.17^{B}	9.25 ± 1.17^{a}	8.47 ± 1.44^{A}
Cr (g kg^{-1})	0.01 ± 0.0002^{a}	0.01 ± 0.002^{B}	0.01 ± 0.001^{b}	0.01 ± 0.0004^{B}	0.02 ± 0.002^{a}	0.02 ± 0.007^{A}
Mn (g kg^{-1})	*0.06 ± 0.002	0.04 ± 0.004^{B}	0.05 ± 0.01	0.05 ± 0.003^{B}	0.08 ± 0.01	0.07 ± 0.01^{A}
Fe (g kg^{-1})	*1.93 ± 0.03^{b}	1.25 ± 0.07^{B}	1.40 ± 0.11^{c}	1.36 ± 0.09^{B}	2.64 ± 0.27^{a}	2.69 ± 0.23^{A}
Cu (g kg^{-1})	*0.01 ± 0.001	0.002 ± 0.0003^{B}	0.005 ± 0.003	0.003 ± 0.001^{AB}	0.01 ± 0.0004	0.01 ± 0.001^{A}
Zn (g kg^{-1})	0.02 ± 0.005	0.01 ± 0.001	0.02 ± 0.01	0.01 ± 0.002	0.02 ± 0.002	0.01 ± 0.002
Total C (g C kg^{-1} soil)	13.29 ± 1.30^{b}	10.45 ± 1.42^{B}	21.57 ± 1.86^{a}	34.45 ± 3.58^{A}	23.63 ± 2.83^{a}	22.80 ± 2.83^{A}
Total N (g N kg^{-1} soil)	1.00 ± 0.06	0.81 ± 0.12	0.83 ± 0.04	0.74 ± 0.03	1.19 ± 0.08	1.07 ± 0.08
CN Ratio	13.60 ± 1.27	13.86 ± 0.95^{B}	27.92 ± 5.48	48.19 ± 5.48^{A}	20.49 ± 2.96	22.10 ± 2.96^{B}
NO_3^- (mg N kg^{-1} soil)	*4.60 ± 0.55^{b}	0.69 ± 0.09^{B}	7.67 ± 0.55^{a}	3.22 ± 0.62^{A}	4.84 ± 0.45^{b}	1.04 ± 0.45^{B}
NH_4^+ (mg N kg^{-1} soil)	1.05 ± 0.13^{a}	1.29 ± 0.22^{A}	0.05 ± 0.02^{b}	0.38 ± 0.11^{B}	0.11 ± 0.18^{b}	0.33 ± 0.18^{B}
Amino acids (mg N kg^{-1} soil)	*0.19 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	0.10 ± 0.01	0.17 ± 0.03	0.12 ± 0.02
Protein (mg N kg^{-1} soil)	5.28 ± 0.37	4.07 ± 0.34	2.53 ± 0.32	4.02 ± 0.69	4.83 ± 0.59	4.28 ± 0.25
DON (mg N kg^{-1} soil)	5.91 ± 0.78	4.67 ± 0.44	6.91 ± 1.12	6.92 ± 1.69	9.64 ± 2.75	6.30 ± 0.80
DOC (mg C kg^{-1} soil)	35.05 ± 6.66	25.18 ±3.74^{B}	39.61 ±8.61	30.77 ±7.66^{AB}	66.45 ± 23.44	48.63 ± 2.34^{A}
Microbial N (mg N kg^{-1} soil)	8.88 ± 0.65^{b}	1.09 ± 0.23	*20.25 ± 1.40^{a}	5.53 ± 0.73	18.51 ± 4.82^{a}	10.54 ± 5.78
Microbial C (mg C kg^{-1} soil)	109.01 ±15.95	58.94 ± 10.65	*171.51 ± 7.23	91.88 ± 4.69	127.12 ± 31.23	73.95 ± 40.49
DOC/DON ratio	5.90 ± 0.84	5.33 ± 0.29^{A}	*5.81 ± 0.21	3.25 ± 0.21^{B}	12.05 ± 5.97	5.70 ± 0.65^{A}
Phenolics (mg kg^{-1} soil)	*5.73 ± 0.12	4.03 ± 0.06	6.64 ± 2.02	6.55 ± 2.57	*4.69 ± 1.30	0.96 ± 0.25
Available P (mg PO_4^{-3} kg^{-1} soil)	*10.03 ± 1.76	3.05 ± 0.81	41.50 ± 3.27	19.23 ± 2.45	*35.70 ± 2.03	4.86 ± 2.03
Soil respiration (µgCO_2 g^{-1} h^{-1})	*0.12 ± 0.005	0.09 ± 0.003^{B}	0.14 ± 0.01	0.12 ± 0.02^{AB}	0.16 ± 0.01	0.15 ± 0.01^{A}

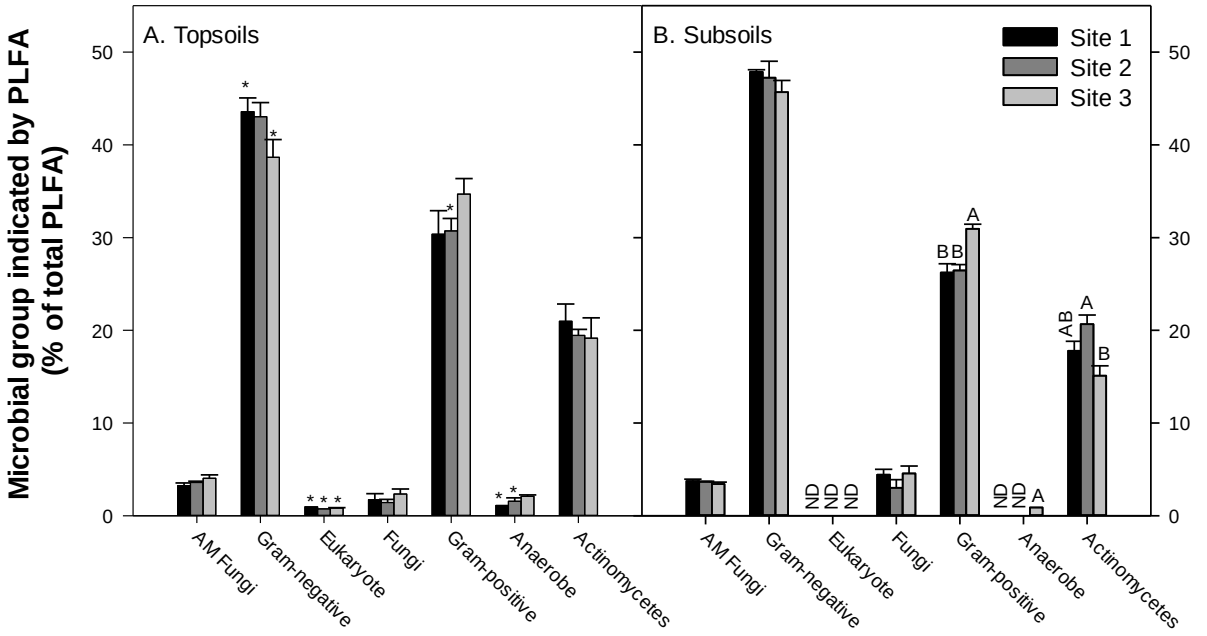


Figure 4.3 Abundance of soil microbial community indicator PLFAs in the either topsoils (Panel A) or subsoils (Panel B) at the different agricultural sites. Values represent means ± SEM (n = 3). ND indicates, not detected. Different letters indicate significant differences between sites ($P \leq 0.05$), while * indicate significant differences between depths. The legend is the same for both panels.

4.3.3 DON compounds mineralization and half-life

Overall, no significant differences were observed between the goodness of fit of the double exponential decay model to the experimental data (R^2 = 0.999 ± 0.001) when comparing different substrate or sites at different temperatures. The effect of temperature on different ^{14}C-substrate mineralization in the topsoils and subsoils are shown in Figure 4.4 and Figure 4.5 respectively, while the kinetic parameters from double first-order exponential decay equation in different sites are presented in Table 4.3. There were no significant effects observed between sites or sampling depths with the production of $^{14}CO_2$ ($P > 0.05$). The production of $^{14}CO_2$ increases with increasing temperature in almost all cases ($P \leq 0.05$), as shown in Figure 4.4 and Figure 4.5 and Table 4.3. The cumulative amount of $^{14}CO_2$ evolved after the addition of glucose to the topsoils and subsoils increased significantly with increasing temperature from 20 to 30°C for all soils, with the pattern showing little difference between sites. Although temperature increases overall mineralization, increasing temperatures from 30 to 40°C for all soils and substrates had little overall effect on the final amount of ^{14}C-substrate mineralized (Figure 4.4 and Figure 4.5).

The shortest $t_{1/2}$ of glucose in both topsoils and subsoils was observed in site 1 (11.7 ± 3.7 h and 18.4 ± 8.5 h respectively). Amino acid $t_{1/2}$ was shortest in topsoils of site 2 and subsoils of site 3 (4.4 ± 0.5 h; 9.1 ± 5.1 h respectively). The shortest $t_{1/2}$ of trialanine was observed in topsoil of site 2 and subsoil of site 3 (2.9 ± 0.3 h; 4.2 ± 1.3 h respectively). There were no significant differences in $t_{1/2}$ between sites, except after the addition of amino acids at 40°C, which led to significant differences between sites in topsoil and followed the pattern of site 1 > site 3 > site 2. In most cases, topsoils exhibited shorter $t_{1/2}$ values than subsoils, with $t_{1/2}$ values decreasing with increasing temperature with some significant differences apparent (Table 4.3). The pool size a_1 describes the relative amount of ^{14}C substrates taken up and used immediately in respiratory processes by the microbial community. In all cases, no significant effects were observed between sites or sampling depths in the size of pool a_1 ($P > 0.05$), but temperature generally increased the amount of ^{14}C allocated to pool a_1. Increasing temperatures from 20 to 30°C for all soils and substrates has some overall effect on the size of pool a_1 ($P \leq 0.05$; Table 4.3). Increasing temperatures from 30 to 40°C for all sites after the addition of ^{14}C amino acids or ^{14}C trialanine has some overall effect on the size of pool a_1 ($P \leq 0.05$). The only exception was an observed decrease in C allocation to pool a_1 after the addition of ^{14}C amino acids to the topsoil of site 2 and the subsoil of site 3 when temperature increased from 30 to 40°C. ^{14}C partitioned into pool a_1 after the addition of ^{14}C glucose decreased in both topsoil and subsoils, with increasing temperature from 30 to 40°C in all soil types. Only a small amount of unmetabolized ^{14}C label was recovered from the soil by 0.5 M K_2SO_4 at the end of 672 h incubation periods (< 1% of the total ^{14}C applied in most cases).

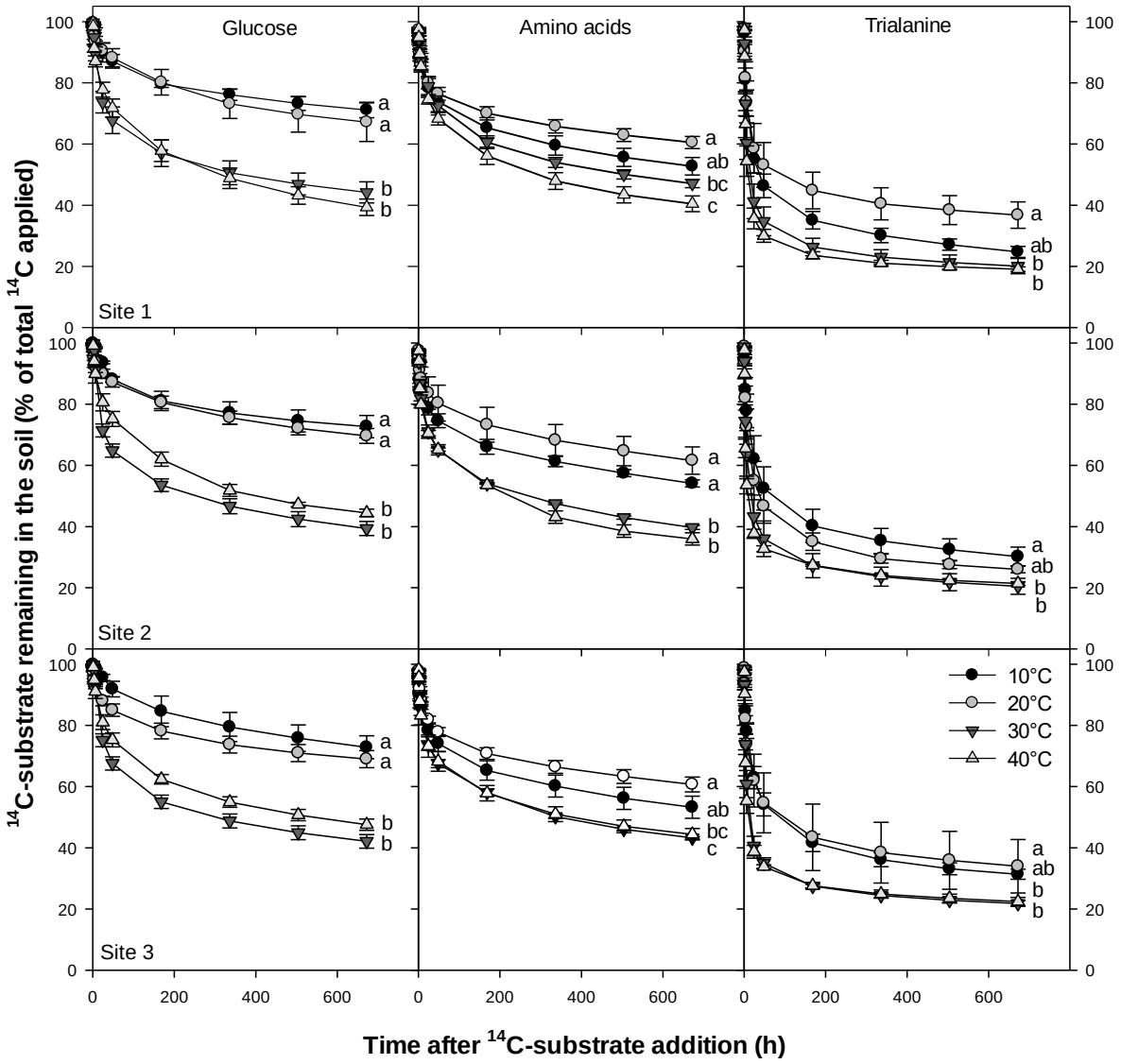


Figure 4.4 Temperature effects on the mineralization of added ^{14}C-substrate in topsoil from three agricultural sites. Points represent means ± SEM (n = 3). Different letters indicate significant differences between temperatures ($P \leq 0.05$). The legend is the same for all panels.

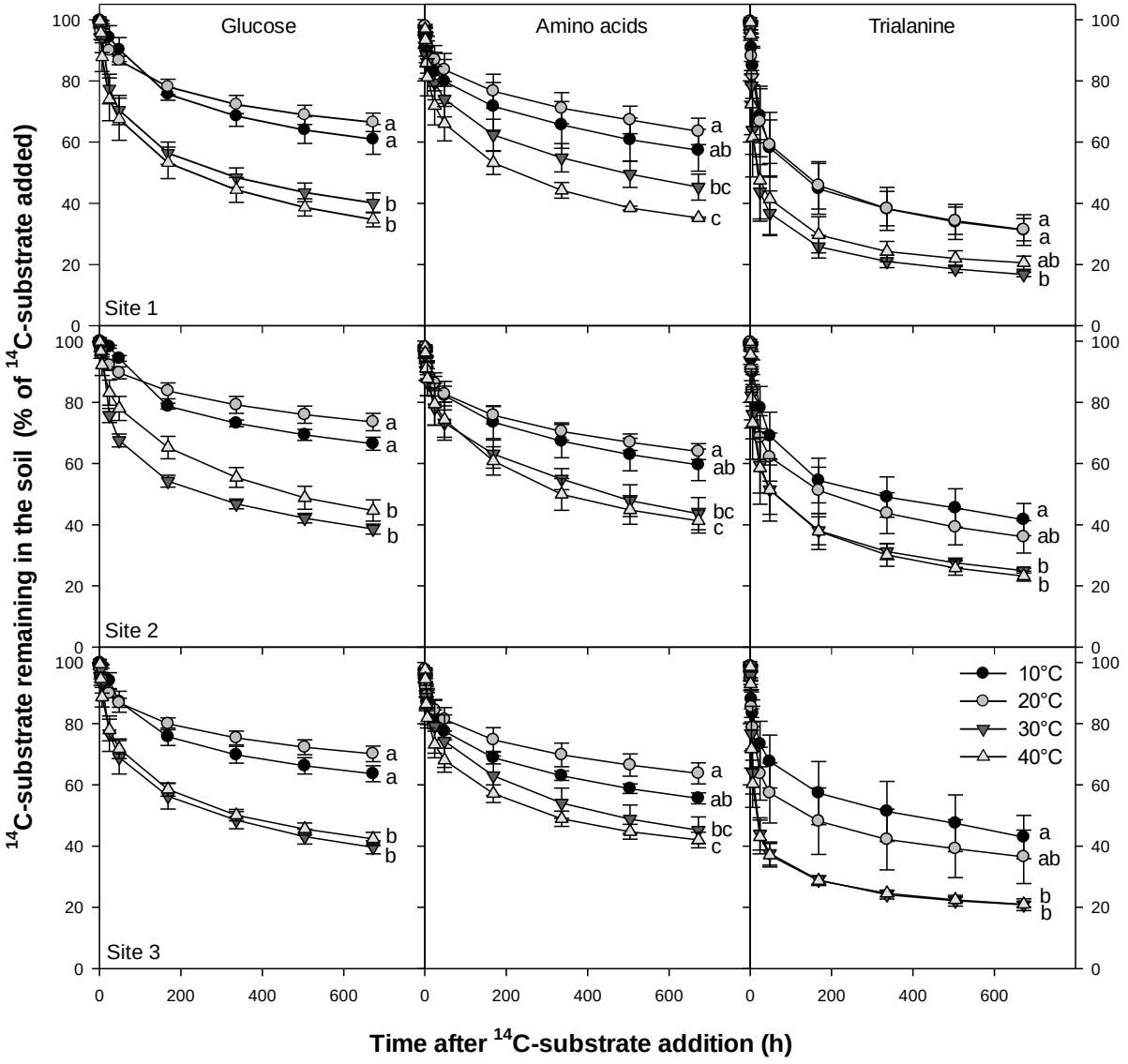


Figure 4.5 Temperature effects on the mineralization of added ^{14}C-substrate in subsoils from three agricultural sites. Points represent means ± SEM (n = 3). Different letters indicate significant differences between temperatures (P ≤ 0.05). The legend is the same for all panels.

Table 4.3 Influence of soil temperature on the modelled kinetic parameters describing the turnover of ^{14}C-labelled glucose, amino acids, and trialanine in the topsoils and subsoils of three different agriculture sites. Values represent mean ± SEM (n = 3). Different letters indicate significant differences between temperatures ($P \leq 0.05$), while no letters indicate there are no significant differences.

Sites	Temperature (°C)	a_1			$t_{1/2}$		
		Glucose (%)	Amino acids (%)	Trialanine (%)	Glucose (h)	Amino acids (h)	Trialanine (h)
Topsoils (0 - 30 cm)							
Site 1	10	19.12 ± 2.71^{b}	21.90 ± 2.20^{bc}	50.23 ± 3.21^{bc}	33.32 ± 22.51^{ab}	12.99 ± 3.97^{ab}	9.00 ± 2.88
	20	17.94 ± 3.64^{b}	20.14 ± 1.81^{c}	45.61 ± 4.99^{c}	71.99 ± 27.63^{a}	5.66 ± 0.79^{b}	8.68 ± 4.30
	30	36.63 ± 7.23^{a}	29.00 ± 1.25^{ab}	66.33 ± 6.84^{ab}	11.71 ± 3.72^{ab}	25.06 ± 6.79^{a}	4.02 ± 0.47
	40	29.29 ± 2.67^{ab}	31.28 ± 2.63^{a}	70.73 ± 4.59^{a}	15.97 ± 1.31^{b}	16.32 ± 1.83^{ab}	3.47 ± 0.57
Site 2	10	18.25 ± 4.06^{bc}	21.00 ± 1.92^{b}	48.30 ± 4.03^{b}	35.63 ± 9.21^{a}	9.33 ± 3.85	13.26 ± 5.77
	20	13.02 ± 1.31^{c}	17.83 ± 4.09^{b}	54.68 ± 4.90^{ab}	22.47 ± 2.85^{ab}	27.31 ± 21.72	10.34 ± 3.40
	30	41.46 ± 2.03^{a}	32.60 ± 0.42^{a}	66.08 ± 6.90^{a}	15.37 ± 1.32^{b}	11.07 ± 5.22	6.03 ± 2.40
	40	28.88 ± 3.86^{b}	30.57 ± 0.95^{a}	70.05 ± 1.59^{a}	18.52 ± 7.09^{ab}	4.37 ± 0.45	2.85 ± 0.31
Site 3	10	40.82 ± 21.14	22.92 ± 2.42^{b}	45.04 ± 2.17^{b}	611.50 ± 560.50	14.18 ± 2.71	12.27 ± 2.95
	20	16.00 ± 2.19	20.09 ± 1.84^{b}	46.16 ± 7.32^{b}	20.82 ± 1.09	11.20 ± 2.86	10.31 ± 6.48
	30	41.13 ± 2.11	30.24 ± 0.69^{a}	66.99 ± 2.62^{a}	19.78 ± 1.74	11.36 ± 2.12	4.26 ± 0.80
	40	30.43 ± 1.00	30.94 ± 2.29^{a}	68.50 ± 1.55^{a}	22.65 ± 6.09	9.99 ± 5.04	3.21 ± 0.62
Subsoils (30 - 60 cm)							
Site 1	10	33.72 ± 9.03	16.57 ± 5.85	46.38 ± 7.10	96.47 ± 41.82^{a}	18.50 ± 6.38	18.24 ± 4.65
	20	17.05 ± 2.60	14.53 ± 4.04	46.12 ± 8.05	30.66 ± 6.92^{ab}	27.50 ± 14.53	18.64 ± 8.18
	30	38.44 ± 5.20	23.58 ± 4.46	66.65 ± 9.36	22.59 ± 9.97^{b}	26.19 ± 11.06	5.95 ± 2.14
	40	37.20 ± 6.29	31.03 ± 3.54	68.66 ± 9.98	18.36 ± 8.51^{b}	11.40 ± 6.95	12.24 ± 9.64
Site 2	10	31.08 ± 4.50^{b}	17.04 ± 4.22^{b}	38.98 ± 7.07	120.69 ± 20.25^{a}	25.49 ± 4.56	26.95 ± 6.04
	20	11.76 ± 2.06^{c}	15.99 ± 2.07^{b}	40.18 ± 6.34	22.87 ± 2.48^{b}	17.35 ± 2.17	12.20 ± 5.81
	30	42.08 ± 1.76^{a}	25.20 ± 3.49^{ab}	51.78 ± 7.44	20.94 ± 1.70^{b}	16.62 ± 6.85	11.88 ± 4.37
	40	25.35 ± 1.73^{b}	29.55 ± 1.64^{a}	52.63 ± 10.67	20.63 ± 7.44^{b}	29.10 ± 16.45	12.21 ± 4.95
Site 3	10	25.25 ± 1.75^{b}	19.43 ± 1.26	32.59 ± 8.33^{b}	60.33 ± 15.27^{a}	17.09 ± 6.95	11.73 ± 6.88
	20	14.80 ± 1.42^{c}	16.41 ± 3.48	42.14 ± 9.95^{ab}	24.12 ± 3.49^{b}	9.14 ± 5.05	8.39 ± 2.70
	30	38.25 ± 3.79^{a}	33.28 ± 7.59	65.08 ± 5.46^{a}	22.25 ± 7.18^{b}	53.67 ± 38.00	5.01 ± 1.48
	40	33.24 ± 0.80^{ab}	30.36 ± 3.32	65.41 ± 5.98^{a}	18.41 ± 6.32^{b}	12.88 ± 5.56	4.18 ± 1.33

4.3.4 Microbial biomass use efficiency of C

Microbial C use efficiency gives an indication of the relative amount ^{14}C immobilized by the microbial community after the addition of DOC or DON substrates (the immobilization-to-mineralization ratio). A comparison of microbial C use efficiency (Mic_{eff}) between the three sites shows some significant differences ($P \leq 0.05$) (Figure 4.6), particularly between topsoil sites after the addition of amino acids at 30°C (where site 1 > site 3 > site 2), and between subsoil sites after the addition of trialanine at 30°C (site 2 > site 3 > site 1). Temperature tended to decrease Mic_{eff}, particularly between 20 and 30°C in all soil types ($P \leq 0.05$).

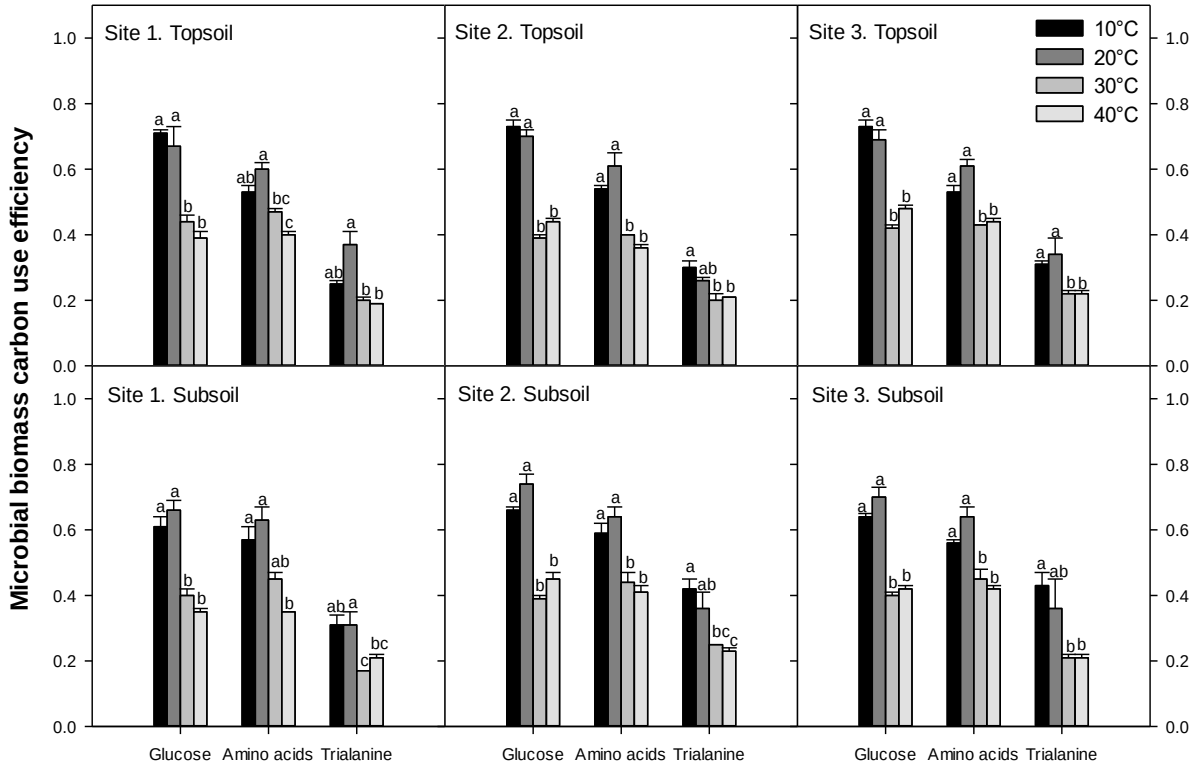


Figure 4.6 Effect of temperature on the microbial carbon use efficiency for different substrates in three agricultural topsoils and subsoils. Values represent means ± SEM (n = 3). Different letters identify significant differences between temperatures ($P \leq 0.05$). The legend is the same for all panels.

4.3.5 Temperature dependency of ^{14}C respiration rate

A square root transformation of ^{14}C respiration rate and temperature (Rousk et al., 2012; Birgander et al., 2013) was used to estimate the effect of temperature on the immediate rate of $^{14}CO_2$ production from the labelled substrates added to soil sites (Figure 4.7). In all the cases, the square root value increased with increasing temperature ($P \leq 0.05$). The square root transformation produced a linear fit when plotted against respiration rate with R^2 values ranging from 0.92 to 0.99 in all cases.

In almost all cases, no effect was observed between soil sites or depths on the square root of ^{14}C respiration rate ($P > 0.05$).

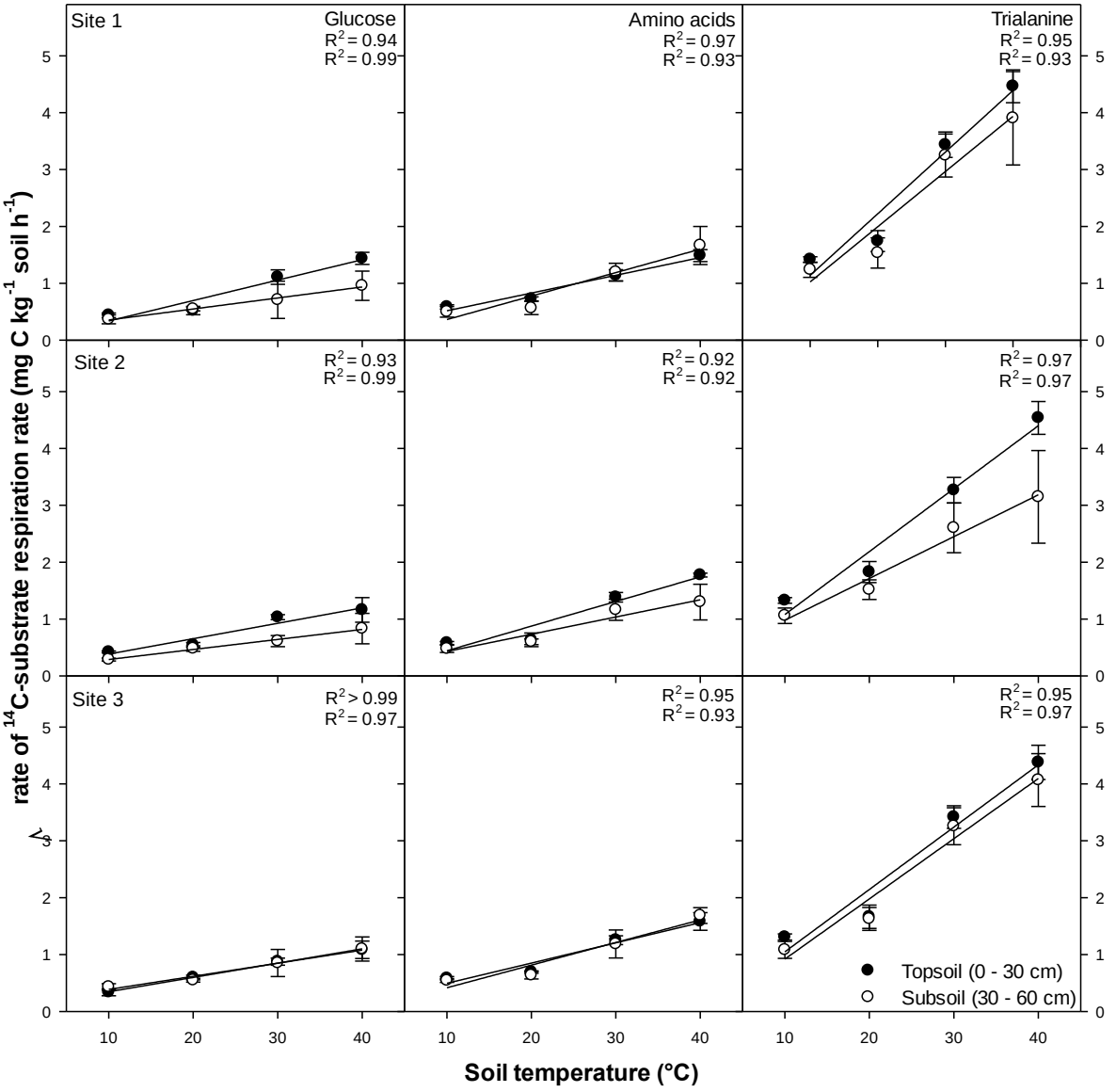


Figure 4.7 Relationship between soil temperature and the square root of mineralization rate for three different ^{14}C-substrates in topsoils and subsoils from three different agricultural sites. Values represent means ± SEM (n = 3). The legend is the same for all panels.

4.4 Discussion

4.4.1 DON compounds mineralization and half-life

To our knowledge, the comparison of DON and DOC mineralization responses to temperature in agricultural oasis soils has not previously been examined. To achieve sustainable agriculture in an oasis system and to maintain N fertility, we need to understand the controlling factors that regulate C and N related processes in soil. This study focuses only on the short-term effects of temperature changes on

DOC and DON mineralization in three agricultural sites from the Al-Hassa eastern oasis, KSA. We know that the depletion of ^{14}C glucose, ^{14}C amino acids and ^{14}C trialanine in soil can only happen as a result of either abiotic mineralization or microbial uptake (Hill et al., 2008; Jan et al., 2009). Here, we assume that the production of $^{14}CO_2$ after the addition of radiolabelled substrate was from microbial uptake, since no evolution of $^{14}CO_2$ was observed from the results of sterile treatments (data not presented). The range of temperature in this study (10 - 40°C) was designed to reflect the actual temperature in soil at the site and those that might arise in the future. In Saudi Arabia, air temperatures might exceed 50°C by 2050 as a result of climate change. However, it should be noted that soil surfaces in these arid regions can exceed 65°C when in direct sunlight (Nobel, 1984; Garratt, 1992; Williams et al., 1999). As we were mainly concerned with the main root proliferation zone for crop plants (0 - 30 cm), we limited our maximum temperature to 40°C due to crop plant cover leading to reduced soil temperature from the direct effect of sunlight.

As expected, our results show an increase in substrate partitioning to C pool a_1 with increasing temperature. Based on the modelling approach used (Glanville et al., 2016), we conclude that this is due to the microorganisms increasingly using ^{14}C for energy production and cell maintenance activities (e.g. generation of heat shock proteins, membrane lipid renewal) rather than for growth and storage. This result is consistent with Boddy et al. (2008), who also showed that the size of C pool a_1 was sensitive to temperature. The results presented in Figure 4.4, Figure 4.5 and Table 4.3 generally demonstrate that the rate of $^{14}CO_2$ evolution from DON > DOC compounds. Neither site nor depth had a major effect on the amount of $^{14}CO_2$ evolution or the amount of C partitioned into C pool a_1. The similarity between agricultural soil sites or depths could be due to their similar microbial communities or to the commonality in metabolic pathways for processing LMW DON or DOC (Hill et al., 2011c).

Although both substrates were rapidly used in soil, the microbial mineralization rate of amino acids tended to be higher than glucose. This is likely to be due to the production of organic acid skeletons (i.e. keto acids) from the rapid internal transamination and deamination of amino acids in the cell, which can then be used directly in the respiration pathway. This result is consistent with Jones et al. (2005a) and Boddy et al. (2008) for a rapid intrinsic rate of amino acid. The $t_{1/2}$ of DON was shorter than DOC, given that the rate of DON turnover is higher than the DOC rate. This result is consistent with Farrell et al. (2013), who show that microbial uptake

rate of trialanine > dialanine > L-alanine, and could be due to higher N and C contents rather than soil microbial community structure defined by PLFA. It is logical to conclude that amino acids have higher microbial N and C mineralization rate than glucose due to higher N and C contents and shorter $t_{1/2}$ of the amino acids. This result supports our hypothesis that mineralization rate of DON is higher than for DOC in arid soils due to higher N and C contents that accumulate more C and N for a rapidly cycling pool.

4.4.2 Microbial biomass C use efficiency

The results indicate that microbial C use efficiency (Mic_{eff}) was very similar between the three sites. Overall, increasing temperature tended to decrease Mic_{eff} after the addition of DON and DOC substrates. We suggest that this could be due to the relative balance of C flow through different metabolic pathways affected by temperature. This result is consistent with Roberts and Jones (2012), who show that the Mic_{eff} of glucosamine tends to decrease with increasing temperature due to changing temperature affecting different metabolic pathways of individual C compounds. These results support our hypothesis that the rate of utilization of LMW DON and DOC compounds by soil microbial communities will be affected by soil temperature and that increasing temperature not only speeds up organic matter cycling but also the way the C is subsequently used by the microbial biomass. Typically, mathematical models describing soil organic matter turnover use a Q_{10} factor of 2 to describe how mineralization increases with temperature but they rarely factor in concurrent changes in Mic_{eff}. Based on our results this would indicate that C will be lost at a greater rates than predicted from these simple C models.

4.4.3 Temperature dependency of ^{14}C respiration rate

Temperature is a regulator of microbial activity in soil and is therefore a key control on increase the rate of soil organic matter turnover. Preserving organic matter in arid soils is central to maintaining soil quality as it's responsible for many beneficial aspects of soil functioning (e.g. increasing aeration, water infiltration, nutrient cycling/retention, providing a habitat for microorganisms etc.). Factors that lead to the accelerated loss of organic matter need to be better understood so that they can be managed either by eliminating or reducing them or putting in place mitigation strategies to minimise them (e.g. replenishing organic matter reserves). In addition, the loss of organic matter will lead to increased emissions of CO_2 and is

likely to lead to enhanced N mineralization, which depending upon the hydrological regime, may lead to excess nitrate leaching. While this may lead to pollution of groundwater and drinking water and indirect N_2O emissions, it also represents an economic loss to farmers.

As expected, our results showed an increase in soil respiration rate with increasing temperature. We suggest that this could be due to microbial communities favouring high temperature for uptake, turnover and metabolism of the added substrate. Agehara and Warncke (2005) show an increase in microbial respiration with increasing temperatures from 5 to 25°C due to the microbial communities favouring catabolic processes of substrate at higher temperature. This could be due to microorganisms using ^{14}C in respiration for maintenance costs at high temperature (to survive) rather than for growth and storage (Boddy et al., 2008).

4.5 Conclusions

As expected, soil temperature increased mineralization rate in the three arid soils examined here. For each low molecular weight substrate, a double exponential decay model conformed well to the experimental mineralization data. This allowed the microbial partitioning of C into catabolic and anabolic processes to be determined for each substrate and soil. Using this approach, we showed significant increases in the amount of substrate-C allocated to microbial catabolic processes with increasing soil temperature. This results in an overall reduction in C use efficiency within the soil microbial community. In addition, this study showed that the rate of amino acid turnover was higher than for glucose. We ascribe this to the production of organic acid skeletons from rapid internal transamination and deamination of amino acids within the microbial biomass, allowing them to be used directly in respiratory pathways. Additional studies are needed to investigate the effect of using alternative sources of irrigation water on soil function, particularly the response of various moisture regimes on soil chemical characteristics and rates of C and N cycling.

Chapter 5. Does soil moisture regimes and irrigation water source affect DOC and DON mineralization in arid agricultural soil?

A.Z. AlMulla, D.L. Jones, P. Roberts

School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd LL57 2UW, UK

Abstract

The provision of nitrogen in a form that plants and microbes can access relies, to a great extent, on the breakdown of plant residues such as leaves and roots to protein, peptide and amino acid components and their conversion to inorganic forms of N. Research to date has largely focused on understanding processes in temperate soils, where temperature and moisture content is known to support microbial growth and function. Other soils need to be studied to gain a better global understanding of N cycling. In this experiment, we used agricultural soil from the Al-Hassa eastern oasis, Saudi Arabia to investigate the influence of short-term effects of moisture regimes and irrigation water source on 1) soil chemical characteristics, and 2) the rate of soluble and insoluble ^{14}C plant materials mineralization by soil microorganisms. Using labelled soluble and insoluble ^{14}C plant materials in laboratory incubation experiments at different soil moisture content (10, 30, 50, 70 and 90% WHC) of different irrigation water source (groundwater (GW), tertiary treated wastewater (TTWW), and mixed water (MW)), we show that different moisture regimes and water irrigation source influenced soil chemical characteristics. Our results showed that the production of $^{14}CO_2$ from insoluble plant materials increased by different irrigation water type and follows a pattern of MW > TTWW = GW due to increasing labile C concentration in irrigation water source (i.e., DOC content) that increase microbial activity. The results from this agricultural site also show increasing respiration of ^{14}C compounds with increasing soil moisture content. Our findings have increased our understanding of the influence of various irrigation water source and moisture content treatments on soil microbial processes that affect C and N cycling in arid agricultural systems.

Keywords: Al-Hassa eastern oasis, ^{14}C plant materials, microbial mineralization, tertiary treated wastewater irrigation, groundwater irrigation, mixed water

5.1 Introduction

Surface soils in arid and semi-arid environments experience extended periods of drying, followed by short rewetting events due to irrigation and infrequency of rainfall events (Fierer et al., 2003; Watts et al., 2007; Xiang et al., 2008; Qiu-Hui et al., 2012). The impacts of wetting and drying (WD) cycles can be expected to be complex, affecting soil physical, chemical and biological conditions, including

changing microbial community activity, soil structure, and soil organic matter (SOM) (Fierer and Schimel, 2002; Agehara and Warncke, 2005; Watts et al., 2007; Qiu-Hui et al., 2012). WD cycles can affect mineralization process by 1) decreasing or increasing bacterial and fungal community through decomposition, death, and/or new growth (Gordon et al., 2008; Bapiri et al., 2010); 2) impacting microbial activity by increasing organic substrate that become available for microbial attack through undergoing osmotic shock (substrate came from death of soil organisms), which release intracellular solutes or induce microbial cell lysis (Fierer et al., 2003; Watts et al., 2007; Xiang et al., 2008; Göransson et al., 2013); and 3) reduction of microbial biomass due to stress-sensitive microbes dying and the resources being allocated to stress survival rather than respiration (Fierer and Schimel, 2002; Watts et al., 2007). Changing soil moisture content can also influence soil microbial C and N mineralization processes through regulation of oxygen diffusion in soil (Agehara and Warncke, 2005). The maximum aerobic microbial activity occurs between 50% and 70% water holding capacity (WHC), where the minimum microbial activity occurs at low soil moisture content due to reducing intracellular water potential, the diffusion of soluble substrate, and therefore, microbial activity (Agehara and Warncke, 2005; Watts et al., 2007).

DON can enter into soil from many sources. These pathways include wet and dry deposition (e.g., irrigation water, rainfall, throughfall; low DON concentrations in arid ecosystem), livestock urine and faeces, organic fertiliser additions, litter fall, microbial exudation, and residues of roots and organisms (varied from low to high concentrations depending on soil site) (Christou et al., 2006; Jones et al., 2008; Ge et al., 2010). Plant and microbial residues are the major inputs of organic matter into soil and most losses are from microbial decomposition of organic C that return CO_2 to the atmosphere (Christou et al., 2006; Jones et al., 2008; Simfukwe et al., 2011). Plant residues can be separated into two fractions (Simfukwe et al., 2011). The soluble fraction generally consists of a mixture of common plant exudate compounds (e.g., organic acids, sugars, and amino acids) that easily turn over in soil within hours. The second fraction, the insoluble fraction, are generally compounds with high molecular weight that contain complex plant polymers (e.g., some proteins, lignin, cellulose, and hemicellulose) which typically turnover over days or months (Simfukwe et al., 2011; Glanville et al., 2012). The soluble fraction is regularly

released to soil from root exudation, microbial and plant cells lysis, and throughfall (Simfukwe et al., 2011), whereas the insoluble fraction tends to be the less easily degraded components of plant residue.

The Al-Hassa Irrigation and Drainage Authority (HIDA) have been utilizing alternative sources of water to irrigate the oasis farms due to increasing demand on limited groundwater supply for agricultural, industrial and domestic uses in arid areas (Al-Kuwaiti, 2010; Aldakheel, 2011). Groundwater (GW), tertiary treated wastewater (TTWW), and a mixture of tertiary treated wastewater, drainage water and groundwater (MW) are used to support agricultural production (Al-Kuwaiti, 2010; Aldakheel, 2011). Utilizing TTWW for irrigation can decrease the pressure on GW supplies significantly. Currently, the Irrigation and Drainage Authority in Al-Hassa Oasis, KSA, sources more than 64% of its total irrigation water from alternative sources, where 24% comes from TTWW and 40% from MW (Al-Kuwaiti, 2010). The changing of water sources is expected to affect soil nutrient status and the environment. Typically, TTWW may supply mineral nutrients and organic matter that are advantageous to crop production, or it may increase heavy metals content and expose crops to toxic substances (Lucho-Constantino et al., 2005). Pescod (1992) shows that the effluent from selected TTWW in California supplied different forms of nitrogen (N) (e.g. 0.4 - 21.3 mg NO_3^--N l^{-1}, 0.1 - 16.6 mg NH_4^+-N l^{-1}, and 0.2 - 2.6 mg organic-N l^{-1}). The addition of organic substrate stimulate the mineralization rate with greater CO_2 release resulting in priming effect (Rukshana et al., 2013) depending on many factors (e.g., soil texture, microbial community, soil temperature see Chapter 3 and 4, moisture content, …etc).

The aim of this study is to simulate the actual period of irrigations at different range of typical moisture content in the Al-Hassa eastern oasis, and to investigate the influence of various types of irrigation water and moisture content treatments on soil microbial processes such as C and N mineralization rate. In addition, agricultural management practice (e.g., fertiliser addition, irrigation water, …etc.) may increase the availability and content of soil organic C (Fisk et al., 2015). Therefore, we need to increase our understanding of the influence of moisture regimes and irrigation water source on mineralization of soluble and insoluble C from plant material in arid agricultural systems such as those found in KSA. We hypothesized that increasing

labile C concentration in irrigation water source will increase the decomposition of the insoluble fractions of plant residues due to increase microbial activity.

To examine these hypotheses, we determine the ^{14}C mineralization of soluble and insoluble fractions of plant components (*Lolium perenne* L. shoots) in response to different water irrigation source or moisture contents from agricultural arid soil, and further determine various soil and water physical and chemical parameters.

5.2 Material and methods

5.2.1 Soils and water sampling

Soil samples used in this study were collected from the topsoil (0 - 30 cm) of an arid agricultural soil in the Al-Hassa eastern oasis (25°25.430'N 049°37.460'E) in the fourth week of April 2013. The date palm (*Phoenix dactylifera*) was the most common crop on this site. *P. dactylifera* were planted in a 4 m X 6 m grid and interplanted with vegetables, fruit, and Hassawi alfalfa (*Medicago sativa* L.) crops. Water samples were collected in clean, labelled bottles from sources of each water source that irrigate Al-Hassa eastern oasis. All soils and water samples were cooled then shipped to the UK for analysis.

The site has a mean annual rainfall of about 70 mm, an annual surface irrigation 27560 m^3 ha^{-1} $year^{-1}$, an average annual temperature range of 28 - 47.5°C with an average of 34.1°C, where the highest temperature recorded is 50°C. The air humidity is remarkably high for arid systems, and could reaching 90% in the summer (Albowarthan et al., 2008; Al-Fredan, 2011; Aldakheel, 2011). The average daily temperature of the soils at 30 cm depth typically varies from 17.1 to 37.6°C with an average of 30°C (data from Agriculture & Veterinary research station and training, King Faisal University, Al-Hassa, KSA).

5.2.2 Laboratory wetting and drying experiments

The soil water holding capacity (WHC) was determined according to OECD Test No. 222 (2004). The soil samples were air dried, sieved through a 2 mm sieve, homogenized, and kept at 6°C for 1 week before use. We applied three different irrigation water source (GW, TTWW, MW) at four different moisture levels:

1) constant 30% WHC incubated for 35 days at 30°C

2) constant 50% WHC incubated for 35 days at 30°C

3) constant 70% WHC incubated for 35 days at 30°C

4) five wetting drying (WD) cycles separated by 7 days. Each cycle is wetted to 70% WHC for a 1-day period, followed by 6-days dried period at 30% WHC. Soil were wetted to 70% WHC by adding sufficient amount of different irrigation waters into each centrifuge tube. The soils dried to 30% WHC by removing the centrifuge tube lid, incubated at 40°C for 24 h (by determined the weight of centrifuge tube), and maintained at 30% WHC (by closing the centrifuge tube lid) for 6 days. This cycle was repeated 5 times over a period of 35 days (Figure 5.1) to simulate the actual period of irrigation on the oasis. Sampling extraction were destructively sampled after rewet the samples on 0, 7, 14, 21, 28, and 35 days for determine soil chemical characteristics (see Section 5.2.3).

Triplicate samples of 40 g of air dried soil were sieved to remove stones and placed in a 50 ml clean labelled centrifuge tube, with a sufficient amount of the different irrigation waters added to each centrifuge tube to maintain different moisture regime (36 samples in 6 sampling events (i.e., 0, 7, 14, 21, 28, and 35 days) + 36 samples for soil respirations; total *n* = 252), and incubated for 35 days at 30°C (representing average soil temperature).

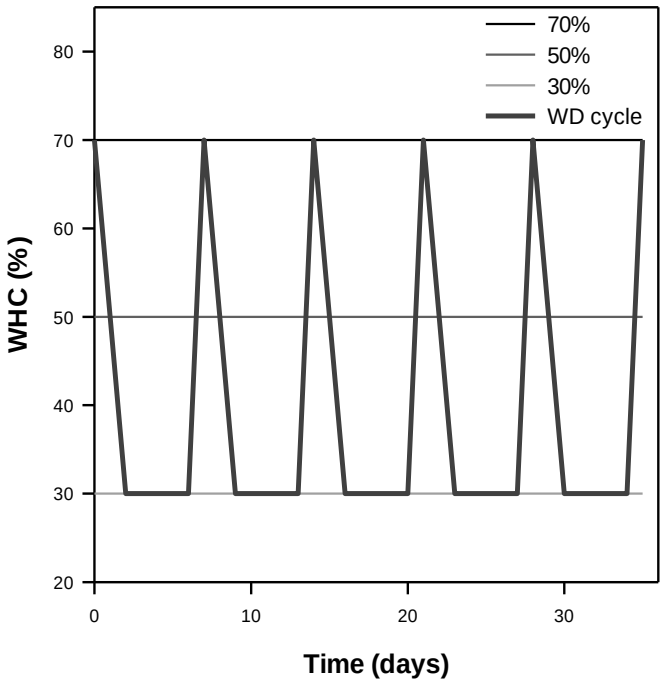


Figure 5.1 Experimental design of the treatment and sampling timeline for the influences of soil moisture regimes and irrigation water source on soil chemical characteristics.

5.2.3 Soil and water samples chemical properties

Soils were destructively sampled after 0, 7, 14, 21, 28, and 35 days incubation at the WHC described in Section 5.2.2. Soil solutions were extracted by centrifuging the samples at 29400 relative centrifugal force (rcf) for 30 min. The soil solutions were either analysed on the day of collection or transferred into clean labelled polypropylene vials and stored in a freezer at -20°C until further analysis. We used standard electrodes to determine pH and EC from soil solutions. Soil solutions were analysed for DOC, amino acids, NH_4^+, NO_3^-, available P, and phenolics as described in Section 3.2.2. Soil respiration (SR) and total element were measured as described in Section 3.2.2. Irrigation water chemistry was analysed using the same methods as those for soil solution described above. TC and TN for water samples were determined using a CHN2000 analyser (Leco Crop., St Joseph, MI).

5.2.4 Determining the influences of soil moisture regimes and irrigation water source on ^{14}C-substrate mineralization

The soluble and insoluble ^{14}C plant materials we used in this study were sourced from ^{14}C-labelled *Lolium perenne* L. shoots with a specific activity of 12.3 kBq g^{-1}. To prepare the soluble and insoluble fraction of plant materials, 4 g of shoots were extracted twice in hot water maintained at 80°C for 4 h (1:10 ratio, plant material:distilled water). After each extraction time, the solution was centrifuged at 2653 rcf for 10 mins and the supernatant (soluble fraction mixture) was filtered through Whatman No. 42 filter paper. The remaining residue (insoluble fraction) was dried at 80°C overnight (Glanville et al., 2012).

In order to keep moisture contents at the desired levels, the soluble fraction was added to soil by drying on sand. To label the sand, 4 ml of ^{14}C soluble fraction mixture was added to 10 g sterile sand and dried overnight at 80°C (Glanville et al., 2012). 100 mg of ^{14}C-labeled sand or insoluble fraction was add to 5 g of soil samples (1.26 kBq g^{-1}). Triplicate samples were incubated at 30°C after addition of sufficient types of the 3 irrigation waters (GW, TTWW, MW) to maintain moisture treatments at 10, 30, 50, 70, and 90% of WHC; (total n = 90) to simulate the rate of irrigation in different period (i.e., high moisture 90% WHC indicate irrigation farm in high rate with small period). The production of $^{14}CO_2$ over time, ^{14}C-labelled soluble fraction mineralization, half-life ($t_{1/2}$) of first pool a_1, and C use efficiency of microbial biomass (Mic_{eff}) were measured as described in Section 3.2.4.

The mineralization of the ^{14}C-labelled insoluble fraction from the soil was described by the single first-order exponential decay model with asymptote:

$$f = y_0 + [a_1 \times exp^{-k_1}] \text{ (Glanville et al., 2012)}$$

Where: y_0 is the asymptote value that represents unavailable pool for microbial mineralization over the duration of the experiment. a_1 is the single pool size of mineralizable (labile C; that recovered in the NaOH traps after 56 days) and k_1 is the corresponding constant rate.

5.2.5 Statistical analysis

Statistical analyses (ANOVA with Tukey's pairwise comparison) were performed using Minitab 17 (Minitab Inc., State College, PA, USA) with significance differences set at $P \leq 0.05$ unless otherwise stated. The double or single first-order exponential decay equations were fitted to experimental results, and linear regression models were applied to find the influences of soil moisture regimes and irrigation water source on ^{14}C-substrate mineralization by using Sigmaplot 12.5 (SPSS Inc., Chicago, IL, USA).

5.3 Results

5.3.1 Irrigation water properties

Chemical characteristics of irrigation water source are presented in Table 5.1. All chemical characteristics were within acceptable national values of water quality for irrigation according to Ayers and Westcot (1985) and Al-Jasser (2011) with the exception of EC value of groundwater and mixed water, which were higher than the standard of 3 mS cm^{-1}. There were significant differences between all chemical characteristics of irrigation waters except Cr, Fe, phenolics, TN, TC, NH_4^+, and DOC/DON ratio content. Groundwater shows the highest pH and Zn contents, but the lowest available P, NO_3^-, DON, DOC, and amino acid contents of the three irrigation water source. Treated wastewater shows the highest available P, DON, and amino acid contents; however, it shows the lowest EC, Na, S, Cl, K, Ca, Mg, and Zn contents of the three irrigation water source. The mixed water shows the highest EC, Na, S, Cl, K, Ca, Mg, NO_3^-, and DOC contents but shows the lowest pH value of the three irrigation water source.

Table 5.1 Physical and chemical characteristics of irrigation water source. Values represent means ± SEM (n = 3). Different letters indicate significant difference between irrigation water source ($P \leq 0.05$) and no letters shows there are no significant differences.

Water/ Irrigation source Parameters	Ground water	Treated wastewater	Mixed
pH	7.27 ± 0.03 [a]	7.22 ± 0.05 [a]	6.83 ± 0.04 [b]
EC (mS cm^{-1})	4.92 ± 0.16 [b]	2.21 ± 0.03 [c]	7.04 ± 0.09 [a]
Na (mg l^{-1})	624.65 ± 35.78 [b]	175.42 ± 2.22 [c]	724.62 ± 7.78 [a]
S (mg l^{-1})	187.79 ± 8.27 [b]	98.99 ± 6.70 [c]	306.20 ± 21.92 [a]
Cl (mg l^{-1})	1103.63 ± 52.75 [b]	406.35 ± 24.31 [c]	1755.29 ± 85.42 [a]
K (mg l^{-1})	17.94 ± 0.89 [b]	15.03 ± 1.25 [b]	47.97 ± 6.86 [a]
Ca (mg l^{-1})	185.20 ± 12.65 [b]	99.56 ± 4.28 [c]	270.72 ± 19.73 [a]
Mg (mg l^{-1})	130.29 ± 6.91 [b]	68.16 ± 0.50 [c]	206.84 ± 3.98 [a]
Cr (mg l^{-1})	0.09 ± 0.01	0.02 ± 0.01	0.05 ± 0.02
Fe (mg l^{-1})	0.18 ± 0.11	0.07 ± 0.03	0.21 ± 0.06
Zn (mg l^{-1})	0.24 ± 0.04 [a]	0.03 ± 0.01 [b]	0.05 ± 0.03 [b]
Available P (mg l^{-1})	0.04 ± 0.001 [c]	4.99 ± 0.46 [a]	2.50 ± 0.11 [b]
Phenolics (mg l^{-1})	1.74 ± 0.58	1.33 ± 0.45	3.10 ± 0.54
TN (%)	0.094 ± 0.019	0.136 ± 0.011	0.141 ± 0.012
TC (%)	0.008 ± 0.001	0.005 ± 0.002	0.005 ± 0.000
NO_3^- (mg N l^{-1})	7.02 ± 0.05 [b]	7.11 ± 0.22 [b]	9.68 ± 0.41 [a]
NH_4^+ (mg N l^{-1})	0.02 ± 0.01	0.02 ± 0.004	0.08 ± 0.04
DON (mg N l^{-1})	0.41 ± 0.01 [b]	0.61 ± 0.09 [a]	0.43 ± 0.18 [b]
DOC (mg C l^{-1})	1.03 ± 0.30 [c]	4.69 ± 0.36 [b]	6.44 ± 0.18 [a]
DOC/DON ratio	2.49 ± 0.70	8.13 ± 0.87	23.84 ± 11.65
Amino acids (mg N l^{-1})	0.01 ± 0.001 [b]	0.05 ± 0.02 [a]	0.03 ± 0.001 [ab]

5.3.2 Influences of soil moisture regimes and irrigation water source on soil chemical characteristics

The influences of soil moisture regimes and irrigation water source on chemical characteristics of soil are presented in Figure 5.2 and Figure 5.3. At the end of the incubation period (35 d), soil parameters responded in different ways dependent on treatment, with no clear pattern except in soil pH and soil respiration for different water irrigation source. There were significant differences between the value of soil pH and the addition of three different water irrigation source across all moisture content treatments on 35 d, following a pattern of MW > GW = TTWW in most cases ($P \leq 0.05$; Figure 5.2 A1 - C1). The value of soil pH on 35 d increased with increasing soil moisture content in almost all cases ($P \leq 0.05$; Figure 5.2 A1 - C1). WD cycles show higher pH value except in soil treated with MW on 35 d ($P \leq 0.05$; Figure 5.2 C1). The addition of different water irrigation source had no significant influence on EC value at different moisture constants, except at 70% WHC treatment, and followed a pattern of GW = MW > TTWW. The value of EC decreased with increasing soil moisture content in all cases ($P \leq 0.05$; Figure 5.2 A2 - C2),

where WD cycles show similar EC value with constant 50% WHC in all cases at 35 d (P > 0.05; Figure 5.2 A2 - C2). Water irrigation source had a significant influence on soil respiration at 35 d and followed a pattern of MW = TTWW > GW in all cases, except at 30% WHC and WD cycles, where no significant differences were observed. There were no significant differences observed at different constant moistures with soil respiration (P > 0.05; Figure 5.2 A3 - C3), but WD cycles show 36% lower soil respiration than constant moistures on 35 d in all cases (P ≤ 0.05).

The addition of different water irrigation source had significant influence on DOC concentrations at constant 50% and 70% WHC treatment by the end of the incubation period. Soil irrigated with MW contain the highest concentration of DOC among all water sources, with about 54% higher than TTWW, 98% higher than GW for constant 50% WHC, 148% more than GW and 174% more than TTWW for constant 70% WHC, whereas the concentration of DOC for constant 30% or WD cycles are broadly similar with no significant differences on 35 d (P > 0.05; Figure 5.3 A1 - C1). The highest DOC concentrations after the application of GW was found after WD cycles treatment, and about 142% more than constant 70%, 219% more than constant 50%, and 275% more than constant 30% on 35 d. By the end of the study, WD cycle shows a significantly higher amount of DOC produced than all constant WHC treatments, except soil treated with MW (P ≤ 0.05; Figure 5.3 A1 - C1). Over 2 weeks' incubation, amino acid concentrations decreased from mean values of 1.03 mg N kg^{-1} on the first day to about 0.13 mg N kg^{-1} soil across all treatment types at an individual treatment scale across incubation times, while NH_4^+ concentrations increased (Figure 5.3 A3 - C3). Over a similar period, we measured increases in NH_4^+ mean values from 0.51 to 4.09 mg N kg^{-1} soil. On day 35, MW had the highest amino acid concentrations in WD cycle among all sites, and about 56% more than TTWW and 172% more than GW sites. The highest concentrations of NH_4^+ at 70% WHC treatment was observed in MW, and about 174% more than TTWW and 447% more than GW site on 35 d, whereas other soil moisture regimes and irrigation water source had no significant influence on amino acid or NH_4^+ concentrations on 35 d (P > 0.05; Figure 5.3 A2 - C3). GW irrigated soil showed the highest NO_3^- concentrations at 70% WHC among all water sources, and about 82% higher than TTWW and 684% more than MW site on 35 d. The constant 50% WHC treatment shows the highest NO_3^- concentration almost in all cases, except soil

treated with GW. WD cycles show the lowest NO_3^- concentrations except soil treated with MW (Figure 5.3 A4 - C4).

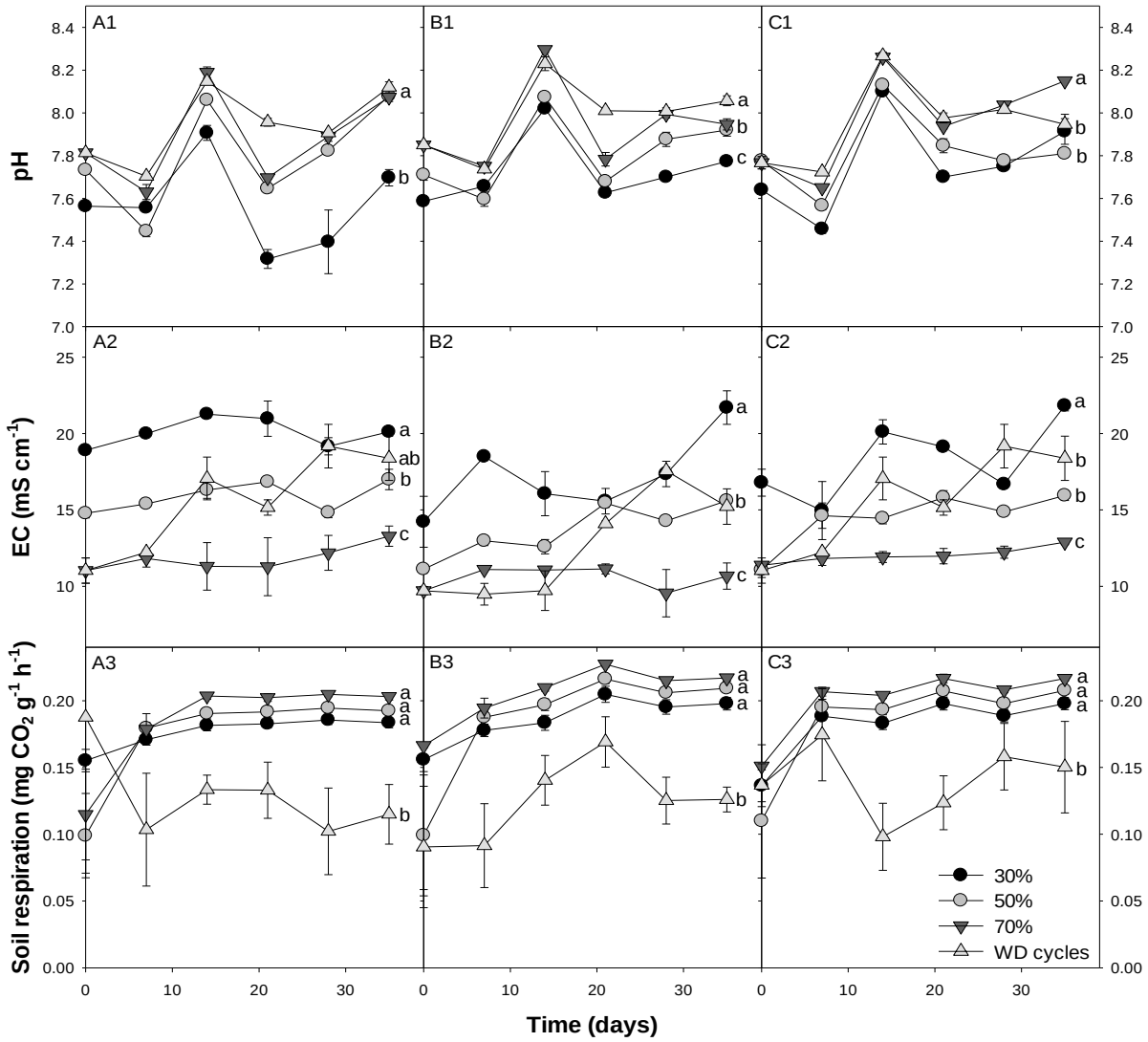


Figure 5.2 Influences of soil moisture regimes and irrigation water source on soil pH (1), EC (2), and soil respiration rate (3), during the periods of incubation 35 days. Points represent means ± SEM (*n* = 3). A, B, and C = soil irrigated with GW, TTWW, and MW type respectively. 30% = constant 30% WHC treatment, 50% = constant 50% WHC treatment, 70% = constant 70% WHC treatment, and WD cycle = five wetting dry cycles. Small letters identify significant differences between moisture content within each water treatment at 35 days ($P \leq 0.05$).

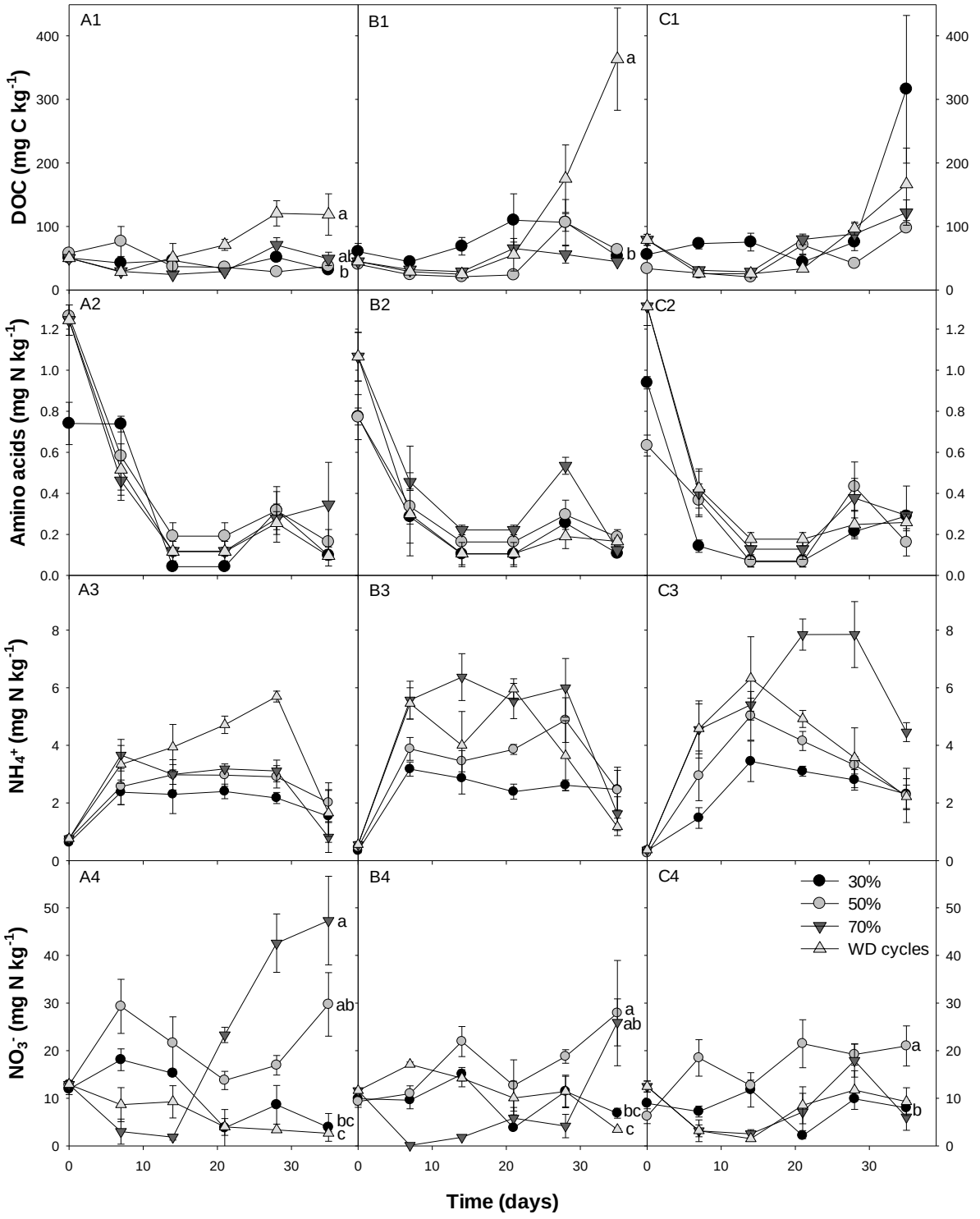


Figure 5.3 Influences of soil moisture regimes and irrigation water source on soluble C and N in soil (dissolved organic C DOC (1), amino acids (2), NH_4^+ (3), and NO_3^- (4) concentrations) during the periods of incubation 35 days. Points represent means ± SEM (n = 3). A, B, and C = soil irrigated with GW, TTWW, and MW type respectively. 30% = constant 30% WHC treatment, 50% = constant 50% WHC treatment, 70% = constant 70% WHC treatment, and WD cycle = five wetting dry cycles. Small letters identify significant differences between moisture content on 35 days ($P \leq 0.05$).

5.3.3 Influences of soil moisture regimes and irrigation water source on ^{14}C-substrate mineralization and half-life

The influences of soil moisture regimes and irrigation water source on $^{14}CO_2$ evolution and Mic_{eff} for soluble and insoluble plant materials are shown in Figure 5.4 and Figure 5.5, while the kinetic parameters reflecting different C allocation pools (e.g. a_1, a_2, and y_0) derived from ^{14}C soluble and insoluble plant materials are presented in Table 5.2. Overall, there was no significant influence on $^{14}CO_2$ evolution from soluble plant materials' fraction between different water irrigation source (P > 0.05; Figure 5.4 and Figure 5.5 A1 - C1). In soils irrigated with GW, the 50% moisture content treatment showed the highest amount of $^{14}CO_2$ evolution from soluble plant material fraction at the end of the incubation period. This was about 25% more than 30% WHC, 28% more than 70% WHC, 33% more than 90% WHC, and 58% more than 10% WHC ($P \leq 0.05$; Figure 5.5 A1). In contrast, irrigating soils with TTWW or MW showed no distinct pattern of overall $^{14}CO_2$ production in soils where soluble substrates were added (P > 0.05; Figure 5.4 and Figure 5.5 B1 and C1).

Irrigation water source had a significant effect on the amount of $^{14}CO_2$ evolution from insoluble plant materials in most cases and followed a pattern of MW > TTWW = GW at the end of the incubation period, except at 30% WHC, where no significant observed. Soil moisture content generally increase overall $^{14}CO_2$ evolution from insoluble plant materials in almost all cases ($P \leq 0.05$). At the end of 56 d, soluble fractions lost more of the initial ^{14}C added as $^{14}CO_2$ (42.58 - 67.47%) compared to insoluble fractions (2.52 - 39.46%) with significant differences (Figure 5.5 A1 - C1).

There were no significant differences observed between C use efficiency of microbial biomass (Mic_{eff}) from soluble plant materials with different addition of water irrigation source or at different moisture constants (P > 0.05; Figure 5.5 A2 - C2). GW showed the highest Mic_{eff} from insoluble plant materials between the addition of three water source and about 4% more than TTWW and 7% more than MW ($P \leq$ 0.05). Moisture content tended to decrease Mic_{eff} from insoluble plant materials with significant differences in most cases. The insoluble plant materials show higher Mic_{eff} than soluble plant materials in all water irrigation sources and content ($P \leq 0.05$; Figure 5.5 A2 - C2).

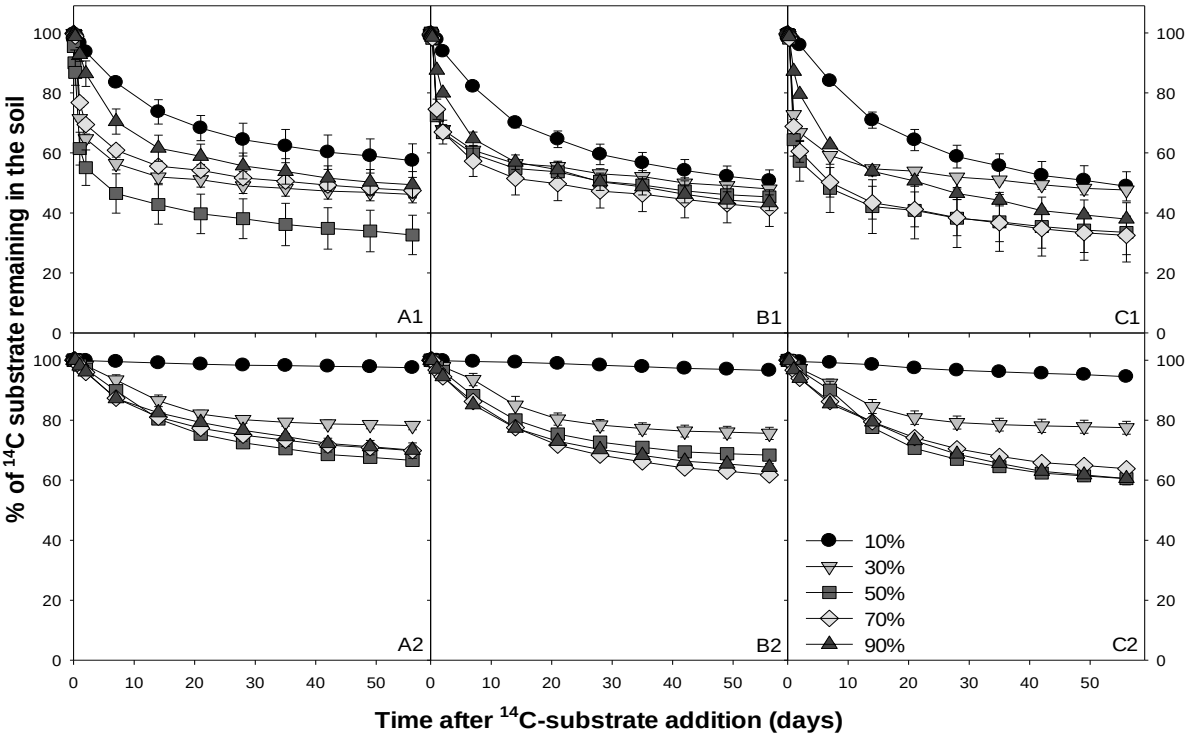


Figure 5.4 Influences of soil moisture regimes and irrigation water source on ^{14}C-substrate mineralization. Points represent means ± SEM (*n* = 3). A, B, and C = soil irrigated with GW, TTWW, and MW type respectively. 1 and 2 = soluble and insoluble plant materials respectively.

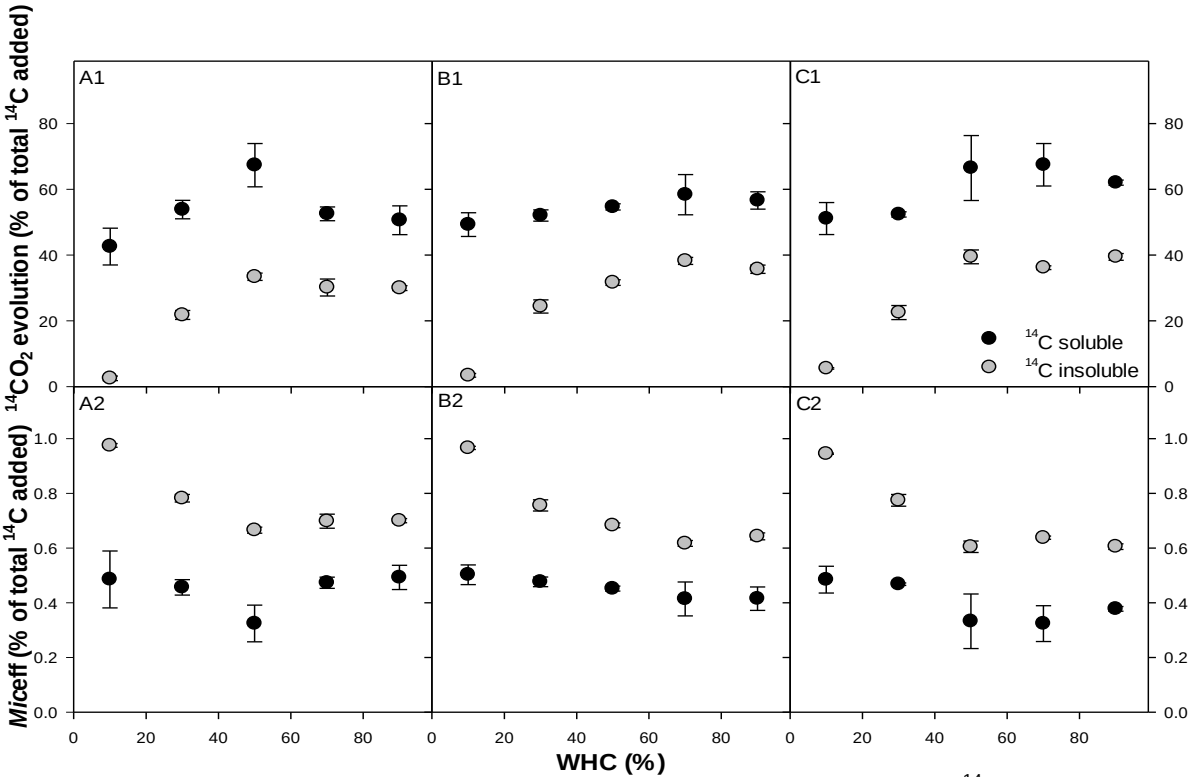


Figure 5.5 Influences of soil moisture regimes and irrigation water source on $^{14}CO_2$ evolution: (1) *Mic*eff (2) for soluble and insoluble plant materials during the periods of incubation 56 days. Points represent means on 56 days ± SEM (*n* = 3). A, B, and C = soil irrigated with GW, TTWW, and MW type respectively.

The double first-order exponential decay equation was the best fit for mineralization data of soluble plant materials fraction (R^2 = 0.995 ± 0.001). Insoluble plant materials fraction best fit a single first-order exponential decay model with asymptote (R^2 = 0.991 ± 0.003). The pool size a_1 derived from ^{14}C soluble plant material described the first rapid phase of $^{14}CO_2$ production from catabolic process (respiration) and a_2 describes the second, slower phase of $^{14}CO_2$ production from immobilized or ^{14}C taken up within the microbial community. In all cases, no significant effects were observed between water irrigation sources or at different moisture constant with the pool size a_1 or a_2 (P > 0.05; Table 5.2). With the exception of 10% WHC, there were no observed significant effects of water irrigation source on the half-life ($t_{1/2}$) of soluble plant material from the first pool a_1. There was a pronounced decrease of approximately 91.5% in $t_{1/2}$ when WHC of all water sources increased from 10% to 30% in all cases ($P \leq 0.05$; Table 5.2).

The addition of different water irrigation source had some significant influence on pool kinetic parameter derived from ^{14}C insoluble plant materials (Table 5.2). Irrigation water source had little or no effect on pool y_0 at low WHC (10% and 30%). At higher WHC's, TTWW and MW reduced the amount of ^{14}C allocated to this pool compared to GW and followed a pattern of GW ≥ TTWW > MW (Table 5.2). In most cases, the asymptotic pool decreases with increasing moisture content. As for soluble material, a_1 describes the pool size of a single mineralizable labile C pool. Water irrigation source affected a_1 pool size (Table 5.2), particularly at 50, 70, and 90% WHC. MW tended to increase the allocation of ^{14}C to the a_1 pool followed by TTWW, but this pattern is by no means consistent for all WHC's. WHC increases the pool a_1 size up to 36% and then remains broadly consistent ($P \leq 0.05$). Irrigation water source affected $t_{1/2}$ of insoluble plant material from first pool a_1 (Table 5.2) at 70, and 90% WHC and followed a pattern of MW > TTWW = GW. $t_{1/2}$ of insoluble plant material did not change with increasing WHC and remained broadly at 16 d.

Table 5.2 Influences of soil moisture regimes and irrigation water source on pool kinetic parameter derived from ^{14}C soluble and insoluble plant materials. Values represent mean ± SEM (n = 3). Letters identify significant differences between moisture content ($P \leq 0.05$). Different numbers indicate there is a significant difference between water irrigation source at same moisture content ($P \leq 0.05$).

Soil moisture content (WHC %)	^{14}C soluble plant materials		
	Pool a_1 (%)	Pool a_2 (%)	$t_{1/2}$ of pool a_1 (d)
Ground water (GW)			
10	34.19 ± 8.98	64.75 ± 8.07	8.34 ± 1.28^{a}
30	46.43 ± 2.02	55.71 ± 2.01	0.81 ± 0.03^{b}
50	50.43 ± 4.90	48.94 ± 5.32	0.57 ± 0.07^{b}
70	41.41 ± 1.77	60.36 ± 1.61	0.93 ± 0.05^{b}
90	36.62 ± 2.42	63.58 ± 2.36	3.69 ± 1.05^{b}
Treated wastewater (TTWW)			
10	39.92 ± 6.11	60.11 ± 6.09	8.43 ± 1.28^{a}
30	40.82 ± 1.49	61.18 ± 1.42	0.73 ± 0.02^{b}
50	42.06 ± 0.53	60.12 ± 0.54	0.73 ± 0.03^{b}
70	44.35 ± 5.29	57.21 ± 4.97	0.93 ± 0.09^{b}
90	37.29 ± 2.92	63.04 ± 2.89	1.93 ± 0.07^{b}
Mixed water (MW)			
10	51.47 ± 8.90	48.30 ± 9.02	11.23 ± 1.60^{a}
30	42.88 ± 0.72	59.06 ± 0.78	0.77 ± 0.01^{b}
50	54.30 ± 8.12	48.34 ± 7.66	0.75 ± 0.02^{b}
70	51.63 ± 4.87	50.60 ± 4.57	0.83 ± 0.03^{b}
90	39.41 ± 2.40	60.97 ± 2.42	2.06 ± 0.20^{b}
Soil moisture content (WHC %)	^{14}C insoluble plant materials		
	Pool y_0 (%)	Pool a_1 (%)	$t_{1/2}$ of pool a_1 (d)
Ground water (GW)			
10	95.24 ± 2.05^{a}	4.70 ± 2.06^{c}	53.41 ± 24.53
30	77.02 ± 1.20^{b}	23.36 ± 1.24^{b}	10.43 ± 2.12
50	65.25 ± 1.73^{c1}	35.04 ± 1.77^{a2}	12.83 ± 1.60
70	70.13 ± 2.44^{bc1}	29.93 ± 2.42^{ab2}	9.63 ± 0.00^{2}
90	70.16 ± 0.83^{bc1}	29.72 ± 0.89^{ab2}	11.23 ± 1.60^{1,2}
Treated wastewater (TTWW)			
10	98.24 ± 0.56^{a}	1.71 ± 0.57^{d}	19.25 ± 4.81
30	74.09 ± 1.27^{b}	26.27 ± 1.27^{c}	12.83 ± 1.60
50	67.50 ± 1.42^{c1}	32.67 ± 1.40^{b2}	11.23 ± 1.60
70	61.10 ± 1.13^{d2}	38.74 ± 1.21^{a1}	9.63 ± 0.00^{2}
90	64.54 ± 1.39^{cd1,2}	35.38 ± 1.35^{ab1,2}	9.63 ± 0.00^{2}
Mixed water (MW)			
10	93.83 ± 2.78^{a}	5.98 ± 2.75^{c}	26.66 ± 11.88
30	76.81 ± 2.00^{b}	23.36 ± 2.12^{b}	9.63 ± 0.00
50	57.89 ± 1.16^{c2}	42.55 ± 0.79^{a1}	14.44 ± 0.00
70	62.94 ± 0.61^{c2}	36.70 ± 0.56^{a1,2}	12.83 ± 1.60^{1}
90	58.32 ± 2.25^{c2}	41.26 ± 2.24^{a1}	14.44 ± 0.00^{1}

5.4 Discussion

5.4.1 Influences of soil moisture regimes and irrigation water source on soil chemical characteristics

The results from this study showed some significant effect on soil properties from the addition of water irrigation source. Soil pH increased with increasing soil moisture content and with the inclusion of WD cycles on 35 d and followed a pattern

of MW > GW = TTWW in most cases (Figure 5.2 A1 - C1). This could be due to either increasing consumption of H^+ ions and OH^- production from organic anion decomposition and ammonification (Rukshana et al., 2014), or increasing basic cations such as Ca^{2+}, Na^+ and Mg^+ content from the different water irrigation source applied (Agehara and Warncke, 2005; Mohammad Rusan et al., 2007). The original high content of Ca^{2+}, Na^+ and Mg^+ from mixed water supported this suggestion ($P \leq 0.05$; Table 5.1).

Increasing soil water content caused a decrease in EC values, irrespective of water irrigation source (Figure 5.2 A2 - C2). This may be due to either the consumption of ions from mineralization/immobilization process with increasing WHC (Zhang and Wienhold, 2002) or dilution factor. NH_4^+ concentrations support this suggestion (Figure 5.3 A3 - C3). Across all water irrigation source, EC values for the WD cycles showed a similar response to the 50% WHC results; this could be due to it is the average of WD cycles (average of 70% and 30% WHC) (Figure 5.2 A2 - C2).

WD cycles showed 36% lower soil respiration than constant moisture regimes, which could be due to either stress-sensitive microbes dying and the resources being allocated to stress survival rather than respiration (Fierer and Schimel, 2002; Watts et al., 2007), or the WD cycles released physically protected the organic nutrient for microbial utilization therefore, reducing soil respiration (Fierer and Schimel, 2002; Xiang et al., 2008). The soil respiration after the application of GW at WD cycle showed decrease after 7 days of incubation (in the first WD cycle; this could be due to microbial died after WD cycle and 35 days is not enough for recovery). whereas, after TWW and MW showed higher concentrations on day 35 than first day this could be due to microbial died after WD cycle and recovered due to priming effect (increasing DOC content from TWW and MW). The high content of DOC after WD cycle support this suggestion (Figure 5.3 A1 - C1).

The highest concentrations of DOC, amino acids, and NH_4^+ were shown after the addition of MW across all water irrigation source, suggesting that it could be due to the original content of these parameters in the water irrigation source applied (Table 5.1) (Mohammad Rusan et al., 2007). Amino acid concentrations decreased over 2 weeks' incubation, while NH_4^+ concentrations increased (Figure 5.3 A2 - C3). This suggests that it could be due to rapid mineralization/immobilization process that converted amino acids to NH_4^+.

5.4.2 Influences of soil moisture regimes and irrigation water source on ^{14}C-substrate mineralization and half-life

The microbial mineralization rate of DON and DOC is controlled by various factors such as temperature, soil moisture content, soil management, vegetation litter, microbial community and soil organic matter (Simfukwe et al., 2011; Glanville et al., 2012; Qiu-Hui et al., 2012; Li et al., 2014). This study focuses only on the short-term effects of moisture regimes and irrigation water source on mineralization of soluble and insoluble ^{14}C plant materials in one arid agricultural soil to ensure uniformity of physical and chemical soil properties. The range of moisture content in this study (10 - 90% WHC) was designed to reflect the actual irrigation regimes and water source applied on this oasis.

Does irrigation water source affect substrate mineralization?

We hypothesized that irrigation water source will increase the mineralization of the insoluble fractions of plant residues depending on the original labile compound from water source that increase microbial activity. Our results show that there were no influences of irrigation water source on mineralization of soluble plant materials and this could be due to microbial usage of different LMW DON or DOC being similar in many microbial communities (Hill et al., 2011c). There were influences of irrigation water source on the mineralization of insoluble plant materials in most cases on 56 d, and followed a pattern of MW > TTWW = GW. This could be due to priming effect from increasing original chemical content (i.e., increasing DOC content from MW; Table 5.1; Rukshana et al. (2013)) and biological factors (e.g., microbial community changes, microbial activity, etc.) (Qualls and Bridgham, 2005; Glanville et al., 2012) applied from insoluble plant materials and different irrigation water source. Increasing labile compounds can affect microbial community composition, microbial size, microbial biomass and microbial activity (Hooker and Stark, 2008; Bowles et al., 2014) that influenced various enzymes' activity related to nutrient transformations and soil health (Elifantz et al., 2011; Liang et al., 2016). This is consistent with other previous studies that show that increased original content of organic matter and substrate applied from organic residue into soil increased microbial community, urease, dehydrogenase (Burgos et al., 2002; Okur et al., 2009), protease (Burgos et al., 2002; Okur et al., 2009; Piotrowska-Długosz and Wilczewski, 2014) and nitrate reductase activity (Piotrowska-Długosz and Wilczewski, 2014) more than control soil.

Elifantz et al. (2011) show that increase in original content of organic C applied from treated wastewater into soil influenced soil microbial activity and increased microbial community respiration more than soil irrigated with fresh water. Anaerobic microbes are activated at high soil water content and these can increase denitrification activity (Zhang and Wienhold, 2002; Li et al., 2014; Liang et al., 2016) this denitrification activity could increase $^{14}CO_2$ production. Barnard et al. (2005) support this suggestion. Water irrigation studied in this study can load different range of DOC content yearly (MW, TTWW and GW can load 148.18 ± 4.19, 107.96 ± 8.26 and 23.77± 6.91 kg ha^{-1} $year^{-1}$ respectively) that increase soil microbial activity leading to increase microbial community respiration. Therefore, further studies are needed to investigate how these different water irrigation source at different soil moisture content influence community composition, microbial biomass, and soil enzymes in the long-term in arid soil for developing better management and reducing N or C gaseous emissions.

Substrate mineralization of water soluble vs. water insoluble fractions

We hypothesized that ^{14}C mineralization of soluble plant materials is faster than insoluble materials. Our results show that the microbial mineralization rate of soluble plant materials is higher than insoluble plant materials with about (42.58 - 67.47%) of $^{14}CO_2$ lost from soluble fractions compared to (2.52 - 39.46%) from insoluble fractions, and that the $t_{1/2}$ of a_1 is 11 times lower (Figure 5.5 and Table 5.2). Therefore, the rate of soluble plant materials turnover is faster than insoluble plant materials rate. This could be due to the mineralization of soluble organic acids, sugars, and other carbohydrates in soluble plant materials being more labile than insoluble plant materials (lignin, cellulose, hemicellulose, insoluble protein, and insoluble residue) (Qualls and Bridgham, 2005; Glanville et al., 2012).

Soil moisture content effects on substrate mineralization

Our results show that there were some influences of increasing WHC on mineralization of soluble plant materials, with highest at 50% and lowest at 10% and 90% (Figure 5.5. A1, B1, and C1). This could be due to low water content at 10% limiting microbial activity by limiting the diffusion of available nutrients and reducing water potential inside the microorganism to prevent dehydration, whereas at 90%, reduced oxygen diffusion due to high water content and therefore oxygen availability can limit microbial activity, thus reducing enzyme production, which is key to

substrate mineralization (Agehara and Warncke, 2005; Glanville et al., 2012). This result is consistent with Li et al. (2014), who found the lowest mineralization rates at 20%, 80%, and 100% WHC. At low WHC, cellular desiccation of microbes occurred, whereas at high WHC, the activity of aerobic microbes decreased due to inhibition of air exchange and increased denitrification process this activity could increase $^{14}CO_2$ production at low value (i.e., denitrification process would increase CO_2; Barnard et al. (2005)).

Insoluble plant materials' mineralization showed significant shifts in kinetic parameter with changing soil moisture regimes. The y_0 pool generally decreases with increasing moisture content for insoluble plant materials (Table 5.2). This occurred with an increase in the rapidly respired pool (a_1), suggesting that water content makes the recalcitrant y_0 pool more prone to microbial decomposition, the response to which is a shift of C into pool a_1, a reduction in $t_{1/2}$, and C use efficiency with increasing moisture content up to the 70% WHC level. This suggests mineralization increase could be due to either the compound becoming more mobile and not protected with the soil matrix at higher moisture content (Glanville et al., 2012) or changes in microbial community compositions at high soil moisture content increasing their ability to metabolize substrates that could not be utilized previously at lower soil moisture (Agehara and Warncke, 2005).

5.5 Conclusions

Three different water irrigation source at different soil moisture content used in this study had some significant influence on soil chemical properties and ^{14}C-substrate mineralization in one arid agricultural site in KSA. Our results show that irrigation water source had no influence on mineralization of soluble plant materials but influenced insoluble plant materials mineralization rate. This could be due to the mineralization affected by labile compounds, microbial community changes, and microbial activity applied from different irrigation water source. This study has shown that increasing WHC had a greater influence on insoluble mineralization rate. Mineralization of ^{14}C soluble plant materials was faster than insoluble materials, thus proving our hypothesis. These findings can extend our understanding of DOC and DON mineralization rate in arid agricultural systems such as those found in KSA. It can help develop a suitable irrigation strategy e.g., reduce the rate of irrigation by

using drip system that can help the government to meeting their target to reduce annual agriculture water demand (reduce 3.7% of agriculture water demand annually), reduce N or C pollution, and support sustainable agriculture systems. This is consistent with Adekoya et al. (2014), who showed that using drip irrigation system in rice cropping enhanced the crop's water and nutrient use efficiency, increased yield, reduced water flooding, reduced nutrient leaching, reduced greenhouse gasses (i.e., methane and CO_2 emissions). Further studies are needed to better understand the long-term effect of different water irrigation source at different soil moisture content on soil microbial processes in arid soil.

Chapter 6. Diffusion of soluble nitrogen away from organic and inorganic fertilisers in an arid soil

A.Z. AlMulla, D.L. Jones, P. Roberts

School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd LL57 2UW, UK

Abstract

Since 2005, the Saudi Ministry of Agriculture (MAO) and Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) started supporting and developing organic agriculture. In this experiment, we used one agricultural soil from the Al-Hassa eastern oasis to investigate the influence of short-term effects of six different N fertiliser treatments on 1) chemical soil properties, 2) the emission of CO_2 and NH_3, and 3) the diffusion of plant-available N compounds. This study focuses on studying the effects of applying different fertilisers (i.e., green manure (GM), hydrolysed protein, ammonium nitrate (NH_4NO_3), and control (no fertilisers applied)) at different fertiliser rates (100 kg N ha^{-1} to simulate the average fertiliser or typical return field rate of 7000 kg N ha^{-1} equivalent to stimulate what happened to microbial population when in direct contact with granular fertiliser) on soil C and N cycling at the millimetre (mm) scale using a microtome. We show that different N fertiliser treatments influence soil chemical properties and N diffusion in one arid agricultural site in KSA. We observed that soil CN ratios significantly decreased after applying NH_4NO_3 at 100 kg N ha^{-1} and 7000 kg N ha^{-1} compared to the control treatment this could be due to N diffused quickly after the addition of fertiliser that changed the CN ratio. GM showed the highest cumulative ammonia (NH_3) emission and CO_2 respiration at 100 kg N ha^{-1} compared to other treatment, but at 7000 kg N ha^{-1}, the highest cumulative NH_3 emission and CO_2 respiration was found after NH_4NO_3 and protein at 7000 kg N ha^{-1} respectively. The highest amino acid concentrations were observed in GM at 100 kg N ha^{-1} among all treatments, where, at 7000 kg N ha^{-1}, the highest amino acid concentrations were observed in protein treatment. NH_4NO_3 showed the highest NH_4^+ and NO_3^- concentrations across all fertilisers at both rates 100 and 7000 kg N ha^{-1}. We observed significant diffusion of amino acids after GM addition at 100 kg N ha^{-1} compared to control, where no diffusion of NH_4^+ and NO_3^- were observed. There was significant diffusion of N compounds after the addition of protein and NH_4NO_3 at both rates 100 and 7000 kg N ha^{-1}, except after the addition of NH_4NO_3 at 100 kg N ha^{-1} on NH_4^+. Therefore, we would recommend farmers and governmental managers to reduce inorganic fertiliser and use more organic N fertiliser that can reduce environmental pollution (e.g. NO_3^- leachate, N_2O emission), cost (due to recycling of farm waste and reduce inorganic fertiliser cost), save more water (due to organic fertiliser can improve water holding capacity), and increased yield. Our finding

increases our understanding of the future influence of using different organic and inorganic fertilisers on C and N cycling in arid soil systems. In addition, it can be valuable to farmers and government in arid and semiarid environment to better understand land management, reduce N or C gaseous emissions, and develop sustainable agriculture systems.

Keywords: Organic N fertilisers, inorganic N fertilisers, *Medicago sativa* L., ammonia

6.1 Introduction

The Ministry of Agriculture in Saudi Arabia (MAO) and Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) started supporting and developing organic agriculture since 2005 (Hartmann et al., 2012). In 2012, 16400 ha was under organic management (78 farms) in Saudi Arabia and around 2200 ha was in development to achieve organic status (Hartmann et al., 2012). These changes in agriculture management practices may influence methods of crop production, and therefore affect soil physical and chemical properties (Chirinda et al., 2010; AlMadini, 2011; Brar et al., 2015), nutrient use efficiency (Chirinda et al., 2010), and soil N transformations (Zhang et al., 2012).

Organic fertilisers are typically associated with the recycling of nutrients from green manure, crop residue, and animal manure into the soil to ensure sustainable agriculture development and to reduce environmental cost (Xiaobin et al., 2001; Chirinda et al., 2010; Brar et al., 2015). The application of organic fertilisers can enhance nutrient cycling through increasing soil organic matter, stimulating microbial activity (Chirinda et al., 2010; Brar et al., 2015) and reducing the need for application of inorganic fertilisers (AlMadini, 2011). In addition, it may improve soil physical properties through improving soil fertility, improving aggregate stability, increasing soil aggregation, improving soil water holding capacity (WHC), increasing the volume of macropores, and decreasing micropores (Chirinda et al., 2010; AlMadini, 2011; Brar et al., 2015). Conversely, the excessive use of inorganic N fertilisers can cause adverse environmental effects, including groundwater pollution from increasing NO_3^- leaching, air pollution from increased emission of greenhouse gasses such as nitrous oxide and ammonia, acidification of soils, decreasing aggregate stability, and promoting soil erosion (Fließbach et al., 2000; Inselsbacher et al., 2009; Chirinda et

al., 2010; López-López et al., 2012; Zhang et al., 2012; Iqbal et al., 2014). Therefore, studies are required to evaluate the environmental effects of using different rates (average and excessive) of organic and inorganic N fertilisers in arid agricultural systems such as those found in KSA.

In this study, we applied six different N fertiliser treatments to investigate N transformations at a high resolution using a custom-made microtome. We used fertiliser applied at 100 kg N ha^{-1} to study its effect on soil chemical properties and the gaseous emissions (CO_2 and NH_3). We then used the microtome method to further investigate the effects of granular fertiliser when in direct contact with soil on microbial population. To achieve this, we applied granular N fertilisers at a typical return field rate of 7000 kg N ha^{-1} equivalent. This was used to simulate the impact on the soil directly adjacent to the fertiliser (i.e., to find out how microbial population will react when direct contact with the fertiliser granule). We hypothesize that: 1) N from inorganic N fertiliser will diffuse faster than N contained in an organic fertiliser due to the rapid increase of inorganic N from inorganic fertiliser; and 2) organic fertiliser can reduce the amount of NO_3^- produced over time that could be due to high microbial N demand from higher available C in organic N fertiliser. This information will be valuable to better understand land management, reduce N or C gaseous emissions, and develop sustainable agriculture systems in arid agricultural systems such as those found in KSA.

6.2 Material and methods

6.2.1 Soils sampling

For this experiment, we used topsoil (0 - 30 cm) of an arid agriculture soil in the Al-Hassa eastern oasis, KSA (25°25.430'N 049°37.460'E). For more details about the soil samples, please see Section 5.2.1.

Treatments

The experiment was designed to determine the effects of using different fertiliser types on the cycling and diffusion of plant-available nitrogen compounds in a typical arid soil. Because soils had been stored for some considerable time since sampling, soil samples were pre-washed with deionized water to remove the accumulated NO_3^-, air dried overnight, sieved through a 2 mm sieve, homogenized, and N free artificial rain water (96 µM NaCl, 10 µM K_2SO_4, 5 µM $CaCl_2$, 6 µM $MgCl_2$,

and 0.1 µM KH_2PO_4) applied to adjust the level of water-holding capacity (WHC) to a constant 50% to ensure uniformity of physical and chemical soil properties. Six different fertiliser treatments were applied: 1. control (no fertiliser addition), 2. ammonium nitrate (NH_4NO_3), 3. hydrolysed protein (Avant Natur®, COMPO, Germany), and 4. green manure (GM) alfalfa or Lucerne (*Medicago sativa* L.) at the same rate of 100 kg N ha^{-1} to simulate the average rate of N applied on the oasis, 5. NH_4NO_3 (N_{7000}) and 6. hydrolysed protein (P_{7000}) (Avant Natur®, COMPO, Germany) at a typical return field rate 7000 kg N ha^{-1} equivalent (covered the whole soil surface with fertiliser granule) to find out what happened to microbial population when in direct contact with granular fertiliser happens when soil is in direct contact with the fertiliser granule (Figure 6.1). *M sativa* was used as a green manure due to it being the major forage crop in KSA (Al-Fredan, 2011). Chemical characteristics of the fertiliser used in this study are presented in Table 6.1.

Table 6.1 Chemical characteristics of the fertilisers used in the experiments. Values represent mean ± SEM (n = 3). Different letters identify significant differences between fertilisers ($P \leq 0.05$).

Fertilisers	NH_4NO_3	Hydrolyzed protein	Green manure
pH	5.35 ± 0.08^{c}	5.54 ± 0.02^{b}	6.17 ± 0.02^{a}
EC (mS cm^{-1})	180.00 ± 2.33^{a}	18.15 ± 0.01^{b}	3.64 ± 0.08^{c}
CN Ratio	-	0.36 ± 0.00^{b}	14.28 ± 0.31^{a}

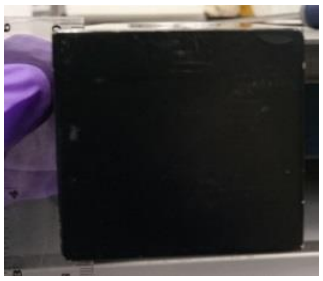
Microtome sampling

Triplicate samples of 40 g of homogenized soil at constant 50% WHC were placed in different tubes cut from centrifuge tubes specifically for microtome sampling with a sufficient amount of fertiliser to each tube to maintain different fertiliser treatments as described above. Samples were destructively collected at 3 sampling events (1, 7, and 28 days (d); Figure 6.1) (90 samples; 54 of the samples have 14 vertical depths 1 to 10, 15, 20, 25, 30 mm = 3 cm. The other 36 samples were in 2 sets of 18 for soil respiration and 18 NH_3 volatilization measurements; total n = 792). Soils were extracted on the day of collection, and soil chemical properties method is described below.

6.2.2 Soil chemical properties

pH and EC were determined using standard electrodes (1:2.5 w/v soil:distilled water extraction). Soils were extracted with 0.5 M K_2SO_4 (1:5 w/v). Total free amino acids, NH_4^+, NO_3^-, CN ratio, and basal soil respiration (SR) were determined as described in Section 3.2.2. Ammonia (NH_3) volatilization was measured by placing a filter paper (Whatman GF/A 2.5 cm diameter) containing 100 µl of 0.15 M phosphoric

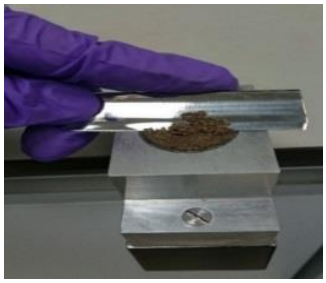
acid into the incubation vessel to trap NH_3 after applying different fertiliser treatments in different set as described above (*n* = 18). After removal, the filter paper was extracted with 1 ml of 0.5 M K_2SO_4 every week for 4 weeks to analyse NH_4^+ as described in Section 3.2.2 (Jones et al., 2012). The solutions were analysed on the day of collection or transferred into clean labelled polypropylene vials and stored in a freezer at -20°C until further analysis. The theoretical linear distance of diffusive movement (*L*) of N over time was calculated according to Jones et al. (2005b) and Abaas et al. (2012).



A) Microtome

B) Specific tube for Microtome

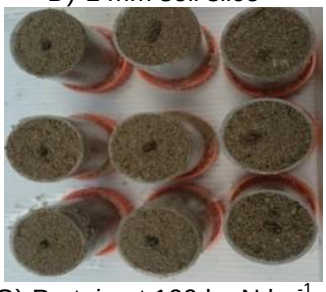
C) Extrusion of the soil 1 mm using the microtome



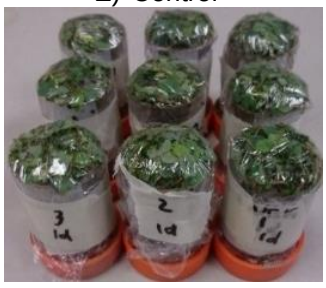
D) 1 mm soil slice

E) Control

F) NH_4NO_3 at 100 kg N ha^{-1}

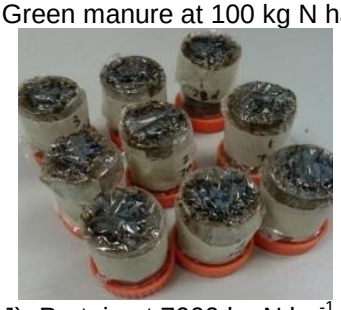


G) Protein at 100 kg N ha^{-1}



H) Green manure at 100 kg N ha^{-1}

I) NH_4NO_3 at 7000 kg N ha^{-1}



J) Protein at 7000 kg N ha^{-1}

Figure 6.1 Experimental design of the treatments and sampling collections at the mm scale using a microtome.

6.2.3 Statistical analysis

Statistical analyses (ANOVA in one column for all factor levels with Tukey's pairwise comparison) were performed using Minitab 17 (Minitab Inc., State College, PA, USA) with significance differences set at $P \leq 0.05$. Cumulative gaseous emissions (NH_3 and CO_2) were determined using the trapezoidal rule. The percentage of substrate N or C evaluated was determined as:

% substrate N or C evaluated = (treatment (NH_3 or CO_2) - control (NH_3 or CO_2) / total N or C applied) X 100%

Nitrogen data scale (amino acids, NH_4^+, and NO_3^-) were transformed by Log base 10 using Sigmaplot 12.5 (SPSS Inc., Chicago, IL, USA) in order to improve the distribution between results.

6.3 Results

6.3.1 Soil pH, EC, and CN ratio responses to fertiliser treatments

Responses to 100 kg N ha^{-1} fertiliser treatments

After the application of fertilisers at 100 kg N ha^{-1}, there were significant effects on the average soil pH (soil pH at different days and distances) and followed a pattern of protein > green manure (GM) > Control > NH_4NO_3 with an average pH value of 9.03, 8.77, 8.51, and 8.31 respectively ($P \leq 0.05$). In most treatments, the average soil pH at different depths showed a significant increase with time, except after applying NH_4NO_3, when it showed no significant difference with time ($P \geq 0.05$; Figure 6.2). The pH value increased by 0.15 pH unit between 1 - 10 mm and showed similar values between 15 - 30 mm after GM addition on day 1 compared to control, increased by 0.26 pH unit between 1 - 30 mm on day 7, and on day 28, increased by 0.35 pH unit and 0.53 pH unit between 1 - 10 mm and 15 - 30 mm respectively compared to control (Figure 6.2). Protein addition increased pH value by 0.57 pH unit and 0.56 pH unit between 1 - 10 mm and 15 - 30 mm respectively compared to control on day 1, 0.63 pH unit between 1 - 30 mm on day 7, and on day 28, increased by 0.50 pH unit between 1 - 30 mm compared to control (Figure 6.2). NH_4NO_3 decreased by 0.23 pH unit between 1 - 10 mm and increased by 0.12 pH unit between 15 - 30 mm on day 1 compared to control, decreased by 0.29 pH unit and 0.10 pH unit between 1 - 10 mm and 15 - 30 mm respectively on day 7, and on

day 28, decreased by 0.36 pH unit between 1 - 10 mm and similar pH value between 15 - 30 mm compared to control (Figure 6.2).

We observed effects of the application of different fertiliser treatments at 100 kg N ha^{-1} on average EC value (EC value at different days and depths) and followed a pattern of NH_4NO_3 > protein > GM = control, with an average of 0.23 - 0.71 mS cm^{-1}. The application of GM increased by 8% and 2% EC value between 1 - 10 mm and 15 - 30 mm respectively compared to control on day 1, increased by 41% and 48% between 1 - 10 mm and 15 - 30 mm respectively on day 7, and on day 28, increased by 67% and 37% between 1 - 10 mm and 15 - 30 mm respectively compared to control (Figure 6.2). The application of protein and NH_4NO_3 showed an immediately increase of EC value in the first 10 mm after 1 d incubation and decreased significantly after protein addition from days 1 to 28 and became similar to EC value of control, but NH_4NO_3 showed no significant decrease with time. Protein increased by 41% and 23% EC value between 1 - 10 mm and 15 - 30 mm respectively compared to control on day 1, increased 11% and 16% between 1 - 10 mm and 15 - 30 mm respectively on day 7, and on day 28, increased by 9% and 4% between 1 - 10 mm and 15 - 30 mm respectively compared to control (Figure 6.2). EC value increased by 75% and 44% between 1 - 10 mm and 15 - 30 mm respectively after NH_4NO_3 application compared to control on day 1, increased by 70% and 56% between 1 - 10 mm and 15 - 30 mm respectively on day 7, and on day 28, increased by 74% and 32% between 1 - 10 mm and 15 - 30 mm respectively compared to control (Figure 6.2).

No effect of different fertiliser treatments at 100 kg N ha^{-1} on CN ratio were observed except after the addition of NH_4NO_3. The average CN ratio (CN ratio at different days and depths) was significantly decreased after applying NH_4NO_3 compared to control treatment ($P \leq 0.05$; Figure 6.2). The CN ratio in control and NH_4NO_3 was stable, while the protein treatment showed a significant decrease after 7 d and then increase again after 28 d. GM showed a significant increase in CN ratio after 7 d and decreased after 28 d. Depth tended to increase CN ratio value in almost all cases ($P \leq 0.05$; Figure 6.2).

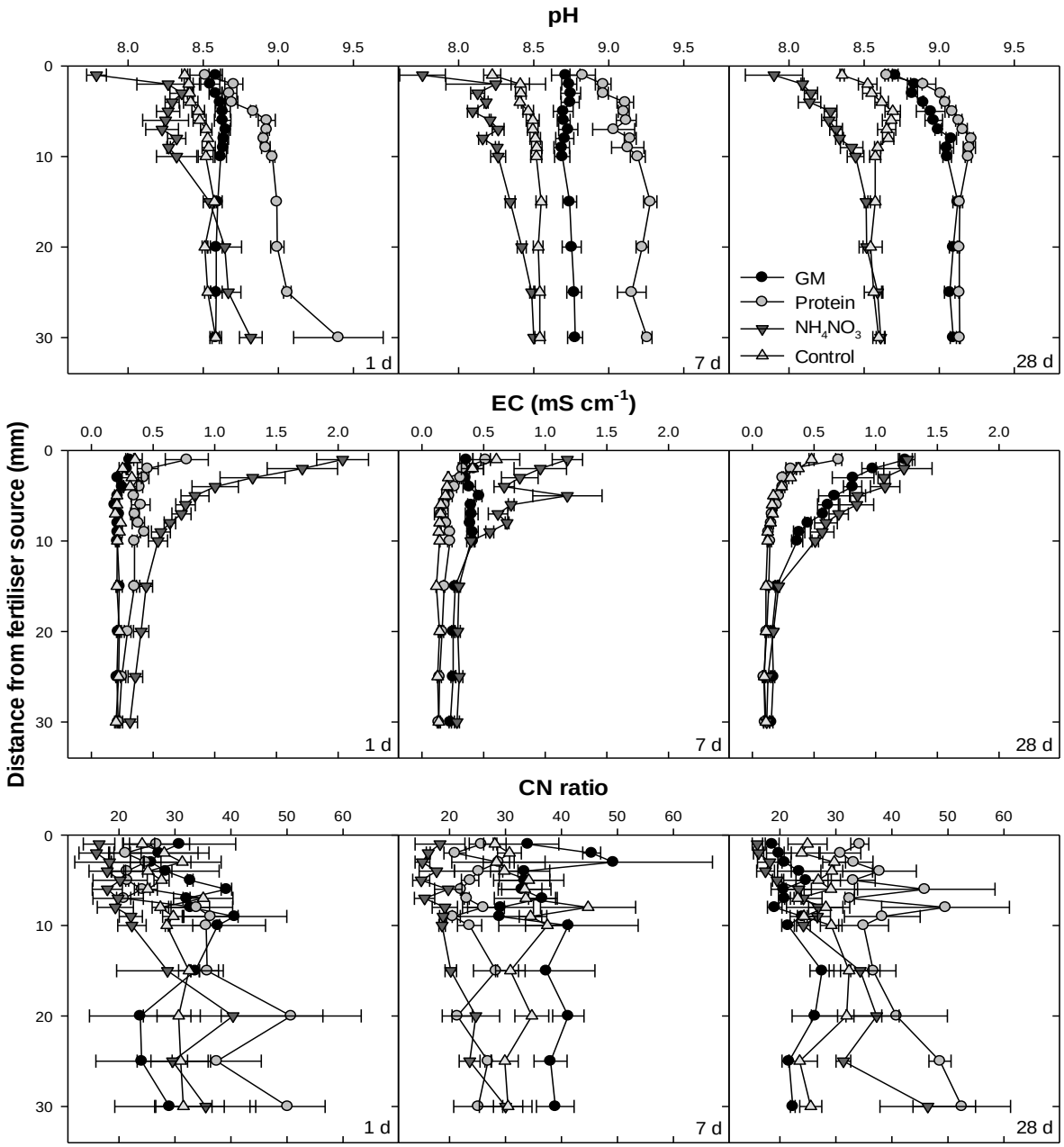


Figure 6.2 Soil pH, EC, and CN ratio responses after addition of 100 kg N ha^{-1} fertilisers. Points represent means ± SEM (*n* = 3).

Responses to 7000 kg N ha^{-1} fertiliser treatments

Fertiliser treatments showed a significant decrease in average soil pH compared to the control treatment ($P \leq 0.05$; Figure 6.3) and followed a pattern of control > P_{7000} > N_{7000} with an average of 7.15 - 8.51. The soil pH in P_{7000} at different depths shows significant increase with time, while N_{7000} slightly dropped with time ($P \leq 0.05$; Figure 6.3). Depth tended to increase soil pH at separate sampling times in almost all cases ($P \leq 0.05$; Figure 6.3). P_{7000} addition immediately decreased by 1.93

pH unit and 0.47 pH unit of soil pH value between 1 - 10 mm and 15 - 30 mm respectively compared to control on day 1 and increased significantly from days 1 to 28 nearly to the original pH (control treatment).

The average EC value (EC value at different days and depths) showed significant differences between fertiliser treatments and followed a pattern of N_{7000} > P_{7000} > control treatment ($P \leq 0.05$; Figure 6.3). The soil EC content in control at different depths remained stable with time, while N_{7000} and P_{7000} treatments showed a significant drop with time ($P \leq 0.05$; Figure 6.3). The depth tended to decrease EC value at separate sampling times in almost all cases ($P \leq 0.05$; Figure 6.3). P_{7000} immediately increased 13-fold and 4-fold of EC value between 1 - 10 mm and 15 - 30 mm respectively compared to control after 1 d incubation then decreased significantly from days 1 to 28 nearly to the control value after P_{7000} addition. EC value increased 72-fold and 27-fold between 1 - 10 mm and 15 - 30 mm respectively after N_{7000} application compared to control on day 1, increased 52-fold and 63-fold between 1 - 10 mm and 15 - 30 mm respectively on day 7, and on day 28, it increased 37-fold and 62-fold between 1 - 10 mm and 15 - 30 mm respectively compared to control (Figure 6.3).

We observed changes in average CN ratio (CN ratio value at different days and depths) after the application of different fertiliser treatments at 7000 kg N ha^{-1} and followed a pattern of control > P_{7000} > N_{7000} (Figure 6.3). The CN ratio in control and N_{7000} at different depths was stable with time, while P_{7000} treatment showed a significant drop with time ($P \leq 0.05$; Figure 6.3). The application of N_{7000} were diffusing more N compounds into soil than P_{7000}. The application of N_{7000} reduced the CN ratio value 30 mm from day 1 compared to control ($P \leq 0.05$; Figure 6.3), while P_{7000} reduced the CN ratio value between 20 and 30 mm on day 1 and 7 respectively compared to control ($P \leq 0.05$; Figure 6.3).

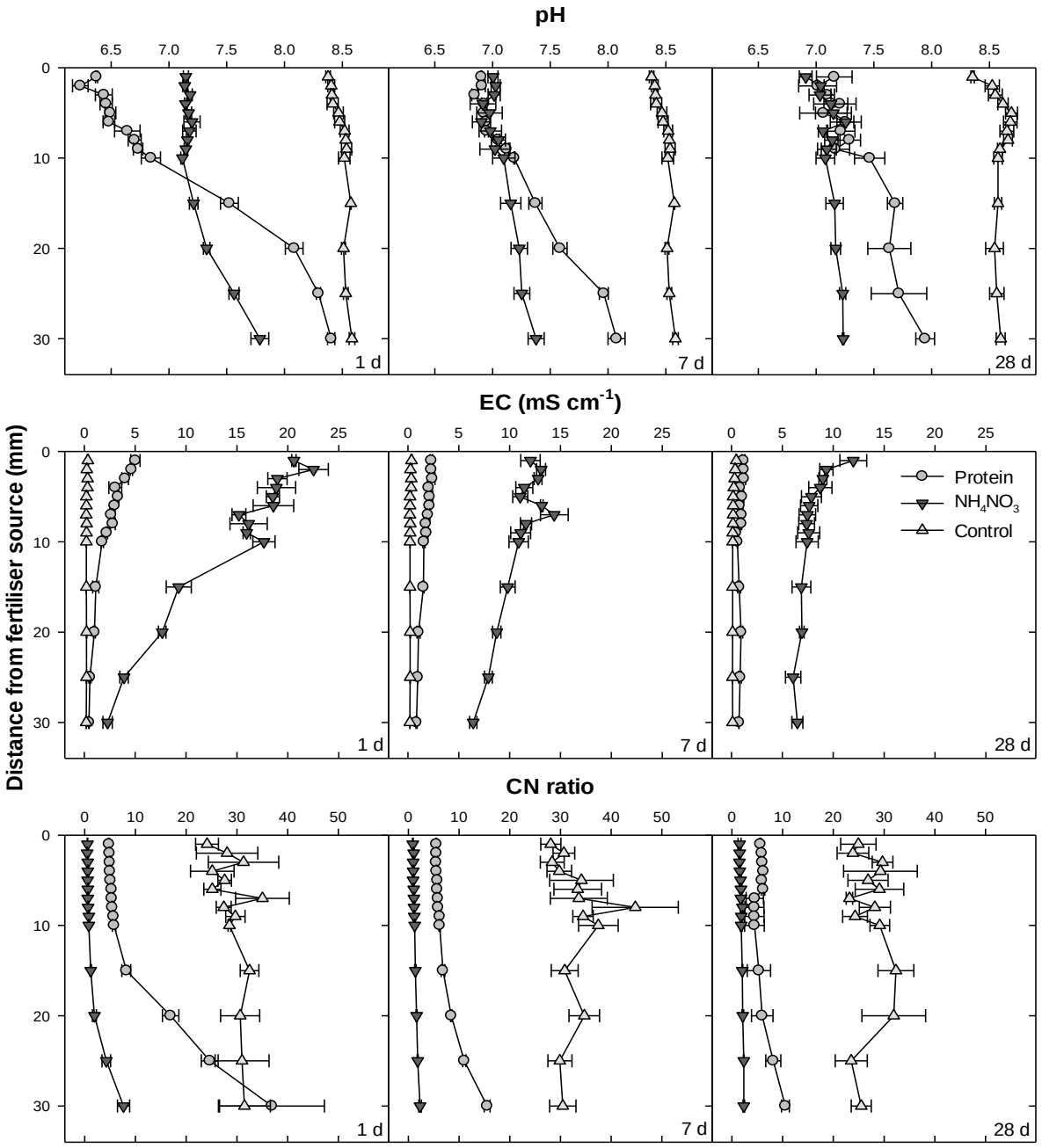


Figure 6.3 Soil pH, EC, and CN ratio responses after addition of 7000 kg N ha^{-1} fertilisers. Points represent means ± SEM (n = 3).

6.3.2 Effects of fertiliser treatments on gaseous emissions

Responses to 100 kg N ha^{-1} fertiliser treatments

The addition of GM produced the highest amount of cumulative NH_3 emission in all treatments and about 18, 28, and 205% higher than and protein, NH_4NO_3 and control treatment, respectively ($P \leq 0.05$; Figure 6.4). GM evaluated N faster than the other fertilisers from the first 21 d with a rate of 5.3, 5.0, and 4.3 kg N ha^{-1} day^{-1} (GM, protein, and NH_4NO_3; respectively). The total percentage of N evaluate from the total

N applied at different fertiliser rate was about 5.4%, 4.2%, and 3.7% (GM, protein, and NH_4NO_3; respectively).

GM had the highest amount of cumulative CO_2 respiration, at about 9-fold higher than protein, 15-fold higher than NH_4NO_3, and 21-fold higher than control treatment ($P \leq 0.05$; Figure 6.4). GM respired CO_2 faster than any other fertiliser treatments from the first 7 d with the rate of 35.3, 4.3, and 2.6 kg C ha^{-1} day^{-1} (GM, protein, and NH_4NO_3; respectively).The total percentage of C respired from the total C applied at different fertiliser rate 73.3%, 4.9% and 1.5% (GM, protein, and NH_4NO_3; respectively).

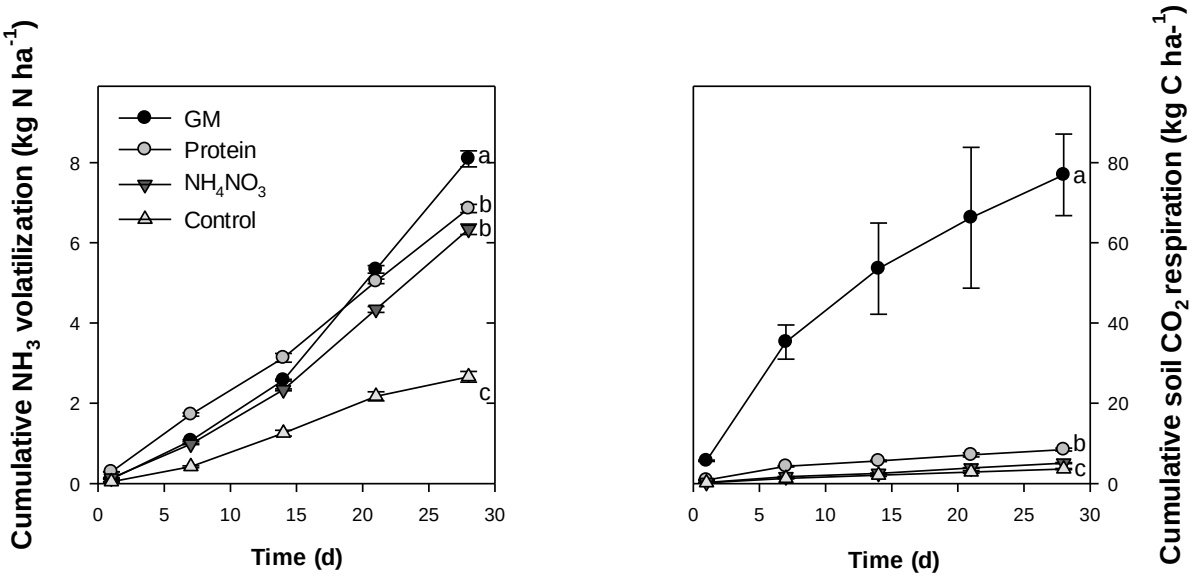


Figure 6.4 Effects of fertilisers' addition at 100 kg N ha^{-1} rate on cumulative gaseous losses during 28 d. Points represent means ± SEM (n = 3). Different letters identify significant differences between fertilisers ($P \leq 0.05$).

Responses to 7000 kg N ha^{-1} fertiliser treatments

N_{7000} showed the highest amount of cumulative NH_3 emission between all treatments, and about 2-fold higher than P_{7000} and 72-fold higher than control ($P \leq 0.05$; Figure 6.5). N_{7000} evaluated N faster than P_{7000} treatment from 7 d with the rate of 45.5 and 30.9 kg N ha^{-1} day^{-1} respectively. The total percentage of N evaluate was about 2.7% and 1.7% from N_{7000} and P_{7000} respectively.

P_{7000} showed the highest amount of cumulative CO_2 respiration between fertilisers' treatment and about 11-fold higher than N_{7000} and 103-fold higher than control ($P \leq 0.05$; Figure 6.5). P_{7000} respired CO_2 faster than N_{7000} treatment from the first 7 d with the rate of 37.9 and 8.3 kg C ha^{-1} day^{-1} respectively. The total

percentage of C respired was about 5.3% and 0.4% from P_{7000} and N_{7000} respectively.

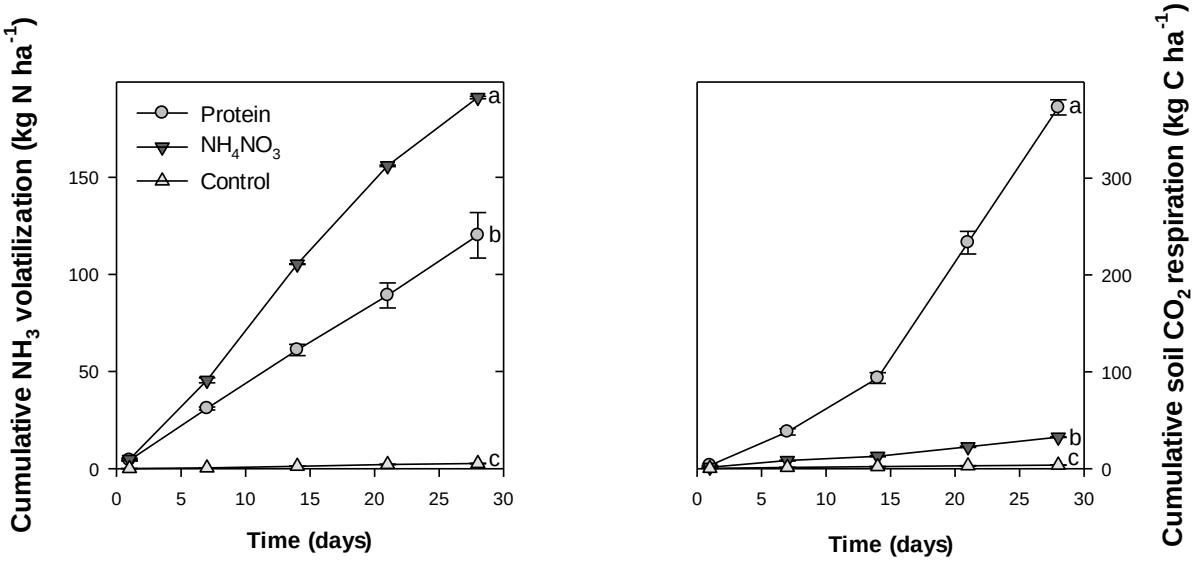


Figure 6.5 Effects of fertilisers' addition at 7000 kg N ha^{-1} rate on cumulative gaseous losses during 28 d. Points represent means ± SEM (*n* = 3). Different letters identify significant differences between fertilisers ($P \leq 0.05$).

6.3.3 Effects of fertiliser treatments on N diffusion

Responses to 100 kg N ha^{-1} fertiliser treatments

GM had the highest average amino acids concentration (amino acids at different days and depths) among all treatments, being about 4-fold higher than protein, 10-fold higher than NH_4NO_3, and 16-fold higher than the control treatment ($P \leq 0.05$; Figure 6.6). The highest average NH_4^+ concentrations (NH_4^+ at different days and depths) occurred in the NH_4NO_3 treatment and was about 2-fold more than protein, and 3-fold more than GM and control ($P \leq 0.05$; Figure 6.6). NH_4NO_3 showed the highest average NO_3^- concentrations (NO_3^- at different days and depths) across all fertilisers and about 34-fold more than control, 43-fold more than protein, and 92-fold more than GM.

GM showed similar amino acid concentrations at different depths with the control on day 1 ($P > 0.05$; Figure 6.6), except at 1 mm ($P \leq 0.05$; Figure 6.6), whereas on days 7 and 28, it showed 33-fold and 2-fold more than the control value, respectively. NH_4^+ value reduced 2-fold on day 1 and was similar to control value on day 7 and 28 after applying GM, while NO_3^- value was similar to control value on day 1 and reduced 2-fold and 8-fold compared to control value on day 7 and 28 respectively. GM caused a 60-fold increase in amino acid concentration at different

depths on day 7 and the concentration decreased 19-fold on day 28, where NH_4^+ increased 6-fold on day 7, then increased 2-fold on day 28, and NO_3^- decreased by 3-fold on day 7 and remained low even after day 28 ($P \leq 0.05$; Figure 6.6). GM addition affected amino acid concentration only in the first 1 mm on day 1, with fast diffusion until 25 mm on day 7 and until 30 mm on day 28 (Figure 6.6). The concentration of NH_4^+ decreased by approximately 54% after GM addition from 2 - 20 mm on day 1 compared to control, by 23% between 1 - 5 mm then decreased by 3-fold between 6 - 30 mm on day 7, and on day 28, it showed similar concentrations as the control value between 1 - 10 mm, then increased 4-fold compared to control between 15 - 30 mm (Figure 6.6). The concentration of NO_3^- after applying GM showed a similar concentration to the control value on day 1, reduced 2-fold between 1 - 10 mm, then decreased 8-fold between 15 - 30 mm compared to control on day 7, and on day 28, NO_3^- value decreased 8-fold between 1 - 30 mm compared to control value (Figure 6.6).

Protein application increased 20-fold, similar to and 2-fold more than amino acid concentrations at different depths on day 1, 7 and 28 respectively compared to control value ($P \leq 0.05$; Figure 6.6). NH_4^+ concentrations increased 5-fold, 2-fold and similar to control value at different depths on days 1, 7, and 28 respectively after application of protein, while NO_3^- concentrations increased 2-fold, similar to, and reduced 3-fold concentrations at different depths on days 1, 7, and 28 respectively compared to control value (Figure 6.6). Protein decreased amino acids 7-fold on day 7 and remained low even after 28 days, NH_4^+ concentrations increased 2-fold on day 7 then decreased 2-fold on day 28, while NO_3^- concentrations decreased about 20% and 29% on day 7 and 28 respectively ($P \leq 0.05$; Figure 6.6). Protein addition increased amino acid concentration only in the first 10 mm on day 1 compared to control, then decreased the value from 15 - 30 mm on day 7 compared to control and similar value on day 28 (Figure 6.6). The concentration of NH_4^+ increased after protein addition until 20 mm on day 1 compared to control, until 30 mm on day 7, while on day 28, it showed similar concentration with control value between 1 - 10 mm, then increased 6-fold compared to control between 15 - 30 mm (Figure 6.6). Protein increased 2-fold the NO_3^- concentration between 1 - 10 mm on day 1 compared to control ($P \leq 0.05$; Figure 6.6), showed similar concentration with control on day 7, while on day 28, it decreased by 3-fold compared to the control.

NH_4NO_3 showed similar amino acid concentration at different depths on day 1 and 7 compared to control, where, on day 28, amino acids increased 2-fold compared to control (Figure 6.6). NH_4NO_3 increased 11-fold, 3-fold and similar concentrations of NH_4^+ compared with control on days 1, 7, and 28 respectively (Figure 6.6). The concentrations of NO_3^- increased 49-fold, 85-fold, and 14-fold after addition of NH_4NO_3 fertiliser compared to control on days 1, 7, and 28 respectively ($P \leq 0.05$; Figure 6.6). NH_4NO_3 increased 3-fold value of amino acid on day 7 compared to day 1 and remained high even after day 28, while NH_4^+ concentrations increased 18% on day 7 then decreased 42% on day 28, whereas NO_3^- concentrations increased 2-fold on day 7 then decreased 2-fold on day 28 (Figure 6.6). NH_4NO_3 showed 2-fold higher amino acid concentration between 1 - 2 mm on day 1 compared to control, while it showed 2-fold higher value between 1 - 15 mm on day 7, whereas on day 28, amino acid concentration was 2-fold higher than control between 1 - 30 mm (Figure 6.6). NH_4NO_3 showed 11-fold higher NH_4^+ concentration between 1 - 30 mm on day 1 compared to control and remained higher than the control until 28 days, except between 1 - 10 mm on day 28, when it showed similar concentration as control (Figure 6.6). NO_3^- concentrations were higher between 1 - 30 mm after applying NH_4NO_3 compared to control on day 1 and remained higher until 28 days, except between 20 - 30 mm on day 28, when it showed similar concentration of NO_3^- as the control (Figure 6.6).

In control samples, the concentrations of amino acid increased 3-fold on day 7 then remained high even after day 28, whereas, NH_4^+ concentrations increased 4-fold on day 7, then increased by 2-fold on day 28. The result of NO_3^- concentrations did not change on day 7, but increased by 3-fold of the concentrations on day 28. There were no N diffusions observed in control across all days (Figure 6.6).

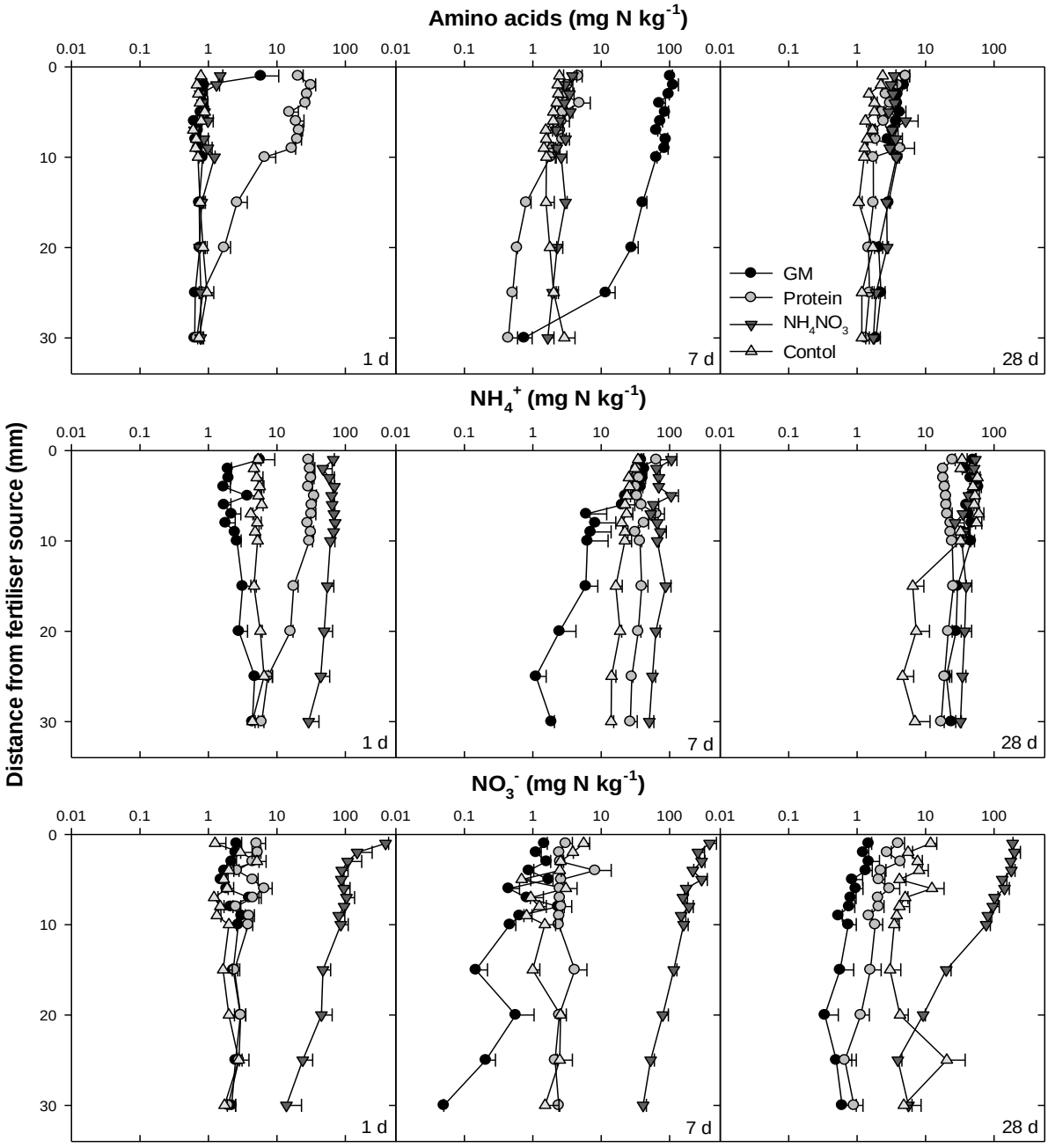


Figure 6.6 Effects of fertilisers' addition at 100 kg N ha^{-1} rate on N diffusion measured after 1, 7 or 28 d. Points represent means ± SEM (*n* = 3). The x-axis is shown on a log scale.

Responses to 7000 kg N ha^{-1} fertiliser treatments

The highest average amino acid concentrations (amino acids at different days and depths) was shown in P_{7000} across all fertilisers, about 130 and 864-fold higher than N_{7000} and control treatment respectively ($P \leq 0.05$; Figure 6.7). N_{7000} had the highest average NH_4^+ concentrations (NH_4^+ at different days and depths) between all fertilisers, about 45 and 158-fold more than P_{7000} and control respectively ($P \leq 0.05$; Figure 6.7). N_{7000} also showed the highest average NO_3^- concentrations (NO_3^- at

different days and depths) across all fertilisers, about 736 and 2059-fold higher than P_{7000} and control respectively ($P \leq 0.05$; Figure 6.7).

P_{7000} increased 264, 966, and 1022-fold amino acid concentrations at different depths compared to control on days 1, 7, and 28 respectively ($P \leq 0.05$; Figure 6.7). P_{7000} increased 3-fold, 8-fold, and showed similar concentrations of NH_4^+ at different depths on days 1, 7 ($P \leq 0.05$; Figure 6.7) and 28 compared to control. NO_3^- concentrations showed similar pattern with NH_4^+ concentrations, increasing 8-fold, 4-fold and staying similar to NO_3^- concentrations at different depths on days 1, 7 ($P \leq 0.05$; Figure 6.7) and 28 compared to control. P_{7000} increased amino acid concentrations 10-fold at different depths on day 7 and remained high even on day 28. NH_4^+ concentrations increased 10-fold at different depths on day 7, then decreased 8-fold on day 28, where the highest NO_3^- concentrations at different depths was on day 1 and decreased 2-fold on day 7 and remained low even after day 28. P_{7000} showed higher amino acid concentrations between 1 - 30 mm on day 1 compared to control and remained high until day 28. P_{7000} showed higher NH_4^+ concentrations between 1 - 20 mm and 1 - 30 on days 1 and 7 respectively compared to control, whereas, on day 28, the NH_4^+ concentrations were lower than control between 1 - 10 mm in most cases and higher than control between 15 - 30 mm ($P \leq 0.05$; Figure 6.7). P_{7000} showed higher NO_3^- concentrations between 1 - 30 mm and 1 - 20 on days 1 and 7 respectively compared to control ($P \leq 0.05$; Figure 6.7), where, on day 28, the NO_3^- concentrations were similar to control between 1 - 30 mm.

N_{7000} increased amino acid concentrations 3, 5, and 10-fold at different depths compared to control on days 1, 7, and 28 respectively ($P \leq 0.05$; Figure 6.7). N_{7000} increased NH_4^+ concentrations 961, 106, and 71-fold at different depths compared to control on days 1, 7, and 28 respectively ($P \leq 0.05$; Figure 6.7). NO_3^- concentrations increased 5828, 1906, and 996-fold at different depths after applying N_{7000} compared to control on days 1, 7, and 28 respectively ($P \leq 0.05$; Figure 6.7). Amino acid concentrations increased 4-fold on day 7 and 2-fold more on day 28 after N_{7000} fertiliser addition, where NH_4^+ concentrations decreased 2-fold on day 7 and remained low even on day 28. NO_3^- concentrations showed 3-fold lower concentrations on day 7 compared to day 1, then increased 2-fold more on day 28.

N_{7000} showed higher amino acids, NH_4^+, and NO_3^- concentrations between 1 - 30 mm from day 1 compared to control ($P \leq 0.05$; Figure 6.7).

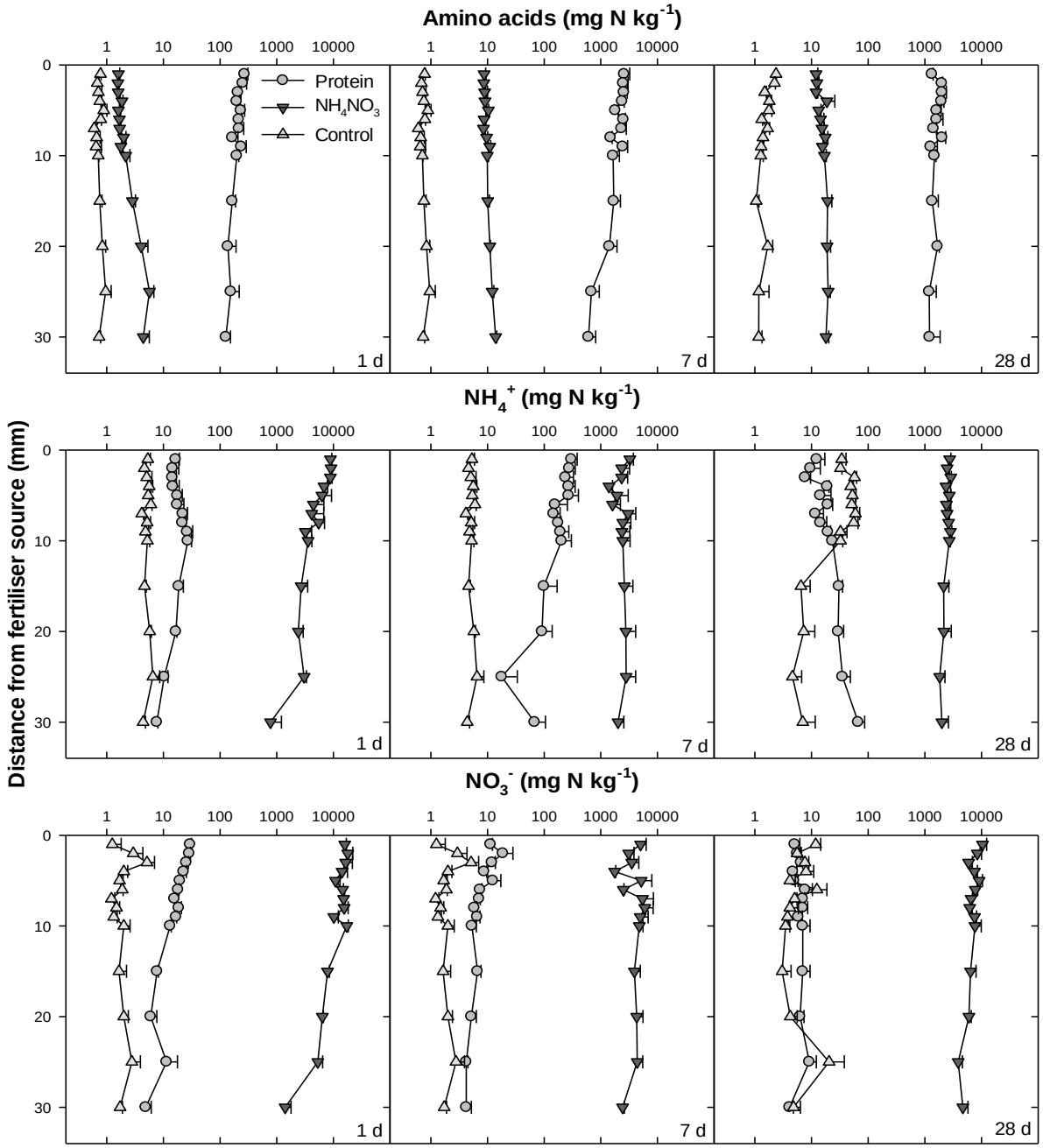


Figure 6.7 Effects of fertilisers addition at 7000 kg N ha^{-1} rate on N diffusion measured after 1, 7 or 28 d. Points represent means ± SEM (n = 3). The x-axis is shown on a log scale.

6.4 Discussion

Many factors influence the concentrations of N in soil, such as soil temperature (Barber, 1995; Deenik, 2006; Butler et al., 2012), soil pH (Barber, 1995; Cookson et al., 2007; Aciego and Brookes, 2008; Filep and Rékási, 2011), EC (Zhang and Wienhold, 2002; Christou et al., 2006; Filep and Rékási, 2011), moisture content

(Barber, 1995; Deenik, 2006; Robertson and Groffman, 2007; Ros et al., 2009; van Kessel et al., 2009), microbial community, soil organic matter (Qiu-Hui et al., 2012; Li et al., 2014), and soil texture (Zhang and Wienhold, 2002; van Kessel et al., 2009; Filep and Rékási, 2011). In this study, we kept all of these factors at a constant with 50% WHC incubated at 30°C by using one homogenized agricultural site from the Al-Hassa eastern oasis, KSA to focus only on the small scale, short-term effects of N fertiliser additions on N cycle. To our knowledge, the comparison of N compounds' diffusion responses to organic and inorganic N fertilisers in agricultural oasis soils at high resolution using a custom-made microtome has not previously been examined. We examined fertiliser factor to extend our understanding of C and N cycles in arid systems for developing more sustainable agriculture systems, better understand land management, and reducing N or C gaseous emissions. The variety of fertilisers used in this study was designed to reflect the actual and future approved organic inputs for organic farming on this oasis.

6.4.1 Influence of fertiliser types on soil chemistry

As expected, our results show there were significant influences of the application of different fertiliser treatments with different N rate on soil pH, EC and CN ratio compared to control in almost all cases. At the 100 kg N ha^{-1} fertiliser rate, soil pH increased after the addition of organic N (GM and protein), whereas inorganic fertiliser addition showed a decrease of pH value compared to control with higher effect at upper 10 mm than between 15 - 30 mm in most cases (Figure 6.2). This could be related to the production of H^+/OH^- after the addition of fertilisers from organic anion decomposition and ammonification (Rukshana et al., 2011, 2014) that can decrease and increase pH value respectively. In contrast, a higher rate of organic N addition (P_{7000}) showed a rapid decrease in soil pH at upper 10 mm more than between 15 - 30 mm on day 1 (Figure 6.3). This could be due to either increase organic acids concentration from the addition of organic fertiliser (Rukshana et al., 2014) or high level of EC after P_{7000} addition on day 1, which reduced microbial enzyme activity that can consume the high concentrations of H^+ ions and decomposes organic matter in soil (Rukshana et al., 2011; Brar et al., 2015). Shah and Shah (2011), found that higher EC value (up to 16 dS m^{-1}) caused a significant decrease of microbial biomass C and N, mineralization of N, nitrification, and soil respiration due to high salinity influence soil microorganisms growth and activity. The

soil pH in most treatments showed significant increase with time and depths in different N rate (Figure 6.2 and Figure 6.3). This could be due to ammonification of organic N (Figure 6.6 and Figure 6.7) and also resulting from organic acids anion decarboxylation that consumes H^+ ions from decomposed organic acids and respires their carbon (CO_2; Figure 6.4 and Figure 6.5) (Rukshana et al., 2011, 2012, 2014). In addition, it could be due to partly decrease from NO_3^- concentration via NO_3^- immobilization or decarboxylation/decomposition of native soil organic matter that increase alkalinity priming from added C and enhanced CO_2 released (Rukshana et al., 2013, 2014). The result of decreasing NO_3^- concentrations (Figure 6.6 and Figure 6.7) and CO_2 emission (Figure 6.4 and Figure 6.5) after organic treatment support this suggestion.

EC value can influence N and C mineralization by affecting soil microbes through osmotic stress that reduce water and nutrient availability to microbes and draw out water from the cell (Shah and Shah, 2011; Yan and Marschner, 2013). EC value increased significantly after the addition of organic and inorganic N fertilisers at different rates (Figure 6.2 and Figure 6.3) suggested that it could be due to the increase in anion and cation concentrations (Zhang and Wienhold, 2002; Filep and Rékási, 2011) at upper 10 mm more than between 15 - 30 mm in most cases. Our results show that the average EC value after inorganic N fertiliser are higher than organic application with higher EC value at upper 10 mm compared to between 15 - 30 mm in most cases this could be due to the original EC value of fertilisers (Table 6.1). In addition, it could be due to higher NO_3^- and NH_4^+ concentrations after inorganic N fertiliser compared to organic application (Figure 6.6 and Figure 6.7) that increase EC value (Figure 6.2 and Figure 6.3). This consist with Zhang and Wienhold (2002), who found there were correlation between soil inorganic N concentration with EC value (R^2 = 0.85 to 0.92). N_{7000} showed the highest average EC value (12.97 mS cm^{-1} and 6.89 mS cm^{-1} at upper 10 mm and between 15 - 30 mm respectively) these values could influence the size and activity of soil microorganisms and reduce microbial biomass C and N, mineralization of N, nitrification, plant-available nutrient and soil respiration (Yuan et al., 2007; Shah and Shah, 2011). Singh (2016) reported that higher salinity can decrease the water availability for microbes and plant, cause death for microbial cells, reduce microbial population, dehydration of plant tissue, and restrict N cycling efficient. Times and

depths tended to decrease EC value after the addition of organic and inorganic N fertilisers at different rates into arid agriculture soil studied. We suggest that this could be due to the consumption of ions from mineralization/immobilization process after the addition of fertilisers (Zhang and Wienhold, 2002).

CN ratio values were not significantly affected after the addition of organic N fertiliser at 100 kg N ha^{-1} (Figure 6.2), where inorganic fertiliser showed decreases of CN ratio compared to control at different fertiliser rate with higher effect at upper 10 mm more than between 15 - 30 mm in most of the cases (Figure 6.2 and Figure 6.3). It is suggested that it could be due to the rapid increase of inorganic N with low availability of C after inorganic fertiliser addition (Robertson and Groffman, 2007) at upper 10 mm more than between 15 - 30 mm in most of the cases. GM showed significant increase in CN ratio after 7 d and decrease after 28 d, while protein showed significant decrease after 7 d and increase after 28 d. This could be explained by increasing amino acid concentrations after applying GM on day 7, then mineralized to inorganic N on day 28 (Figure 6.6) and CO_2 release (Figure 6.4) which reduced the added C. This result is consistent with Dong et al. (2012), who show that CN ratio increased with time due to both total N (TN) and soil organic carbon (SOC) accumulations with time, and the build-up of TN was slower than SOC over time. In addition, it could be due to increased C and N compounds, microbial community compositions, size and activity from the application of GM than protein fertilisers (Table 6.1). At high fertiliser rate, CN ratio value were significant decreased after organic and inorganic N application compared to control (Figure 6.3), suggesting that it could be due to N diffused quickly after the addition of fertiliser that changed the CN ratio and rapid mineralization/immobilization process after fertiliser application (Robertson and Groffman, 2007). The original CN ratio value of fertilisers (Table 6.1) support this suggestion. The result of CN ratio at high fertiliser rate showed that inorganic fertiliser diffuses N compound between 1 - 30 mm faster than organic fertiliser due to the fast reduction of CN in inorganic compared to organic fertiliser (Figure 6.7).

6.4.2 Influences of fertiliser on N cycling

Our results show that the average concentrations of amino acid after organic N fertiliser are higher than inorganic application whereas, higher amino acid concentrations were found at upper 10 mm compared to between 15 - 30 mm in

most cases, except after N_{7000} when it showed higher concentrations between 15 - 30 mm compared to upper 10 mm. This could be due to the original chemical content of organic N fertiliser increasing more C compound input than inorganic N that increases the microbial community compositions, size and activity. Increased microbial community activity, compositions, and size will release more CO_2 ($P \leq 0.05$; Figure 6.4 and Figure 6.5) and reduced the added C with time due to mineralization process. Previous studies showed that applied organic manure increased the cumulative CO_2 flux more than inorganic fertiliser due to increasing microbial activity from the original organic matter content in organic manure (Chirinda et al., 2010). Chen et al. (2014) showed that the amount of C mineralized to CO_2 doubled after the addition of wheat residue due to increasing microbial community size, activity, and composition. In addition, it could be due to priming effect after the addition of organic fertiliser that stimulated the decomposition and ammonification of native soil organic matter from the added C or increased microbial NO_3^- uptake (Rukshana et al., 2012, 2014). Amino acid concentrations increased 60-fold on day 7 then decreased 19-fold on day 28 after applying GM, while NH_4^+ concentrations increased 7-fold and 2-fold on day 7 and 28 respectively with high accumulation of CO_2 and NH_3 (92.1 kg C ha^{-1} and 7.7 kg N ha^{-1}; Figure 6.4). This resulted from decomposition of GM that increase amino acids in the first 7 days, then mineralization/immobilization of organic N that increased NH_4^+ after that volatilize it or decrease NO_3^- (Figure 6.6) that will release more CO_2 and NH_3 (Figure 6.4). This is consistent with Fisk et al. (2015), who show that inorganic N pool decreased due to the immobilization process after short-term application of plant residues. Organic N fertiliser at both rates show decreasing NO_3^- concentrations with lower concentration between 15 - 30 mm than at upper 10 mm compared to control (Figure 6.6 and Figure 6.7); this could be due to high microbial N demand from higher available C in organic N fertiliser. Our result is consistent with other studies that show increasing glucose (Rukshana et al., 2012) or DOC content (Cookson et al., 2007), which increases microbial mineralization/immobilization of N (Cookson et al., 2007; Chirinda et al., 2010; Rukshana et al., 2012) and decreases available N for nitrification (Cookson et al., 2007). This result support our hypothesis that organic fertiliser can reduce the amount of NO_3^- produced over time that could be due to high microbial N demand from higher available C in organic N fertiliser. The application of

protein fertiliser affected amino acid concentration more than NH_4^+ and NO_3^- concentrations; this could be due to increasing mineralization process in first 7 d with high accumulation of CO_2 (10.7 and 381.6 kg C ha^{-1} at 100 and 7000 kg N ha^{-1} respectively; Figure 6.4 and Figure 6.5). The increased NH_4^+ concentrations 2-fold and 10-fold on day 7 at 100 and 7000 kg N ha^{-1} respectively (Figure 6.6 and Figure 6.7) due to rapid mineralization/immobilization process that converted amino acids to NH_4^+. NH_4^+ concentration decreased on day 28 due to either immobilization or NH_3 volatilization pathway after application of protein fertiliser at both rates this could be due to high soil pH above 7 and high NH_4^+ concentration present (Barber, 1995). The result of increasing amino acid concentrations (Figure 6.6 and Figure 6.7) and NH_3 losses (Figure 6.4 and Figure 6.5) support this suggestion.

Inorganic fertiliser shows increasing average NH_4^+ and NO_3^- concentrations compared to organic N at different times and rates, with higher concentration at upper 10 mm than between 15 - 30 mm compared to control in most cases. The application of NH_4NO_3 increased the concentrations of NH_4^+ and NO_3^- from the first day at both rates compared to control, then decreased with time, while amino acids show similar or higher value at 100 and 7000 kg N ha^{-1} respectively on day 1, then increased with time (Figure 6.6 and Figure 6.7). This could be due to the rapid increase of inorganic N from NH_4NO_3 with low availability of C in soil affecting C-cycling enzyme activity (Bowles et al., 2014) therefore, increasing amino acid concentrations with time. NH_4^+ and NO_3^- decrease with time after NH_4NO_3 application this could be due to high NH_4^+ content present with high pH above 7 after 7 d that activated NH_3 volatilization (Barber, 1995) and immobilization process, the high NH_3 losses (Figure 6.4 and Figure 6.5) and the increasing of amino acids (Figure 6.6 and Figure 6.7) support this suggestion.

The theoretical linear distance of diffusive movement of amino acids, NH_4^+ and NO_3^- over 28 d was calculated as 20.1, 3.7 and 56.5 mm, respectively. In most cases, our observed diffusive distance of amino acids and NH_4^+ was greater than theoretical distance after organic and inorganic N fertiliser at both rates, whereas the diffusion of NO_3^- was lower and higher than theoretical distance after organic and inorganic N fertiliser at both rates, respectively. This could be due to rapid mineralization/immobilization process (increase amino acids and NH_4^+) and reduce NO_3^- to NH_4^+ after the NO_3^- had diffused 15 - 30 mm. Inorganic fertiliser shows

higher NO_3^- leachate than organic fertiliser at both rates that cause fast reduction of CN ratio and rapid mineralization/immobilization process due to higher inorganic N with low availability of C after inorganic fertiliser addition (Robertson and Groffman, 2007). This results support our hypothesis that N from inorganic N fertiliser will diffuse faster than N contained in an organic fertiliser due to the rapid increase of inorganic N from inorganic fertiliser.

6.5 Conclusions

Six different N fertiliser treatments used in this study had a significant influence on soil chemical properties and N diffusion in one arid agricultural site in KSA. Our results show that soil pH in most treatments showed significant increase with time and depths for different N rates. This could result from ammonification of organic N and also from organic acids anion decarboxylation that consumes H^+ ions from decomposed organic acids and respired their carbon (CO_2). EC value decreased with time and depths at different rate of N fertiliser treatments; this could be due to the consumption of ions from mineralization/immobilization process after the addition of fertilisers. Inorganic fertiliser decreased CN ratio and diffused more N compounds than organic N fertiliser at different rates; this could be due to the rapid increase of inorganic N with low availability of C in inorganic fertiliser and rapid mineralization/immobilization process, causing fast reduction of CN in inorganic compared to organic fertilisers. Our results show that organic N fertilisers affect amino acid concentrations more than inorganic application at different times and rates ($P \leq 0.05$) with higher effect at upper 10 mm than between 15 - 30 mm. This could result from the original chemical content of organic N fertiliser increasing C compound input than inorganic N that increases microbial community compositions, size and activity. Increased microbial community activity, compositions, and size will release more CO_2. Inorganic fertiliser shows higher NO_3^- leachate than organic fertiliser at both rates, which cause fast reduction of CN ratio and rapid mineralization/immobilization process due to higher inorganic N, with low availability of C after inorganic fertiliser addition. Therefore, we would recommend farmers and governmental managers to reduce inorganic fertiliser and use more organic N fertiliser that can reduce environmental pollution (e.g. NO_3^- leachate, N_2O emission), cost (due to recycling of farm waste and reduce inorganic fertiliser cost), save more

water (due to organic fertiliser can improve water holding capacity), and increased yield. Further studies are needed to investigate how these different fertiliser types influence microbial community composition, microbial biomass, and soil enzymes for the long-term in arid soil for developing better management and reducing N or C gaseous emissions.

Chapter 7. General discussion

The aim of this PhD thesis was to investigate and understand the impacts of changing soil temperature, water irrigation source and fertiliser type on soil N cycling in arid systems. Findings from each factor controlling the rate of C and N turnover in arid soils was discussed individually in their respective Chapters 3, 4, 5, and 6. This final chapter aims to summarize the PhD thesis findings and to address the overall objectives and hypotheses of the thesis in the following sections; the specific hypotheses were:

1) Increasing silt, clay, and salinity will reduce the rate of utilization of DON and DOC compounds due to the reduction of microbial activity.
2) The mineralization rate of DON is higher than for DOC in arid soils because they are likely to be processed by different metabolic pathways inside the cell.
3) The mineralization rate of DON and DOC increases with increasing temperature due to temperature tends to increase maintenance cost of microorganisms.
4) Increasing labile C concentration in irrigation water source will increase the decomposition of the insoluble fractions of plant residues due to increase microbial activity.
5) N from inorganic N fertiliser will diffuse faster than N contained in an organic fertiliser due to the rapid increase of inorganic N from inorganic fertiliser.
6) Organic fertiliser can reduce the amount of NO_3^- produced over time that could be due to high microbial N demand from higher available C in organic N fertiliser.

7.1 Do soil properties influence DON and DOC utilization?

Arid and semiarid lands covers about 41% of the total global area. More than 35% of world population depend on these lands (Sinsabaugh et al., 2015) for food, water etc. One of these arid countries is KSA (Al-Hammad et al., 2014). Studies into N and C cycles are key to understanding biogeochemical cycles in these areas. Typical agricultural practices in KSA focus on the production of dates. In 1996, the total production of date was 620,695 tonnes with the highest production comes from

eastern and central regions (Alkolibi, 2002). The largest oasis and date supplier in Eastern KSA is Al-Hassa oasis therefore this thesis focusses on understanding N and C cycles in this area to further understand fundamental processes in arid soils, to provide information that will help develop a more sustainable agriculture system and reduce environmental pollution (e.g., reduce CO_2 emission, NO_3^- leachate).

To give us a fuller picture of soil microbial community responses to DON and DOC substrates, we initially studied a range of non-agricultural and agricultural soils. Results from non-agricultural soil sites studied in Chapter 3 have shown that the rate of utilization of DON and DOC compounds was soil dependent and probably due to differences in the properties of the soils at the different sites (e.g., soil texture, chemistry and microbial community). The results showed that soils with the highest content of silt, clay, and salt had the lowest $^{14}CO_2$ evolution. The addition of DON and DOC in high clay content soil can increase the potential C subject to immobilization processes through electrostatic interaction, hydrophobic interaction, van der Waals forces, hydrogen bonding, and covalent bonding between clay minerals and enzyme molecules (An et al., 2015). This binding and adsorption of enzymes in clay minerals after the addition of organic compounds can improve the immobilization process so as to increase the stability (e.g. storage stability), activity, loading, and reusability of enzymes (An et al., 2015). In addition, it could be due to the potential increase of C contents after the application of organic residue in sandy loam soil and simulated microbial biomass and activity with greater N demand, thus promoted immobilization of available N (Singh, 2015). Watts et al. (2007) reported that soil with higher silt and clay contents showed higher N immobilization than soils with a sandy texture. This could be due to differences in soil porosity, water holding capacity, and bulk density that reduce microbial activity therefore, reducing $^{14}CO_2$ evolution. This result supports our hypothesis that increasing silt, clay, and salinity contents will reduce the rate of utilization of DON and DOC compounds due to the reduction of microbial activity.

Our results from non-agricultural soil sites studied in Chapter 3 showed that soil with higher salts content (mountain soil 2) had the lowest CO_2 evolution at different temperature, this could be due to higher salinity influencing N and C mineralization. Higher soil salinity can cause a decline in N and C mineralization. This can occur via a reduction in the size and activity of the microbial biomass as a result of reduced

extracellular enzyme activity and an inhibition of microbial growth due to osmotic stress, ion toxicity, and reduction of essential nutrient absorption (Shah and Shah, 2011; Yan and Marschner, 2013). This is consistent theoretically with Gao et al. (2014), who found that microbial mineralization of N decreased at higher salinities through an inhibition of microbial activity. Singh (2016) reported that increasing in salt content can reduce water for plant and microbial growth, cause dehydration of plant tissue, death for microbial cells, reduced microbial population, reduced vegetation cover (e.g., tree, grass, and crop), reduced organic input (e.g., litter, fine root, and crop residue), restricted N cycling efficiency (due to reduction of microbial population and enzyme activity), and inhibits other soil processes. Further work is needed to investigate the soil salinity implication (using one type of soil and increase salinity content) on soil microbial process (e.g., using metagenomics DNA extraction; Devi et al., 2015; Narayan et al., 2016, or using extracellular enzyme assay (EEA); Schindlbacher et al., 2015; Cenini et al., 2016; Moorhead et al., 2016) in arid soil to fully understand the processes involved. To examine the soil salinity implication on microbial C or N acquiring enzymes (Sinsabaugh et al., 2015; Cenini et al., 2016) we can use three extracellular enzymes (i.e., ß-1,4-glucosidase (BG) that catalyse cellulose degradation, ß-1,4-N-acetylglucosaminidase (NAG) that hydrolyses amino sugars in fungal and bacterial cell walls, or leucine aminopeptidase (LAP) that produce amino acids from protein/peptide substrate).

Temperature, soil moisture content, soil management, vegetation litter, microbial community and soil organic matter can all influence the rate of microbial mineralization of DON and DOC in soil (Simfukwe et al., 2011; Glanville et al., 2012; Qiu-Hui et al., 2012; Li et al., 2014). Results from non-agricultural soil sites studied in Chapter 3 showed there were significant differences between the abundance of soil microbial community structures (i.e., Gram-negative bacteria, Gram-positive bacteria, actinomycetes) in the different soils. Actinomycetes were the only soil microbial community group observed in mountain 2; this could reduce $^{14}CO_2$ evolution at lower temperature, since most of actinomycetes are either mesophilic (20 to 42°C) or thermophilic (45 to 60°C) species and may have higher carbon use efficiencies (Kurapova et al., 2012). Results from non-agricultural soil sites studied in Chapter 3 showed that the highest affected value of Mic_{eff} with increasing temperature was found in mountain 2 with increases of $^{14}CO_2$ evolution even beyond

40°C. This could be due to increasing temperature would potential increase and activate the growth of the mesophilic or thermophilic actinomycetes species (Kurapova et al., 2012), which in turn, leading to potential increase the size and activity of microbial community in this site with higher temperature (temperature adaption) and, consequently, decreases Mic_{eff} with increases $^{14}CO_2$ evolution.

Results from Chapter 4 showed, there were no significant differences effect on the rate of utilization of DON and DOC compounds with neither agricultural soil site nor depth studied and probably due to the similarity in soil microbial community structures that indicated by PLFAs. Hill et al. (2011c) reported that similar microbial communities responding in similar patterns to different LMW DON or DOC (i.e. common metabolic pathways). In addition, there were significant differences between agricultural soil sites or depth studied in Chapter 4 on the initial a diverse microbial community and followed the similar pattern of Gram-negative bacteria > Gram-positive bacteria > actinomycetes > AM fungi = fungi = anaerobic bacteria = eukaryote with no significant effect on the rate of utilization of DON and DOC compounds. This could be due to a diverse microbial community having similar substrate uptake transporters from soil (Glanville et al., 2016). The results of soil depth from agricultural soil site studied in Chapter 4 was differ from Rukshana et al. (2013), who showed that soil depth can influence substrate utilization due to higher microbial activity, biomass, organic matter, C content, and soil pH in topsoil compared to subsoils. This could be due to there being no significant differences between microbial community, soil pH and initial C content within different soil depth studied in Chapter 4. Most of non-agricultural soil sites studied in Chapter 3 showed only slightly lower $^{14}CO_2$ evolution than agricultural soil sites studied in Chapter 4 and probably due to common metabolic pathways whereas, mountain 2 showed the lowest $^{14}CO_2$ evolution compared to all soil sites studied in Chapter 3 and Chapter 4 ($P \leq 0.05$). This could be due to differences in silt, clay, salinity, microbial community content of the agricultural compared to non-agricultural soil sites as described above.

7.2 The rate of DON and DOC turnover in arid soil sites

Within this thesis, in most cases, the rate of DON turnover was higher than the rate of DOC turnover (Chapter 3 and Chapter 4) as evidenced by higher amounts of $^{14}CO_2$ production and a shorter $t_{1/2}$ for DON substrates. This could be due to the

higher N and C content of DON substrates than DOC substrate for rapidly cycling pool rather than different soil microbial community structure defined by phospholipid fatty acid (PLFA). Farrell et al. (2013) reported that the microbial uptake rate of trialanine > dialanine > alanine and could be due to higher N and C contents in trialanine compared to free amino acids. It is also likely that amino acids have higher $^{14}CO_2$ respiration values than glucose due to either amino acids is larger sources of N and C than glucose (no N source) therefore, creating higher and rapidly cycling pool of N and C or their processing by different metabolic pathways inside the cell. After uptake into the cell, amino acids can be transaminated or deaminated leading to the production of organic acid skeletons (keto acids), which can be used directly in respiratory pathways (Jones et al., 2005a; Boddy et al., 2008) therefore, higher $^{14}CO_2$ respiration after amino acids application that glucose. In contrast, we suggest that glucose-derived C is typically preferentially used for producing new cell biomass. This suggestion is consistent with Boddy et al. (2008) who showed that amino acids had lower mean resident time through soil microbial biomass compared to glucose (20 ± 1 days and 40 ± 6 days respectively). This could be due to the processes of desorption/sorption on microbial residues (e.g., dead microbes, cell wall components), different partition of substrate within microbial cells or specific cohorts of microbial community that influence the substrate turnover rate through microbial biomass (i.e., formatting new biomass; Boddy et al., 2007, 2008). This result supports our hypothesis that the mineralization rate of DON is higher than for DOC in arid soils due to higher N and C contents that accumulate more C and N for a rapidly cycling pool.

7.3 The turnover rate of water soluble and insoluble fractions in arid soil sites

Plant residues typically dominate C inputs into soil. They can be operationally separated into two fractions based on their solubility in water (Simfukwe et al., 2011). To increase our understanding of the mineralization of soluble and insoluble fractions of plant components in arid sites it is useful to study these two fractions independently (Chapter 5). The soluble fraction generally consists of a mixture of common plant metabolites present inside the cell or released in root exudates (e.g., organic acids, sugars, and amino acids). Generally, these are easily turned over in the soil (i.e. within hours). The second fraction, the insoluble fraction, are generally

compounds with high molecular weight and include plant polymers such as proteins, lignin, cellulose, and hemicellulose. These typically turn over in soil within days or months (Simfukwe et al., 2011; Glanville et al., 2012). The soluble fraction is regularly released into soil from root exudation, microbial and plant cells lysis, and canopy throughfall (Simfukwe et al., 2011), whereas the insoluble fraction tends to be the less easily degraded components of plant residues (e.g. plant litter, plant polymers, cereal straw). Our results from Chapter 5 showed that the rate of mineralization of insoluble plant residues was slower than the soluble component with lower amounts of $^{14}CO_2$ lost and about an 11-fold longer substrate half-life ($t_{1/2}$) of rapidly mineralized substrate (pool a_1) compared with the same pool of the soluble fraction which could be due to the likely distribution of component fraction as described above. We can conclude that soluble plant material can release more C to the atmosphere in the short-term whereas, insoluble plant material can increase the C-store in soil with small release of C to the atmosphere. The increase in soil stored C resulting from the addition of insoluble plant material might increase microbial activity in long-term by increasing native soil organic matter. Increasing soil microbial activity may increase the mineralization rate of soil stored C (the priming effect) and could increase the potential to cause environmental pollution (e.g., increase CO_2 emission, N_2O emission). Increasing microbial activity may also increase the amount of mineral N that can potential leach to groundwater or denitrify to atmosphere (Mitchell et al., 2016), in addition to environmental concerns, this is an economic loss to the farmer.

7.4 Does temperature affect DON and DOC mineralization in arid soil?

The most serious problems facing the world in general and in arid land in particular are global warming, increasing populations and water and food scarcity (Alkolibi, 2002). Climate change is already negatively affecting agriculture and water supply in KSA (Alkolibi, 2002). Temperatures on soil surfaces receiving direct sunlight are predicted to exceed 65°C in these arid regions (Nobel, 1984; Garratt, 1992; Williams et al., 1999). This future change in soil temperature can greatly influence soil processes, impact on soil nutrient and organic matter cycling in these arid regions. In addition, the increase in soil temperature may decrease soil moisture, increase evapotranspiration across the kingdom (Chowdhury and Al-

Zahrani, 2013), increase water requirement for agriculture in Middle East and North Africa (Qadir et al., 2010). Therefore, it is important to increase our understanding of the impact of potential increases in temperature and climate change in KSA on DON or DOC mineralization rate in arid soils. Chapters 3 (i.e., non-agriculture soil sites) and Chapter 4 (i.e., agriculture soil sites) focused only on the short-term effects of temperature changes on DOC and DON mineralization in arid soils. The results show that, in most cases, temperature tends to increase the allocation of C to the fast mineralization pool (a_1) and decrease the $t_{1/2}$ of a_1. In addition, there was a negative correlation between $^{14}CO_2$ respiration rate and microbial use efficiency (Mic_{eff}) after the addition of DON and DOC substrate into soil (Chapters 3 and Chapter 4); this could be due to:

1) Temperature-induced increases in solute diffusion and the mass flow of water making the substrates and exoenzymes more mobile and less protected within the soil matrix (Baumhardt et al., 2000). Therefore, increase $^{14}CO_2$ respiration rate due to either the substrate becoming more mobile and not protected with the soil matrix at higher temperature or changes in microbial community compositions (i.e., increase mineralization).
2) Microbial communities favouring higher temperature for uptake, turnover and metabolism of the added substrate. This is consistent theoretically with Agehara and Warncke (2005), who showed an increase in microbial respiration with increasing temperatures from 5 to 25°C due to a shift in microbial community composition and activity with increasing temperature that increases the ability for metabolizing more substrate at a higher temperature than lower temperature. In this study, the results of $^{14}CO_2$ respiration continually increased even after 25°C (Chapters 3 and Chapter 4) suggesting that it could be due to most of actinomycetes are mesophilic or thermophilic (Kurapova et al., 2012) in arid soil at higher temperature that increases $^{14}CO_2$ evolution.
3) The increased amount of ^{14}C partitioned into the a_1 pool as temperature increases in arid soil studied is theoretical consistent with the results presented by Boddy et al. (2008) but with a lower temperature range (4 - 20°C) applied in different soil sites (i.e., Arctic tundra soils). This is potentially due to an increase in the maintenance cost of microorganisms,

which means that microbes will use more ^{14}C for respiration processes at higher temperature rather than for growth and storage (Boddy et al., 2008).

4) Temperature tended to decrease Mic_{eff} in the arid soils studied here. This could be due to temperature affecting the relative balance of microbial C gain through different metabolic pathways. This result is consistent with Schindlbacher et al. (2015) who showed that the microbial substrate (mixture of amino acids, amino sugars, sugars, and organic acids) use efficiency tended to decrease with increasing temperature because changing temperature may affect different processes such as increasing C respiration and microbial turnover rate.

This result supports our hypothesis that the mineralization rate of DON and DOC increases with increasing temperature due to temperature tends to increase maintenance cost of microorganisms. Mineralization process can produce inorganic N forms (i.e., NH_4^+ and NO_3^-) by using C as a source of energy from DON substrate used in soil, fulfilling needs for nutrients (e.g., N) and supporting growth (Robertson and Groffman, 2007) or by soil enzymes (Jones et al., 2008; Tan, 2009). Many soil enzymes are responsible to breaking down HMW or LMW organic residues into inorganic N (e.g., proteases enzymes that break down protein to peptide, peptidase enzyme produce amino acids from peptide, and deaminases or urease enzymes that produce NH_4^+ from breaking down LMW DON; Jones et al., 2008; Tan, 2009; Hill et al., 2011b). Our results from Chapters 3 (i.e., non-agriculture soil sites) and Chapter 4 (i.e., agriculture soil sites) showed that soil microbial communities will mineralize LMW DON and DOC compounds at different rates depending on temperature in short-term. Therefore, the amount of inorganic N produced from OM decomposition will increase during summer periods; this could increase the potential of NO_3^- leachate to groundwater or N_2O losses. This is consistent with Fisk et al. (2015), who showed the greatest risk in semi-arid soil is the losses of N by accumulation of NO_3^- due to OM decomposition during summer periods which will cause to increase NO_3^- leachate to groundwater during rain or irrigation period. Therefore, to mitigate the N loss, the farmers need to reduce fertiliser rate under summer periods or planting crop in the late summer and early autumn to reduce NO_3^- leachate and CO_2 evaluations. Further work is needed to investigate the long-term effect of increasing temperature

on soil microbial processes and soil enzymes in arid soil using extracellular enzyme assay (EEA) to fully understand the processes involved.

7.5 Do soil moisture regimes and irrigation water source affect DOC and DON mineralization in arid agricultural soils?

Global warming is increasing the global temperature and influencing water availability and quality (e.g., increasing evaporation can decrease water availability and increase salts content) (DeNicola et al., 2015). It challenges the sustainability of global development and environmental health (Hanjra et al., 2012). The global population will increased to approximately between 9 to 10 billion between 2015 and 2050 (Wichelns and Qadir, 2014) whereas, in the last 40 years, the population of KSA increased between 7 million to 27 million. The combination of rapid increases in population and climate change can influence both the water quality and availability and result in water and food scarcity (Hanjra et al., 2012; Wichelns and Qadir, 2014; DeNicola et al., 2015). KSA is one of the largest arid countries in the world without lakes or rivers and low in natural renewable water resources (DeNicola et al., 2015). As a results, KSA is addressing the challenge of providing sufficient sustainable water sources for irrigation by using non-conventional water resources (Ouda, 2014a). KSA government encourages the reuse of treated wastewater in parkland and for crop irrigation in many areas (Ouda, 2014b). Since 1980, Al-Hassa irrigation and drainage authorities have been utilizing alternative sources of water (e.g., TTWW, agriculture drainage, MW) to irrigate the oasis for achieving sustainable irrigation (Al-Kuwaiti, 2010; Aldakheel, 2011). The reuse of alternative water sources had both positive and negative implications. There are many positive implications of reusing alternative water sources e.g. it can help to balance food and water security, reduce cost of energy for disposal pumping, potential to reduce chemical fertilisers (Hanjra et al., 2012; DeNicola et al., 2015), reduce C emissions, increase C credit, increase crop yield, and nutrient recycling (Hanjra et al., 2012; DeNicola et al., 2015). However, using alternative sources may also have negative longer term environmental and public health implications, e.g., increased microbial pathogen or antibiotic-resistant bacteria, heavy metals, salt, and nutrient content (Qadir et al., 2010; Hanjra et al., 2012; Wichelns and Qadir, 2014; DeNicola et al., 2015; Singh, 2015; Sinsabaugh et al., 2015; Bob et al., 2016). Therefore, quantification water

parameters and investigating the implications of using alternative water resources and moisture regimes on soil microbial processes such as C and N mineralization rate are very helpful in understand the management changes needed to develop sustainable irrigation system in arid soil.

The results from Chapter 5 showed that changing irrigation water source influenced the mineralization of insoluble plant materials. This could be due to either the initial chemical and biological properties of different irrigation water source, or due to increasing labile compound (i.e., DOC content) and biological factors (e.g., microbial community changes, microbial activity, free enzymes etc.; Qualls and Bridgham, 2005; Glanville et al., 2012; Fraser et al., 2016) after the applications of insoluble plant materials that influence the mineralization rate. Our results showed that the highest production of $^{14}CO_2$ from insoluble plant materials was observed after the application of MW irrigation compared to other water sources (i.e., TTWW and GW). This could be due to MW having a high intrinsic DOC content relative to the two other irrigation water source. This is consistent with Elifantz et al. (2011), who showed that when applied to soil, C in treated wastewater can influence soil microbial activity and increase microbial respiration more than soil irrigated with fresh water. Water irrigation studied in this thesis can load different range of DOC content yearly (MW, TTWW and GW can load 148.18 ± 4.19, 107.96 ± 8.26 and 23.77± 6.91 kg l^{-1} ha^{-1} $year^{-1}$ respectively) that increase soil microbial activity leading to increase microbial community respiration. In addition, future application of poor quality water resources can influence public and environmental health. Therefore, further studies are needed to investigate how these different water irrigation source at different soil moisture content influence community composition, microbial biomass, and soil enzymes in the long-term in arid soil for developing better management and reducing N or C gaseous emissions. This result supports our hypothesis that irrigation water source will increase the mineralization of the insoluble fractions of plant residues depending on the original labile compound (i.e., DOC content) from water source that increase microbial activity. In addition, the application of insoluble plant materials could potential increase labile compound, organic matter, and biological factors (e.g., increasing free enzymes). This is consistent with other studies that showed the application of organic residue from plant residue can increase the native soil organic matter and substrate turnover due to the increases of microbial and

enzyme activity such as, urease, dehydrogenase, and protease activity in soil (Burgos et al., 2002; Okur et al., 2009; Piotrowska-Długosz and Wilczewski, 2014).

The results from Chapter 5 showed that changing soil moisture regimes would influence the mineralization rate with the highest rate observed at a WHC of 50% and the lowest at 10% and 90% after application of soluble plant materials. This result is consistent with Li et al. (2014) who also found the lowest mineralization rates at the moisture extremes (< 20% WHC and > 80% WHC). At low WHC, cellular desiccation of microbes occurs, whereas at high WHC, the activity of aerobic microbes declines due to inhibition of air exchange and increased denitrification (denitrification process can increase CO_2 production; Rukshana et al. (2013)).

Changing soil moisture regimes showed significant shifts in modelled kinetic parameters after applying insoluble plant materials into soil (Chapter 5). This could be due to either changes in microbial community composition at higher soil moisture content, which increasing their ability to metabolize substrates that could not be utilized previously at lower soil moistures (Agehara and Warncke, 2005), or the insoluble compound becoming more mobile and not protected with soil matrix at a higher moisture content (Glanville et al., 2012). The results from Chapter 5 showed that the production of $^{14}CO_2$ from insoluble plant materials increased and Mic_{eff} decreased at higher moisture content this could be due to the potential increase of labile compound and biological factors as described above. Therefore, at high rate of irrigation water in short period (e.g., high moisture content in soil), the insoluble plant materials could become more liable to decomposition and releasing more C to the atmosphere. This result supports our hypothesis that increasing soil moisture content will increase the mineralization rate of insoluble plant residues due to increasing labile compound after the applications of water and insoluble residues that increase microbial activity.

7.6 Does inorganic and organic N fertiliser input change effect N and C cycles?

One of the most serious problems facing the world in general and in arid land in particular is food scarcity (Alkolibi, 2002). The availability of N and water resources influences cropping system and can cause soil, air and water pollution (Lenka et al., 2013). The application of inorganic N to cornfield had higher residual soil NO_3^-

content to a depth at 120 cm in comparison to the compost and manure treatment (Lenka et al., 2013). Therefore, it is important to evaluate the effect of using different organic and inorganic fertilisers on arid soils to better understand land management, reduce N or C gaseous emissions, and develop sustainable agriculture systems in arid agricultural systems such as those found in KSA. Concentrations of available N for plant growth was affected by applying different inorganic and organic N fertiliser onto soil (Chapter 6). We observed that inorganic N reduces C:N ratio more than organic N fertiliser with highest effect seen in the upper 10 mm compared to 15 - 30 mm in most cases. This could be due to inorganic N fertiliser addition increasing inorganic N content (NH_4^+ and NO_3^-) with no C addition that will reduce C:N ratio (Robertson and Groffman, 2007). Organic N fertiliser addition increase both inorganic N (NH_4^+ and NO_3^-) and C content into soil. C:N ratio results from Chapter 6 showed increases with time; this could be due to the accumulating of both total N (TN) and soil organic carbon (SOC) over the experimental period, but the build-up of TN was slower than SOC over time. Dong et al. (2012) support this suggestion.

Results in Chapter 6 showed that amino acid concentration increased after organic N fertiliser addition more than inorganic fertiliser with higher concentration at upper 10 mm compared to between 15 - 30 mm. This could be due to organic N fertiliser increasing C compound input (from the original chemical content) and organic matter into the soil more than inorganic fertiliser, in turn, leading to potential increases the microbial activity, compositions and size and, consequently, increase mineralization process that released more CO_2 and reduced the added C with time. Chirinda et al. (2010) reported that the cumulative CO_2 flux increased more after the application of organic manure than inorganic fertiliser due to the original organic matter content of organic manure increasing microbial activity. In addition, it could be due to the addition of organic fertiliser stimulating the decomposition and ammonification of native soil organic matter from the added C or increased microbial NO_3^- uptake (e.g., immobilization) by priming effect (Rukshana et al., 2012, 2014). Our results from Chapter 6 showed that the addition of organic N fertiliser decreased the NO_3^- concentrations with lower concentration between 15 - 30 mm than at upper 10 mm compared to control. This could be due to the original C compound content from organic N fertiliser will potential increase C availability into soil that cause high microbial N demand. This result is consistent with previous studies that showed

increasing DOC (Cookson et al., 2007) or glucose content (Rukshana et al., 2012), increases microbial mineralization/immobilization of N (Cookson et al., 2007; Chirinda et al., 2010; Rukshana et al., 2012) and decreases available N for nitrification (Cookson et al., 2007). This result supports our hypothesis that organic fertiliser can reduce the amount of NO_3^- produced over time that could be due to high microbial N demand from higher available C in organic N fertiliser.

Inorganic N fertilisers increased the concentration of NH_4^+ and NO_3^- more than organic N fertilisers with higher concentration at upper 10 mm than between 15 - 30 mm compared to control in most of the cases. Inorganic N fertilisers increased the concentration of amino acids with time; this could be due to the rapid increase of inorganic N from NH_4NO_3 with low availability of C in soil affecting C-cycling enzyme activity (Bowles et al., 2014). The high pH above 7 and high NH_4^+ content present after inorganic fertiliser application can activate immobilization process and NH_3 volatilization that reduce NH_4^+ and NO_3^- and increase amino acids content with time, our result support this suggestion (i.e., decrease NH_4^+ and NO_3^- concentrations, high NH_3 losses, increase amino acids concentration).

Our result from Chapter 6 showed that the organic and inorganic N fertiliser at both rates had greater diffusive distance of amino acids and NH_4^+ than the theoretical linear distance (20.1 and 3.7 mm respectively), whereas the diffusion of NO_3^- was lower and higher than theoretical distance (56.5 mm) after organic and inorganic N fertiliser at both rates, respectively. This could be due to increases amino acid and NH_4^+ concentrations through rapid mineralization/immobilization process, whereas the NO_3^- concentrations reduced due to the reduction of NO_3^- to NH_4^+ after the NO_3^- had diffused into 15 - 30 mm.

Inorganic fertiliser results in Chapter 6 showed a higher potential for NO_3^- leaching in comparison to the organic fertiliser that causes a fast reduction of CN ratio and rapid mineralization/immobilization process due to higher inorganic N with low availability of C after inorganic fertiliser addition (Robertson and Groffman, 2007). This is consistent with Lenka et al. (2013), who showed that NO_3^- content at all depth under organic treatment was lower than inorganic N treatment. This result supports our hypothesis that N from inorganic N fertiliser will diffuse faster than N contained in an organic fertiliser due to the rapid increase of inorganic N from inorganic fertiliser. We would recommend that farmers investigate the available N

content in their soils before applying different organic or inorganic fertiliser to reduce NO_3^- leachate and protect groundwater.

7.7 General conclusions and future works

Arid and semiarid ecosystems are important to study because they cover one-third of the total global land area (Cable et al., 2008) and relatively little is known about DON and DOC cycling in these ecosystem, particularly in the Middle East. This thesis increased our understanding of the impacts of changing soil temperature (Chapter 3 and Chapter 4), water irrigation source (Chapter 5) and fertiliser types (Chapter 6) on soil C and N cycle within arid ecosystem. It shows that chemical, biological, change of temperature, water irrigation source and fertiliser types would influence DON and DOC cycling differently in the arid soil studied in this thesis. Our results showed that soil with higher silt, clay, and salinity contents had the lower CO_2 emissions. Therefore, further study are needed to investigate the influence of increasing silt+clay (e.g., using different type of soil that contain deferent soil texture) or salinity content (e.g., using one type of soil and increase salinity content) on microbial activity (e.g., using extracellular enzyme assay (EEA); Schindlbacher et al., 2015; Cenini et al., 2016; Moorhead et al., 2016) in arid soil.

7.7.1 Climate change implications

Climate change is expected to affect KSA with a predicted rise in temperature from 1.8 - 4.1°C by 2050. The subsequent increase in soil temperature may influence water availability and decrease soil moisture (43.80 - 237.25 mm $year^{-1}$), reduce relative humidity (0.8 - 2.3%), increase evapotranspiration (76.1 - 195.6 mm $year^{-1}$) and increase precipitation (15 - 25 mm $year^{-1}$) across the kingdom (Chowdhury and Al-Zahrani, 2013). Our results showed an increase in soil temperature tends to increase the rate of low molecular weight (LMW) DON and DOC compound utilization by soil microbial communities in arid soil studied, thereby increasing losses of soil organic matter and greenhouse gases. Therefore, it is important to focus the attention of HIDA and KSA farmers on the effect of future soil temperature rises in arid systems and to develop appropriate soil management practices that reduce N or C gaseous emissions in those scenarios (e.g. reduce using fertiliser on hot months). Our studies are based on laboratory works, further

field studies are needed to investigate the long-term effects of changing temperature on soil microbial processes in arid soil.

7.7.2 Water irrigation source implications

The water availability and quality of water resources in KSA will be affected by climate change with a predicted combination of 8.8% increase of annual total water demand and 2.5% annual growth population of the country (DeNicola et al., 2015). As a result of increasing annual total water demand with limited groundwater supply for agricultural, industrial and domestic uses, the government has been utilizing alternative sources of irrigation water, such as tertiary treated wastewater and mixtures of drainage water and groundwater (Al-Kuwaiti, 2010; Aldakheel, 2011). The government encourages the reuse of treated wastewater in park and crop irrigations. KSA government used 240 million m^3 $year^{-1}$ in 2010 as TTWW and their target is to increase this to 400 million m^3 $year^{-1}$ in 2014 and increasing 10% in annual production of TTWW after 2014 (1838 million m^3 $year^{-1}$ in 2030) (Ouda, 2014b). Our results from Chapter 5 showed that changing water irrigation sources at different moisture regimes in the arid soil studied influenced mineralization rate and CO_2 emissions. This could be due to the increase in original content of labile compounds (i.e., DOC content) applied from different irrigation water source influencing soil microbial activity and increased microbial community respiration. This study is based on laboratory works, laboratory works are in controlled environment (e.g., there are no leaching effect, plant uptake, wind, day and night temperature etc.) that may overestimate the actual effect if we compare it with the same experiment in the field. Therefore, further field studies are needed to investigate the long-term effects of changing water irrigation sources and different moisture regimes on soil microbial processes in arid soil for developing better water irrigation strategy to reduce N or C pollution, and support sustainable agriculture systems.

Human, animal, and plant health may be affected by long-term irrigation with poor quality water sources containing potentially higher salinity, microbial pathogen, antibiotic-resistant bacteria, or heavy metal concentrations than their acceptable national values for irrigation (Hanjra et al., 2012; DeNicola et al., 2015). In addition, more than 76% of the crop products from various food markets located in Taif city, KSA were affected by many bacteria populations that resistant to at least one antibiotic (due to irrigation with poor water quality of TTWW which contain antibiotic-

resistant bacteria and microbial pathogens; DeNicola et al., 2015) this results could potential increase public health risks. We would recommend the government to investigate the quality of alternative water sources and compare them to the Ministry of Water and Electricity (2006-MWE) standards after improve it (e.g., add standard for microbial pathogen and remove antibiotic-resistant bacteria) before supplying it to farmers until the implications of using alternative water sources are better understood. Our results in Chapter 5 showed that all chemical characteristics of waters used in this study were within acceptable national values of water quality for irrigation according to Ayers and Westcot (1985) and Al-Jasser (2011) with the exception of EC value of MW. The government can reuse all alternative water source to irrigate parkland after provided a regulatory monitoring program that guarantees the quality of alternative water sources (i.e., TTWW and MW) is in place. In addition, encourage the government to reuse MW for planting salt-tolerant crops or fruits.

7.7.3 Fertiliser types implications

Since 2005, the government has been supporting and developing organic agriculture (Hartmann et al., 2012). In arid and semiarid regions organic agriculture has become more important because its focus on increasing organic matter improves water holding capacity and it can improve food safety production by reducing chemical fertilisers and pesticides, minimise farm waste, decrease water and soil pollutions (Hartmann et al., 2012). Only a few farms in KSA are currently under organic management. Conversely, the excessive use of inorganic N fertilisers can cause adverse environmental effects (e.g., increase NO_3^- leachate and greenhouse gases) (Fließbach et al., 2000; Inselsbacher et al., 2009; Chirinda et al., 2010; López-López et al., 2012; Zhang et al., 2012; Iqbal et al., 2014). Our results in Chapter 6 showed that the amount of NO_3^- produced over time after applying organic N fertiliser into arid soil studied reduced more than inorganic fertiliser and control soil. This could be due to low NO_3^- concentration after organic N compared to inorganic N fertiliser and control soil. In addition, it could be due to greater C availability with greater N demand after organic N fertiliser compared to inorganic N fertiliser thus increasing microbial mineralization/immobilization of N and decrease available N for nitrification. This result supports our hypothesis that organic fertiliser can reduce the amount of NO_3^- produced over time that could be due to high microbial N demand from higher available C in organic N fertiliser. It will be worth

studying a wider range of fertiliser types and their short and long term effect on soil enzymes and microbial community composition in order to develop better fertiliser management protocols and reducing N or C gaseous emissions.

All our studies are based on laboratory works. Further field studies about the impacts of changing soil temperature, water irrigation source and fertiliser types on soil N cycle in arid systems will be beneficial for improving water and fertiliser managements in the arid environment

7.8 Recommendations for sustainable water and fertiliser management

- Using alternative water source (e.g., TTWW, agriculture drainage, MW, rainfall harvesting, desalination of seawater and brackish groundwater) can decrease the pressure on GW supplies significantly, mitigate water scarcity, reduced chemical fertilizer (due to it is reach source of nutrient), save energy cost that reduce carbon emission. In contrast, it had some negative impact if water quality is not accounted for (e.g., increasing salt, heavy metal, nutrient, and pathogens). Our results showed (Chapter 5) that the alternative water source influenced microbial mineralization due to the potential for increasing the amount of labile compounds (i.e., DOC content) applied from this water source. Therefore, we would encourage the government to further investigate the implications of using alternative water sources in order to minimize human and environmental risks.
- Encourage farmers who are using MW to irrigate their soils to plant salt-tolerant crops or fruits due to MW had the highest EC content that can accumulate more salts at long-term irrigation that influence plant growth (plant growth will reduce due to high salts which can reduce the amount of water taking up by plant through osmotic potential) (Bob et al., 2016). This consist with Singh (2016), who showed high salinity will influence soil physical, chemical, biological properties, and crop productivity significantly. In addition, the differences in salinity content depends on irrigation frequency, irrigation water quality, nature of crop cultivated, field application of organic and inorganic fertiliser (Singh, 2015). Therefore, more research on the alternative water quality are required to reduce environmental and public risks.

- Recommend the farmers and HIDA reduce the rate of irrigation water to reduce the potential amount of labile compounds applied from different irrigation water source and reduce the potential to cause environmental pollution (e.g., NO_3^- leachate, CO_2 emissions). This is consistent with Lenka et al. (2013), who showed that using high rate of water to irrigate different crops (i.e. maximum irrigation rate) increased NO_3^- leachate into lower depth more than other irrigation rate (i.e., minimum and medium irrigation).
- Convert traditional irrigation method to alternative irrigation system (e.g., drip irrigation systems) to reduce water losses, cost, and enhance water use efficiency. In addition, due to relative small water addition and careful irrigation scheduling by drip irrigation system, the soil profile at 50 cm is not often becoming saturated this will allow more available N that mineralized from soil organic matter to stay in the root zone and reduce the leachate of NO_3^- and other N source to groundwater (Shock, 2005). This is consistent with Adekoya et al. (2014), who showed that using drip irrigation system in rice cropping enhanced the crop's water and nutrient use efficiency, increased yield, reduced water flooding, reduced nutrient leaching, reduced greenhouse gasses (i.e., methane and CO_2 emissions). This recommendation will help the government to achieve their goal to reduce 3.7% of agriculture water demand each year (current consuming is 85% of total national water demand in KSA) from 2010 to 2014 (Ouda, 2014b) and reduce environmental pollutions (e.g., reduce NO_3^- leachate to groundwater).
- Encourage farmer and the government managers to use more greenhouse farming in arid and semiarid environment as it can reduce evaporation rate (by controlling temperature and light inside greenhouse), well-managed irrigation system, and save water cost (due to well-managed irrigation system and low evaporation rate).
- Encourage farmers and farm managers in arid and semiarid areas to reduce inorganic fertiliser and use more organic N fertiliser that can reduce environmental pollution (e.g. NO_3^- leachate, N_2O emission), cost (due to recycling of farm waste and reduce inorganic fertiliser cost), save more water (due to organic fertiliser can improve water holding capacity), and increased yield. Using more organic N fertiliser increased corn yield, N uptake, water

and N use efficiency whereas, at low organic N fertiliser resulted in low yields even at 105 kg N ha^{-1} of inorganic N fertiliser (Xiaobin et al., 2001). Lenka et al. (2013) showed that NO_3^- content at all depth under organic treatment was lower than inorganic N treatment. This recommendation will help the government to meeting their target to reduce annual agriculture water demand (reduce 3.7% of agriculture water demand annually), cost, and minimise farm waste.

References

Abaas, E., Hill, P.W., Roberts, P., Murphy, D.V. and Jones, D.L., 2012. Microbial activity differentially regulates the vertical mobility of nitrogen compounds in soil. *Soil Biology and Biochemistry*, 53, pp.120–123.

Aciego, J.C. and Brookes, P.C., 2008. Nitrogen mineralisation along a pH gradient of a silty loam UK soil. *Soil Biology and Biochemistry*, 40(3), pp.797–802.

Adekoya, M.A., Liu, Z., Vered, E., Zhou, L., Kong, D., Qin, J., Ma, R., Yu, X., Liu, G., Chen, L. and Luo, L., 2014. Agronomic and Ecological Evaluation on Growing Water-Saving and Drought-Resistant Rice (Oryza sativa L.) Through Drip Irrigation. *Journal of Agricultural Science*, 6(5), pp.110–119.

Agehara, S. and Warncke, D.D., 2005. Soil Moisture and Temperature Effects on Nitrogen Release from Organic Nitrogen Sources. *Soil Science Society of America Journal*, 69(6), p.1844.

Al-Fredan, M.A., 2011. Nitrogen Fixing Legumes in the Plant Communities. *American Journal of Environmental Sciences*, 7(2), p.166.

Al-Hammad, B.A., Abd El-Salam, M.M. and Ibrahim, S.Y., 2014. Quality of wastewater reuse in agricultural irrigation and its impact on public health. *Environmental Monitoring and Assessment*, 186(11), pp.7709–7718.

Al-Jabr, M.A., 2002. *The agricultural situation in the Al-Hassa oasis (in Arabic)* 1st ed., King Fahd National Library, Riyadh.

Al-Jasser, A.O., 2011. Saudi wastewater reuse standards for agricultural irrigation: Riyadh treatment plants effluent compliance. *Journal of King Saud University - Engineering Sciences*, 23(1), pp.1–8.

Al-Kuwaiti, K.A., 2010. *Efforts Drainage and Irrigation Authority and its plans to provide irrigation water from different sources (in Arabic)*, Ministry of Agriculture, Al-Hassa Irrigation and Drainage Authority.

Al-Rawahi, M.N., Brinkmann, K., Schlecht, E. and Buerkert, A., 2014. Effects of changing water availability on land use in irrigated mountain oases of Al Jabal Al Akhdar, northern Oman. *DIE ERDE–Journal of the Geographical Society of Berlin*, 145(4), pp.197–211.

Albowarthan, A., Aldhofar, M. and Bojabarah, A., 2008. *The experiment of follow up and monitor the amount of irrigation water consumed at the farm level by using the counter hours of operation, Irrigation and Drainage Authority in Al-Hassa*

Oasis, KSA (in Arabic), Al-Hassa Irrigation and Drainage Authority.

Aldakheel, Y.Y., 2011. Assessing NDVI Spatial Pattern as Related to Irrigation and Soil Salinity Management in Al-Hassa Oasis, Saudi Arabia. *Journal of the Indian Society of Remote Sensing*, 39(2), pp.171–180.

AlKhouly, A., AlMusa, K. and AlFalag, Y., 2004. Environmental assessment of the deterioration of the trees from bumpers of arid agriculture in Al-Hassa national park (in Arabic). In *1st International Conference on Water Resources and Arid Environments*.

Alkolibi, F., 2002. Possible effects of global warming on agriculture and water resources in Saudi Arabia: Impacts and responses. *Climatic Change*, 54(1–2), pp.225–245.

AlMadini, A., 2001. Spatial Variability of Nitrate and Phosphate Concentrations in Waters from Main Drainage Canals at Al-Hassa Oasis, KSA. *Damascus University Journal for Agricultural Science*, 17(2), pp.98–109.

AlMadini, A.M., 2011. Physico-chemical properties of composts produced from various agricultural residues common in the Kingdom of Saudi Arabia. *European Journal of Scientific Research*, 57(4), pp.635–645.

AlMulla, A., 2012. *Quantification of Inorganic Nitrogen in North Wales Soil Samples*. Bangor University.

An, N., Zhou, C.H., Zhuang, X.Y., Tong, D.S. and Yu, W.H., 2015. Immobilization of enzymes on clay minerals for biocatalysts and biosensors. *Applied Clay Science*, 114, pp.283–296.

Ayers, R.S. and Westcot, D.W., 1985. *Water Quality for Agriculture*, Food and Agriculture Organization of the United Nations, Rome, Italy.

Bapiri, A., Bååth, E. and Rousk, J., 2010. Drying – Rewetting Cycles Affect Fungal and Bacterial Growth Differently in an Arable Soil. *Microbial Ecology*, 60(2), pp.419–428.

Barber, S.A., 1995. Nitrogen. In *Soil Nutrient Bioavailability: A Mechanistic Approach*. New York, USA: Wiley, pp. 180–201.

Barnard, R., Leadley, P.W. and Hungate, B.A., 2005. Global change, nitrification, and denitrification: A review. *Global Biogeochemical Cycles*, 19(1), pp.1–13.

Bashour, I. and Sayegh, A., 2007. *Methods of Analysis for Soils of Arid and Semi-Arid Regions*, Rome: Food and agriculture organization of the United Nations.

Batlle-Aguilar, J., Brovelli, A., Porporato, A. and Barry, D.A., 2011. Modelling soil carbon and nitrogen cycles during land use change. A review. *Agronomy for Sustainable Development*, 31(2), pp.251–274.

Baumann, K. and Marschner, P., 2013. Effects of salinity on microbial tolerance to drying and rewetting. *Biogeochemistry*, 112(1–3), pp.71–80.

Baumhardt, R.L., Lascano, R.J. and Evett, S.R., 2000. Soil Material, Temperature, and Salinity Effects on Calibration of Multisensor Capacitance Probes. *Soil Science Society of America Journal*, 64(6), p.1940.

Birgander, J., Reischke, S., Jones, D.L. and Rousk, J., 2013. Temperature adaptation of bacterial growth and ^{14}C-glucose mineralisation in a laboratory study. *Soil Biology and Biochemistry*, 65(0), pp.294–303.

Bob, M., Abd Rahman, N., Elamin, A. and Taher, S., 2016. Assessment of groundwater suitability for irrigation in Madinah City, Saudi Arabia. *Arabian Journal of Geosciences*, 9(1), p.38.

Boddy, E., Roberts, P., Hill, P.W., Farrar, J. and Jones, D.L., 2008. Turnover of low molecular weight dissolved organic C (DOC) and microbial C exhibit different temperature sensitivities in Arctic tundra soils. *Soil Biology and Biochemistry*, 40(7), pp.1557–1566.

Boddy, E., Hill, P.W., Farrar, J. and Jones, D.L., 2007. Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. *Soil Biology and Biochemistry*, 39(4), pp.827–835.

Boulding, J.R., 1994. *Description and Sampling of Contaminated Soils: a Field Guide* 2nd ed., CRC Press.

Bowles, T.M., Acosta-Martínez, V., Calderón, F. and Jackson, L.E., 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biology and Biochemistry*, 68, pp.252–262.

BP, 2016. BP Statistical Review of World Energy: Carbon dioxide emissions. , June, pp.1–2.

Brar, B., Singh, J., Singh, G. and Kaur, G., 2015. Effects of Long Term Application of Inorganic and Organic Fertilizers on Soil Organic Carbon and Physical Properties in Maize–Wheat Rotation. *Agronomy*, 5(2), pp.220–238.

Buerkert, A., Nagieb, M., Siebert, S., Khan, I. and Al-Maskri, A., 2005. Nutrient

cycling and field-based partial nutrient balances in two mountain oases of Oman. *Field Crops Research*, 94(2), pp.149–164.

Burden, A., Garbutt, R.A., Evans, C.D., Jones, D.L. and Cooper, D.M., 2013. Carbon sequestration and biogeochemical cycling in a saltmarsh subject to coastal managed realignment. *Estuarine, Coastal and Shelf Science*, 120(0), pp.12–20.

Burgos, P., Madejón, E. and Cabrera, F., 2002. Changes in soil organic matter, enzymatic activities and heavy metal availability induced by application of organic residues. *Developments in Soil Science*, 28, pp.353–362.

Butler, S.M., Melillo, J.M., Johnson, J.E., Mohan, J., Steudler, P.A., Lux, H., Burrows, E., Smith, R.M., Vario, C.L. and Scott, L., 2012. Soil warming alters nitrogen cycling in a New England forest: implications for ecosystem function and structure. *Oecologia*, 168(3), pp.819–828.

Buyer, J.S. and Sasser, M., 2012. High throughput phospholipid fatty acid analysis of soils. *Applied Soil Ecology*, 61, pp.127–130.

Cable, J.M., Ogle, K., Williams, D.G., Weltzin, J.F. and Huxman, T.E., 2008. Soil texture drives responses of soil respiration to precipitation pulses in the sonoran desert: Implications for climate change. *Ecosystems*, 11(6), pp.961–979.

Cai, A., Xu, H., Shao, X., Zhu, P., Zhang, W., Xu, M. and Murphy, D.V., 2016. Carbon and Nitrogen Mineralization in Relation to Soil Particle-Size Fractions after 32 Years of Chemical and Manure Application in a Continuous Maize Cropping System. *PLoS ONE*, 11(3), p.e0152521.

Cenini, V.L., Fornara, D.A., McMullan, G., Ternan, N., Carolan, R., Crawley, M.J., Clément, J.C. and Lavorel, S., 2016. Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. *Soil Biology and Biochemistry*, 96, pp.198–206.

Certini, G. and Scalenghe, R., 2006. *Soils: Basic Concepts and Future Challenges*, The Edinburgh Building, Cambridge, UK: Cambridge University Press.

Chen, X., Wang, X., Liebman, M., Cavigelli, M. and Wander, M., 2014. Influence of residue and nitrogen fertilizer additions on carbon mineralization in soils with different texture and cropping histories. *PLoS ONE*, 9(7), pp.1–11.

Chirinda, N., Olesen, J.E., Porter, J.R. and Schjønning, P., 2010. Soil properties, crop production and greenhouse gas emissions from organic and inorganic fertilizer-based arable cropping systems. *Agriculture, Ecosystems and*

Environment, 139(4), pp.584–594.

Chowdhury, S. and Al-Zahrani, M., 2013. Implications of Climate Change on Water Resources in Saudi Arabia. *Arabian Journal for Science and Engineering*, 38(8), pp.1959–1971.

Christou, M., Avramides, E.J. and Jones, D.L., 2006. Dissolved organic nitrogen dynamics in a Mediterranean vineyard soil. *Soil Biology and Biochemistry*, 38(8), pp.2265–2277.

Cole, J., 2008. *Quantification of the Long-Term Effects from Nutrient Reductions on Groundwater Nitrate Concentrations in an Agricultural Setting*. University of Waterloo.

Cookson, W.R., Osman, M., Marschner, P., Abaye, D.A., Clark, I., Murphy, D.V., Stockdale, E.A. and Watson, C.A., 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. *Soil Biology and Biochemistry*, 39(3), pp.744–756.

Deenik, J., 2006. *Nitrogen Mineralization Potential in Important Agricultural Soils of Hawai'i*,

DeNicola, E., Aburizaiza, O.S., Siddique, A., Khwaja, H. and Carpenter, D.O., 2015. Climate change and water scarcity: The case of Saudi Arabia. *Annals of Global Health*, 81(3), pp.342–353.

Devi, S.G., Fathima, A.A., Radha, S., Arunraj, R., Curtis, W.R. and Ramya, M., 2015. A rapid and economical method for efficient DNA extraction from diverse soils suitable for metagenomic applications. *PLoS ONE*, 10(7), pp.1–16.

Dong, W., Zhang, X., Wang, H., Dai, X., Sun, X., Qiu, W. and Yang, F., 2012. Effect of Different Fertilizer Application on the Soil Fertility of Paddy Soils in Red Soil Region of Southern China. *PLoS ONE*, 7(9), pp.1–9.

Dungait, J.A.J., Cardenas, L.M., Blackwell, M.S.A., Wu, L., Withers, P.J.A., Chadwick, D.R., Bol, R., Murray, P.J., Macdonald, A.J. and Whitmore, A.P., 2012. Advances in the understanding of nutrient dynamics and management in UK agriculture. *Science of the Total Environment*, 434, pp.39–50.

Egboka, B.C., 1984. Nitrate contamination of shallow groundwaters in Ontario, Canada. *Science of the Total Environment*, 35(1), pp.53–70.

Elifantz, H., Kautsky, L., Mor-Yosef, M., Tarchitzky, J., Bar-Tal, A., Chen, Y. and Minz, D., 2011. Microbial Activity and Organic Matter Dynamics During 4 Years

of Irrigation with Treated Wastewater. *Microbial Ecology*, 62(4), pp.973–981.

Ewing, S.A., Macalady, J.L., Warren-Rhodes, K., McKay, C.P. and Amundson, R., 2008. Changes in the soil C cycle at the arid-hyperarid transition in the Atacama Desert. *Journal of Geophysical Research*, 113(G2), p.G02S90.

FAO, 2006. *Near East Fertilizer-Use Manual*, Food and Agriculture Organization of the United Nations.

Farrell, M., Hill, W., Farrar, J., Wanniarachchi, D., Bardgett, D. and Jones, D.L., 2011. Rapid peptide metabolism: A major component of soil nitrogen cycling? *Global Biogeochemical Cycles*, 25, p.11.

Farrell, M., Hill, P.W., Farrar, J., DeLuca, T.H., Roberts, P., Kielland, K., Dahlgren, R., Murphy, D.V., Hobbs, P.J., Bardgett, R.D. and Jones, D.L., 2013. Oligopeptides Represent a Preferred Source of Organic N Uptake: A Global Phenomenon? *Ecosystems*, 16(1), pp.133–145.

Ferguson, S.D. and Nowak, R.S., 2011. Transitory effects of elevated atmospheric CO_2 on fine root dynamics in an arid ecosystem do not increase long-term soil carbon input from fine root litter. *New Phytologist*, 190(4), pp.953–967.

Fierer, N., Schimel, J.P. and Holden, P.A., 2003. Influence of Drying–Rewetting Frequency on Soil Bacterial Community Structure. *Microbial Ecology*, 45(1), pp.63–71.

Fierer, N. and Schimel, J.P., 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, 34(6), pp.777–787.

Filep, T. and Rékási, M., 2011. Factors controlling dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and DOC/DON ratio in arable soils based on a dataset from Hungary. *Geoderma*, 162(3), pp.312–318.

Findlay, R.H., 2004. Determination of microbial community structure using phospholipid fatty acid profiles. In Kowalchuk et al.eds. *Molecular Microbial Ecology Manual*. Dordrecht, the Netherlands: Kluwer Academic Publishers, pp. 983–1004.

Fisk, L.M., Barton, L., Jones, D.L., Glanville, H.C. and Murphy, D.V., 2015. Root exudate carbon mitigates nitrogen loss in a semi-arid soil. *Soil Biology and Biochemistry*, 88, pp.380–389.

Fließbach, A., Mäder, P. and Niggli, U., 2000. Mineralization and microbial

assimilation of ^{14}C-labeled straw in soils of organic and conventional agricultural systems. *Soil Biology and Biochemistry*, 32, pp.1131–1139.

Fraser, F.C., Corstanje, R., Deeks, L.K., Harris, J.A., Pawlett, M., Todman, L.C., Whitmore, A.P. and Ritz, K., 2016. On the origin of carbon dioxide released from rewetted soils. *Soil Biology and Biochemistry*, 101, pp.1–5.

Frostegård, Å., Bååth, E. and Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, 25(6), pp.723–730.

Gao, H., Bai, J., He, X., Zhao, Q., Lu, Q. and Wang, J., 2014. High Temperature and Salinity Enhance Soil Nitrogen Mineralization in a Tidal Freshwater Marsh. *PLoS ONE*, 9(4), p.e95011.

Garratt, J.R., 1992. Extreme Maximum Land Surface Temperatures. *Journal of Applied Meteorology*, 31(9), pp.1096–1105.

Ge, T.D., Huang, D.F., Roberts, P., Jones, D.L. and Song, S.W., 2010. Dynamics of Nitrogen Speciation in Horticultural Soils in Suburbs of Shanghai, China. *Pedosphere*, 20(2), pp.261–272.

Ghani, A., Dexter, M., Carran, R.A. and Theobald, P.W., 2007. Dissolved organic nitrogen and carbon in pastoral soils: the New Zealand experience. *European Journal of Soil Science*, 58(3), pp.832–843.

Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E. and Jones, D.L., 2016. Combined use of empirical data and mathematical modelling to better estimate the microbial turnover of isotopically labelled carbon substrates in soil. *Soil Biology and Biochemistry*, 94, pp.154–168.

Glanville, H.C., Hill, P.W., Maccarone, L.D., Golyshin, P.N., Murphy, D. V and Jones, D.L., 2012. Temperature and water controls on vegetation emergence, microbial dynamics, and soil carbon and nitrogen fluxes in a high Arctic tundra ecosystem. *Functional Ecology*, 26(6), pp.1366–1380.

Gordon, H., Haygarth, P.M. and Bardgett, R.D., 2008. Drying and rewetting effects on soil microbial community composition and nutrient leaching. *Soil Biology and Biochemistry*, 40(2), pp.302–311.

Hanjra, M.A., Blackwell, J., Carr, G., Zhang, F. and Jackson, T.M., 2012. Wastewater irrigation and environmental health: Implications for water governance and public policy. *International Journal of Hygiene and*

Environmental Health, 215(3), pp.255–269.

Hartmann, M., Khalil, S., Bernet, T., Ruhland, F. and AlGhamdi, A., 2012. *Organic Agriculture in Saudi Arabia*, Deutsche Gesellschaft für Internationale Zusammenarbeit GIZ.

Hill, P.W., Farrar, J.F. and Jones, D.L., 2008. Decoupling of microbial glucose uptake and mineralization in soil. *Soil Biology and Biochemistry*, 40(3), pp.616–624.

Hill, P.W., Quilliam, R.S., DeLuca, T.H., Farrar, J., Farrell, M., Roberts, P., Newsham, K.K., Hopkins, D.W., Bardgett, R.D. and Jones, D.L., 2011a. Acquisition and Assimilation of Nitrogen as Peptide-Bound and D-enantiomers of Amino Acids by Wheat. *PLoS One*, 6(4), p.e19220. doi:10.1371/journal.pone.0019220.

Hill, P.W., Farrar, J., Roberts, P., Farrell, M., Grant, H., Newsham, K.K., Hopkins, D.W., Bardgett, R.D. and Jones, D.L., 2011b. Vascular plant success in a warming Antarctic may be due to efficient nitrogen acquisition. *Nature Climate Change*, 1(4), pp.50–53.

Hill, P.W., Marsden, K.A. and Jones, D.L., 2013. How significant to plant N nutrition is the direct consumption of soil microbes by roots? *New Phytologist*, 199(4), pp.948–955.

Hill, P.W., Farrell, M., Roberts, P., Farrar, J., Grant, H., Newsham, K.K., Hopkins, D.W., Bardgett, R.D. and Jones, D.L., 2011c. Soil-and enantiomer-specific metabolism of amino acids and their peptides by Antarctic soil microorganisms. *Soil Biology and Biochemistry*, 43(12), pp.2410–2416.

Hooker, T.D. and Stark, J.M., 2008. Soil C and N cycling in three semiarid vegetation types: Response to an in situ pulse of plant detritus. *Soil Biology and Biochemistry*, 40(10), pp.2678–2685.

Hussain, G., Alquwaizany, A. and Al-Zarah, A., 2010. Guidelines for Irrigation Water Quality and Water Management in the Kingdom of Saudi Arabia: An Overview. *Journal of Applied Sciences*, 10, pp.79–96.

Hussain, G. and Al-Saati, A.J., 1999. Wastewater quality and its reuse in agriculture in Saudi Arabia. *Desalination*, 123(2), pp.241–251.

Inselsbacher, E., Ripka, K., Klaubauf, S., Fedosoyenko, D., Hackl, E., Gorfer, M., Hood-Novotny, R., Von Wirén, N., Sessitsch, A., Zechmeister-Boltenstern, S.,

Wanek, W. and Strauss, J., 2009. A cost-effective high-throughput microcosm system for studying nitrogen dynamics at the plant-microbe-soil interface. *Plant and Soil*, 317(1–2), pp.293–307.

Iqbal, Z., Khalid, M., Soleman, N. and Jones, D.L., 2014. Differential Utilization of Dissolved Organic and Inorganic Nitrogen by Wheat (*Triticum aestivum* L.). *Journal of Plant Nutrition*, 37(13), pp.2094–2107.

Jan, M.T., Roberts, P., Tonheim, S.K. and Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. *Soil Biology and Biochemistry*, 41(11), pp.2272–2282.

Javid, M., Bahramifar, N., Younesi, H., Taghavi, S.M. and Givehchi, R., 2015. Dry deposition, seasonal variation and source interpretation of ionic species at Abali, Firouzkouh and Varamin, Tehran Province, Iran. *Atmospheric Research*, 157, pp.74–90.

Jones, D.L., Nguyen, C. and Finlay, R.D., 2009. Carbon flow in the rhizosphere: Carbon trading at the soil-root interface. *Plant and Soil*, 321(1), pp.5–33.

Jones, D.L., Shannon, D., Murphy, V.D. and Farrar, J., 2004. Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biology and Biochemistry*, 36(5), pp.749–756.

Jones, D.L., Farrar, J.F., Macdonald, A.J., Kemmitt, S.J. and Murphy, D.V., 2008. Dissolved Organic Nitrogen and Mechanisms of Its Uptake by Plants in Agricultural Systems. In Liwang et al.eds. Quantifying and Understanding Plant Nitrogen Uptake for Systems Modeling. CRC Press Taylor and Francis Group, pp. 95–126.

Jones, D.L., Owen, A.G. and Farrar, J.F., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. *Soil Biology and Biochemistry*, 34(12), pp.1893–1902.

Jones, D.L., Kemmitt, S.J., Wright, D., Cuttle, S.P., Bol, R. and Edwards, A.C., 2005a. Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy. *Soil Biology and Biochemistry*, 37(7), pp.1267–1275.

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H. and Murphy, D. V, 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biology and Biochemistry*, 45, pp.113–124.

Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F. and Hodge, A., 2005b. Dissolved organic nitrogen uptake by plants - An important N uptake pathway? *Soil Biology and Biochemistry*, 37(3), pp.413–423.

van Kessel, C., Clough, T. and Groenigen, J.W., 2009. Dissolved Organic Nitrogen: An Overlooked Pathway of Nitrogen Loss from Agricultural Systems? *Journal of Environmental Quality*, 38(2), pp.393–401.

Kurapova, A.I., Zenova, G.M., Sudnitsyn, I.I., Kizilova, A.K., Manucharova, N.A., Norovsuren, Z. and Zvyagintsev, D.G., 2012. Thermotolerant and thermophilic actinomycetes from soils of Mongolia desert steppe zone. *Microbiology*, 81(1), pp.98–108.

Lenka, S., Singh, A.K. and Lenka, N.K., 2013. Soil water and nitrogen interaction effect on residual soil nitrate and crop nitrogen recovery under maize-wheat cropping system in the semi-arid region of northern India. *Agriculture, Ecosystems and Environment*, 179, pp.108–115.

Li, C., Li, Y. and Tang, L., 2013. The effects of long-term fertilization on the accumulation of organic carbon in the deep soil profile of an oasis farmland. *Plant and Soil*, 369(1), pp.645–656.

Li, Y., Liu, Y., Wang, Y., Niu, L., Xu, X. and Tian, Y., 2014. Interactive effects of soil temperature and moisture on soil N mineralization in a Stipa krylovii grassland in Inner Mongolia, China. *Journal of Arid Land*, 6(5), pp.571–580.

Liang, Y., Li, F., Nong, M., Luo, H. and Zhang, J., 2016. Microbial Activity in Paddy Soil and Water-Use Efficiency of Rice as Affected by Irrigation Method and Nitrogen Level. *Communications in Soil Science and Plant Analysis*, 47(1), pp.19–31.

López-López, G., Lobo, M.C., Negre, A., Colombàs, M., Rovira, J.M., Martorell, A., Reolid, C. and Sastre-Conde, I., 2012. Impact of fertilisation practices on soil respiration, as measured by the metabolic index of short-term nitrogen input behaviour. *Journal of Environmental Management*, 113(0), pp.517–526.

Lucho-Constantino, C.A., Álvarez-Suárez, M., Beltrán-Hernández, R.I., Prieto-García, F. and Poggi-Varaldo, H.M., 2005. A multivariate analysis of the accumulation and fractionation of major and trace elements in agricultural soils in Hidalgo State, Mexico irrigated with raw wastewater. *Environment International*, 31(3), pp.313–323.

Miranda, K.M., Espey, M.G. and Wink, D.A., 2001. A Rapid, Simple Spectrophotometric Method for Simultaneous Detection of Nitrate and Nitrite. *Nitric Oxide: Biology and Chemistry*, 5(1), pp.62–71.

Mitchell, E., Scheer, C., Rowlings, D.W., Conant, R.T., Cotrufo, M.F., van Delden, L. and Grace, P.R., 2016. The influence of above-ground residue input and incorporation on GHG fluxes and stable SOM formation in a sandy soil. *Soil Biology and Biochemistry*, 101, pp.104–113.

Mohammad Rusan, M.J., Hinnawi, S. and Rousan, L., 2007. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination*, 215(1–3), pp.143–152.

Moorhead, D.L., Sinsabaugh, R.L., Hill, B.H. and Weintraub, M.N., 2016. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. *Soil Biology and Biochemistry*, 93, pp.1–7.

Mulvaney, R.L., 1996. Nitrogen – Inorganic Forms. In Sparksed. Methods of Soil Analysis: Part 3: Chemical Methods. Madison, Wisconsin, United States of America: Soil Science Society of America, Inc., American Society of Agronomy, Inc., pp. 1123–1184.

Murphy, D.V., Macdonald, A.J., Stockdale, E.A., Goulding, K., Fortune, S., Gaunt, J.L., Poulton, P.R., Wakefield, J.A., Webster, C.P. and Wilmer, W.S., 2000. Soluble organic nitrogen in agricultural soils. *Biology and Fertility of Soils*, 30(5–6), pp.374–387.

Murphy, J. and Riley, J.P., 1962. A Modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27(0), pp.31–36.

Narayan, A., Jain, K., Shah, A.R. and Madamwar, D., 2016. An efficient and cost-effective method for DNA extraction from athalassohaline soil using a newly formulated cell extraction buffer. *3 Biotech*, 6(1), p.62.

Nobel, P.S., 1984. Extreme temperatures and thermal tolerances for seedlings of desert succulents. *Oecologia*, 62(3), pp.310–317.

OECD Test No. 222, 2004. Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*). In *OECD Guidelines for the Testing of Chemicals, Section 2*. Organisation for Economic Co-operation and Development, Paris.

Okur, N., Altindisli, A., Cengel, M., Gocmez, S. and Kayikcioglu, H.H., 2009.

Microbial biomass and enzyme activity in vineyard soils under organic and conventional farming systems. *Turkish Journal of Agriculture and Forestry*, 33(4), pp.413–423.

Ouda, O.K.M., 2014a. Impacts of agricultural policy on irrigation water demand: a case study of Saudi Arabia. *International Journal of Water Resources Development*, 627(June 2015), pp.1–11.

Ouda, O.K.M., 2014b. Water demand versus supply in Saudi Arabia: current and future challenges. *International Journal of Water Resources Development*, 30(2), pp.335–344.

Paul, E.A., 2007. *Soil Microbiology, Ecology, and Biochemistry* 3rd ed., Elsevier Inc.

Pescod, M.B., 1992. *Wastewater treatment and use in agriculture - FAO irrigation and drainage paper 47*, Food and Agriculture Organization, Rome, Italy.

Piotrowska-Długosz, A. and Wilczewski, E., 2014. Assessment of soil nitrogen and related enzymes as influenced by the incorporation timeof field pea cultivated as a catch crop in *Alfisol*. *Environmental Monitoring and Assessment*, 186, pp.8425–8441.

Qadir, M., Bahri, A., Sato, T. and Al-Karadsheh, E., 2010. Wastewater production, treatment, and irrigation in Middle East and North Africa. *Irrigation and Drainage Systems*, 24(1–2), pp.37–51.

Qiu-Hui, C., Ying, F., Zhang, Y.P., Zhang, Q.C., Shamsi, I.H., Zhang, Y.S. and Xian-Yong, L.I.N., 2012. Short-term Responses of Nitrogen Mineralization and Microbial Community to Moisture Regimes in Greenhouse Vegetable Soils. *Pedosphere*, 22(2), pp.263–272.

Qualls, R.G. and Bridgham, S.D., 2005. Mineralization rate of ^{14}C-labelled dissolved organic matter from leaf litter in soils of a weathering chronosequence. *Soil Biology and Biochemistry*, 37(5), pp.905–916.

Roberts, P. and Jones, D.L., 2008. Critical evaluation of methods for determining total protein in soil solution. *Soil Biology and Biochemistry*, 40(6), pp.1485–1495.

Roberts, P. and Jones, D.L., 2012. Microbial and plant uptake of free amino sugars in grassland soils. *Soil Biology and Biochemistry*, 49(0), pp.139–149.

Robertson, G.P. and Groffman, P.M., 2007. Nitrogen Transformations. In Pauled. *Soil Microbiology, Ecology, and Biochemistry*. Elsevier, New York: Academic

Press, pp. 341–364.

Ros, G.H., Hoffland, E., Van, K.C. and Temminghoff, E., 2009. Extractable and dissolved soil organic nitrogen – A quantitative assessment. *Soil Biology and Biochemistry*, 41(6), pp.1029–1039.

Rousk, J., Frey, S.D. and Bååth, E., 2012. Temperature adaptation of bacterial communities in experimentally warmed forest soils. *Global Change Biology*, 18(10), pp.3252–3258.

Rui, L.I., Yu, L.I.U. and Gui-xin, C.H.U., 2015. Effects of different cropping patterns on soil enzyme activities and soil microbial community diversity in oasis farmland. *Yingyong Shengtai Xuebao*, 26(2), pp.490–496.

Rukshana, F., Butterly, C.R., Xu, J.M., Baldock, J.A. and Tang, C., 2013. Soil organic carbon contributes to alkalinity priming induced by added organic substrates. *Soil Biology and Biochemistry*, 65, pp.217–226.

Rukshana, F., Butterly, C.R., Baldock, J.A., Xu, J.M. and Tang, C., 2012. Model organic compounds differ in priming effects on alkalinity release in soils through carbon and nitrogen mineralisation. *Soil Biology and Biochemistry*, 51, pp.35–43.

Rukshana, F., Butterly, C.R., Xu, J.M., Baldock, J.A. and Tang, C., 2014. Organic anion-to-acid ratio influences pH change of soils differing in initial pH. *Journal of Soils and Sediments*, 14(2), pp.407–414.

Rukshana, F., Butterly, C.R., Baldock, J.A. and Tang, C., 2011. Model organic compounds differ in their effects on pH changes of two soils differing in initial pH. *Biology and Fertility of Soils*, 47(1), pp.51–62.

Safar, Z., 2011. *Migration of Heavy Metals in Soils by Evaporation*. Utrecht university.

Schindlbacher, A., Schnecker, J., Takriti, M., Borken, W. and Wanek, W., 2015. Microbial physiology and soil CO_2 efflux after 9 years of soil warming in a temperate forest - no indications for thermal adaptations. *Global Change Biology*, 21(11), pp.4265–4277.

Schulten, H.R. and Schnitzer, M., 1997. The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils*, 26(1), pp.1–15.

Shah, S.A. and Shah, Z., 2011. Changes in soil microbial characteristics with elevated salinity. *Sarhad Journal of Agriculture*, 27(2), pp.233–244.

Shock, C.C., 2005. Nitrogen management with drip and sprinkler irrigation. In *Western Nutrient Management Conference, Salt Lake City, Utah*. pp. 66–71.

Simfukwe, P., Hill, P.W., Emmett, B.A. and Jones, D.L., 2011. Soil classification provides a poor indicator of carbon turnover rates in soil. *Soil Biology and Biochemistry*, 43(8), pp.1688–1696.

Singh, K., 2015. Microbial and Enzyme Activities of Saline and Sodic Soils. *Land Degradation and Development*, 718(May 2015), pp.706–718.

Sinsabaugh, R.L., Belnap, J., Rudgers, J., Kuske, C.R., Martinez, N. and Sandquist, D., 2015. Soil microbial responses to nitrogen addition in arid ecosystems. *Frontiers in Microbiology*, 6(JUL), pp.1–12.

Sollins, P., Homann, P. and Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma*, 74(1), pp.65–105.

Swain, T. and Hillis, W.E., 1959. The phenolic constituents of *Prunus domestica.* I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10(1), pp.63–68.

Tan, K.H., 2009. *Environmental Soil Science* Third., CRC Press Taylor and Francis Group.

US-EPA, 2007. Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils, and oils. In *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. EPA Publication SW-846 (3rd edition - Final Update IV).

Watts, D.B., Torbert, H.A. and Prior, S.A., 2007. Mineralization of Nitrogen in Soils Amended with Dairy Manure as Affected by Wetting/Drying Cycles. *Communications in Soil Science and Plant Analysis*, 38(15–16), pp.2103–2116.

White, R.E., 2009. *Principles and Practice of Soil Science: The Soil as a Natural Resource* 4th ed., Hoboken, New Jersey, USA: John Wiley & Sons, Inc. Available at: http://www.bangor.eblib.com/

Wichelns, D. and Qadir, M., 2014. Achieving sustainable irrigation requires effective management of salts, soil salinity, and shallow groundwater. *Agricultural Water Management*, 157, pp.31–38.

Williams, J.B., Tieleman, B.I. and Shobrak, M., 1999. LIZARD BURROWS PROVIDE THERMAL REFUGIA FOR LARKS IN THE ARABIAN DESERT. *The Condor*, 101(3), pp.714–717.

Xiang, S.R., Doyle, A., Holden, P.A. and Schimel, J.P., 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biology and Biochemistry*, 40(9), pp.2281–2289.

Xiaobin, W., Dianxiong, C. and Jingqing, Z., 2001. Land application of organic and inorganic fertilizer for corn in dryland farming region of North China. In Stott et al.eds. *Sustaining the Global Farm – Selected papers from the 10th International Soil Conservation Organization Meeting, May 24-29, 1999*. West Lafayette, Indiana USA: International Soil Conservation Organization in cooperation with USDA-ARS National Soil Erosion Research Laboratory and Purdue University, pp. 419–422.

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

Yang, R., Su, Y. and Yang, Q., 2015. Crop Yields and Soil Nutrients in Response to Long-Term Fertilization in a Desert Oasis. *Agronomy Journal*, 107(1), pp.83–92.

Yuan, B.-C., Li, Z.-Z., Liu, H., Gao, M. and Zhang, Y.-Y., 2007. Microbial biomass and activity in salt affected soils under arid conditions. *Applied Soil Ecology*, 35(2), pp.319–328.

Zhang, R. and Wienhold, B.J., 2002. The effect of soil moisture on mineral nitrogen, soil electrical conductivity, and pH. *Nutrient Cycling in Agroecosystems*, 63(2–3), pp.251–254.

Zhang, X., Wang, Q., Gilliam, F.S., Bai, W., Han, X. and Li, L., 2012. Effect of nitrogen fertilization on net nitrogen mineralization in a grassland soil, northern China. *Grass and Forage Science*, 67(2), pp.219–230.