DYNAMICS OF NITROGEN AND CARBON CYCLING ASSOCIATED WITH GREENHOUSE GAS EMISSIONS IN THE SALT-AFFECTED SOILS

by

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Candidate's Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge, it contains no material previously published or written by another person, except where due reference is made in the text.

DANG DUY MINH Date:

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Abstract

Salinity is one of the most severe environmental factors limiting the productivity of aquaculture and agriculture. The worldwide area of salt-affected soils is predicted to become even more widespread in the future due to climate change and sea-level rise. However, the soil nitrogen and carbon dynamics associated with soil-induced gas emissions under salinity are not well understood. The main objective of this study was to investigate changes of soil carbon and nitrogen cycling associated with greenhouse gas emissions, plant growth and fertilizer recovery under effects of different salinity levels. This study addressed research issues with the following main objectives. The main aim of the study reported in Chapter 2 was to analyse greenhouse gas production from different soils with different times of lid closure and to assess the effects of different activation time on gas emissions from soils. The results showed that the 20-min sampling interval at the closure time of maximum 80 minutes had good results with less variance either for soil types or monitored gases. Lengthening activation times for the incubation study may affect emission rates due to differences in soil properties. The study in Chapter 3 examined the effects of salinity and additional sources of nitrogen and carbon on soil nitrogen and carbon cycling in an acid sulphate soil (ASS) and an alluvial soil. The findings of this study demonstrated that salinity significantly decreased N_2O emissions from the acid sulphate soil but did not affect emissions from the alluvial soil. The addition of glucose and nitrate enhanced N_2O production in both salt-affected soils. This investigation indicated that salinity altered the carbon and nitrogen cycles in the acid sulphate soil; it recommends that future fertiliser and crop management will need to account for the changed nutrient cycling caused by saline water intrusion and climate change. The objective of the study reported in Chapter 4 was to identify a relationship between induced-soil gas emissions and the abundance of denitrification genes in a salt-affected soil. Increased salinity caused a decrease in both flux and cumulation of the N_2O-N production and soil respiration from the incubated soil. The study result also showed that elevated salinity increased the denitrifying genes in the incubated acid sulphate soil. Abundance of the *nir* genes was usually high between the first and second week of incubation, while number of copies of the *nosZ* gene were significantly low at those times. Another study presented in Chapter 5 investigated changes in soil properties, the dynamics of N and its effects on rice growth and yield under different salinity levels by using a ^{15}N label fertilizer technique. Flooding soils for two weeks by saline water greatly decreased rice yield and yield components in the acid sulphate soil. High salinity significantly lowered the recovery of fertilizer N by rice plants, especially in the acid sulphate soil where the crop did not produce any grain. The loss of fertilizer nitrogen was highly controlled by the interaction effect of soil types and salinity. Findings

from the thesis substantially and originally contribute to the literature on salt-affected soils and will assist in developing new managemental interventions and strategies for soils where increased salinity is a real possibility in the future.

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List of Acronyms and Abbreviations

ACIAR	Australian Centre for International Agricultural Research
ANCA	Automated Nitrogen Carbon Analysis
ANOVA	Analysis of variance
ASS	Acid sulphate soil
CEC	Cation exchange capacity
CH_4	Methane gas
CLRRI	Cuu Long Delta Rice Research Institute
CLUES	Climate Change affecting Land Use in the Mekong Delta: Adaptation of Rice-based Cropping Systems.
CO_2	Carbon dioxide gas
DAS	Days after seeding
EC	Electrical conductivity
ECD	Electron capture detector
EC_e	Electrical conductivity of saturation extract
ESP	Exchangeable sodium percentage
FAO	Food and Agriculture Organisation
GC	Gas chromatograph
GHG	Greenhouse gas
HSD	Honest significant difference
IPCC	Intergovernmental Panel on Climate Change
IRMS	Isotope Ratio Mass Spectrometer
MD	The Mekong River Delta
MONRE	Ministry of Natural Resources and Environment
N_2O	Nitrous oxide gas
NRE	Nitrogen recovery efficiency
PCR	Polymerase Chain Reaction
qPCR	Quantitative real-time PCR
SAR	Sodium adsorption ratio
SLR	Sea-level rise
SOM	Soil organic matter
US-EPA	United States Environmental Protection Agency
WRB	World Reference Base for Soil Resources

Chapter 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Climate change, one of the most serious problems facing the world today, will cause increased frequency and intensity of drought and floods, more storms and rising sea levels plus the extinction of species and the loss of whole ecosystems (Truong et al., 2011). An increase in future global temperatures, accelerated melting of ice sheets and glaciers, would cause further sea-level rise (SLR) (Smajgl et al., 2015). Sea-level rise exacerbated by climate change has already begun, severely affecting coasts and river estuaries in low-income countries (Vineis and Khan, 2012). Coastal lowlands less than a metre above sea level will be flooded by the end of the 21st century and delta areas that are at risk of flooding will increase by 50% (Syvitski et al., 2009; Giosan et al., 2014). Most impacts of climate change will be transferred to human and ecological communities through sea level rise, storms, flooding, and drought (Truong et al., 2011). Sea-level rise will flood and inundate occupied lands much more rapidly and much more extensively and alter hydrology leading to salinization of fresh water aquifers and agricultural land (Oliver-Smith, 2009). Rising sea-levels will also affect natural systems by wetland loss, erosion, saltwater intrusion into surface waters and groundwater and rising water tables (Nicholls and Tol, 2006). Therefore, this issue requires more empirical research that will inform better management of land and water resources in which human communities are adapting to climate change (Truong et al., 2011).

The rise of sea level will cause soil salinization through seawater intrusion into surface water, particularly irrigation water (IPCC, 2007a; Pereira *et al.*, 2015). Seawater is mostly constituted by free ions of sodium (31%) and chloride (55%); the addition of these ions to soil alters the soil chemistry, water holding properties and ultimately plant productivity (Wang and Li, 2013). Saline soils are often recognized by the presence of white salt encrustations on the surface and predominant chlorides and sulphates of Na, Ca, and Mg. Saline soils usually have a saturation paste of pH < 8.2, an electrical conductivity of saturation extracts (EC_e) > 4 dS m^{-1} at 25°C and a sodium adsorption ratio (SAR) of the soil solution < 15 (Gupta and Abrol, 1990). Saline soils are found worldwide, and soil salinization has been identified as a major process of land degradation. The total area of saline soil and sodic soil is more than 350 million ha and over 500 million ha, respectively (Ali, 2011). Out of the current 1.7 thousand million ha of irrigated and dry agricultural land, ~ 80 million ha are salt-affected soils (Ghassemi et al., 1995). The area of salt-affected soils will become more widespread in the future due to climate change and sea-level rise.

Salinity is one of the most severe environmental factors limiting the productivity of aquaculture and agriculture. For example, changes in salinity in aquatic environments represent major ecological disturbances in tropical fish farming (Ahmadi et al., 2016). In agriculture, most crops are sensitive to salinity due to the effects of the high concentrations of salts in the soil and irrigated water (Pitman and Läuchli, 2002). The growth and development of rice are affected when the soil EC > 3 dS m^{-1} due to Na^{+} toxicity. High content of salt in the soil also affects rice yield components and the production of perennial trees such as citrus, amongst the most susceptible of all trees to salt stress (Ahmadi et al., 2016). Crop productivity and production losses caused by salinization have a considerable impact on farm and irrigation system economics (Zinck and Metternicht, 2008). The cost of salinity impact to agriculture is globally estimated be about $US 12 billion per year and profitability loss is expected to increase as soils are continually affected (Ghassemi *et al.*, 1995; Pitman and Läuchli, 2002).

Climate change and sea level rise have recently emerged as serious challenges facing Vietnam's low-lying aquaculture and agricultural regions. Mekong Delta (MD) communities are located in one of the most globally vulnerable deltas, exposed to the combined effects of rising sea levels, salinity intrusion and an increased frequency of extreme climate events such as tropical storms (Smajgl et al., 2015). Sea level has been predicted to rise by up to 1 m by the end of the 21st century and would inundate 40% of the whole MD area. By 2100, sea level rise may cause inundation of 12 of the 13 provinces in the Mekong River Delta, affecting approximately 12,377 sq. km in this region (Ministry of Natural Resources and Environment (MONRE), 2009). Rising sea levels are likely to infiltrate groundwater aquifers and increase salinity gradients in large parts of the Mekong Delta, in particular during the dry-season months of October to May (Carew-Reid, 2007). During this season, flows from the upper catchment drop significantly, enabling salt water to intrude into half of the delta, 50 km up the main channel (Truong et al., 2011). This is significant as the Mekong Delta is an important rice production region and is crucial to Vietnam´s food security. With about 1.8 million ha of rice production land, the Mekong Delta annually provides approximately 23 million tonnes of rice for both domestic consumption and export (Nhan et al., 2011). However, increasing salinity levels in the MD have already substantially reduced agricultural productivity and caused declining rice production (Wassmann et al., 2004; Le Dang et al., 2014; Smajgl et al., 2015). Changes in environmental conditions, such as reduced flows, severe storms, and saline water intrusion, would threaten coastal regions and lead to an adjustment of agricultural systems.

1.2 Literature review

1.2.1 Salt affected soils

Global distribution of salt-affected soils

Soil salinity and alkalinity problems occur in many regions of the world, with over 900 million hectares of land suffering from salinization and alkalinisation (Table 1.1) (Zinck and Metternicht, 2008; Carrow and Duncan, 2012). Causes of soil salinization include primary or natural salinization that naturally occurs or secondary salinization which is a result of human activities (Duncan et al., 2009).

Table 1.1 Global distribution of salt-affected soils (million ha)

Area	Saline soil	Sodic soil	Total	Total (%)
Australasia	17.6	340.0	357.6	38.4
Asia	194.7	121.9	316.5	33.9
America	77.6	69.3	146.9	15.8
Africa	53.5	26.9	80.4	8.6
Europe	7.8	22.9	30.8	3.3
World	**351.2**	**581.0**	**932.2**	**100.0**

Source: Adapted from Carrow and Duncan (2012); and Zinck and Metternicht (2008)

Primary salinization is associated with the accumulation of salts in the soil over long periods from weathering of salt-laden parent materials (Pannell and Ewing, 2006). Intrusion of seawater into soils of coastal lands can result in salt accumulation (Rengasamy, 2010b). Salt movement into the root zone from a naturally high saline water table in coastal swamps or marshes also causes salinization (Carrow and Duncan, 2012). Secondary or human-induced salinization involves human activities such as irrigation and drainage practices. Poor practices of irrigation and drainage in areas with high evaporation rates are the main causes of secondary salinization (Lambers, 2003). Understanding the causes of secondary salinization is important to indicate preventative measures that can minimize adverse effects (Carrow and Duncan, 2012).

Classification and characteristics of salt-affected soils

Salt-affected soils are classified into three groups depending on the amounts and kinds of total soluble salts present (estimated by electrical conductivity), exchangeable sodium percentage (ESP) and soil pH (Table 1.2) (US Salinity Laboratory Staff, 1954). Saline soil is classified by electrical conductivity of the saturation extract (EC_e) > 4 dS m^{-1}, ESP < 15, and soil pH < 8.5. The soil is characterised by high concentrations of soluble

cations (sodium, calcium, and magnesium) and anions (chloride, sulphate, carbonate, and bicarbonate) in a soil solution (Rengasamy, 2010b). High salt concentration in the soil solution causes low osmotic potential, ion toxicity or ion imbalance leading to adverse effects on soil biota and crop growth (Marschner, 2012).

Table 1.2 Generalized classification of salt-affected soils

Soil class	**Criteria**		
	EC_e (dS m^{-1}) (*)	ESP (%) (**)	pH
Non-saline soil	< 4	< 15	< 8.5
Saline soil	> 4	< 15	< 8.5
Sodic (alkali) soil	< 4	> 15	> 8.5
Saline-sodic soil	> 4	> 15	> 8.5

Note: (*) Electrical conductivity of the saturation extract; (**) Exchangeable sodium percentage
Source: Adapted from US Salinity Laboratory Staff (1954)

A sodic soil is characterized by a high proportion of exchangeable sodium (ESP > 15%) on the CEC (Cation Exchange Capacity), but relatively low total soluble salt levels. Accumulation of Na on CEC sites and in Na carbonates in the sodic soil causes soil degradation owing to a loss of structure. Degradation of soil physical properties occurs with displacement of Ca and Mg by Na ions on the negatively charged CEC sites of clay colloids (Carrow and Duncan, 2012). A saline-sodic soil exhibits both high salt levels (EC_e > 4 dS m^{-1}) and high exchangeable sodium (ESP > 15%). All problems presented by saline soil can occur in saline-sodic soil because both contain high amounts of total soluble salt.

1.2.2 Effects of salinity on soil nitrogen cycling

Nitrogen cycling is the sequence of chemical and biological processes in which nitrogen atoms move from the atmosphere into plant, soil, water and other living organisms and are transformed from one form to another. In soil, the transformation of nitrogen form can alter or limit the availability of the nitrogen source to both crop and soil microorganisms. Elevated salinity in soils changes a number of soil processes associated with the soil nitrogen cycle including volatilization, mineralization, nitrification and ammonification. The gaseous loss of ammonia increases with salinity and more than 30% of added N is lost at high salinity levels (EC_e > 45 dS m^{-1}) while salinity and pH correlates negatively with the N mineralization and positively with the gaseous losses of NH_3 (Gandhi and Paliwal, 1976). Similarly, McClung and Frankenberger (1985b) found that increasing salinity promoted the amount of N lost through NH_3 volatilization. A decrease in N

mineralization was found under saline conditions and at higher moisture regimes (Lodhi et al., 2009). The study also concluded that salinity retards the nitrification process resulting in negative effects on the normal N transformation in soil. Akhtar et al. (2012) also found that increased salinity levels have adversely effects on the nitrification process. However, Laura (1977) revealed that the effects of salinity on nitrification depend on the degree of salinity and type of amendment. Elevation of salinity impacts on soil microbe activities in the nitrification process and this leads to a reduction in the conversion of ammonium to nitrate (Irshad *et al.*, 2005; Kumar *et al.*, 2007). Activity and growth of N_2 fixing bacteria declines under salt stress (Zahran, 1999). A better understanding of the soil nitrogen dynamics associated with soil-induced gas emissions under salinity impacts would result in better approaches to the management of nitrogen cycling to maintain soil fertility and plant productivity. The aim of this study is to contribute to this understanding.

1.2.3 Salinity, soil microbial activity and denitrification

Salinity impacts soil microbial activity mainly by lowering osmotic potential. Microbe tolerance to osmotic potential varies between species. Some adapt to the low osmotic potential while others are highly sensitive and die. This change in salinity, therefore, alters the community, functional diversity and activity of soil microorganisms (Pankhurst et al., 2001). High salt concentration usually reduces the efficiency of microbes in utilizing carbon (Oren, 1999; Rietz and Haynes, 2003; Wichern et al., 2006).

Salinity alters the structure of the soil microbial community due to differences in tolerance of soil microbial genotypes to osmotic stress (Nelson and Mele, 2007; Chowdhury *et al.*, 2011b; Baumann and Marschner, 2013). This has impacts on soil nutrient cycling because of the reduced ability of most bacteria to decompose the complex molecules of organic matter (Sardinha et al., 2003; Chowdhury et al., 2011b). The fluctuating salinity changes the osmotic potential and may impact on the activity and growth of soil microbes (Wichern et al., 2006; Setia et al., 2010; Chowdhury et al., 2011a). The size of the microbial biomass was reported as not affected by soil salinity (Sarig and Steinberger, 1994; Wong et al., 2008) whereas many other studies found that salinity depresses the microbial biomass (Laura, 1974; Pathak and Rao, 1998; Rietz and Haynes, 2003; Elgharably and Marschner, 2011). Recently, Morrissey et al. (2014) found that salinity is positively related to bacterial abundance and tightly linked with community composition. These inconsistencies could be due to soil type, salinity level, and water content and indicates a need for a more mechanistic understanding of how salinity affects soil microbial activity and nutrient cycling.

Denitrification is a key process regulating N cycling in natural environments. This process allows nitrates to be reduced to nitrogen gas by facultative anaerobic bacteria due to the

combination of low O_2 availability and high organic C content (Knowles, 1982; White and Reddy, 1999; Valiela et al., 2000). Denitrifiers are present in almost all soils and come from a wide range of microorganisms, including *Pseudomonas spp.*, *Alcalignes spp.*, *Flavobacterium spp.*, *Paracoccus spp.*, and *Bacillus spp.* The bacterial denitrification process consists of four reactions catalysed by nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos) (Hayatsu et al., 2008). Many prior studies have reported that carbon content, O_2 concentration in wetland soils and nitrate supply become the limiting factors for denitrification (Cooper, 1990; White and Reddy, 1999). Overall increase or variation of in situ denitrifying activity in soils has been associated with an increase in nitrate concentration (Thompson *et al.*, 1995; Gardner and White, 2010). Mineralization of available C has been positively correlated with denitrification (Reddy et al., 1982) while denitrification rates in terrestrial soils increase with temperature (Knowles, 1982). Wang et al. (2007) also concluded that oxygen availability, organic matter, nitrate supply, and temperature have the most significant influence over biological denitrification in wetland sediments.

The effects of salinity on denitrification have been highlighted in a number of previous studies (Antheunisse et al., 2007; Seo et al., 2008; Wu et al., 2008; Marks et al., 2016). Elevated salinity has been shown to decrease denitrification activity (Seo et al., 2008). In mangrove microcosms inundated with wastewater, a high salinity treatment resulted in a reduction of potential denitrification (Wu et al., 2008). However, the results reported by Antheunisse et al. (2007) showed no significant correlation between the reintroduction of salt water to semi-natural and agricultural soils on denitrification enzyme activity or potential denitrification. Intermediate salinity water has stimulated denitrification rates in fresh marsh soil by 75%, while higher salinity seawater (35 ppt) suppressed potential denitrification by 73%. This indicated the sensitivity of the denitrifying microbial communities to rapid shifts in salinity (Marks et al., 2016). However, little is known about the salinity effects on denitrifiers from salt-affected soils and the earlier mixed results point to a need for further investigation into the influence of salinity on denitrification in wetland soils addressing denitrifying genes and soil-induced GHG emissions.

1.2.4 Salinity effects on soil carbon cycling

Carbon dynamics in salt-affected soils may raise more concerns in the future because of the extent of salinization and sodicification globally. The properties of saline soils alter biochemical processes which impact the soil microbial biomass and microbial activity, changing CO_2 fluxes and the nature and delivery of nutrients to vegetation (Wong et al., 2010). Several studies have concluded that the reduction of CO_2 emissions (Laura, 1974; Pathak and Rao, 1998; Setia *et al.*, 2010) with increasing salinity is due to the decreasing osmotic potential. Other studies have shown that an increase in soil respiration with

salinity is due to a combination of high salinity and sodicity that increase carbon availability (Chandra *et al.*, 2002; Wong *et al.*, 2008). High pH caused by Na hydrolysis in saline soil may increase the solubility of organic matter and promote an organic C loss (Pathak and Rao, 1998). Recently, Setia *et al.* (2011a) found that salinity has a pronounced negative effect on soil organic matter decomposition, irrespective of soil texture. The contradictory results of these studies might be explained by the differences in soil type, water content and microbial community structure. Therefore, further studies are needed to investigate to better understanding the salinity impacts on soil carbon processes.

1.2.5 Salinity stress and plant/crop growth

A large part of the world's agricultural land is impacted by salinity and this forces serious limitations on crop growth and productivity (Tanji, 2002; Guo et al., 2013) and consequently on N use efficiency (Fageria, 2013). Läuchli and Grattan (2011) investigated the principal mechanisms and crop responses to salinity and sodicity stress (Figure 1.1). Salinity depresses the external water potential (osmotic effect), and the predominant ions in the solution may have chemical or specific-ion effects.

Firstly, a reduction in the osmotic potential of the medium is one of the primary causes of the adverse effects of salinity on plant growth (Maas and Nieman, 1978). At high salinities which give rise to an increase of solute concentration in the root zone, the external osmotic potential may be depressed below that of the cell water potential (Läuchli and Grattan, 2011; Yadav *et al.*, 2011). The osmotic effect of salinity is an important factor in reducing the plant water uptake and yield to uneconomical levels under dry land conditions when the soil solution osmotic pressure is below 1000 kPa (Rengasamy, 2010b).

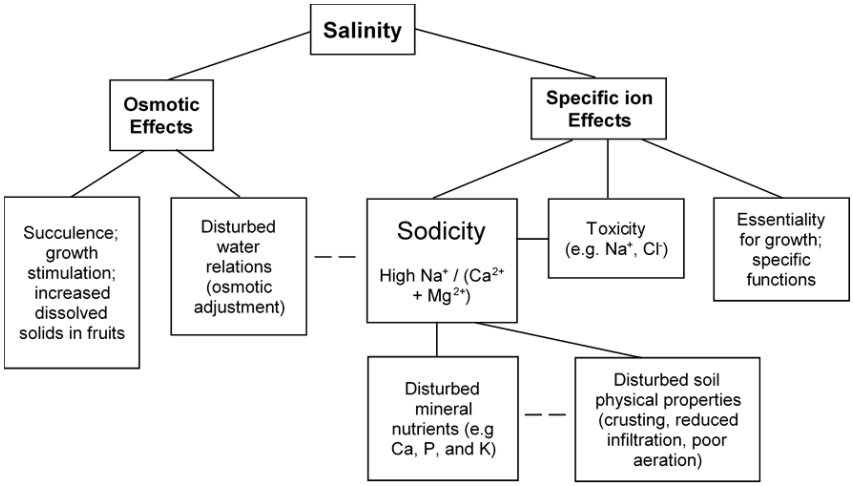

Figure 1.1 Effects of salinity and sodicity on plant growth.

Source: Adapted from Läuchli and Grattan (2011)

Secondly, specific ion effects cause ion toxicity (Na^+ and $Cl^{-)}$ and nutrient deficiency (N, P, Ca^{2+} and K^+) in a plant that lead to a negative impact on plant metabolism (Munns and Tester, 2008; Marschner, 2012). Ion competition between Na^+ and NH_4^+ and/or Cl^- and NO_3^- causes a reduction of N uptake in a saline soil (Fisarakis et al., 2001). A combination of reduced nitrate uptake and low osmotic potential can exhibit inhibitory effects on plant photosynthesis (Yadav et al., 2011). Relative crop yield often exhibits a linear decrease after a threshold of salinity has been reached shown in Figure 1.2. The relative yield, therefore, varies greatly depending on the salinity levels and the degree of tolerance (Hasanuzzaman et al., 2013). However, there has been little discussion on effects of salinity on nitrogen recovery and rice growth in the field soil-plant system.

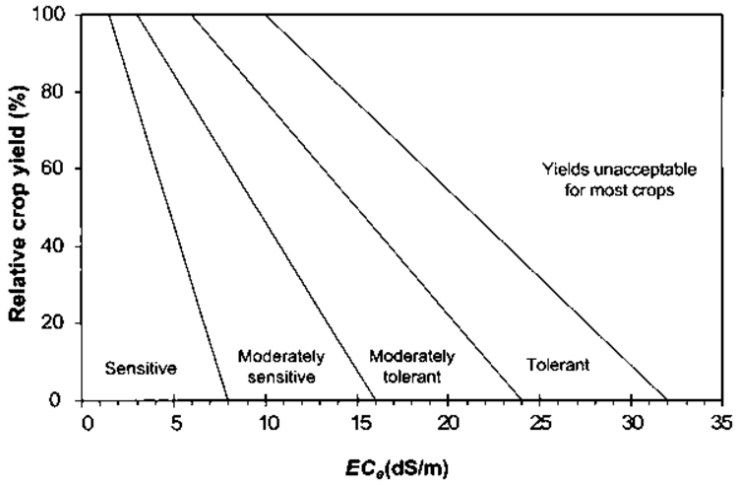

Figure 1.2 Relative crop yield in response to various salinity levels and degree of salt tolerance.

Source: Adapted from Hasanuzzaman *et al.* (2013)

1.2.6 Greenhouse gas emissions induced by agricultural soils

Global warming influenced by greenhouse gases (GHGs) has become a worldwide concern. Current GHG emissions are contributed by anthropogenic activities including land use and land use change in agricultural land forest systems, industrial development, urban expansion, and other sources (IPCC, 2007b). Agricultural activities are the major contributors to GHGs and emit 58% of total anthropogenic emissions of N_2O and 47% of CH_4 (US-EPA, 2006b; Smith *et al.*, 2007). Both N_2O and CH_4 have 298 and 25 times, respectively, more global warming potential (GWP) as compared to CO_2 (IPCC, 2007a). Future population increase positively relates to increased emissions from agricultural activities in most countries (van Beek et al., 2010). Net emission of CO_2 is small through agricultural cropping systems in comparison to its total cycling in agriculture and is mainly due to energy use on-farm and in the manufacture and transport of agricultural products (Snyder et al., 2009). Methane is mostly released from rice cultivation and ruminant

livestock while N_2O production results from agriculture linked to soil management and fertilizer use through two biochemical processes: soil nitrification and denitrification. Although the processes of GHG production and emission are controlled by biological factors, soil physical conditions also influence biology by their effect on the physical environment (Gregorich et al., 2006). Understanding the mechanisms of GHG emissions and developing technologies and practices to mitigate their effects are crucial strategies for sustainable and productive crop systems.

Salinity is suggested as one of the soil factors to influence gas emissions from soil by affecting soil microbial activity and processes. Soil nitrous oxide (N_2O) and carbon dioxide (CO_2) driven by denitrification and metabolism may be significantly affected by salt concentrations leading to lower emissions (Setia et al., 2011b). However, cumulative CO_2 emission from soils did not differ significantly due to the complex interactions of salinity and sodicity while saline-sodic soils can be a significant contributor of N_2O emissions (Ghosh et al., 2017). Salinity negatively affects to CH_4 emissions by influencing methanogenesis (Pattnaik et al., 2000). However, the addition of NaCl to alluvial soil caused an increase in CH_4 production relative to the control (Ramakrishnan et al., 1998) while CH_4 emission did not differ significantly between the inside saline patch and outside saline patch (Supparattanapan et al., 2009). Mechanism of CH_4 emission in saline condition is not clearly understood. In addition, contradicting results on GHG emissions may be due to differences in carbon substrate, soil chemical properties at various soil types. There certainly is necessary of investigating effects of different salinity levels on GHG emissions of different soil types and amendments.

1.2.7 Laboratory incubation and lid closure time for assessing GHG emissions from soil

Laboratory incubation has been suggested for good estimates of greenhouse gas emissions under controlled conditions. Schaufler et al. (2010) reported that the comparison of GHG flux of land-use types is difficult to address because of climate factor variation and to overcome these interactions, the incubation of soil cores in the laboratory is a potential approach. To derive the effect of a single parameter on gas emissions from field measurements is difficult because spatial and temporal parameters such as climate, N and C deposition, litterfall and nitrogen availability often co-vary or interact (Davidson *et al.*, 2000; Pilegaard *et al.*, 2006). Laboratory incubation provides the best and least biased basis for estimating the temperature dependence of organic matter decomposition which can be applied to the measurement of other GHGs (Kirschbaum, 2006).

Different gas sampling times have been used in many incubation studies in which gas samples were collected after 30 minutes (Singh et al. 2010; Inselsbacher et al. 2011), 1

h (Dobbie and Smith 2001; Velthof et al. 2002; Schaufler et al. 2010), 2 h (Wang et al. 2011), 3 h (Nguyen et al. 2014a), 4 h (Dobbie and Smith 2001), 6 h (Tenuta and Sparling 2011) or 24 h (Lang et al., 2011) after closing the incubation jars or chambers. Nguyen et al. (2014b) indicated that the concentration of GHGs is saturated if the closure time for incubation is longer than 3 h. This leads to a reduction in oxygen in the headspace volume of the incubated jars and limits microbial activity. In addition, Beauchamp et al. (2007) suggested that incubation time for denitrification experiments should be restricted to 5 h, especially in the denitrification potential assay, as nonlinear emission rates occur when new enzymes are produced. In this particular case, a shorter sampling timeline is suitable. Thus, research on the effects of closure time is essential for those looking particularly at denitrification and nitrification rates from an incubation experiment.

1.2.8 Current research on salinity effects and soil-induced gas emissions in the Mekong Delta

Current research on climate change and salinity effects

The Mekong Delta (MD) in Vietnam has been identified as one of the most vulnerable areas to the potential impacts of global climate change (Nijssen *et al.*, 2001; Hoanh *et al.*, 2003; IPCC, 2007a). Changes in the Mekong River flow and sea level rise are identified as the two most disruptive factors impacting on agricultural production (Khang *et al.*, 2008). Dun (2012) explored how the changing environmental conditions have impacted on agricultural change and the increasing salinization has been linked to the switch to shrimp aquaculture in the MD. Using a model to simulate flow and salinity intrusion in the MD, Khang *et al.* (2008) indicated that an area of triple rice crop will be reduced by 72,000 ha while the area yielding only single crops will increase by 180 thousand ha by the mid-2090s. The high floods in the future will cause deep inundation and severe damage to infrastructure and production in the delta. In addition, salinity intrusion results in not only a deficit of fresh water flows to the estuaries, but also causes problems for production and human health (Tuan et al., 2007). The CLUES project (Climate Change affecting Land Use in the Mekong Delta: Adaptation of Rice-based Cropping Systems) was implemented from 2011 to 2015 to increase the capacity of rice production systems in the MD. This project was aimed to provide to farmers and management agencies the technologies and knowledge to adapt to climate change and improve food security in the Mekong Delta. Findings of this project showed that severe impacts of climate change on rice production are increasing not only by the increase of saltwater intrusion and flood inundation but also by the shift in the rainfall regime (Hien et al., 2016). The results from the CLUES project also indicated that in the rice-shrimp farming system, a short-duration rice variety could give higher yield and help famers avoid salinity stress on their crop at the end of a season (Hoa et al., 2016). Although

there have been a number of studies on the effects of climate change and saltwater intrusion in the MD, little information is known about the salinity effects on soil nitrogen and carbon cycling in agricultural production. This present study aims to fill that gap.

Current knowledge about greenhouse gas emissions

Vietnam has participated in and implemented the Kyoto Protocol under the United Nations Framework Convention on Climate Change. To proactively respond to climate change, Vietnam approved the National Target Program to Respond to Climate Change since 2008. The objectives of this program are to assess climate change impacts on sectors and regions in specific periods and to develop feasible action plans to effectively respond to climate change in the short-term and long-term (Phung et al., 2016). One of the National Target Program objectives is to assess greenhouse emissions in agricultural systems. However, the majority of emission factors used are default values accepted from the Revised 1996 IPCC Guidelines. There have been few studies directly measuring greenhouse gas emissions (Arai et al., 2015; Izumi et al., 2016). For example, straw used as mushroom beds for straw-mushroom cultivation exhibited lower GHG emissions than straw burning (Arai et al., 2015). Improved management of livestock manure through installation of domestic biogas digesters can reduce GHG emissions (Izumi et al., 2016). Reliable and synchronous data on the parameters of GHG emissions are sparse, and the data collection process is slow. Moreover, the data collection system for greenhouse gas inventory is incomplete and there is a shortage of technical experts (Phung et al., 2016). The CLUES project (2011 – 2015) was a pioneer project to collect baseline data on GHG emissions from paddy rice in the Mekong Delta. This contemporary baseline data will be used as input data to compare GHG mitigation methods. However, the development of standardized protocols for measurement of GHG emissions in the MD is critical to ensure further studies in both laboratory and field ecosystems are comparable.

1.3 Study rationale and thesis outline

The literature review indicates that sea level rise will adversely affect crop production systems in tropical mega deltas around the world. The mega-deltas in Vietnam (the Mekong Delta), Myanmar (Irrawaddy) and Bangladesh (Ganges–Brahmaputra), the backbone of the rice economy in their respective countries, will experience specific climate change impacts due to sea level rise (Wassmann *et al.*, 2009b). In particular, sub-lethal salinity levels which will cause reductions in crop production are likely to change soil carbon and nitrogen cycling leading to increased losses.

Due to limited resources in developing countries like Vietnam, Myanmar or Bangladesh, it is crucial to develop incubation techniques that allow rapid, robust scientific and

economic assessment of carbon and nitrogen cycle changes. Currently there are many incubation methods being applied to assess respiration and denitrification on untested soil types. This leads to the **first research question in Chapter 2**, a methodological question: how do lid closure time, gas sampling interval and activation of a soil incubation influence greenhouse gas emissions? This information is needed to design future research on management of carbon and nitrogen cycling in farming systems.

Alluvial and acid sulphate soils are common production soils in Australia and Vietnam. Acid sulfate soils are commonly distributed in low-lying areas and are vulnerable to sea-level rise (Bush et al., 2010). The two soil types will respond differently to salinity effects. Using rapid assessment of laboratory incubation technique (developed in the methodological Chapter 2), an assessment of the effect of salinity on these soil types was undertaken. This allows the rapid quantification of denitrification and respiration changes within the soils. The information from this study will answer the **second research question in Chapter 3**: does elevated soil salinity change greenhouse gas emission from soils?

Principle roles of soil microbes in soil carbon and nitrogen cycling have been addressed. However, the effects of salinity on the genetic make-up of the soil bacteria driving carbon and nitrogen cycling in soil are poorly understood. Applying the incubation method and suggested sampling interval in Chapter 2, and research findings from the Chapter 3, the **third research question in Chapter 4** is: how do different salinity levels change the abundance of denitrifier genes in acid sulphate soil? The information generated by this study will clarify the relationship between denitrifying gene abundance and greenhouse gas emissions under the salt-affected soil environment. The finding contributes to our current understanding of the biological pathway of soil gas emissions and this contribution is needed to identify the most effective mitigation approaches.

The Mekong Delta elevation is only slightly (< 2 m) above mean sea level and more than 2.7 million ha of land are at present affected by tidal flooding and salt water intrusion (Wassmann et al., 2004). Typical rice production soils in the Mekong Delta are alluvial and acid sulphate soils. Future sea-level rise will adversely affect not only rice production systems in this region, but also differentiate soil properties due to the responses of these soils to salinity. Using the findings from the incubation studies, a further greenhouse study was performed to address the **fourth research question in Chapter 5**: how does salinity alter soil properties and rice nutrient efficiency in the field soil-plant system? The information generated by this study is crucial for designing and managing current and future farming systems in the Mekong Delta and other similar tropical deltas worldwide.

1.4 Research questions and objectives of study

In summary, this study addressed the following research questions:

1. How does incubation lid closure time affect the emission of GHGs?
2. Does elevated soil salinity change greenhouse gas emission from soils?
3. Does salinity alter abundance of denitrifying genes of a salt-affected soil?
4. How does salinity alter soil properties and rice nutrient efficiency in the field soil-plant system?

The correspondent overall objective of this study was to understand changes of soil carbon and nitrogen cycling associated with greenhouse gas emissions, plant growth and fertilizer recovery under effects of different salinity levels. To obtain the overall objective, the present study attempted to achieve following specific objectives:

- To analyse greenhouse gas production from different soils with different times of lid closure and to assess the effects of different activation times on gas emissions from soils (Chapter 2).
- To investigate carbon and nitrogen release from soils under the effects of saltwater submergence (Chapter 3).
- To identify a relationship between induced-soil gas emissions and abundance of denitrification genes in a salt-affected soil (Chapter 4).
- To investigate changes of soil properties, the N dynamic and the collective effect on rice growth and yield under various salinity levels by using a ^{15}N label fertilizer technique (Chapter 5).

Reviewing these objectives, it is clear that the outputs of this thesis can make a significant contribution to the design and management of current and future farming systems in the Mekong Delta. It is clear also from these objectives that they are applicable to many other similar tropical deltas worldwide, particularly given the underlying threat of sea level rise that is common to all low-lying deltas.

Chapter 2: DIFFERENT LID CLOSURE TIMES ALTER FLUXES OF GREENHOUSE GASES FROM INCUBATED SOILS

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Authorship statement

Manuscript title: Fluxes of greenhouse gases from incubated soils using different lid-closure times.

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I implemented the experiment, analysed soil and gas samples, interpreted and analysed data, wrote the manuscript and acted as the corresponding author. I hereby certify that the statement of contribution to the manuscript is accurate.

Signature: _____ Date: _____

Ian White
I evaluated the manuscript, provided comments and corrections. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Sören Warneke
I provided comments and suggestions the manuscript. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Ben Macdonald
I supervised development of work, evaluated the manuscript, provided comments and corrections. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signature: _____ Date: _____

Chapter 2: DIFFERENT LID CLOSURE TIMES ALTER FLUXES OF GREENHOUSE GASES FROM INCUBATED SOILS

Abstract

Different sampling times for greenhouse gas measurements have been proposed in many incubation studies. Little has been known about effects of closure time on denitrification and nitrification rates from incubation experiments. The objectives of the present study were to analyse greenhouse gas production from different soils with different times of lid closure and to assess effects of different activation time on gas emissions from soils. To quantify greenhouse gas emissions from three soil types, 40g of air-dried soil samples (0-10 cm) were incubated in a 125-ml jar at 25^{o}C with the addition of glucose and nitrate. The first experiment aimed to measure greenhouse gas fluxes at different lid-closure time (40, 80, 120 and 1440 minutes). The second experiment was to assess the effects of soil activation (40, 80, 120 and 1440 minutes) on gas emissions. Our findings showed closure time <1 hour or >2 hours may cause an underestimation of greenhouse gas emissions. The 20-min sampling interval at the closure time of maximum 80-minute produced good results that showed less variance for either soil types or monitored gases. Lengthening activation times may result in different emission rates in line with soil characteristics although deployment time of headspace gas samples was the same. To measure gas fluxes based on a linear regression model, we suggest that 4 or 5 sampling points should be taken, with sampling at 20-minute intervals over a maximum period of 80 minutes for estimating gas fluxes from soil. Because activation time for incubated soils is critical and a driving factor in the measurement of soil-induced gas emissions, a standardized procedure to quantify gas fluxes is needed for incubation studies.

Keywords: lid closure, greenhouse gas emissions, incubation, amendment, and nitrogen cycle.

2.1 Introduction

The most important greenhouse gases contributing to global warming from the biosphere are carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). Soils are a major terrestrial source of the greenhouse gases to the atmosphere (Schaufler et al., 2010). The measurement of greenhouse gas emissions (GHGs) from agricultural soils is currently a “hot topic” because agricultural activities release 10–12% of total global anthropogenic emissions of greenhouse gases (US-EPA, 2006b; Smith *et al.*, 2007) and

the earth's atmosphere is warming. Both laboratory and field studies have been conducted to quantify the rates of processes producing GHGs from agricultural soils and to assess mitigation strategies.

A number of approaches and strategies exist for the field measurement of greenhouse gas emissions each having its own strengths and weaknesses (Collier et al., 2014). Mass balance techniques rely on wind-based dispersion of gases and are used to measure fluxes from small, well-defined sources (Denmead et al., 1998). Micrometeorological approaches based on real-time direct measurement of vertical gas fluxes can provide direct measurements over large areas (Smith et al., 1994). However, the need for costly infrastructure can limit the deployment possibilities of this method. Chamber-based methods focus on change in gas concentration at the soil surface by sampling from a restricted above-ground headspace and obtain measurements from small areas and numerous treatments (Davidson et al., 2008; Phillips et al., 2009; Collier et al., 2014). However, this method can be labour-intensive and time-consuming. Savage et al. (2014) recently deployed an automated soil respiration system with a newly-available quantum cascade laser to measure simultaneously the three most important greenhouse gases from soils.

A laboratory incubation approach can access one or more expected factors to address the research questions and meet the objectives. For example, greenhouse gas emissions can be investigated by running an incubation study covering a wide spectrum of temperature, soils and moisture conditions (Schaufler et al., 2010). In addition, researchers can implement and monitor a greater quantity of samples, treatments and/or replicates. With the advantage of homogenized samples (Bandibas et al., 1994), variations in the results can be minimised and the cost to run laboratory incubation is lower than a measurement in the field. Laboratory incubations providing valuable information on the production of GHGs (Nguyen et al., 2014b) have been used extensively, but a leading question is what is the effect of lid closure time on the GHG production.

Many incubation studies have used different gas sampling times to collect gas samples after closing the incubation jars or chambers (Velthof et al., 2002; Singh et al. 2010; Inselsbacher et al. 2011; Lang et al. 2011; Wang et al. 2011). Nguyen et al. (2014b) indicated that the concentration of GHGs is saturated if the closure time for incubation is longer than 3 h. This leads to a reduction in oxygen in the headspace volume of the incubated jars and limits microbial activity. Moreover, Beauchamp et al. (2007) suggested that incubation time for denitrification experiments should be restricted to 5 h, especially in the denitrification potential assay, as nonlinear emission rates occur when new enzymes are produced. In this particular case, a shorter sampling timeline is

suitable. Thus, study on the effects of closure time is essential for those looking particularly at denitrification and nitrification rates from an incubation experiment.

Pre-incubation, hereinafter named activation time, has been applied to settle and standardise the soil microbial community following disturbance of sampling and sieving (Creamer et al., 2014). Storage of soil samples is inevitable, and this causes an extra variation in the results. Hence, activation of soil samples is suggested before conducting an experiment (Bloem et al., 2006). The activation of re-wetted soils with substrates can also help to activate microbial activity, as microbes generally survive in a dormancy period under a dried condition (Mondini et al. 2006). However, there is much less information about the effects of activation time on soil-induced gas emissions.

In this study, we hypothesized that longer closure time would affect greenhouse gas emissions from soils. The objectives of the incubation study were: i) to analyse greenhouse gas production from different soils with different times of lid closure; and ii) to assess the effects of different activation times on gas emissions from soils. The findings from the present study will be used to discuss the preferable lid closure time for laboratory experiments and to evaluate whether data from studies using different lid closure times could be comparable.

2.2 Materials and methods

2.2.1 Sampling sites and soil collection

Description of sampling sites

Three soil types were used to represent a range of common production soils in Australia. An acid sulphate soil (ASS) managed as a pasture soil was collected from south coast Nowra, New South Wales, Australia (34°49'S, 150°39'E). The elevation of this area varies from 0.5 to 2.5 m above sea level; average annual rainfall is 1,135 mm. The soil collected was classified as a Hydrosol (Isbell, 2002) and the site has a dark loamy topsoil (Lawrie and Eldridge, 2004). The soil surface of the sampling site was covered by ryegrass. Soil pH through the 2 m soil profile ranged from 3.09 to 5.63, and < 4 within one meter below the soil surface. Total soil nitrogen was 0.60% and total carbon was 7.31%.

A pasture soil sample was collected from a site (36º1' S, 146°22' E) of unimproved pasture. The elevation of this site is 143 m above sea level; average annual rainfall is 541 mm. Collected soil was classified as a Red Dermosol (Isbell, 2002). Smith et al. (2001) reported that clay content of this soil was 290 mg kg^{-1} soil (~0.029%) in the surface 10 cm. Properties of the soil surface (10 cm) were soil pH (1:5 soil:water) of 4.79; total carbon of 1.70% and total nitrogen of 0.15%.

A soil sample on which cotton (*Gossypium hirsutum* L.) has been grown was collected from an experimental field at the Cotton Research Institute, Narrabri, New South Wales,

Australia (150ºE, 30ºS). The climate at this site is subtropical, with annual rainfall of 645 mm, but this is highly variable (420 – 870 mm). The site has been cultivated for cotton for almost 40 years. This soil type is classified as Vertosols (Isbell, 2002). Soil surface (30 cm) with 53% clay content, 22% each silt and sand content (Rochester, 2011). Soil surface pH was 7.06 while soil total N and organic carbon were 0.18% and 2.07%, respectively. The properties of the three soil types are summarized in Table 1.

Table 2.1 Basic characteristics of incubated soil samples (0 – 10 cm)

Soil	EC (dS m^{-1})	pH (1:5)	Total C (%)	Total N (%)	NO_3 – N (mg kg^{-1})	NH_4 – N (mg kg^{-1})
Acid sulphate soil	2.34	3.93	7.31	0.60	0.34	214.18
Red Dermosol soil	0.19	4.79	1.70	0.15	6.23	41.13
Vertosol soil	0.25	7.06	2.07	0.18	41.90	60.27

Soil collection

Surface soil samples (0-10 cm) from the three soil types were collected into plastic bags, stored in isolated containers and delivered to the laboratory within 5 hours. The samples were dried at 40ºC, sieved (<2mm) and mixed well before representative subsamples were collected and used for the incubation experiment.

2.2.2 Soil extraction and analysis

Soil pH and EC were measured in 1:5 soil:water extract after end-over-end shaking at 25°C in a closed system for 1 h (Rayment and Lyons, 2011). Soil ammonium and nitrate were extracted with 2 M KCl solution and determined following the method described by Keeney and Nelson (1982). Total carbon and nitrogen were analysed based on Dumas high-temperature combustion by using a Europa 20-20 isotope ratio mass spectrometer with an Automated Nitrogen Carbon Analysis (ANCA) preparation system. Nitrogen and carbon content were measured by a mass spectrometer for the N_2 and CO_2 peaks sequentially (Nelson and Sommers, 1996; Rutherford *et al.*, 2007; Rayment and Lyons, 2011).

2.2.3 Incubation experiment for greenhouse gas measurement

Two incubation experiments were undertaken to quantify greenhouse gas emissions from the three soil types. The two incubation experiments received the same pre-treatment. Forty grams (40g) of air-dried soil samples (0-10 cm) with moisture content ranged 2.31 – 2.57% were weighed in a 125 mL jar, with a solution (12 mL) of glucose (300 µg glucose-C g^{-1} soil) and nitrate (50 µg NO_3-N g^{-1} soil) added. This treatment was applied to ensure that denitrification was not limited by nitrogen or carbon supply (Luo et al., 1996). Incubation jars were mixed well and left open at 25ºC to activate microbial

activities for 24 hours before starting greenhouse gas emission measurement. The incubations were maintained at 25^{o}C at a constant temperature without any light source.

Experiment 1: to measure greenhouse gas fluxes in different lid-closure time

Incubated jars were closed for 40, 80, 120 minutes and 24 hours (1440 minutes) as study treatments. The experiment was conducted as a randomized complete block design with each treatment replicated three times. A sample of headspace gas (6 mL) was collected using a syringe at 0 min and another 4 times at each quarter of the total closed period. The headspace gas sample was injected into evacuated vials (3.75 mL) for storage and analysed within 24 hours. Helium (6 mL) was returned into each jar after each sample was collected.

Experiment 2: to assess the effects of soil activation on GHG emissions

The second jar set was tested to ascertain whether additional 'pre-incubation time' (activation time) influenced gas fluxes. The second jar set was also pre-incubated for 24 h at 25^{o}C. Then, these jars were left open for additional duration of 40, 80, 120 minutes, and 24 hours (1440 minutes) as activation treatments. The second experiment was also performed as a randomized complete block design with each treatment replicated three times. A sample of headspace gas (6 mL) was collected using a syringe at 0 min and then every 10-minute interval, making a total of five sampling times. Helium return was accomplished as for the first experiment.

Gas samples and standards were analysed for N_2O, CH_4, and CO_2 concentrations using a GC-2014 Shimazu gas chromatograph (Shimazu, Kyoto, Japan) equipped with an electron capture detector (ECD) to determine nitrous oxide emission from the incubated jars. A flame ionization detector was also connected to the gas chromatograph to detect other gases such as CH_4 and CO_2.

2.2.4 Data calculation and statistical analysis

Atmospheric pressure and temperature in the laboratory were recorded at each sampling point. As helium gas was returned after headspace sampling, volumetric gas concentration was re-corrected before it was converted to mass gas concentration, following equation 1:

$$C_{mass} = (C_{volumetric} \times P \times MW_{gas}) / (T \times R) \qquad (1)$$

where C_{mass} is mass concentration ($\mu g\ L^{-1}$), $C_{volumetric}$ is volumetric concentration (ppm), P is ambient air pressure (atm), MW_{gas} is molecular weight of the gas (g mole^{-1}), T is ambient air temperature (^{o}K), and R is the ideal gas constant (L atm K^{-1} mole^{-1}).

Emission data were calculated by fitting a linear regression model through at least three of the 5 sampling points, removing any outliers to achieve a minimum R^2 of 0.85 (Petersen et al., 2006; Gao et al., 2014). Slopes of the regression were used to estimate gas fluxes. The gas fluxes were converted to the gas emissions per gram soil basis following equation 2. The data for gas flux were based on oven-dried weight. The linear regression was only presented for N_2O fluxes (Appendix 1) and similar calculations were performed for fluxes of other gases and for fluxes of all gases in the second experiment (data not shown).

$$F = S * V * W_{soil}^{-1} \qquad (2)$$

where F is flux of greenhouse gases ($\mu g\ hr^{-1}\ g^{-1}$), S is slope of the regression ($\mu g\ L^{-1}\ hr^{-1}$), V is headspace volume (L), and W_{soil} is weight of soil used in an incubated jar (g).

Statistical analysis was conducted with R statistical software version 3.0.2 (The R Foundation, Vienna). Prior to the analysis, data were tested for homogeneity and normality and the results of data skewness tests were accepted. One-way ANOVA and Tukey's test were used to identify differences among closure time or activation treatments at $P<0.05$ and $n=3$.

2.3 Results

2.3.1 Effects of different lid closure time (Experiment 1) on GHG emissions

N_2O fluxes

Average of N_2O fluxes from the acid sulphate soil was less than 0.16 µg kg^{-1} soil $hour^{-1}$. Absorption of N_2O was found within 40 minutes which was significantly different from the emissions in other treatments (Figure 2.1*a*). Among the three soil types nitrous oxide fluxes from Red Dermosol soil were relatively high and varied from 5.92 to 6.74 µg kg^{-1} soil $hour^{-1}$. However, differences of closure time did not result in any significant difference in the emissions (Figure 2.1*b*). In the Vertosol soil, the closure time of 24 hours resulted in the least emissions (0.03 µg kg^{-1} soil $hour^{-1}$) compared to those at 40 and 80 minutes 0.37 µg kg^{-1} soil $hour^{-1}$. The nitrous oxide emission rate of the 120-minute closure treatment (0.17 µg kg^{-1} soil $hour^{-1}$) was not significantly different to those from the 40 and 80-minute closure treatments, nor from the 24-hour treatment (Figure 2.1*c*).

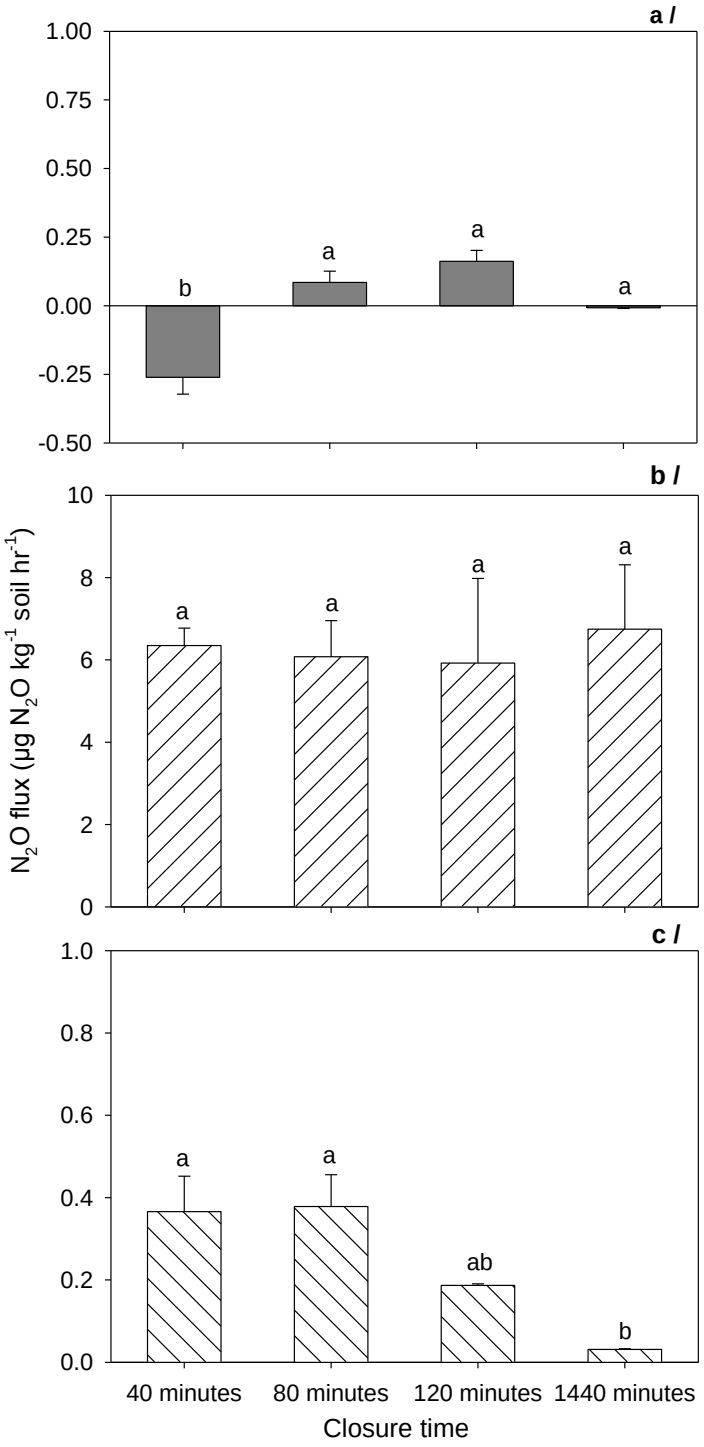

Figure 2.1 Fluxes of N_2O from acid sulphate (a), Red Dermosol (b) and Vertosol (c) soils for closure times 40, 80, 120 and 1440min. Error bars represent standard error and different letters on the bars show significant difference at $P < 0.05$, $n = 3$.

CO_2 fluxes

Acid sulphate soil showed similar CO_2 fluxes among the different closure treatments. The emissions were from 0.43 to 0.62 mg CO_2 kg^{-1} soil $hour^{-1}$ (Figure 2.2*a*). In the Vertosol soil, the CO_2 flux of the 40-minute closure treatment was 1.06 mg CO_2 kg^{-1} soil $hour^{-1}$, but this emission was not significantly different compared that of the 120-minute closure treatment. The emissions of the 80-minute and 24-hour closure treatments were significantly less than 40 minutes but not significantly different from each other (Figure 2.2*c*). The flux from the 80-minute treatment was 0.56 mg CO_2 kg^{-1} soil $hour^{-1}$, whereas release from the 24-hour closure treatment was 0.45 mg CO_2 kg^{-1} soil $hour^{-1}$. Carbon dioxide fluxes from the Red Dermosol soil ranged from 2.29 to 5.01 mg CO_2 kg^{-1} soil $hour^{-1}$ and there were no significant differences among the treatments with closure time >1 hour. However, absorption of CO_2 was recorded in the 40-minute closure treatment, at 3.51 mg kg^{-1} soil $hour^{-1}$ (Figure 2.2*b*).

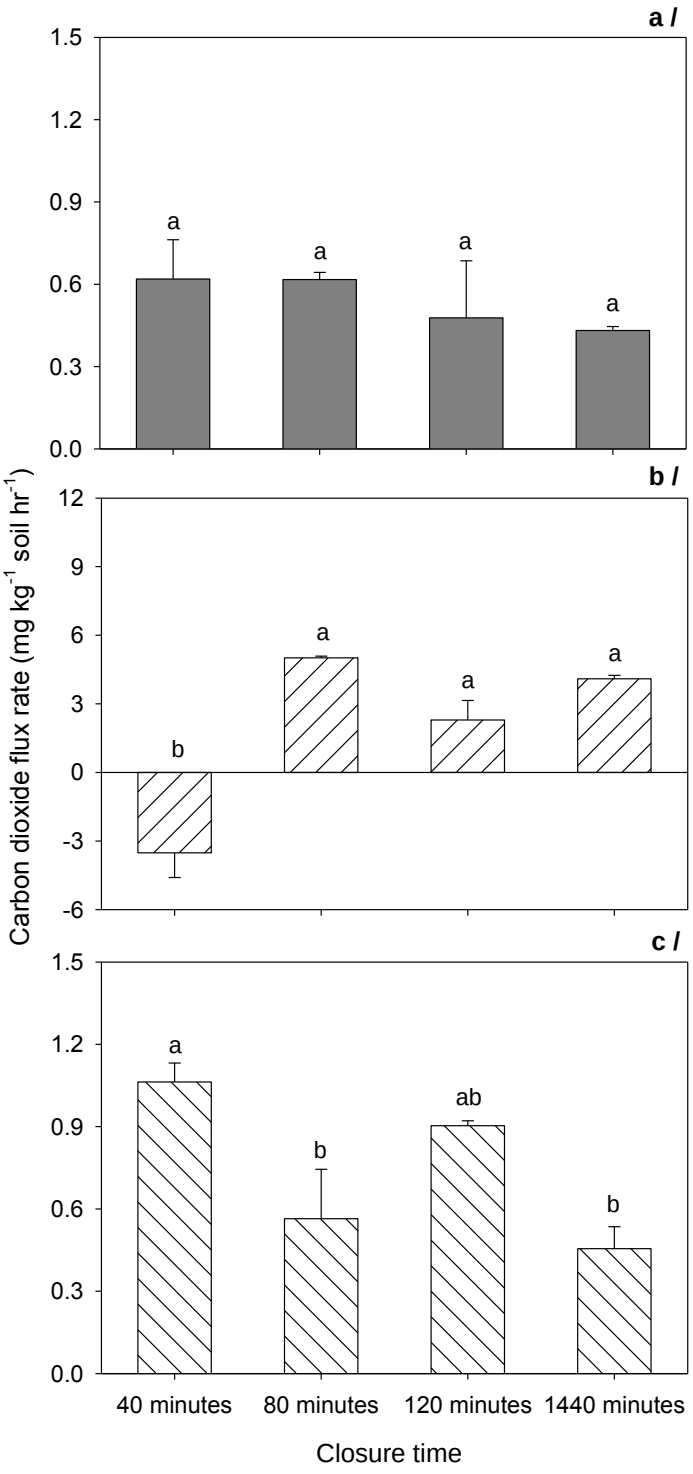

Figure 2.2 Fluxes of CO_2 measured from treatments for closure times 40, 80, 120 and 1440min in acid sulphate (a), Red Dermosol (b) and Vertosol (c) soils. Error bars represent standard error and different letters on the bars show significant difference at $P < 0.05$, n = 3.

CH_4 fluxes

Methane absorption occurred in most treatments of the three soils. In the acid sulphate soil, absorption was recorded in 3 treatments except for the 80-minute treatment. In the Red Dermosol soil, the greatest absorption was 13.15 µg kg^{-1} soil $hour^{-1}$ in the 40-minute closure (Figure 2.3*b*). The absorption in this treatment was significantly different with the 120-minute treatment, at 4.69 µg kg^{-1} soil $hour^{-1}$. Fluxes of CH_4 were not significantly different between the 80-minute and 24-hour closure treatments. Similar to the trend in the acid sulphate soil, results for CH_4 fluxes did not differ among the different closure treatments. However, in all soils, positive CH_4 fluxes were only found in the 80-minute closure treatment and average emissions from this treatment were: 1.02 µg kg^{-1} soil $hour^{-1}$ for the acid sulphate soil, 3.90 µg kg^{-1} soil $hour^{-1}$ for the Red Dermosol soil, and 0.60 µg kg^{-1} soil $hour^{-1}$ for the Vertosol soil (Figure 2.3).

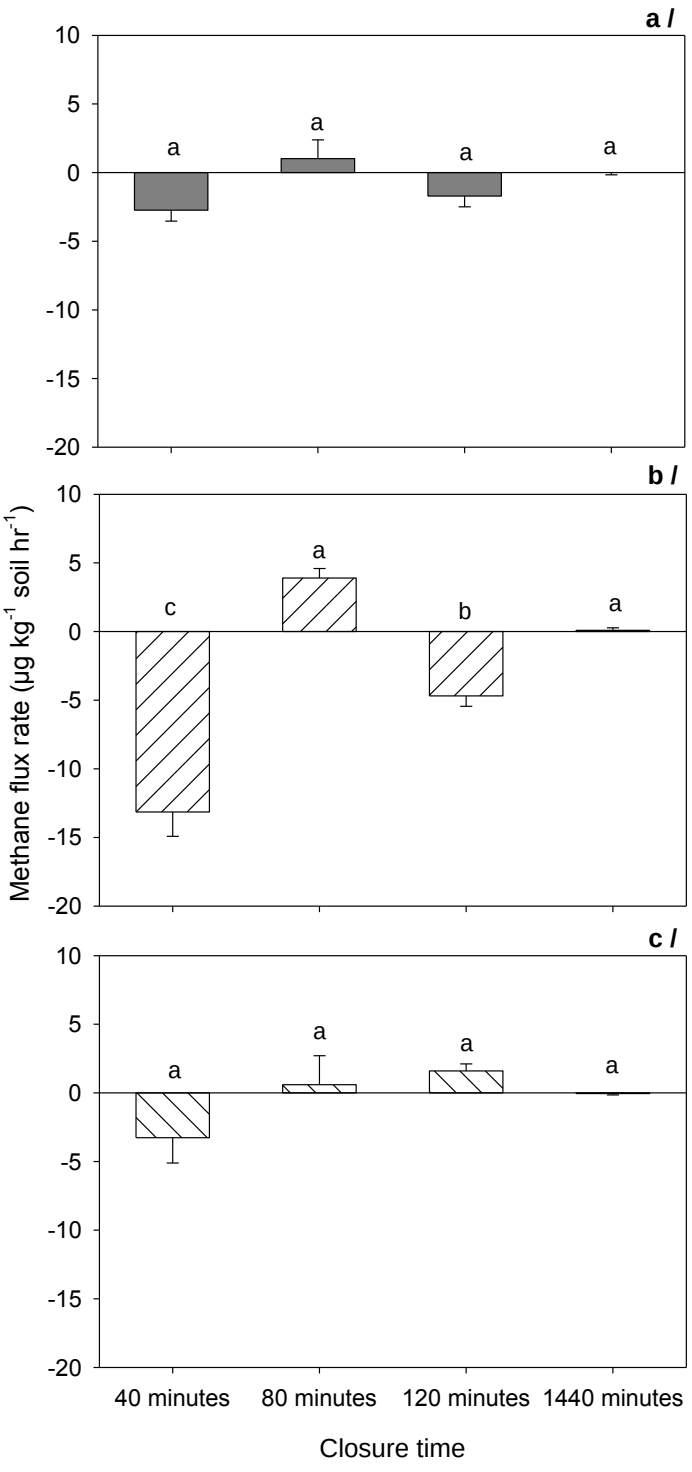

Figure 2.3 Fluxes of CH_4 in acid sulphate (a), Red Dermosol (b) and Vertosol (c) soils for closure times 40, 80, 120 and 1440 min. Error bars represent standard error and different letters on the bars show significant difference at $P < 0.05$, $n = 3$.

2.3.2 Effects of different activation time (Experiment 2) on fluxes of gas emissions

N_2O fluxes

Difference of soil N_2O production from different activation times was not significant in the acid sulphate soil (Figure 2.4a). The 1.3-hour activation of the acid sulphate soil had a positive flux at 0.32 µg kg^{-1} soil $hour^{-1}$ (Figure 2.4*a*) whereas the other treatments showed N_2O absorption. Conversely, the finding from the Vertosol soil showed that N_2O was absorbed only in the 1.3-hour treatment while the other treatments had positive emissions, ranged from 0.04 to 0.16 µg kg^{-1} soil $hour^{-1}$. Greater N_2O fluxes were recorded in the Red Dermosol soil (13.53–16.77 µg kg^{-1} soil $hour^{-1}$); in particular, the highest emission was recorded for the 24-hour activation (57.96 µg kg^{-1} soil $hour^{-1}$).

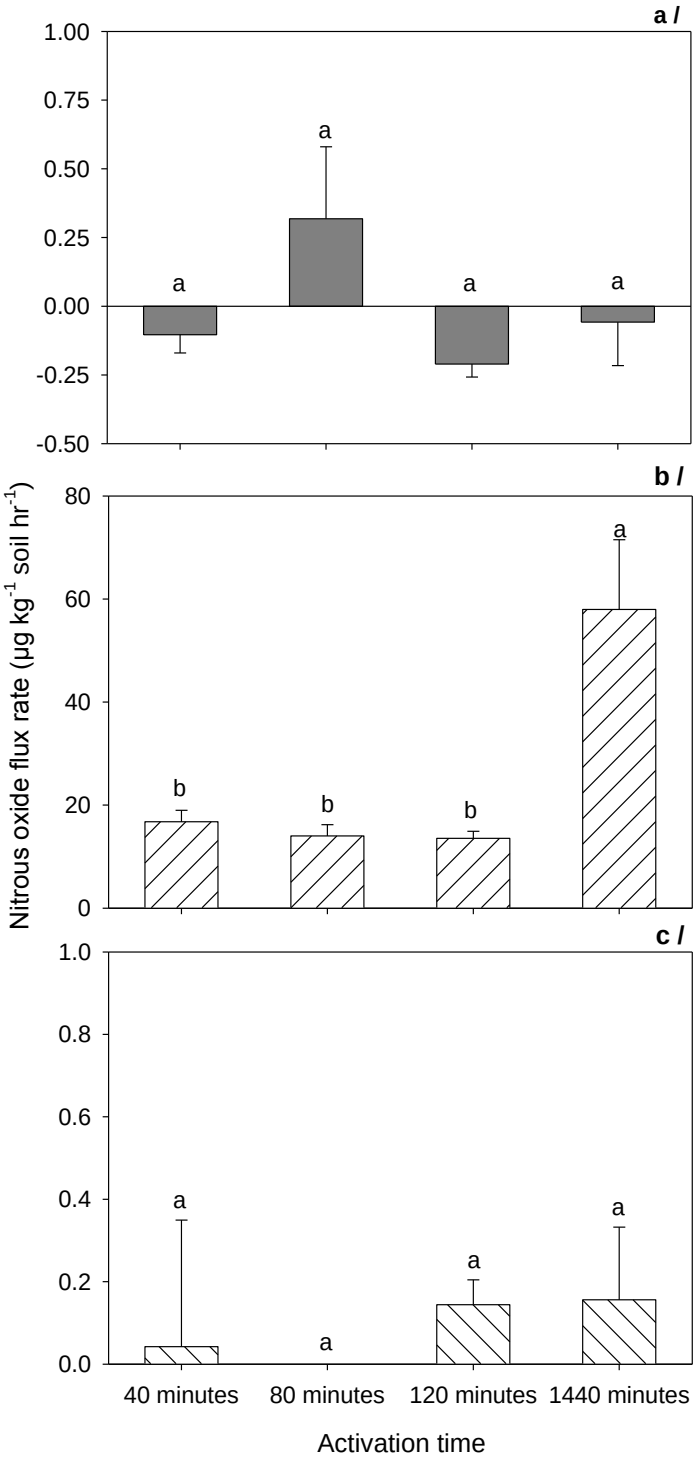

Figure 2.4 N_2O fluxes at 10-min intervals from different treatments of activation time (i.e. different durations of soil activation) with the addition of glucose and nitrate. Fluxes of N_2O from acid sulphate (a), Red Dermosol (b) and Vertosol (c) soils. Error bars represent standard errors and different letters on the bars show significant difference at $P < 0.05$, $n = 3$.

CO_2 fluxes

Different activation times did not result in significant differences in fluxes of CO_2 in the acid sulphate soil (Figure 2.5*a*). The fluxes from this soil ranged from 0.37 to 0.72 mg kg^{-1} soil $hour^{-1}$. Respiration from the Red Dermosol soil was greater than that from the other two soils (Figure 2.5). The treatment of 24-hour activation increased the emission of CO_2 to 9.55 mg kg^{-1} soil $hour^{-1}$, however the flux in this treatment was not significantly different from that in the 1.3-hour activation, at 6.46 mg kg^{-1} soil $hour^{-1}$. Fluxes of the other two treatments in Red Dermosol soil were ~4.70 mg kg^{-1} soil $hour^{-1}$ (Figure 2.5*b*). In the Vertosol soil, although CO_2 flux in the 24-hour activation was low (0.02 mg kg^{-1} soil $hour^{-1}$), it was not significantly different from the fluxes in the 0.7- and 1.3-hour activation (Figure 2.5*c*).

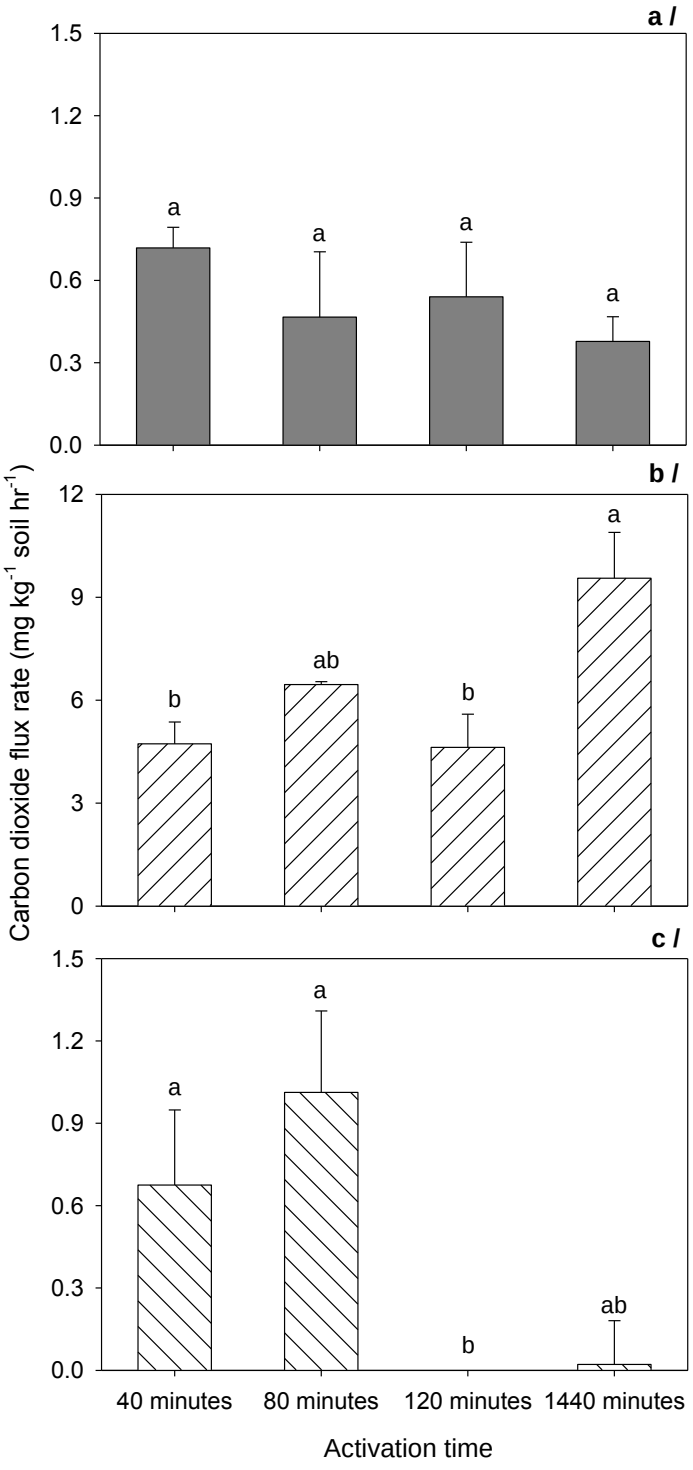

Figure 2.5 Fluxes of CO_2 sampled at 10-min intervals from different treatments of activation time (i.e. different durations of soil activation) with the addition of glucose and nitrate. Fluxes of CO_2 are shown for acid sulphate (a), Red Dermosol (b) and Vertosol (c) soils. Error bars represent standard error and different letters on the bars show significant difference at $P < 0.05$, $n = 3$.

CH_4 fluxes

Lengthen activation time (>2 hours) caused methane absorption in all soils whereas positive fluxes were recorded in the short activation treatments (<1.3 hour) (Figure 2.6). Also, fluxes of CH_4 were not significantly different among activation treatments. The results for CH_4 flux were mainly high in the 1.3-hour activation while the 2-hour activation had high absorption of CH_4. This trend was similar in all the experimental soils (Figure 2.6).

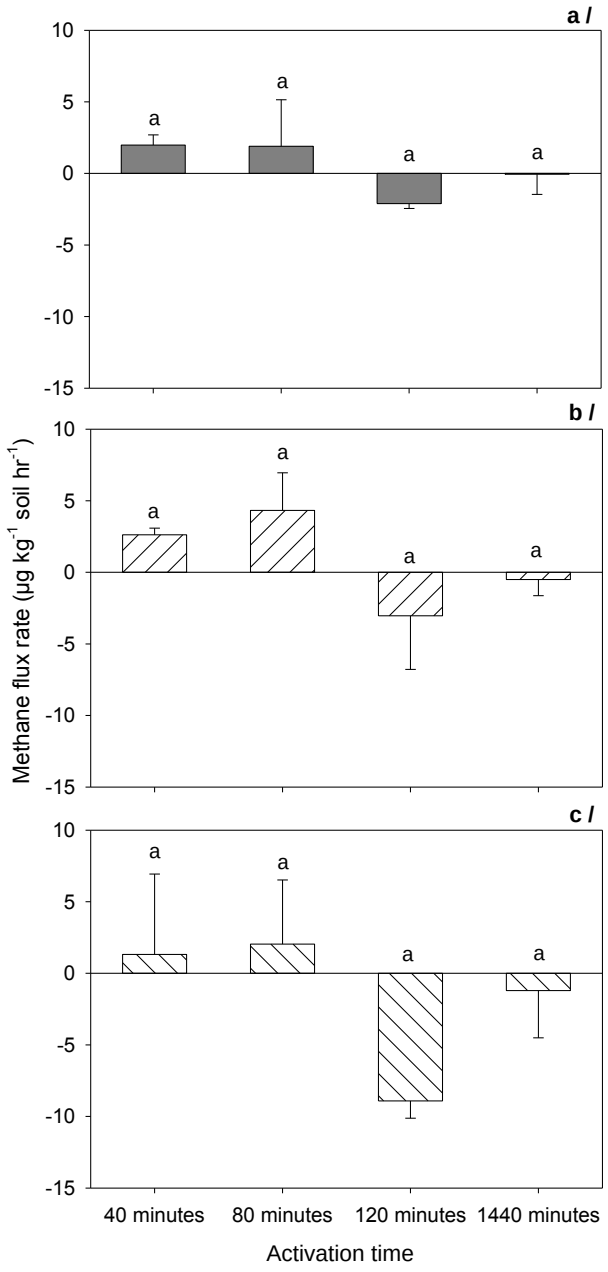

Figure 2.6 Effects of different activation times (i.e. different durations of soil activation) with the addition of glucose and nitrate on fluxes of CH_4 in acid sulphate (a), Red Dermosol (b) and Vertosol (c) soils. Error bars represent standard error and different letters on the bars show significant difference at $P < 0.05$, $n = 3$.

2.4 Discussion

2.4.1 Effects of closure time on soil gas fluxes

Different closure times altered GHG emissions from the three incubated soils. Nguyen et al. (2014b) showed that the highest emission of N_2O and CO_2 was at 1-hour closure time and they continuously reduced over 24 hours. In our study, a similar pattern was recorded for N_2O (Figure 2.1) and CO_2 (Figure 2.2) emissions after an 80-minute closure while longer closure time reduced or did not significantly change N_2O emission. Similarly, methane emission was only recorded at 80-minute closure in the Red Dermosol and all other treatments showed either no production or consumption of CH_4 (Figure 2.3). Our findings implied that short closure time (<1 hour) may cause an absorption or uptake of gases. This was observed for the N_2O measurement in the acid sulphate soil (Figure 2.1*a*); in CO_2 measurement for the Red Dermosol soil (Figure 2.2*b*); and in the measurement of CH_4 fluxes (Figure 2.3). On the other hand, longer closure time (2 hours in the ASS and Red Dermosol or 24 hours in the Vertosol) can also result in CH_4 consumption (Fig. 2.3). For all soil types or monitored gases, the results for the 80-min closure were mostly positive rates. A deployment time for gas sampling should be no shorter than 4-5 minutes and no longer than 1 hour (Holland et al., 1999). In addition, the period over which sampling should occur is the period of a linear increase in gas concentration over time. Healy et al. (1996) also reported that long deployment times lead to significant underestimations of the flux. (Nakano *et al.*, 2004) found that calculating the flux by linear regression of the concentration change over a 10-min period considerably underestimated the flux at some sites. To calculate gas fluxes based on a linear regression model, the results indicate that 4 or 5 sampling-points should be used for other gas flux measurements because samples at more than three times can reduce uncertainty in flux calculations, but with additional labour cost (Parkin and Venterea, 2010).

2.4.2 Effect of soil activation on gas emissions

The second experiment aimed to test how additional activation time prior to closure in the 1st experiment affected gas emissions from soil incubation. Increasing the activation time prior to chamber closure produced different results between soils and gas types. Our study showed that rates of greenhouse gas emissions were significantly similar for the acid sulphate soil and Vertosol soil. The emission rates also showed a similar trend for all gases measured (Figures 2.4, 2.5 and 2.6). However, different times of additional activation caused significantly different gas fluxes from the Red Dermosol soil (Figures 2.4*b* and 2.5*b*). The different activation in the second experiment may have created variations in easily oxidisable C and resulted in higher N_2O and CO_2 fluxes in the Red Dermosol soil. Azam et al. (2002) have reported that the addition of glucose in different

amounts and pre-incubation of soil for different lengths of time changed the pattern of N_2O emissions, which was ascribed to changes in soil respiration. Moreover, soils pre-incubated for 25 and 49 hours showed high N_2O emissions compared to soil samples pre-incubated for 1 hour. In our study, the Red Dermosol soil had low carbon and nitrate content, therefore the application of additional glucose and nitrate prior to measurement enhanced both N_2O emission and respiration, especially in the long activation treatment (24 hours) (Figures 2.4*b* and 2.5*b*). The addition of easily oxidisable organic matter increases N_2O emissions even under apparently aerobic conditions (Beauchamp et al., 1989). Because the presence of large quantities of denitrifying enzymes in aerobic soils is evidence of microsite anaerobiosis (Azam et al., 2002), high N_2O fluxes occurred in the Red Dermosol soil in the present study. After 24 hours, the rate of bacterial growth increased almost two-fold in the rewetted soil with glucose addition (Iovieno and Bååth, 2008). This presents another explanation for the high gas emissions in the current study. Our findings indicated that additional activation times may result in different emission rates according to soil characteristics although deployment time of headspace gas samples was the same.

2.4.3 Soil properties effects on gas fluxes

N_2O fluxes

In our study, the condition of well-aerated incubation allowed both nitrification and denitrification because many soil denitrifiers can produce N_2O over a wide range of oxygen pressures (Khalil et al., 2004). Nitrification depends strictly on aerobic conditions since the NH_4^+ oxidation enzyme requires oxygen for activation (Wood, 1986). N_2O emissions from the three soils were quite different due to the differences in the controlling factors affecting both processes from the experiment samples. Although nitrate was added to all incubation jars, the emission of N_2O from the ASS was lowest (Figure 2.1*a*) because the soil nitrate concentration is very low (Table 2.1). In addition, the low emission of N_2O from the ASS could be caused by the limited nitrification due to acidity and the mineralized N accumulating entirely as ammonium-N (Sahrawat, 1980). Above a threshold of soil pH (4.4), nitrification dominates N_2O production (Cheng et al., 2015). In our study, both Red Dermosol and Vertosols soil had soil pH higher than the threshold, so nitrification might be the dominant process. However, the low content of NO_3 in the Red Dermosol soil could increase possibility for the nitrification, the transformation ammonia to nitrate, occurring in this soil and lead to higher N_2O emissions than that in the Vertosols soil (Figures 2.1*b, c*).

CO_2 fluxes

In the laboratory incubation, microbial respiration causes soil CO_2 emission and the CO_2 emission has been used as an index to assess soil microbial activity (Janssens *et al.*,

2001; Lang *et al.*, 2011). Under the same closure time, CO_2 fluxes were different among the different soils in the present study (Figure 2.2). The CO_2 release was higher in the Red Dermosol than in the other soils. The differences among the three soil types can be attributed to the soil organic matter and nitrogen content, pH, and microbial activity. Wang et al. (2003) also reported that the variations in soil respiration could be due to variations in the chemistry of soil organic matter, the activity of microbial biomass carbon, the extent of physical protection afforded by the mineral matrix. The differences in soil respiration were also caused by variations in substrate availability and drying and re-wetting effects (Kaiser and Heinemeyer, 1993). On the other hand, soluble organic carbon had a significant influence on soil biological activity (Chantigny, 2003). In the present study, the Red Dermosol soil had lower C, N, and nitrate content and the addition of glucose and nitrate could be more effective on microbial respiration than on the other two soils because microbial respiration is mainly controlled by the supply of readily decomposable SOM (Rustad et al., 2000) and increases with soluble organic carbon content in soil (Lou et al., 2007).

CH_4 fluxes

Methane emissions were low in the incubated soils and CH_4 consumption was found in many treatments of our study. The result of low emissions is similar to that reported in a review of Le Mer and Roger (2001) that CH_4 emission in unplanted upland soils temporarily submerged is around a few g ha^{-1} d^{-1}. Methanogenic activity is generally low in non-flooded soils because the redox potential (Eh) is not favourable for methanogens (van Cleemput et al., 1983). Aerobic conditions do not favour CH_4 production because CH_4 formation is usually caused by microbial breakdown of organic compounds in strictly anaerobic conditions (Smith et al., 2003). On the other hand, CH_4 consumption occurred because nitrate application causes competition for H_2 between denitrifying bacteria and methanogens. In addition, nitrate reduces CH_4 emission by increasing soil Eh (Jugsujinda et al., 1995). The high methane consumption occurred in the 40-minute closure treatment for all incubation soils and CH_4 production slightly increased in longer closure time treatments (Figure 2.3). This is because the addition of nitrate increased soil Eh and almost completely inhibited CH_4 production. However, soon after the consumption, CH_4 releases due to nitrate reduction and loss through denitrification (Wang et al., 1992). West and Schmidt (1999) reported that atmospheric CH_4 consumption by a well-drained soil increased four times when carbon substrate was added to soils. This is similar to the findings of the present study because addition of glucose was applied to all incubation treatments.

In the acid-sulphate soil, CH_4 emission is normally lower than in the other soil types due to competition for H_2 between methanogens and sulphate reducers (Jermsawatdipong

et al., 1994). The activity of methanogens is usually sensitive to variations in soil pH (Wang et al., 1993). Moreover, the bacterial reducers of nitrate and Fe^{3+} are more highly competitive for electron donors than methanogens (Ma and Lu, 2011).

2.5 Conclusion

In this incubation study, gas emissions from soils mostly decreased with longer lid closure. Positive fluxes of N_2O and CO_2 were greatest after an 80-minute closure but longer closure time would reduce or did not significantly change the emissions. Similarly, methane rate was only recorded in the 80-minute closure treatment, and other treatments showed CH_4 consumption or negligible CH_4 production. A deployment time of maximum 80 minutes with 4 or 5 samplings, at each quarter of the total closed period, would be applicable to estimate gas fluxes from soil incubation. Lengthening activation times resulted in different emission rates according to soil characteristics. Additional 24-hour activation caused greater N_2O and CO_2 fluxes in the Red Dermosol soil relative to other soils, and long activation time (≥ 2 hours) showed no statistical differences in CH_4 flux rate in any of the soils. The findings of this study also suggest that a comparison between GHG flux results from different incubation techniques and studies is not possible due to experimental artefacts. Thus, a full description of soils being tested would enable comparison of results from different soils. In addition, activation time was critical and drove gas emissions, so a standardized procedure is needed to quantify gas fluxes from soil in laboratory experiments.

Chapter 3: AVAILABLE CARBON AND NITRATE INCREASE GREENHOUSE GAS EMISSIONS FROM SOILS AFFECTED BY SALINITY

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I implemented the experiment, analysed soil and gas samples, interpreted and analysed data, wrote the manuscript and acted as the corresponding author. I hereby certify that the statement of contribution to the manuscript is accurate.

Signature: _____ Date: _____

Ian White
I evaluated the manuscript, provided comments and corrections. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Sören Warneke
I provided comments and suggestions the manuscript. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Ben Macdonald
I supervised development of work, evaluated the manuscript, provided comments and corrections. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signature: _____ Date: _____

Chapter 3: AVAILABLE CARBON AND NITRATE INCREASE GREENHOUSE GAS EMISSIONS FROM SOILS AFFECTED BY SALINITY

Abstract

Sea-level rise and saline water intrusion have caused a shortage of fresh water and affected agricultural areas globally. Besides inundation, salinity can alter soil nitrogen and carbon cycling in coastal soils. To examine the effect of salinity, an incubation experiment was used to investigate soil nitrogen and carbon cycling from an acid sulphate soil and an alluvial soil with and without additional nitrogen and carbon sources. Four levels of saline solution of 0.03, 10, 16 and 21 dS m^{-1} were used to submerge acid sulphate and alluvial soil samples in a 125-mL jar. The experimental jars were incubated in the dark at 25°C. Gas samples were collected over 4 weeks and analysed for nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4). The results showed that salinity significantly decreased N_2O emissions from the acid sulphate soil but did not affect emissions from the alluvial soil. The addition of glucose and nitrate enhanced N_2O production in both salt-affected soils. Emissions of CO_2 were not different among the salinity treatments, whereas available carbon and nitrate promoted soil respiration. Changes of CH_4 fluxes over the 4-week incubation period were similar for both soils and substrate addition did not affect emissions in either soil. The findings indicate that salinity has altered carbon and nitrogen cycles in the acid sulphate soil, and future fertiliser and crop management will need to account for the changed nutrient cycling caused by saline water intrusion and climate change.

Keywords: salinity, acid sulphate soil, greenhouse gas emission, incubation experiment, submergence, denitrification, osmotic potential, electron donor, and methanogenesis.

3.1 Introduction

Sea-level rise caused by global climate change presents problems for many coastal and agricultural areas worldwide. Church et al. (2013) indicated that sea-level rise could range from 0.52 to 0.98 m by the end of the 21st Century; however, for the high warming scenario, the increase could be 1.2 m by 2100 (Horton et al., 2014). Almost globally, coastlines will be affected by rising sea level by the end of the 21st Century (Cazenave and Cozannet, 2014). The sea-level rise could affect >55 million people, and in developing countries, ~0.4% of the total agricultural land would be affected by a 1-m rise and 2.1% by a 5-m rise (Dasgupta et al., 2009). Rising sea levels would have both direct

and indirect impacts on agricultural land through inundation, altered flood dynamics or erosion, all of which cause modification of groundwater dynamics. In addition, seawater intrusion would cause a shortage of freshwater resources and a reduction in irrigation water (Snoussi et al., 2008).

Acid sulphate and salt-affected soils are two common groups of globally degraded soils. Salt-affected soil comprises almost 10% of total global land area (Pessarakli, 2011; Abd-Elgawad et al., 2013) and reduces both plant growth and crop yields (Suarez, 2011). High salt concentration in the soil leads to low water uptake by plants due to the effects of low osmotic potential (Harris, 1981) and causes competition for nutrient uptake and an increase in the toxicity of ions such as sodium, chloride and boron (Keren, 2011). Acid sulphate soil is characterised by severe acidification (pH <4) and the mobilisation of toxic metals such as aluminium, iron and copper, and other hazards including hydrogen sulphide, and sulfuric acid as a result of acidification (Sullivan et al., 2011). The global extent of acid sulphate soils is ~17 Mha, most commonly found in Africa, Australia, Asia and Latin America (Andriesse and Mensvoort, 2005). Acid sulphate soils have been reclaimed for agricultural activities such as rice cultivation (Minh et al., 1997), rice–shrimp systems, *Melaleuca leucadendra* or *M. cajuputi* forest, and other annual and perennial crops (Sullivan et al., 2011). Because acid sulphate soils are commonly distributed in low-lying areas (<5 m a.s.l.), these landscapes are particularly vulnerable to sea-level rise caused by global warming and climate change (Bush et al., 2010). Tidal inundation of acid sulphate soils can change the geochemistry of the soil from conditions of oxidation to reduction and establish new reductive geochemical processes (Johnston et al., 2009). Moreover, seawater intrusion on areas of acid sulphate soils can increase release of aluminium via cation exchange processes (Wright et al., 1988), as well as ammonium (Portnoy and Giblin, 1997), into the pore water. The effects of climate change on nitrogen and carbon cycles of salt-affected and acid sulphate soils are not quantified.

Several studies have investigated the impacts of salinity on soil properties and processes. A decrease in the mineralisation of nitrogen occurs under saline conditions and under higher moisture regimes (Lodhi et al., 2009). A higher salinity level promotes the loss of nitrogen in ammonia form (Akhtar et al., 2012). Elevation of salinity affects soil microbe activities in nitrification processes and this leads to a reduction in the conversion ammonium to nitrate (Irshad et al., 2005; Kumar et al., 2007). Regarding soil carbon dynamics, saline conditions can enhance the decomposability of soil organic matter (Wong et al., 2010). Some studies have shown a decrease in carbon dioxide (CO_2) emission with increasing salinity, due to decreasing osmotic potential or depressed microbial activity (Laura, 1974; Pathak and Rao, 1998; Setia et al., 2010). Although the effects of salinity on carbon and nitrogen decomposition have been addressed, these

studies focused on soil samples with pH >6. Information about carbon and nitrous oxide (N_2O) emissions from acid sulphate soil is scarce.

Soil micro-organisms must use inputs of fresh, labile substrate such as animal and plant residues and root exudates. But, these substrates usually present in irregular pulses in soil. Hence, growth and dormancy of microorganism depend on the availability of readily degradable fresh substrates (Mondini et al., 2006). Denitrification is the main process of nitrogen transformation in soil containing sufficient organic carbon under anaerobic conditions and a high NO_3^- concentration (Ha et al., 2015). Denitrification requires available organic carbon as an electron donor; a substrate of NO_3^- as an electron acceptor; and the absence of oxygen or soil moisture contents at > 60% water-filled pore space. To our knowledge, effects of salinity and substrates on greenhouse gas soil emissions remain uncertain.

In this study, we hypothesised that interaction of salinity and substrates would increase the decomposition of organic matter and transformation of nitrogen compounds, leading to increased emissions of greenhouse gases from soil. The aim of this work was to investigate carbon and nitrogen release from acid sulphate soil under the effects of saline solution submergence with and without the addition of nitrogen and carbon sources.

3.2 Materials and methods

3.2.1 Description of sampling site

Plate 3.1 Acid sulphate soil with a jarosite layer 50 cm below soil surface

An acid sulphate soil (ASS) managed as a pasture soil was collected from the south coast at Nowra, New South Wales, Australia (34°49'14.8"S, 150°39'8.0"E). The elevation of the area varies from 0.5 to 2.5 m a.s.l., with average annual rainfall of 1135 mm (Lawrie and Eldridge, 2004). The acid sulphate soil is in low-lying, former backswamps and has dark loamy topsoil. The soil was classified as Entisols (Soil Survey Staff, 2010) or Thionic Fluvisol (FAO-WRB, 2006). At sampling time, the water table was 1.2 m below the surface, and the soil surface of the irrigation area was covered by ryegrass. Field pH testing was conducted in a paste of soil and deionised water at 10-cm intervals on a soil profile by using a pH meter. The soil pH through the 2-m soil profile varied from 3.09 to 5.63, and in particular was <4 in the top 1 m of the profile. Jarosite, formed by oxidation of sulfidic material, was found at a depth of 50 cm. The site of the alluvial soil had field pH values of 4.20–4.86 to 50 cm depth, and pH <4 below 50 cm depth. An oxidation layer to 50 cm from the soil surface was found with a brownish colour (Munsell colour 10YR 4/3), and a jarosite layer observed at 1.2 m depth. A black organic layer was appeared below 1.7 m depth.

3.2.2 Soil and saline water sample collection

Samples (0–15 cm depth) of the two types of soil were collected in plastic bags, stored in insulated containers and returned to the laboratory within 5 hours. Within 24 h, soil samples were extracted for laboratory analysis of mineral nitrogen. Part of each sample was dried at 40°C for analysis of soil physical and chemical characteristics. Field-moist soil samples were sieved (<2 mm) and mixed well, then representative subsamples were collected and used for the incubation experiment. In the laboratory, these homogenised samples were submerged with distilled water or with saline solutions made up of collected saline water diluted with distilled water. Soil cores in the 0–15 cm depth were also taken for measurement of soil bulk density. There was no compaction and the soil bulk density was measured by using the core method (Blake and Hartge, 1986; Hao et al., 2007). Saline water from a tidal canal around the farm was collected for the incubation study; this canal is connected to the sea. The saline water had an electricity conductivity (EC) of 23.3 dS m^{-1} (total dissolved solids ~18.6 g L^{-1}) and pH 6.64. Concentrations (g L^{-1}) of the six most abundant ions in the saline water sample were: chloride (Cl^-) 8.00, sodium (Na^+) 5.02, sulfate (as S) 0.40, magnesium (Mg^{2+}) 0.57, calcium (Ca^{2+}) 0.17, and potassium (K^+) 0.20.

3.2.3 Soil extraction and analyses

Soil pH and EC were measured in 1:5 soil:water after end-over-end shaking at 25°C in a closed system for 1 hour (Rayment and Lyons, 2011). Soil ammonium and nitrate analyses were carried out on a 2 M KCl solution and measured following the method of Keeney and Nelson (1982). A CNS-2000 (LECO, St. Joseph, MI, USA) was used to measure total carbon, nitrogen and sulphur. In the combustion process, any compound consisting of carbon, nitrogen and sulfur was converted to CO_2, N_2 and SO_2. These gases were then flowed through infrared cells to detect the carbon and sulfur content and through a thermal conductivity cell to determine nitrogen content. Soil particle size was measured using the method of Kettler et al. (2001). This method uses a combination of sieving and sedimentation steps to evaluate soil particle distribution. The results of the chemical and physical analyses are presented in Table 3.1.

3.2.4 Incubation experiment for measurement of greenhouse gases

As discussed in the Chapter 2, there were some advantages in laboratory incubation for estimating gas fluxes from soil, particularly controlling environmental factors. In this chapter, an incubation experiment was also conducted to quantify greenhouse-gas emissions from acid sulphate and alluvial soils under different salinity concentrations. Field-moist soil samples (20 g, 0–15 cm depth) were submerged in a 125-mL jar with 15 mL of different saline solutions: 0.03 dS m^{-1} (distilled water), 10 dS m^{-1} (low salinity), 16 dS m^{-1} (medium salinity) and 21 dS m^{-1} (high salinity). Based on the suggestion for

salinity conversion put forward by Tanji and Wallender (2012), total dissolved solids (g L^{-1}) of these solutions were equal to 0.02 (fresh water), 8 (low salinity), 12.8 (medium salinity) and 16.8 (high salinity). The jar was swirled for 1 minute to ensure adequate mixing. There were two batches of the incubation jars: those treated with and those without substrate addition. In the first batch, 5 mL of solution providing both 300 µg glucose-C and 50 µg NO_3-N g^{-1} soil was added to the soil in each jar. This treatment was applied to ensure that denitrification was not limited by nitrogen or carbon supply (Luo et al., 1996). In the second batch, 5 mL of distilled water was added to each jar, so that all jars in both batches had the same water content. Each treatment was replicated with three jars, and a number of replications were the same for both batches. Soil samples in all jars were therefore submerged with 20 mL solution; this resulted in a water level of 5 mm above the soil surface. This constant water level was used because a difference in water level above soils can affect gas emissions, rates of movement through water being much slower than through air. Soil slurries were activated for 24 hours as presented in the chapter 2 before they were incubated in the dark at 25°C. Distilled water was added to the jars to maintain the same soil moisture over time. Headspace gas was sampled with a syringe after 0, 1, 2, 3, 5, 7, 14, 21 and 28 days of incubation. A 6-mL headspace sample was collected by using a gas syringe while air was allowed back into the jar via another needle. The headspace gas sample was injected into evacuated vials (3.75 mL) for storage.

Gas samples and standards were analysed by a GC-2014 gas chromatograph (Shimazu, Kyoto, Japan) equipped with an electron capture detector to determine N_2O emission from the soil slurries. A flame ionisation detector was also connected to the gas chromatograph to detect other gases such as methane (CH_4) and CO_2.

3.2.5 Data calculation and statistical analyses

Gas samples collected when the incubation jars were closed represented time zero. The concentration of each consequent gas sample was standardised to the time zero concentration. After the gas sampling was completed, the jars were opened, and a small fan was used to flush all the gas from the jars. At subsequent sampling times, these steps were repeated to prevent double-calculation of ambient gas concentration. Atmospheric pressure and temperature were recorded at each sampling time. These data were used to convert gas concentrations from volumetric to mass-based:

$$C_{mass} = (C_{volumetric} \times P \times MW_{gas}) / (T \times R) \quad (1)$$

where C_{mass} is mass concentration (μg L^{-1}), $C_{volumetric}$ is volumetric concentration (ppm), P is ambient air pressure (atm), MW_{gas} is molecular weight of the gas (g mol^{-1}), T is ambient air temperature (K), and R is the ideal gas constant (L atm K^{-1} mol^{-1}).

The mass concentrations of gas were used to calculate the gas emissions per kg of soil. The cumulative emissions from the same treatment at later sampling time were determined by adding the emission to that of the previous sampling time. Flux rates of emitted gases were calculated by dividing cumulative emissions at each sampling time by the number of days the incubation jars:

$$F = (C_{mass\ d} - C_{mass\ d0}) \times V \times W_{soil}^{-1} \times t^{-1} \quad (2)$$

where F is flux of greenhouse gases (in μg day^{-1} kg^{-1}), $C_{mass\ d}$ is gaseous concentration at sampling time d (μg L^{-1}), $C_{mass\ d0}$ is gaseous concentration at time zero (d0) (μg L^{-1}), V is headspace volume (L), W_{soil} is weight of soil used in an incubated jar (kg), and t is time interval (days).

Statistical analysis was conducted with R statistical software version 3.0.2 (The R Foundation, Vienna). Prior to analysis, data were tested for homogeneity and normality and results of data skewness tests were accepted. Data analysis for soil chemicals was performed by using a paired t-test. The results of cumulative emissions from soils were analysed using the repeated-measures analysis of variance (ANOVA) procedure. Tukey's test was used to identify significant differences among treatments. Three-way ANOVA was used to examine the interaction effects of salinity, substrate amendment and soil types on cumulative N_2O emissions. Two-way ANOVA was performed to test the interaction effects of salinity and amendment on N_2O fluxes. Repeated Measures ANOVA was also carried out to analyse CO_2 and CH_4 flux data.

3.3 Results

3.3.1 Properties of soil samples

The bulk density of the soil samples was 0.85 g cm^{-3} for acid sulphate soil and 1.02 g cm^{-3} for alluvial soil. The samples were very strongly acidic, with soil pH values <5. Total nitrogen of the acid sulphate soil was 0.51% and that of the alluvial soil was 0.48%; NH_4^+-N contents were 92.50 mg kg^{-1} for the acid sulphate soil and 96.75 mg kg^{-1} for the alluvial soil, and respective NO_3^--N contents were 12.51 and 14.25 mg kg^{-1} soil. Total carbon contents were >6.5% in both the acid sulphate and alluvial soils, and total sulphur content was much greater in the acid sulphate soil (0.65%). The carbon : nitrogen ratio was ~14 for both soils, indicating good conditions for rapid decomposition of soil organic matter. The soil texture was sandy loam for the acid sulphate soil and loam for the alluvial soil (Table 3.1).

Table 3.1 Characteristics of field soil samples (0 – 15 cm) from the field located in Nowra. Means in the same row followed by the different letters are significant. For t-test analysis between two soils: (*) P <0.05; (**) P < 0.01; (***) P <0.001; (ns) not significant.

Soil parameters	Acid sulphate soil	Alluvial soil	Significant level
pH	3.92b	4.61a	**
EC (dS m^{-1})	1.79a	0.21b	***
Total C (%)	7.11	6.70	ns
Total N (%)	0.51	0.48	ns
Total S (%)	0.65a	0.09b	**
NO_3^- – N (mg kg^{-1})	12.51	14.25	ns
NH_4^+ – N (mg kg^{-1})	92.50	96.75	ns
Bulk density (g cm^{-3})	1.02a	0.85b	*
Soil texture			
Sand (%)	54.84a	49.21b	*
Silt (%)	35.54	37.65	ns
Clay (%)	9.62b	13.14a	*

3.3.2 Production of N_2O

Cumulative N_2O-N emission from incubated soils

In the acid sulphate soil, cumulative N_2O-N emissions from elevated salinity treatments after 7 days ranged from 2.03 to 2.29 mg N_2O-N kg^{-1} soil and were less than those from the fresh water (FW) treatment, at 3.46 mg kg^{-1} soil (Figure 3.1*a*). However, when nitrate and glucose were added to the incubation jars, emissions from the salinity treatments were >5 mg N_2O-N kg^{-1} soil after 3 days and significantly greater than emissions from the FW treatment, whose emissions increased only slightly to 3.50 mg N_2O-N kg^{-1} soil (Figure 3.1*b*). The different levels of salinity did not result in significant differences in cumulative N_2O-N emission from the acid sulphate soil; however, there was a significant salinity × time interaction effect (F = 7.33, P < 0.001) on emission when carbon and nitrogen were added (Figure. 3.1*b*).

Although there were significant differences between treatments for cumulative emissions from the alluvial soil during the second and third days, subsequent gas production was similar for all treatments and little emission occurred after day 5 (Figure 3.1*c*). As in the acid sulphate soil, glucose and nitrate amendment significantly increased the emissions from treatments with saline water over those of the FW treatment (Figure 3.1*d*). Emissions from elevated salinity treatments were 4.88–5.42 mg N_2O-N kg^{-1} soil, whereas release from the FW treatment was 2.91 mg N_2O-N kg^{-1} soil. Similarly, a salinity × time

interaction effect (F = 74.3, P < 0.001) was recorded in this soil with carbon and nitrogen addition (Figure 3.1*d*).

The data show that N_2O-N was released rapidly during the first week of incubation, after which there was almost no further release. Addition of nitrate and glucose created good conditions for denitrification which resulted in greater N_2O-N emissions from treatments with elevated salinity than from the FW treatment.

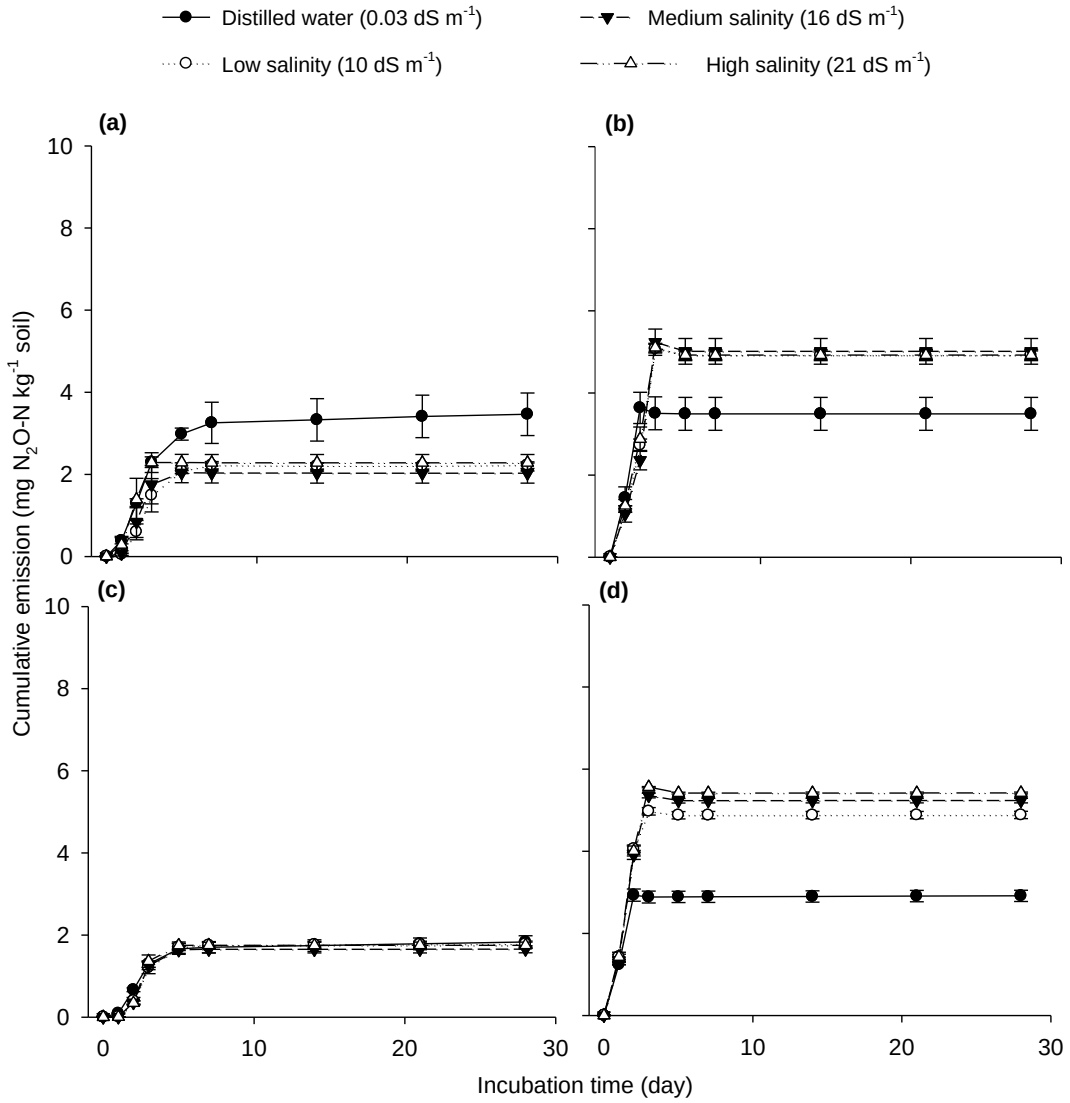

Figure 3.1 Cumulative N_2O-N emission during four-week incubation of four salinity levels applied to two soils: acid sulphate soil without (a) and with (b) glucose and nitrate addition; alluvial soil without (c) and with both the addition of nutrients. Bars indicate standard errors of means (n=3).

Interaction effects of factors on cumulative N_2O-N production

During the first and second days, cumulative N_2O-N emissions in the same treatment of substrate amendment were similar for all salinity treatments. This indicates that salinity did not affect soil processes in the early stages of incubation. When N_2O-N emissions

from the two soil types were analysed separately, total cumulative N_2O-N emissions for the first week were similar between salinity levels but less than those from the FW treatment (3.26 mg N_2O-N kg^{-1} soil) in acid sulfate soil with no substrate addition (Figure 3.2*a*). However, the addition of available nitrogen and carbon increased by ~30% total cumulative emissions from salinity treatments compared with the FW treatment. A similar effect of substrate addition was found in the alluvial soil after the first week of the incubation experiment (Figure 3.2*b*). Although the effect as a function of salinity, soil and amendment occurred on the second day, cumulative N_2O-N emissions were dominated only by the main effect of salinity and amendment and by their interactions for the remaining times ($P < 0.01$).

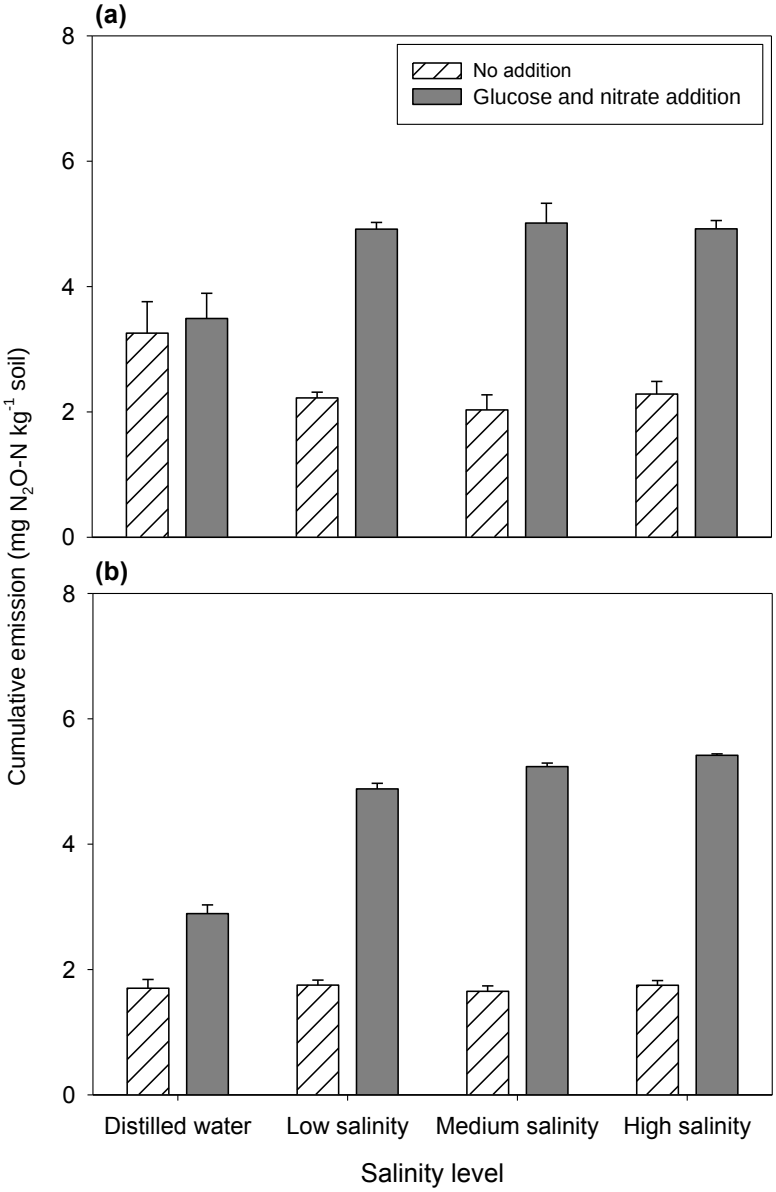

Figure 3.2 Effects of different salinity levels, nutrient addition and soils on total cumulative N_2O-N emission in the first-week incubation. Graphs are average emissions on acid sulphate soil (a) and alluvial soil (b). Error bars represent standard errors of means (n = 3).

3.3.3 Emission rate of N_2O-N from incubated soils

The various salinity levels altered the N_2O-N flux, the effect being particularly evident on the third day (Figure 3.3). Average maximum N_2O fluxes varied from 0.36 mg N_2O-N kg^{-1} soil day^{-1} for the FW treatment to 1.53 mg N_2O-N kg^{-1} soil day^{-1} for the medium salinity treatment. Increased salinity levels resulted in higher emission rates and significant differences between salinity and FW treatments. For other sampling times in the first week, no significant difference was recorded.

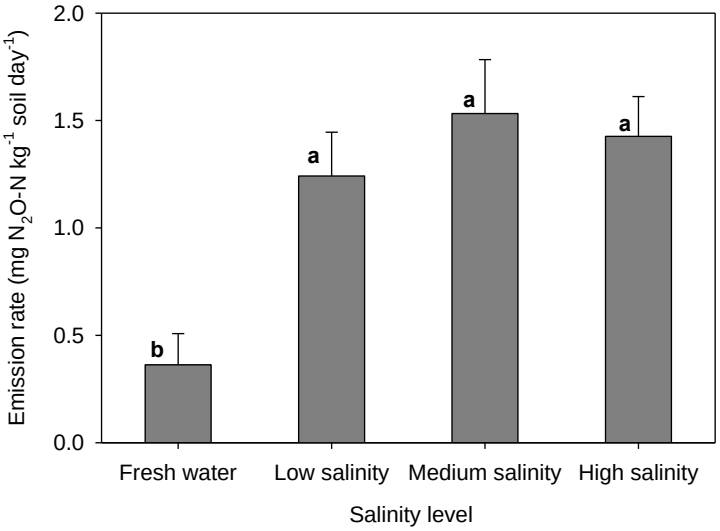

Figure 3.3 Fluxes of nitrous oxide emissions from salt-affected soil on the third day of incubation. Letters on the bars show statistical significances between treatment means and error bars represent standard errors of means ($F = 7.095$, $P < 0.001$, $n = 12$).

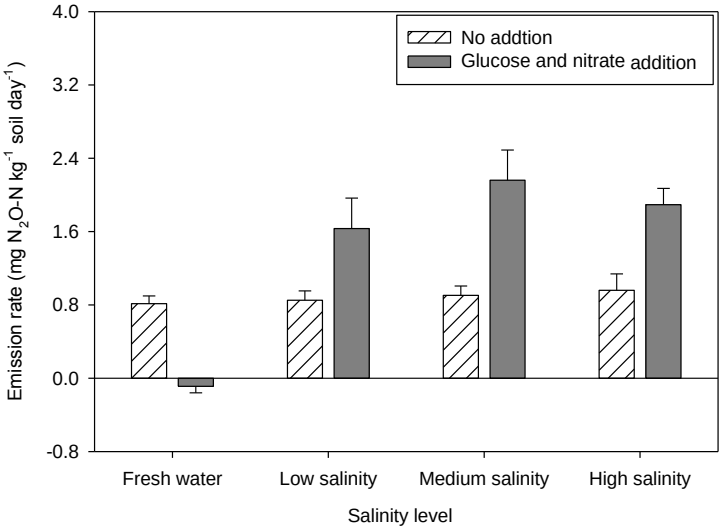

Figure 3.4 Effects of available carbon and nitrogen on the flux of nitrous oxide emission from salt-affected soil on the third day of the incubation. Error bars indicate standard errors of means ($n = 6$).

There was a significant interaction effect of substrate addition and level of salinity on maximum flux of N_2O-N emission (Figure 3.4). In the absence of amendments, the fluxes for all salinity treatments were ~0.85 mg N_2O-N kg^{-1} soil day^{-1}. However, large differences in flux were found when the substrate was added (Figure 3.4). The negative value in the graph indicates that an uptake of N_2O occurred in the FW treatment, whereas other treatments had higher fluxes varying between 1.63 and 2.16 mg N_2O-N kg^{-1} soil day^{-1}.

3.3.4 Cumulative CO_2 and CH_4 emission from soil during the 4-week incubation

Cumulative CO_2 emissions increased over time (Figure 3.5). In both soils, total emissions without substrate amendment were <400 mg CO_2 kg^{-1} soil after 4 weeks (Figure 3.5*a*, *c*), except for the high salinity treatment in acid sulphate soil (Figure 3.5*a*). Glucose and nitrate addition caused a 2-fold increase in cumulative CO_2 over the 4-week incubation period in both soil types (Figures 3.5*b*, *d*). In the alluvial soil with the addition of substrate, there were significant differences between salinity treatments and the FW treatment for CO_2 released on day 3. Similarly, the FW treatment also resulted in different CO_2 emissions to the treatment of medium and high salinity on day 7 (Figure 3.5*d*).

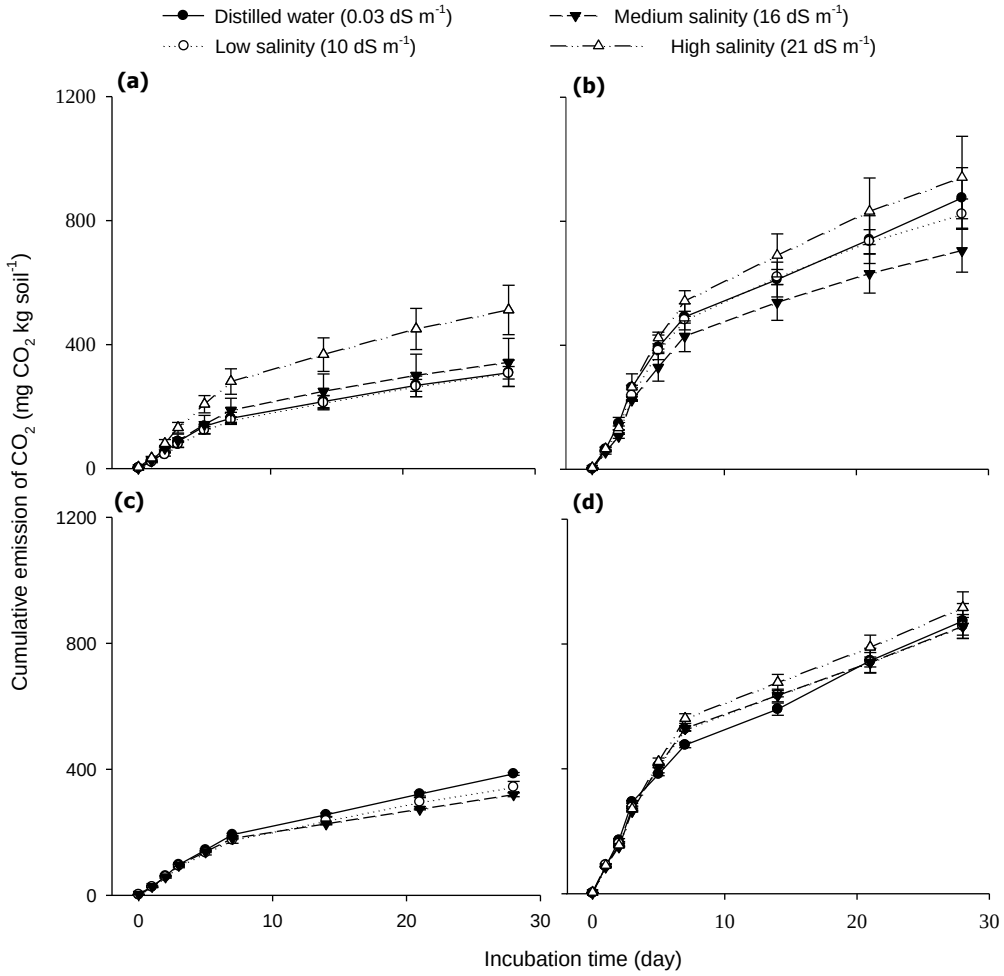

Figure 3.5 Cumulative CO_2 emissions during four-week incubation of four salinity levels applied to two soils: acid sulphate soil without (a) and with (b) glucose and nitrate addition; alluvial soil without (c) and with both the addition of nutrients. Bars indicate standard errors of means (n=3).

Table 3.2 Cumulative CH_4 emission during four-week incubation of four salinity levels applied to two soils: acid sulphate soil and alluvial soil without and with addition of carbon and nitrate. Numbers in brackets are standard errors of means.

Soil	**Salinity (dS m^{-1})**	**Cumulative emission of methane (µg CH_4 kg $soil^{-1}$)**					
		Incubation time (day)					
		0	**3**	**7**	**14**	**21**	**28**
Acid sulphate soil				Without C and N addition			
	0.03	14 (5)	7 (4)	57 (27)	62 (24)	70 (26)	51 (22)
	10	19 (11)	12 (5)	42 (34)	35 (40)	36 (40)	31 (41)
	16	11 (1)	16 (7)	94 (101)	75 (112)	77 (114)	81 (110)
	21	11 (1)	13 (4)	57 (39)	44 (40)	54 (43)	38 (19)
				With C and N addition			
	0.03	10 (1)	72 (1)	124 (51)	115 (58)	126 (61)	88 (85)
	10	12 (1)	76 (2)	124 (28)	124 (28)	125 (34)	103 (57)
	16	14 (2)	74 (3)	56 (30)	53 (31)	62 (44)	61 (101)
	21	10 (1)	72 (1)	130 (36)	132 (35)	150 (44)	230 (147)
Alluvial soil				Without C and N addition			
	0.03	13 (3)	68 (6)	85 (21)	74 (17)	77 (17)	52 (25)
	10	12 (3)	69 (2)	70 (11)	72 (8)	81 (10)	54 (23)
	16	10 (1)	69 (4)	62 (5)	61 (5)	60 (3)	61 (2)
	21	12 (1)	73 (1)	77 (2)	72 (1)	69 (3)	30 (24)
				With C and N addition			
	0.03	10 (1)	68 (2)	73 (5)	75 (6)	69 (5)	27 (21)
	10	9 (1)	79 (4)	100 (16)	98 (14)	101 (15)	101 (21)
	16	12 (2)	75 (1)	105 (10)	99 (7)	96 (6)	30 (10)
	21	10 (1)	76 (5)	203 (60)	202 (61)	203 (62)	162 (84)

Methane emissions in this study were highly variable, indicating the complex processes involved in CH_4 release from soil (Table 3.2). The different salinity concentrations gave rise to a wide range of cumulative CH_4 emissions, although they were not significantly different. The production of CH_4 emissions ranged from 6.7 to 230 µg CH_4 kg^{-1} soil. The addition of carbon and nitrogen substrate did not contribute to significantly different CH_4 emissions in the soil types over the 4 weeks.

3.3.5 Methane and CO_2 fluxes from soil during the 4-week incubation

The flux rate of CO_2 emission peaked after 3 days of incubation for both soils (Figure 3.6). Mean maximum CO_2 fluxes varied from 31.9 to 46.7 mg kg^{-1} soil day^{-1} for the acid sulfate soil (Figure 3.6*a*) and from 31.5 to 37.1 mg kg^{-1} soil day^{-1} for the alluvial soil (Figure 3.6*c*). Maximum fluxes of CO_2 emissions increased ~2.5-fold when glucose and nitrate were added (Figure 3.6*b*, *d*). Fluxes of CO_2 changed over the duration of the experiment; however, no significant interaction effect of salinity × time on emission fluxes was found during the 4-week incubation. Fluxes of CH_4 also peaked at their maximum rate on day 3; however, substrate addition did not affect emissions in either soil. Changes of CH_4 fluxes over the 4-week incubation were similar for both soils with or without substrate amendment (Figure 3.7).

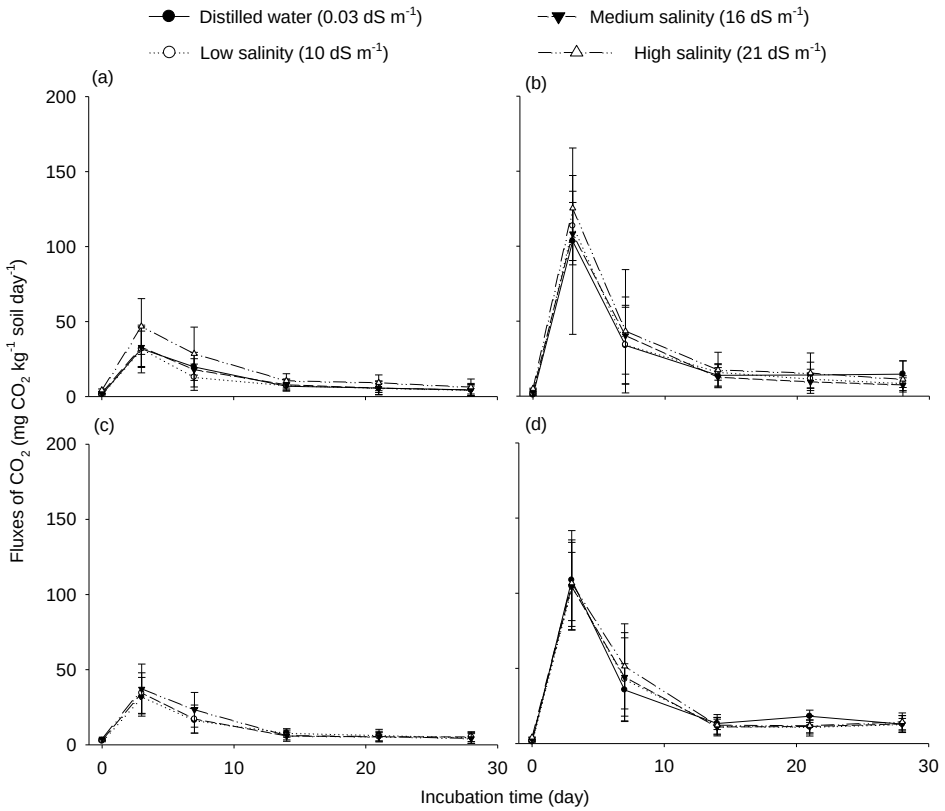

Figure 3.6 CO_2 fluxes during four-week incubation of four salinity levels applied to two soils: acid sulphate soil without (a) and with (b) glucose and nitrate addition; alluvial soil without (c) and with both the addition of nutrients. Bars indicate standard errors of means (n=3).

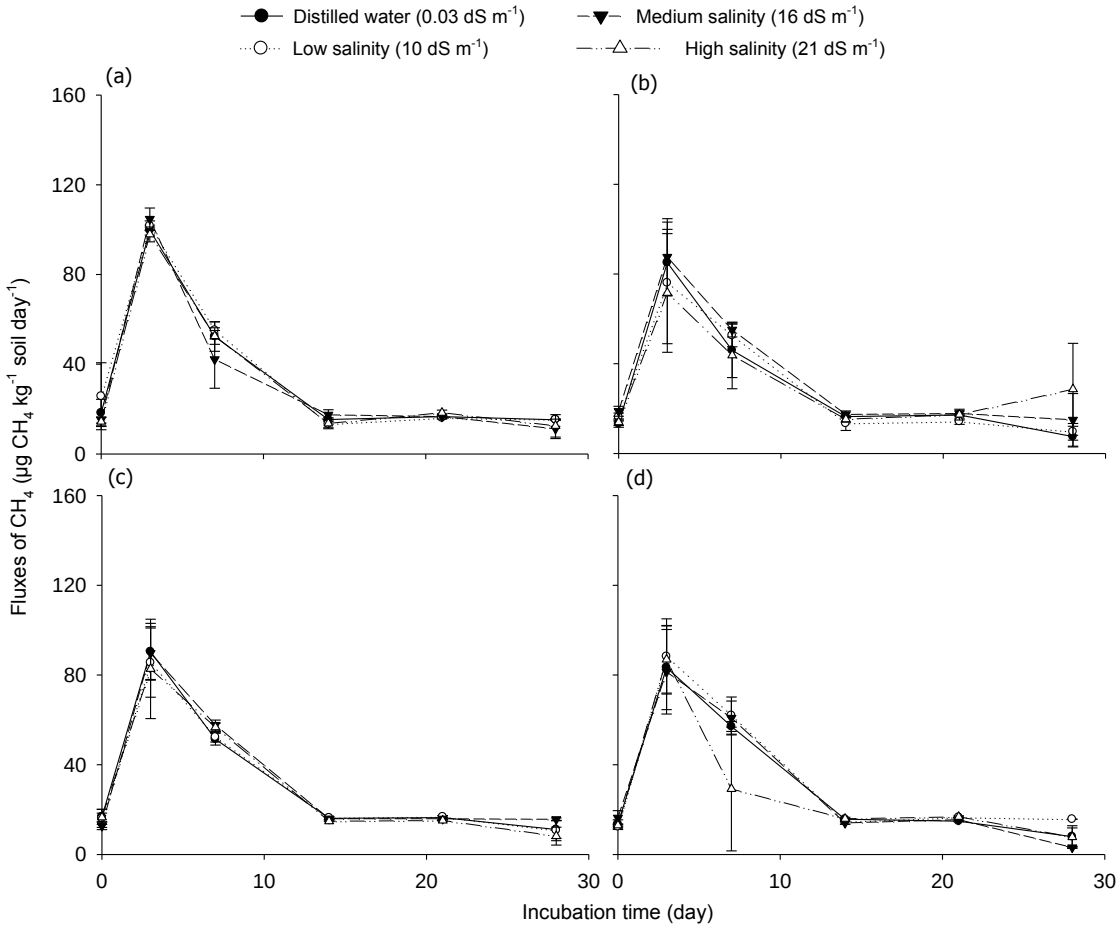

Figure 3.7 Methane fluxes during four-week incubation of four salinity levels applied to two soils: acid sulphate soil without (a) and with (b) glucose and nitrate addition; alluvial soil without (c) and with both the addition of nutrients. Vertical bars indicate standard errors of means (n=3).

3.4 Discussion

3.4.1 Effect of salinity on soil nitrogen and carbon processes

One of the main objectives of this study was to investigate how elevated salinity influences soil nitrogen and carbon processes. In this incubation study, soil samples were flooded with saline and fresh water solution in jars; therefore, a denitrification process, in which nitrate serves as a terminal electron acceptor and is reduced to gaseous end products, would be dominant in this anoxic condition (Buresh et al., 2008). Higher cumulative N_2O-N evolution occurred from the acid sulphate soil than from the alluvial soil (Figure 3.1*a*, *c*). This is caused by the sensitivity of N_2O reductase to proton activity (Ussiri and Lal, 2013) and the lower pH of the acid sulphate soil (Table 3.1). Moreover, ferrous and ferric iron in the acid sulphate soil can be oxidised by soil nitrate in further denitrification, which releases more N_2O (Macdonald et al., 2010). The rate of

N_2O-N production was greatest at the beginning of incubation (Figure 3.3) when soil pore spaces became anaerobic after the addition of saline solutions, leading to an increase of denitrification rate (Inubushi et al., 1999). After 2 or 3 days, the emission rate quickly decreases to zero with the reduction of N_2O to N_2 (Sahrawat and Keeney, 1986).

Salinity caused a decrease in cumulative N_2O-N production in acid sulphate soil relative to fresh water (Figure 3.1*a*). This is due to the inhibition of nitrification and denitrification processes, resulting from the physiological influences of salinity at a microbial level (Inubushi *et al.*, 1999; Rysgaard *et al.*, 1999a). When N_2O-N emission rates peaked after day 3 of incubation, significant differences in the emissions from the saline and FW treatments were observed (Figure 3.3). This phenomenon can be explained by the inhibition of nitrous oxide reductase under saline conditions, resulting in N_2O accumulation from the denitrification process (Menyailo et al., 1997) or under aerobic conditions (Marton et al., 2012).

Although Weston et al. (2006), Poffenbarger et al. (2011) and Marton et al. (2012) found that increasing sulphate decreases CH_4 emissions from soil sediments through decreased methanogenesis, CH_4 production among the treatments in our study did not differ significantly. This result was due to the high variability among replicates. However, the study showed that CH_4 evolution tends to increase in the initial stage of the incubation when methanogenesis occurred and was involved in organic material decomposition (Table 3.2).

Many studies have concluded that elevated salinity has adverse effects on microbial processes in soil through the reduction of enzyme activities through osmotic stress (Pathak and Rao, 1998; Rietz and Haynes, 2003; Setia *et al.*, 2010; Setia *et al.*, 2011a). However, the findings of the present study indicate that soil respiration was only significantly higher in salinity treatments early in the incubation, whereas respiration later in the incubation showed no differences among treatments (Figure 3.5*a*, *c*). This result occurred because the high carbon content in the experiment soils (>6.5%) assisted soil microbes to adapt to adverse environmental conditions and multiply rapidly; therefore, there was no significant difference in soil respiration. A similar result was found by Wong et al. (2009).

3.4.2 Effect of carbon and nitrogen on gas production

The availability of carbon and nitrate in soil has the greatest effect on denitrification (Knowles, 1982). In the present study, glucose and nitrate were added to investigate the effect on gas production from incubated soil slurry. Although both soils contained high amounts of organic carbon (6–7%), the results indicated that the addition of available carbon and nitrogen had strong effects on greenhouse-gas production (Figures 3.1, 3.2, 3.4 and 3.5). This means that substrate was one of the co-limiting factors affecting soil

processes under saline conditions. Glucose is highly decomposable for soil microorganisms (Yamane and Sato, 1964) and is a source of readily available organic carbon. Glucose provides more energy for denitrification than other sources of carbon substrates (e.g. acetate and propionate) due to the presence of more C-C bonds, and requires less energy than these substrates during synthesis of cell materials (Paul et al., 1989). Available carbon stimulates growth and activity of microbes (Zumft, 1997), but carbon is used for energy rather than growth under saline conditions (Mavi and Marschner, 2013). The available carbon in glucose enhances the ability of the microbes to tolerate low osmotic potential (Pathak and Rao, 1998; Mavi and Marschner, 2013) , and the presence of nitrate as an electron donor in anoxic conditions inhibits the reduction of N_2O to N_2, resulting in a high proportion of N_2O emission (Mavi and Marschner, 2013). Therefore, the addition of glucose and nitrate in our study resulted in an increase in the cumulative N_2O-N emission (Figure 3.1*b*, *d*). Lloyd (1993) reported a similar finding, that increased water content and nitrate as well as low pH can increase the $N_2O/(N_2 + N_2O)$ product ratio.

The supply of glucose and nitrate in the present study increased soil respiration, leading to the greater emissions of CO_2 (Figure 3.5*b*, *d*). Because of the effects of available carbon in promoting microbial growth and increasing microbial community tolerance to low osmotic potential, the respiration increased, in accordance with the result of McGill et al. (1981) who proposed that an immediate source of carbon for soil microbial activity resulted in CO_2 evolution. Similarly, higher cumulative CO_2 emissions were found in treatments with high labile organic matter (Tejada et al., 2006). In the present study, microorganisms active in denitrification may utilise carbon substrates for energy and release CO_2, as concluded by Robertson and Groffman (2007).

3.4.3 Implications

Agricultural land covers ~40–50% of Earth's land surface and agricultural activities cause 58% of total anthropogenic emissions of N_2O and 47% of CH_4 (US-EPA, 2006a; Smith *et al.*, 2007). While climate change alters water regime in soils causing huge soil acidification due to exposure of oxidizable sulphide materials to air (Rengel, 2011), future sea-level rise will continue to increase saline water intrusion and the salinization of agricultural lands (Smajgl et al., 2015). Demand for food security results in the exploitation of marginal land, such as saline and acid sulphate soils, for agricultural production. The findings of the present study suggest that salinity reduces greenhouse-gas emissions from acid sulphate soils, but inorganic and organic fertiliser applications could increase N_2O emissions and respiration. This will lead to not only loss of soil nitrogen and carbon, which reduces fertiliser efficiency and crop yield, but also to higher production costs for agricultural activities. Our study shows that salinity and substrate

amendment can alter the soil carbon and nitrogen cycles, suggesting that increasing saline water intrusion and climate change could change the carbon and nitrogen cycles of agricultural production systems in acid sulphate soils.

Although greenhouse-gas emissions responded to salinity and available substrates during the laboratory incubation, actual emissions may differ under field conditions. We conducted the experiment at the constant temperature of 25°C, which is not similar to the field temperature at the sampling sites, and natural saline water was used. The controlled environment of the laboratory allowed us to avoid environmental variability, and the study results can quantify the short-term response of the gas emissions. However, this work could be expanded to predict long-term effects by additional studies under field conditions.

3.5 Conclusions

Our study concluded that a saline water solution reduced the emissions of N_2O in acid sulphate soil, whereas CO_2 emissions were not affected by the salinity. However, the addition of available carbon and nitrate significantly increased both cumulative N_2O and soil respiration over 4 weeks. Methane fluxes reached their maximum within the first 3 days of incubation, but there was no significant difference between salinity treatments over 4 weeks. This study could predict only the short-term effects of salinity on greenhouse-gas emissions under controlled conditions. Further studies under natural conditions need to be conducted for long-term predictions.

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Chapter 4: QUANTIFYING GAS EMISSIONS AND DENITRIFYING GENES IN A SALT-AFFECTED SOIL

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The material contained in this chapter was prepared in a manuscript.

Authorship statement

Manuscript title: Quantifying gas emissions and denitrifying genes in a salt-affected soil.

Dang Duy Minh (PhD Candidate)

I implemented the experiment, analysed of soil and gas samples, interpreted and analysed data, wrote the manuscript and acted as the corresponding author. I hereby certify that the statement of contribution to the manuscript is accurate.

Signature: _____ Date: _____

Craig Strong

I evaluated and provided comments and corrections on the manuscript. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Cao Van Phung

I provided comments and suggestions on development of work. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

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I provided comments and suggestions on development of work. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Sören Warneke

I evaluated and provided comments and corrections on the manuscript. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Ben Macdonald

I supervised development of work, evaluated the manuscript, provided comments and corrections. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signature: _____ Date: _____

Chapter 4: QUANTIFYING GAS EMISSIONS AND DENITRIFYING GENES IN A SALT-AFFECTED SOIL

Abstract

Salinity effects on microbial community relative to greenhouse gas emissions are not well understood in salt-affected soils. A better understanding of this interaction would be useful for agricultural practices to reduce nitrogen gas losses and manage environmental pollution. We hypothesized that interaction of elevated salinity and substrate addition would increase the abundance of denitrifier genes resulting in a high rate of denitrification. The objectives of this study were to measure induced-soil greenhouse gas emissions and to quantify denitrifying genes in a salt-affected soil over a 3-week incubation period. This incubation study was conducted by submerging field-moist samples of an acid sulphate soil in different saline solutions: 0.03 dS m^{-1} (distilled water), 10 dS m^{-1} (low salinity), 16 dS m^{-1} (medium salinity) and 21 dS m^{-1} (high salinity). A quantitative real-time PCR was used to quantify the abundance of resident bacterial denitrification genes in the salt-affected soil. It was found that increased salinity caused a decrease in both flux and cumulative emission of N_2O from the incubated soil, relative to fresh water. Soil respiration was significantly reduced in salinity treatments compared to the distilled water treatment. The study results showed that elevated salinity increased the denitrifying genes in the incubated acid sulphate soil. Abundance of the *nir* genes was usually high between the first and second week of incubation, while number copies of the *nos*Z gene were significantly low at those times. The study concludes that salinity modifies the biological aspects of denitrification leading to a reduction of greenhouse gas emissions. Findings from this investigation extend our knowledge about the underlying molecular ecological mechanisms of denitrification that manage nitrogen cycling in salt-affected soils.

Keywords: salinity effects, gas emissions, denitrifying genes, nitrous oxide, qPCR.

4.1 Introduction

Sea-level rise due to global climate change is increasing salt water intrusion into freshwater resources and would cause serious impacts for many coastal and agricultural areas worldwide in the future. DeConto and Pollard (2016) indicated that sea-level rise could be more than 1 m by 2100 and 15 meters by 2500 due to ice cliffs in Antarctica. Coastlines around the world will be affected by rising sea level by the end of the 21st Century (Cazenave and Cozannet, 2014). A 1-m rise in sea-level could affect ~0.4% of

the total agricultural land, while a rise 5-m could extend this effect to 2.1% (Dasgupta et al., 2009). Seawater intrusion by sea-level rise and freshwater discharge reduction would cause a shortage of freshwater resources and a decrease in irrigation water (Snoussi et al., 2008). Hence, the area available for crop production will be negatively impacted by salinity with increases in the future. Salinity effects and land-use change can alter soil nutrient cycling and lead to soil degradation and a lowering of soil fertility. On marginal soils including salt-affected soil, the addition of fertilizers and/or alternative amendments such as agrochemicals or lime will be required to achieve higher yields (Baligar and Fageria, 2015), adding to the costs of food production.

Nitrogen (N) is the most important plant nutrient required and determined the crop production (Dass et al., 2015) and it is also one of the most yield-limiting nutrients on crop growing regions globally (Fageria et al., 2015). The recovery efficiency of N is lower than 50% in most cropping systems (Fageria, 2014). Losses and transformations of nitrogen within the soil-plant system affect N availability to plants and N transfer into the wider environment (Cameron et al., 2013). In agricultural systems, mineral-N in soil is mainly lost through volatilization, leaching, denitrification, and soil erosion (Fageria and Baligar, 2005a; Cameron *et al.*, 2013). In wet soils where anaerobic conditions or low oxygen availability condition, denitrification - a microbial process of reducing nitrate and nitrite to gaseous forms of nitrogen, principally nitrous oxide (N_2O) and nitrogen (N_2) is a dominant process in soil nitrogen transformation (Firestone, 1982; Bateman and Baggs, 2005).

Many studies have investigated the impacts of salinity on soil nitrogen cycling (Irshad *et al.*, 2005; Kumar *et al.*, 2007; Lodhi *et al.*, 2009; Akhtar *et al.*, 2012). A decrease in the mineralization of N was found under saline conditions and higher moisture regimes (Lodhi et al., 2009). Higher salinity level promotes the loss of nitrogen in the form of ammonia (Akhtar et al., 2012). Irshad et al. (2005) observed decrease in nitrification with increasing salinity possibly due to adverse osmotic effects on autotrophic nitrifiers. One study reported the sensitivity of denitrifying microbial consortia to rapid shifts in salinity; pulses of intermediate saline water (15 ppt) increased denitrification by 75%, while similar pulse of seawater (35 ppt) suppressed potential denitrification by 73% (Marks et al., 2016). Salinity reduces microbial biomass mainly due to the osmotic stress leading to drying and lysis of cells (Pathak and Rao, 1998). On the other hand, salinity also decreases microbial activity, microbial biomass and changes microbial community structure (Setia et al., 2011b). Activities of urease, alkaline phosphatase, β-glucosidase were strongly inhibited by salinity (Pan et al., 2013).

In environmental investigations and laboratory studies, among denitrifying genes the *nirS*, *nirK* and *nosZ* genes have been received more scientific interest than other

denitrifying genes (e.g. *napA*, *narG* and *cnorB*) (Hu et al., 2015) because their abundance and structure could be potential indicators of denitrification-derived N_2O fluxes in soils (Morales et al., 2010). The reduction of nitrite (NO_2^-) to nitric oxide is catalysed by two different types of nitrite reductases (Nir), encoded by *nirS* or *nirK* (Kandeler et al., 2006). The reduction of nitrous oxide in the final step of the denitrification is catalysed by nitrous oxide reductase encoded by *nosZ* gene (Zumft, 1997). Yoshie et al. (2004) investigated the diversity of *nirK* and *nirS* in denitrifying bacteria and concluded that salinity decreased *nir* gene diversity in a nitrate-containing saline wastewater treatment system. In another study Miao et al. (2015) characterized the alteration of various denitrifying genes, functional gene abundance and nitrogen metabolic pathways in an expanded granular sludge bed reactor treating high-nitrate wastewater. The study found that a decrease of salinity stress enhanced the biodiversity of the denitrifying bacteria carrying the functional genes. Despite the many studies on denitrifying genes, the effects of salinity on a microbial community relative to greenhouse gas (GHG) emissions are not well understood in a salt-affected soil. A better understanding of this interaction in agricultural systems might help reduce soil gas emissions, enhance our knowledge on nitrogen reduction pathway in the salt-affected soil, and support for management efforts on agricultural nutrient input in the future.

Findings from the chapter 3 showed that the substrate addition enhanced the GHG emissions from salt-affected soils. However, mechanism of the emission increase has not been observed. In this chapter, interaction of elevated salinity and substrate addition was hypothesized to increase the activity of denitrifier genes leading to high rate of GHG emissions. This study measured GHG emissions over time and analysed denitrification genes including *nirK*, *nirS* and *norZ* in a salt-affected soil over a 3-week incubation period. Quantitative real-time PCR was used to quantify the abundance of these bacterial genes in the study soil.

4.2 Materials and methods

4.2.1 Sampling sites and soil collection

Description of sampling sites

An acid sulphate soil (ASS) managed as a pasture soil was collected from Nowra, on the south coast of New South Wales, Australia (34°49'S, 150°39'E). The elevation of this area varies from 0.5 to 2.5 m above sea level with average annual rainfall of 1,135 mm. The soil collected was classified as a Hydrosol (Isbell, 2002) and the site has a dark loamy topsoil (Lawrie and Eldridge, 2004). The soil surface of the sampling site was covered by ryegrass. Soil pH through the 2-m soil profile varied from 3.09 to 5.63, and < 4 within one meter below the soil surface. Total soil nitrogen was 0.60% and total carbon was 7.31%.

Collection of soil samples

Surface soil samples (0-15 cm) from the sampling site were collected and placed in plastic bags, stored in isolated containers and delivered to the laboratory within 5 hours. The collected samples were sieved (< 2 mm) and mixed well before representative subsamples were collected and used for the incubation experiment.

4.2.2 Soil extraction and analysis

Soil pH and EC were measured in 1:5 soil:water extract after end-over-end shaking at 25°C in a closed system for 1 h (Rayment and Lyons, 2011). Soil ammonium and nitrate were extracted with a 2 M KCl solution and determined following the method proposed by Keeney and Nelson (1982). Total carbon and nitrogen were analysed based on Dumas high-temperature combustion by using a Europa 20-20 isotope ratio mass spectrometer with an Automated Nitrogen Carbon Analysis (ANCA) preparation system. Nitrogen and carbon content were measured by a mass spectrometer for the N_2 and CO_2 peaks sequentially (Nelson and Sommers, 1996; Rutherford *et al.*, 2007; Rayment and Lyons, 2011).

4.2.3 Incubation experiment for measurement of greenhouse gases

Field-moist samples of an acid sulphate soil (20 g) were submerged in a 125-mL jar with 15 mL of different saline solutions: 0.03 dS m^{-1} (distilled water), 10 dS m^{-1} (low salinity), 16 dS m^{-1} (medium salinity) and 21 dS m^{-1} (high salinity). The jar was swirled for 1 min to ensure adequate mixing. To the incubation jar we added 5 mL of solution providing both 300 µg glucose-C and 50 µg NO_3-N g^{-1} soil. This treatment was applied to ensure that denitrification was not limited by nitrogen or carbon supply (Luo et al., 1996). Each treatment was replicated with three jars. Soil samples in all jars were therefore submerged in a 20-mL solution; this resulted in a water level of 5 mm above the soil surface. This constant water level was used because a difference in water level above soils can affect gas emissions, rates of movement through water being much slower than through air. The experiment was conducted with a completely randomized design, and each treatment was replicated with three jars. After substrate addition, activation time of 24 hours in the dark at 25°C was set for soil slurries to activate microbial activities before starting GHG emission measurement. Jars were left opened for the duration of the experiment and only closed when sampling gas. Loss of water in a jar was compensated every day by adding deionized water.

Plate 4.1 Soil with different salinity level was incubated in jars at constant temperature (25^{o}C). The photo was taken before sampling headspace gas.

4.2.4 Gas sampling and analysis

Headspace gas was sampled using a gas-tight syringe after 0, 1, 2, 3, 5, 7, 14, 21 days of incubation. Results from the chapter 3 of the present thesis showed that sampling deployment of sampling interval was 20 minutes with maximum 80-minute closure. Therefore, in this chapter 4 the study applied the suggestion from the chapter 3 for headspace gas sampling. Jars were sealed with a butyl rubber stopper for 60 minutes and the samples of headspace gas (6 ml) were collected at 0, 20, 40, and 60 minutes after closing the experiment jar. The headspace samples were transferred to an evacuated gas-tight vial (3.7 ml) and analysed within a week. Helium (6ml) was returned to each jar after collecting the headspace gas. Gas samples and standards were analysed by a GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an electron capture detector to determine N_2O emission from the soil slurries. A flame ionisation detector was also connected to the gas chromatograph to detect other gases such as methane (CH_4) and CO_2. A detailed description of the configuration and working condition of the gas chromatograph is presented in Poole (2012).

4.2.5 Soil microbial gene analysis

DNA extraction

Soil samples from destructive jars were collected from the incubation jars for molecular analysis after gas sampling at day 0, 7, 14 and 21. Soil samples were stored at -80^{o}C until the extraction of soil DNA and analyzing soil microbial genes. Three replicates were used per one experimental treatment and the number of incubated jars were sufficient to 4 sampling times. Soil DNA was extracted from 0.25g soil samples of the destructive samples using a PowerSoil™ DNA Isolation Kit following the manufacturer's instructions (MO Bio Laboratories Inc., Carlsbad, California USA). DNA quality was verified by running the DNA extract on a 1% (w/v) agarose gel, stained with SYBR Safe Gel stain (Invitrogen) and visualized under UV light (Bio-Rad Gel Doc XR). DNA quantitation was conducted by using a Qubit® Fluorometer with the Qubit® ds DNA BR Assay Kits (Thermo Fisher Scientific).

Real-time PCR of soil genes

The copy numbers of microbial genes were quantified by quantitative real-time PCR (qPCR) which is a specific, highly sensitive, and rapid method (D'haene et al., 2010). Fragments of soil genes were amplified from the extracted DNA with following primer pairs nirSCd3aF/nirSR3cd for *nirS* documented by Throbäck et al. (2004) and Kandeler et al. (2006); nosZ1F/nosZ2R for *nosZ* and nirKF1aCu/nirK5R for *nirK* described in Hallin and Lindgren (1999) and in Kloos et al. (2001). All primer sets and sequences used for amplifying each gene in qPCR are presented in Table 4.1. Quantitative PCR was performed on a Bio-Rad CFX96™ Real-Time system (Biorad Laboratories, USA) with SsoAdvanced™ SYBR® Green SuperMix (premix of dNTPs, Taq DNA polymerase, PCR buffers and SYBR green) (Biorad). Each qPCR reaction contained 2 µL of 2 ng genomic DNA, 200 nM each primer and the 2x SsoAdvanced supermix in a final volume of 10 µL. Each qPCR reaction was accompanied by triplicates and 3 negative (no DNA) controls. Standard curves of templates were made by 10-fold serial dilutions of linearized recombinant plasmids harbouring amplicon amplified from soil DNA.

The qPCR programmes consisted of an initial denaturing temperature of 98^{o}C for 2 minutes followed by 40 cycles of denaturing at 98^{o}C for 5s and a 30s combined annealing and extension step at 60^{o}C for *nirS*, 53^{o}C for *nosZ* and 61^{o}C for *nirK*. Product melt curves were calculated at the end of each qPCR reaction using a continuous thermal gradient of 65^{o}C to 95^{o}C. At the end of the melt curve analysis, amplified products were analysed on 1% agarose gel to confirm the expected size of gene fragments.

Table 4.1 Specific primer sets used for gene amplification in qPCR assays.

Gene	Primer	Sequence (5' – 3')	Referrence
nirS	nirSCd3aF	GTS AAC GYS AAG GAR ACS GG	(Kandeler *et al.*, 2006)
	nirSR3cd	GAS TTC GGR TGS GTC TTG A	(Throbäck *et al.*, 2004)

nirK	nirKF1aCu	ATC ATG GTS CTG CCG CG	(Hallin and Lindgren, 1999)
	nirK5R	GCC TCG ATC AGR TTR TGG TT	(Hallin and Lindgren, 1999)
nosZ	nosZ1F	CGY TGT TCM TCG ACA GCC AG	(Kloos *et al.*, 2001)
	nosZ1622R	CGS ACC TTS TTG CCS TYG CG	(Throbäck *et al.*, 2004) (Enwall *et al.*, 2005)

4.2.6 Data calculation and statistical analysis

Gas emissions

Atmospheric pressure and temperature in the laboratory were recorded at sampling point. As helium gas was returned after headspace sampling, volumetric gas concentration was recorded at each time of sampling before it was converted to mass gas concentration by the following equation 1:

$$C_{mass} = (C_{volumetric} \times P \times MW_{gas}) / (T \times R) \qquad (1)$$

where C_{mass} is mass concentration ($\mu g\ L^{-1}$), $C_{volumetric}$ is volumetric concentration (ppm), P is ambient air pressure (atm), MW_{gas} is molecular weight of the gas (g $mole^{-1}$), T is ambient air temperature (^oK), and R is the ideal gas constant (L atm K^{-1} $mole^{-1}$).

Emission data were calculated by fitting a linear regression model (Petersen et al., 2006; Gao et al., 2014). Slopes of the regression were used to estimate gas fluxes. The gas fluxes were converted to the gas emissions per gram soil basis following equation 2. Cumulative gas emissions from each replication were calculated from the integrated daily fluxes, assuming a constant flux rate, beginning with the date of each gas sampling until the next gas sampling. This is the best approximation of gas emission rates and is commonly used (Chao et al., 2000; Merino et al., 2004; Yang et al., 2015). The data of gas fluxes and cumulative emissions were based on oven-dried weight.

$$F = S * V * W_{soil}^{-1} \qquad (2)$$

where F is flux of greenhouse gases ($\mu g\ hr^{-1}\ g^{-1}$), S is slope of the regression ($\mu g\ L^{-1}\ hr^{-1}$), V is headspace volume (L), and W_{soil} is weight of soil used in an incubated jar (g).

Copy number of genes

The efficiency and data of qPCR was calculated by an absolute method and the copy number of a targeted gene was determined following the instruction in Videmšek et al. (2009) and Brzoska and Hassan (2014). In a spreadsheet, an XY scatter plot of the Cq values versus the log of the DNA standard was performed to calculate equation 3 of the linear regression line in the form:

$y = mx + b$ (3)

In this standard-curve based copy analysis, *y* is the Cq value, *x* is log of the copies of the DNA standard, *m* is slope and *b* is constant. Therefore, the qPCR efficiency (*e*) and copy number (*N*) were calculated by the following equations 4 and 5, respectively. The data for gene copies were based on an oven-dried weight of soil.

$e = [10^{-(1/m)}] - 1$ (4)

$N = \text{Antilog}\ [(Cq - b) / m)]$ (5)

Statistical analysis

Statistical analysis was conducted with the SPSS software version 16 (IBM SPSS, New York) or R statistical software version 3.0.2 (The R Foundation, Vienna, Austria). Prior to the analysis, data were tested for homogeneity of variances and normality and the results of data skewness tests were accepted. When normality of data and homogeneity of variances were not found, a data transformation was conducted to stabilize the variance prior to an analysis of variance (ANOVA). The results of N_2O and CO_2 fluxes from soils were analyzed by using the repeated measures ANOVA procedure. A two-way ANOVA was performed to test the interaction effects of salinity and times for cumulative N_2O emission, cumulative CO_2 emission, and denitrifying gene copies. Tukey's HSD test was used to identify differences among treatments at $P<0.05$. Linear regression was also performed between Σnir / *nosZ* and the N_2O fluxes.

4.3 Results

4.3.1 Soil properties

The bulk density of the collected sample was 1.02 g cm^{-3}. The ASS sample was strongly acidic, with soil pH values <5. Total N of the top soil (0-15 cm depth) was 0.51%. The content of NH_4^+-N and NO_3^--N was 92.50 and 12.51 mg kg^{-1}, respectively. Total C was higher, at 6.5%, resulting in a C/N ratio of 14. Total S of the experimental soil was 0.65% and the soil texture of the ASS was determined to be sandy loam (Table 4.2).

Table 4.2 Characteristics of the experimental sample (0–15 cm). Data are means (n = 3) followed by standard errors of means.

Soil parameters	Acid sulphate soil
pH	3.92 ± 0.01
EC (dS m^{-1})	1.79 ± 0.01
Total carbon (%)	7.11 ± 0.12
Total nitrogen (%)	0.51 ± 0.01
Total sulphur (%)	0.65 ± 0.02

NO_3^- - N (mg kg^{-1})	12.51 ± 1.09
NH_4^+ - N (mg kg^{-1})	92.50 ± 6.42
Bulk density (g cm^{-3})	1.02 ± 0.07
Soil texture (%)	
Sand	54.84 ± 1.07
Silt	35.54 ± 0.71
Clay	9.62 ± 0.58

4.3.2 Gas fluxes

N_2O emissions

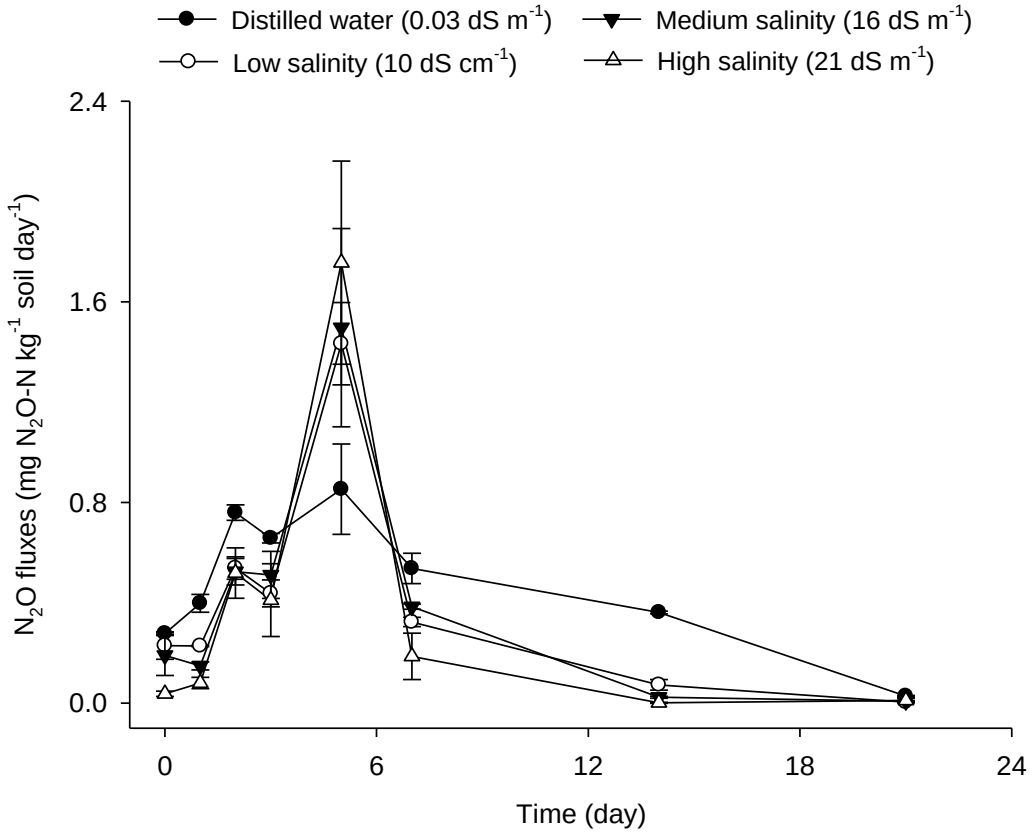

Figure 4.1 Fluxes of N_2O-N emission from soils treated with different salinity during the three-week incubation. Capped lines are standard errors of means (n = 3), which are not always visible because the standard error is smaller than the symbol.

Fluxes of N_2O-N from elevated salinity treatments were usually less than those from the distilled water (DW) treatment (Figure 4.1). However, the fluxes from the salinity treatments were greater than 1.43 mg N_2O-N kg^{-1} soil day^{-1} at day 3 and significantly greater than that for the DW treatment, at 0.85 mg N_2O-N kg^{-1}. After a 1-week incubation, the fluxes rapidly reduced in the salinity treatments, ranging from 0.19 to 0.38 mg N_2O-N kg^{-1} soil day^{-1} while the decrease was less in the DW (0.54 mg N_2O-N kg^{-1} soil day^{-1}). The DW treatment continued to release N_2O gas for up to day 14 (0.36 mg N_2O-N kg^{-1}

soil day^{-1}) while the other salinity treatments show significantly lower gas production rates (< 0.073 mg N_2O-N kg^{-1} soil day^{-1}). After 3-week incubation period, all study treatments were similar in the gas fluxes (Figure 4.1).

Cumulative N_2O-N emission in the DW significantly increased over the 3-week incubation period while the salinity treatments only showed a significant increase in cumulative N_2O-N by the second week (Figure 4.2). A large difference in the cumulative N_2O-N was found within the first week of incubation. An approximately 20-fold increase in emissions was recorded from the beginning of the incubation (week 0) to week 1 but there were no significant differences between treatments at these times. The salinity treatments did not result in an increase in cumulative gas between week 2 and week 3; the cumulative emissions were 6.27 – 7.51 mg N_2O-N kg^{-1} soil. The DW showed significantly higher emissions compared to the salinity treatments in both week 2 and week 3. The cumulative emissions of N_2O in the DW were 8.22 and 10.75 mg N_2O-N kg^{-1} soil at weeks 2 and 3, respectively (Figure 4.2).

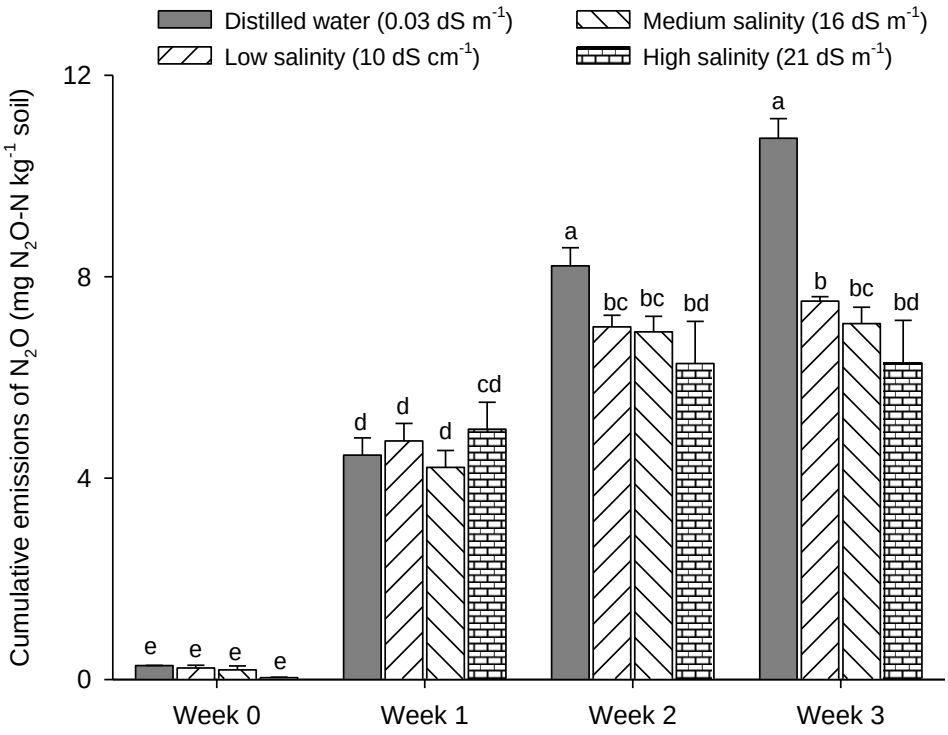

Figure 4.2 Cumulative N_2O-N emission from soils treated with different salinity during the three-week incubation. Capped lines are standard errors of means (n = 3), which are not always visible due to the small data set.

CO_2 emissions

Flux rates of CO_2 emission in all treatments peaked after 2-day incubation (Figure 4.3). High variation in the CO_2 fluxes was found before the peak time. The average of

maximum CO_2 fluxes on day 2 ranged from 20.9 to 28.8 mg kg^{-1} soil day^{-1}. The CO_2 fluxes slightly increased on day 5 after a rapid decline on day 2. The low and high salinity treatments continually decreased at one week's incubation and the fluxes of these treatments were significantly less than that from the DW at 14.65 mg kg^{-1} soil day^{-1}. This difference was also presented in the second week, however CO_2 fluxes in all treatments were not significant at the end of the experiment (Figure 4.3).

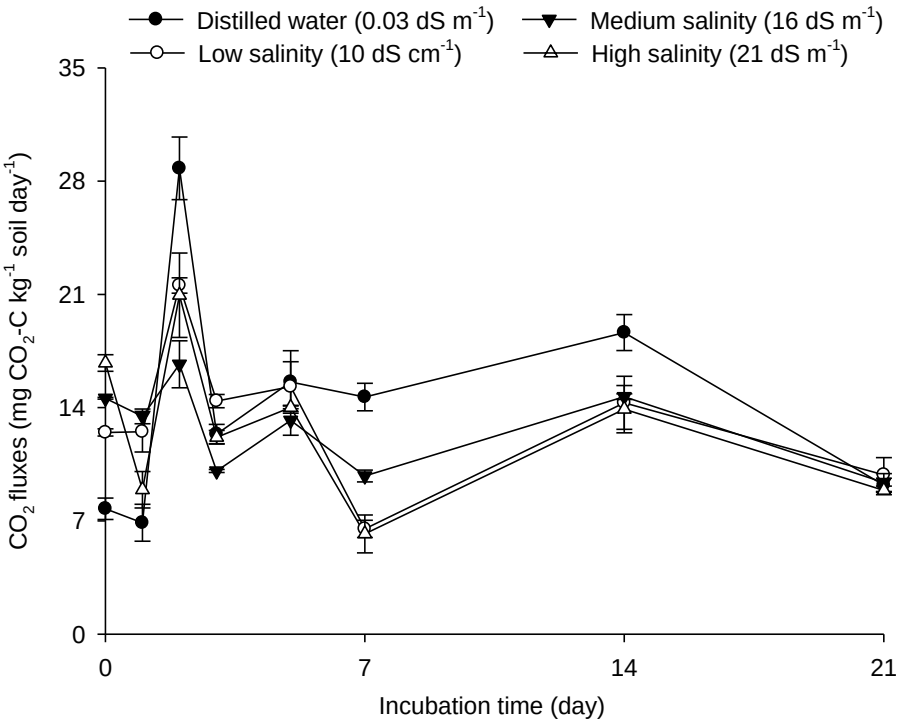

Figure 4.3 Fluxes of CO_2-C emission from soils treated with different salinity during the three-week incubation. Capped lines are standard errors of means (n = 3), which are not always visible because the standard error is smaller than the symbol.

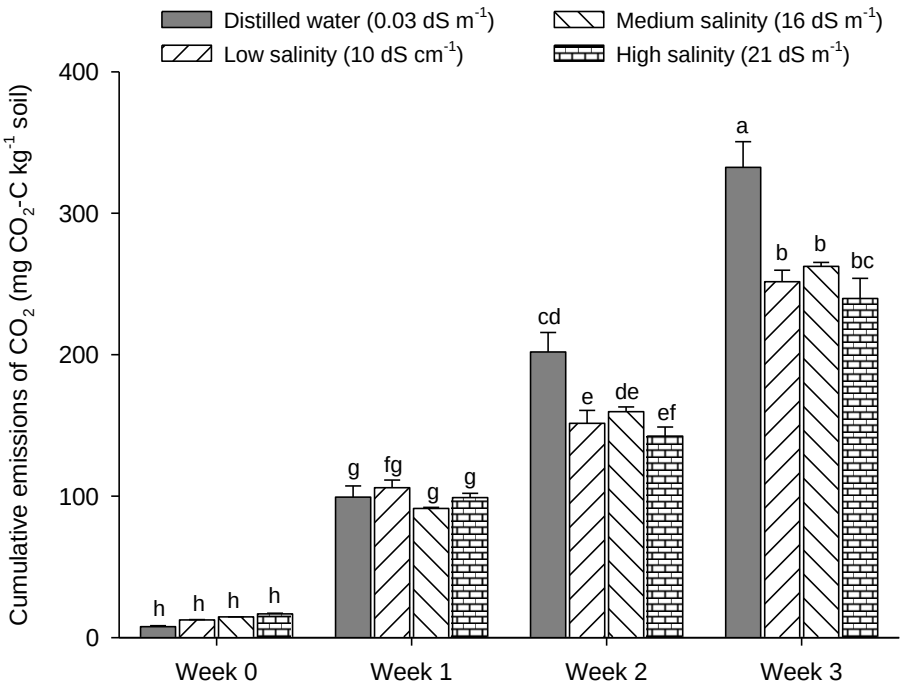

Figure 4.4 Cumulative CO_2-C emission from soils treated with different salinity during the three-week incubation. Capped lines are standard errors of means (n = 3), which are not always visible due to the small data set.

Cumulative CO_2 emission increased over the time of the experiment (Figure 4.4). Among the elevated salinity treatments, there were no significant differences in the cumulative CO_2 emissions at any week of the incubation. The cumulative CO_2 emissions in the DW were significantly higher than in the other elevated salinity treatments after 2 and 3 weeks. The emissions in the DW were 202 and 332 mg CO_2-C kg^{-1} soil for the second and third week, respectively. From the beginning of the incubation, the cumulative emissions in the DW increased more than 40-fold while the other treatments showed a 15- to 20-fold time increase at the end of the incubation period (Figure 4.4).

4.3.3 Soil microbial genes

Abundance of nirK gene

The abundance of *nirK* genes was not significant among the experimental treatments at the beginning and after 3 weeks (Figure 4.5). Number copies of the *nirK* gene ranged from 5.32 x 10^5 to 9.73 x 10^5 copies g^{-1} soil at the beginning and between 4.36 x 10^5 and 8.55 x 10^5 copies g^{-1} soil at the end of the study. The fresh water and low salinity treatment in the end of week 1 recorded significantly low scores (2.04 x 10^7 and 2.07 x 10^7 copies of *nirK* g^{-1} soil, respectively) relative to high salinity. The abundance of *nirK* ranged from 1.98 x 10^7 to 2.35 x 10^7 copies g^{-1} soil for the salinity treatments while the DW treatment had a significantly high copy number at 3.19 x 10^7 copies of *nirK* g^{-1} soil in the second week (Figure 4.5).

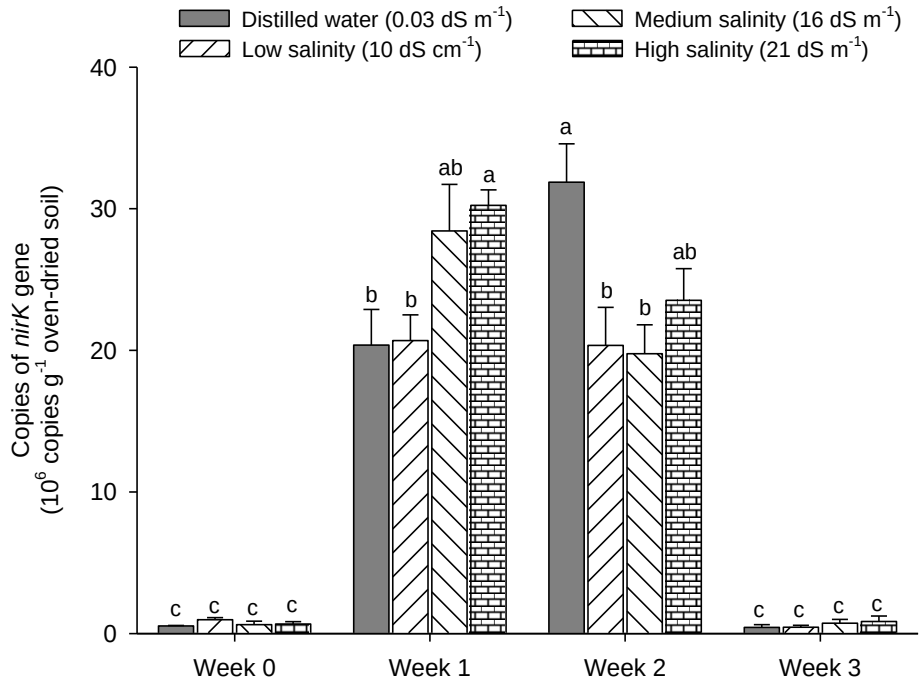

Figure 4.5 Abundance of *nirK* gene (copies g^{-1} dried soil) from soils treated with different salinity over the three-week incubation. Capped lines are standard errors of means (n = 3), which are not always visible due to the small data set.

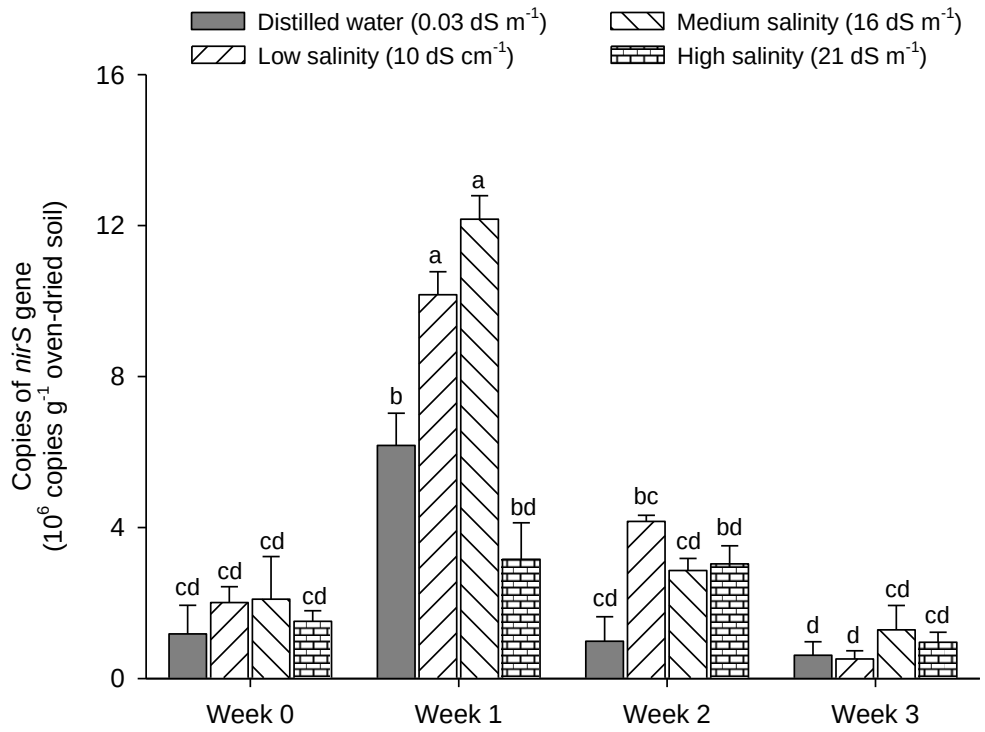

Figure 4.6 Abundance of *nirS* gene (copies g^{-1} dried soil) from soils treated with different salinity over the three-week incubation. Capped lines are standard errors of means (n = 3), which are not always visible due to the small data set.

Abundance of nirS gene

Generally, the copies of *nirS* gene increased in all treatments, but except the high salinity treatment in the 1st week and then quickly reduced thereafter (Figure 4.6). The abundance of the *nirS* gene was similar among the treatments over the duration of experiment, except in week 1. In this first week, the low and medium salinity had the greatest copies of the *nirS* gene, at 1.02×10^7 and 1.22×10^7 copies g^{-1} soil. On the other hand, a significant number of low copies were found in the DW and high salinity treatment. There were only 6.18×10^6 and 3.16×10^6 copies of *nirS* gene g^{-1} soil for the DW and high salinity (Figure 4.6), respectively.

Abundance of nosZ gene

The abundance of the *nosZ* gene ranged from 2.74×10^7 to 5.27×10^7 copies g^{-1} soil among the treatments at the beginning of the incubation, but there was no statistically significant difference among them (Figure 4.7). The abundance of this gene increased markedly between week 2 and week 3. The high salinity treatment showed significantly higher numbers of copies of the gene at all measured times, varying from 1.52×10^7 to 8.29×10^7 copies of *nosZ* gene g^{-1} soil. Although other treatments also increased in *nosZ* copies after the first week, there were no significant differences among them (Figure 4.7).

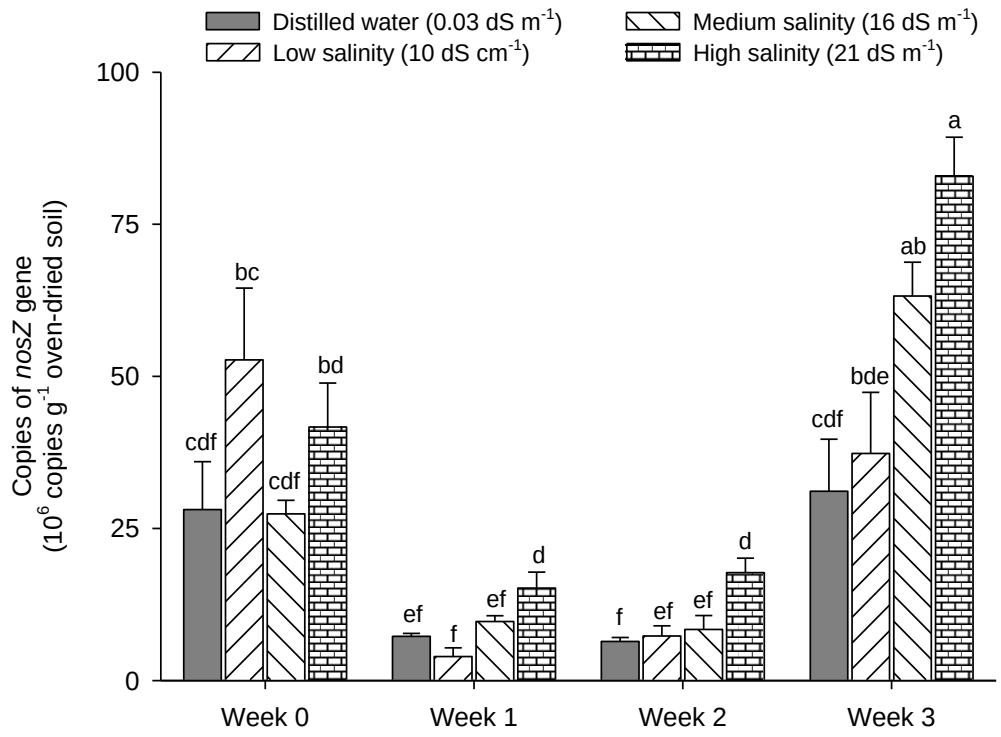

Figure 4.7 Abundance of *nosZ* gene (copies g^{-1} dried soil) from soils treated with different salinity over the three-week incubation. Capped lines are standard errors of means (n = 3), which are not always visible due to the small data set.

Correlation between N_2O fluxes and denitrifying genes as a function of salinity

Linkages between N_2O fluxes (mg N_2O-N kg^{-1} soil day^{-1}) and Σ*nir* / *nosZ* ratio were dependent on the concentration of soil salinity (Figure 4.8). The distilled water treatment had a significant correlation between N_2O fluxes and ratios of denitrifying genes ($R = 0.62$, $F = 26.01$, $P < 0.01$) (Figure 4.8*a*). Similarly, N_2O fluxes from incubated soil was also significantly correlated with the ratio of Σ*nir* / *nosZ* gene (Figure 4.8*b*) in the low level of salinity ($F = 6.39$, $P < 0.05$). Correlation between the N_2O fluxes and the ratios of Σ*nir* / *nosZ* gene could not be explained by a linear model because of low Pearson Coefficient of Determination ($R = 0.05$, in the medium salinity and $R = 0.32$, in the high salinity).

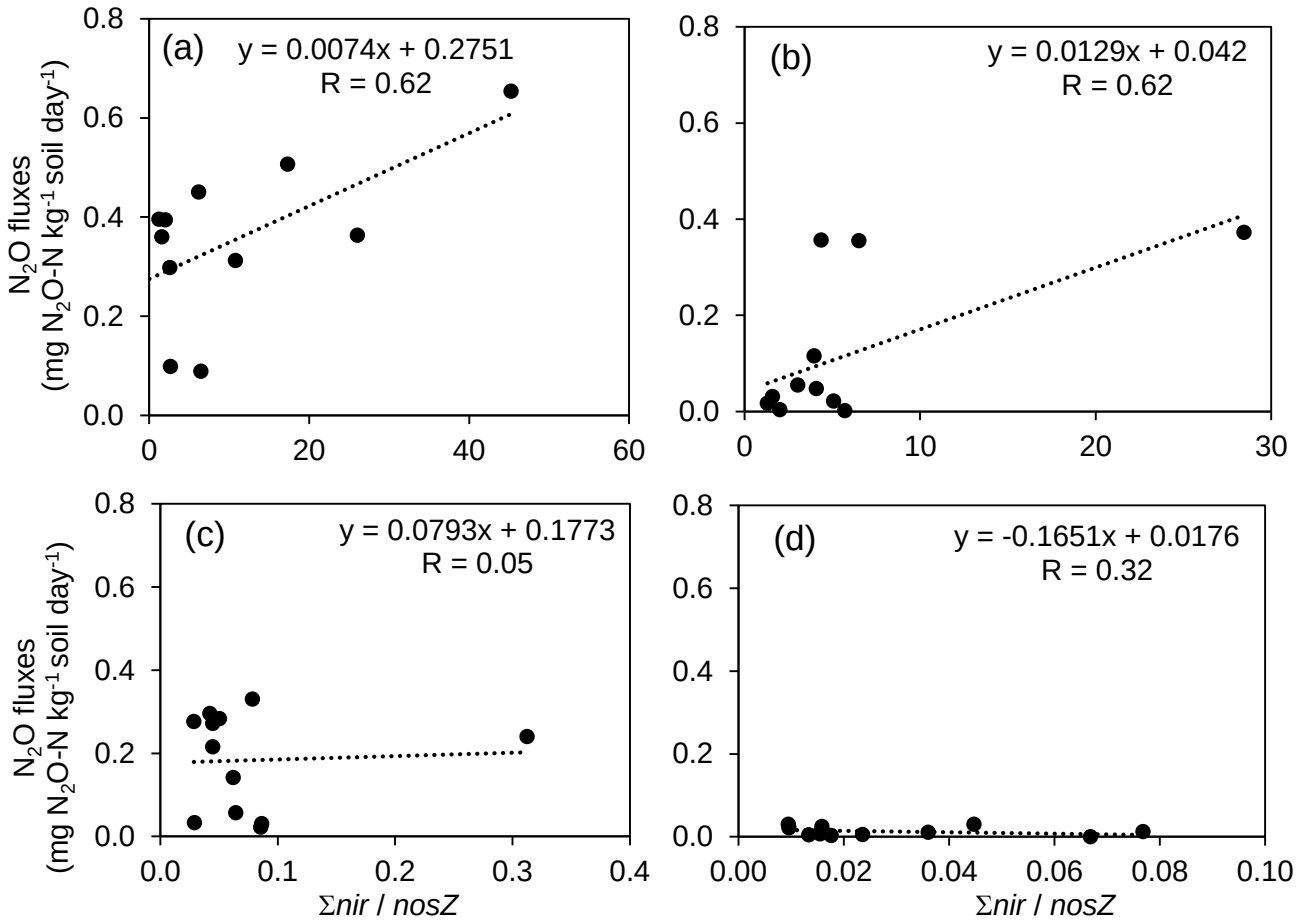

Figure 4.8 Linkages between N_2O fluxes (mg N_2O-N kg^{-1} soil day^{-1}) and Σ*nir* / *nosZ* ratio as a function of soil salinity over the three-week incubation. Graph (*a*) presents for the treatment of distilled water; (*b*) for the treatment of low salinity; (*c*) for the treatment of medium salinity; and (*d*) for the treatment of high salinity. Linear regression statistics are reported in text.

4.4 Discussion

4.4.1 Effects of salinity on soil gas fluxes

One of the main objectives of this study was to investigate how elevated salinity influences soil nitrogen and carbon processes leading to the greenhouse gas emissions. An experimental incubation was conducted by inundating the acid sulfate soil with different saline solutions leading to oxygen shortage; as a consequence, soil denitrification would be dominant in this anaerobic condition. The rate of N_2O-N production was high at the beginning of incubation (Figure 4.1) when soil pore spaces likely became anaerobic after the addition of saline solutions, leading to an increase in the denitrification rate (Inubushi et al., 1999). After peaking at day 5, however, the N_2O-N fluxes quickly decreased to zero because of the reduction of N_2O to N_2 (Sahrawat and Keeney, 1986). Elevated salinity caused a decrease in both flux and cumulation of the N_2O-N production relative to fresh water (Figures 4.1, 4.2). The decrease in the N_2O production from the salinity most likely occurred due to due to inhibiting denitrification processes, resulting from the physiological influences of salinity at a microbial level (Inubushi *et al.*, 1999; Rysgaard *et al.*, 1999b). When N_2O-N emission rates peaked on day 5, significantly high emissions were recorded from the saline treatments relative to DW treatments. This finding can be explained as being due to the inhibition of nitrous oxide reductase under saline conditions, resulting in N_2O accumulation from the denitrification process or under aerobic conditions (Menyailo et al., 1997; Marton et al., 2012). The result is consistent with the finding of Dang *et al.* (2017). Because a significant flux difference was found between the DW treatment and salinity treatments after day 7, a significant difference in the cumulative production was recorded in the second week and the end of the incubation (Figure 4.2).

Available carbon plays a role in promoting microbial growth and increasing microbial community tolerance to low osmotic potential (Tejada et al., 2006; Dang *et al.,* 2017). An application of glucose in the present study may have supported an increase in soil respiration on day 2 (Figure 4.3). McGill et al. (1981) proposed that applying an immediate source of carbon for soil microbial activity resulted in CO_2 evolution. Although the fluxes of CO_2-C declined after they peaked, the rate immediately increased over 7 to 14 days due to longer-term resilience of microbial communities to salinity pulsing events. Chambers et al. (2013) reported that rates of soil organic C cycling typically returned to pre-pulsed levels within 9 days of an event, even when the salinity difference was higher or lower than the typical ambient salinity of the soil.

Many studies have concluded that elevated salinity has adverse effects on microbial processes in soil, by reducing enzyme activities through osmotic stress (Pathak and Rao, 1998; Rietz and Haynes, 2003; Setia *et al.*, 2011b). Conversely, Chambers et al. (2011)

indicate that microbial populations rebound quickly from ionic stress; therefore, the intrusion of diluted seawater into freshwater wetlands can accelerate organic C mineralization through the short-term increase in respiration without inhibiting methanogenesis. However, findings from the present study showed that soil respiration reduced significantly in salinity treatments relative to the DW treatment. This result can be explained by salinity interrupting cellular function, growth, and can even lead to cell lysis (Frankenberger and Bingham, 1982; Saviozzi *et al.*, 2011; Rath and Rousk, 2015). Although microbial respiration directly correlates with environmental parameters, bioavailable carbon, pH and abundance of Archaea and Bacteria are important in explaining CO_2 flux rates (Lammel et al., 2015). The present study has not analyzed for the abundance of Archaea and Bacteria genes, therefore further studies should be taken into consideration to explain the relationships between these genes and the CO_2 fluxes under condition of salinity effects.

4.4.2 Effect of salinity on soil microbial genes

The increase of the emission of N_2O in the first week of soil incubation and the decrease of N_2O in the following weeks (Figure 4.1) correspond with the increase of *nirS* and *nirK* abundance within the first week and its subsequent decrease (Figures 4.5, 4.6). Furthermore, this pattern of N_2O emission is enhanced due to the decrease of the abundance of *nosZ* genes (coding for N_2O reductase) within the first week and the increase of *nosZ* within the following two weeks (Figure 4.7). The significant dependence of the N_2O flux to the ratio of Σ*nir* / *nosZ* (Figure 4.8) has been shown previously in other soil incubation studies (Philippot *et al.*, 2009; Čuhel *et al.*, 2010; Warneke *et al.*, 2011). In the present study, N_2O emission was associated with the ratio of Σ*nir* / *nosZ* depending on the different levels of salt concentration in soil solution. In a condition of no salinity (Figure 4.8*a*), the emissions of nitrous oxide were significantly and positively correlated with the gene ratio. This is similar to the finding of Saarenheimo et al. (2015) who found that N_2O accumulation was connected to the relative abundance of nitrite versus N_2O reductase genes, particularly the (nirS+nirK) / nosZ gene ratio. Over time of the experiment, the increased total of *nir* gene or lower *nosZ* led to an increase of N_2O fluxes. This correlation was similarly found in the treatment of low salt concentration (Figure 4.8*b*). However, there was no evidence to conclude that abundance of denitrifying genes was a controlling factor of the N_2O emissions in condition of salinity > 15 dS m^{-1} although a trend of weak negative relation between gas fluxes and the gene ratio occurred in the high salinity treatment (Figure 4.8*d*).

Over incubation time of our study, the three different salt concentrations most likely had an impact on the abundance of *nirS* and *nirK* genes and differed from the control of fresh water between the 1st and 2nd week (Figures 4.5, 4.6). The abundance of *nosZ* showed

a significant relationship with increasing salt concentration. Significantly high copies of *nosZ* genes with the high salinity were measured after the first week of incubation (Figure 4.7). Microbial gene variation in this study showed that these denitrifying genes in wetland soils varies with salinity levels due to differences in adaptation of microorganisms to the extracellular osmotic pressure (Oren, 1999; Piao *et al.*, 2012). The *α*-subdivision of *nosZ*-community tending to adapt and sustain in high salinity environments whereas *β*- and *γ*-subdivisions tended to be sustained in low salinity environments (Piao et al., 2012). Similarly, denitrifying community with a dynamic *nosZ* relative expression level can adapt to frequent salinity changes and shows high resistance with salinity increases (Zaghmouri et al., 2018). However, Wang et al. (2017) reported that α- and γ-Proteobacteria was active and the metabolic activity of β-Proteobacteria was inhibited by increasing salinity. Therefore, the finding of the present study can precisely be explained based on adaptation mechanism of microorganisms only with evidence of sequencing the *nosZ* functional gene to determine the taxonomic identity of the *nosZ* genes present in the studied soil. After the first week, the increase of *nosZ* gene copies in the salinity treatments resulted in a low N_2O emissions in these treatments due to the complete conversion of N_2O to N_2 product. Whilst the effect of salinity on the composition of denitrifying bacteria could not be completely separated from soil characteristics, carbon source and nitrogen content (Baneras et al., 2012), this study reports the short-term temporal effects salinity has on the abundance of denitrification genes which can be related to GHG emission only at low salinity levels (Figure 4.8 *a* and *b*).

5. Conclusion

This study showed both flux and cumulation of the N_2O-N production and soil respiration were reduced by increasing the salt concentration from the incubated soil. The study found that elevated salinity increased the denitrifying genes in the incubated acid sulphate soil. Changes in gene abundance were clearly observed between the first and second weeks of the incubation. The abundance of *nosZ* was significantly related to the increasing salt concentration leading to a low N_2O emission. The study confirmed that there was significant correlation between the *nosZ* bacteria gene relative to the sum of *nirK* and *nirS* communities and the emission of nitrous oxide in the condition of low salinity. Overall, short-term exposure to salinity led to a reduction of CO_2 and N_2O emissions due to the biological aspect of denitrification being controlled. Findings from this investigation extend our knowledge about the underlying molecular ecological mechanisms of denitrification and will assist in managing nitrogen cycling in salt-affected soils.

Acknowledgements

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Chapter 5: SALINITY EFFECTS ON SOIL CHARACTERISTICS, NITROGEN RECOVERY AND RICE GROWTH ON TWO PADDY SOILS IN THE MEKONG DELTA

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Authorship statement

Manuscript title: Salinity effects on soil characteristics, nitrogen recovery and rice growth on two paddy soils in the Mekong Delta.

Dang Duy Minh (PhD Candidate)
I implemented the experiment, analysed soil and gas samples, interpreted and analysed data, wrote the manuscript and acted as the corresponding author. I hereby certify that the statement of contribution to the manuscript is accurate.

Signature: _____ Date: _____

Ian White
I provided comments and corrections on development of work. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Chau Minh Khoi
I provided comments and suggestions on development of work. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

Signed Date: _____

Ben Macdonald
I supervised development of work, evaluated the manuscript, provided comments and corrections. I hereby certify that the statement of contribution to the manuscript is accurate and I give permission for the inclusion of the manuscript in the thesis.

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Chapter 5: SALINITY EFFECTS ON SOIL CHARACTERISTICS, NITROGEN RECOVERY AND RICE GROWTH ON TWO PADDY SOILS IN THE MEKONG DELTA

Abstract

Future sea level rise will increase the area affected by salinity and threaten rice producing river deltas throughout Asia. Currently, salinity is one of the major biotic stresses on rice and affects rice production and in the future, this will challenge the world's food security. Acid sulfate soil is a ubiquitous feature of many Asian river deltas and research on impacts of salinity on nitrogen (N) cycling and fertilizer efficiency in this soil type is limited. Improving N fertiliser efficiency in salt-affected soils is required to improve rice yield and increase farm profitability under future environmental conditions. The present pot experiment investigated changes in soil properties, the dynamics of N and their effects on rice growth and yield under various salinity levels by using a ^{15}N label fertilizer technique. High salinity resulted in higher soil inorganic N after the final application of nitrogen fertilizer. Differences in soil type and salinity significantly altered height and the number of rice tillers that developed in all measurements. Overall, 50% of fertiliser loss occurred because of crop yield failure and for the remaining treatments losses ranged from 28-38% with N fertiliser recovery of 37-50%. Growing the salt tolerant rice variety, salinity did not alter rice yield or N fertiliser losses on the alluvial soil. However, the high salinity (8-ppt) on the ASS caused significant loss of crop yield and decreased in nitrogen recovery due to increased N losses, most likely as denitrification. Findings from this study showed that rice production and N fertiliser application in a conventional cultivation may not be sustainable on the ASS that are likely to be inundated by saline water due to conditions associated with climate change. Therefore, new cropping systems and appropriate interventions should be noticed in the climate change context.

Keywords: salinity, acid sulphate soil, glasshouse experiment, rice, nitrogen recovery.

5.1. Introduction

Rice is an important staple food for half of the world's population (FAOSTAT, 2009; Fageria et al., 2015). Rice occupies about 23 % of the total area under cereal production in the world (Wassmann *et al.*, 2009a; Jagadish *et al.*, 2010) and is produced and consumed in all continents, but the majority of rice is produced and consumed in Asia. It

is also consumed in large quantities in North America and Europe by native populations and immigrants from Asia, Africa, and South America (Fageria et al., 2015). Rice demand will increase by ~60% by the year 2025 due to the increase in the world's population (Fageria, 2014). Similarly, Normile (2008) predicted that an increase of 1.2 % per year of rice production will be required to meet the growing demand for food due to population growth and economic development in the next decade. Enhancement of rice production is an important feature of grain production that will benefit the world's 3.5 billion people who depend on rice for their livelihood and as their basic food. Modern rice cultivars need improved cultural practices to achieve higher yields. In this context, efficient use of inputs is vital to safely produce the additional food from the limited resources with minimal adverse impacts on the environment (Fageria et al., 2015).

Salinity is a major abiotic stress on rice production at all growth stages (Moradi and Ismail, 2007) and presents problems over large areas in Asia (Khan and Abdullah, 2003). Kumar et al. (2015) reported that millions of hectares in the humid regions of South and Southeast Asia are technically suited for rice production but are left uncultivated or produce very low yields due to salinity and problem soils. Currently, about 12 million ha of land are salt affected and half of this area is in India. Church et al. (2013) indicated that sea level rise will be a global issue for many coastal and agricultural areas by 2100. Sea level rise could affect >55 million people and in the developing countries: ~0.4% of the total agricultural land will be impacted by a 1m rise and 2.1 % by a 5 m rise (Dasgupta et al., 2009). Rising sea levels would have direct or indirect impacts on agricultural land through inundation, altered flood dynamics or erosion, which would cause modifications of groundwater dynamics. In addition, seawater intrusion will cause a shortage of freshwater resources and a reduction in availability of irrigation water (Snoussi et al., 2008). Hence, the area of rice producing land impacted by salinity will increase in the future.

The Mekong Delta is an important region of rice production for Vietnam´s food security. With about 1.8 million ha of rice production land, the Mekong Delta (MD) provides approximately 23 million tons of rice annually for both domestic consumption and export (Nhan et al., 2011). Most of the rice area in the Mekong is located in alluvial and acid sulphate soils, which are the two most widespread soil groups in the region (Buu et al., 1995). Acid sulphate soils have been reclaimed for agricultural activities such as rice cultivation (Minh et al., 1997), rice - shrimp systems and other annual and perennial crops (Sullivan et al., 2011). Drought and salinity have been considered more important than flood damage to rice productivity in the Mekong Delta (Buu and Lang, 2004). Tuyen (2011) indicated that low flows and sea-level rise may result in an ongoing increase of the salinity level in the MD.

Nitrogen is one of the most yield-limiting nutrients in crop production including rice in all growing regions worldwide (Fageria et al., 2015) and recovery efficiency of N is lower than 50 % in most cropping systems (Fageria, 2014). The major part of N in soil is lost through volatilization, leaching, denitrification, and soil erosion (Fageria and Baligar, 2005b). A number of studies have investigated the impacts of salinity on soil nitrogen cycling (Irshad et al., 2005; Kumar et al., 2007; Lodhi et al., 2009; Akhtar et al., 2012). A decrease in mineralization of N was found under saline conditions and at higher moisture regimes (Lodhi et al., 2009). Higher salinity levels promote the loss of nitrogen in ammonia form (Akhtar et al., 2012). The elevation of salinity impacts on soil microbe activities in the nitrification process and this leads to a reduction in the conversion of ammonium to a nitrate (Irshad et al., 2005; Kumar et al., 2007). Results in the chapter 3 show that salinity significantly decreased N_2O emissions from the acid sulphate soil but did not affect emissions from the alluvial soil. However, available carbon and nitrate promoted soil respiration under salinity effects. In addition, findings from the chapter 4 indicate that salinity controls the biological aspects of denitrification leading to a decrease of greenhouse gas emissions. Zeng et al. (2015) found that soil salinity causes differences in emergence rate, yield, and nitrogen use efficiency in sunflowers and cotton. Low recovery of N is not only responsible for the higher cost of crop production but also for environmental pollution (Fageria et al., 2015). However, our understanding of the impacts of salinity on N cycling and N recovery efficiency (NRE) in salt-affected paddy soils is still limited.

In the context of sea level rise, more and better information on NRE in salt-affected soils is required to improve fertiliser management and enhance rice yields in river delta environments. We hypothesized that salinity in combination with acidity increases plant stress, lowers N fertiliser uptake and increases N loss. Therefore, the aim of the experimental pot study was to investigate the impact of salinity on soil properties, nitrogen recovery and rice growth.

5.2 Materials and methods

5.2.1 Soil and saline water collection

Undisturbed profiles (0 – 30 cm) of acid sulphate and alluvial soil were collected from fields at the beginning of the wet season in the Mekong Delta. The soils were collected from the Hoa An Research Station (11°N, 107°E) (acid sulphate soil) and a farmer's field in Thoi Lai (10°N, 105°E) (Can Tho state) (alluvial soil). At the time of sampling, the ASS soil profile was classified as a Typic Sulfaquept (Soil Survey Staff, 1993) or Thionic Fluvisol (FAO-WRB, 2006). The type of the alluvial soil was identified as Mollic Gleysols (FAO-WRB, 2006). Neither of the sampling sites are located in the saline tidal areas of

the Mekong Delta. Saline water was collected at 9°25'N 105°59'E and the distance from the collecting site to the coast line is about 25km.

Undisturbed soil cores (20-cm diameter) were collected for the pot experiment to ensure the representation of field conditions. The soil cores were delivered to the glasshouse and the pot experiment was started the same day. The bulk density of the field soil was measured at 0 – 10 cm and 10 – 20 cm below the soil surface. The chemical and physical soil properties are summarized in Table 5.1.

Table 5.1 Characteristics of soil samples used in the pot experiment. Different superscript letters along a row indicate significant differences between means of soil samples based on a one-way ANOVA (*P*-value < 0.001, n = 3)

	Acid sulphate soil		Alluvial soil	
Depth (cm)	**0 – 10**	**10 – 20**	**0 – 10**	**10 – 20**
pH	3.53^{b}	3.51^{b}	6.14^{a}	6.09^{a}
EC (dS cm^{-1})	1.90^{a}	1.54^{b}	0.21^{c}	0.21^{c}
Total N (%)	0.630^{a}	0.531^{b}	0.268^{c}	0.150^{d}
Total P (%)	0.066^{a}	0.049^{a}	0.044^{ab}	0.020^{b}
Total K (%)	0.845^{c}	0.737^{d}	1.062^{b}	1.112^{a}
Total C (%)	10.30^{a}	10.25^{a}	3.085^{b}	1.744^{c}
NH_4^+ - N (mg kg^{-1})	59.99^{a}	52.67^{a}	57.41^{a}	27.36^{b}
NO_3^- - N (mg kg^{-1})	0.151^{a}	0.143^{a}	0.038^{b}	0.180^{b}
Bulk density (g cm^{-3})	0.562^{d}	0.675^{c}	0.865^{b}	1.226^{a}

5.2.2 Experiment design

The soil cores were flooded to 5 cm above the soil surface by three different salinity concentrations: 0, 4 and 8 g L^{-1} (ppt - parts per thousand) for 14 days. Before sowing rice seeds, the saline solution was drained by manual pumping with a 120 mL syringe and replaced by fresh water for 7 days, which is standard practice by rice cultivators in the MD. The surface soil was maintained in a moist wet condition to aid growth of the young plants. At 10 days after sowing (DAS), the first fertiliser was applied. The level of fresh water in the pots was increased to and maintained at 5 cm above soil surface. One week before the harvesting time (98 DAS) the surface water was manually drained from the pots by a 120 mL syringe to allow the soil surface to dry out.

A salt and acidity tolerant rice variety (OM 10252) from Cuu Long Rice Research Institute (CLRRI) of Vietnam with 90–100-day growth duration was used for this experiment. Each pot was sown with 20 seeds and five healthy rice plants were re-selected before the first nitrogen fertilization. The plants were retained and used for agronomical and yield measurement during one crop season. The rate of fertilization applied for the present study was equivalent to 100 kg of ^{15}N-urea (10% atom enrichment) nitrogen (N), 60 kg of phosphorus (P_2O_5) and 60 kg of potassium (K_2O) per hectare. All the phosphorus and 50% of the potassium were applied before seed was sown in the preparation stage. Nitrogen fertilizer was applied at a rate of 40% 40 days after seeding (DAS); the nitrogen was combined with 50% potassium, and another 60% of nitrogen was top dressed in three splits at 10, 20 and 65 DAS. The pot experiment was implemented with a random completed design. The experiment had three treatments of different salinity 0, 4 and 8 ppt for each soil type and 3 replicates for each treatment.

Plate 5.1 The pot experiment with different salinity levels was performed in a greenhouse in the Mekong Delta.

5.2.3 Data collection and variables measurement

Agronomical data collection:

The number of tillers and plant height were measured at 20, 23, 27, 43, and 90 DAS. At harvesting time, yield and yield components (number of panicles/m^2, the number of spikelets per panicle, the percentage of filled grains, 1000-grain weight) were also recorded (Yoshida et al., 1976). Plant sample analysis was conducted at the end of the experiment. Three uprooted plants were collected and oven-dried at 70^0C to obtain a constant dry weight for plant analysis. Total ^{15}N content was analysed in the plant samples harvested. Analysis of ^{15}N content in plants was carried out using an isotope ratio mass spectrometer (IRMS). Recovery of fertilizer N by rice plant was calculated according to suggestion from Asagi and Ueno (2009) and Wang et al. (2011b). Nitrogen loss was calculated by subtracting total N recovery and N remained in soil from total N application.

Soil sample collection, extraction, and analysis

At the beginning of the experiment, soil pH, EC, total C, N, P and inorganic nitrogen (ammonia and nitrate) were determined. Two samplings in the middle of the crop season occurred at 27 and 43 DAS to analyse inorganic nitrogen. At the end of the experiment, soil pH, EC, total C, N and inorganic nitrogen were analysed again. Water pH and EC were monitored at the same time as soil sampling events and also at the time of the final drainage event (90 DAS). The pH and EC of soil (0 – 10 cm) and water were directly measured in the rice pots from the beginning to the drainage event. At harvesting time, soil pH and EC were measured in a 1 : 5 soil to water ratio (Rayment and Lyons, 2011). Soil ammonium and nitrate was extracted with 2 M KCl solution and measured following the method of Keeney and Nelson (1982). Total nitrogen, ^{15}N, and total carbon content were measured using a mass spectrometer for the N_2 and CO_2 peaks sequentially (Nelson and Sommers, 1996; Rutherford et al., 2007; Rayment and Lyons, 2011). The calculation of nitrogen uptake and efficiency was followed the explanation of Wang *et al.* (2011c) and Motior et al. (2011). Soil bulk density was measured based on the core method (Blake and Hartge, 1986; Hao et al., 2007).

5.2.4 Data statistical analysis

R statistical software version 3.3.1 was used for statistical data analysis. Prior to the analysis, data homogeneity and normality were tested and if required the data were transformed. Field soil properties were statistically analysed by using a one-way ANOVA. A two-way ANOVA tested the interaction effects of salinity and soil types on soil nitrogen, nitrogen efficiency, agronomical data, yield components and yield. Significance was set at $p<0.05$ and post-hoc ANOVA was used to compare the differences between

treatments with a Tukey HSD test. The one-way ANOVA identified differences in water pH and EC. Differences in soil pH and EC at the beginning of the experiment, 43 and 98 DAS, were also analysed by conducting the two-way ANOVA and Tukey tests.

5.3 Results

5.3.1 Soil and water chemical attributes at rice planting

Water pH and salinity over crop season

At 20 DAS, water pH was significantly high in the alluvial soil and highest in the no salinity treatment (6.48 ± 0.07) (Figure 5.1*a*). Water pH increased around the neutral value (7.0) in the alluvial soil between 23 and 43 DAS while the readings for the ASS were in most cases < 4.5, except in the non-salinity treatment (5.15 ± 0.22). Before drainage, water pH was significantly higher in the alluvial soil compared to the ASS for all salinity treatments. At 90 DAS, water pH in the 8-ppt treatment of the ASS was 4.78 ± 0.27 and lowest in all treatments while other treatments had water pH ranging from 5.58 to 7.40 (± 0.08 – 0.18) (Figure 5.1*a*).

In the 8-ppt salinity treatment, surface water EC for both ASS and alluvial soils at 20 DAS was significantly higher than the other treatments, at 5.79 ± 0.15 and 5.35 ± 0.26, respectively (Figure 5.1*b*). The surface water EC in all salinity treatments declined, but was always higher than that in the non-salinity treatment for both soils between 23 DAS and 43 DAS. However, the water EC was not significant between treatments in the alluvial soil at 90 DAS while it was still significantly high (1.43 ± 0.37) in the 8-ppt treatment in the ASS (Figure 5.1*b*).

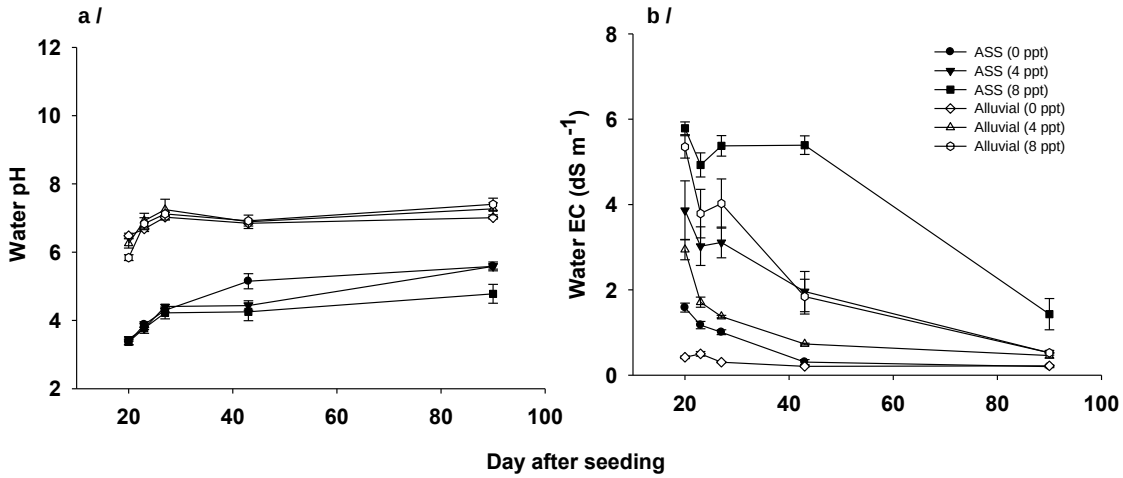

Figure 5.1 Changes of surface water pH (a) and EC (b) in the experiment pots over 90 days after seeding. ASS and Alluvial in the legend mean acid sulphate soil and alluvial soil, respectively. Number and text in legend brackets show different salinity treatments. Data presents mean and standard error of mean (error bar, n = 3).

Soil pH and salinity

At the beginning, the ASS had significantly lower soil pH than the alluvial soil. The ASS pH ranged from 3.62 to 3.78 while the alluvial pH was between 5.70 and 6.19 (Table 5.2). There was no significant difference between the salinity treatments applied to the two soil types at the beginning. The soil pH of all treatments increased at 43 DAS and then decreased at 98 DAS. At harvesting time, the 8-ppt treatment of the alluvial soil had soil pH (5.36 ± 0.11) significantly higher than other treatments of the ASS (Table 5.2).

The EC value recorded for the alluvial soil was less than the ASS (Table 5.2). At the beginning of the experiment, soil EC of the salinity treatments was significantly higher than in the non-salinity treatment and this result was same for both experimental soils. Soil EC declined over the stages of the rice growth, but the EC of the alluvial soil was usually lower than in the ASS. At the end of the crop season, all salinity treatments had the same soil EC range, from 0.59 – 0.96 for the ASS, and 0.13 – 0.46 for the alluvial soil (Table 5.2).

Table 5.2 Changes in soil pH and EC in the pot experiment (0 – 10 cm). Values are means ± standard error, n = 3 per treatment group. Different superscript letters in a column indicate significant differences between treatment groups, according to a two-way ANOVA ($p < 0.05$).

Soil	Salinity (ppt)	Beginning	43 DAS [i]	98 DAS [ii]
			pH	
ASS	0	3.62 ± 0.16^{b}	4.89 ± 0.06^{b}	4.29 ± 0.18^{b}
	4	3.83 ± 0.29^{b}	4.56 ± 0.11^{b}	4.28 ± 0.37^{b}
	8	3.78 ± 0.15^{b}	4.77 ± 0.33^{b}	4.32 ± 0.15^{b}
Alluvial	0	6.19 ± 0.09^{a}	6.85 ± 0.03^{a}	5.16 ± 0.15^{ab}
	4	5.88 ± 0.14^{a}	6.70 ± 0.07^{a}	5.23 ± 0.16^{ab}
	8	5.70 ± 0.10^{a}	6.49 ± 0.16^{a}	5.36 ± 0.11^{a}
P-value	Soil	< 0.05	< 0.05	< 0.05
	Salinity	0.61	0.25	0.84
	Interaction	0.17	0.44	0.92
			EC (dS m^{-1})	
ASS	0	1.32 ± 0.10^{d}	1.00 ± 0.01^{c}	0.59 ± 0.06^{abc}
	4	3.53 ± 0.51^{c}	1.29 ± 0.06^{b}	0.84 ± 0.10^{ab}
	8	7.54 ± 0.08^{a}	3.48 ± 0.03^{a}	0.96 ± 0.15^{a}
Alluvial	0	0.39 ± 0.05^{d}	0.15 ± 0.02^{f}	0.13 ± 0.02^{d}
	4	3.26 ± 0.37^{c}	0.43 ± 0.01^{e}	0.39 ± 0.05^{cd}
	8	5.82 ± 0.20^{b}	0.81 ± 0.02^{d}	0.46 ± 0.00^{bd}
P-value	Soil (S1)	< 0.05	< 0.05	< 0.05
	Salinity (S2)	< 0.05	< 0.05	< 0.05
	Interaction effects	0.07	< 0.05	0.93

(i) DAS: Day after seeding
(ii) Soil pH and EC were measured in a 1:5 soil to water ratio.

Total and inorganic soil nitrogen

Total soil nitrogen

The results presented in Figure 5.2 show a significantly higher level of total soil nitrogen for the ASS in comparison to the alluvial soil. The total N content of the ASS was two times higher than that in the alluvial soil. Different salinity levels did not result in differences between means of total N in either top soil. An interaction effect of soil and salinity was also found in the ASS (10 – 20 cm) at the beginning ($F = 52.65$, $P<0.05$) (Figure 5.2*c*). However, the subsoil (10 – 20 cm) in the 4-ppt and 8-ppt treatments of the ASS had total N significantly lower than the 0-ppt treatment at the beginning.

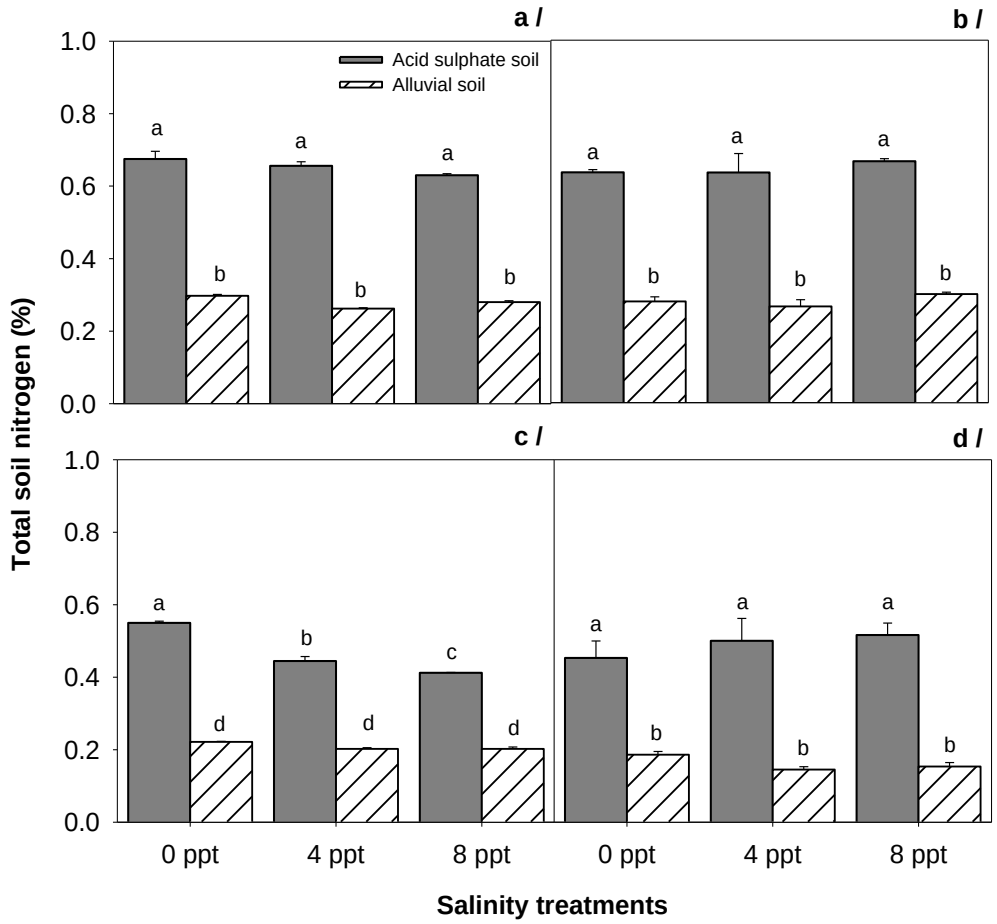

Figure 5.2 Total soil N in pots from the 0-10 cm layer of ASS and alluvial soils at the beginning of the experiment (a) and at harvest (b) and at 10-20 cm for the sampling times (c) and (d), respectively. The presented data are means of total N, and the error bars represent standard errors of means (n = 3). Different letters on bars show a significant difference between means of total soil N.

Soil inorganic nitrogen

Soil inorganic nitrogen was significantly different when soils and salinity levels were compared prior to rice seedings being planted. At 0 DAS the concentration (mg kg^{-1}) of inorganic N in the ASS ranged from 81 to 100 and was significantly higher than the concentration in the alluvial soil (33 – 59 mg kg^{-1}) (Figure 5.3, 0 DAS). The means of soil inorganic N between treatments in each soil depth differed due to an interaction effect of soil type and salinity treatment ($F = 11.16$, $P<0.002$ for 0 – 10 cm samples, and $F = 31.62$, $P<0.001$ for 10 – 20 cm samples) (Figure 5.3, 0 DAS).

At 27 DAS, soil inorganic nitrogen was increased in salinity treatments of the ASS for both experimental soil layers (Figure 5.3, 27 DAS). In this soil type, the inorganic N of the salinity treatments were significantly higher than in the non-salinity treatment and similar for both subsoil levels. The alluvial soil had significantly lower inorganic N

compared to the ASS and there was also differences between salinity treatments in this soil (Figure 5.3, 27 DAS). After the third fertilizer (43 DAS), the 8-ppt salinity treatment had significantly higher inorganic N content and a strong interaction effect of soil and salinity on inorganic N levels was also found in both soils at this sampling time ($F = 224.3$ and $P<0.001$). At the end of the pot experiment, all acid sulphate soil treatments (0 – 10 cm) had soil nitrate nitrogen below a detection limit of an auto-analyser (0.001 ppm), so inorganic N was only ammonium nitrogen (Figure 5.3, 98 DAS upper). There was also no significant difference in inorganic N between salinity treatments within each soil; however, differences in soil types and salinity had a significant interaction with inorganic N in 10 – 20 cm soil samples ($F = 6.53$, $P<0.05$) (Figure 5.3, 98 DAS below) although concentrations were negligible.

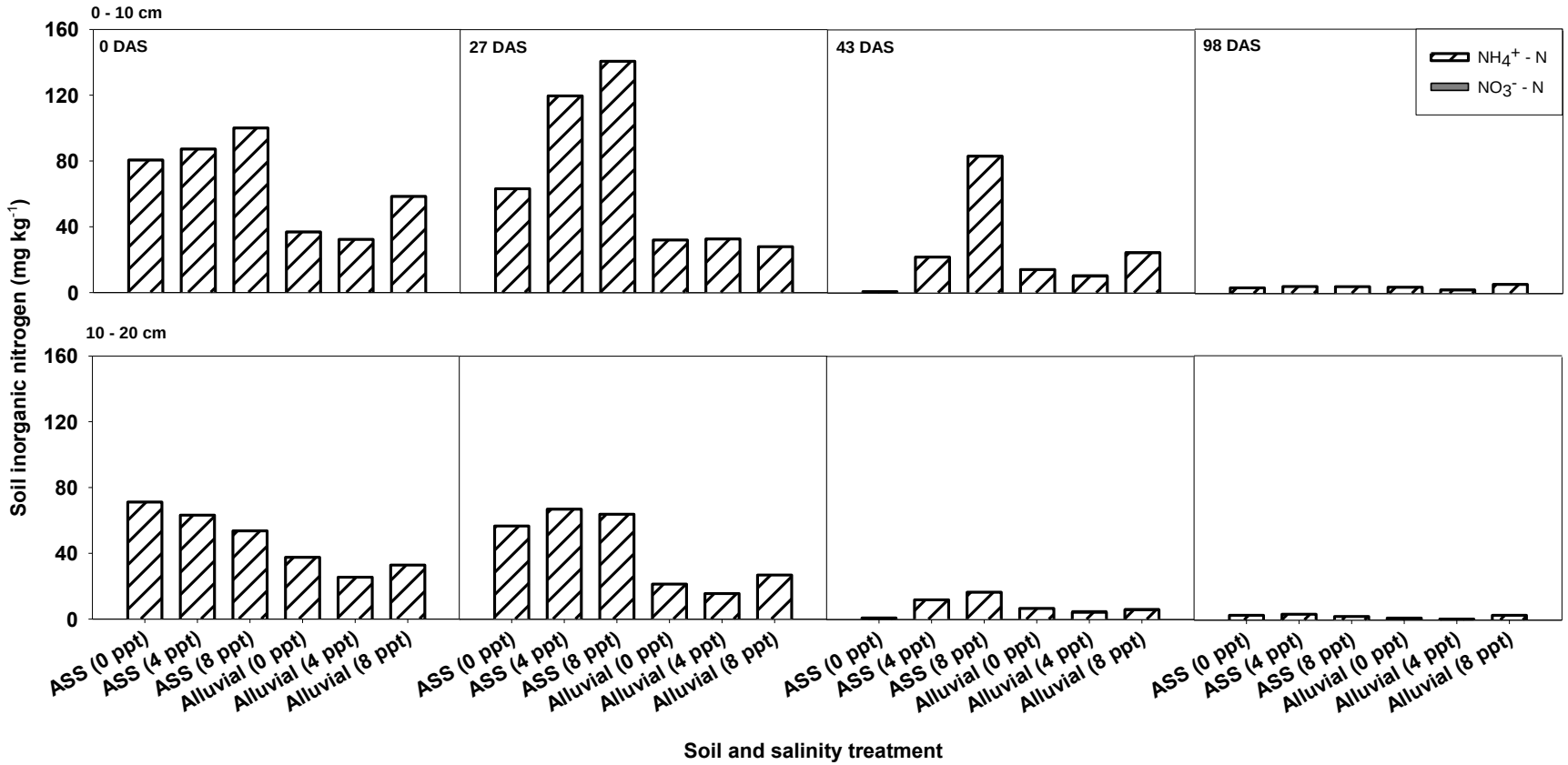

Figure 5.3 Soil inorganic nitrogen as a total content of NH_4^+ - N and NO_3^- - N (mg kg^{-1}) over stages of rice growth. The four upper graphs show the results of inorganic nitrogen in soil samples at 0 – 10 cm depth. The lower four graphs show results of inorganic nitrogen in soil samples at 10 – 20 cm depth. Soil nitrate is extremely low (< 0.1 mg kg^{-1}) and invisible on bars. A name of a treatment includes soil types (ASS and alluvial soils) and salinity level in brackets. The height of a stacked bar presents total ammonium and nitrate nitrogen extracted by KCl 2M in the same treatment (n=3).

5.3.2 Effects of salinity on rice growth performance, yield and yield components

Rice growth performance

The differences in soil type and salinity significantly altered the height of the rice plants in all measurements. At the day 27, in the non-salinity treatment the height of the rice plants was significantly greater compared to that under salinity treatments in both soils (Figure 5.4*a*). The height of rice plants showed significant differences between the 4 and 8-ppt treatments in both experimental soils. However, the no salinity and low salinity treatment (4 ppt) showed similar results in plant height after the third fertilizer application (43 DAS). High salinity (8ppt) significantly lowered the rice plant growth in the ASS. The plant height in the ASS and alluvial soil was 65 and 84 cm, respectively, at 90 DAS (Figure 5.4*a*).

Both the salinity treatment and different soil types strongly affected the number of tillers per hill and the total number of tillers in pots. Until 27 DAS, the number of tillers per hill was significantly lower in the salinity treatments in the ASS while this parameter was only low in the 8-ppt salinity of the alluvial soil (Figure 5.4*b*). After the third fertilizer application (65 DAS), the number of tillers per hill were not significantly different between the salinity treatments in the alluvial soil although the tillers per hill reduced at 90 DAS. In contrast, an increase of salinity still caused a lower number of tillers per hill in the ASS and the number of tillers per hill in the ASS non-salinity treatment was significantly greater than under other treatments at 90 DAS (Figure 5.4*b*). The number of tillers per hill and per pot was strongly affected by the interaction effect of soil type and salinity treatment ($P<0.05$) (Figures 5.4*b* and *c*).

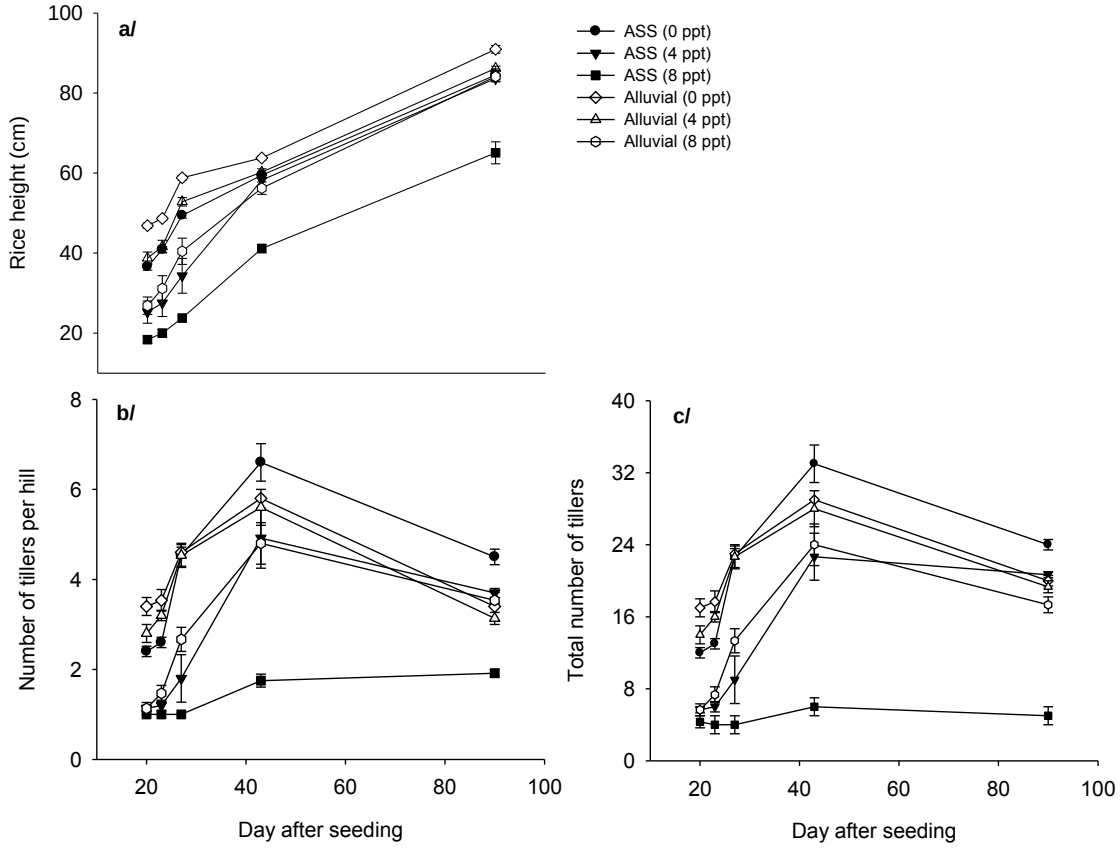

Figure 5.4 Rice height (a), number of tillers per hill (b), and tillers in pots (c) from the second fertilizer application to 90 days after seeding. The data presented show means of plant height, tillers/hill, tillers/pot, and the error bars are standard errors of means (n = 3).

The number of rice tillers present at harvesting time was significantly different between the soil and salinity treatments (Figure 5.5). The 8-ppt salinity of the ASS had 5 unproductive tillers and was significantly different with 1 unproductive tiller in the non-salinity treatment of the alluvial soil. An interaction effect of soil and salinity treatment was also found in data for productive tillers ($F = 66.4$, $P<0.001$). There were no productive tillers in the ASS 8-ppt salinity while 15 tillers were present under a similar treatment of the alluvial soil. On the other hand, similar salinity treatments resulted in the same number of productive tillers when two soils were compared (Figure 5.5).

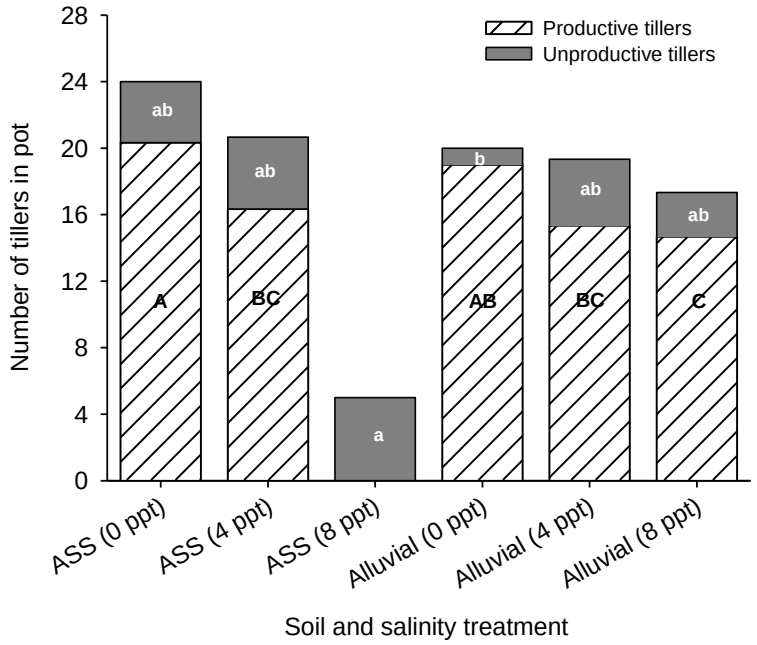

Figure 5.5 Means of number of rice tillers at harvesting time (98 DAS). Different lowercase on bars shows a significant difference in the mean of unproductive tillers. Bars with different capital letters show significant difference in means of productive tillers ($P < 0.05$) as analysed by two-way ANOVA (n = 3).

Rice yield and yield components

The high salinity treatment (8-ppt) strongly impacted rice yield and yield components in the ASS. The rice yield of all alluvial soil treatments was not significantly different with the rice yield of the non-salinity treatment in the ASS (21.72 – 30.11 g/pot) (Table 5.3). The highest salinity treatment (8-ppt) resulted in no rice yield in the ASS. Higher salinity significantly reduced the number of panicles/pot in both soils. The numbers of panicles/pot in the non-salinity treatments were 19 and 20 panicles for the alluvial and ASS, respectively, compared with about 14 – 16 panicles in other salinity treatments. Except for the no rice yield in the ASS 8-ppt salinity, the percentage of filled spikelet and 1,000-grain weight were not altered by differences in soil and salinity level (Table 5.3).

Table 5.3. Rice yield and yield components (mean ± standard error of mean) in the pot experiment. Different letters in a column indicate significant differences between treatments as analysed by two-way ANOVA and the TUKEY test ($p < 0.05$).

Soil	Salinity (ppt)	Yield (14%) (g / pot)	No. of panicles per pot	No. of spikelets per panicle	Filled spikelet (%)	1,000-grain weight (g)
ASS	0	21.72 ± 2.23^{ab}	20.33 ± 1.20^{a}	90.60 ± 2.64^{ab}	74.96 ± 2.77^{a}	20.63 ± 0.32^{a}
	4	16.31 ± 1.93^{b}	16.33 ± 0.33^{bc}	76.47 ± 11.64^{b}	47.18 ± 11.23^{a}	18.48 ± 0.67^{a}
	8[(i)]	NA	NA	NA	NA	NA
Alluvial	0	30.11 ± 2.29^{a}	19.00 ± 0.01^{ab}	107.40 ± 5.72^{a}	69.96 ± 3.93^{a}	19.90 ± 1.04^{a}
	4	25.29 ± 1.33^{ab}	15.33 ± 0.88^{bc}	98.60 ± 3.78^{ab}	73.09 ± 1.20^{a}	19.77 ± 0.14^{a}
	8	24.06 ± 3.14^{ab}	14.67 ± 1.20^{c}	85.53 ± 2.81^{ab}	68.92 ± 8.88^{a}	18.60 ± 1.29^{a}

[(i)] NA: data were not available as no grain yield collected in this treatment.

5.3.3 Nitrogen recovery efficiency

High salinity level significantly lowered the recovery of fertilizer N by rice plants in the ASS. The 4 and 8-ppt salinity showed recovery of N fertilizer of 36.63 and 19.92%, respectively (Table 5.4). The recovery of N fertilizer in the alluvial soil was the same for all treatments and ranged from 39.06 to 49.12%. The amount of fertilizer N remaining in soils was not significantly different for all study treatments. The average of nitrogen remaining in the ASS was 23.53 and 27.71% for the alluvial soil. The percentage of fertilizer nitrogen loss was significantly high (59%) in the highest salinity of the ASS compared to other treatments for both soils. The fertilizer N loss was about 30% in the alluvial soil and 39% for the non-salinity in the ASS. A two-way analysis of variance shows an interaction effect of soil and salinity on the fertilizer nitrogen loss ($F = 5.68$, $P = 0.02$) (Table 5.4).

Table 5.4 Nitrogen recovery and balance (%) in the rice pot experiment at harvesting time (98 DAS). The data presents mean and standard errors. Different superscript letters indicate significant differences between treatments, according to a two-way ANOVA ($p < 0.05$) and Tukey-HSD test.

Soil	Salinity (ppt)	Recovery of fertilizer N by rice plant (%)	Fertilizer N remaining in pot (%)	Fertilizer N Loss (%)
ASS	0	38.84 ± 5.04^{a}	22.32 ± 2.65	38.84 ± 3.61^{b}
	4	36.43 ± 1.07^{ab}	26.58 ± 4.47	36.99 ± 3.89^{b}
	8	19.92 ± 4.55^{b}	21.16 ± 1.25	58.92 ± 3.96^{a}
Alluvial	0	49.12 ± 1.38^{a}	22.04 ± 4.02	28.83 ± 5.39^{b}
	4	40.14 ± 4.69^{a}	28.86 ± 1.86	31.01 ± 3.59^{b}
	8	39.06 ± 3.02^{a}	32.23 ± 2.64	28.71 ± 1.80^{b}
P-value	Soil	< 0.05	0.10	< 0.05
	Salinity	< 0.05	0.19	< 0.05
	Interaction	0.15	0.19	< 0.05

5.4 Discussion

5.4.1 Effects of salinity on soil properties and nitrogen recovery of rice

Water pH increased up to 27 DAS after which there was no significant change. Within one soil group, the water pH between salinity treatments was not significant while the water pH in pots of alluvial soil was significantly more alkaline than the acid sulphate soil over the crop season (Figure 5.1*a*). Kawahigashi et al. (2012) reported that the pH value of water in a rice field varied from 2.8 to 6.3 and the soil pH from each horizon was around 3.0. The pH value of soil water extracts was also strongly acidic due to the formation of sulphuric acid, which can decrease the soil pH to less than 4 (David, 1986; Dent and Pons, 1995; Jayalath et al., 2016). The ionic composition of acid sulphate soils

causes a higher concentration of acidic and basic metals (Hartikainen and Yli-Halla, 1986). In fact, acidic conditions and higher EC were found in the ASS surface water than those in the alluvial soil surface water (Figure 5.1*b* and Table 5.2). However, soil pH was almost the same in all treatments for both soils at harvesting time while the EC of the alluvial soil was still lower than that in the ASS. The salinity decreases over time because the pots were continuously flooded and replenished with fresh water. Salt can move down to lower depth of a soil profile and redistribute because of an application of fresh water irrigation (Kara and Willardson, 2006). In addition, the decrease of salinity could be explained with sodium uptake by plants which is controlled by overall mechanism of sodium uptake through root properties and the subsequent distribution of sodium in the vegetative plant and panicle (Asch et al., 1998). On the other hand, excessive Na^+ in the rooting solution enlarges the apoplastic pathways causing Na^+ intrusion into the xylem vessels and resulting in an excessive accumulation of Na^+ in rice shoots (Ochiai and Matoh, 2002).

Salinity did not affect total nitrogen in soils (Figure 5.2) because there was no rapid mineralisation or loss of the organic nitrogen; however, between two soils, the alluvial soil showed low content of total nitrogen compared to the ASS. This means either a short-term flooding (two weeks) by saline water or that the experiment did not change total soil N. However, the salinity treatment and soil type both affected the dissolved soil inorganic N pools. In both the alluvial and acid sulphate soil treatments, salinity increased the accumulation of NH_4-N. However, increasing ammonium resulted from salinity increase was greatest in the ASS because of the reduced conversion of NH_4-N to NO_3-N (Irshad et al., 2005). Strongly acidic soil conditions as in the acid sulphate soil inhibit the nitrification process (Roelofs, 1983). Reduced sulphur and iron in acid soils consume soil oxygen, preventing ammonium oxidation to nitrates (Straub et al., 1996). In the ASS of the present study, these findings might explain the higher ammonium concentration compared to that in the alluvial soil.

The recovery of nitrogen fertilizer by plants was low in the high salinity treatment leading to a high N loss only in the ASS. The plants in the highest salinity treatment in ASS soils recovered only 20% of the nitrogen fertilizer while in the non-salinity treatments fertiliser uptake approached 40%, similar to other reported values (Table 5.4) and by Fageria and Baligar (2005b). Total fertiliser N losses approached 40% for all treatments except the ASS with 8 ppt salt, which experienced a 60% N loss (Table 5.4). This loss was due to the plants within the treatment not producing any grain. The combination of acidity and salinity in this treatment was a constraint that the rice plants could not overcome. Only 21 to 32 % of the applied fertiliser remained in the soil after harvest. The final fate of the lost N is unknown, but it was either lost to the atmosphere by denitrification (Seitzinger,

1988) from the water column or the soil surface after drainage, or lost through ammonia volatilisation (Akhtar et al., 2012) and surface run-off. However, water and soil pH of the ASS would not favour the ammonia volatilisation, therefore this evidence can provide more support for losses by denitrification. This is also consistent with the finding in the chapter 3 in which nitrate and available carbon addition has increased the N_2O emission in an anaerobic condition via denitrification.

5.4.2 Effect of salinity and soils on rice physiology

In the present study, high salinity (8 ppt) significantly decreased the height of rice plants. The most common whole-plant response to salt stress is a general stunting of growth (Maas and Grattan, 1999). Chlorophyll pigments in rice are sensitive to salt stress especially in salt susceptible varieties (Ali et al., 2004). The chlorophyll, involving photosynthetic electron transport, carbon metabolism, and photosynthesis, degrades during a salt stress event leading to a reduction in plant growth (Razzaque et al., 2010; Hakim et al., 2014b). Hakim et al. (2014a) found that dry weight of rice shoot varied significantly under different salt concentrations. The authors also reported that Na accumulation in the rice plant increased with the increase of salinity (Hakim et al., 2014a). Sodium derived from a saline solution directly inhibits plant growth and development (Mansour and Salama, 2004; Chinnusamy et al., 2005). The numbers of plants per hill, total plants and tillers in pots were significantly lower in the high (8ppt) salinity level soil, particularly in the acid sulphate soil (Figures 5.4*b*, *c* and 5.5). Over 30 days after seeding, soil pH in all treatments in the ASS was <4, so the constraints on rice growth in this soil were also soluble Fe and Al which are toxic to rice plants (Husson et al., 2000). Acid sulphate soils are generally unproductive or exhibit low productivity due to one or more of the following unfavourable factors: soil acidity, salinity, aluminium toxicity, iron toxicity, low content of major nutrients, low base status, and hydrogen sulphide toxicity. A high level of aluminium affects cell division, disrupts certain enzyme systems, and hampers uptake of phosphorus, calcium and potassium (Attanandana and Vacharotayan, 1986).

5.4.3 Effects of salinity on the rice yields and components

Only the 8-ppt treatment of the ASS resulted in significantly lower grain yield compared to other treatments for each soil (Table 5.3). Diluted salt water only flooded experimental pot soils at the beginning. The salt solution increased soil salinity, but only the 8-ppt in the ASS maintained high salinity (3.48 dS m^{-1}) until 43 DAS. Other treatments significantly reduced soil salinity and salt-tolerant rice variety was used in the present study. Consequently, rice yield of most treatments was not affected at the harvesting stage. The result is similar to those reported in Verma and Neue (1984) that lower rice yields of salt-tolerant variety only occurred under highest salinity level (EC_e 8.7). Grain

yield of rice has been widely shown to be significantly reduced under salinity stress (Mahmood et al., 2009; Nejad et al., 2010). The reduction of grain yield can be explained as being due to salts modifying the metabolic activities of the cell wall and a decrease of turgor pressure efficiency in cell enlargement (Hakim et al., 2014a). Other studies have shown that salt might cause a decrease of photosynthesis resulting in shrinkage of cell contents. Furthermore, salinity also inhibits the development of tissues, unbalanced nutrition and damage to membranes. Salinity lowers rice yield because of an increase in the number of sterile florets per panicle of rice and a reduction in percentage germination of rice pollen grains (Narale et al., 1969).

5.5 Conclusions

This study has examined the effects of salinity on changes in soil properties, plant performance and nitrogen efficiency in paddy soils. The evidence from this study indicates that the interaction between soil and salinity significantly altered rice growth performance. Crop yield was greater in the alluvial soil relative to the acid sulphate soil treatments. The impact of acidity was most evident in the acid soil treatment, where yields were reduced despite higher soil carbon and nitrogen content. Ammonium N remained in the acid sulphate soil and salinity treatments until 43 DAS, reflecting overall poor crop performance relative to the alluvial soil treatments. The combination of acidity and elevated salinity (8-ppt) treatment resulted in no yield. This highlights the need to develop strategies to improve crop yield under such scenarios. Overall the greatest fertiliser loss of 59% occurred when crop yield failed and for the remaining treatments losses ranged from 28-38% with N fertiliser recovery of 37-50%. Using the salt tolerant rice variety, salinity did not alter rice yield or N fertiliser losses on the alluvial soil. However, the high salinity (8-ppt) on ASS resulted in significant loss of crop yield and decreased in nitrogen recovery due to increased N losses, most likely as denitrification. Therefore, rice production and N fertiliser application in a conventional cultivation may not be sustainable on the ASS that are likely to be inundated by saline water due to conditions associated with climate change. New cropping systems and appropriate interventions should be noticed in the climate change context.

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Chapter 6: CONCLUSION AND FUTURE WORK

6.1 Synthesis and conclusion

Sea level rise will continue to cause soil salinization through saltwater intrusion and mixing with irrigation water. This will increase the area of salt-affected soil in the future and severely impact the productivity of aquaculture and agriculture. Salinity changes in aquatic environments cause ecological disturbances mainly on fish farming. Agriculture and horticulture crops are also sensitive to salinity due to the effects of high concentrations of salts in the soil and irrigation water. The cost of salinity to agriculture and economic losses are expected to increase because the area of salt-affected soils is becoming more widespread due to global climate change.

Understanding soil nutrient processes to ameliorate the salt-affected soil is crucial to enhance crop growth and ensure food security for the growing population. Sea level rise compounds the sediment crisis and will adversely affect agricultural production systems in tropical mega deltas around the world. Some agricultural areas would no longer be productive (Chown and Duffy, 2017) and people have to leave the lower delta because saltwater-soaked soils make agricultural difficult (Giosan et al., 2014). Similarly, the global mega-deltas in Vietnam (the Mekong Delta), Myanmar (Irrawaddy) and Bangladesh (Ganges–Brahmaputra), whose national economies are largely based on agricultural production, will also experience specific climate change impacts due to sea level rise. Sublethal levels of salinity causing a reduction of crop production are likely to change soil carbon and nitrogen cycle resulting in an increase of nutrient loss. Yet in these countries and other developing countries there is an urgent need to develop incubation techniques that allow rapid, robust scientific and economic assessment of soil carbon and nitrogen cycle changes.

Studies have reported that elevated salinity levels change many soil processes associated with the soil nitrogen cycle including volatilization, mineralization, nitrification and ammonification (Gandhi and Paliwal, 1976; McClung and Frankenberger, 1985a; Lodhi *et al.*, 2009; Akhtar *et al.*, 2012). Increased salinity also had adverse effects on soil microbial structure (Nelson and Mele, 2007; Chowdhury *et al.*, 2011b; Baumann and Marschner, 2013). Effects of salinity on soil denitrification have been addressed in a number of previous studies (Antheunisse et al., 2007; Seo et al., 2008; Wu et al., 2008; Marks et al., 2016). Increasing salinity showed contradictory results on soil carbon cycling in several previous studies (Laura, 1974; Pathak and Rao, 1998; Chandra et al., 2002; Wong et al., 2008; Setia et al., 2010). However, the studies agree that salinity stress forces serious limitations on crop growth and productivity (Tanji, 2002; Guo et al., 2013) and N use efficiency (Fageria, 2013). Nevertheless, there have been

inconsistencies in the results of salinity effects in previous studies that might be due to soil type, salinity level, and water content. On the other hand, little information is known about salinity effects on denitrifiers associated with greenhouse gas emissions, and the nitrogen efficiency of plants in salt-affected soils. Investigation of this aspect of salinity impacts requires more mechanistic understanding and further exploration of the effects of elevated salinity on soil nitrogen and carbon cycling. The studies presented in this thesis have addressed the effects of increased salinity on nitrogen and carbon dynamics, and on rice nitrogen efficiency in salt-affected soils. This chapter provides a summary of the advances made in this thesis and discusses the broader scale impacts. A conceptual model of the agricultural floodplain illustrating the findings of the study is also presented (Figure 6.1). A synthesis of the conclusions is presented for each research objective below:

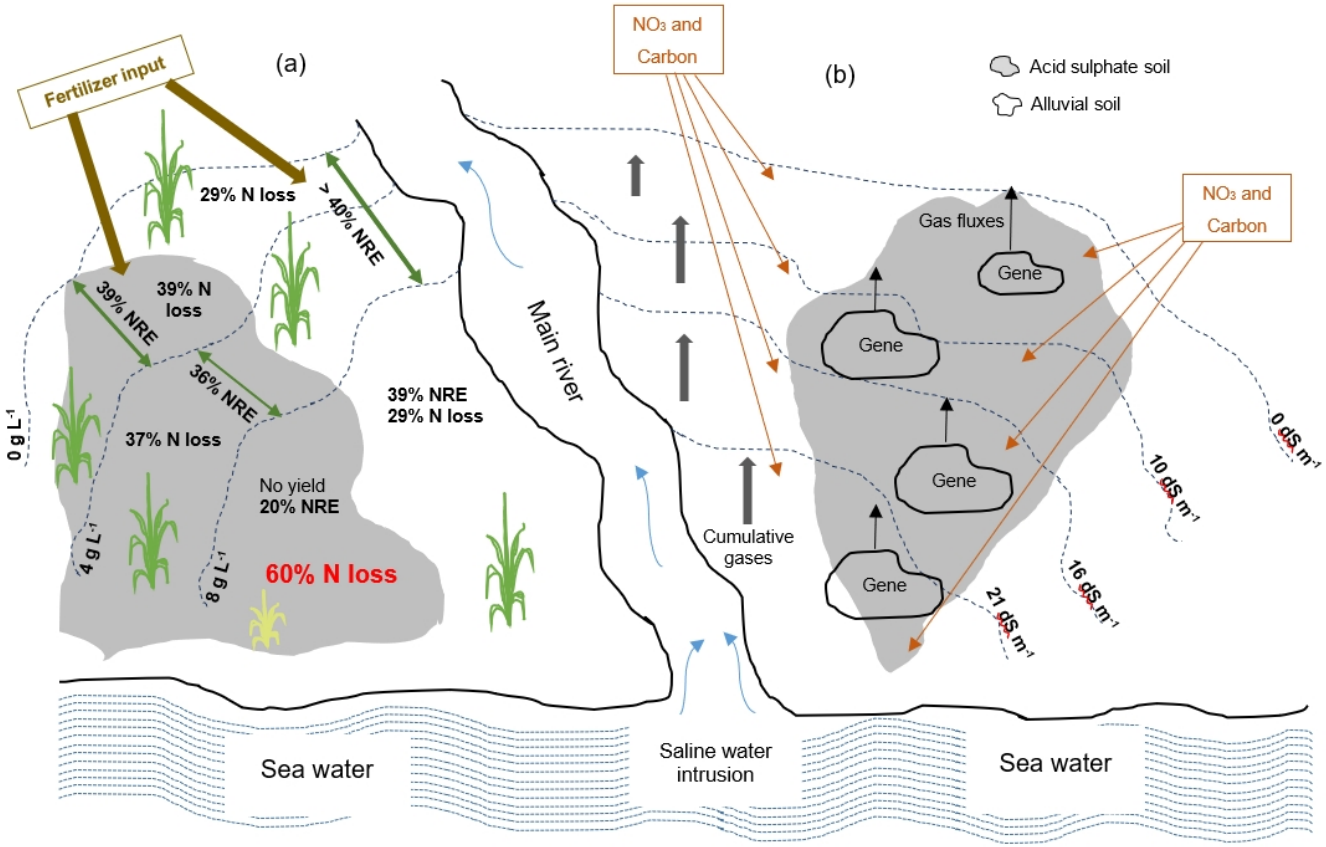
The methodological study in Chapter 2 was conducted to answer the **first research question**: how do incubation lid closure times influence greenhouse gas emissions? This study aimed to investigate greenhouse gas releases from different soils with different times of lid closure and to understand the effects of different activation times on gas emissions from soils. Findings from the study will assist in designing future research on the management of carbon and nitrogen cycling in farming systems. The result showed that the 80-minute closure time produced good results with less variance for either soil type or measured gases. Lengthening activation time may cause different emission rates according to soil properties. To analyse gas fluxes based on a linear regression model, the study suggested 4 or 5 sampling-points should be taken over a maximum of 80 minutes.

The information in Chapter 3 was used answer the **second research question**: does elevated soil salinity change greenhouse gas emission from soils? The study objective in this chapter was to examine the effect of salinity on soil N and C cycling in an acid sulphate soil and an alluvial soil. The N and C cycling in these soils was also investigated with and without additional nitrogen and carbon sources. Salinity significantly reduced N_2O emissions from the acid sulphate soil but did not affect emissions from the alluvial soil. Emissions of CO_2 were not different among the salinity treatments. Although methane fluxes peaked within 3 days of incubation, changes of CH_4 fluxes occurred over the 4-week incubation period. The addition of glucose and nitrate enhanced N_2O and soil respiration in both salt-affected soils. However, substrate addition did not affect CH_4 emissions in either soil. The findings in Chapter 3 indicate that salinity had altered carbon and nitrogen cycles in the acid sulphate soil, and future fertiliser and crop management will need to account for the changed nutrient cycling caused by salt water intrusion and climate change.

Using the incubation method and the research findings from Chapters 2 and 3, the experiment in Chapter 4 was designed to respond to **the third research question**: how does salinity change the denitrifying community in the acid sulphate soil? The nature and activity of the microbial community relative to greenhouse gas emissions has not been addressed in salt-affected soils. The study in Chapter 4 was to clarify the interaction between denitrifying gene abundance and greenhouse gas emissions within the salt-affected soil environment. The study hypothesis was that elevated salinity would increase the abundance of denitrifying genes leading to a high rate of denitrification. It was found that increased salinity caused a reduction in both flux and cumulation of the N_2O-N production in the incubated soil, relative to fresh water. Soil respiration was significantly different in salinity treatments compared to the FW treatment. The study results showed that elevated salinity increased the denitrifying genes in the incubated acid sulphate soil. The abundance of *nir* genes was usually high between the first and second week of incubation, while number copies of the *nosZ* gene were significantly low at those times. This confirmed that salinity alters the biological aspects of denitrification leading to a reduction of greenhouse gas emissions. Findings from Chapter 4 extend our understanding about the underlying molecular ecological mechanisms of denitrification that manage nitrogen cycling in salt-affected soils.

The Mekong Delta of Vietnam has been identified as one of the areas most vulnerable to potential impacts of sea level rise and salt-water intrusion. Two typical soil types for rice production in the MD are alluvial and acid sulphate soils. Salinity is a major biotic stress affecting rice production and this will severely challenge Vietnam's national food security in the future. Salinity may also change soil properties due to the response of these soils to salt concentration. Because research on the impacts of salinity on N cycling and fertiliser efficiency on these soils is limited, the greenhouse study in Chapter 5 was conducted to respond to the **fourth research question**: how does salinity alter soil properties and rice nutrient efficiency in the field soil-plant system? The study objective was to investigate changes in soil properties, the dynamics of N and their effects on rice growth and yield with different salinity levels. Higher cumulation of soil inorganic N was found in the high salinity treatment of the acid sulphate soil. The study showed that the interaction between soil and salinity significantly altered rice growth performance. The combination of acidity and elevated salinity affects to plant growth such that fertilizer N could not be taken up thereby lowering the recovery of applied N and resulting in yield limitation. The greatest fertilizer loss was 60% while other losses ranged from 28-38% with fertilizer uptake of 19-50%. The result of this study highlights the need to develop strategies to improve both soil productivity and crop yield under such scenarios.

Figure 6.1 An integrated model of the agricultural floodplain undergoing salinity changes. Figure (*a*) presents the model based on the results from the pot experiment in the chapter 5. Figure (*b*) shows the conceptual model following the results from incubation studies in the chapters 3 and 4. The thickness of brown and green arrows (a) and grey arrows (b) indicates the amount of fertilizer input, the percentage of nitrogen recovery efficiency (NRE), and cumulative gases emitted respectively. The different sizes of gene (b) show different abundance of denitrifying genes. Nitrate and available carbon addition is same amount for both studied soils (b). Different length of grey and dark arrows (b) indicates different gas emissions relative to salinity levels.



The world's agricultural floodplains in low-lying areas are subject to changes of sea level. Sea level rise causing salt water intrusion in agricultural land will increase salinity effects on soil properties and crop growth. The integrated model in Figure 6.1 illustrates major impacts of salinity changes on a typical floodplain with two common soils: acid sulphate soil and alluvial soil. Based on the results of the studies undertaken for this research, it is concluded that about 60% of nitrogen input is lost when it is applied to acid sulphate soil with high salinity (8 g L^{-1}). Nitrogen recovery efficiency of rice planted in the alluvial soil was around 40% at either salinity level. Crop yield from the alluvial soil may not be affected by salinity while crops could fail to produce yield due to the adverse effects of high salt concentration in the acid sulphate soil. On the other hand, the salinity reduces cumulative GHG emissions, but the emissions will increase with the addition of nitrogen and carbon sources. The abundance of denitrifying genes did not reduce under the effects of water salinity. This leads to completed denitrification and results in a decrease of nitrous oxide flux and soil respiration.

The present study has assessed effects of salinity on nitrogen and carbon cycling, soil-induced gas emissions in agricultural soils. Under salt water impacts, key issues including GHG emissions, changes of soil nutrients, nitrogen recovery efficiency, abundance of denitrifying genes, crop growth and yield have been discussed. Salinity alters carbon and nitrogen cycling in flooded soils leading to changes in number of denitrifying genes and gas emissions when carbon and nitrogen source are available. Therefore, effective mitigations such as salt leaching or washing to reduce soil and water salinity of soil plant systems; biochar application to reduce gas emissions and organic matter amendment to improve soil properties and plant nutrient uptake should be paid attention when arable soils are used for agricultural production under context of saline water intrusion increased by climate change. Right time of nitrogen fertilizer application based on crop nutrient demand can reduce nitrogen overload in ecosystems and increase nutrient use efficiency. Moreover, use of nitrification/denitrification inhibitors can be efficient in regulating soil microbial activity and N transformation by blocking the first stage of nitrification resulting in decrease nitrate availability and nitrous oxide fluxes. However, cost and benefit from the use of nitrification inhibitors need to be analysed when this method is applied in regions or areas lack of capital resources. In particularly vulnerable areas to salinity and climate change impact similar to the Mekong Delta, to maintain and improve agricultural production either change of cropping calendar to avoid a period of high salinity or diversification of farming systems by introducing shrimp and salt-tolerant crops to adapt with natural conditions can be alternative land-use options.

6.2 Limitations and future research

The results reported in this thesis will improve current understanding of the nitrogen and carbon cycling, and of soil-induced gas emissions, in salt-affected soils. However, there are some limitations in the study and they also prompt new research questions which can be addressed in future studies.

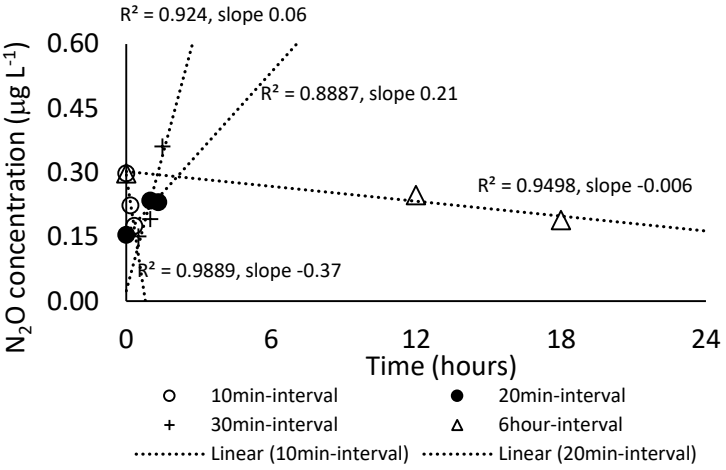
1. Field measurements: Most results reported in this thesis were achieved only from short term incubations in controlled laboratory conditions. The controlled environment of the laboratory allowed the studies to avoid environmental variability and tested the desired experimental factors such as soil types and salinity effects. Soil slurries were maintained under completely inundated condition resulting in a difference of hydrology from nature where soil can be impacted by a change of tidal regimes. Therefore, this work could be expanded to predict long-term effects by additional studies under field conditions.
2. In Chapter 3, the different levels of salinity did not result in significant differences in cumulative N_2O-N emission from the alluvial soil. This suggested that N_2O-N emissions from this soil after saline water application may have been affected by the availability of other components such as dissolved organic carbon, and microbial biomass C and N. Further studies on the interaction of these components with salinity and their effects on N and C cycling in soils should be addressed.
3. Intervention to ameliorate the adverse effects of saline soil will not only enhance microbial activity, but also improve crop productivity. Typical ameliorations suggested for saline soil include applying optimum rates of fertilizer to crops, and additional organic carbon to improve soil physio-chemical and biological properties. In this research, a better understanding of the adverse effects of salinity were gained. But the further application of the amelioration has not been tested. Future work could investigate the dynamics of soil N and C in saline soils with the application of these ameliorations.
4. The present incubation study used only nitrate and glucose as the available sources of energy and as electron donors for microbial activity. The microbes can quickly use these substrates to complete their function in soil processes. Other types of nitrogen and carbon sources such as plant residues, compost or commercial organic fertilizers might cause different responses in the salt-affected soil. Because of the low content of carbon in the salt-affected or saline soil, this suggests further investigations into different types of nitrogen and carbon, and repeated addition of these substrates to changes of properties in the salt-affected soil.

5. Chapter 5 investigated the nitrogen fertilizer recovery of rice plants in salt-affected soil. The result also quantified how much fertilizer nitrogen was lost. However, the study in this chapter does not indicate which was the most important pathway of fertilizer nitrogen loss either gas or drainage loss. Additional studies could analyse ^{15}N labelled nitrogen in gas samples and drainage water to collect data from each pathway of the loss.

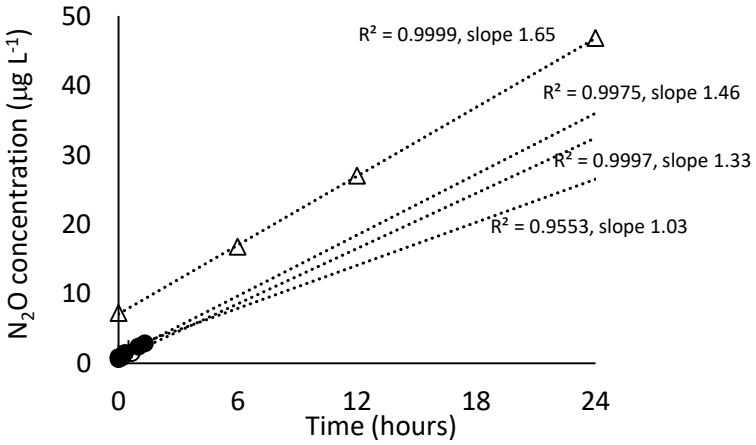
Appendix 1: Linear regression for N_2O fluxes in the ASS (a), Red Dermosol (b), and Vertosol (c).

These graphs support for the method in Chapter 2.

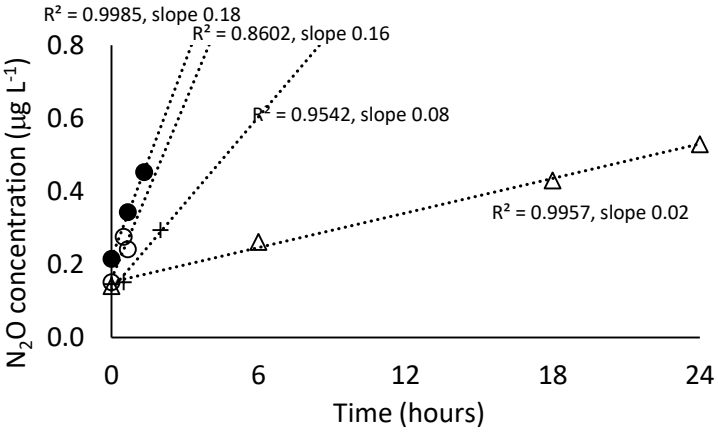
(a)



(b)



(c)



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