Influence of gypsum application on methane production and emission from saline paddy soils in relation to growth and root exudation of rice plants

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DECLARATION OF ORIGINALITY

I hereby declare that this thesis and the work reported herein was composed by and originated entirely from me. Information derived from the published and unpublished work of the others has been acknowledged in the text and references are given in the list of sources.

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Abstract

Paddy rice fields are identified as one of the major sources of global warming methane (CH_4) gas. However, the extent of CH_4 emission is largely influenced by the soil condition to which rice is planted and management practices associated with rice cultivation. Among the world total rice areas, about 30% contains too high levels of salts for normal rice growth. In general, soil salinity is caused by occasional or periodic intrusion of sea water, or by surface evaporation of soil water initially high in salt content. Thus, CH_4 emission from rice cultivation was evaluated under irrigation water salinity and saline soil. To improve the rice growth in Na^+ saturated soil or water, gypsum fertilizer application is one of the common practices. Because of current increasing rate (about 1.0% each year) of global CH_4 concentration in the atmosphere (IPCC, 2007) and its strong infrared absorption characteristic, it also needs to evaluate the impact of paddy field management practices on CH_4 emission to avoid climate change impact (Rogner et al., 2007). Therefore, the effect of gypsum fertilizer addition to saline condition on CH_4 emission was also studied in relation to rice growth.

In chapter (1), CH_4 production potential of soil was evaluated under five different irrigation water salinity levels; 0, 10, 30, 60 and 90 mmol L^{-1} NaCl for 3 weeks. The addition of NaCl up to 30 mmol L^{-1} increased in CH_4 production. The increase of CH_4 production in salinity 10 and 30 mmol L^{-1} NaCl was 1.2 times and 1.5 times higher than control, respectively. This addition of saline water up to 30 mmol L^{-1} to soil might fulfils the

requirement of Na^+ by methanogenic bacteria because Kalmokoff (1988) reported that sodium is required for amino acid transport, growth, methanogenesis, and internal pH regulation in methanogenic bacteria. At higher salinity levels (S60 and S90), CH_4 production was 19 to 33% lower than control. It might be due to the Cl^- toxicity at higher salinity levels.

The salinity levels such as 30 and 90 mmol L^{-1} NaCl which showed maximum and minimum CH_4 production were selected and studied in rice cultivation with or without gypsum fertilizer addition. It was conducted as pot experiment. The continuous application of saline water significantly suppressed the rice growth at both salinity levels. The rice plants in salinity 90 mmol L^{-1} could survive until 7 weeks after transplanting. The higher total numbers of dead leaves were observed in saline condition with or without gypsum fertilizer addition. The CH_4 emission in salinity level 30 mmol L^{-1} NaCl without gypsum fertilizer (296 kg CH_4 ha^{-1}) was not significant different with that of control (316.213 kg CH_4 ha^{-1}) but the amount of emission was numerically lower. It might be due to its lower above plant biomass yield. The lowest CH_4 emission was observed in salinity 90 mmo L^{-1}NaCl (44.2-55.5 kg CH_4 ha^{-1}). It might be due to its lower CH_4 production potential and lower above biomass yield and the shortest growth duration period. Gypsum fertilizer addition reduced CH_4 emission either in saline or non-saline condition. The reduction rate due to gypsum fertilizer addition was about 53-83% in saline condition and about 56% in non-saline condition. It might be due to the competition of SO_4^{2-} reducing bacteria and methanogenic bacteria for the organic carbon substrate.

Thus, the effect of different rates of gypsum fertilizer addition in to rice cultivation on CH_4 emission was evaluated in chapter with pot experiment. The gypsum application rates were 0, 1, 2.5 and 5 ton ha^{-1} and irrigation water salinity levels were 0 and 25 mmol L^{-1} NaCl. The results showed that the irrigation of water with salinity level 25 mmol L^{-1}NaCl could not suppress CH_4 emission. Higher application of gypsum resulted in higher reduction in CH_4 emission. However, the reduction of CH_4 emission due to gypsum was higher in non-saline condition than saline condition. It might be due to the higher carbon availability that was contributed from dead plant materials in saline condition compared to non-saline condition.

As saline soil is composed of a range of dissolved salts such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3, CH_4 emission was also studied in coastal saline soil in chapter (4). The coastal saline soil was collected from the tsunami flooded paddy field in Sendai city, Tokyo, Japan. Three different rates of gypsum fertilizer such as gypsum 0.5, 1 and 2 ton ha^{-1} were applied to the coastal saline soil to provide the appropriate Na/Ca ratios in the soil. Methane emission was evaluated during vegetative stage for 30 days. Gypsum fertilizer addition significantly reduced the soil pH and increased soil EC. However, the increase in K^+ concentration of plants and decrease in Na^+ concentration of plants were observed in gypsum 0.5 and 1 ton ha^{-1}. The total organic carbon concentration in gypsum 0.5 ton ha^{-1} was the highest among the treatments at 30 days after transplanting. There was no significant difference in the shoot dry weight except gypsum 2 ton ha^{-1}. The significant highest CH_4 emission was observed in gypsum 0.5 ton ha^{-1} and it might relate with its highest total carbon concentration, the added amount of SO_4^{2-} that that did not reach the threshold limit necessary for a successful competition of carbon substrates between sulphate reducers with methanogens. The lowest CH_4 emission was observed in gypsum 2 ton ha^{-1}. This lowest CH_4 emission might relate with its lowest pH value (about 5.82), highest input of SO_4^{2-} concentration from gypsum fertilizer and lowest above dry matter yield.

Rice plants supply carbon substrate to methanogens via root exudates and dead plant materials. Plants release about 40% of net carbon fixed during photosynthesis as root exudates. As high salinity reduces photosynthetic efficiency and plant growth, it may limit the extent of carbon contribution to the soil via root exudation. Thus, root exudates were collected from plants which are grown in chapter (4) at maximum tillering stage. After that, organic acids concentrations of root exudates were analyzed in chapter (5). Six organic acids species such as Citric, Tartaric, Malic, Lactic, Formic and Acetic acids were observed in the root exudates of control, gypsum 0.5, and 1 ton ha^{-1}. Only four organic acids species, Citric, Tartaric, Malic, and Lactic acids were observed in gypsum 2 ton ha^{-1}. It might relate with its lower shoot dry weight. Furthermore, the higher values of organic acid exudation potential were observed in gypsum fertilizer treatments over that control. It might relate with higher EC values in gypsum fertilizer treatments over that of control. Under the salt stress, most of the plants accumulate low molecular weight organic solutes (Jouyban, 2012), and) plants can secret more metabolites and some unknown substances under environmental stress Bucio et al. (2000).

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List of Abbreviations

C: Carbon

Ca: Calcium

CH_4: Methane

CO_2: Carbon dioxide

DAT: Days After Transplanting

EC: Electrical conductivity

Eh: Redox potential

K: Potassium

LSD: Least Significant Difference

N: Nitrogen

NH_4: Ammonium

NO_3: Nitrate

SO_4: Sulphate

SOC: Soil Organic Carbon

TOC: Total Organic Carbon

Chapter 1

General Introduction

1. General Introduction

1.1 Rice production around the world

Rice is the most loved cereal of Asia. It feeds more than half of the world's population (IRRI, 2006). In 2007, the average annual consumption per capita was about 197 kg (FAOSTAT, 2012) and provided 49% of the calories and 39% of the protein in the diet (FAOSTAT, 2012). According to the FAOSTAT online data base, approximately164.72 million hectares were harvested worldwide in 2013. Among them, about 88.9% (146.46 million ha) were in Asia and 34.9% (51 million ha) of the cultivation area were harvested in Southeast Asia alone.

1.2 Salinity in rice growing regions of the world

In the present, salinity is the second most widespread soil problem in rice growing countries next to drought and considered as a serious constraint to increased rice production (Amaranatha et al., 2014). About 30% of the 130 million hectares where rice is grown contain salt that is too high to allow normal rice yield (Mishra, 2004). Rice is cultivated in more than 115 countries, of which Asia's share is more than 91% of the world total. Majority of the Asian rice production zone is confined to South and South East Asia wherein about 48 Mha of potentially useful agricultural land is unusable for growing rice in Southern Asia and South East Asia due to saline soils (Vinod et al., 2013). Although rice is considered as a sensitive crop to salinity, it is one of the most widely grown crops in coastal areas

(Amaranatha et al., 2014). Redfern et al. (2012) reported that in the humid regions of Southeast Asia, there are many hectares that are technically appropriate for rice production but are left uncultivated or are grown with very low yields because of salinity and soil problem. In general, soil salinity is caused by occasional or periodic intrusion of sea water, or by surface evaporation of soil water initially high in salt content. Thus, some saline land is found along every coast, but the extent of affected areas varies greatly. Generally, salt accumulation increases in the drier climates and diminishes strongly in an equatorial climate without a pronounced dry season. Extremely saline deltas are found in arid areas (Datta et al., 1981). In view of current levels of the growing world population, it is estimated that there will be a need to increase food production up to 38 % by 2025 and 57 % by 2050 to maintain the food supply. Green revolution helped to solve the world's demand for food, but is not enough to meet the 21st century's exploding population (Barus et al., 2013). Therefore, it is necessary to promote rice production not only to increase yield per unit of fertile land but also to expand the area on uncultivated land efficiently.

1.2.1 Impact of salinity on rice growth

Soil salinity is caused by an increased activity of soluble salts. Generally, soils with electrical conductivity higher than 4 dS m^{-1} are generally considered as saline soils (Munns, 2005). According to the USDA (1954), soils can be classified as non-saline/non-sodic soils (ESP ≤15%; EC ≤ 4 mmhos cm^{-1}), saline soil (ESP ≤ 15%; EC > 4 mmhos cm^{-1}), sodic soil (ESP > 15%; EC ≤ 4 mmhos cm^{-1}) and as saline sodic soil (ESP > 15%; EC >4 mmhos

cm^{-1}). The pH of saline soils is generally less than 8.5, of saline sodic soils about 8.5 and of sodic soils more than 8.5.

Salinity reveals adverse effects on plants. Elevated levels of salt ions in soil solution surrounding plant roots induce an imbalance in water potential between plant root cells and ambient soil solution and result in cellular dehydration (Kosová et al., 2013). It is known as osmotic stress. In addition, elevated salts lead to a passive salt ion penetration via plasma membrane and to an accumulation of salt ions in cell cytoplasm which can lead to inhibition of intracellular enzyme activity (Munns, 2008). If excessive amount of salt enter the plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence and reduced the photosynthetic leaf area of a plant to a level that can not sustain growth (Shereen et al., 2005). Thus, salinity appears to affect two plant processes such as water relations and ionic relations. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves (Amirjani, 2011). Therefore, it can be said that salinity has three potential effects on plants:

i. Lowering of the water potential
ii. Direct toxicity of any Na^+ and Cl^- absorbed
iii. Interference with the uptake of essential nutrients (Flowers and Flowers, 2005)

Rice is relatively salt-sensitive amongst cereals (Kavitha et al., 2012). According to the classification of salt tolerance to salinity, the rice crop is within the sensitive division from 0 dsm^{-1} to 8 dsm^{-1} (Mass and Hoffman, 1986). There are two essential parameters sufficient for expressing salt tolerance; threshold and slope values. Threshold value means the maximum allowable salinity without yield reduction and slope value means the percent of yield reduction per unit increase in salinity beyond the threshold. The threshold and slope values of rice (*Oryza sativa*) are 3.0 dsm^{-1} and 12% per dsm^{-1} of saturated soil extract (ECe), respectively (Mass and Hoffman, 1977). Relative salt tolerance of rice at 50 % yield and at 50 % emergence are 3.6 dsm^{-1} and 18 dsm^{-1} of ECe, respectively (Wahhab, 1961).

Although, salinity affects all stages of the growth and development of rice plant and the crop responses to salinity varies with growth stages, concentration and duration of exposure to salt (Shereen et al., 2005). The symptoms of salt injury in rice are stunted growth, rolling of leaves, white tips, drying of older leaves and grain sterility. Soil salinity limits the rice plant's growth and development, resulting in yield losses of more than 50% (Zeng and Shanon, 2000). Sensitivity of rice to salinity stress varies with the growth stage. Though salinity affects all stages of the growth and development of the rice plant, when the rice is at the young seedling stage it becomes even more sensitive to salinity (Shereen et al., 2005; Deepa Sankar, Saleh and Selvaraj, 2011). Rad et al. (2012) also reported that the primary stages of rice growth such as tillering and panicle initiation stages are more sensitive to salinity stress.

1.2.2 Amelioration practices used in salt affected area

As salinity is one of the most serious abiotic stresses on rice growth of the main rice growing area especially in saline deltas of arid areas, it needs to find out low-cost, efficient treatment strategies to reduce the salt toxicity of soils and to improve the soil properties. There are many procedures that can be used to improve salt affected land, such as, water leaching, chemical remediation and phytoremediation (Ghafoor et al., 2008; Sharma and Minhas, 2005; Qadir et al., 2007; Feizi et al., 2010). The remediation of saline soil using Ca amendments such as ($CaSO_4$ $2H_2O$), calcite ($CaCO_3$), calcium chloride ($CaCl_2$) and organic matter (farmyard manure, green manure, organic amendment and municipal solid waste), is a fruitful topic of investigation and can be applied worldwide, being low cost, effective and simple to implement (Mitchell et al., 2000; Hanay et al., 2004; Sharma and Minhas, 2005; Tejada et al., 2006; Makoi and Verplancke, 2010). Among those Ca amendments, gypsum is typically used as a source of calcium (Ca) (Shaaban et al., 2013). High Ca^{2+} concentrations can reduce the permeability of plasma membrane to Na^+. The reduction in membrane permeability to Na^+ by Ca^{2+} reduces the accumulation of Na^+ by passive influx (Cramer et al., 1985). The Ca^{2+} ions in the gypsum fertilizer will replace Na^+ ions that adsorbed to the soil particles and $NaSO_4$ will be reached away from the root zone.

In addition, the application of Ca^{2+}amendment can improve various soil properties and act as soil modifiers that prevent the development of sodicity which is directly related to plant growth, crop productivity and crop yields (Muhammad and Khattak, 2011). Many studies such as Ghafoor et al. (2001), Choudhary et al. (2004), and Wong et al. (2009) approved that the physical, chemical and biological properties of salt affected soil are improved by the application of gypsum and/or farmyard manure (FYM) as remediation for sustainable land usage and crop productivity, leading to enhanced plant growth and development. Since organic materials improve the soil physicochemical properties that accelerate exchange of cations on soil solids and leaching of salts from the root zone (Clark et al., 2007), hence preventing root from salt injuries and roots can grow more smoothly, gypsum is usually applied in combination to organic materials such as farmyard manure and humid acids etc. Ullah and Bhatti (2007) found that the conjunctive uses of farm manure with gypsum significantly improve soil physicochemical properties of sodic soils as compared to their alone application (Ullah and Bhatti 2007). Cha-um et al. (2011) observed that the application of gypsum and FYM to saline soil had 79.6% spikelet fertility, while rice grown in soil without the gypsum and FYM treatment had only 46.4%. Thus, this author suggested that gypsum and FYM treatment should effectively remedy the saline soil problem, resulting in yield improvement from paddy fields.

1.3 Global warming and methane gas emission

Methane (CH_4) is the second most important greenhouse gas after carbon dioxide (CO_2). Its global warming potential is 25 times greater than CO_2. It contributes to the greenhouse effect is about 1.7 W m^{-2} (Purkait et al., 2006) that accounts for 15 to 20% of global warming (IPCC, 1996). Moreover, global concentration of greenhouse gas CH_4 has been increasing at the rate of ~1% each year (IPCC, 2007). If the current rate of increase is maintained, in the next 50 years, it is estimated to contribute an additional 0.5 W m^{-2} in radiative heating (Purkait et al., 2006). Thus, global warming due to increasing greenhouse gases emission is current great environmental concern.

Methane is produced under anaerobic environments by obligate anaerobic microorganisms through either CO_2 reduction or transmethylation processes (Hou et al., 2000). There are two main sources for CH_4 production; anthropogenic sources and natural sources. More than 50% of global CH_4 is related to human activities (US EPA, 2006). Anthropogenic sources include fossil fuel production, animal husbandry (enteric fermentation in livestock and manure management), rice cultivation, biomass burning, and waste management, etc. Among the anthropogenic sources, rice fields occupied 10% of global anthropogenic CH_4 emission (IPCC, 2007) because of the flooding condition required for the rice cultivation. However, there are several factors influencing upon CH_4 emission in paddy fields such as soil type, climatic conditions, agronomic practices including water and fertilizer management, organic matter amendment, and application of pesticides, etc. (Zou et al., 2005; Xiong et al., 2007; Yan et al., 2005; Aulakh et al., 2001; Liesack et al., 2000).

1.3.1 Factors affecting methane emission

Methane emission from rice fields is affected by various factors such as prevalent weather conditions, water regime, various cultural practices, and soil properties (Jian et al., 2004). Among those factors, soil factor is largely influenced on CH_4 emission from paddy fields where rice plants are grown associated with specific management practices. The nature of paddy rice fields require for flooded condition. When soil is saturated with water, pH drops at first due to organic acid produced from fermentation of easily decomposable organic materials. Then, pH gradually starts to rise as a result of the buffering capacity of the soil (Kyuma, 2004). Methane production in flooded rice soil is very sensitive to pH and Setyanto et al. (2002) reported that the optimum pH of paddy soils required for methanogenic bacteria to produce CH_4 is around 6 to 6.6. At the same time, the redox potential (Eh) of flooded soils also decrease as a result of the production of reduced forms. The sequence of the redox reaction and approximate values for redox potentials associated with specific oxidation-reduction process are as follows (Kyuma, 2004);

Eh values	**Reactions**
+330 mV	$\frac{1}{2} O_2 + 2e^- + 2H^+ \rightarrow H_2O$ (by facultative anaerobes and aerobes)
+220 mV	$2NO_3^- + 12 H^+ + 10e^- \rightarrow N_2 + 6H_2O$ (by denitrifiers)

+200 mV $MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$ (by manganese reducing bacteria)

+120 mV $Fe(OH)_3 + 3 H^+ + 2e^- \rightarrow Fe^{2+} + 2H_2O$ (by iron reducing bacteria)

-150 mV $SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$ (by sulfate reducing bacteria)

-250 mV $CO_2 + 8 H^+ + 8e^- \rightarrow CH_4 + 2 H_2O$ (by methanogens)

Based on this explanation, CH_4 production can be occurred below the redox potential value (-150 mV) (Jain et al., 2004). As CH_4 is an end product of microbial decomposition organic materials under anaerobic condition, the availability of soil organic carbon (SOC) is an important factor for CH_4production in rice fields. Jiao et al. (2005) proved that there was significant positive correlation between SOC and CH_4 emission in rice soil. In addition, the decomposition rate of organic matter from which methanogenic substrates are produced is affected by soil temperature. In Japanese paddy fields, Hattori et al. (2001) recorded that the optimum temperature for CH_4 production was 40 ˚C due to dominance of methanogenic population at this temperature. Methane formation was very slow at temperature below 20 ˚C (Yamane and Sato, 1961). Furthermore, availability of electron acceptors such as Fe (III), SO_4^{2-} is also an important indicator in CH_4 emission in rice fields. Seasonal CH_4 emission in Fe-amended pots was lower than the control treatment (Huang et al., 2009). Van der gon and Neue (1995) also proved that the application of SO_4^{2-} containing fertilizer to paddy fields reduced in CH_4 emission. Beside these above soil factors, salinity is considered as one of the soil factors to influence upon CH_4 emission by affecting soil microbial activities including methanogens (Pattnaik et al., 2000).

1.3.2 Impact of salinity on methane emission

Biswas et al. (2006) have studied CH_4 emission from the saline rice fields of Sunderban mangroves of east coast of India and reported significant reduction of CH_4 emission from the rice fields reclaimed from mangrove swamp compared to upland rice fields and mangrove forest area. Supprattanapan et al. (2009) have studied the effect of organic amendment on CH_4 emission from saline rice soil and reported no significant difference in CH_4 emission from soils amended with organic matter as compared to unamended soils. Generally, salinity is known to inhibit CH_4 production. However, saline soils are composed of different levels of carbonates/bicarbonates, sulphates and chlorides of sodium (NaCl), potassium (KCl), calcium ($CaCl_2$), and magnesium ($MgCl_2$). These different components of salinity inhibit CH_4 production followed the order of sulphate> bicarbonate> chloride (Mishra et al., 2003). Thus, among these anion species, the addition of NaCl showed the least inhibitiononCH_4 production and the reduction rate was 38% compared with control at pore water EC 8 dSm^{-1} (Mishra et al., 2003). Some of the methanogenic strains showed inhibition only at high concentrations (>0.17 M), well above the concentrations normally found in saline rice soils (0.04-0.1 M) (Van der gon and Neue, 1995) because Na is required for amino acid transport, growth, methanogenesis, and internal pH regulation in methanogenic bacteria. The amount of sodium requirement is different among methanogens including very high values for halophilic methanogens (Jarrell and Kalmokoff, 1988). The mechanism might be osmotic imbalance by affecting the cation-anion equilibrium that in turn may adversely affect the activity of methanogens and/or microorganisms producing substrates for methanogens.

1.3.3 Impact of gypsum amendment on methane emission

For Na-saturated soil or water, gypsum fertilizer is a commonly suggested fertilizer as the nutrient Ca^{2+} source (Davitt et al., 1981; Aslam et al., 2001; Mahmood et al., 2009). The chemical composition of gypsum fertilizer ($CaSO_4$. $2H_2O$) is 23.8% Ca^{2+} and 18% S. Regarding with the CH_4 emission, sulphate (SO_4^{2-}) containing fertilizers including gypsum fertilizer is frequently suggested as CH_4 mitigation option. Van der gon and Neue (1994) proved that the application of gypsum (6.66 ton ha^{-1}) to rice paddy soil could reduce CH_4 emission in the wet season. At the successive growing period, Van der gon and Neue (1995) studied the salinity effect on CH_4 emission by adding NaCl to the same paddy fields which is amended with or without gypsum fertilizer. The authors observed that addition of NaCl reduced in CH_4 emission. The reduction value of CH_4 emission was considerably smaller in the rice paddies where the soils have low in SO_4^{2-} than that of CH_4 emission where the soils have high in SO_4^{2-}. Nevertheless, there are still contradictory report on rice fields fertilized with SO_4^2 containing fertilizers such as ammonium sulphate (NH_4 $(SO_4))_2$, calcium sulphate ($CaSO_4.2H_2O$)etc. on CH_4 emission; CH_4 emission either increased (Cicerone and Shetter, 1981), stayed constant (Wassmann et al., 1993) or decreased (Schutz et al., 1989a; Van der gon, 1995; Lindau et al., 1993). These contradicting results may be due to differences in C substrate and SO_4^2 availability at various field sites.

1.4 The role of root exudates in methane emission

Plant roots have ability to synthesize, accumulate and secrete a diverse array of compounds. More than 200 C compounds released from plant roots in the form of exudates (Bowen, 1999). On the basis of molecular weight, they can be grouped into high molecular-weight compounds such as mucilage, gelatinous material, covering root surfaces, ectoenzymes and low molecular-weight compounds such as organic acids, sugars, phenolics, amino acids, phytosiderophores, flavonoids and vitamins. Secretion of compounds into the rhizosphere is one of the most remarkable metabolic features of plant roots (Kumar et al., 2006). Cereals transfer 20–30% of total assimilated C into the soil, and half of this amount is subsequently found in the roots and about one-third is lost in the form of CO_2 by root respiration and microbial utilization of root-borne organic substances (Gregory and Atwell, 1991).

The remaining part of underground translocated C is incorporated into the soil microorganisms and SOC. Researchers argue that photosynthetically fixed C in cereals and grasses is transported rapidly towards the root and can reach the external environment of the root within an hour (Kuzyakov, 1999). During one vegetative period, cereals and grasses allocated below the ground are about 1500 and 2200 kg C/ha respectively (Bowen, 1999).

This accounted for nearly 5 to 21% of all photosynthetically fixed C transferred to the rhizosphere through root exudates and ranged from 20 to 50% of plant biomass.

Rice plants play an important role in different levels of the CH_4 budget of rice fields. Various studies have reported about root exudates in regulating the CH_4 budget of rice field (Nakićenović, 2000). Wassmann and Aulakh (2000) reported that CH_4 production in rice fields largely depends on plant-borne material that can be either decaying tissue or root exudates. Holzapfel-Pschorn et al. (1986) observed that methanogenesis in submerged soil when rice plants were stimulated and concluded that a second peak observed in CH_4 emission at the end of tillering stage was due to the release of root exudates or products of root autolysis. By contrast, high in situ fluxes of CH_4 during the reproductive period of rice plants have been attributed to the supply of organic C from rice root exudation (Lindau et al., 1991; Minoda and Kimura, 1994; Neue and Sass, 1994). By using a ^{13}C-trace technique, Chidthaisong and Watanabe (1997) indicated that the contribution of plant-supplied organic material to the formation and emission of CH_4 sharply increase after heading. However, the amount of knowledge available on exudation from rice plants is minute and the literatures about the impact of quality and quantity of root exudation on CH_4 production and emission are also limited.

1.4.1 Factors affecting root exudation

The qualitative and quantitative composition of root exudates is affected by environmental factors such as pH, soil type, oxygen status, light intensity, soil temperature, plant growth, nutrient availability and microorganisms (Mimmo et al., 2011). Generally, it was reported that plant roots would excrete more metabolites and some unknown substances under environmental stress (López-Bucio et al., 2000). However, there is limited information about all possible factors influencing on root exudation of rice crop. Furthermore, there is also limited knowledge to explain how physiological process involved in exudation process. The possible factors influencing on quantity and composition of root exudates in different crops and different situation are summarized and described as follows.

(a) Soil types

Neumann et al., (2014) studied that the effect of three soil types on root exudation of the lettuce plant (*Lactucasativa* L. cv.Tizian (BBCH19)). Apart from variability in root development, huge quantitative differences particularly for sugars and amino acids were detected in the root exudates samples of lettuce plants. Mutual interactions between plant roots and soil- specific microbes seem to be important determinants for these quantitative differences of root exudation.

(b) Soil moisture

Katznelson et al. (1954) found that temporarily wilting of plants greatly increased the release of amino acids from roots, which would be important under field conditions.

(c)Temperature

Husain and McKeen (1963) found more amino acids in exudates of strawberry plants (*Fragariavesca* L.) grown at 5 to 10 ^{o}C than at 20 to 30 ^{o}C; this markedly influenced the pathogenicity of Rhizoctonia fragariae which attacks strawberries at low soil temperature.

(d) Nutrient deficiency

Lilia et al. (2012) compared the root exudates from axenically grown maize plants exposed to nitrogen (N), potassium (K), phosphate (P) or iron (Fe) deficiency. The results showed that a higher release of glutamate, glucose, ribitol, and citrate from Fe-deficient plants. Phosphate deficiency stimulated the release of C-amionobutyric acid and carbohydrate. Potassium-starved plants released less sugar, in particular glycerol, ribitol, fructose, and maltose, while under N deficiency lower amounts of amino acids were found in root exudates.

(e) Ion toxicity

According to Ma and Furukawa (2003), plant roots under Al stress might exude various organic acids, including citric and Malic acids, and these organic acids could play a very important role in alleviating aluminium toxicity in soil.

(f) Plant species

Within same rice species but among 4 different cultivars including Supanburi 1, Supanburi 60, Supanburi 90 and Chainat 1, Pusatjapong (2003) observed higher quantity of sugars and organic acids in the root exudates of Supanburi 60 and Supanburi 90 compared to the rest 2 rice cultivars.

Between legume and grass species, the use of ^{15}N-labelled amino acids has shown that efflux of glycine and serine from roots of legumes is higher than from roots of grasses among amino acids found in root exudates (Lesuffleur et al., 2007).

(g) Microorganisms

Shi et al. (2011) studied the effects of artificial root exudates solutions, based on those collected *in situ*, on the community composition and diversity of metabolically active soil bacteria in a soil microcosm experiment. This study showed that organic acids caused significantly greater increases than sugars in the detectable richness of the soil bacterial community. Therefore, organic acids play a significant role in shaping soil bacterial communities.

Understanding the factors that affect on root exudation in various crops may insight an effective approach for increasing crop production and mitigating CH_4 emission from paddy rice soil since root exudation is known as the significant C cost for crop production and C pool for CH_4 production in paddy rice cultivation.

1.4.2 Function and mechanism of organic acids in plant

Based on the reference of (López-Bucio, 2000), organic acid metabolism is of fundamental importance at the cellular level for several biochemical pathways, including energy production, formation of precursors for amino-acid biosynthesis and at the whole plant level in modulating adaptation to the environment. A striking feature of plant tissues is that the total content of organic acids is higher than in other organisms. The high accumulation of organic acids in plant tissues is most probably due to their important role as photosynthetic intermediates. However, organic acids have a potential role as metabolically active solutes for the osmotic adjustment and the balance of cation excess. Organic acids also participate as key components in the mechanisms that some plants use to cope with nutrient deficiencies, metal tolerance and plant–microbe interactions operating at the root–soil interface (Lo´pez-Bucio, 2000). Organic acids are mainly produced in mitochondria through the tricarboxylic acid or Krebs cycle and to a lesser extent in the glyoxysome as part of the glyoxylate cycle. Because of the catalytic nature of the Krebs cycle, organic acids are present only in very small pools in the mitochondria and are preferentially stored in the vacuole (López-Bucio, 2000).

Chapter 2

Influence of different levels of saline irrigation water on methane production and emission in paddy rice soil with or without addition of gypsum fertilizer

The objectives of this study are;

1. To clarify the influence of irrigation water salinity levels on CH_4 emission from paddy rice soil
2. To evaluate the effect of gypsum fertilizer as Ca^{2+} on CH_4 emission in relation to plant growth.

2.1 Materials and methods

2.1.1 Laboratory Incubation Experiment

(a) Effect of salinity levels on methane production potential of soil

A composite soil sample was collected from a depth of 0–15 cm at FM Fuchu Honmachi, Field Science Center of the Tokyo University of Agriculture and Technology. The soil was kept in a refrigerator for one week until the incubation experiment. The background salinity level of soil (EC 1:1) was 0.23 d Sm^{-1}. The methane production potential of this soil was studied at 5 salinity levels: control, 10 (S10), 30 (S30), 60 (S60) and 90 (S90) mmol L^{-1} NaCl. The objective of this study was to evaluate the CH_4 production potential of soil by the addition of different concentration of NaCl solution. Twenty gram of wet soil was put into 100-ml conical flasks and then flooded with 20 ml of tap water for the control or with different levels of saline water for the salinity treatments. The conical flasks were fitted with rubber stoppers possessing two tubes to facilitate flushing with nitrogen gas (N_2) and the collection of gas samples. These flasks were kept in an incubator at 30^{o}C for 21 days. There were 4 replications for each salinity level.

Three replications were used to collect the CH_4 gas samples and another one replication was used to monitor the changes of soil pH under each salinity level during the incubation period by using Beckman, Φ 260 pH/ Temp/ mV meter. To check the redox potential value (Eh) of incubated soil whether it could support for anaerobic condition or not, the extra one control sample was prepared and platinum Eh probe was permanently inserted into that soil. The value of Eh was recorded by using SWC-201RP, Sanyo water checker on each sampling day. Gas samples were collected on 2, 4, 7, 14 and 21 days after incubation.

The flushing with N_2 gas was carried out one day before and immediately after the sampling at 250 ml min^{-1} for 3 minutes. The conical flasks were shaken just before sampling to release the CH_4 entrapped within the soil. Gas samples were drawn with 20 ml plastic disposable syringe fitted with a needle and a three-way valve to prevent diffusion of the gas sample. During sampling, the air in headspace of each conical flask was thoroughly mixed by pushing the plunger of the syringe up and down for ten times at first, and then about 5 ml of gas sample was collected. After collecting the samples, the beakers were flushed with nitrogen gas immediately at 250 ml per minute for 3 seconds. Methane gas samples were analyzed by using a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan) with a flame ionization detector (FID). The GC machine was equipped with a Porapak N (80/100 mesh) column and used He gas as carrier gas. The temperature of column, and injector were maintained at 60 ˚C and 180 ˚C. The amount of CH_4 flux was calculated by using the following equation;

$$C_i = C_a \times V_{hs} \times MW \times {}^{MV}/_{WS} \times d \times 1000 \qquad (1)$$

Where C_i: CH_4 production (μgCH_4/g soil /day)

C_a: CH_4 concentration in the head space (ppm)

V_{hs}: the volume of head space (ml)

MW: the molecular mass of CH_4 = 16.123 mg $mmol^{-1}$

MV: the molar volume of CH_4 at 30 ˚C = mmol mL^{-1}

W_s: the wet weight of soil (g)

2.1.2 Pot experiment

Pot experiment was conducted in the open field of FM Fuchu Honmachi, Field Science Center of the Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan from 15th June 2010 to 30th September 2010.

(a) Preparation of soil and cultivation of rice

The soil used in pot experiment was also collected from paddy fields in FM Fuchu Honmachi, Field Science Center of the Tokyo University of Agriculture and Technology. The physico-chemical properties of each soil are shown in Table 1 (Tanaka et al., 2008). A salt tolerant Indica rice variety, Dorfak cultivar which is originated from Iran, was used in this study for both experiments.

About 8 kg of soil was placed in 30 × 20 cm plastic pots. About one week before transplanting, puddling was conducted by irrigating the pots twice on alternate days with specific salinity levels of NaCl solutions or tap water (control). The chemical fertilizers such as 40 kg P ha^{-1} and 70 kg K ha^{-1} were applied one day before transplanting. 70 kg N $ha^{-1}$was applied in 3 equal splits at basal (one day before transplanting), active tillering stage (2 weeks after transplanting) and panicle initiation stage (4 weeks after transplanting). Urea, ammonium phosphate and potassium chloride were used as a source of N, P and K, respectively.

Two salinity levels 30 mmol L^{-1} NaCl (S30), and 90 mmol L^{-1} NaCl (S90) were tested in this pot experiment. The treatments comprised 1) control (tap water), 2) 30 mmol L^{-1} NaCl (S30), 3) 90 mmol L^{-1} NaCl (S90), 4) gypsum 1 ton ha^{-1} (G1), 5) 30 mmol L^{-1} NaCl plus gypsum 1 ton ha^{-1}(S30-G1), and 6) 90 mmol L^{-1} NaCl plus gypsum 1 mmol L^{-1} ha^{-1}(S90-G1). All treatments were laid out in a completely randomized design with 3 replications. Twenty-one-day-old seedlings were transplanted with 3 seedlings per pot on 23rd June, 2010 and harvested on 30th September, 2010. A water level of about 2–3 cm was maintained in pots throughout the growing seasons by irrigating regularly with the assigned NaCl concentration until crop maturity. The purposes of this experiment was to examine the effect of those saline levels (S30 and S90) upon CH_4 emission related with rice growth and to evaluate the CH_4 emission by applying gypsum amendment that is commonly used into saline soil, to Na-salinized paddy soil under rice cultivation.

(b) Measured parameters and analytical methods

Data regarding agronomic characters such as plant height, tiller number, number of dead leaves, and soil environment were collected at weekly interval. The pH and EC of the flooded water was directly measured by portable meters (Beckman, Φ 260 pH/ Temp/ mV meter, and ES-51 COND METER, Horiba, Japan, respectively). The Eh value of soil was monitored by inserting platinum electrodes permanently into the soil during the rice growing season at a depth of 5 cm in each plot using Eh probes (SWC-201RP, Sanyo, Japan). Soil temperature was also measured at 5cm depth by inserting OPTEX Thermometer TBW-3 at each sampling time. Flowers and Flowers (2005) pointed that salinity affects on plants by lowering water potentials and interfering the uptake of essential nutrients including N. Furthermore, as there is an interactive effect between NH_4^+ and Na^+ and/or between Cl^- and NO_3^-, surface water samples were also collected at weekly intervals for the analysis of ammonium (NH_4^+) and nitrate (NO_3^-) ions concentrations (Hu and Schmidhalter, 2005). Both NH_4^+ and NO_3^- concentrations were determined by using a UV spectrophotometer (UV–VI Mini 1240, Shimadzu Corporation, Kyoto, Japan). Before analysis of these ions, water samples were filtered with 0.45-μm filter paper. For NH_4^+ analysis, 5 ml of water samples, 2 ml of solution A and 3 ml of solution B were mixed and allowed to stand for about 45 minutes. After 45 minutes, NH_4^+ was determined at the wavelength 630 nm. Solution A contains the chemicals such as 30g of $Na_2HPO_4.H_2O$, 30g $C_6H_5Na_3O_7.2H_2O$, 3g of EDTA.2Na, 60 g of Phenol 60 g and 0.02 g of $Na_2Fe(CN)_5NO.2H_2O$ which is prepared to get the final volume 1 Liter by adding distilled water. Solution B contains 16 g of NaOH, 20 ml of NaClO which is prepared to get the final volume 1Liter by adding distilled water. For NO_3^- analysis, no reagent was added to the 5-ml water samples and measurements were made directly at the wavelength 230 nm.

(c) Gas sampling, analysis and calculation

Gas sampling was taken at 09:00 – 12:00 am by a closed-chamber method (Lu et al., 1999). The chambers used were 100 cm in height, 30 cm in length, 30 cm in width, and made of acrylic transparent sheets. A plastic tray with a length of 40 cm, width of 40 cm, and height of 5 cm was filled with 3 cm of water and placed under the pot. The chamber was put into the tray by covering the pot, and the tray water sealed the surrounding area of the chamber to form an airtight chamber. A battery-operated fan and Tedlar bag were installed at the chamber to mix the air inside the chamber and regulate the pressure, respectively. The temperature inside the chamber was recorded using a micro-temperature thermometer (PC-9125, AS ONE Co., Tokyo, Japan) fitted with rubber septum inserted into the small hole of the chamber. To assess the linear rate increase of gas concentration emitted from the surface area of soil inside the pot with time, gas samples were drawn from the chambers through a three-way stop cock using a 50-ml airtight syringe at intervals of 15 min (0, 15 and 30 min). The air inside the chamber was thoroughly mixed before collecting gas samples by flushing the syringe 3 times. Approximately 45 ml of gas samples were then taken with the 50-ml plastic syringe, adjusted to 40 ml and then transferred into a 20-ml pre-vacuumed glass vial. The amount of CH_4 flux was calculated using the following equation:

$$Q = (V/A) \text{ x } (\Delta c/\Delta t) \text{ x } (M/22.4) \text{ x } (273/K) \quad (2)$$

Where Q: flux of the CH_4 gas (mg m^{-2} min^{-1})

V: volume of gas chamber

A: gas emitted surface area (m^2)

Δc: increased or decreased change of the gas concentration (mg m^{-3})

Δt: unit of time intervals (min)

M: molar weight of the gas

K: Kelvin temperature of air inside the chamber

The cumulative amount of CH_4 gas measured during one crop season was calculated by multiplying the daily gas flux at each measurement with the time interval and then adding all of these values. All the data were evaluated by an analysis of variance (ANOVA) by using CropStat 7.0 statistical software. Comparison of treatment means was performed using least significant differences (LSD) at p=0.05. Correlation and regression analysis was performed using Sigma Plot 11.0 statistical software.

2.2 Results

2.2.1 Laboratory incubation experiment

(a) Effect of salinity levels on methane production potential of soil

The addition of NaCl kept the pH lower than that of the non-saline control (Table 2.2). The Eh values were in the range -320 to -435 mV. The addition of NaCl up to S30 increased CH_4 production. The increase of CH_4 production in salinity S10 and S30 was 1.2 and 2 times higher than that of control, respectively, although they were not significantly different. At higher salinity levels (S60 and S90), CH_4 production was 19 to 33% lower than the control.

2.2.2 Pot Experiment

(a) Soil environment during the rice growing season

Soil temperature throughout the growing season ranged from 20 to 35°C (Fig. 2.1b). There was no large difference in temperature between the treatments. The desired EC level was maintained by adding saline water or tap water. However, the desired EC level during the first 3 weeks could not be achieved due to continuous rainy days (Fig. 2.1c). Soils of saline condition showed higher EC values compared to those of non-saline conditions (Fig. 2.1c). High fluctuation of pH was observed among the treatments until 5 weeks after transplanting (Fig. 2.1d). The pH was then between 6 and 7.4 throughout the subsequent growing period.

(b) Impact of different levels of irrigation water salinity on rice growth with or without addition of gypsum fertilizer

Continuous irrigation with saline water severely suppressed rice growth for both S30 and S90 salinity levels. A significant difference in the level of growth suppression was observed between S90 and S30 from immediately after transplanting because rice growth was much more seriously affected by salinity level S90 compared to S30 and the non-saline control in terms of tillering pattern (Fig. 2.3a) and above-ground plant biomass yield (Table

2.3). The survival period of rice plants in S90 was until 7 weeks after transplanting (Fig. 2.3a) and the lowest number of dead leaves was observed in S90 (Table 2.3). Rice plants in S30 survived until the harvesting period, but the above-ground plant biomass yield in S30 was significantly lower and the total number of dead leaves was significantly higher compared to the non-saline control (Table 2.3). The addition of gypsum as a source of Ca^{2+} under continuously flooded conditions with saline water suppressed the above-ground plant biomass yield significantly compared with the non-saline condition with or without the addition of gypsum.

(c) Impact of different levels of irrigation water salinity on methane emission with or without addition of gypsum fertilizer

A distinct higher CH_4 flux was observed during the reproductive stage of rice growth in both non-saline and saline conditions (Fig. 2.4a, b). Methane emissions gradually increased during the reproductive stage (Fig. 2.4a), while the rate of dead leaves also increased about 63 days after transplanting and the tiller numbers decreased after the maximum tillering period at about 56 days after transplanting. The daily CH_4 fluxes under saline conditions were lower than those under non-saline conditions, especially during the vegetative stage of rice growth. Under saline conditions, the highest total CH_4 emission (296 kg CH_4 ha^{-1}) was found in S30 without gypsum amendment. However, the amount of CH_4 emission in S30 without gypsum amendment was 6% lower than that of the non-saline control, although the difference was not significant. The lowest CH_4 emission among the treatments was observed in S90 with (55.5 kg CH_4 ha^{-1}) or without gypsum amendment (44.2 kg CH_4 ha^{-1}). The addition of gypsum suppressed CH_4 emission under saline and non-saline conditions throughout the rice-growing period. The reduced rates due to gypsum addition were about 56% for non-saline conditions and 53–83% for saline conditions (S30-G1, and S90-G1) compared to CH_4 emission in the non-saline control (Fig. 2.5a). Although rice plants are known as major CH_4 transport conduits, the number of tillers in S30-G1 was significantly lower than that in G1, even though the level of the reduced CH_4 emission rate did not differ significantly. However, the total number of dead leaves, which can contribute as a source of organic carbon (C) for methanogens, was higher in S30-G1 than G1 (Table 2.3).

2.3 Discussion

2.3.1 Laboratory incubation experiment

(a) Effect of salinity levels on methane production potential of soil

The higher CH_4 production in S10 and S30 compared with non-saline control might be due to the Na^+ ion concentration of those salinity levels which meet the Na^+ requirement of methanogenic bacteria. Ramakrishnan et al. (1998) also found that the addition of 27 mM NaCl to illuvial soil caused a two fold increase in CH_4 production over that of control and higher addition of NaCl (54, 135 and 274mM) causing about 50% reduction in CH_4 production. According to the report of Jarrell and Kalmokoff (1988), sodium is required for amino acid transport, growth, methanogenesis, and internal pH regulation in methanogenic bacteria. However, the quantum of sodium requirement varies widely among methanogens. The lower CH_4 production over S30 level (S60 and S90) might be due to Cl^- toxicity under higher additions of NaCl.

2.3.2 Pot experiment

(a) Impact of different levels of irrigation water salinity on rice growth with or without addition of gypsum fertilizer

The concentration of salts in normal soil is generally lower than that in the cells of plants' roots. Water is absorbed by plant roots through a process called osmosis, which involves movement of water from soil with lower concentration of salts to a place that has a higher concentration of salts, inside the cells of plants' roots (Alex, 2006). In the pot experiment, the maintaining of continuous flooded condition with specific salinity level either S30 or S90 inhibited the rice growth by lowering the tiller numbers, above-ground plant biomass yield and enhancing the numbers of dead leaves (Table 2.3). The addition of saline water with salinity levels (S30) or (S90) may have disturbed the uptake of water by plants. Moreover, higher concentration of NH_4^+ and NO_3^- in flooded water was observed in saline condition compared with non-saline condition especially in early stage of vegetative growth (3 weeks after transplanting) (Fig. 2.2a and b). Neves-Piestun and Bernstein (2001), and Homaei (2002) pointed that as the salinity and osmotic pressure increase, this process leads to less water and nutrient absorb including N and potassium by plants hence the plants growth will get stunted. Thus, this higher NO_3^- concentration (especially in S90) and NH_4^+

concentration of flooded water under saline condition might be due to less water and N nutrient uptake by the plants under osmotic stress. Although N fertilizer was applied as the NH_4^+ form in this experiment, the existence of NO_3^- in flood water might be contributed from the rain water or the conversion of NH_4^+ to NO_3^- through nitrification process in the flood water. In comparison of S30 and S90, higher NH_4^+ and NO_3^- concentrations of S90 indicated that the osmotic stress in S90 levels was much more severe than that in S30. Thus, the shortest growth duration was observed in S90. Therefore, it can be assumed that the growth reduction under saline condition might be due to the osmotic stress, and interference with the uptake of essential nutrient such as N.

Although many studies showed that the addition of Ca^{2+} has beneficial effects either in saline soil (Cha-um et al., 2011) and saline solution with hydroponic culture (Song and Fujiyama, 1996), the present study did not show any improvement in rice growth in terms of tiller numbers or total number of dead leaves following irrigation with saline water (Table 2.3). When irrigation water with a high Na^+ concentration relative to divalent cations is used, there will be a great tendency for adsorption of exchangeable Na^+ around soil particles (Alobaidy et al., 2010). Naeem and Qureshi (2005) suggested that an adequate amount of Ca^{2+} in proportion to Na^+ must be present in the system to improve the selectivity of ion. The purpose of adding gypsum was to replace the monovalent Na^+ ion by increasing the Ca^{2+} ion concentration in the root zone and to maintain appropriate Na/Ca concentration in the root zone as shown in the following equation:

$$Ca^{2+}\ \text{Soil water} + Na^{+}\ \text{Soil particle} ---> Na^{+}\text{Soil water} + Ca^{2+}\ \text{Soil Particle} \quad (3)$$

The use of gypsum ($CaSO_4.2H_2O$) as a source of Ca^{2+} is a well-established practice for the amelioration and management of Na^+-saturated waters/soils (Mathad and Hiremath, 2010). Soil water laden with those Na ions is then drained or leached out of the soil. However, the present study was conducted as pot experiment and the depth of a pot was only 30 cm. The water outlets of the pots were also covered with rubber stoppers during the whole rice-growing period, and there was no space for regular leaching of the soil solution with Na^+ ions as occurring in ordinary field conditions. Therefore, the regular addition of saline water could not provide sufficient Na^+/Ca^{2+} in the root zone, and a clear effect due to Ca amendment was not found.

(b) Impact of different levels of irrigation water salinity on methane emission with or without addition of gypsum fertilizer

In the pot experiment, the results showed that a salinity level of 90 mmol L^{-1} NaCl (S90) suppressed CH_4 emission (Fig. 2.5) through a lower CH_4 production potential as shown in the laboratory incubation experiment (Table. 2), and a lower biomass yield (Table. 2.3) and shorter growth duration period (Fig. 3). Although the extent of above biomass in S30 was 49% lower than that of non-saline control (Table. 2.2), the amount of CH_4 emission in S30 was only 6% lower than that of non-saline control that was also not statistically significantly different (Fig. 5). Therefore, S30 did not suppress CH_4 production potential of soil. The addition of saline irrigation with salinity levelS30 might provide the Na required by methanogens, thereby favouring CH_4 production as shown in laboratory incubation experiment (Table. 2.2). However, the lower value of CH_4 emission in S30 over that of the non-saline control under rice cultivation might relate with its lower above biomass yield. The observed reduction of CH_4 emission under gypsum application might be due to its high SO_4^{2-} content (Fig. 2.5). In the presence of SO_4^{2-}, SO_4^{2-}-reducing bacteria will out compete methanogens for the same substrates such as hydrogen (H_2) or carbon dioxide (CO_2) or Acetate (CH_3COO^-) that methanogens use in methane production (Lindau et al., 1993; Van der gon and Neue, 1994; Epule et al., 2011). Furthermore, Gauci (2004) mentioned that SO_4^{2-}-reducing bacteria have a higher affinity for both H_2 and CH_3COO^- than methanogens, which enables them to maintain the pool of these substrates at concentrations too low for methanogens to use. Therefore, the reduction of CH_4 emission following the addition of gypsum (Fig. 2.5) might be due to the competition between SO_4^{2-}-reducing bacteria and CH_4-producing bacteria for substrates as stated by Lindau et al. (1993), Van der gon and Neue (1994) and Epule et al. (2011).

(c) Relationship between soil parameters, rice growth, and gypsum fertilizer addition and methane emission

There was positive relationship between soil temperature and CH_4 emission (p=0.614**) because temperature plays an important role in the rate of activity of soil microorganisms including those involved in CH_4 production and consumption. According to the Van Hulzen et al. (1999), temperature influences CH_4 production by regulating anaerobic carbon mineralization, availability of alternative electron acceptors and methanogenic activity. At higher temperature, mineralization increases and more carbon substrate becomes available, result in faster depletion of the alternative electron acceptor pool. Moreover, Temperature also CH_4 transport through the rice plant (Hosono and Touchi, 1997)

As many studies pointed that the rice plants are the main conduit for CH_4 transport through well-developed system of inter cellular air space (aerenchyma), the above plant biomass yield may play a significant role in CH_4 emission (Schutz et al., 1989). Therefore, the positive relationship were observed between total CH_4 emission (p = 0.704**) and dry matter yield, and between total CH_4 emission and tiller numbers per pot (p= 0.819**). There was negative relationship between total CH_4 emission and gypsum fertilizer application although it was not significantly different.

2.4 Conclusion

Our study showed that saline conditions do not suppress CH_4 emission, but favour CH_4 emission since Na^+ is required for methanogens in terms of the CH_4 production potential. However, excessive addition of NaCl (>30 mmol L^{-1}NaCl) can inhibit CH_4 production. Although gypsum was added as Ca^{2+} source to maintain appropriate Na^+/Ca^{2+} in the soil, no improvement of rice growth due to gypsum addition in saline condition might be due to the high Na^+/Ca^{2+} ratio under addition of saline water into the limited space of pot. The amendment with gypsum resulted in a reduction of CH_4 emission. Sulfate-containing fertilizer can be used as a CH_4 mitigation option either in saline or non-saline conditions. In gypsum treatments, the extent of CH_4 emission depended not only on the SO_4^{2-} content of the soil, but also on the availability of a C source from dead plant materials. Finally, it can be concluded that CH_4 emission under saline conditions was influenced by salinity levels and rice growth in relation to nutrient absorption.

Table 2.1 Soil properties of Honmachi field (Tanaka et al., 2008)

Parameters	Values
Soil Type	Inceptisols
Soil Texture	Sandy loam
Clay %	16%
Silt %	33%
Sand %	51%
pH	6.0
Total N	3.6 g kg^{-1}
Available P_2O_5	51.8 g kg^{-1}
Available K_2O	198.3 mg kg^{-1}
CEC	19.8 cmol kg^{-1}

Table 2.2 Results of laboratory incubation experiment

Treatments	Soil pH		Methane production potential of soil ($\mu g\ CH_4$/g soil/day)	
	(2DAI)	(21DAI)		
Control	6.4	7.0	130 ± 26	ns
S10	6.3	6.7	165 ± 57	
S30	6.0	6.0	200 ± 57	
S60	6.0	6.0	105 ± 32	
S90	6.0	6.0	100 ± 21	

Values are the means ± standard deviation, n=3. ns stands for no statistically significant difference among the treatments within each column. DAI means days after incubation.

Table 2.3 Effect of different salinity levels and gypsum fertilizer addition on rice growth

Treatments	Maximum Tiller numbers (Numbers pot^{-1})	Numbers of dead leaves (Numbers pot^{-1})	Above ground plant biomass yield (g pot^{-1})
Control	67 ± 1.7 ^{a}	143 ± 8.9 ^{b}	177± 8.0 ^{a}
G1	64 ± 0.6^{b}	141 ± 9.8 ^{b}	174± 15.0 ^{a}
S30	60 ± 0.6 ^{c}	223 ± 11.6 ^{a}	91 ± 2.6 ^{b}
S90	14 ± 1.7 ^{f}	54 ± 2.1 ^{d}	0 ^{c}
S30-G1	52 ± 4.0 ^{d}	208 ± 9.5 ^{a}	64± 2.6 ^{b}
S90-G1	19 ± 0.0 ^{e}	85 ± 11.0 ^{c}	0 ^{c}

Values are the means ± standard deviation (n= 3 replications). In each column, means followed by a common letter are not significantly different by using least significant difference (LSD) at p=0.05.

Table 2.4 Results of ANOVA test for CH_4 emission and crop growth

Pot experiment				
Source of vriation	**Maximum tiller numbers (numbers pot^{-1})**	**Total numbers of dead leaves (numbers pot^{-1})**	**Above biomass yield (g pot^{-1})**	**CH_4 emission in one crop season (kg CH_4 ha^{-1})**
Salinity	**	**	**	**
Gypsum application	**	ns	ns	**
Salinity* Gypsum application	**	*	ns	**

**, * and ns stand for significance at 1%, 5% and non-significance respectively.

Table 2.5 Spearman rank order correlation analysis

Pot experiment							
Parameters	**Gypsum application**	**pH**	**EC**	**Soil Temp (˙C)**	**Maximum Tillers (numbers pot^{-1})**	**Total numbers of dead leaves (numbers pot^{-1})**	**Above biomass yield (g pot^{-1})**
Methane emission per crop season (kg CH_4 ha^{-1})	-0.332 ^{ns}	-0.278 ^{ns}	0**	0.614**	0.819**	0.0836^{ns}	0.704**

**, * and ns stand for significance at 1%, 5% and non-significance respectively.

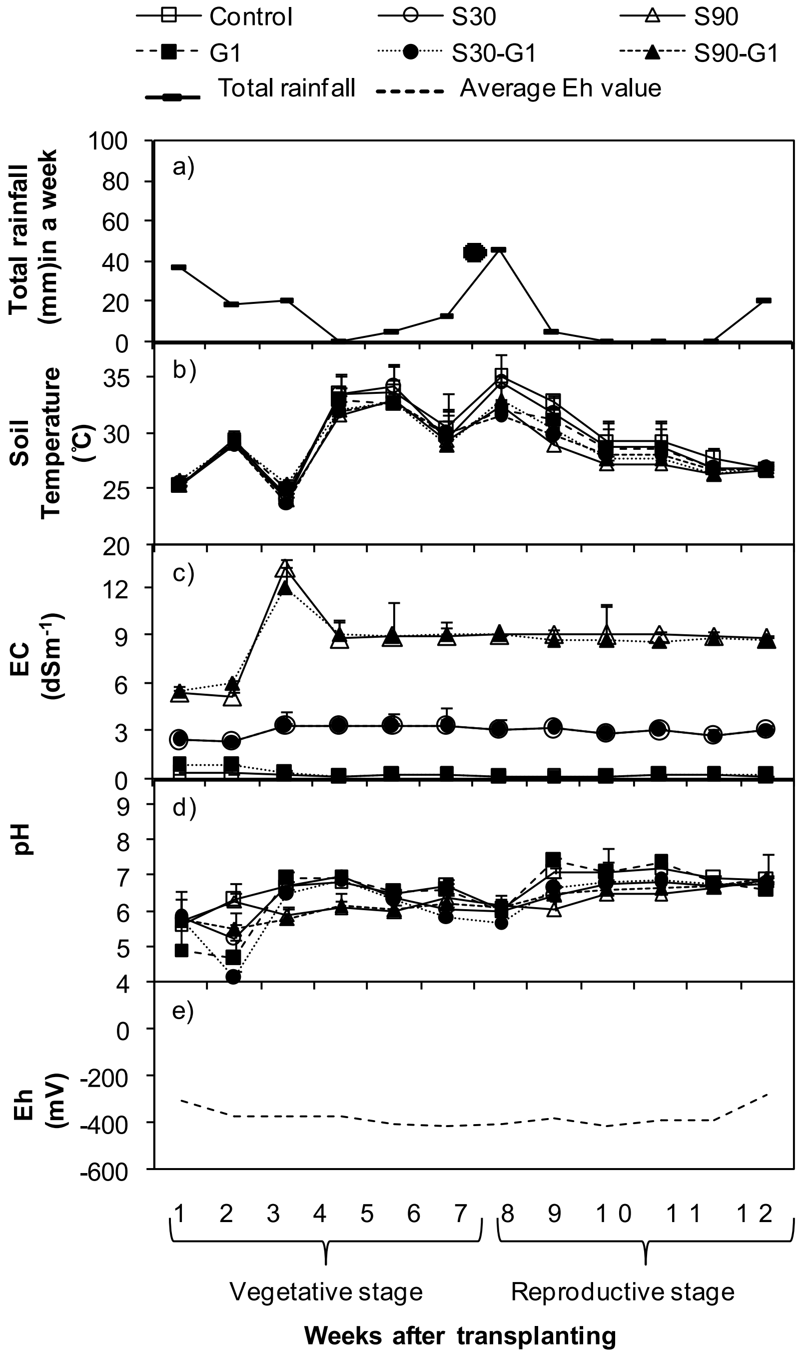


Fig.2.1 Distribution of rainfall and changes of soil environment under different salinity levels with or without addition of gypsum fertilizer. Error bars indicate standard deviation (n=3 replications).

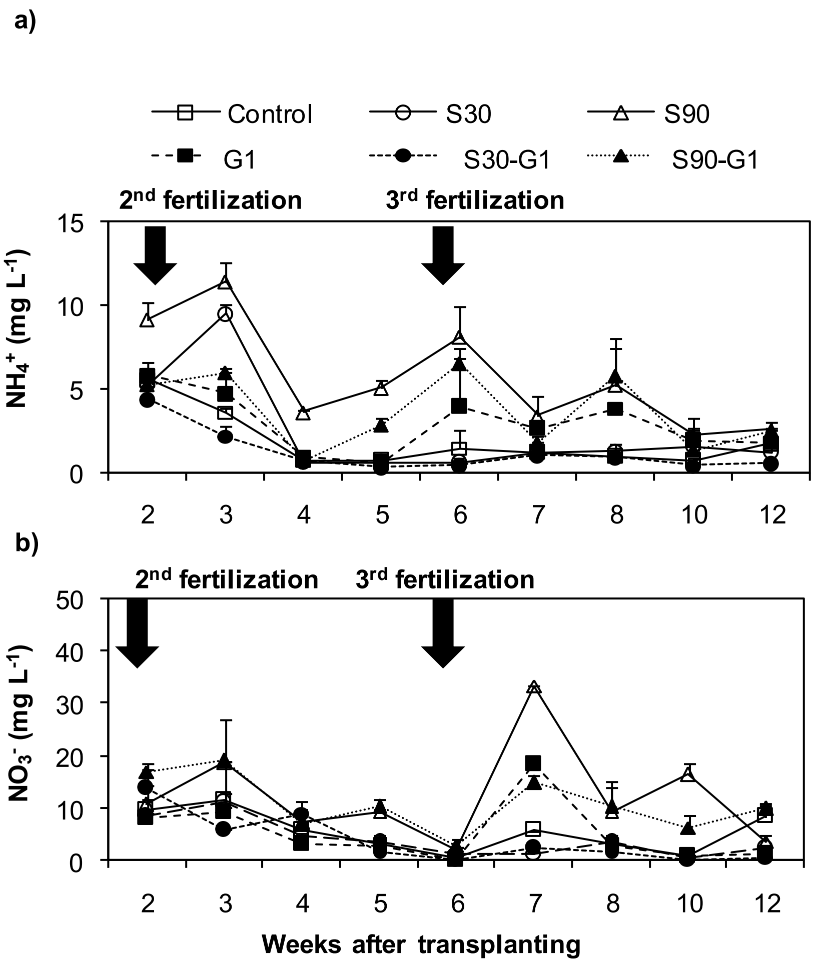


Fig.2.2 Changes of NH_4^+ and NO_3^- concentration under different salinity levels with or without addition of gypsum fertilizer. Error bars indicate standard deviation (n=3 replications).

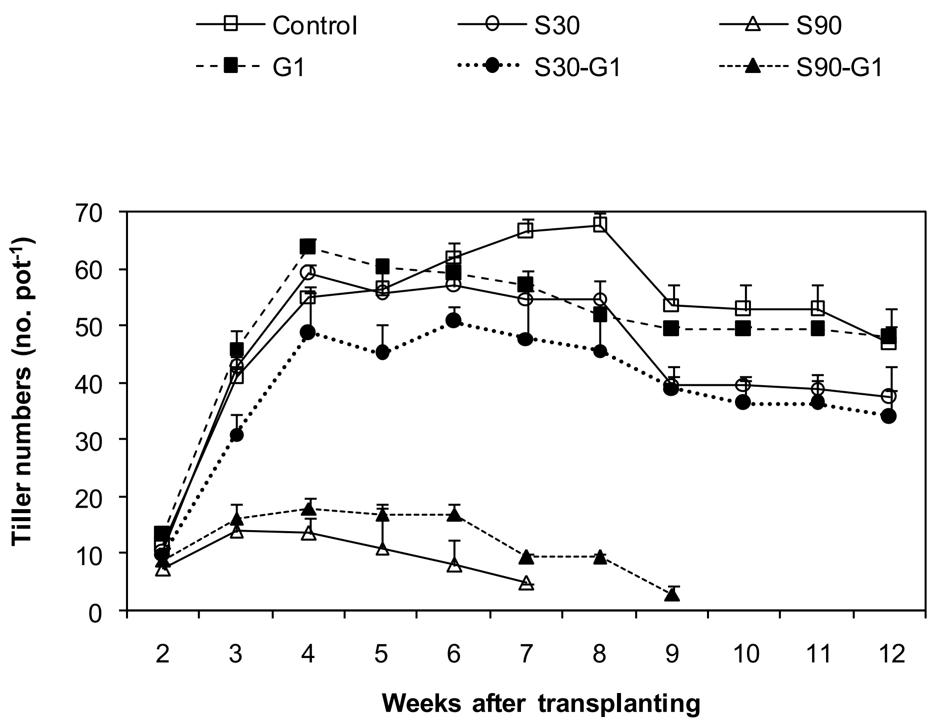


Fig.2.3 Tillering pattern under different salinity levels with or without addition of gypsum fertilizer. Error bars indicate standard deviation (n=3 replications).

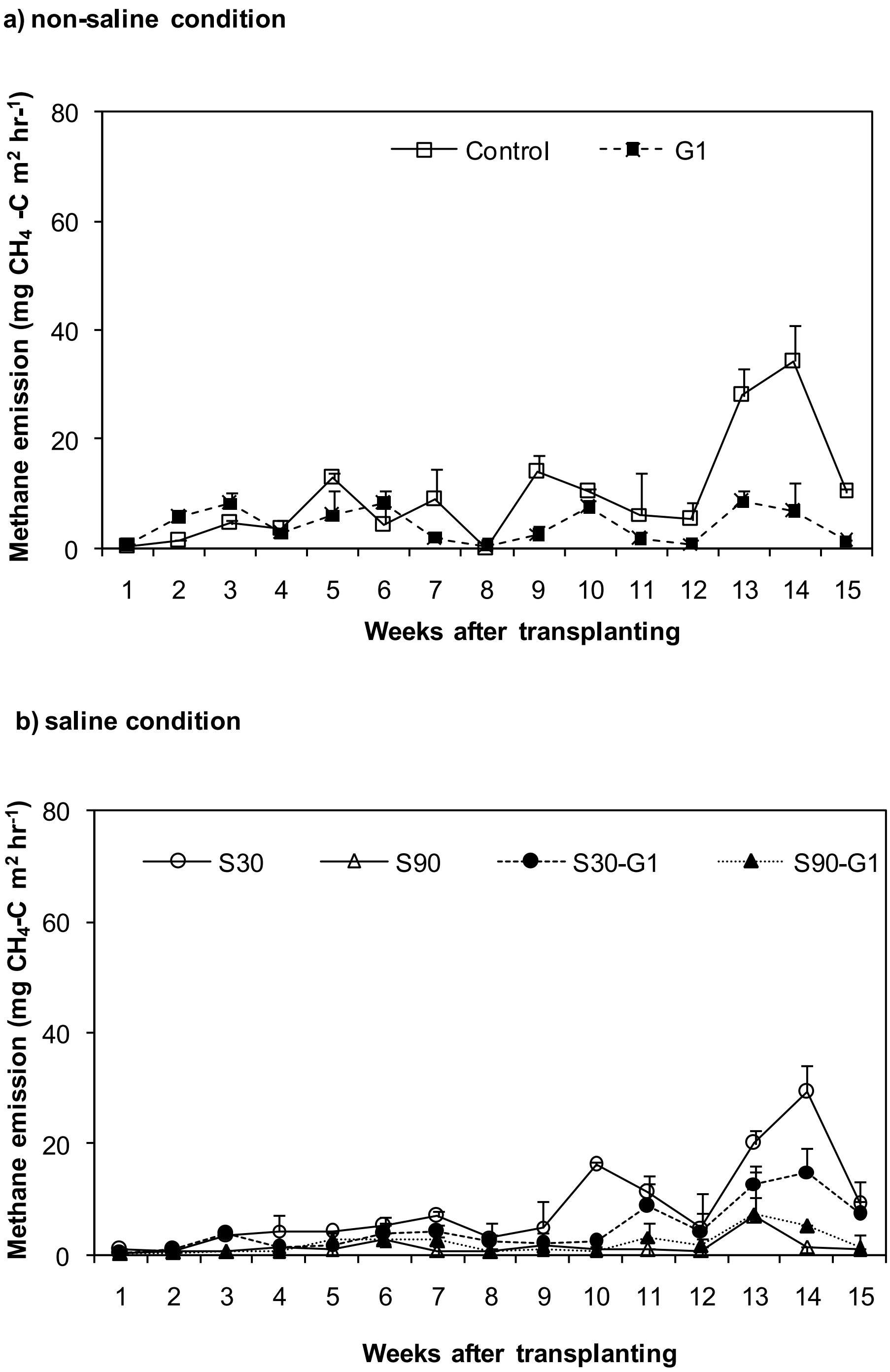


Fig.2.4 Methane emission pattern under (a) non-saline and (b) saline condition. Error bars indicate standard deviation (n=3 replications).

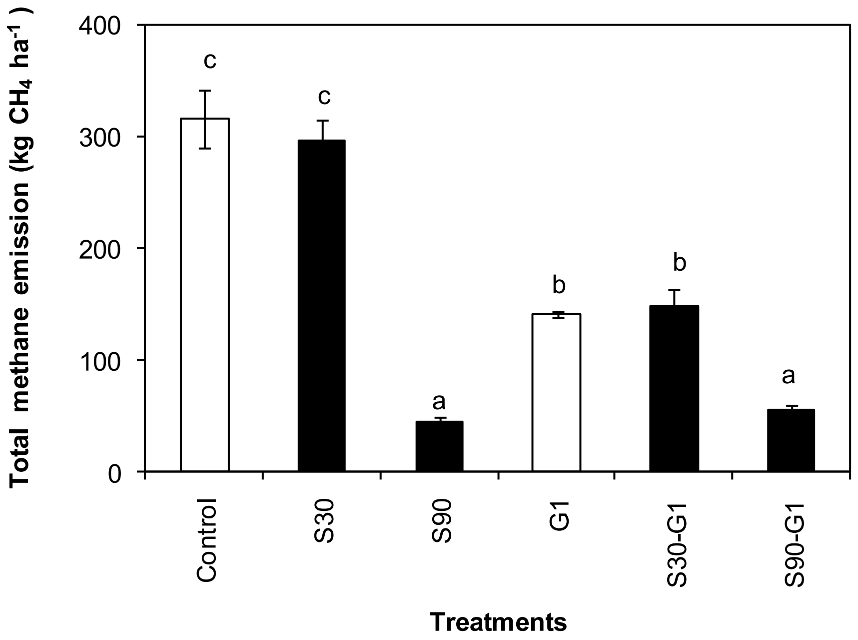


Fig.2.5 Total CH_4 emission under different salinity levels with or without addition of gypsum fertilizer. Error bars indicate standard deviation (n=3 replications).

Chapter 3

Influence of different rates of gypsum fertilizer addition on methane emission in paddy rice soil affected by saline irrigation water

The objectives of this study are;

1. To evaluate CH_4 emission due to the application of gypsum fertilizer to salt affected soil related with rice growth
2. To compare the effect of different rates of gypsum fertilizer on CH_4 emission between saline condition and non-saline condition

3.1 Materials and methods

3.1.1 Experimental site and Treatments

To evaluate the different rates of gypsum application upon CH_4 emission under Na-salinized paddy soil, pot experiment was conducted with (3) different gypsum application rates either in non-saline or saline condition. The chemical composition of gypsum fertilizer is shown in Table 3.1. The treatments were assigned according to a randomized complete block design with 3 replications and comprised 1) control (tap water), 2) 25 mmol L^{-1} NaCl (S25), 3) gypsum 1 ton ha^{-1} (G1), 4) gypsum 2.5 ton ha^{-1} (G2.5), 5) gypsum 5 ton ha^{-1} (G5), 6) 25 mmol L^{-1} NaCl plus gypsum 1 ton ha^{-1}(S25-G1), 7) 25 mmol L^{-1} NaCl plus gypsum 2.5 ton ha^{-1}(S25-G2.5), and 8) 25 mmol L^{-1} NaCl plus gypsum 5 ton ha^{-1}(S25-G5). The layout of experiment was as shown in Fig.3.1. It was conducted at Field Museum, Honmachi farm, Field Science Center, Tokyo University of Agriculture and Technology from 21st June, 2011 and to 23rd September, 2011.

3.1.2 Preparation of soil and cultivation of rice in pot experiment

The soils used in this experiment were also collected from paddy fields in FM Fuchu Honmachi, Field Science Center of the Tokyo University of Agriculture and Technology (see for detail Chapter 2). Its texture sandy loam composing with 16% clay, 33% silt, 51% sand. The pH and EC of soil at soil water ratio (1:2.5) is 6 and 0.231. A salt tolerant Indica rice variety, Dorfak cultivar which is originated from Iran, was cultivated in this study.

About 8 kg of soil was placed in 30 × 20 cm plastic pots. About one week before transplanting, puddling was conducted by irrigating the pots twice on alternate days with specific salinity level 25 mmol L^{-1} NaCl solutions or tap water (control). The chemical fertilizers such as 40 kg P ha^{-1} and 70 kg K ha^{-1} were applied one day before transplanting. 70 kg N ha^{-1} was applied in 3 equal splits at basal (one day before transplanting), active tillering stage (2 weeks after transplanting) and panicle initiation stage (4 weeks after transplanting). Urea, ammonium phosphate and potassium chloride were used as a source of N, P and K, respectively. Twenty-one-day-old seedlings were transplanted with 2 seedlings per pot. A water level of about 2–3 cm was maintained in pots at each irrigation time. Irrigation was performed when the soil water reached the saturation level (i.e., no standing water) as intermittent irrigation.

3.1.3 Measured parameters and analytical methods

The agronomic characters such as tiller numbers, numbers of dead leaves were collected at weekly interval. Soil environmental data such as soil pH, EC, NH_4^+ concentration, NO_3^- concentration in flooded water were also monitored at weekly interval. Methane gas samples were collected by using the closed chamber method weekly. The analytical methods for all the above parameters are conducted similar as the pot experiment in chapter (2). Total organic carbon content flooded water was also checked (2) times at 6 weeks and 10 weeks after transplanting by using Total Organic Carbon analyser (TOC-VCPH, Shimadzu Corp., Japan).

3.1.4 Statistical analysis

All the data were evaluated by an analysis of variance (ANOVA) by using CropStat 7.0 statistical software. Comparison of treatment means was performed using least significant differences (LSD) at p=0.05. Correlation and regression analysis was performed using Sigma Plot 11.0 statistical software.

3.2 Results

3.2.1 Soil environment during the rice-growing season

Soil temperature throughout the growing season ranged from 27 to 34°C (Fig. 1b). There was no large difference in temperature between the treatments. However, a sudden drop of soil temperature at 4 weeks after transplanting was observed following a period of rain on that sampling day. The intermittent application of saline water resulted in higher EC levels during the latter growth period from 9 weeks after transplanting. The range of flood water' EC value under saline condition was observed from 1.11- 2.58 dSm^{-1} in the vegetative stage of rice growth (until 7 weeks after transplanting) and from 1.13 - 4.8 dSm^{-1} in the reproductive stage of rice growth. Gypsum application under saline conditions showed higher EC values compared to those of non-saline conditions (Fig. 1h). The pH was in the range of 6.7–8.4 throughout the growing season (Fig. 3.1d).

The 2 highest peaks of NH_4^+ and NO_3^- concentration were observed at and immediately after fertilizer addition. The $NH_4^+$and NO_3^- concentration in flooded water was 0.02–18.7 mg L^{-1} (Fig. 3.2a) and 0.02–18.5 mg L^{-1}(Fig. 3.2b), respectively. The

NH_4^+concentrations in the non-saline control and S25 were lower than those in other treatments at the time of the second fertilization. There was no large difference in NH_4^+ concentration during the latter growth period (from 4 weeks after transplanting), except for S25-G5. In the early growth period (until 5 weeks after transplanting), the concentration of NO_3^- was higher in S25 than in other treatments.

3.2.2 Impact of different rates of gypsum fertilizer addition on rice growth

The significant reduction of tiller number was observed in S25 and S25-G5 over that of non-saline control at maximum tillering stage (Table 3.2). Moreover, salinity suppressed rice growth in terms of the number of dead leaves (Table 3.2). There were no significant differences in above-ground plant biomass yield under different rates of gypsum application in non-saline or saline conditions. However, significant reduction of above-ground biomass yield was observed in the addition of gypsum G5 to saline condition (Table 3.2).

3.2.3 Impact of different rates of gypsum fertilizer addition on methane emission

Methane emission was slightly higher during the vegetative growth stage (Fig. 3.4a, b). Total CH_4 emission under intermittent irrigation using saline water with salinity level S25 did not differ significantly from that of the non-saline control (Fig. 3.5). The addition of gypsum suppressed CH_4 emission under non-saline and saline conditions. Under non-saline conditions, CH_4 emission in G1 was about 22% lower than that of the non-saline control, although the difference was not significant. Methane emission in G2.5 and G5 was significantly lower than that of the non-saline control. Methane emissions were reduced in G2.5 and G5 compared with the non-saline control by 52% and 73%, respectively. Under saline conditions, reductions of CH_4 emission due to the addition of gypsum were 23%, 27% and 61% in G1, G2.5 and G5, respectively. The results show that a higher rate of gypsum addition produces a higher reduction of CH_4 emission. Although the above-ground plant biomass yield, which is known as a major CH_4 transport conduit, did not differ significantly except for S25-G5, the reduction of CH_4 emission due to the addition of gypsum was lower under saline conditions than non-saline conditions. Our findings also demonstrated a negative relationship between gypsum application rates and CH_4 emission ($p < 0.001$) based on the result of regression analysis (Fig 3.6).

3.3 Discussion

3.3.1 Impact of different rates of gypsum fertilizer addition on rice growth

At maximum tillering stage, the lower tiller numbers in S25 compared with non-saline control might relate with the mild osmotic stress in vegetative stage of rice growth. Homaei (2002) pointed that as the salinity and osmotic pressure increase, this process leads to less water and nutrient absorb including N and potassium by plants. Similar as this statement, the higher NO_3^- concentration was also observed in S25 over that of non-saline control in the present study, although there was no significant difference in NH_4^+ concentration in the flood water. As ammonium phosphate fertilizer was applied as nitrogen source in this study, this higher NO_3^- concentration in S25 compared with non-saline control treatment might be converted from NH_4^+ which was less absorbed by rice plants due to osmotic stress and the antagonistic effect of NH_4^+ and Na^+ ions in S25.Furthermore, Abdelgadir et al. (2005) also observed less NO_3- N uptake of shoots and roots of rice plants due to antagonistic effect of Na^+ and Cl^- ion under NaCl salinity stress. The higher numbers of dead leaves in S25 over that of control might be due to the salt (NaCl) load exceeding the ability of the cells to compartmentalize salts in the vacuole. The salt taken up by the plant concentrates in the old leaves; continued transport of salt into transpiring leaves over a long period of time eventually results in very high Na^+ and Cl^- concentrations, and the leaves died.

In non-saline condition, there was no significant difference in maximum tiller numbers, numbers of dead leaves, and above-ground plant biomass yield under different rate of gypsum application such as gypsum 1 ton ha^{-1} (G1), 2.5 ton ha^{-1} (G2) and 5 ton ha^{-1} (G3). It was indicating that Ca^{2+} concentration in normal soil is enough for plant growth. Even though the Ca^{2+} concentration is exceeded over plant's requirement due to the addition of gypsum fertilizer, it could not disturb the plant growth in non-saline condition.

In saline condition, the addition of gypsum 5 ton ha^{-1} (G5) significantly suppressed maximum tiller numbers and above-ground biomass yield. The growth reduction in S25-G5 might be due to the highest EC level under the addition of G5 during the vegetative growth stage. Furthermore, The higher Ca^{2+}concentration in G5 in saline condition might not allow much absorption of NH_4^+ ion as it is indicated by significantly higher NH_4^+ concentration of flood water in S25-G5 in the reproductive stage of rice growth due to antagonistic effect of NH_4^+ and Ca^{2+} (Fig. 3.2b).

3.3.2 Impact of different rates of gypsum fertilizer addition on methane emission

There was also no statistically significant difference in total CH_4 emission between S25 and the non-saline control. However, the extent of CH_4 emission in S25 was numerically lower than that in non-saline control (Fig. 3.5b), although the aboveground plant biomass yields were slightly higher in S25 compared with the non-saline control (Table 3.3). This lower value of CH_4 emission in S25 might be due to the existence of a higher NO_3^- concentration in S25 in the early growth stage (Fig. 3.2d). Furthermore, Dubey (2005) mentioned that soils containing greater amounts of readily decomposable organic substrates (acetate, formate, methanol, methylated amines, etc.) and low amounts of electron acceptors such as ferric ion, manganese, NO_3^- and SO_4^{2-} are likely to show high production of CH_4. It can therefore be concluded that the extent of CH_4 emission under saline conditions is influenced not only by the above-ground plant biomass yield, but also by the existence of electron acceptors such as NO_3^- that accumulate in rice-growing environments due to the osmotic stress of rice plants.

The observed reduction of CH_4 emission under gypsum application either in saline and non-saline condition might be due to the result of substrate competition between sulphate reducers and methanogens as stated by Van der Gon and Neue (1994). The higher value of CH_4 reduction under higher rates of gypsum addition might be related with the amount of SO_4^{2-} added to the soil. According to Van der gon et al. (2001), one mole of SO_4^{2-} is needed to reduce one mole of CH_4 produced. These considerations support the notion of a direct and proportional correlation between the amount of SO_4^{2-} added and the reduction of CH_4 production. However, the amount of SO_4^{2-} added to rice cultivation in pot culture might be lost from the soil through plant uptake. It may be one possible factor that the level of CH_4 reduction was not directly proportional to the amount of SO_4^{2-} added in the present study.

In addition, Chin and Conrad (1995) and Rothfuss and Conrad (1993) reported that 20–30% of CH_4 production in rice fields is derived from the conversion of H_2 or CO_2 and 70–80% is derived from CH_3COO^-. Although sulfate-reducing bacteria can outcompete methanogens for H_2 or CO_2, it is impossible to completely outcompete them for CH_3COO^- that was derived from a native soil organic C source and dead plant materials. Pangala et al. (2010) also indicated that level of CH_4 suppression in the wetland is influenced by the ratio of SO_4^{2-} and organic matter. Therefore, the lower reduction of CH_4 emission under saline conditions in this study was due to the higher availability of organic C sources from leaf senescence under these conditions and their contribution as a source of nutrients for methanogens.

3.3.3 Relationship between rice growth and methane emission

The result of spearman rank order correlation analysis showed that the soil temperature positively correlated with total CH_4 emission (p=0.04*) (Table 3.3). Methane is the final product of organic matter decomposition by soil microorganism under strict anaerobic condition. The increase in soil temperature may accelerate the decomposition rate of soil organic matter and enhance carbon availability for methanogenic bacteria to produce more CH_4. However, there was no significant relationship between total CH_4 emission and rice growth such as maximum tiller numbers per pot, total numbers of dead leaves, above-ground biomass yield because the gypsum application was the most prominent impacting factor upon CH_4 emission rather than the rice growth as shown by significant negative relationship between total CH_4 emission and amount of gypsum fertilizer added (p= -0.754**) (Table 3).

3.4 Conclusion

As there was no significant reduction of above-plant biomass yield by intermittent irrigating with saline water in this study, saline water with salinity level 25 mmol L^{-1} NaCl can be used in rice cultivation if no fresh water is available. Under non-saline condition, gypsum fertilizer can be used as CH_4 mitigation option without disturbing the rice growth. In case of saline condition, addition of gypsum fertilizer up to 2.5 ton ha^{-1} can be recommended as CH_4 mitigation option in paddy rice fields. Significant growth reduction in gypsum 5 ton ha^{-1} in saline condition might not only due to excessive concentration of Ca^{2+} ion alone but also due to the total cations concentration such as Na^+ concentration plus Ca^{2+} which might led to reduction of essential nutrient's uptake NH_4- N. Although the addition of SO_4^{2-} containing fertilizer can suppress CH_4 emission, the level of suppress depend not only on the amount of SO_4^{2-} added but also on carbon availability in the soil.

Table 3.1 Chemical composition of gypsum fertilizer

Purity percentage of gypsum fertilizer	Chemical composition of gypsum			
	Calcium	Hydrogen	Sulphur	Oxygen
98.5% of ($CaSO_4 .2H_2O$)	23.80%	2.34%	18.62%	55.76%

Table 3.2 Effect of irrigation water salinity and different rates of gypsum fertilizer addition on rice growth

Treatments	Maximum Tiller numbers (numbers pot^{-1})	Numbers of dead leaves (Numbers pot^{-1})	Above-ground plant biomass yield(g pot^{-1})
1. Control	54 ± 2.5 a	62 ± 6.4 b	95.9 ± 10.6 a
2. S25	48 ± 9.5 bc	86 ± 5.9 a	101.3 ± 19.7a
3. G1	54 ± 3.8 a	58 ± 3.5 b	106.4 ± 1.1 a
4. G2.5	52 ± 5.0 ab	61 ± 5.3 b	95.6 ± 22.1 a
5. G5	55 ± 3.1 a	63 ± 5.1 b	103.2 ± 16.8 a
6. S25-G1	52 ± 3.5 ab	99 ± 21.8 a	90.6 ± 27.2 a
7. S25-G2.5	51 ± 4.2 ab	101 ± 10.0 a	104.8 ± 10.4 a
8. S25-G5	46 ± 7.0c	95 ± 4.6 a	65.0 ± 14.6 b

Values are the means ± standard deviation (n= 3 replications). In each column, means followed by a common letter are not significantly different by using least significant difference (LSD) at p=0.05.

Table 3.3 Results of ANOVA test for CH_4 emission and crop growth

Source of vriation	Maximum tiller numbers (no. pot^{-1})	Total numbers of dead leaves (no. pot^{-1})	Above biomass yield (g pot^{-1})	CH_4 emission in one crop season (kg CH_4 ha^{-1})
Salinity	**	**	ns	ns
Gypsum application rate	ns	ns	ns	**
Salinity* Gypsum application rates	ns	ns	*	*

**, * and ns stand for significance at 1%, 5% and non-significance respectively.

Table 3.4 Spearman rank order correlation analysis

Parameters	Gypsum application	pH	EC	Soil Temp ·C	Maximum Tillers (no. pot^{-1})	Total numbers of dead leaves (no. pot^{-1})	Above biomass yield (g pot^{-1})
Methane emission per crop season (Kg CH_4 ha^{-1})	-0.754 **	-0.15 Ns	-0.074 ns	0.44 *	0.00 ns	0.084 ns	0.17 ns

**, * and ns stand for significance at 1%, 5% and non-significance respectively.

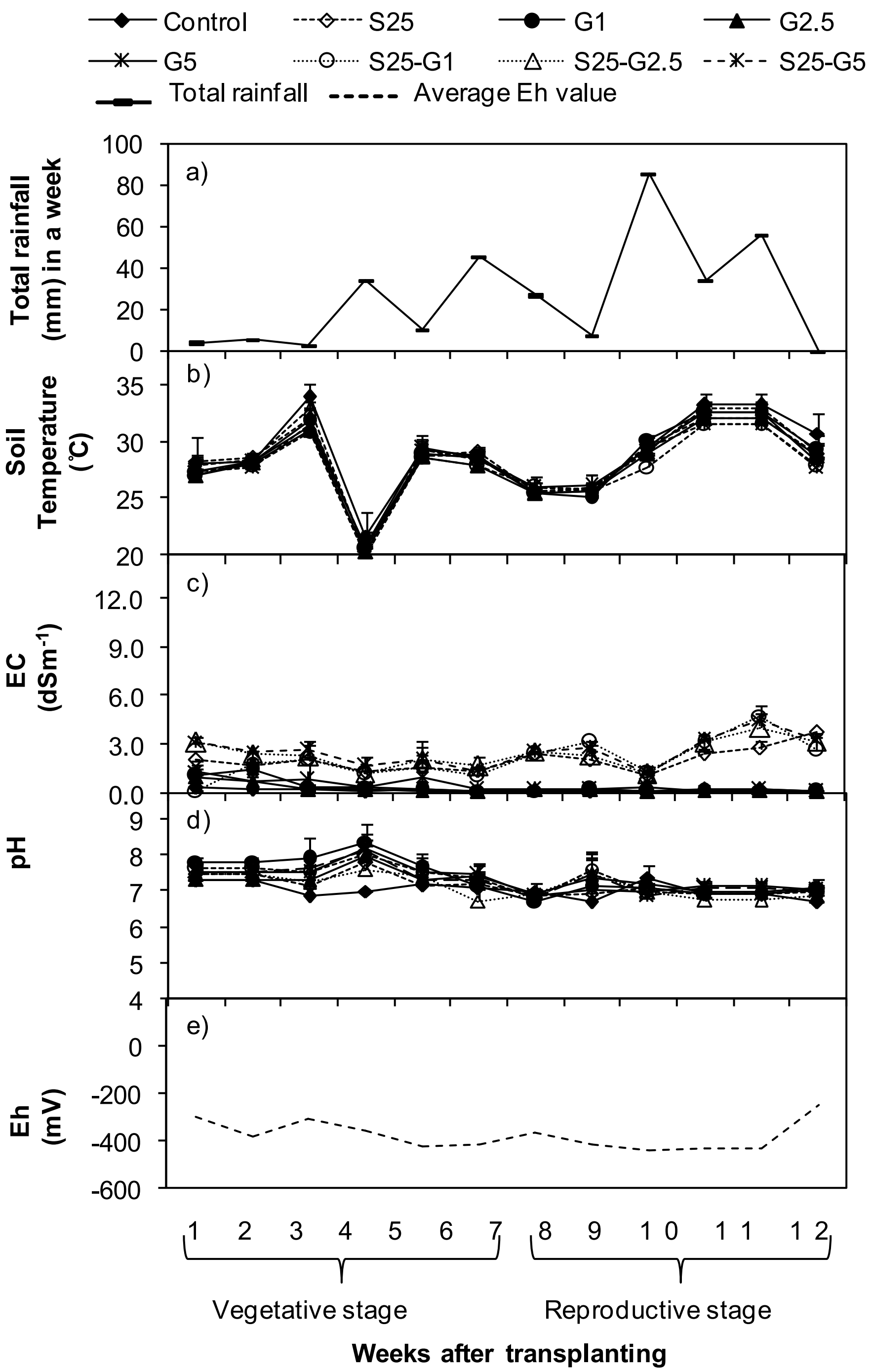
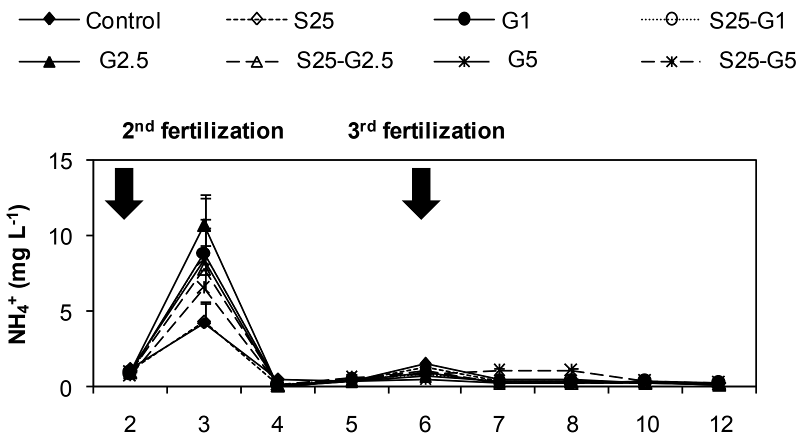


Fig.3.1 Distribution of rainfall and changes of soil environment under different gypsum application rates. Error bars indicate standard deviation (n=3 replications).

(a)



(b)

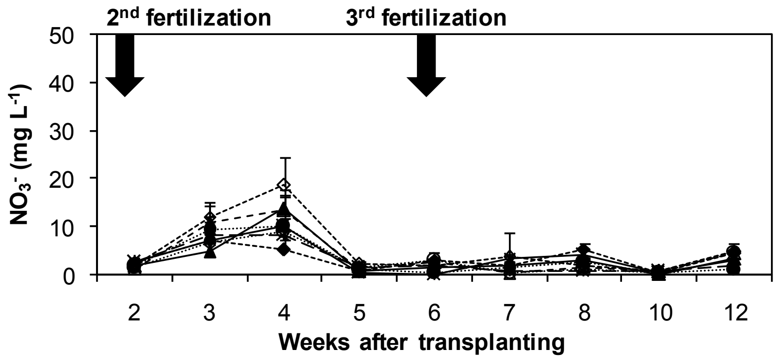


Fig.3.2 Changes of NH_4^+ and NO_3^- concentration under different rates of gypsum application to non-saline and saline condition. Error bars indicate standard deviation (n=3 replications).

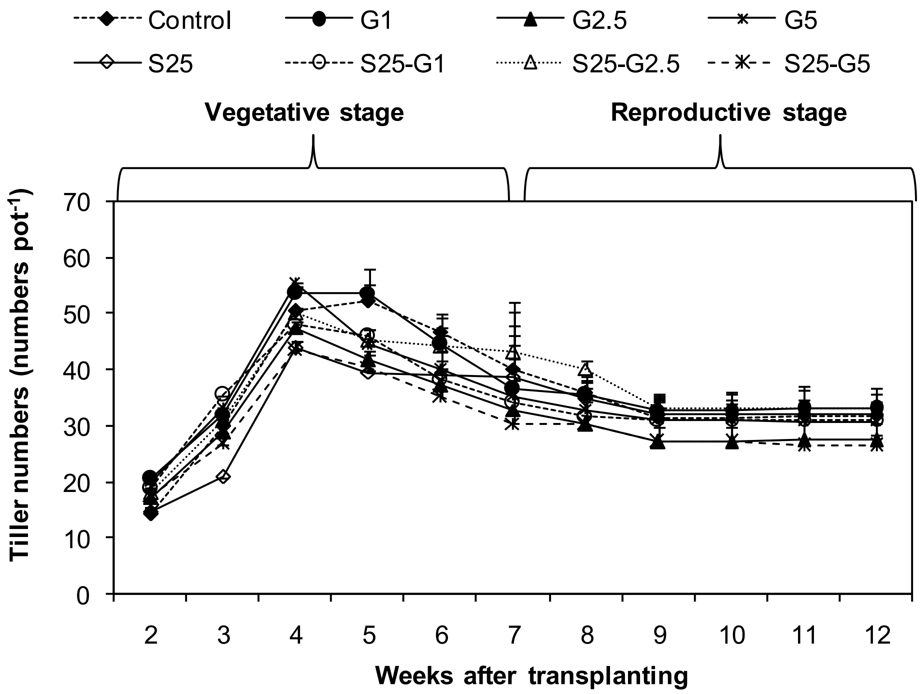
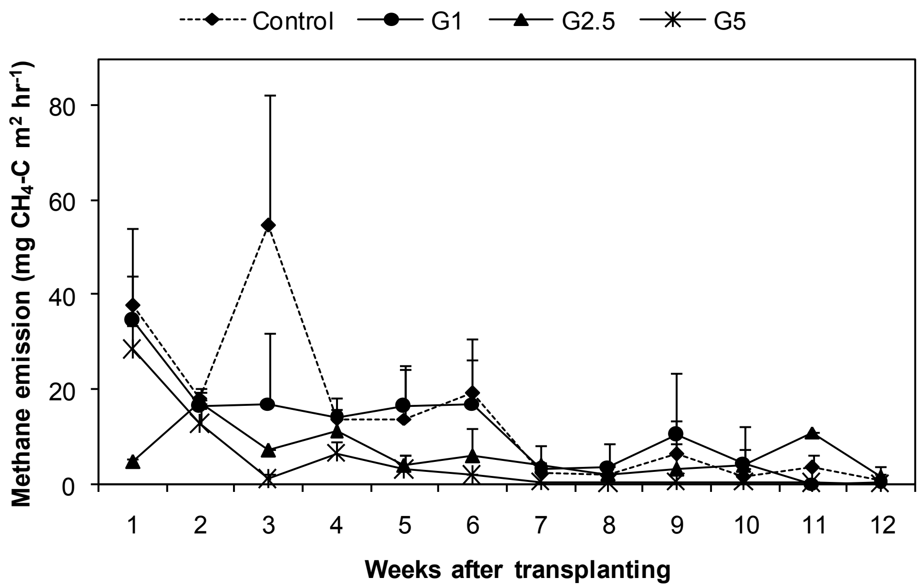


Fig.3.3 Tiller pattern under different gypsum application rates to non-saline and saline condition. Error bars indicate standard deviation (n=3 replications).

(a) Non-saline condition



(b) Saline condition

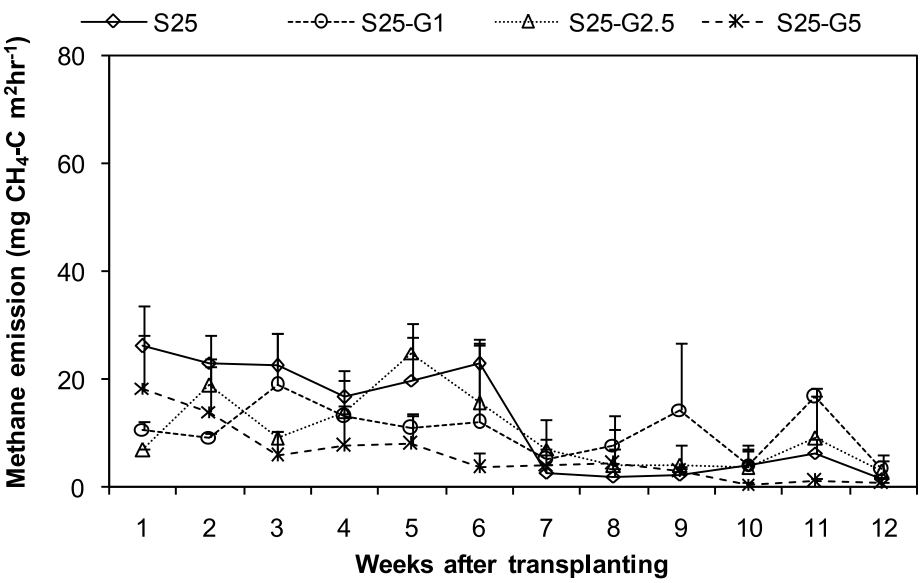


Fig.3.4 Methane emission pattern under different gypsum application rates to non-saline and saline condition. Error bars indicate standard deviation (n=3 replications).

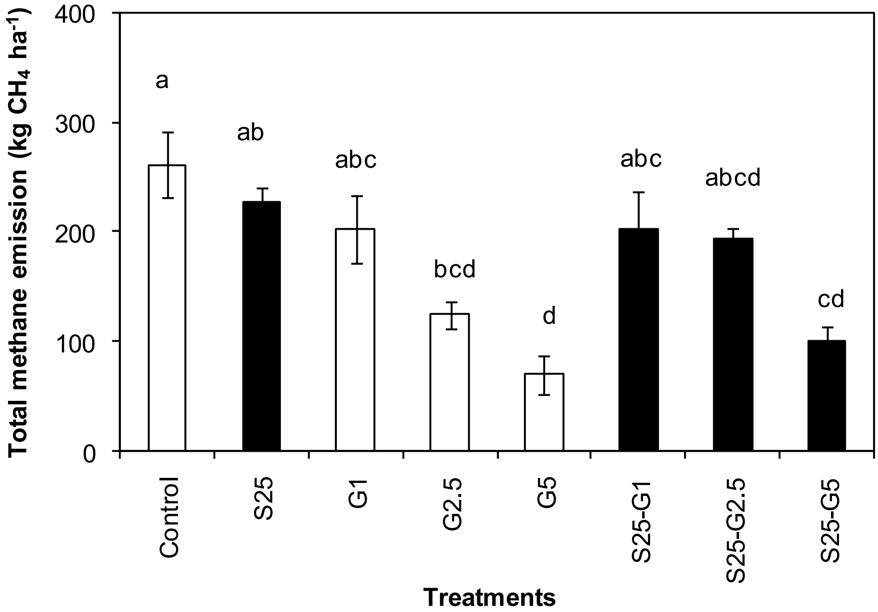


Fig.3.5 Total CH_4 emission under different gypsum application rates to non-saline and saline condition. Means followed by a common letter are not statistically significant different by using least significant differences (LSD) at p=0.05. Error bars indicate standard error (n=3 replications).

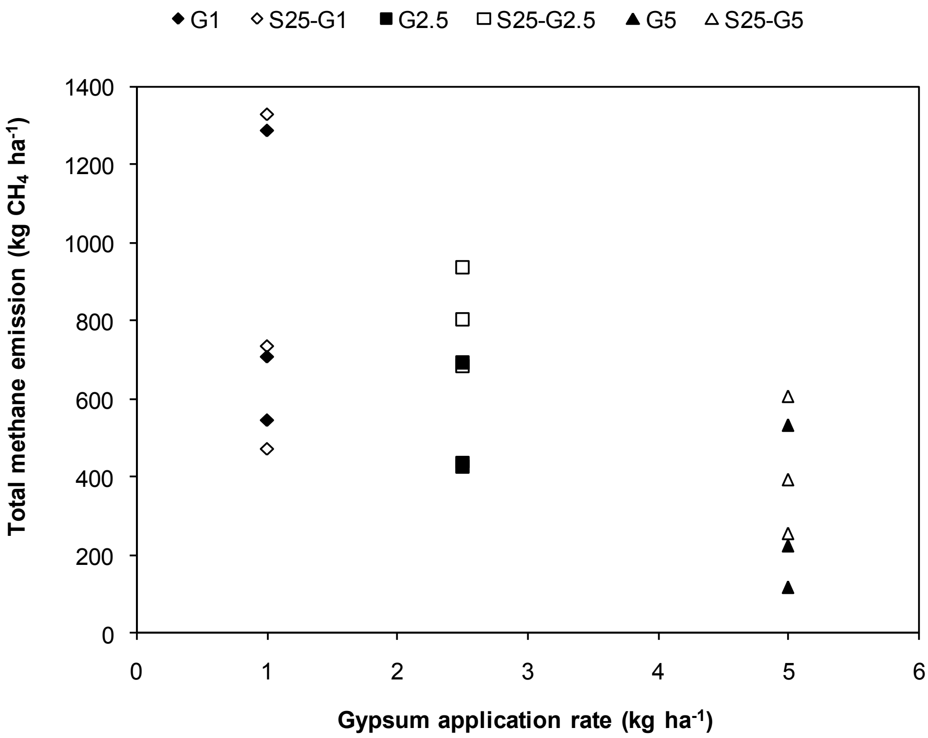


Fig.3.6 Relationship between total CH_4 emission and gypsum fertilizer application rates

Chapter 4

Evaluation of methane emission in coastal saline paddy soil with different Na/Ca ratios by adding gypsum fertilizer

The objectives of this study are;

1. To evaluate rice growth in coastal saline soil with different Na/Ca ratios by adding gypsum fertilizer
2. To identify the different rates of gypsum fertilizer application on CH_4 emission in coastal saline soil related with rice growth

4.1 Materials and methods

4.1.1 Experimental site

The pot experiment was conducted inside a Phytotron at Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan. Natural daylight was used. The day and night temperature inside the Phytotron was maintained as 25° C and 30°C respectively.

4.1.2 Preparation of soil and cultivation of rice

The saline soils used in this experiment were collected on 28th July 2012 from the upper 5cm depth of tsunami-flooded paddy field in Sendai, in Japan (38.23°N Latitude and 140.96°E Longitude). The soil texture was loam, the pH was 6.6, EC 5.87, CEC 23.51 meq 100g $soil^{-1}$, where 4.3 meq 100g $soil^{-1}$ was exchangeable sodium, 0.8 meq 100g $soil^{-1}$ was exchangeable calcium. The total nitrogen content was51.6 g $kg^{-1}$and total carbon 5.6g kg^{-1}. The sodium adsorption ratio (SAR) value was 16.

About 3.5 kg of soil was placed into Wagner pots with the area of 0.02 m^2. Puddling was done by irrigating the pots with tap water at about one week before transplanting. Chemical fertilizers were applied one day before transplanting on 30th October 2013 at the rate of 35 kg N ha^{-1}, 40 kg P ha^{-1} and 70 kg K ha^{-1}. Urea, ammonium phosphate and potassium sulfate were used as a source of N, P and K, respectively. As it is required to maintain the appropriate Na/Ca and roots supplied with elevated levels of external Ca^{2+} (5-

10 mM) are often to maintain their K^+ reported by Aslam et al. (2001) , different rates of gypsum fertilizer were added (Table 4.1).

There were four treatments; no gypsum (control), gypsum 0.5 ton ha^{-1} (G0.5), gypsum 1 ton ha^{-1} (G1) and gypsum 2 ton ha^{-1} (G2). All the treatments were laid out in a completely randomized design with 3 replications. The gypsum fertilizer treatments were given on 30th October 2013. Thirty days old seedlings of a salt tolerant Indica rice variety, Dorfak were transplanted with one seedling per pot on 31st October, 2013 and harvested at maximum tillering stage on 30th November, 2013. A water level of about 2–3 cm was maintained in pots throughout the growing seasons by irrigating regularly with tap water.

4.1.3 Measured parameters

Soil redox potential value (Eh), total organic carbon (TOC), ammonium (NH_4^+) ion concentration, nitrate ion concentration (NO_3^-) in flooded water, and CH_4 gas sampling were collected at ten days interval. At the end of experiment, shoot weight, root weight, soil pH and soil EC value were recorded. The root length and total numbers of root tips were also measured by scanning with an Epson perfection V700 photo scanner and Win RHIZO Pro v.2009c software. Sodium and potassium ion concentrations in the pants were also detected after harvesting rice plants.

4.1.4 Analytical method for soil environment data

The Eh value of soil was monitored by platinum electrodes, which were inserted at 5 cm depth in each plot throughout the rice growth (SWC-201RP, Sanyo, Japan). For TOC, NH_4^+ -N and NO_3^- -N analysis, water samples were filtered with 0.45-μm filter paper at first. Total organic carbon content in flooded water was detected by Total Organic Carbon analyzer (TOC-VCPH*,* Shimadzu Corp*.,* Japan). Both NH_4^+ -N and NO_3^- -N concentrations were determined by using a UV spectrophotometer (UV–VI Mini 1240, Shimadzu Corporation, Kyoto, Japan), by Indophenol method at 630 nm and absorption at 230 nm, respectively. At the end of experiment, the pH and EC of the soil was measured by portable meters (Beckman, Φ 260 pH/ Temp/ mV meter, and ES-51 COND METER, Horiba, Japan, respectively). To analyse the Na^+ and K^+ ion concentration in plants, the above ground plant samples were dried at 60-70 °C. About 5 g of each grounded plant samples were digested by wet digestion method (Jones and Case, 1990). In the digest, Na^+ and K^+ were determined by Hitachi Z-5010 polarized Zeeman atomic absorption spectrophotometer.

4.1.5 Gas sampling, analysis and calculation

Gas sampling was taken at 10-days interval by closed-chamber methods mentioned in chapter (2).

All the data were evaluated by an analysis of variance (ANOVA) by using CropStat 7.0 statistical software. Comparison of treatment means was performed using least significant differences (LSD) at $p=0.05$.

4.2 Results

4.2.1 Soil environment during the experiment

The average NH_4^+-N concentration ranged from 2.8 to 3.9 mg N L^{-1}, the average NO_3^--N concentration from 0.6 to 1.0 mg N L^{-1} (Table 4.2). Total organic carbon concentration of flooded water in all treatments becomes higher at 30 days after transplanting. At 30 days after transplanting, TOC in control was significantly lower than that of G0.5 and G2 but not significantly different with that of G1.The average redox potential (Eh) value lied between -365.5 to -434.0 mV during the experiment. At the end of experiment, the soil pH values were observed as 6.5, 6.0, 6.0, and 5.8 in control, G0.5, G1, and G2 respectively. The soil EC values were observed as 4.9, 6.5, 7.5 and 7.7 in control, G0.5, G1 and G2, respectively.

4.2.2 Sodium and potassium concentration of rice plants

The range of Na^+ concentrations in plants was observed from 0.2 to 0.4 mg g dry plant $sample^{-1}$ (Fig. 4.1a). The values of Na^+ concentration of plants were lower in gypsum treatments than that of control. The significant lower Na^+ concentration was observed only in G2 treatment. The range of K^+ concentration in plants was observed from 1.2 to 1.5 mg g dry plant $sample^{-1}$ (Fig. 4.1b). The higher K^+ ion concentration was observed in plants of gypsum treatments except G2 compared with control.

4.2.3 Rice growth in coastal saline with different Na/Ca ratios

The range of shoot dry weight was 5.4 to 6.4 g pot^{-1} (Fig. 4.2a). The range of root dry weight as observed from 1.4 to 1.7 g pot^{-1} (Fig. 4.2b). Although the roots dry weight in G2 was not significantly different with that of control, the lowest shoot dry weight was observed in G2. There was no improvement in shoot dry weight by adding gypsum fertilizer to saline soil. However, the total numbers of root length and root tips in G0.5 was significantly higher that of other treatments (Fig. 4.3a and b).

4.3.3 Methane emission in coastal saline soil

The CH_4 emission was monitored during vegetative stage of rice growth. The CH_4 flux was suppressed in all treatments until 20 days after transplanting (Fig. 4.4a). The highest CH_4 flux was observed at 30 days after transplanting (Fig. 4.4a). The total CH_4 emission was ranged from -18 to 150 mg m^{-2}over 30 days growth period (Fig. 4.4b). The significant higher total CH_4 emission was observed in G0.5 and G1 compared with control and G2 .The lowest and negative value of total CH_4 emission was observed in G2.

4.3 Discussions

4.3.1 Ion concentration of plants and plant growth

The addition of gypsum as a source of Ca^{2+} decrease Na^{+} ion concentrations in plants under saline condition (Fig.4.1a), because addition of Ca^{2+}protect the integrity and permeability of plasma membranes against Na^{+} toxicity (Tester and Davenport, 2003).

Moreover, the existence of high Na^+ level in the experimental soil seems to interfere with K^+ metabolism in control treatment and the addition of Ca^{2+} also reduced the interference of Na^+ in G0.5 and G1. While K^+ concentration was higher in G0.5 and G1 compared to control, the lowest value of K^+ concentration was observed in G2. The excessive concentration of Ca^{2+} in G2 seems to cause a competition between Ca^{2+} and K^+ uptake by rice plants.

Even though the ion balance was improved in G0.5 and G1 compared to control, there was no significant improvement in shoot dry weight in gypsum fertilizer addition as Ca^{2+} source (Fig.4.2a). No significant changes of chlorophyll content among all treatments (Fig.4.1c) indicated that available nitrogen contents (NH_4^+ -N and NO_3^- -N) are not limited for plant growth. Thus, the growth reduction under gypsum fertilizer addition was due to the higher EC values under gypsum fertilizer addition.

The value of SAR of experimental soil was 16 (Section 4.1.2) which was higher than 13, a limit for saline-sodic or sodic soil described by US Salinity Lab Staff, Agriculture hand book (Richard, 1954). Calcium concentration of experimental soil was about 0.80 meq 100g soil^{-1} (4.1mM) (Section 4.1.2). The Ca^{2+} level should be maintained at 5-10 mM in the external solution of saline condition (Aslam et al., 2001). High Ca^{2+}concentrations can reduce the permeability of plasma membrane to Na^+. The reduction in membrane permeability to Na^+ by Ca^{2+}reduces the accumulation of Na^+ by passive influx (Cramer et al., 1985). Thus, the gypsum fertilizer was applied as Ca^{2+} source. The Ca^{2+} ions in the gypsum fertilizer will replace Na^+ ions that adsorbed to the soil particles and $NaSO_4$ will be leached away from the root zone. In case of pot experiment, there was limited space to wash away Na^+ along with water, and thus, the addition of gypsum led to increase in soil EC value. Flowers and Flowers (2005) stated that salinity has three potential effects on plants such as (a) lowering of the water potential, (b) direct toxicity of any Na and Cl absorbed and (c) interference with the uptake of essential nutrients. No improvement of rice growth under G0.5 and G1 might not exactly relate with ion toxicity and deficiency because of the lower Na^+ and higher K^+ concentration of plants as shown in Fig. 4.1a and b and NH_4^+ -N and NO_3^- -N of flooded water (Table. 4.2). Furthermore, the higher values of root length and root tips were observed in G0.5 and G1 although the total root dry weight was not significant among the treatments. Probably, these higher values of root length and root tips in G0.5 and G1 might be due to the formation fine roots under higher EC values of gypsum treatments compared to the control because Neumann and Römheld, (2002) found that root growth stimulation particularly of fine root structures is frequently induced by moderate limitations of P, N, Fe, and water. In case of G2, the reduction in the growth of rice plants under gypsum treatments especially in G2 treatment might more relate with osmotic effect that may reduce the ability of plants to take up water (Rad et al., 2012).

4.3.2 Methane emission

The higher total CH_4 emission in gypsum fertilizer treatments; G0.5 and G1 might be due to its higher organic carbon content of flooded water that might be contributed either from native soil organic matter or root exudates. In the presence of SO_4^{2-}, sulphate reducers are stronger competitors than methanogens for carbon substrate leading to a reduced production of CH_4 in the rice soil (Van der gon and Neue, 1994). However, the observed significant highest CH_4 emission in G0.5 might also relate with the amount of added SO_4^{2-} ion that did not reach the threshold limit necessary for a successful competition of carbon substrates between sulphate reducers with methanogens. Furthermore, methanogens and SO_4^{2-} reducing bacteria can use more than one substrate and the affinities for these substrates differ. As a result, higher CH_4 emission was observed in G0.5 and G1 compared to control. The optimum pH of paddy soils required for methanogenic bacteria to produce CH_4 is around 6 to 6.6 (Setyanto et al., 2002). The lowest CH_4 emission in G2 might relate with its lowest pH value (about 5.82), highest input of SO_4^{2-} concentration from gypsum fertilizer and lowest above dry matter yield.

4.4 Conclusion

It can be concluded that the Na/Ca ratio around 6 is the optimum ratio in coastal saline soil which contains excess Na^+ concentration. Methane emission in coastal saline soil was closely related with the root growth and total carbon content of flooded water. Moreover, this study proved that although sulphate containing fertilizers are frequently recommended as CH_4 mitigation option, the extent of CH_4 emission was mainly depend on the extent of SO_4^{2-} ions added and carbon availability of those soils. Moreover, as the higher TOC concentrations were observed in gypsum treated soils, it might relate with carbon contribution of the root exudates which is the important carbon source for methanogens.

Therefore, further research should more emphasize on carbon contribution of root exudates under those gypsum treated soils.

Table4.1 Different Na^+/Ca^{2+} ratios of coastal saline soil after adding gypsum fertilizer

Ion conc:	Initial soil	Final soil (After adding gypsum)		
		Gypsum (0.5 tonha^{-1})	Gypsum (1 tonha^{-1})	Gypsum (2 tonha^{-1})
Na(mM)	33	33	33	33
Ca(mM)	4	5.5	7	10
Na/Ca	**8**	**6**	**4**	**3**

Table 4.2 Soil environmental data under different rates of gypsum fertilizer addition

Treatment	Average NH_4^+ -N (mg L^{-1})	Average NO_3^- -N (mg L^{-1})	TOC (mg L^{-1})		Soil Eh	Soil pH	Soil EC (dSm^{-1})
			20 DAT	30 DAT			
Control	3.9 ± 0.3a	0.6 ± 0.2a	7 ± 2a	11 ± 3 b	-385 ± 26a	6.5± 0.1a	4.9 ± 0.7a
G0.5	3.8 ± 0.2a	0.8 ± 0.1a	9 ± 1a	26 ± 11a	-356 ± 63a	6.0± 0.1b	6.3 ± 1.0a
G1	2.8 ± 0.2a	1.0 ± 0.2a	4 ± 1b	17 ± 2 ab	-425 ± 26a	5.9 ± 0bc	7.5 ± 0.2a
G2	4.5 ± 0.2a	0.7 ± 0.3a	8 ± 0a	25 ± 6 a	-451 ± 14a	5.8 ± 0c	7.7 ± 0.3a

Values are the means ± standard deviation (n=3 replications). In each column, means followed by a common letter are not statistically significant different by using least significant difference (LSD) at p= 0.05.

Table 4.3 Pearson product moment correlation analysis among measured parameters

Parameters	pH	EC	Shoot dry weight (g pot^{-1})	TOC in flooded water at 20 DAT (mg C L^{-1})	TOC in flooded water at 30 DAT (mg C L^{-1})	Total CH_4 emission for 30 days (mg CH_4 m^2)
Amount of SO_4^{2-} added in each treatments	**-0.83** *	**0.782** **	**-0.704** **	-0.0446 Ns	0.463 ns	-0.346 ns
pH		**-0.815** **	0.552 ns	0.14 Ns	-0.549 ns	-0.155 ns
EC			-0.39 ns	-0.275 Ns	0.324 ns	-0.072 ns
Shoot dry weight (g pot^{-1})				-0.04 Ns	**-0.655** *	0.09 ns
TOC in flooded water at 20 DAT (mg C L^{-1})					0.282 ns	0.19 ns
TOC in flooded water at 30 DAT (mg C L^{-1})						0.382 ns

**, * and ns stand for significance at 1%, 5% and non-significance respectively.

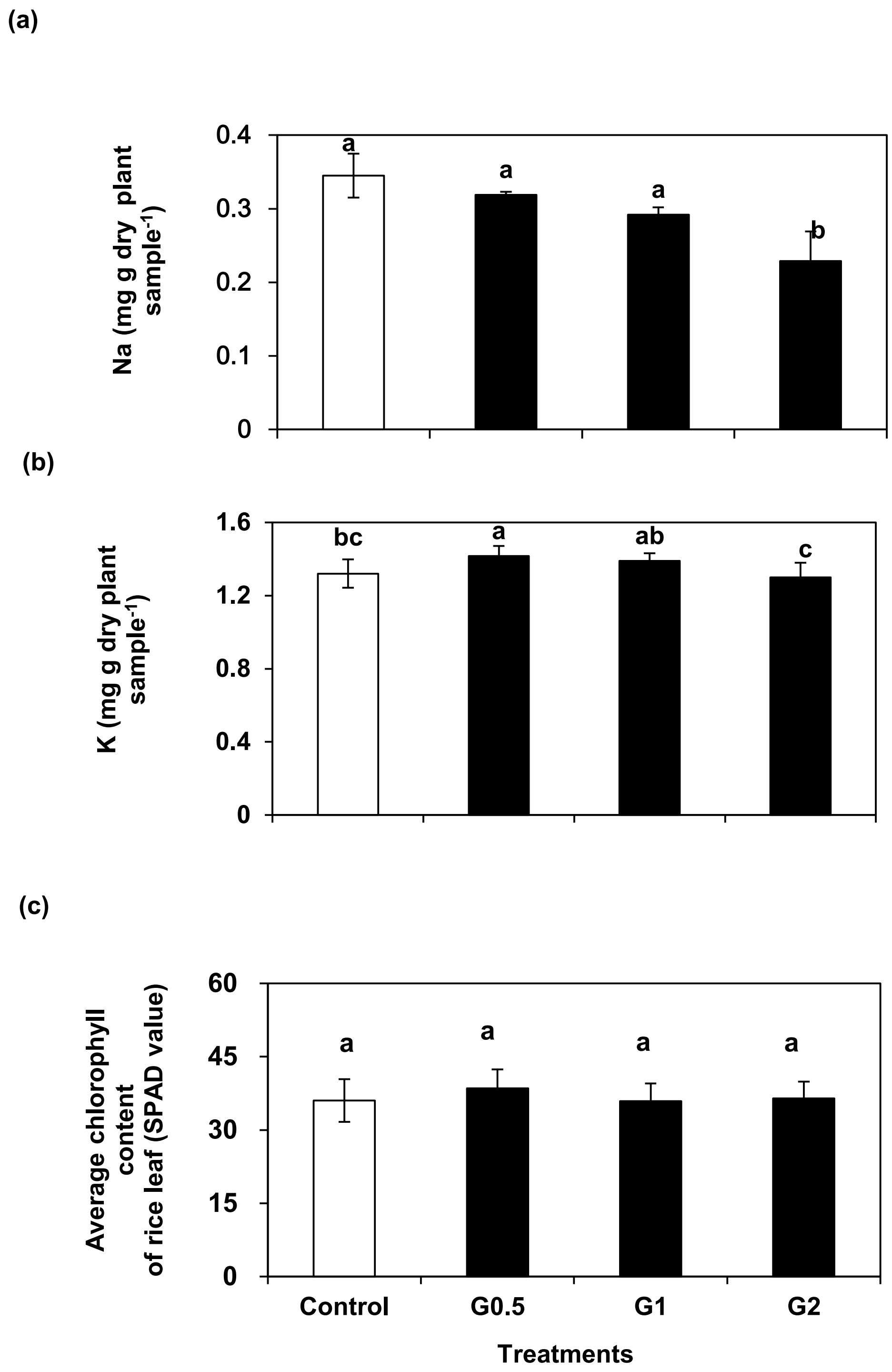
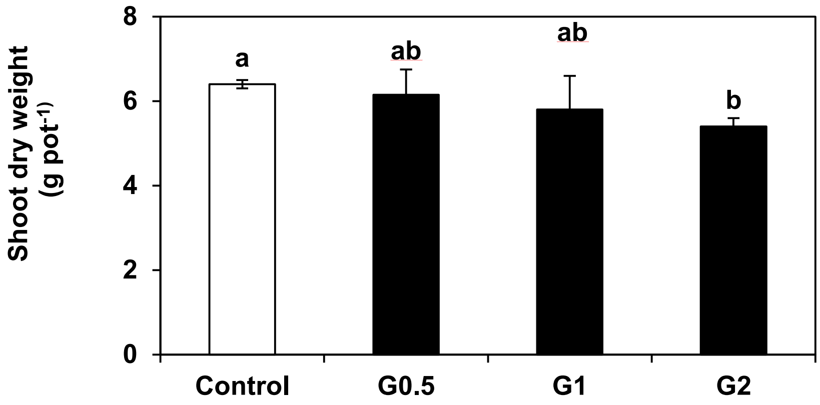


Fig.4.1 Effect of different rates of gypsum fertilizer application on (a) Na^+, (b) K^+ concentration in plant samples and (c) average chlorophyll content of leaf. Mean followed by a common letter are not statistically significant different by using least significant difference (LSD) at p=0.05. Error bars indicate standard deviation, n=3.

(a)



(b)

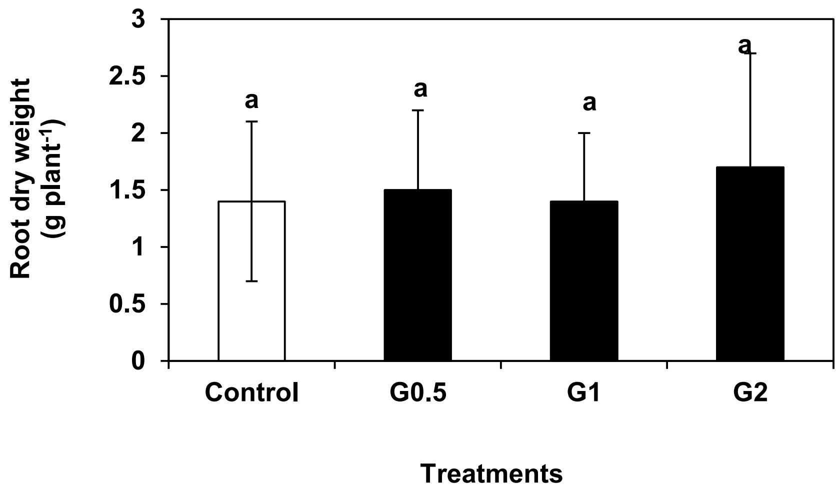
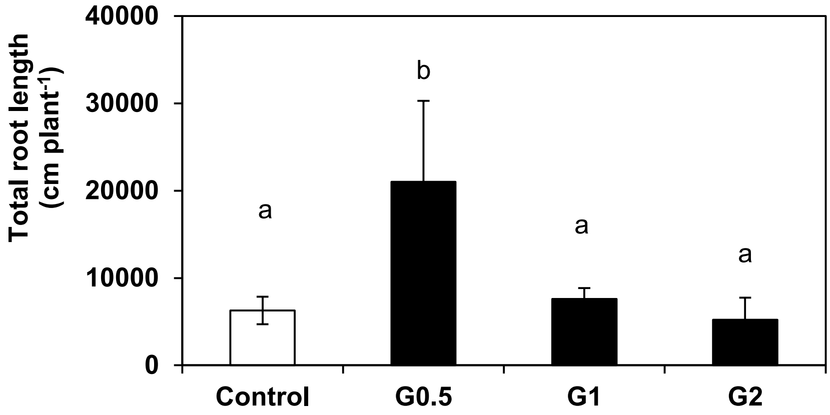


Fig.4.2 Effect of different rates of gypsum fertilizer application on (a) shoot dry weight, and (b) root dry weight. Means followed by a common letter are not statistically significant different by using least significant difference (LSD) at p=0.05. Error bars indicate standard deviation, n=3.

(a)



(b)

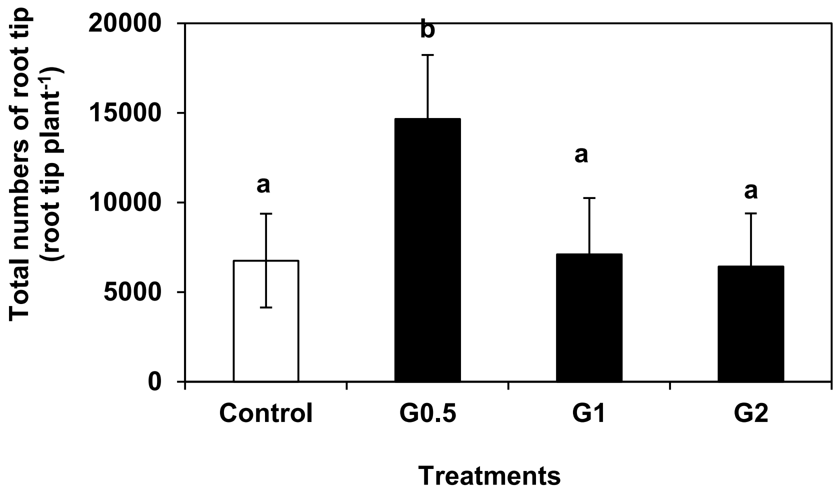
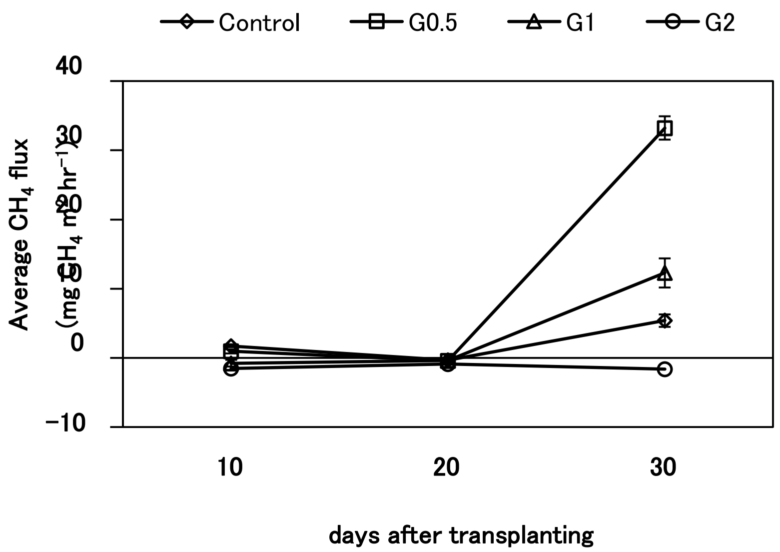


Fig.4.3 Effect of different rates of gypsum fertilizer application on (a) total root length, (b) total numbers of root tips. Means followed by a common letter are not statistically significant difference by using least significant difference (LSD) at p=0.05. Error bars indicate standard deviation, n=3.

(a)



(b)

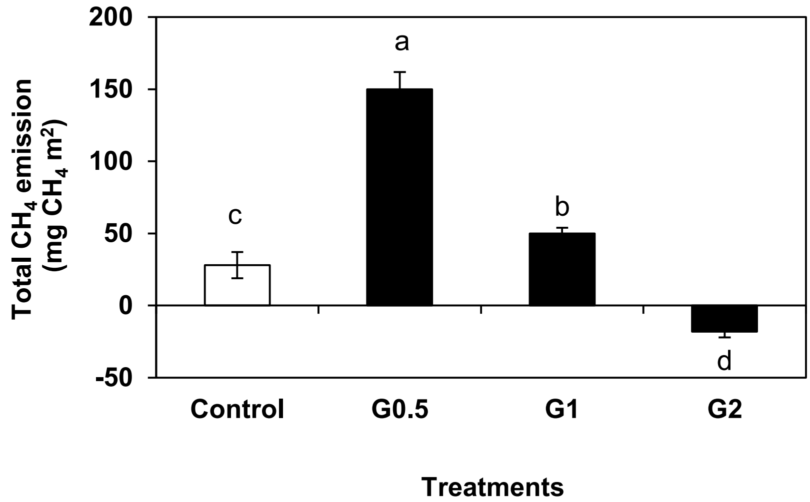


Fig.4.4 Effect of different rates of gypsum fertilizer application on total CH_4 emission; (a) average CH_4 flux pattern, and (b) total CH_4 emission for 30 days. Means followed by a common letter are not statistically significant different by using least significant difference (LSD) at $p=0.05$. Error bars indicate standard deviation, $n=3$.

Chapter 5

Study on organic acids exudation by rice plants in coastal saline soil with different Na and Ca ratios by adding gypsum fertilizer

The objective of this study is;

To identify the quantity and quality of root exudation of rice plants grown in coastal saline soils with different Na/Ca ratios

5.1 Materials and methods

5.1.1 Root exudates collection method

This study was conducted at the end of rice cultivation experiment with pot in chapter (4). The rice plants from pot experiment of chapter (4) were taken out with the intact soil pot and immediately used for the root exudates collection. Firstly, the soils around the roots were removed by shaking gently inside the water in a plastic box. Then, the remaining soils attached to the roots were washed gently with water. The soil washing process was conducted carefully to avoid damage to the roots. The root exudates of rice plants under each treatment were collected by using filter paper method which is slightly modified to the method of Neumann (1999). The rice plants were taken out with the intact soil from the pot. Subsequently, ten not damaged root tips were selected to collect the root exudates. The tip of the root was sandwiched between two pieces of 7 mm diameter filter paper (4A, ADVANTEC). After that, the filter papers with root were wetted by putting one drop of milli-Q water. After 30 minutes, the total 20 pieces of filter papers were put into centrifugation tube fitted with 0.45 µm filter paper (Centricut ultra-mini W-50, 50000MW, Kurabo, Japan). About (0.2 ml) of distilled water was added to the ultracentrifugation tube and the diluted root exudates was centrifuged for 10 minutes at 12000 rpm. The centrifuge tubes containing the filtrate were kept in the freezer until analysing the organic acids.

5.1.2 Root exudates analysis and calculation method

High performance liquid chromatography (HPLC) was used for determination of low molecular weight organic acids in soil (Van Hees et al., 1999). HPLC was performed on a Shimadzu Organic Acid Analysis System LC-10AD (Non-suppresser, post column type) equipped with electric conductivity detector CDD-6A and two Shim-pack 102H columns at a temperature of 40 °C. A 5 mM *p*-toluenesulfonic acid (pH 2.8) was used as a carrier solution at a flow rate of 0.8mL min^{-1}. The analysis of organic acids was carried out by injecting a 100 µl of solution in the system. Individual organic acids were identified and calibrated by comparing retention times with those of standards prepared with the known amounts of Citric, Tartaric, Malic, Succinic, Lactic, Formic, Acetic, and Propionic acids. The concentration of each organic acid under each treatment was calculated by dividing the area of each organic acid with the value of recovery rate. To know the recovery rate, one sample was randomly selected and 4 solutions were prepared such as standard plus milli-Q water (1:1), sample plus milli-Q water (1:1) and standard plus sample (1:1). The recovery rate of each organic acid in a selected sample was calculated as follows.

$$F = 100^* (D-B)/A \qquad (1)$$

F = recovery rate (%)

A= the area of standard plus milli-Q water

B= the area of selected sample plus milli-Q water

D= the area of standard plus selected sample

5.2 Results and discussion

5.2.1 Organic acids exudation from coastal saline soil with different Na/Ca ratios

Six organic acids species such as Citric, Tartaric, Malic, Lactic, Formic and Acetic acids were observed in the root exudates of control, G0.5, and G1 (Fig. 5.1). Only four organic acids species, Citric, Tartaric, Malic, and Lactic acids were observed in G2 (Fig. 5.2). Among the 6 organic acid species, Lactic acid exudation was the highest and the range of lactic acid exudation was observed as 3.9 to 7.9 μmol ml^{-1} root $tip^{-1}day^{-1}$. The minimum amount of exudation was observed in Acetic acid and the range of its exudation was found as 0.08 to 0.4 μmol ml^{-1} root $tip^{-1}day^{-1}$. The order of organic acid exudation in root exudates was observed as Lactic acid > Malic acid >Tartaric acid >Formic acid >Citric acid >Acetic acid. Furthermore, the higher values of organic acid exudation potential were observed in gypsum fertilizer treatments over that control (Fig. 5.3).

The higher concentration of total organic acids exudation (Fig. 5.3) might relate with higher EC values in G0.5 and G1 (Table 4.2). Under the salt stress, most of the plants accumulate low molecular weight organic solutes inside (Jouyban, 2012), because plants need to maintain internal water potential below that of soil and maintain turgor and water uptake for growth (Tester and Davenport, 2003). Organic acids can serve as an active metabolic solute, regulating osmotic pressure and balancing excess cation (Sun et al., 2002). In order to maintain their charge balance, roots release protons whenever they take up more cations than anions, and take up protons when the opposite occurs (Hinsinger et al., 2003). Lo´pez-Bucio et al. (2000) also pointed that plants would activate more metabolites and some unknown substances under environmental stress. The quantities and qualities of root exudates can be determined by plant species, the ages of individual plants, and external factors such as biotic and abiotic stressors (Badri and Vivanco, 2009). Many studies have shown that plants' roots under Aluminium stress exude higher concentration of Malic acid in wheat crop (Delhaize et al., 1993b, Basu et al., 1994). In our study, higher concentration of Malic acid and Tartaric acid was observed in gypsum fertilizer treatments that showed higher EC values, which might be due to the metabolic regulation of organic acids under salinity stress.

Plants discharge about 5 to 21% of photosynthetic carbon as root exudates (Kumar et al., 2006) and root exudation clearly represents a significant carbon cost to the plant (Walker et al., 2014). Therefore, higher amount of carbon contribution from root exudates under gypsum fertilizer addition as shown in Fig.5.3 may induce numerically lower shoot dry weight in G0.5 and G1, and statistically lower shoot dry weight in G2 compared to control. The lowest concentrations and less species of organic acid in root exudates of G2 compared with G0.5 and G1 might also relate with its lower shoot dry weight because the significant reduction of shoot dry weight in G2 may reduce the extent of photosynthetic carbon which may result in less species and lower concentrations of organic acids in G2.

5.1 Conclusion

It can be concluded that if the soil is more saline, the rice plants will secrete more organic acids via root exudation. Under saline condition, the amount of organic acids exuded by rice plants to the soil may depend on shoot and root growth especially root tips of rice plants. Although the highest amount of Malic acid and Tartaric acids was observed in gypsum fertilizer treatments, the cellular mechanism of those acids in rice plants was unknown yet.

Table 5.1 Different Na^+/Ca^{2+} ratios of coastal saline soil after adding different rates of gypsum fertilizer

Ion conc:	**Initial soil**	**Final soil (After adding gypsum)**		
		Gypsum (0.5 tonha^{-1})	**Gypsum (1 tonha^{-1})**	**Gypsum (2 tonha^{-1})**
Na(mM)	33	33	33	33
Ca(mM)	4	5.5	7	10
Na/Ca	**8**	**6**	**4**	**3**

Table 5.2. Soil environment under different Na/Ca ratios

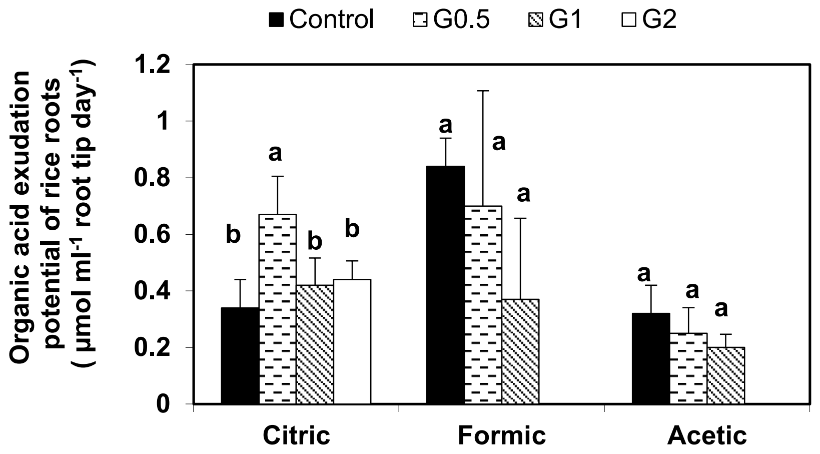
Treatments	Soil pH	Soil EC (dSm^{-1})
Na/Ca = 8 (Control)	6.5 ± 0.1a	4.9 ± 0.7b
Na/Ca= 6 (G0.5)	6.0 ± 0.1b	5.3 ± 1ab
Na/Ca=4 (G1)	5.9 ± 0.0bc	7.5 ± 0.2a
Na/Ca=3 (G2)	5.8 ± 0.0c	7.7 ± 0.3a

ANOVA test for soil pH and EC

Source of variation	Soil pH	Soil EC (dSm^{-1})
Treatments	**	**

**, * and ns stand for significance at 1%, 5% and non-significance respectively.

(a)



(b)

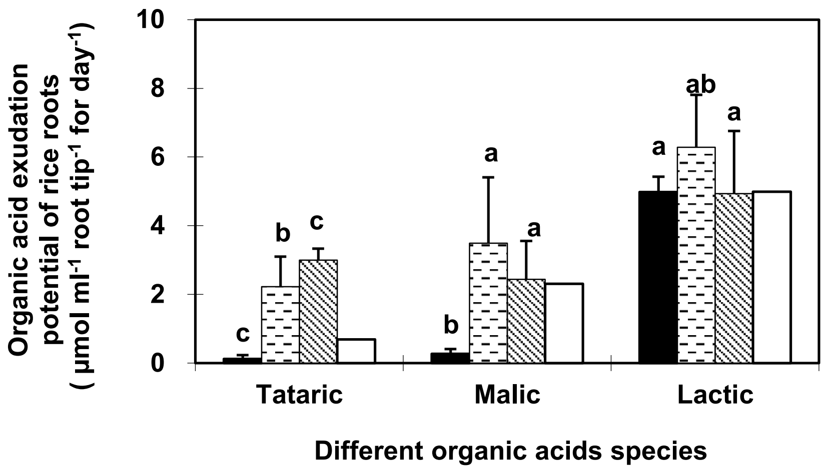


Fig.5.1 Organic acids exudation potential of rice under different Na/Ca ratios. Means followed by a common letter within each acids are not statistically significant different by using least significant difference (LSD) at p=0.05. Error bars indicate standard deviation, n=3.

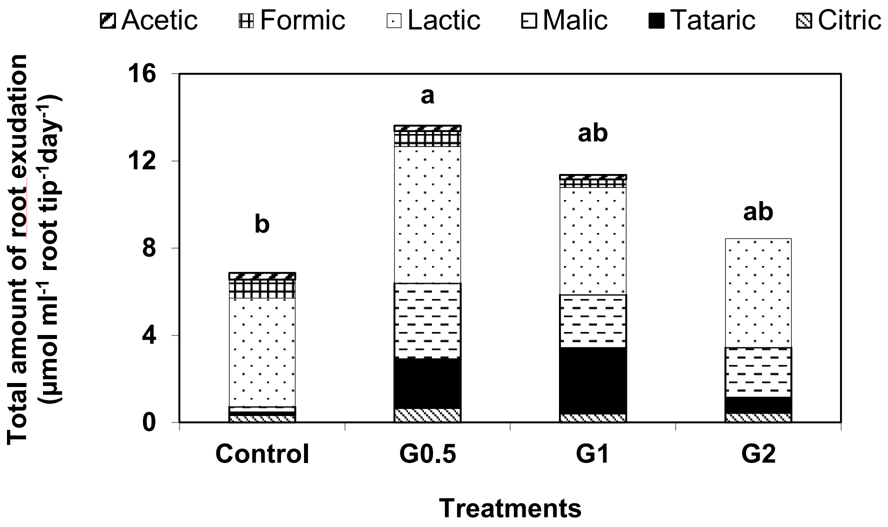


Fig.5.2 Effect of different rates of gypsum fertilizer addition on total amount of organic acids exudation potential per root tip per day. Means comparison is done for the total organic acid exudation of rice plants under each treatment. Means followed by a common letter are not statistically significant different by using least significant difference (LSD) at p=0.05.

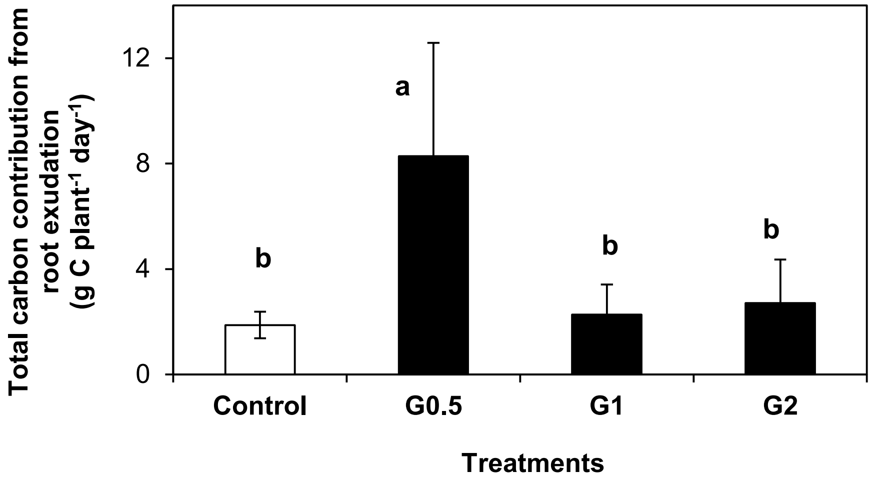


Fig. 5.3 Effet of different rates of gypsum fertilizer addition on total carbon contribution from rhizosphere exudation of rice plants. Means followed by a common letter are not statistically significant different by using least significant difference (LSD) at p=0.05.

Chapter 6

General discussion and overall conclusion

6.1 Methane emission in paddy rice soil affected by saline irrigation water

Paddy rice fields are identified as one of the major sources of global warming CH_4 gas (IPCC, 2007). The extent of CH_4 emission is largely influenced by the soil condition to which rice is planted and management practices associated with rice cultivation. Rice is widely grown in coastal line of humid tropic (Reddy, 2014). In those regions, rice fields are affected by salinity due to periodic sea water intrusion during the rainy season and irrigation water salinity during the dry season. The most prevalent salt in the soil solution of coastal saline soil is NaCl which show the most detrimental effect on rice growth. Therefore, CH_4 emission was evaluated in paddy rice soil affected by saline irrigation water with NaCl in chapter 2 and 3.

Methane emission from paddy field results from a balance of CH_4 production by methanogenic bacteria, methane oxidation by methanotrophic bacteria and transportation by rice plant. (Win et al., 2012). The complexity of the influencing factors of CH_4 emission under different irrigation water salinity levels from the current experiment is summarized in Fig. 6.1.

Under saline conditions (S25, S30 and S90), rice growth are decreased as shown in Table 2.3 and Table 3.2. The significant higher dead leaves were observed in all salinity levels such as S25 and S30 and S90. Wassmann and Aulakh (2000) reported that CH_4 production in rice fields largely depends on plant-borne material that can be either decaying tissue or root exudates. Thus, the increase in total numbers of dead leaves under saline condition may contribute the carbon source for methanogenic bacteria. In another side, salinity significantly suppressed the above-ground biomass yield in S30 and S90. As rice plants are the major transporters of CH_4 gas from soil to atmosphere, the decrease in above-ground biomass yield in S30 and S90 may reduce CH_4 transport capacity of rice plants.

According to the results of laboratory incubation experiment, CH_4 production was increased up to salinity 30 mmol L^{-1} NaCl (Table 2.2). This increase in CH_4 production in S30 might be due to the Na requirement of methanogens. According to the report of Jarrell and Kalmokoff (1988), sodium is required for amino acid transport, growth, methanogenesis, and internal pH regulation in methanogenic bacteria. In case of CH_4 emission in paddy rice cultivation, CH_4 emission in S30 was not significant different with CH_4 emission in control. Although CH_4 production is enhanced in S30, no difference of CH_4 emission value between S30 and control might be due to lower above-ground biomass yield of S30. As rice plants serve as major CH_4 transporters from soil to atmosphere, the reduced above ground biomass yield may limit the CH_4 transport capacity by rice plants. The addition of S25 to paddy rice cultivation could not suppress above-ground biomass yield. Thus, CH_4 transportation factor is not limited in S25. Although enhancing effect of CH_4 production was observed up to salinity level S30 in laboratory incubation experiment, no difference of CH_4 emission between S25 and control might be due to the existence of a higher NO_3^- concentration in S25 in the early growth stage (Fig. 3.2). At the highest salinity level (S90), CH_4 production was decreased. It might be due to Cl^- toxicity under higher additions of NaCl. The lowest CH_4 emission in paddy rice cultivation was also observed in S90. Thus, this lowest CH_4 emission in S90 might be due its lowest CH_4 production potential, the shortest growth duration period and the lowest above ground biomass yield.

To improve the rice growth in Na^+ saturated soil/water, gypsum fertilizer is commonly applied as Ca^{2+} source. Therefore, the effect of gypsum fertilizer addition to paddy rice soil on rice growth and CH_4 emission was also studied in non-saline and saline condition. In chapter 1, the gypsum 1 ton ha^{-1} was applied to both non-saline and saline conditions (S30 and S90). The addition of gypsum fertilizer significantly reduced in CH_4 emission in both non-saline and saline condition (Fig. 2.5). In the presence of SO_4^{2-}, SO_4^{2-}-reducing bacteria will out compete methanogens for the same substrates such as hydrogen (H_2) or carbon dioxide (CO_2) or Acetate (CH_3COO^-) that methanogens use in methane production (Lindau et al., 1993; Van der gon and Neue, 1994; Epule et al., 2011). Therefore, the reduction of CH_4 emission following the addition of gypsum (Fig. 2.5) might be due to the competition between SO_4^{2-}-reducing bacteria and CH_4-producing bacteria for substrates as stated by Lindau et al. (1993), Van der gon and Neue (1994) and Epule et al. (2011). In chapter 3, three different rates of gypsum fertilizer are applied to the paddy field under non-saline and saline condition with salinity level S25. The higher application gypsum resulted higher reduction in CH_4 emission. However, the amount of reduction due to gypsum fertilizer addition is lower in saline condition than that of non-saline condition. It might be due to the availability of C source from the dead plant materials under saline condition.

6.2 Methane emission in coastal saline soil

Although NaCl is the most abundant ion in most saline soils, the soil solution of saline soils is composed of a range of dissolved salts, such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3. Thus, CH_4 emission was evaluated in coastal saline soil in chapter 4 and 5. To provide appropriate Na/Ca ratios for rice growth, different rates of gypsum fertilizer such as 0.5, 1 and 2 ton ha^{-1} were added to the coastal saline soil. After adding gypsum fertilizers treatment, the estimated Na/Ca ratios were 8, 6, 4, and 3 in control, G0.5, G1 and G2, respectively.

The addition of gypsum fertilizers ($CaSO_4.2H_2O$) resulted to decrease soil pH and increase in soil EC. At the same time, it will also increase in soil SO_4^{2-} concentration. Furthermore, The K^+ ion concentration of plants was increased and Na^+ ion concentration of plant was decreased in G0.5 and G1 (Fig. 4.1). However, no improvement in ion balance and the significant reduction of shoot dry weight was observed in G2. It might be due to the excess Ca^{2+}/SO_4^{2-} ion concentration in G2. Although gypsum fertilizer is known to inhibit CH_4 emission, the higher total CH_4 emission in G0.5 was observed over 30 days study. The lowest CH_4 emission was observed in G2. Thus, the possible mechanism of CH_4 emission in coastal saline soil will be explained as shown in Fig. 6.2.

Under the salt stress, most of the plants accumulate low molecular weight organic solutes (Jouyban, 2012). Organic acids can serve as an active metabolic solute, regulating osmotic pressure and balancing excess cation (Sun et al., 2002). In order to maintain their charge balance, roots release protons whenever they take up more cations than anions, and take up protons when the opposite occurs (Hinsinger et al., 2003). As a result, higher organic acids exudation was observed in gypsum fertilizer treatments, which showed higher EC values, compared to that of control (Fig. 5.2). Among the gypsum fertilizer treatments, the significant highest organic acids exudation potential was observed in G0.5. The less organic acids species and lowest organic acids exudation potential was observed in G2. It might be due to the lowest shoot dry weight which may turn to reduce photosynthetic carbon of plant because plants discharge about 5 to 21% of photosynthetic carbon as root exudates (Kumar et al., 2006). Thus, the highest CH_4 emission in G0.5 might be due to the level of SO_4^{2-} input was not enough to inhibit CH_4 production, the highest C availability from root exudation. The lowest CH_4 emission in G2 might relate with its lowest shoot dry weight, pH value, and highest level of SO_4^{2-} input.

6.3 Overall conclusion

The continuous irrigation of saline water can severely affects the rice growth. To reduce the irrigation water salinity problem in rice cultivation, irrigation water with salinity level up to 25 mmo L^{-1} NaCl can be used intermittently if no fresh water is available for rice cultivation. To improve the ion balance of rice plants which are grown in saline soil, the appropriate Na/Ca ratio around 6 should be maintained.

Although salinity is generally known to suppress CH_4 emission, the addition of water with salinity level up to 30 mmol L^{-1} NaCl favours CH_4 production and could not suppress

CH_4 emission in rice cultivation. In the paddy rice cultivation, Although gypsum fertilizer is known to reduce CH_4 emission from paddy rice soil, the level of CH_4 emission under gypsum fertilizer addition depends on both the level of SO_4^{2-} added and the carbon availability of the soil. As rice plants excrete more organic acids to adjust the osmotic stress under saline condition, the organic acids of root exudates clearly represent the main carbon pool for CH_4 emission in saline condition. However, the extent of organic acids exudation may depend on the shoot and root growth of rice plants.

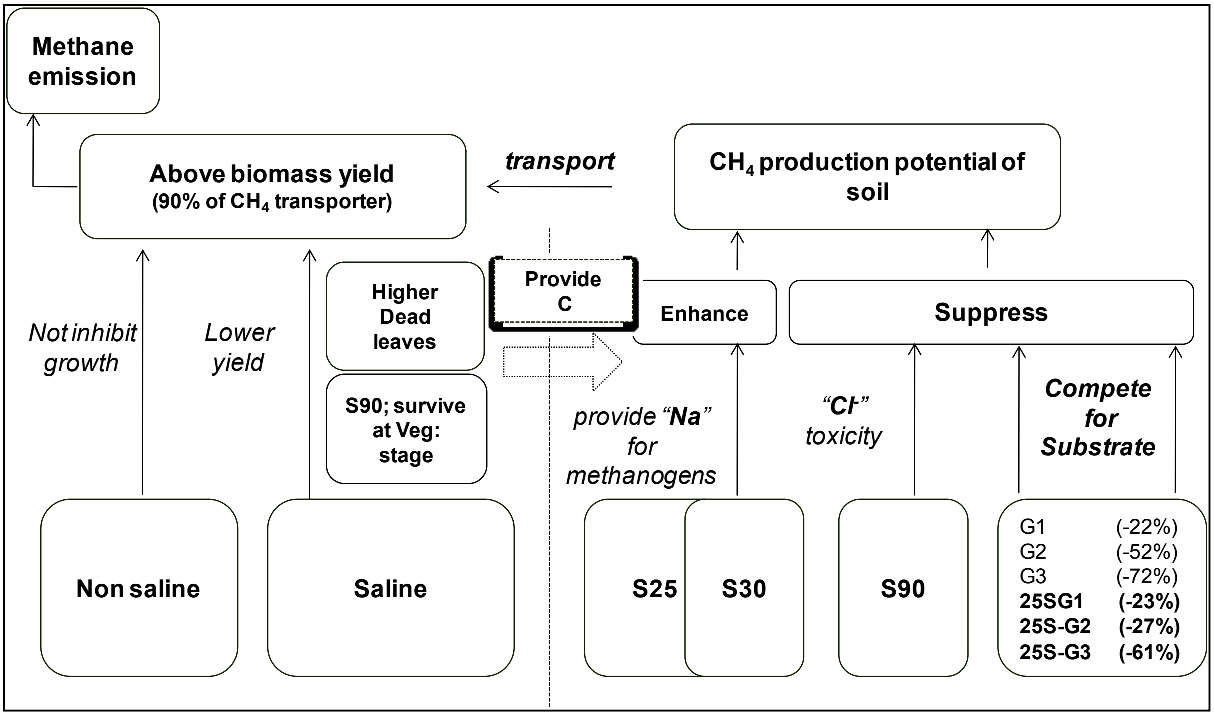


Fig.6.1 A schematic diagram for chapter 2 and 3 explaining the mechanism of CH_4 emission relation C and SO_4^{2-} availability under different levels of irrigation water salinity and gypsum application rates

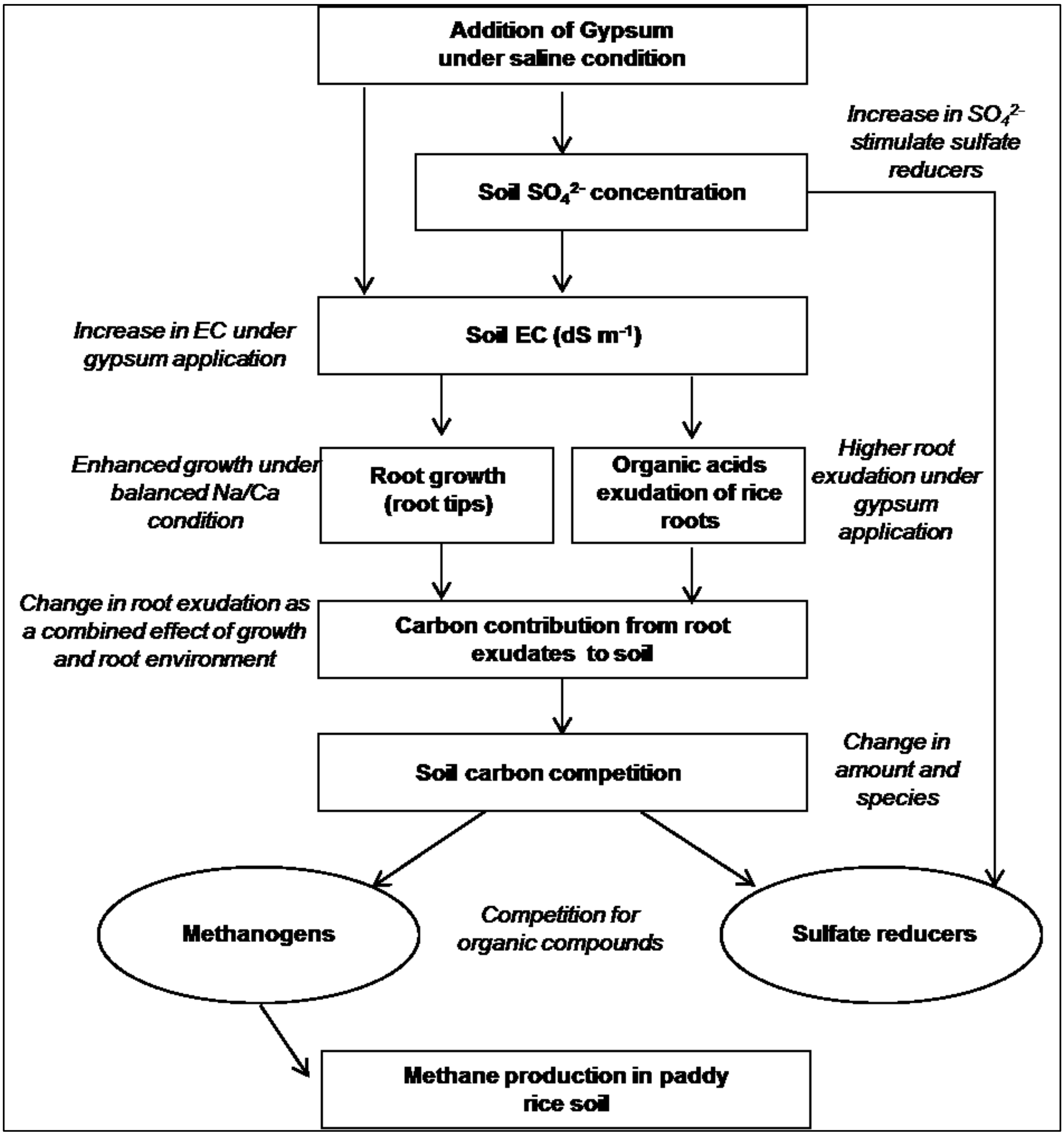


Fig.6.2 A schematic diagram for chapter 4 and 5 explaining the mechanism of CH_4 emission in relation to C and SO_4^{2-} availability under different rates of gypsum fertilizer application

References

References

Abdelgadir, E.M., Oka, M., Fujiyama, H., 2005. Nitrogen nutrition of rice plants under salinity. Biologia Plantarum 49 (1), 99-104.

Alex, C. W., 2006. Plant nutrition.Infobase publishing, NewYork.

Alobaidy, A., Al-Sameraiy, M., Kadhem, A., Majeed, A., 2010. Evaluation of treated municipal wastewater quality for Irrigation. Journal of Environmental Protection 1 (3), 216-225.doi:10.4236/jep.2010.13026.

Amaranatha, R.M., Rose, M.F., Rasool, SK.N., and Venkata, R.P., 2014. Breeding for tolerance to stress triggered by salinity in rice. International Journal of Applied Biology and Pharmaceutical Technology 5 (1), 167-176.

Amirjani, M.R., 2011. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. International Journal of Botany 7, 73-81.

Aslam, M., Mahmood, I.H., Qureshi, R.H., Nawaz, S., Akhtar, J., and Ahmd, Z., 2001. Nutritional role of calcium in improving rice growth and yield under adverse conditions. International Journal of Agriculture and Biology 3 (3), 292-297.

Aulakh, M.S., Wassmann, R., Rennenberg, H., 2001. Methane emission from rice fields—quantification, mechanisms, role of management and mitigation options. Advanced Agronomy 70, 193–260.

Badri, D. V., and Vivanco, J. M., 2009. Regulation and function of root exudates. Plant, Cell and Environment. 32, 666-681.

Barus, W. A., Rauf, A., Damanik, S. J. B., Rosmayati, 2013. Screening and Adaptation in Some Varieties of Rice under Salinity Stress (Case Study at Paluh Merbau, Deli Serdang District, North Sumatera, Indonesia). Journal of Rice Research 1(2). doi: 10.4172/jrr.1000112.

Basu, U., Godbold, D., and Taylor, G.J., 1994. Aluminium resistance in Triticum aestivum associated with enhanced exudation of malate. J. Plant Physio. 144, 747-753.

Biswas, H., Mukhopadhyay, S. K., De, T. K., Sen, S., Jana, T. K., 2006. Methane emission from wetland rice fields of Sagar Islands, NE coast of Bay of Bengal. International Journal of Agricultural Research 1 (1), 76 - 84.

Bowen, G. D. and Rovira, A. D., 1999. The rhizosphere and its management to improve plant growth. Advanced Agronomy 66, 1–102.

Cha-um, S., Pokasombat, Y., Kirdmanee, C., 2011. Remediation of salt-affected soil by gypsum and farmyard manure importance for the production of Jasmine rice, Australian Journal of Crop Science 5 (4), 458-465.

Chidthaisong, A., Watanabe, I., 1997. Changes in concentrate and ∂ 13C value of soil entrapped CH_4 and CO_2 in flooded rice soil. Biology and Fertility of Soils 24, 70–75.

Chin, K., Conrad, R., 1995. Intermediary metabolism in methanogenic paddy soil and the influence of temperature. FEMS Microbiology Ecology 18 (2), 85–102.

Choudhary, O. P., Josan, A.S., Bajwa, M.S., Kapur, L., 2004.Effect of sustained sodic and saline-sodic irrigation and application of gypsum and farmyard manure on yield and quality of sugarcane under semi-arid conditions. Field Crops Research 87,103–116.

Cicerone, R.J., and Shetter, J.D., 1981. Sources of atmospheric methane: Measurements in rice paddies and a discussion. Journal of Geophysical Research 86, 7203-7209.

Clark, G., Dodgshun N., Sale P., Tang C. 2007. Changes in chemical and biological properties of a sodic clay subsoil with addition of organic amendments. Soil Biology and Biochemistry 39, 2806–2817.

Cramer, G. R., Lauchli, A., Polito, V. S., 1985. Displacement of Ca^{2+} by Na^{+} from the plasma membrane of root cells. A primary response to salt stress? Plant Physiology 79, 207-211.

Datta, S. K., 1981. Changes of landscape and soil on which rice is grown: problems soils on which lowland rice is grown; In Principles and practices of rice production. Wiley Interscience Publication. P.76.

Davitt, D., Jarrell, W.M., and Stevens, K.L.,1981. Sodium-Potassium ratio in soil solution and plant response under saline conditions. Soil Science Society of American Journal 45, 80–86.

DeepaSankar, P., Saleh, Arabi Mohamed Sale, M.A., Selvaraj, Immanuel, 2011. Rice breeding for salt tolerance. Research in Biotechnology 2(2): 1-10.

Delhaize, E., Ryan, P.R., and Randall, P.J., 1993. Aluminum Tolerance in Wheat (Triticum aestivum L.) (II. Aluminum-Stimulated Excretion of Malic Acid from Root Apices). Plant Physiology 103, 695-702.

Dobermann, A., Fairhurst, T., 2000. Rice: nutrient disorder and nutrient management (VOl.1). International Rice Research Institute.

Dubey, S.K., 2005. Microbial ecology of methane emission in rice agroecosystem: A review. Applied Ecology and Environmental Research 3 (2), 1-27.

Epule E.T., Peng, C., Mongoh, N., 2011. Methane emissions from paddy rice fields: Strategies towards achieving a Win-Win sustainability scenario between rice qroduction and methane emission reduction. Journal of Sustainable Development 4(6), 188-196.

FAOSTAT. 2012. (available at: www.faostat.fao.org/)

Feizi, M., Hajabbasi, M. A., Mostafazadeh-Fard, B., 2010. Saline irrigation water management strategies for better yield of safflower (*Carthamus tinctorius* L.) in an arid region. Australian Journal of Crop Science 4, 408–414.

Flowers, T.J. and S.A. Flowers, 2005. Why does salinity pose such a difficult problem for plant breeders? Agriculture and Water Management 78, 15-24.

Gauci, V., Matthews, E., Dise, N., Walter, B., Koch, D., Granberg, G., andVile, M., 2004. Sulfur pollution suppression of the wetland methane source in the 20th and 21st centuries. Proceedings of the National Academy of Sciences of the United States of America 101 (34), 12583–12587.

Ghafoor, A., Qadir, M., Murtaza, G., 2004. Salt–affected Soils: Principles of management. Allied Book Center: Urdu Bazar, Lahore. 38–53.

Ghafoor, A., Murtaza, G., Ahmad, B., Boers, T.M., 2008. Evaluation of amelioration treatments and economic aspects of using saline sodic water for rice and wheat production on salt-affected soils under arid land conditions. Irrigation and Drainage 57, 424-434.

Ghafoor, A., Gill, M.A., Hassan, A., Murtaza, G., Qadir, M., 2001. Gypsum: An economical amendment for amelioration of saline-sodic waters and soils and for improving crop yields. International Journal of Agricultural Biology 3, 266–275.

Gregory, P. J. and Atwell, B. J., 1991. The fate of carbon in pulse labelled crops of barley and wheat. Plant and Soil 136, 205–213.

Grieve, C.M., Fujiyama, H., 1987. The response of two rice cultivars to external Na/Ca ratio. Plant and Soil 103, 245-250.

Grünberger, O., Maeght, J.L, Montoroi, J.P., Rattana-Anupap, S., Wiengwongnam, J., Hammecker, C., 2005. Assessment of salinity hazard by time domain reflectometry in flooded sandy paddy soils. Proc. of the Management of Tropical Sandy Soils for

Sustainable Agriculture (Session 3). 27, 112–117.

Hanay, A., Büyüksönmez, F., Kızıloglu, F.M., Canbolat, M.Y., 2004. Reclamation of saline-sodic soils with gypsum and MSW compost. Compost Science and Utilization 12, 175–179.

Hattori, C., Ueki, A., Seto, T., Ueki, K., 2001. Seasonal variations in temperature dependence of methane production in paddy soil. Microbes and Environment 16 (4), 227-233.

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints, a review. Plant and Soil. 248, 43-59.

Holzapfel-Pschorn A., Conrad R., and Seiler W., 1986. Effects of vegetation on the emission of methane from submerged paddy soil. Plant and Soil 92, 233-391.

Homaei, M., 2002. Plant reaction to salinity. National Irrigation & Drainage Committee Publication (in Farsi).

Hosono, T., Nouchi, I. 1997. The dependence of methane transport in rice plants on the root zone temperature. Plant and Soil 197, 233-240.

Hou, A. X., Wang Z. P., Chen, G. X., and Patrick, Jr. W. H., 2000. Effect of organic and N fertilizers on methane production in a Chinese rice soil and its microbiological aspects. Nutrient Cycling in Agroecosystem 58, 333-337.

Hu, S., Tao, H., Qian, Q., Guo, L., 2012. Genetics and molecular breeding for salt-tolerance in rice. Rice Genomics and Genetics 3 (7), 39-49. doi:10.5376/rgg.2012.03.0007.

Hu, Y.andSchmidhalter, U., 2005. Drought and salinity: A comparison of their effects on mineral nutrition of Plants. Journal of Plant Nutrition and Soil Science 168 (4), 541–549.

Huang, B., Yu, K., and Gambrell, R. P., 2009. Effects of ferric iron reduction and regeneration on nitrous oxide and methane emissions in a rice soil. Chemosphere, 74(4), 481-486.

Husain, S. S., and Mckeen, W. E., 1963. Interactions between strawberry roots and *Rhizoctonia /ragariae.* Phytopathology 53, 541-545.

Hussain, K., Nisar, M.F., Majeed, A., Nawaz, K., Bhatti, K.H., Afghan, S., Shahazad, A., Zia-ul-Hassnian, S., 2010. What molecular mechanism is adapted by plants during salt stress tolerance? African Journal of Biotechnology 9, 416-422.

IPCC-Intergovernmental panel on climate change, 1995. The Science of Climate Change, Cambridge (UK): Cambridge University Press, 1996.

IPCC, (1996): XII summary for policy makers. In: (Houghton, IT., Meira, F., Callander, LG., Harris, B.A., Kattenberg, A., Maskell, K., (Eds.), Climate Change 1995: The Scientific Basis of Climate Change. − Cambridge University Press, Cambridge, UK. p. 572.

IPCC-Intergovernmental panel on climate change, 2007. Changes in atmospheric constituents and in radiative forcing, IPCC Fourthly Assessment Report, p. 141–142.

IRRI . 2006. Bringing hope, improving lives: Strategic Plan 2007-2015, Manila .61 p.

Ivanov, V. P., 1962. Mutual effect through the root system of a mixed crop of corn and broad beans. Fiziol. Rast. 9(2), 179-188.

Jain, N., Pathak, H., Mitra, S., and Bhatia, A., 2004. Emission of methane from rice fields- A review. Journal of Scientific and Industrial Research 63, 101-115.

Jarrell, K.F., Kalmokoff, M.L., 1988. Nutritional requirements of the methanogenic archaebacteria. Canadian Journal of Microbiology 34, 557–576.

Jiao, Y., Huang, Y., Zong, L., Zheng, X., and Sass, R. L., 2005. Effects of copper concentration on methane emission from rice soils. Chemosphere 58(2), 185-193.

Jones, J. B., Case, V. W., 1990. Sampling, handling and analyzing plant tissue samples, in: Westerman, R.L. (Ed.), Soil Testing and Plant Analysis, third ed., Soil Science Society of America, Book Series No. 3, Madison, Wisconsin, pp. 389–427.

Jouyban, Z., 2012. The effects of salt stress on plant growth. Technical Journal of Engineering and Applied Science 2 (1), 7-10.

Kader, M.A., Lindberg, S., 2008. Cellular traits for sodium tolerance in rice (*Oryza sativa* L.). Plant Biotechnology 25, 247-255.

Katznelson H., Rouatt, J. W., Payne, T. M. B., 1954. Liberation of Amino-acids by Plant Roots in relation to Desiccation. Nature 174, 1110-1111.

Kavitha, P. G., Miller, A. J., Mathew, M. K., Maathuis, F. J. M., 2012. Rice cultivars with differing salt tolerance contain similar cation channels in their root cells. Journal of Experimental Botany. doi:10.1093/jxb/ers052 .

Kerdchoechuen, O., 2005. Methane emission in four rice varieties as related to sugars and organic acids of roots and root exudates and biomass yield. Agriculture, Ecosystem and Environment 108 (2), 155-163.

Khosa, K.M., Sidhu, B.S., Benbi, D.K., 2011. Methane emission from rice fields in relation to management of irrigation water. Journal of Environment and Biology 32, 169-172.

Kosová, K., Prási I. T., Vítámvás, P., 2013. Protein Contribution to Plant Salinity Response and Tolerance Acquisition. International Journal of Molecular Sciences 14, 6757-6789.

Kumar, R., Pandey, S., and Pandey, A., 2006. Plant roots and carbon sequestration. Current Science 91(7), 885-890.

Kuzyakov, Y., Kretzschmar, A. and Stahr, K., 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil.Plant and Soil231, 127–136.

Kyuma, K., 2004. Paddy Soil Science. Kyoto University Press, Kyoto, Japan.

Lesuffleur, F., Paynel, F., Bataille, M.P., Le Deunff, E., Dliquet, J. B., 2007. Root amino acid exudation: measurement of high efflux rates of glycine and serine from six different plant species. Plant and Soil 294, 235-246.

Liesack, W., Schnell S., Revsbech, N.P., 2000. Microbiology of flooded rice paddies. FEMS Microbiological Reviews 24, 625–645.

Lilia, C. C., Dennis, P. G., Fedoseyenko, D., Hajirezae, M., Borriss, R., Wiren, N. V., 2012. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. Journal of Plant Nutrition and Soil Science 174, 3-11.

Lindau, C. W., Bollich, P. K., DeLaune, R. D., Patrick Jr., W. H., Law, V. J., 1991. Effect of urea fertilizer and environmental factors on CH_4 emissions from a Louisiana, USA, rice field. Plant and Soil 136, 195–203.

Lindau, C.W., Bollich, P.K., Delaune, R.D., Mosier, A.R., Bronson, K.F., 1993. Methane mitigation in flooded Louisiana rice fields. Biology and Fertility of Soils 15 (3), 174–178.

López-Bucio J., Nieto-Jacobo, M.F., Ramirez-Rodriguez, V., Herrera-Estrella, L., 2000. Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Science 160 (1), 1-13.

Lu Y., Wassmann, R., Neue, H.U., Huang, C., 1999. Impact of phosphorus supply on root exudation, aerenchyma formation and CH_4 emission of rice plants. Biogeochemistry 47, 203-218.

Ma, J.F., Furukawa, J., 2003. Recent progress in the research of external Al detoxification in higher plants, a mini review. Journal of Inorganic Biochemistry 97, 46-51.

Maas, E. V., and Hoffman, G. J., 1977. Crop salt tolerance\-current assessment. Journal of the Irrigation and Drainage Division 103(2), 115-134.

Makoi, J. H. J. R., Verplancke, H., 2010. Effect of gypsum placement on the physical properties of a saline sandy loam soil. Australian Journal of Crop Science 4, 556–563.

Mahmood, I.A., Salim, M., Ali, A., Arshadullah, M., Zaman, B., Mir, A., 2009. Impact of calcium sulphate and calcium carbide on nitrogen use efficiency of wheat in normal and saline sodic soils. Soil and Environment 28 (1), 29-37.

Mathad, P. and Hiremath, S., 2010. Alleviation of Saline Stress by Gypsum in *Chlorella vulgaris* BEIJERINCK. Journal of Algal Biomass Utilization (3), 43 – 53.

Mimmo, T., Hann, S., Jaitz, L., Cesco, S., Gessa, C. E., Puschenreiter, M., 2011. Time and substrate dependent exudation of carboxylates by *Lupinus albus* L. and *Brassica napus* L. Plant Physiology and Biochemistry 49 (2011) 1272-1278.

Minoda, Y., Kimura, M., 1994. Contribution of photosynthesized carbon to the methane emitted from paddy fields. Geophysical Research Letters 21, 2007–2010.

Mishra, S.R., Pattnaik, P., Sethunathan, N., Adhya, T.K., 2003. Anion-mediated salinity affecting methane production in a flooded alluvial soil. Geomicrobiology Journal 20, 579 - 586.

Mishra, B., 2004. Present status issues and future strategies for increasing quality rice production and export. In: National Symposium on strategies for enhancing export of quality rice held at NBPGR, New Delhi, pp1-16.

Mitchell, J.P., Shennan, C., Singer, M.J., Peters, D.W., Miller, R.O., Prichard, T., Grattan, S.R., Rhoades, J.D., May, D.M., Munk, D.S. 2000. Impacts of gypsum and winter cover crops on soil physical properties and crop productivity when irrigated with saline water. Agricultural Water Management 45, 55–71.

Muhammad, D., Khattak, R. A. 2011. Wheat yield and chemical composition as influenced by integrated use of gypsum, pressmud and FYM in saline-sodic soil. Journal of the Chemical Society of Pakistan 33, 82–86.

Munns, R., 2005. Genes and salt tolerance: bringing them together. New Phytologist 167, 645-665.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59, 651–681.

Naeem, M.A., and Qureshi, R.H., 2005. Rice growth and ionic composition under saline hydroponic conditions: I. Supplemented with Ca^{2+}: Na^{+} Ratios. Pakistan Journal of Agricultural Science 42, 1-2.

Nakic´enovic´, N., Davidson, O., Davis, G., Gru¨ bler, A., Kram, T., La Rovere, E. L., Metz, B., Morita, T., Pepper, W., Pitcher, H., Sankovski, A., Shukla, P., Swart, R., Watson, R., and Dadi, Z., 2000. Special report Mechanisms of crop management impact on methane emissions on emissions scenarios: A special report of Working Group III of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Neue, H.U., Sass, R.L., 1994. Trace gas emission from rice fields. IG Activities 17, 3–11.

Neumann, G., Massonneau, A., Martinoia, E., Römheld, V., 1999. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. Planta 208, 373-382.

Neumann, G., and Römheld,V., 2002. Root-induced changes in the availability of nutrients in the rhizosphere, in: Waisel, Y., Eshel, A., Kafkafi, U., (Eds), Plant roots the hidden half, third Ed. NY: Marcel Dekker, Newyork, pp. 617–649.

Neumann, G., Bott, S., Ohler, M., Mock, H. P., Lippmann, R., Grosch, R., Smalla, K., 2014. Root exudation and root development of lettuce (Lactucasativa L. cv. Tizian) as affected by different soils. Frontiers in Microbiology 4 (2) 1, doi: 10.3389/fmicb.2014.00002

Neves-Piestun, B.G., and Bernstein, N., 2001. Salinity-induced inhibition of leaf elongation in maize is not mediated by changes in cell wall acidification capacity. Plant Physiology 125 (3), 1419-1428.

Nouchi, I., Hosono, T., Aoki, K., Minami, K., 1994. Seasonal variation in methane flux from rice paddies associated with methane flux from rice paddies associated with methane concentration in soil-water, rice biomass and temperature, and its modelling. Plant and Soil 161,195-208.

Pangala, S.R., Reay, D.S., and Heal, K.V., 2010. Mitigation of methane emissions from constructed farm wetlands. Chemosphere 78 (5), 493-499.

Pattnaik, P., Mishra, S.R., Bharati, K., Mohanty, S.R., Sethunathan, N., Adhya, T.K., 2000. Influence of salinity on methanogenesis and associated microflora in tropical rice soils. Microbiological Research. 155, 215–220.

Purkait, N.N., Saha, A.K., and Sanghamitra, D., 2006. Behavior of methane emission from a paddy field of high carbon content. Indian Journal of Radio and Space Physics. 36, 52-58.

Pusatjapong, W., Kerdchoechuen, O., Towprayoon, S., 2003. Sugars and organic acids of root and root exudates from 4 rice varieties. Proceedings of the 2nd Regional Conference on Energy Technology towards a Clean Environment, 12th – 14th February, 200, Phuket, Thailand.

Qadir, M., Oster, J. D., Schubert, S., Noble, A.D., Sahrawat, K.L., 2007. Phytoremediation of sodic and saline-sodic soils. Advanced Agronomy 96, 197-247.

Rad, H. E., Aref, F., Rezaei, M., 2012. Response of rice to different salinity levels during different growth stages. Research Journal of Applied Sciences, Engineering and Technology 4 (17), 3040-3047.

Ramakrishnan, B., Kumaraswamy, S., Mallick, K., Adhya, T.K., Rao, V.R., Sethunathan, N., 1998. Effect of various anionic species on net methane production in flooded rice soils. World Journal of Microbiology and Biotechnology 14, 743-749.

Redfern, S.K., Azzul, N., and Binamira, J.S., 2012. Rice in Southeast Asia: facing risks and vulnerabilities to respond to climate change. In Building resilience for adaptation to climate change in the agriculture sector. Rome: Food and Agriculture Organization.

Richard, L., 1954. Diagnosis and Improvement of Saline and Alkali Soils. Agriculture handbook No.60. United States Department of Agriculture, Washington.

Rothfuss, F., Conrad, R., 1993. Vertical profiles of CH_4 concentrations, dissolved substrates and processes involved in CH_4 production in a flooded Italian rice field. Biogeochemistry18 (3), 137–152.

Schütz, H.,Holzapfel-Pschorn, A.,R. Conrad, R., Rennenberg, H.,*and*Seiler, W.,1989. A 3-year continuous record on the influence of daytime, season, and fertilizer treatment on methane emission rates from an Italian rice paddy. Journal of Geophysical Research 94 (13), 16405–16416.

Setyanto, P., Rosenani, A. B., Makarim, A. K., Fauziah, C., Bidin, A., and Suharsih, 2002. Soil controlling factors of methane gas production from flooded rice fields in pati district, central Java. Indonesian Journal of Agricultural Science 3 (1), 1-11.

Shaaban, M., Abid, M., Abou-Shanab, R. A. I., 2013. Amelioration of salt affected soils in rice paddy system by application of organic and inorganic amendments. Plant, Soil and Environment 59 (5), 227-233.

Sharma, B. R., Minhas, P.S. 2005. Strategies for managing saline/alkali waters for sustainable agricultural production in South Asia. Agricultural Water Management 78,136–151.

Shereen, A., S. Mumtaz, S. Raza, M.A. Khan and S. Solangi, 2005. Salinity effects on seedling growth and yield components of different inbred rice line. Pakistan Journal of Botany 37, 131-139.

Shi, S., Richardson, A. E., O'Callaghan, M., DeAngelis, K. M., Jones, E. E., Stewart, A., Firestone, M. K. and Condron, L. M., 2011. Effects of selected root exudate components on soil bacterial communities. FEMS Microbiology Ecology 77: 600–610.

Song, J.Q., and Fujiyama, H., 1996. Difference in response of rice and tomato subjected to sodium salinization to the addition of Calcium. Soil Science and Plant Nutrition 42 (3), 503-510.

Sun, Q., Ni, W.Z,, Yang, X.E., 2002. Role of organic acid in detoxification of aluminum in higher plants and its mechanisms. Chinese Bull. Bot. 19, 496-503.

Supparattanapan, S., Saenjan, P., Quantin, C., Maeght, J.L., and Grünberger, 2009. Salinity and organic amendment effects on methane emission from a rain-fed saline paddy field. Soil Science and Plant Nutrition 55, 142–149.

Tanaka, H., Nakamura, Y., Motobayashi, T., 2008. Detailed soil map of paddy fields located in field museum honmachi, tokyo university of agriculture and technology. Journal of Field Science 7, 1-10.

Tavakkoli, E., Rengasamy, P., MacDonald, G.K., 2010. High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of Faber bean under salinity stress. Journal of Experimental Botany 61 (15), 4449-4459.

Tejada, M., Garcia, C., Gonzalez, J.L., Hernandez, M.T., 2006. Use of organic amendment as a strategy for saline soil remediation: Influence on the physical, chemical and biological properties of soil. Soil Biology and Biochemistry 38, 1413–1421.

Tester, M., Davenport, R., 2003. Na^+ tolerant and Na^+ transport in higher plants. Annals of Botany 91, 503-527.

Ullah W., Bhatti A., 2007. Physico-chemical properties of soils of Kohat and Bannu districts NWFP Pakistan. Journal of the Chemical Society of Pakistan, 29, 20–25.

US EPA, 2006. Global anthropogenic non-CO_2 greenhouse gas emissions: 1990-2020. Office of Atmospheric Programs, Climate Change Division, U.S. Environmental Protection Agency, Washington.

Van der Gon, D.H.A., and Neue, H.U., 1995. Methane emission from a wetland rice field as affected by salinity. Plant and Soil 170, 307–313.

Van der Gon, D.H.A., and Neue, H.U., 1994. Impact of gypsum application on the methane emission from a wetland rice field. Global Biogeochemical Cycles 8 (2), 127–134.

Van Hees P. A. W., Dehl én, J., Lundström U. S., Borén, H., Allard, B., 1999. Determination of low molecular weight organic acids in soil solution by HPLC. Talanta 48, 173-179.

Van Hulzen, J.B., Segers, R, Van Bodegom, P.M., Leflelaar, P.A., 1999. Temperature effect on soil methane production: An explanation for observed variability. Soil Biology and Biochemistry 31, 1919-1929.

Van der Gon, D.H.A., Bodegom, P.M., Wassman, R., Lantin, S.R., and Metra-corton, M.T., 2001. Sulfate-containing amendment to reduce methane emissions from rice fields: mechanisms, effectiveness and cost. Mitigation and Adaptation Strategies for Global Climate Change 6 (1), 71-89.

Vinod, K. K., Krishnan, S.G., Babu, N.N., Nagarajan, M., Singh, A.K., 2013. Improving salt tolerance in rice: Looking beyond the conventional. In: Azooz, M.M., Prasad, M. N. V. (eds) Ahmad, P., Salt Stress in Plants, Springer New York. pp 219-260.

Walker, T. S., Bais, H. P., Grotewold, E., Vivanco, J. M., 2014. Root Exudation and Rhizosphere Biology. Plant Physiology 132 (1), 44-51.

Wahhab A., 1961. Salt tolerance of various varieties of agricultural crops at the germination stage. In: 'Salinity Problems in the Arid Zone. (Proc. Teheran Symposium on Arid Zone Research, 14'. UNESCO. pp. 185-192.

Wassmann, R., Schütz, P. H., Rennenberg, H., Seiler, W., Dai, A.G., Shen, R.X., Shangguan, X.J., Wang, M.X., 1993. Quantification of methane emissions from Chinese rice fields (Zhejiang Province). Biogeochemistry 20, 83-101.

Wassmann, R., and Aulakh, M. S. 2000. The role of rice plants in regulating mechanism of methane emission. Biology and Fertility of Soils 31, 20-29.

Win, K. T., Nonaka, R., Win, A. T., Sasada, Y., Toyota, K., Motobayashi, T., Hosomi, M., 2012. Comparison of methanotrophic bacteria, methane oxidation activity, and methane emission in rice fields fertilized with anaerobically digested slurry between a fodder and a normal rice variety. Paddy and Water Environment 10 (4), 281-289.

Wong, V. N. L., Dalal, R. C., Greene, R. S. B., 2009. Carbon dynamics of sodic and saline soil following gypsum and organic material additions: A laboratory incubation. Applied Soil Ecology 41, 29–40.

Wu, G.Q., and Wang, S.M., 2012. Calcium regulates K^+/Na^+ homeostasis in rice (*Oryza sativa* L.) under saline conditions. Plant, Soil and Environment 58 (3), 121–127.

Xiong, Z.Q., Xing, G.X., and Zhu, Z.L., 2007. Nitrous oxide and methane emissions as affected by water, soil and nitrogen. Pedosphere17(2),146-155.

Yamane I., and Sato K., 1961. Effect of temperature on the formation of gases and ammonium nitrogen in the water-logged soils. Science Reports of the Research Institutes, Tohoku University D (Agr.) 12, 31-46.

Yan, X., Yagi, K., Akiyama, H. and Akimoto, H., 2005. Statistical analysis of the major variables controlling methane emission from rice fields. Global Change Biology 11, 1131–1141.

Zeng, L., Shannon, M.C. 2000. Salinity effects on seedling growth and yield components of rice. Crop Science 40, 996–1003.

Zou, J., Huang, Y., Jiang, J., Zheng, X. and Sass, R.L. 2005. A 3-Year field measurement of methane and nitrous oxide emissions from rice paddies in China: Effects of water regime, crop residue and fertilizer application. Global Biogeochemical Cycles 19(2), DOI: 10.1029/2004GB002401.