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Influence of different rates of gypsum application on methane emission from saline soil related with rice growth and rhizosphere exudation

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ABSTRACT

The effect of different rate of gypsum fertilizer addition on rice plant performance and methane (CH_4) emission was evaluated for saline paddy rice soil by a pot experiment for 30 days. There were four treatments; control, gypsum 0.5 (G0.5), 1 (G1), and 2 (G2) ton ha^{-1} with 3 replications. Gypsum application led to a significant decrease in pH and an increase in EC. Although no significant improvement in rice growth was observed between control and gypsum fertilizer treatments, the addition of gypsum fertilizers significantly improved the potassium ion concentration except G2 and significantly decreased the sodium ion concentration of plants. The addition of gypsum G0.5 and G1 resulted in higher concentration and larger species of organic acids of rice rhizosphere exudates. Furthermore, the addition gypsum fertilizer G0.5 and G1 enhanced CH_4 emission compared to control while lowest CH_4 emission was observed in G2. The highest CH_4 emission in G0.5 might be due to the highest availability of organic carbon which was contributed from the rhizosphere exudates of rice plants. The lowest CH_4 emission in G2 might be due to its lower above dry matter yield, lowest pH value, and excessive sulfate (SO_4^{2-}) concentration in the soil.

1. Introduction

Saline soils are widely distributed around the world. According to the data of Mishra (2004), about 30% of world total rice soils contain too high level of salts for normal rice growth. This high level of salts in paddy rice fields influences plant metabolism directly or indirectly, there by affecting plant growth. Reduction in growth and photosynthesis are among the most conspicuous effects of salinity stress (Pattanagul and Thitisaksakul, 2008). During vegetative growth, cereal plants discharge about 5 to 21% all photosynthetically fixed C transferred to the rhizosphere as root exudates, which can range from 20 to 50% of plant biomass (Kumar et al., 2006). The root exudates are important C source for methanogenic bacteria to produce CH_4 in flooded rice soils (Lu et al., 2000). Thus, saline condition may directly affect rice growth which serve as major CH_4 transporter and also indirectly affect root or rhizosphere exudation which contributes C substrates for CH_4 production.

On the other hands, salinity is suggested as one of the soil factors to influence methane (CH_4) emission by affecting soil microbial activity including methanogenesis (Pattnaik et al., 2000). However, in laboratory incubation study of Ramakrishnan et al. (1998), the addition of 27 mM NaCl to alluvial soil caused an almost two-fold increase in CH_4 production relative to the control, and higher addition of NaCl resulted in an approximate 50% reduction of CH_4 production. Supparattanapan et al. (2009) also studied CH_4 emission in coastal saline rice fields and observed that CH_4 emission in no organic matter treatment did not differ significantly between inside saline patch and outside saline patch. In the experiment, the inside saline patch and outside saline patch were defined based on the previous soil data of Grünberger et al. (2005) which indicated that inside saline patch, the electrical conductivity of soil solution is 10 dSm^{-1} that is sufficient to decrease rice yield; in contrast with outside saline patch the electrical conductivity of soil solution was suitable for rice production. Thus, CH_4 emission mechanism in saline condition is not clearly understood.

To improve the ion imbalance for rice plants under salinity stress, gypsum is typically used as a source of calcium ion (Ca^{2+}) to remove the exchangeable sodium (Shaaban et al., 2013). The gypsum fertilizer ($CaSO_4 \cdot 2H_2O$) contains 23.8% Ca^{2+} and 18% S (Table 1). Muhammad and Khattak (2011) stated that the application of Ca^{2+} amendment can act as soil modifiers that would prevent the development of sodicity which is directly related to plant growth, crop productivity and crop yields. Besides this, regarding with the CH_4 emission, sulfate (SO_4^{2-}) containing fertilizers including gypsum fertilizer is frequently suggested as CH_4 mitigation option. Nevertheless, there are still contradicting

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Table 1
Chemical composition of gypsum.

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

results in rice fields fertilized with SO_4^2 containing fertilizers such as (NH_4 (SO_4))$_2$, $CaSO_4 \cdot 2H_2O$, etc. CH_4 emission either increased (Cicerone and Shetter, 1981), stayed constant (Wassmann et al., 1993) or decreased (Schütz et al., 1989; Van der Gon and Neue, 1994; Lindau et al., 1993). These contradicting results may be due to differences in carbon substrate and $SO_4^{2\,-}$ availability at various field sites. As CH_4 emission is a net result of soil and plant interaction, the addition of Ca^{2+} fertilizer may not only affect soil chemical properties but also affect rice growth and plant performance and these may in turn affect CH_4 emission.

Until now, there was little information about CH_4 emission related with rice growth and C contribution from rhizosphere exudates under saline condition with the addition of different rates of gypsum fertilizers. Therefore, the objective of this study was to evaluate the effect of salinity and common amelioration practice for saline soils like gypsum fertilizer addition to saline soil on CH_4 emission related with rice growth and its performance especially rhizosphere exudation.

2. Materials and methods

2.1. Experimental site

The pot experiment was conducted inside a phytotron at Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan. According to the critical temperature for rice crop during vegetative growth (Yoshida, 1978), the day and night temperature inside the Phytotron was maintained as 25 °C and 30 °C respectively.

2.2. Preparation of soil and cultivation of rice

The saline soils used in this experiment were collected from the upper 5 cm depth of tsunami-flooded paddy field in Sendai, in Japan (38.23°N latitude and 140.96°E longitude). The physico-chemical properties of this experimental soil have loam in soil texture, 6.6 in pH, 5.87 dSm^{-1} in EC, 23.51 meq 100 g $soil^{-1}$ in CEC, 4.3 meq 100 g $soil^{-1}$ in exchangeable sodium, 0.8 meq 100 g $soil^{-1}$ in exchangeable calcium, 5.6 g kg^{-1} in total nitrogen and 51.6 g kg^{-1} in total carbon. A salt tolerant Indica rice variety, Dorfak cultivar was used in this experiment.

About 3.5 kg of soil was placed into Wagner pots with the area of 0.02 m^2(inside dimension: 159 mm, height: 250 mm). 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. 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 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.

2.3. 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 day interval. At the end of experiment, shoot weight, root weight, soil pH and soil electrical conductivity value (EC) were recorded. The Na^+ and K^+ ion concentration in plants were analyzed at harvest. The rhizosphere exudates was collected at immediately after harvest and the root length and total numbers of root tips were also measured from each sample. Total carbon contribution from rhizosphere exudates in each treatment was estimated by multiplying total numbers of root tip and total carbon contributed from organic acids of rhizosphere exudates.

2.3.1. 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 pot 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 analyze 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 was 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.

2.3.2. The method of rhizosphere exudates collection and analysis

At the end of experiment, the rhizosphere 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. 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. Subsequently, ten not damaged root tips were selected to collect the rhizosphere 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 min, the total 20 pieces of filter papers were put into centrifugation tube fitted with 0.45 μm filter paper (Centricut ultra-mini W-50, 50,000 MW, Kurabo, Japan). About (0.2 mL) of distilled water was added to the ultracentrifugation tube and the diluted rhizosphere exudates were centrifuged for 10 min at 12,000 rpm. The centrifuge tubes containing the filtrate were kept in the freezer until analyzing the organic acids. 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-10 AD (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 0.8 mL 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 porpionic 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 3 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.

$$F = 100^{*}(D - B)/A \tag{1}$$

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

2.3.3. Root tips and length measurement

Immediately after collecting the rhizosphere exudates, the roots parts of rice plants were cut and kept in 70% ethanol solution until before root tips and length measurement. The roots were scanned by using an Epson perfection V700 photo scanner (Epson, Nagano, Japan), and the images *were* analyzed by *WinRHIZO Pro* v.2009c software. Before analyzing the root tips and length, the roots were cut right below the crown section of the rice plants and divided into three equal volumes to facilitate to scan all of the roots.

2.3.4. 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 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 was then taken with the 50-mL plastic syringe, adjusted to 40 mL and then transferred into a 20-mL pre-vacuumed glass vial.

The cumulative amount of CH_4 gas measured for 30 days 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 difference (LSD) at $p = 0.05$.

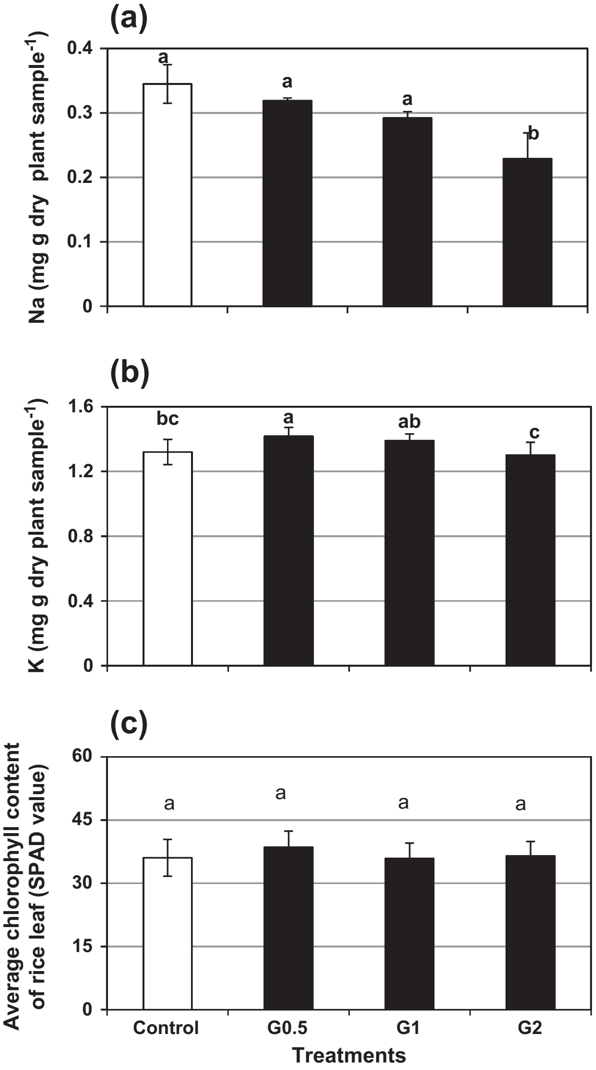

Fig. 1. Effect of different rates of gypsum fertilizer addition on (a) Na^+, (b) K^+ concentration in plant samples and (c) average chlorophyll content of leaf. Means followed by a common letter are not significantly different by using least significant differences (LSD) at $p = 0.05$. Error bars indicate standard deviation, $n = 3$.

Table 2
The effect of different rates of gypsum fertilizer addition on soil environment.

Treatments	Average NH_4^+-N ($mg\ N\ L^{-1}$)	Average NO_3^--N ($mg\ N\ L^{-1}$)	TOC ($mg\ C\ L^{-1}$) 20 DAT	TOC ($mg\ C\ L^{-1}$) 30 DAT	Soil Eh	Soil pH	Soil EC (dSm^{-1})
Control	3.9 ± 0.3a	0.6 ± 0.2a	6.8 ± 2.0a	10.6 ± 2.7b	−385 ± 26a	6.5 ± 0.1a	4.9 ± 0.7b
G0.5	3.8 ± 0.2a	0.8 ± 0.1a	8.6 ± 0.8a	26.2 ± 11a	−356 ± 63a	6.0 ± 0.1b	6.3 ± 1ab
G1	2.8 ± 0.2a	1.0 ± 0.2a	4.3 ± 1.0b	17.2 ± 2 ab	−425 ± 26a	5.9 ± 0.0bc	7.5 ± 0.2a
G2	4.5 ± 0.2a	0.7 ± 0.3a	8.1 ± 8.1a	24.9 ± 6 a	−451 ± 14a	5.8 ± 0.0c	7.7 ± 0.3a

ANOVA test for all of these above soil environment data

Source of variation	Average NH_4^+-N ($mg\ N\ L^{-1}$)	Average NO_3^--N ($mg\ N\ L^{-1}$)	TOC at 20DAT ($mg\ C\ L^{-1}$)	TOC at 30DAT ($mg\ L^{-1}$)	Soil Eh	Soil pH	Soil EC (dSm^{-1})
Treatments	ns	ns	**	*	ns	**	**

Values are the means ± standard deviation ($n = 3$ replications). In each column, means followed by a common letter are not statistically significantly different by using least significant difference (LSD) at $p = 0.05$. **, * and ns stand for significant at 1%, 5% and non-significant respectively.

3. Results

3.1. Effect of gypsum fertilizer addition upon soil environment

The range of average NH_4^+-N concentration was 0.02 to 2.04 mg N L^{-1}, that of NO_3^--N concentration 1.52 to 5.18 mg N L^{-1} (Table 2). Total organic carbon concentration of flooded water in all treatments becomes higher at 30 days after transplanting (Table 2). 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 and −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 (Table 2). The soil EC values were observed as 4.9, 6.5, 7.5 and 7.7 in control, G0.5, G1 and G2, respectively (Table 2).

3.2. Effect of gypsum fertilizer addition upon plant performance

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

The range of shoot dry weight was observed from 5.4 to 6.4 g pot^{-1} (Fig. 2a). The range of root dry weight was observed from 1.4 to 1.7 g pot^{-1} (Fig. 2b). Although the root 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 were significantly higher that of other treatments (Fig. 2c and d).

Six organic acids species, citric, tartaric, malic, lactic, formic and acetic acids were observed in the rhizosphere exudates of control, G0.5, and G1. Only four organic acids species, citric, tartaric, malic, and Lactic were observed in G2 (Fig. 3). The total carbon contribution from organic acids was significantly higher in G0.5 compared with that in control, G1 and G2 (Fig. 4).

3.3. Effect of gypsum fertilizer addition upon CH_4 emission

The CH_4 emission was monitored during vegetative stage of rice growth. The total CH_4 emission for 30 days ranged from −18 to 150 mg m^2 (Fig. 5). The significantly 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. Discussions

4.1. Ion concentration of plants and plant growth

The addition of gypsum as a source of Ca^{2+} decreases Na^+ ion concentrations in plants under saline condition (Fig. 1a), because addition of calcium protects the integrity and permeability of plasma membranes against Na^+ toxicity (Tester and Davenport, 2003). Moreover, the existence of high level Na^+ 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. 2a). No significant changes of chlorophyll content among all treatments indicated that available nitrogen contents (NH_4^+-N and NO_3^--N) are not limited for plant growth (Fig. 1c). Thus, no improvement of rice growth under gypsum fertilizer addition was due to the higher EC values under gypsum fertilizer addition. The value of sodium adsorption ratio (SAR) of experimental soil was 16.1 (Section 2.2) which was higher than 13, a limit for saline-sodic or sodic soil described by US Salinity

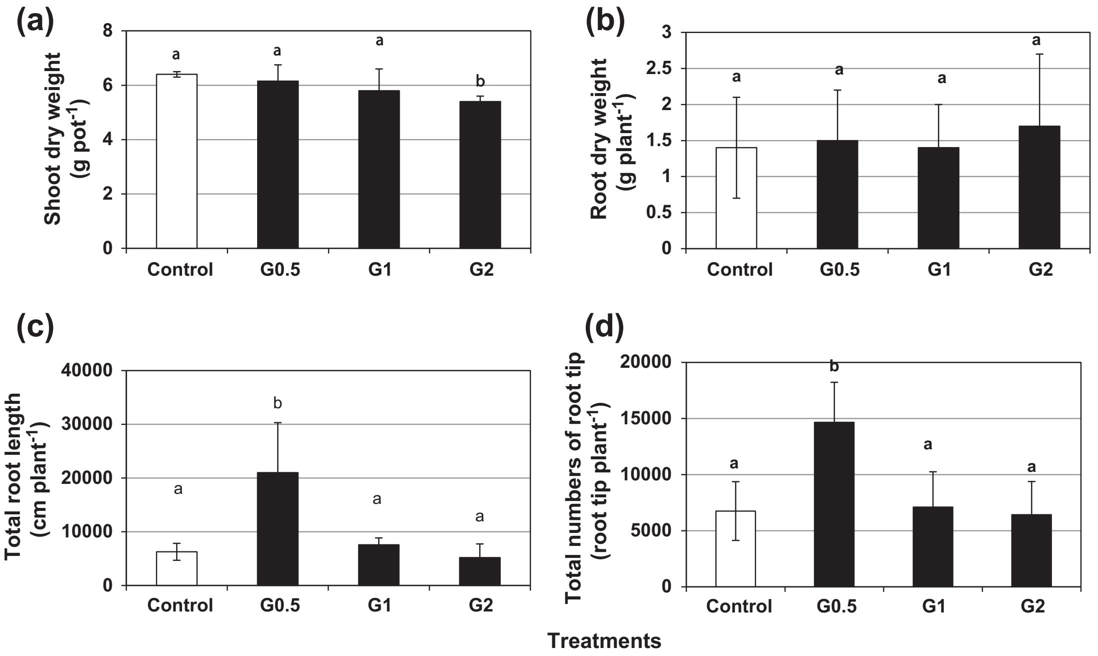

Fig. 2. Effect of different rates of gypsum fertilizer addition on (a) shoot dry weight, (b) root dry weight, (c) total root length and (d) total numbers of root tips. Means followed by a common letter are not significantly different by using least significant differences (LSD) at $p = 0.05$. Error bars indicate standard deviation, $n = 3$.

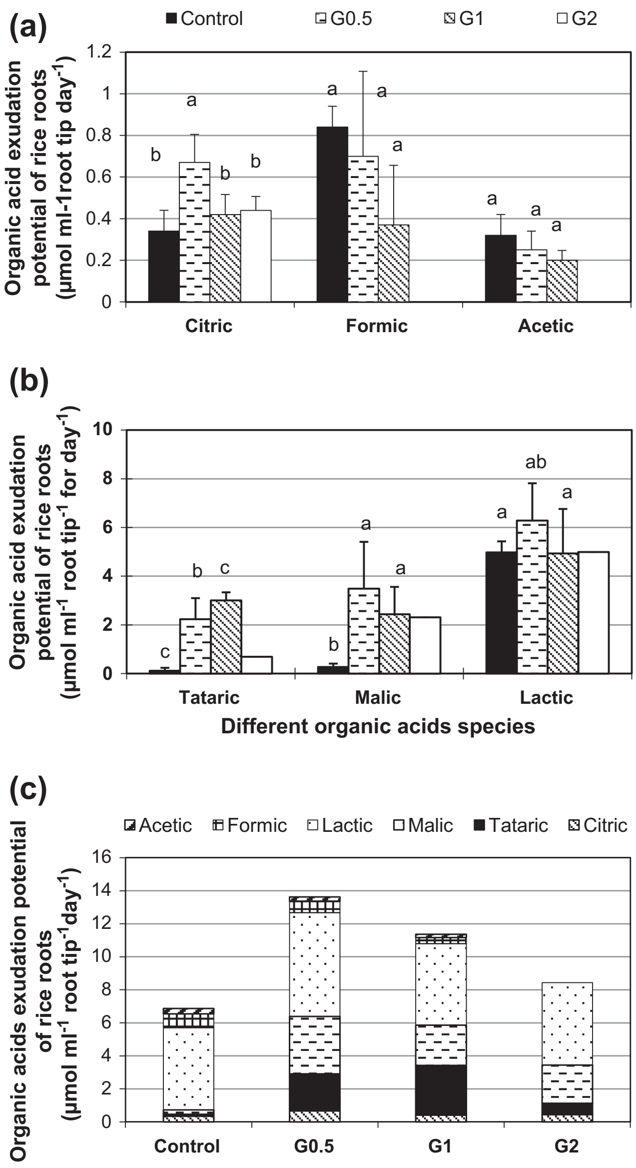

Fig. 3. Effect of different rates of gypsum fertilizer addition on organic acids exudation potential of rice; (a) citric, formic and acetic acid concentration of rhizosphere exudates, (b) tartaric, malic and lactic acid concentration, and (c) the total root amount of rhizosphere exudation per root tip per day. Means followed by a common letter within each acids are not significantly different by using least significant difference (LSD) at $p = 0.05$. Error bars indicate standard deviation, $n = 3$.

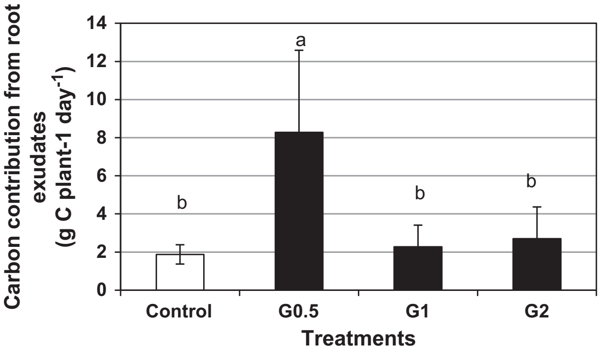

Fig. 4. Effect of different rates of gypsum fertilizer addition on carbon contribution from rhizosphere exudates. Means followed by a common letter are not significantly different by using least significant difference (LSD) at $p = 0.05$. Error bars indicate standard deviation, $n = 3$.

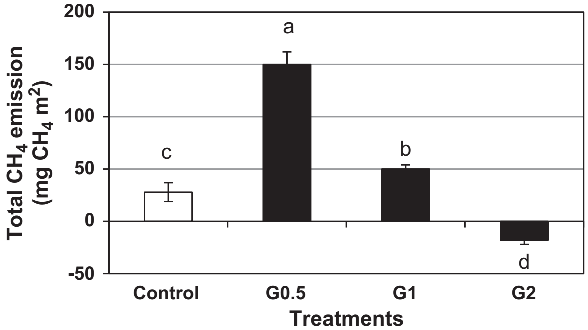

Fig. 5. Effect of different rates of gypsum fertilizer addition on total methane emission for 30 days. Means followed by a common letter are not significantly different by using least significant differences (LSD) at $p = 0.05$. Error bars indicate standard deviation, $n = 3$.

Lab Staff, Agriculture hand book (Richard, 1954). Calcium concentration of experimental soil was about 0.80 meq 100 g $soil^{-1}$ (4.1 mM) (Section 2.2). The Ca^{2+} level should be maintained at 5–10 mM in the external solution of saline condition (Muhammad, 1998). 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 reached 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 gypsum fertilizer treatments might not exactly relate with ion toxicity and deficiency because of the lower Na^+ and higher K^+ concentration of plants as shown in Fig. 1a and b and NH_4^+-N and NO_3^--N of flooded water (Table 2). Furthermore, the higher values of root length and root tips in G0.5 and G1 might relate with the formation of fine roots under moderate water stress because root growth stimulation particularly of fine root structures is frequently induced by moderate limitations of P, N, Fe, and water (Neumann and Römheld, 2002). Therefore, no improvement of rice growth under gypsum treatments in this study might more relate with osmotic effect that may reduce the ability of plants to take up water (Rad et al., 2012).

4.2. Organic carbon contribution from rhizosphere exudates

The higher concentration of total organic acids exudation potential (Fig. 3c) and total carbon contribution of rhizosphere exudates in G0.5 and G1 compared to control might relate with higher EC values in G0.5 and G1 (Fig. 4). 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). López-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 aluminum stress exude higher concentration of malic acid in wheat crop (Kitagawa et al., 1986; Delhaize et al., 1993;

Table 3
Pearson product moment correlation analysis among measured parameters.

Parameters	pH	EC	Shoot dry weight (g pot^{-1})	Carbon contribution from root exudates (g C plant^{-1} day^{-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 sulfate added in each treatments	**−0.83***	**0.782****	**−0.704****	−0.139 ns	−0.0446 ns	0.463 ns	−0.346 ns
pH		**−0.815****	0.552 ns	−0.187 ns	0.14 ns	−0.549 ns	−0.155 ns
EC			**−0.39***	−0.0457 ns	−0.275 ns	0.324 ns	−0.072 ns
Shoot dry weight (g pot^{-1})				0.02 ns	−0.04 ns	**−0.655***	0.09 ns
Carbon contribution from root exudates (g C plant^{-1} day^{-1})					0.206 ns	0.152 ns	**0.723****
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 significant at 1%, 5% and non significant respectively.

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 (Table 2), 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 organic acids exudation under gypsum fertilizer addition may induce lower above dry matter weight in all gypsum fertilizer treatments compared to control. The lowest concentrations and less species of organic acid in rhizosphere exudates of G2 compared with G0.5 and G1 might also relate with its lower above dry matter yield because the significant reduction of above dry matter yield in G2 may reduce the extent of photosynthetic carbon which may result in less species and lower concentrations of organic acids in G2 (Table 3).

4.3. Effect of gypsum fertilizer addition upon CH_4 emission

The significantly highest CH_4 emission in G0.5 might mainly relate with the highest carbon availability which was contributed from

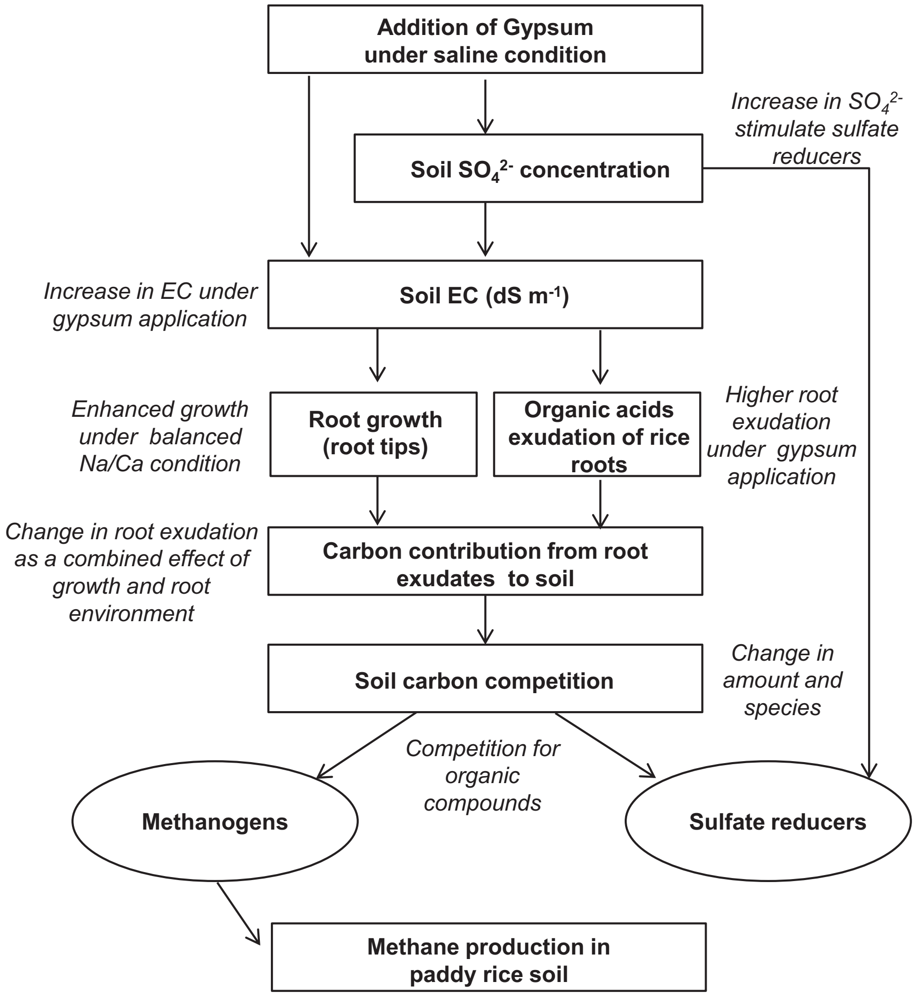

Fig. 6. A schematic diagram for the mechanism of CH_4 emission related with carbon and sulfate availability under different rates of gypsum fertilizer applications.

rhizosphere exudates of rice plants. In the presence of sulfate, sulfate reducers are stronger competitors than methanogens leading to a reduced production of CH_4 in the rice soil (Van der Gon and Neue, 1994). However, sulfate reducing bacteria do not fully outcompete the methanogens for substrate in the present study because of the highest carbon contribution from rhizosphere exudates in G0.5. Furthermore, methanogens and sulfate reducing bacteria can use more than one substrate and the affinities for these substrates differ (Van der Gon et al., 2001). Although the total carbon contribution from rhizosphere exudates were not statistically significantly different between control and G1, the higher total CH_4 emission was observed in G1 over that of control. According to the statement of Van der Gon et al. (2001), the significantly higher concentration of malic and tartaric acid in rhizosphereexudation potential of G1 (Fig. 3b) might favor CH_4 emission of G1 over that of 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), and highest input of SO_4 concentration added from gypsum fertilizer. Van der Gon and Neue (1994) also found the reduction effect of CH_4 emission in the paddy rice field due to the application of gypsum 6.66 ton ha^{-1}. Although carbon contribution from rhizosphere exudates among control, G1, and G2, was not statistically significant different, the amount of SO_4^{2-} added in G2 was the highest level which may probably encounter the high SO_4^{2-} to carbon ratio in the soil and leads to methane reduction as shown in Fig. 6. 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 content of the soil.

5. Conclusion

Our study showed that higher EC levels result in higher amount of organic acids exudation in the rhizosphere of rice plants. Higher amount of organic acids exudation in gypsum fertilizer treatments may be one of the carbon costs which reduce the rice growth and one of the carbon pools for methanogenic bacteria to produce more CH_4. Methane emission was closely related with the organic carbon contribution from rhizosphere exudates. At the same time, gypsum application was also a source for SO_4^{2-}. In the presence of SO_4^{2-}, sulfate reducing bacteria might compete carbon substrate with methanogens but sulfate reducers could not fully outcompete the carbon substrates which were provided by rice plants such as rhizosphere exudation. Thus, the present results of increased or decreased CH_4 emission due to gypsum fertilizer addition might more relate to the ratio of SO_4^{2-} and carbon availability of soils where rice plants are grown.

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