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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.

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## 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

**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.