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Mitigate CH_4 emission by suppressing methanogen activity in rice paddy soils using ethylenediaminetetraacetic acid (EDTA)

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ABSTRACT

Methane (CH_4) is the second most potent greenhouse gases after carbon dioxide. More than 90% of world rice is cultivated under submerged condition, which facilitates CH_4 production in soil. In this pot experiment, different doses of EDTA were applied in rice paddy soils to evaluate their effects on CH_4 emission and plant growth during rice cultivation. Application of EDTA at small doses (up to 5.0 ppm) significantly ($P < 0.05$) suppressed CH_4 emission without compromising rice grain yield. Higher doses (10.0 ppm) of EDTA application extended vegetative growth stage of rice plants, which not only reduced ripening percent of rice grains but also increased CH_4 emission (even more than control). Therefore, based on this pot experiment data it could be concluded that EDTA application at 5.0 ppm was probably the most rational treatment to mitigate CH_4 emission from rice paddy soils.

1. Introduction

Methane (CH_4) is considered as the second most potent greenhouse gas after carbon dioxide (CO_2). It has 25 times higher global warming potential (IPCC, 2007) and 20% higher radiative forcing (IPCC, 2001) as compared to CO_2. Approximately 1 billion tons of CH_4 is globally formed per year by methanogens. Therefore, about 1.5% of the total 70 Gt of CO_2, fixed annually into biomass by photosynthesis, is converted into CH_4 (Thauer, 1998). Methanogenesis is an enzyme-mediated multi-step process by methanogens. Though methanogens may differ in terms of their preference for initial substrates (formate, acetate and/or CO_2–H_2); all of them synthesize a common compound namely coenzyme M (Co-M: 2-mercaptoethane sulfonate) (Ferry and Kastead, 2007; Grahame and Gencic, 2000). In the penultimate step, methylated Co-M is reduced by methyl Co-M reductase (MCR) enzyme to CH_4 involving a nickel-containing cofactor F_{430} (Kaster et al., 2011). The activity of MCR enzyme is dependent on the F_{430} (Thauer et al., 2008) and therefore, the bioavailability of Ni to methanogens is expected to influence MCR activity and CH_4 production in soil. Pramanik and Kim (2013a) revealed that incubation of soil with EDTA under anaerobic condition significantly decreased Ni bioavailability, reduced Co-M concentration and mcrA gene (responsible for synthesizing MCR enzyme) copy numbers in methanogens and hence suppressed CH_4 production in soil.

Rice is one of the most staple foods in the world and is generally cultivated under flooded condition. Submerged rice paddy fields are recognized as an important source of CH_4 emission (Xiong et al., 2006). The hypothesis of this study was that EDTA application might be effective to mitigate CH_4 emission from rice paddy soils; however, its effect on soil nutrient status and rice plant growth was not known as it was the first attempt to use EDTA in rice paddy soil for mitigating CH_4 emission. In this experiment, three different doses of EDTA were applied in rice paddy soil under greenhouse condition and changes in CH_4 emission fluxes were correlated to the soil chemical and biochemical properties. The objective of this study was to evaluate the possibility of using EDTA for mitigating CH_4 emission from rice paddy soils.

2. Materials and methods

2.1. Experiment setup

The experiment was conducted in the greenhouse at agricultural field of Gyeongsang National University (36° 50′ N and 128° 26′E), Jinju, South Korea. The soil of this experiment was poorly drained with clay loam texture and was classified as andiaquands. Organic carbon (C) and nitrogen contents of initial soil were 11.79 ± 1.51 g kg^{-1} and 0.94 ± 0.09 g kg^{-1}, respectively with C/N ratio of 12.54 and soil pH was 6.32 ± 0.43 (soil: water = 1:5, w/v). That soil was first homogenized, air-dried and 13 kg of that soil was packed in pots having surface area equal to 1/2000 acre land. Pots were then flooded with water and allowed to stand for stabilization (filling up of capillary pores with water). After stabilization of soil conditions, fertilizers and EDTA were applied and 3 rice (Dongjinbyeo cultivar, Japonica type)

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seedlings (25 days old) were transplanted in each pot. The chemical properties of initial soil were presented in Table 1. Chemical fertilizers were applied in each pot (including control) at N–P_2O_5–K_2O = 1.12–1.63–0.56 g pot^{-1} following the recommended doses (N–P_2O_5–K_2O = 90–45–58 kg ha^{-1}) for Korean rice paddy soils. EDTA was applied at four doses, 0 ppm, 2.5 ppm, 5.0 ppm and 10.0 ppm (ppm amounts of EDTA were calculated considering the weight of pot soil). The 'bases' of cylindrical chambers were fixed in each pot and then the pots were arranged in the greenhouse following completely randomized design. Water management practices were also followed according to the recommendation for Korean rice paddy fields and levels of water in each pot were maintained at 5–7 cm above the soil surface. Gas samples, soil temperatures and E_h readings were collected after every 4 days, while soil samples were collected from each pot at 20 day intervals. After rice harvesting, above-ground portions were air-dried and grain and straw yields were recorded for all the treatments.

2.2. CH_4 gas sampling and analysis

A closed-chamber method (Ali et al., 2009) was used to estimate CH_4 flux from soil for the entire cultivation period. Cylindrical acryl chambers having diameter 21 cm and height 100 cm were placed on 'bases' of each pot during gas sample collections. The gas samples were collected by using 50 ml air-tight syringes at 0, 15 and 30 min after placing the chambers to check the linearity and daily gas sampling was carried out at 0800, 1200 and 1600 h to find the optimum time for gas sample collection. Based on these data, gas samples were collected initially and 30 min after closing the chamber within 1030 and 1200 h at 4 day intervals throughout the rice cultivation period.

Methane concentrations in the collected gas samples were measured by gas chromatography (Shimadzu, GC-2010, Japan) equipped with Porapak NQ column (Q 80–100 mesh) and a flame ionization detector (FID). The temperatures of column, injector and detector were adjusted at 100 °C, 200 °C, and 200 °C, respectively. Helium and H_2 were used as carrier and burning gases, respectively.

Methane emission from soil was calculated from the increase in CH_4 concentrations per unit surface area of the chamber within a specific time interval. A closed-chamber equation (Rolston, 1986) was used to estimate CH_4 fluxes from each treatment.

$$F = \rho \times (V/A) \times (\Delta c/\Delta t) \times (273/T)$$

where F was the CH_4 flux (mg CH_4 m^{-2} h^{-1}), ρ was the gas density (0.714 mg cm^{-3}), V was the volume of chamber (m^3), 'A' was the surface area of chamber (m^2), Δc/Δt was the rate of increase of CH_4 gas concentration in the chamber (mg m^{-3} h^{-1}) and T (absolute temperature) was calculated as 273 + mean temperature in (°C) of the chamber.

Total CH_4 flux for the entire cultivation period was computed following the equation proposed by Singh et al. (1999):

$$\text{Total } CH_4 \text{ flux} = \sum_i^n (R_i \times D_i)$$

where R_i was the CH_4 emission flux (g m^{-2} d^{-1}) in the *i*th sampling interval, D_i was the number of days in the *i*th sampling interval, and n was the number of sampling intervals.

2.3. Coenzyme M concentration in soil

Coenzyme M (Co-M), an intermediate compound of methanogenesis, was quantified as a biomarker of methanogens in soil (Pramanik and Kim, 2012). The fresh soil was homogenized with lysis buffer (100 mM Tris–HCl solution (pH 8.0), 100 mM EDTA solution (pH 8.0) and 1.5 M NaCl solution) (Soil: buffer = 1: 2, w/v basis) and sonicated for 2 min (1 minute sonication followed by 10 second vertex and then 1 minute sonication again). The soil suspension was centrifuged at 4000 rpm for 10 min. Required amount of ethanol was added to the supernatant to make it 80% ethanol solution. The solution mixture was allowed to stand for 2 h at 4 °C and centrifuged again at 4000 rpm for 10 min. The precipitate was dissolved in deionized water and diluted to the suitable volume for HPLC analysis using UV detector at 270 nm (Pramanik and Kim, 2012). The mixture of acetonitrile and 0.05 M trichloroacetic acid solution (30:70, v/v) was used as mobile phase for Co-M quantification.

2.4. Microbial biomass C in soil

Soil microbial biomass was estimated by the fumigation–extraction method of Vance et al. (1987). The fresh soil was fumigation with chloroform and C from fumigated (C_f) and non-fumigated (C_{nf}) soils were extracted by 0.5 M K_2SO_4 solution. The differences in these C contents give the measure of soil microbial biomass

$$MBC = (C_f - C_{nf})/K_c$$

where K_c, the correction factor, is equal to 0.45 for an agricultural soil (Wu et al., 1990).

2.5. Methanogen activity in soil

To measure methanogen activity, fresh soil (10 g) was incubated with 1 ml of 1% glucose solution and 25 ml deionized water under anaerobic condition at 30 °C for 5 h (Pramanik and Kim, 2013b). The methanogen activity was measured by estimating CH_4 concentration at the head-space of the bottles and the values were expressed as μg CH_4 produced g^{-1} soil h^{-1}.

Table 1
Soil nutrient status, rice grain yield and yield attributes as affected by different EDTA treatments.

Categories	Parameters	Control	2.5 ppm EDTA	5.0 ppm EDTA	10.0 ppm EDTA
Soil chemical properties	Soil organic C (%)	1.206 ± 0.273	1.086 ±0.174	1.288 ± 0.227	1.183 ± 0.325
	Total N (%)	0.071 ± 0.005	0.071 ± 0006	0.074 ± 0.003	0.069 ± 0.004
	Nitrate–N (mg kg^{-1})	16.94	15.38	17.43	17.25
	Available P_2O_5 (mg kg^{-1})	26.5 ± 3.6	32.1 ± 2.8	29.5 ± 3.1	31.4 ± 3.2
	Exchangeable K_2O (mg kg^{-1})	247.2 ± 31.6	264.0 ± 33.7	255.2 ± 42.8	230.9 ± 26.7
Rice yield	Grain yield (t ha^{-1})	1.80 ± 0.09	1.91 ± 0.12	2.06 ± 0.08	1.56 ± 0.12
	Straw yield (t ha^{-1})	2.98 ± 0.22	2.94 ± 0.06	2.63 ± 0.29	1.68 ± 0.20
	Harvest index	0.61	0.65	0.79	0.75
Yield attributes	Plant height (cm)	95.8 ± 3.3	101.0 ± 1.0	100.0 ± 3.5	87.7 ± 5.3
	Tiller numbers	24.3 ± 1.5	26.7 ± 1.5	27.3 ± 3.1	22.7 ± 1.2
	Number of grains $panicle^{-1}$	124.5 ± 39.1	113.1 ± 29.0	140.9 ± 40.2	99.2 ± 28.3
	1000 grain weight	22.22 ± 1.19	22.77 ± 0.61	22.07 ± 1.92	21.96 ± 1.59
	Ripening percent	82.1 ± 3.9	85.2 ± 1.8	86.7 ± 0.7	74.1 ± 2.6