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Impact of Aquatic Earthworms on Methane Emission Reduction from the Paddy Field Soil in Japan

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Abstract

Methane (CH_4) is one of the major greenhouse gases that significantly contributes to global warming. Therefore, substantial efforts are being made to reduce CH_4 emissions. Paddy fields make a major contribution to atmospheric CH_4 concentration because of their anoxic soil environment. Paddy field is habitat of many aquatic earthworms which can play a crucial role in reducing CH_4 emissions from paddy field, because their bioturbation activities influence the soil structure and increase oxygen penetration and hence the activity of methanotrophs. Therefore, it was hypothesized that aquatic earthworms may accelerate oxidation of CH_4 through their bioturbation activity. This incubation study evaluated the effects of earthworms on the activity of methanotrophs. Soil was incubated with a full factorial combination of two levels of aquatic earthworm (with and without earthworm) and two levels of fertilizer (with and without urea) for 35 days. The addition of urea increased the earthworm density in paddy soil by nearly doubled of without urea. At 28 days after incubation (DAI) the CH_4 flux decreased from 2055 mg m^{-2} h^{-1} in only urea received soil to 425 mg m^{-2} h^{-1} in soil received both urea and earthworm treatment. Phospholipid fatty acid analysis showed that the presence of aquatic earthworms contributed to an increase in the biomass of methanotrophs. The study implies that aquatic earthworms may play vital role to reduce CH_4 emission form paddy fields by creating favorable environment for methanotrophs, even in the soils fertilized with urea.

Keywords: bioturbation, *Branchiura sowerbyi*, urea, PLFA analysis, methanotrophs

1. Introduction

The Paris Agreement has set a target to limit global warming to well below 2 °C (UNFCCC, 2015). Diversified efforts and actions to reduce emissions of major greenhouse gases (GHGs) are critical to achieve this ambitious, but important target. Methane (CH_4) is one of the most potent GHGs as its contribution to the greenhouse effect is almost half of that of carbon dioxide (CO_2), even though the atmospheric concentration of CH_4 is 1.8 ppm, lower than that of CO_2 (399 ppm) (Gavin, 2004; Blasing, 2016). CH_4 has been recognized as the second most important GHG after CO_2, because of its higher global warming potential, which is 25 times higher than CO_2 (Shindell et al., 2009). A recent report showed, over the last 200 years, the atmospheric CH_4 concentration increased to double (Blasing, 2016). Wetland paddy fields significantly contribute to CH_4 emission, which is equivalent to 11% of total emission (Smith et al., 2007).

Rice is the staple food of more than half of the world's population, which is mainly grown in flooded field condition (Kögel-Knabner et al., 2010). Continuous flooding condition for over the rice cultivation period leads to the anoxic soil environment, which favors growth of unique microbial communities (Yao et al., 1999; Lüdemann et al., 2000; Kögel-Knabner et al., 2010). In absence of oxygen, biodegradation of organic matter results in the methanogenesis, which is the main terminal microbiological process in anoxic environment (Thauer, 1998).

The major methanogenic substrates are molecular hydrogen (H_2), CO_2, formate, acetate, methyl alcohol, and methylamine; the dominant reactions of methanogenesis in soil are the reduction of CO_2 using molecular H_2 and the transmethylation of acetate (Takai, 1970). During methanogenesis, carbon substrates are supplied to soil from soil organic matter, sloughed tissues of rice plants, applied organic matter, and root exudates (Watanabe et al., 1999; Kimura et al., 2004). Methanotrophs consume some of the CH_4 produced under oxidative conditions in the rhizosphere of rice plants and in the thin layer where the soil and surface water meet (Dubey, 2005). The rest of the produced CH_4 is released from paddy fields into the atmosphere via diffusion, ebullition from soil, and by diffusion and mass flow through the continuous intercellular gas space system between the rice rhizosphere and leaves. Inubushi et al. (1989) reported that 90% of total CH_4 from paddy field is released through rice plant and ebullition and diffusion from soil contributes 10% and < 1%, respectively. The physiological structure of rice plants favors greater emissions of CH_4. A path of gaseous exchange exists between the root zone and atmosphere; intercellular channels through the aerenchyma connect the rhizosphere to the leaves, where gases are released into the atmosphere via stomata in the leaves and micropores in the leaf sheaths. In submerged paddy fields, this allows the diffusion of CH_4 from the roots, to higher parts of the plant and then into the atmosphere (Nouchi et al., 1990).

Above discussions revealed that methanogenesis is the main process of CH_4 formation in the wetland rice field and dropping of oxygen supply is one of the main factors to stimulate this process. There are many management measures including change in tillage practice, modify irrigation and drainage practice, change of nutrient supply sources has been tested and found some potential measures to improve oxidation status of soil environment of wetland paddy field. For example, a single mid-season drainage results in significant reduction of CH_4 emissions (Kimura et al., 1992; Minamikawa et al., 2005). However, this management system increases emission risk of N_2O (Bronson et al., 1997; Chen et al., 1997; Suratno et al., 1998). In a study, Yagi and Minami (1990) reported that chemical fertilizers strongly affect CH_4 emissions from paddy field soils. Urea is the most widely used nitrogenous fertilizer for growing wetland rice because it is easy to handle and reasonably priced (Jones et al., 2013). In 1961, worldwide consumption of urea was 2 million tons, whereas in 1998, worldwide consumption of urea was 86 million tons (Soh, 2001). Use of huge amount of urea for growing rice may have impact on CH_4 emission from paddy fields. Number of studies has been conducted to evaluate urea's impact on CH_4 emission in paddy fields. However, the effects of urea on CH_4 emissions from soils were found to be context-dependent. In some studies, CH_4 emissions increased with increasing urea application rates because of increasing soil pH and ammonium inhibited the growth of methanotrophs (Wang et al., 1992; Dubey et al., 2003). In contrast, other study reported that ammonium enhanced oxidation of CH_4 and the activities of methanotrophs (Bodelier et al., 2000).

Aquatic earthworms are a major group of invertebrate fauna in paddy field ecosystems and maintain soil quality (Simpson et al., 1993). More specifically, aquatic earthworms in paddy soils improve soil health by stimulating the release of NH_4^+-N from the surface waters to the soils (Kikuchi & Kurihara, 1977, 1982; Fukuhara et al., 1980; Grant & Seegers, 1985) as well as PO_4^{3-} (Kikuchi & Kurihara, 1982). The earthworm gut and fresh cast of earthworms accelerate activity of methanogens (Depkat-Jakob et al., 2012). On the other hand, burrows made by earthworms may aerate the soil and stimulate methanotrophic activity (Kernecker et al., 2015). The bioturbation activity of aquatic earthworms affects microorganism community by enhancing oxidation (Yingst & Rhoads, 1980). Thus, aquatic earthworms may mitigate CH_4 emissions from flooded paddy soils through inhibiting methanogenesis process and stimulating growth of CH_4-consuming methanotrophs, a biological sink of CH_4. However, there has been no study conducted to examine the effect of aquatic earthworms in CH_4 emission from wet land paddy fields. It is also important to consider effect of fertilizer application on earthworms. However, low concentration of urea was reported as positive and beneficial for earthworm growth (Xiao et al., 2004).

Considering the above-mentioned background, we hypothesized that controlled application of urea and bioturbation activity by aquatic earthworms would enhance the biomass of methanotrophs, ultimately leading to a decrease in CH_4 emissions. To test this hypothesis, we designed a research study with a full factorial combination of two aquatic earthworm levels (with earthworm and no earthworm) and two levels of fertilizer (with urea and no urea). As of our knowledge, this is the first study, which examined potential role of aquatic earthworms in CH_4 mitigation process. The main objectives of our study were as follows:

(i) To investigate effect of urea application on abundance of aquatic earthworms and CH_4 emission.

(ii) To see effect of aquatic earthworms on methanotrophs and CH_4 emission from wetland paddy soil.

2. Method

2.1 Soil Preparation

The laboratory experiment was conducted in Soil Ecology Laboratory of Yokohama National University, Japan. A 2X2 factorial experiment with 6 replications was designed involving two levels in the aquatic earthworm treatment: the presence or absence of aquatic earthworms (EW and NW, respectively), and two levels of urea treatment (UR and NU, respectively). Soil was collected from a paddy field in Kamakura, Kanagawa (35°20′N, 139°31′E; 56 m.a.s.l.). The soil contained 40% sand, 58% silt, and 2% clay. The soil was sun-dried, plant debris and stones were removed, and then the soil was sieved using 2 mm sieve to achieve a homogenous texture. A 100 g of soil was added in each of 24 transparent glass bottles. Then 120 ml water was poured on soil in each bottle.

2.2 Collection of Aquatic Earthworms

Aquatic earthworms (*Branchiura sowerbyi* Beddard, 1892, Clitellata, Tubificidae) were collected from the flooded paddy soil, because it is one of the most dominant aquatic earthworm species in the paddy field (Yachi et al., 2012). The paddy soil was flooded for 8 weeks before collecting aquatic earthworms. To collect aquatic earthworms, muddy soils were sieved by a 500-µm mesh sieve. The earthworms were kept in deionized water for 48 h to excrete intestinal residues. Then, the live earthworms were placed on filter paper to remove extra water from the body and each earthworm was weighed to record their biomass. Collected aquatic earthworms were incubated in soil for 7 days.

2.3 Experimental Set-Up

Rice straw powder (2500 kg ha^{-1}) was added to soil in each bottle as a source of organic matter and mixed gently using a spoon. As fertilizer treatment, granular urea (46% N) was added in respective 12 bottles at a rate of 90 kg N ha^{-1}. Pre-incubated 6 earthworms were inoculated in six urea treated bottles and six no-urea treated bottles. Number of inoculated aquatic earthworms was decided based on representative density of earthworms in the Kamakura paddy field (unpublished data), which was 2525 earthworms m^{-2}.

All bottles were incubated at 25 °C temperature and under 14 hours photoperiod to provide a nearly natural lighting environment for the earthworms for 35 days. At the end of the incubation period, the aquatic earthworms were collected from the soil in the bottles by sieving through a 500-µm mesh size sieve and the numbers and biomass of earthworms were recorded.

2.4 Gas Sampling and CH_4 Measurement

Gas samples were collected once per week, starting from day 0, during the experiment. The first sample was collected 2 h after preparing the soil and the bottles were placed in the sampling area 30 min before sampling. Then, the mouth of each bottle was closed with a rubber septum that had plastic lids running from it (to collect the gas produced). The first gas sample was collected 10 min after closing a bottle, and then after 20 and 30 min, with a plastic disposable 50 ml syringe. Gas samples were collected into 30-ml glass vials. Methane concentration was measured using a gas chromatograph (GC-2014, SHIMADZU, Japan) and CH_4 flux was calculated using the flux package (Jurasinski et al., 2014) in R software (R Development Core Team, 2015).

2.5 Monitoring Soil Properties

Each week soil pH and soil oxidation reduction potential (Eh) were measured at 1 cm and 5 cm depth from surface immediately after gas sample collections. Soil pH and Eh were measured using pH/COND Meter D-54 instrument (Horiba, Kyoto, Japan) and a pH/ORP/DO Meter D-75 instrument (Horiba, Kyoto, Japan), respectively.

2.6 Measuring Soil Properties at the End of Incubation Period

After collection of the final gas sample, soil samples were collected to measure total carbon (T-C) and total nitrogen (T-N). Collected samples were oven-dried at 40 °C for 48 h, and then T-C and T-N were measured using a CN Macro Corder (JM1000CN, J-Science Lab. Kyoto, Japan).

2.7 Phospholipid Fatty Acid (PLFA) Analysis

A phospholipid fatty acid (PLFA) analysis was conducted to determine the biomass of methanotrophs in this experiment. Lipids were extracted using a method modified from Frostegård et al. (1991), and Niwa et al. (2008). Briefly, lipids were extracted from 7-8 g fresh wet soil samples in a one-phase chloroform-methanol-phosphate buffer. After condensation of the lipids, the phospholipids were fractionated using a silicic acid column (BOND ELUT LRC-SI; Varian, Palo Alto, CA, USA) and then fatty acid methyl esters were separated from the

phospholipids after mild alkaline methanolysis. An internal standard, methyl nonadecanoate (19:0), was added to all samples. Fatty acid methyl esters were identified using the Sherlock Microbial Identification System (MIDI, Newark, DE, USA). The fatty acid 18:2ω6c was used to estimate the biomass of fungi (Frostegård & Baath, 1996); 15:0iso, 15:0antesio, and 17:0iso were used to estimate the Gram-positive bacteria (Olsson & Alström, 2000; Moore-Kucera & Dick, 2008); and 17:0cyclo was used to estimate the Gram-negative bacteria (Wilkinson, 1988). The lipid biomarkers of methanotrophs often overlap with other groups, but in this study the fatty acid 16:1ω7c and 16:1ω5c were used to estimate the biomass of type I methane-oxidizing bacteria and 18:1ω7c was used to estimate the biomass of type II methane-oxidizing bacteria (R. S. Hanson & T. E. Hanson, 1996; Bodelier et al., 2009; Zigah et al., 2015).

2.8 Statistical Analysis

The homogeneity of variance and normality of the data collected were determined with Bartlett's test and the Shapiro-Wilk test, respectively. When required, data were log-transformed to meet the assumptions of normality. The main and interaction effects of fertilizers and aquatic earthworms were assessed by generalized linear model (GLM) with Gamma distribution. The effects of the aquatic earthworms on the biomass of methanotrophs were assessed by two-way ANOVA. Statistical analyses were performed using R 3.2.3 for Microsoft Windows (R Development Core Team, 2015) using the package lme4 (Bates et al., 2015) to fit the model.

3. Result and Discussion

3.1 Soil Environment

The soil Eh value was higher at 1-cm than at 5-cm below soil surface irrespective of treatments throughout the incubation period (Table 1). At 1 cm below soil surface, Eh value was recorded between -35 mV to -73 mV, indicated oxidation reduction potential is much higher than required Eh value to initiate methanogenesis process. In principle, Eh value -150 mV or less leads to a significant CH_4 production. A sharp decrease of Eh values were observed with increasing soil depth. Eh value decreased to -250 mV at 5 cm below soil surface within 7 DAI and same level maintained until the end of the incubation period. The results indicate that Eh value at 5 cm soil depth was favorable for CH_4 formation. Observed soil pH values showed slightly alkaline soil condition over the incubation period irrespective of treatments. Neutral to slightly alkaline pH is beneficial for growth aquatic earthworm (Lou et al., 2013). Table 2 also shows total-C and total-N data of 35 DAI. Total-C ranges from 18.39 g kg^{-1} in only earthworm treated soil to 19.52 g kg^{-1} in control treatment (NW NU). However, difference of total-C is not significant among the treatments. Total-N content also showed similar trend. CN ratio was 25:1. It implies that the soil has almost perfect balance of carbon to nitrogen for soil microbial growth. USDA (2011) reported that CN ratio 24:1 is favourable for soil microorganism.

Table 1. The Soil Eh at different depth during incubation period

Treatments	Eh at 1 cm depth (mV)						Eh at 5 cm depth (mV)					
	DAI						DAI					
	0	7	14	21	28	35	0	7	14	21	28	35
EW UR	-38	-45	-51	-53	-57	-60	-41	-258	-240	-235	-252	-242
EW NU	-35	-45	-48	-50	-57	-62	-38	-252	-247	-232	-246	-237
NW UR	-35	-44	-54	-58	-64	-67	-37	-240	-230	-230	-254	-249
NW NU	-38	-48	-56	-62	-66	-73	-40	-244	-236	-228	-247	-245

Table 2. The Soil properties during incubation period

Days	pH				Soil total C (g kg^{-1})				Soil total N (g kg^{-1})			
	EW UR	EW NU	NW UR	NW NU	EW UR	EW NU	NW UR	NW NU	EW UR	EW NU	NW UR	NW NU
0	7.16	7.17	7.17	7.21	18.78	18.37	18.4	18.47	1.43	1.34	1.61	1.7
7	7.44	7.48	7.44	7.47	-	-	-	-	-	-	-	-
14	7.47	7.41	7.35	7.31	-	-	-	-	-	-	-	-
21	7.48	7.42	7.39	7.35	-	-	-	-	-	-	-	-
28	7.61	7.56	7.52	7.52	-	-	-	-	-	-	-	-
35	7.51	7.42	7.29	7.29	18.89	18.39	18.41	18.52	1.44	1.37	1.62	1.71

3.2 Effect of Urea Fertilizer on Abundance of Branchiura Sowerbyi

Figure 1 shows abundance of *Branchiura sowerbyi* in the soil received both treatments (earthworm + Urea) and in the soil received only earthworm treatment at 35 DAI. The data clearly shows that urea application was beneficial for growth of *Branchiura sowerbyi* during the incubation period. Average density of *Branchiura sowerbyi* was approximately 4050/m^2 in earthworm inoculated and urea treated soils. Whereas, the density of *Branchiura sowerbyi* in soil without receiving urea was 2400/m^2. The result indicated that applying urea at rate of 90 kg N ha^{-1} was beneficial for growth of *Branchiura sowerbyi* in paddy field soil. Previous studies reported that low dose of urea is beneficial for aquatic earthworms (Xiao et al., 2004; Bhattacharya & Sahu, 2014). Bhattacharya and Sahu (2014) showed that with 100 mg urea kg^{-1} soil, earthworm mortality rate was 0 % and gradually the mortality rate was increased with increase of urea dose. In our study urea dose was very limited and it was 0.00012 mg urea kg^{-1} soil which increased *Branchiura sowerbyi* density in soil. Therefore, further study is required to identify optimal dose of urea that is beneficial for *Branchiura sowerbyi*.

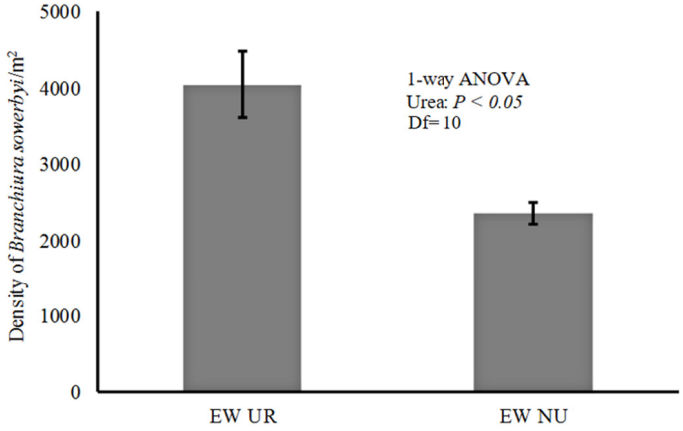

Figure 1. Influence of urea fertilizer on abundance of *Branchiura sowerbyi* in paddy field soil

Note. Vertical bars indicate standard error of mean.

3.3 Influence of Branchiura Sowerbyi and Urea Fertilizer on CH_4 Flux

A clear increase in CH_4 flux from 7 DAI was observed and reached to peak at 28 DAI, irrespective of treatments, except EW NU treatment, where the peak was at 21 DAI (Figure 2). At 28 DAI the highest CH_4 flux (2055 mg m^{-2} h^{-1}) was measured from soil received only urea without earthworm inoculation followed by 1378 mg m^{-2} h^{-1} in soil without receiving earthworm and urea. The results indicated urea application in paddy soil might increase CH_4 emission. In previous studies (Wang et al., 1992; Banik et al., 1996; Yang & Chang, 1997; Dubey, 2003) showed that urea increased CH_4 production from anaerobic soil; they also observed a direct relationship among CH_4 production, lower Eh values and higher pH levels in a flooded paddy soil. After urea is applied to paddy soils, it is hydrolyzed by microbial ureases, resulting in an alkaline soil pH and lowering the soil Eh. However, in our study, when paddy field soil received both urea and *Branchiura sowerbyi*, CH_4 flux was reduced significantly, which was nearly 5 times less than that of soil with only urea treatment. Figure 1 in the earlier section showed that urea application at 90 kg N ha^{-1} rate stimulated density of *Branchiura sowerbyi* in paddy field. As a result, bioturbation activities increased, which might lead to increase penetration of oxygen in soil layer. These phenomena combinedly contributed to increase methanotrophs activities in paddy filed, consequently lower CH_4 flux. Although, the lowest CH_4 flux (120 mg m^{-2} h^{-1}) without urea fertilizer in *Branchiura sowerbyi* inoculated soil, fertilizer application was required to get paddy yield at a satisfactory level. Therefore, we suggest future research should focus on optimization of urea rate that will be beneficial for earthworm growth and CH_4 oxidation without compromising satisfactory level of crop yield.

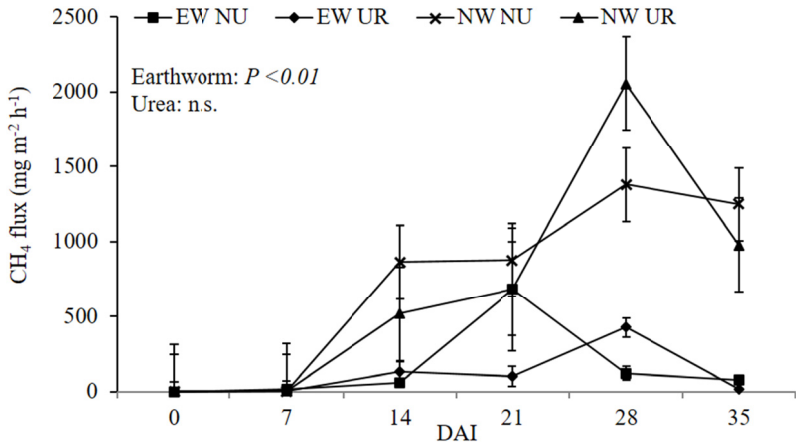

Figure 2. The effect of *Branchiura sowerbyi* and urea application on CH_4 flux

Note. Vertical bars indicate standard error of mean.

During the incubation period, there was no significant effect of urea on CH_4 flux, but the presence of urea increased the abundance of aquatic earthworms. The highest cumulative CH_4 emission was observed in the NW-UR treatment (18.0 g m^{-2} day^{-1}) and the lowest were in the EW-UR treatment (3.2 g m^{-2} day^{-1}). Aquatic earthworms significantly ($P < 0.01$) decreased the CH_4 emissions after 35 days of incubation. On day 35, in the treatment where the aquatic earthworm density decreased to 1684 earthworms m^{-2}, the CH_4 flux was 116 mg m^{-2} h^{-1}, but in the treatment where the aquatic earthworm density increased to 5471 earthworms m^{-2}, the CH_4 flux was only 5.32 mg m^{-2} h^{-1}. *Branchiura sowerbyi* is buried in the soil for feeding, while its tail remains on the surface for respiration; through this bioturbation activity *Branchiura sowerbyi* can introduce O_2-rich water into the lower soil layers and it also produces a layer of fecal pellets on the soil surface. This modification of the soil habitat might lead to an increase in the biomass of methanotrophs, which accelerated the oxidation of CH_4 produced by the methanogens. There are some previous studies which confirmed that macropores which are created through burrowing of earthworms and oxygenating water by bioturbation (Fox & Taylor, 1955), earthworms diffuse O_2 to the burrow linings and casts which is favorable microsite for methanogens and from these anoxic microsites CH_4 would be consumed by methanotrophs which are available in relatively O_2-rich habitat (Kernecker et al., 2015).

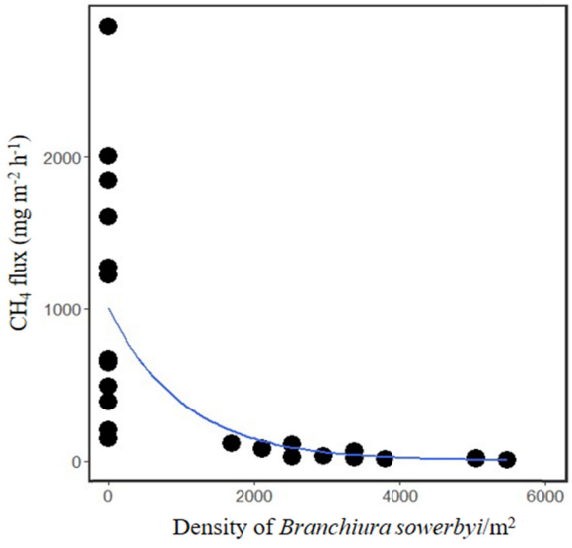

Figure 3. Relation between density of *Branchiura sowerbyi* and CH_4 emission at 35 DAI

3.4 Aquatic Earthworms and Biomass of Methanotrophs

The PLFA profile in the soils is shown in Figure 4. Methanotrophs were dominant in the earthworm-treated soils, whereas Gram-positive bacteria were dominant in the soils where there were no earthworms (Figure 4a). The amount of PLFAs was compared among treatments to estimate the biomass of methanotrophs (Figure 4b).

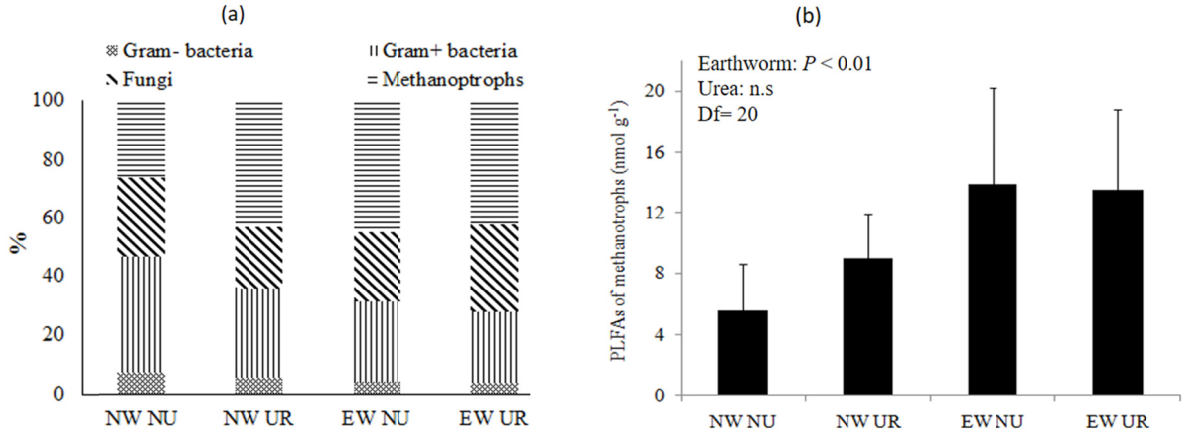

Figure 4. The composition of soil microbes and biomass with methanotrophs in the incubated paddy soil at 35 DAI

Note. Vertical bars indicate standard error of mean.

The highest biomass levels of methanotrophs, based on the PLFAs contents, were measured in the EW-NU and EW-UR treatments (13.8 and 13.5 nmol g^{-1}, respectively) and the lowest levels were observed in the NW-NU treatment (5.61 nmol g^{-1}). The results of the GLM confirmed that the presence of aquatic earthworms affected the biomass of methanotrophs ($P < 0.001$), whereas urea did not have any significant effect. Increasing biomass of methanotrophs translated to reduction of the CH_4 flux from the incubated soils ($P < 0.01$) (Figure 5a). Figure 5b implies that the presence of aquatic earthworms accelerates the growth of methanotrophs ($P < 0.001$), which leads to reduction of the CH_4 flux ($P < 0.01$). Kernecker et al. (2015) also confirmed that the reduction of CH_4 production rate in anoxic soil with the presence of earthworms is caused by methanotrophy which is stimulated by bioturbation activity of earthworms.

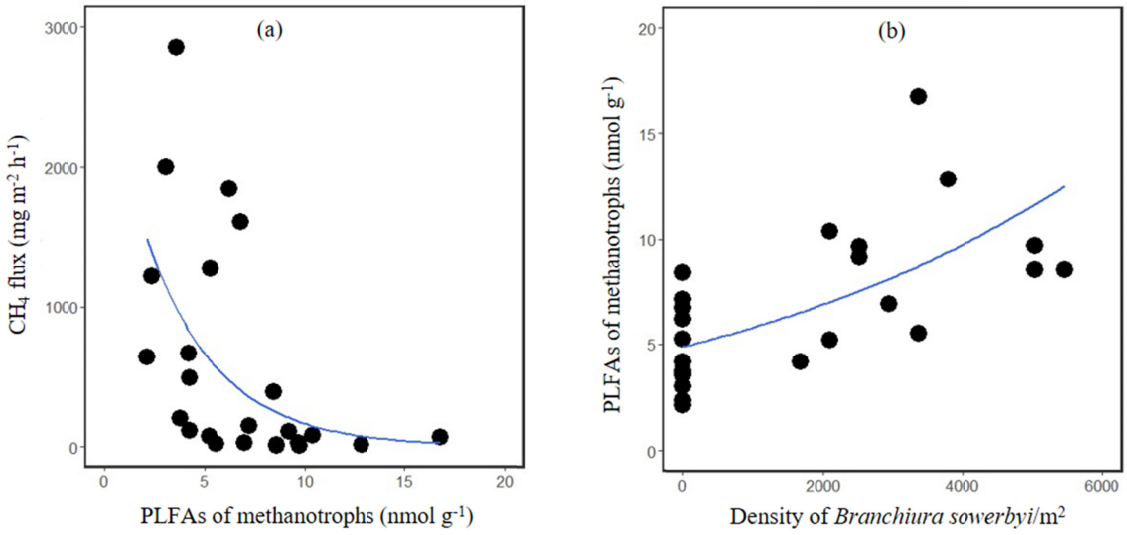

Figure 5. The relation between methanotrops and CH_4 flux (a) and between *Branchiura sowerbyi* density and growth of methanotrophs (b)

Results of GLM have been summarized in Table 3 and it implies: (i) increasing density of *Branchiura sowerbyi* has significant positive impact on growth of methanotrophs; (ii) increasing methanotrophs activity leads to significant CH_4 emission reduction; and (iii) as a result *Branchiura sowerbyi* significantly contributes to mitigate CH_4 in paddy soil environment.

Table 3. Parameter summary results of generalized linear model (GLM)

Model		Estimates	Std. error	P value
CH_4 emission~*Branchiura sowerbyi* density	Intercept	6.914	1.95E-01	< 0.001
	Branchiura sowerbyi	-9.656E-04	7.77E-05	< 0.001
CH_4 emission~PLFA of methanotrophs	Intercept	7.9		< 0.001
	PLFA of methanotrophs	-0.2796	0.07489	< 0.01
PLFA of methanotrophs~ *Branchiura sowerbyi* density	Intercept	1.59615	0.11192	< 0.001
	Branchiura sowerbyi	0.07169	0.01877	< 0.001

4. Conclusions

Remarkable increase of atmospheric CH_4 concentration over the centuries has puzzled climate scientists. Flooded rice cultivation has been blamed as one of the main sources of anthropogenic CH_4 emission. Anaerobic methanogenesis is the main process of CH_4 production in paddy field soil. It is obvious that wetland rice cultivation will be intensified to feed growing populations, which will also lead increasing contribution to atmospheric CH_4 concentration, our findings showed that bioturbation of aquatic earthworms helps to reduce CH_4 emission to the atmosphere by increasing methanotrophic activities in paddy field soil. Though earlier studies argued that urea application increases CH_4 emission from paddy field, our study showed that in the presence of aquatic earthworms, urea fertilizer reduced CH_4 emission by 90% by stimulating earthworm abundance in soil. Hence, it is important to conserve suitable environment for optimal growth of aquatic earthworms. We also recommend future research should focus on optimization of urea application rate that will be beneficial for earthworm growth and CH_4 oxidation without compromising satisfactory level of crop yield.

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