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ORIGINAL PAPER

Can abundance of methanogen be a good indicator for CH_4 flux in soil ecosystems?

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Abstract Methane, which is produced by methanogenic archaea, is the second most abundant carbon compound in the atmosphere. Due to its strong radiative forcing, many studies have been conducted to determine its sources, budget, and dynamics. However, a mechanistic model of methane flux has not been developed thus far. In this study, we attempt to examine the relevance of the abundance of methanogen as a biological indicator of methane flux in three different types of soil ecosystems: permafrost, rice paddy, and mountainous wetland. We measured the annual average methane flux and abundance of methanogen in the soil ecosystems in situ. The correlation between methane flux and the abundance of methanogen exists only under a specific biogeochemical conditions such as SOM of higher than 60 %, pH of 5.6–6.4, and water-saturated. Except for these conditions, significant correlations were absent. Therefore, microbial abundance information can be applied to a methane flux model selectively depending on the biogeochemical properties of the soil ecosystem.

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Introduction

CH_4, an important greenhouse gas, has 25 times more radiative force than carbon dioxide on a molecular basis. Its atmospheric concentration has increased again 2006 (Rigby et al. 2008). Most CH_4 is produced under an anaerobic condition by a specific class of archaea known as methanogen. Due to the strong warming potential of CH_4, many studies have been conducted for an accurate determination of its sources and budget (Conrad 1996, 1998; Lelieveld et al. 1998; Wang et al. 2004; Chen and Prinn 2005; Kirschke et al. 2013). Around half of all CH_4 sources (548–678 Tg CH_4 $year^{-1}$) are natural sources (218–347 Tg CH_4 $year^{-1}$) (Kirschke et al. 2013). Several studies have attempted to construct mechanistic models of CH_4 flux (Cao et al. 1996; Van Bodegom et al. 2001; Morrissey et al. 2014), but they are applicable only to specific types of ecosystems, and their explanatory power is relatively low. An integrated dynamics of CH_4 flux has yet to be clearly determined; hence, indicators that can represent actual CH_4 flux in diverse ecosystems are strongly required to develop an advanced CH_4 flux model. Recently, soil carbon cycle models have utilized microbial kinetic variables such as microbial community structure or extracellular enzyme to refine the

model accuracy (Allison et al. 2010; Wieder et al. 2013). In CH_4 flux models, however, microbial traits have not been widely incorporated while edaphic factors were primarily considered as key controlling variables (Van Bodegom et al. 2001).

Previous studies have revealed key controlling edaphic variables for CH_4 flux, which include the water table (Frenzel and Karofeld 2000; Ding et al. 2010; Berger et al. 2013), the methanogenic substrate (Shannon and White 1996; Saarnio et al. 1998; Liu et al. 2012), and the temperature (Westermann 1993; Kim et al. 2012; Yvon-Durocher et al. 2014). A high water table induces a strict anaerobic condition, which enhances CH_4 production (Frenzel and Karofeld 2000; Ding et al. 2010; Berger et al. 2013). Shannon and White (1996) suggested that a considerable amount of CH_4 production occurs in conjunction with a high substrate supply. The temperature is positively correlated with the CH_4 flux at various scales of the population and community, up to the ecosystem level (Yvon-Durocher et al. 2014). In the case of microbial variables, however, the associations between the two factors, specifically the CH_4 flux and the abundance of methanogen, under various environmental conditions have not been fully clarified. Traditionally, abundance of methanogen is believed to be directly correlated with CH_4 emission from soil ecosystems. However, Liu et al. (2011) and Thauer (1998) argued that methanogen abundance determined by DNA-based methods may not be significantly correlated with CH_4 production from soil ecosystems because of the large proportion of inactive methanogen population. However, they did not provide enough quantitative analysis for the effects of surrounding environment to support their argument. In this study, we investigated the biogeochemical conditions of various soil ecosystems in addition to molecular analysis to clarify as to what extent the information about methanogen can explain variations in methane emission.

Methanogen in soil environment is estimated to emit 175–217 Tg $year^{-1}$ of CH_4 globally, which is almost 70 % of the annual CH_4 emission from natural sources (218–347 Tg CH_4 $year^{-1}$) (Kirschke et al. 2013). However, large uncertainties remain with regard to global biogenic CH_4 flux from soil environment (Kirschke et al. 2013), as it is accurately difficult to estimate the global distribution of soil environment and the CH_4 flux in each type of soil environments. Specifically, CH_4 flux diverges tremendously across the various types of ecosystems (Melling et al. 2005) because of the different biogeochemical conditions, such as the different soil organic matter (SOM) and pH characteristics.

In this study, we measured the abundance of methanogen and the CH_4 flux from three types of soil environment in situ (a rice paddy, permafrost, and a mountainous wetland) in four seasons and investigated the relationship between the two measurements in each site. However, we measured the summer data only in permafrost site due to limited accessibility to the site. These three soil ecosystems were selected for following reasons: (1) Mountainous wetland (or freshwater wetland) is a major CH_4 source in nature, because it is flooded through entire year and is rich in organic matter, (2) rice paddies emit 10 % of global annual anthropogenic CH_4 (Kirschke et al. 2013), and (3) permafrost is a minor CH_4 source currently, but CH_4 emission in this area may have been enhanced by climate change (Nauta et al. 2015). We have focused on the effects of SOM and pH on the correlation between CH_4 flux and abundance of methanogen in each soil ecosystem. Typical temporal scale of most CH_4 budget and flux models is a year; thus, we have conducted the annual average data of methanogen and CH_4 flux for the correlation analysis. The result of the analysis will provide better understandings of methane dynamics and fundamental information about microbial methane flux model.

Materials and methods

Sampling sites

The soil samples were collected in three different types of soil ecosystems: a rice paddy, permafrost, and a mountainous wetland (Fig. 1; Table 1). The rice paddy site is located near Haean-myeon, Kangwon-do, South Korea (38°17′08″N, 128°08′41″E). Haean-myeon is located in a mountainous basin, which is surrounded by mountains of heights which exceed 1100 m above the mean sea level. The average height of the Haean-myeon basin ranges from 400 to 500 m, and various crops (e.g., onion, grape, rice, potato, and cabbage) are grown in the basin. The two permafrost sites are located in near Dasan station at Ny-Ålesund on the Svalbard archipelago in Norway (78°55′00″N, 11°56′00″E) and at the Nome Complex on the Seward

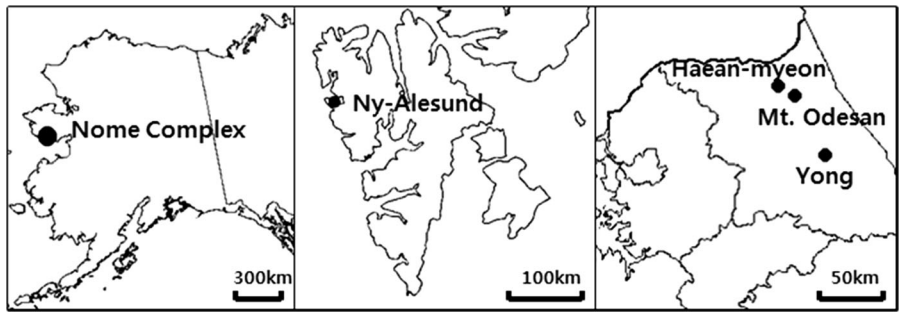

Fig. 1 Location of sampling sites

Table 1 Study site characteristics

Site name	Ecosystem types	Location	Temperature (annual mean) (°C)
Haean-myeon	Rice paddy	38°17′08″N, 128°08′41″E	+4.7 to +16.2 (+10.2)
Ny-Ålesund	Permafrost	78°55′00″N, 11°56′00″E	−12.0 to +3.8 (−5.2)
Nome Complex	Permafrost	64°30′14″N, 165°23′58″W	−14.9 to +11.2 (−2.6)
Mt. Odesan	Mountainous wetland	37°47′52″N, 128°32′36″E	+4.7 to +16.5 (+10.1)
Yong	Mountainous wetland	38°13′24″N, 128°07′20″E	+4.7 to +16.2 (+10.2)

Temperature data are 30-year average from 1981 to 2010. Data sources are 'Korean Meteorological Administration' for Haean-myeon, Mt. Odesan, and Yong, 'Forland et al. (2011)' for Ny-Ålesund, and 'NOAA' national climate data center for Nome Complex. (Forland et al. 2011)

Peninsula of Alaska (64°30′14″N, 165°23′58″W). Ny-Ålesund is a high arctic ecosystem (elevation of around 500 m) with poor vegetation. Lichen is one of the dominant species in this area. The Nome Complex is a subarctic area; hence, this area is slightly warmer than Ny-Ålesund. It has a long, cold winter (70 days of below −18 °C) and a short growing season (around 80 days with melted ice), and most of the vegetation is the shrub type. The Mt. Odesan wetland (37°47′52″N, 128°32′36″E) and the Yong wetland (38°13′24″N, 128°07′20″E) on Mt. Daeam are temperate mountainous wetland areas in Korea. Both are submerged throughout the year (with a water table level of approximately 1 m) and are rich in various vegetation of temperate mountainous wetland. Sea-level altitudes are 1170 and 1280 m for the Mt. Odesan wetland and the Yong wetland, respectively. These two wetlands are protected by the Ramsar Convention.

Sample collection and chemical analysis

We collected soil samples in four different seasons (spring, summer, fall, and winter), except permafrost. We collected soil samples from permafrost only in growing season (summer). Each sample had three replicated soil cores at a depth of 5 cm. The soil cores were sealed by polybags and stored in 4 °C less than a week, before analyzing. The SOM was determined as the weight loss after burning the fresh soil samples in a furnace at 600 °C for 24 h; these values are expressed in weight percentages. The soil pH was measured by a pH meter in situ.

Molecular analysis

Soil microbial DNA was extracted from each 500 mg of sample using the NucleoSpin® Soil kit (Macherey–Nagel GmbH & Co. KG, Germany) with two replicates. To estimate the abundances of methanogen in the soil DNA samples, we performed real-time quantitative polymerase chain reactions (rt-qPCR) using the SYBR Green Real-time PCR Master Mix (Toyobo Co., Japan) as a detector. The target gene for the analysis was the mcrA gene. The mcrA gene is involved in the terminal step of methanogenesis, which appears in every class of methanogenic archaea (Hales et al. 1996; Thauer 1998). Thus, the abundance of mcrA is commonly applied during quantitative

analyses in the methanogen community. The reaction was performed by the CFX96TM Real-Time PCR Detection System (BIO-RAD, USA). The total volume of each reaction mixture was 20 μL, with 6.4 μL of distilled water, 2 μL of extracted soil DNA, 10 μL of the SYBR Green Master Mix, 0.8 μL of each forward (5′-GGT GGT GTM GGD TTC ACM CAR TA-3′) (Steinberg and Regan 2008) and reverse (5′-TCA TKG CRT AGT TDG GRT AGT-3′) (Hales et al. 1996) primer (10 μM). Three-step PCR was used for amplification (Heid et al. 1996), with 50 cycles with denaturation at 94 °C for 5 s, primer annealing at 50 °C for 30 s, and strand extension at 72 °C for 30 s. Two independent rt-qPCR assays were performed on each DNA sample for replication. The gene copy number was calculated by the standard curve method. The standard curve was deduced using tenfold diluted series of plasmids containing the mcrA gene.

CH_4 flux measurement

Gas samples were collected at the same time with soil collection. The static chamber method was conducted to collect gas samples. We installed three acrylic cylinders (20 cm height and 19.5 cm diameter) 5 cm deep in the soil at each site. One hour after the installation, we closed the cylinder using an acrylic cap with a septum. Next, we collected 10 mL of gas samples from the chamber through the septum at 0, 5, 10, and 15 min using 10-mL syringes. The concentrations of CH_4 from the collected gas samples were measured by means of FID (flame ionization detector) gas chromatography (gas chromatograph: CP-3800 Varian, USA). From the concentration differences over time, the CH_4 flux was calculated by following equation.

$$\mathrm{CH_4\ flux} = \frac{\mathrm{dCH_4}}{\mathrm{d}t} \times \frac{V}{A} \times \frac{P \times 100 \times \mathrm{MW}}{R} \times \frac{273.15\,^{\circ}\mathrm{C}}{273.15\,^{\circ}\mathrm{C} + T}$$

(where dCH_4/dt is a CH_4 concentration gradient over time, V is a volume of the static chamber, A is a horizontal area of the static chamber, P is an atmospheric pressure (1 atm), MW is a molecular weight of CH_4 (16 g mol^{-1}), R is an ideal gas constant (8.314 J mol^{-1} K^{-1}), and T is the temperature in Celsius degree)

Statistical analysis

To determine differences in the abundances of methanogen, the CH_4 flux, and the soil chemical properties (SOM and pH) among the sites, data were analyzed via one-way analysis of variation (ANOVA). We used the Pearson correlation coefficient to investigate the correlation between the abundances of methanogen and the other factors. All analyses were conducted with SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

There was a weak positive correlation ($r = 0.304$) between the annual average CH_4 flux and the abundance of methanogen when all data were combined (Fig. 2; Table 2). However, a significant correlation ($r = 0.327$) was observed only in the mountainous wetland, whereas it was absent in other soil ecosystems. The mountainous wetland exhibited the highest level of the abundance of methanogen ($19.0 \pm 3.8 \times 10^9$ mcrA gene copy g^{-1} dry soil) and the highest CH_4 flux (570 ± 223 mg CH_4 m^{-2} day^{-1}) (Fig. 2). The permafrost and rice paddy showed CH_4 flux of -3.4 ± 2.5 and 75.3 ± 40.2 mg CH_4 m^{-2} day^{-1}, respectively; these were statistically identical, whereas the rice paddy exhibited a higher value for

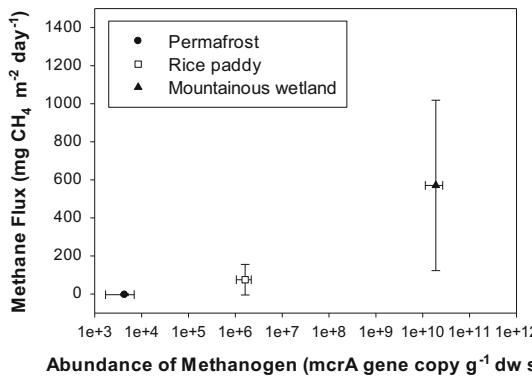

Fig. 2 Correlation between CH_4 flux and abundance of methanogen. *Circle*, *square*, and *triangle* represent permafrost, rice paddy, and mountainous wetland, respectively. *Error bars* represent 95 % confidence range

Table 2 Pearson correlation coefficient between CH_4 flux and abundance of methanogen in different types of soil ecosystems

Ecosystem types	*r*	*P* value	*n*
Permafrost	−0.079	0.504	73
Rice paddy	0.188	0.127	67
Mountainous wetland	0.327	0.018	52
Total	0.304	<0.000	192

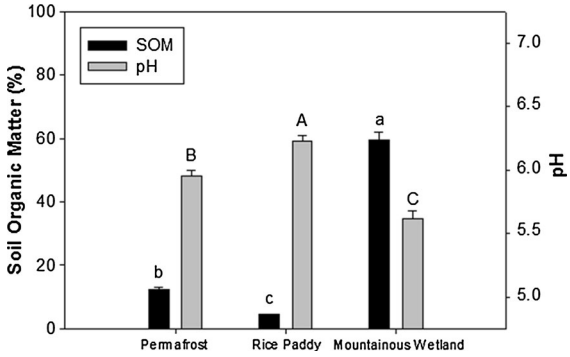

Fig. 3 Soil pH and SOM in each type of soil ecosystems. *Alphabet* denotes a significant difference in SOM and pH ($P < 0.05$). *Error bars* represent standard errors

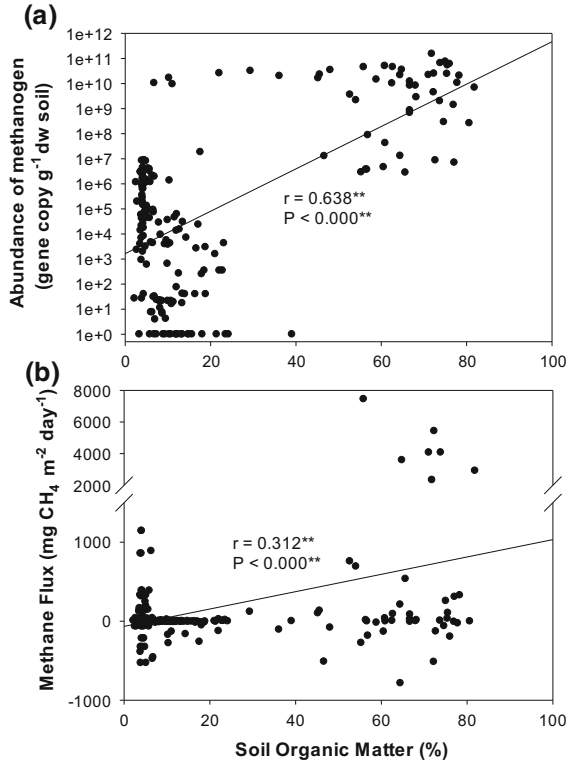

Fig. 4 Correlations between soil organic matter (SOM) and abundance of methanogen (**a**), or CH_4 flux (**b**) across all sites

the abundance of methanogen ($1.6 \pm 0.3 \times 10^6$ mcrA gene copy g^{-1} dry soil) than the permafrost ($4.3 \pm 1.3 \times 10^3$ mcrA gene copy g^{-1} dry soil) (Fig. 2).

The highest SOM value was found in the mountainous wetland (59.4 ± 2.6 %), followed by the permafrost (12.2 ± 0.7 %) and the rice paddy (4.5 ± 0.1 %) (Fig. 3). The SOM exhibited a strong positive correlation ($r = 0.638$) with the abundance of methanogen (Fig. 4a) and a weak positive correlation ($r = 0.312$) with the CH_4 flux (Fig. 4b). Samples with SOM levels that exceeded 60 % (peat soil) exhibited a positive correlation ($r = 0.359$) between the abundance of methanogen and the CH_4 flux, while samples with lower SOM (organic soil and mineral soil) levels did not show a relationship (Table 3).

The pH of the soil ranged from 5.25 to 6.34. In contrast to the SOM, the pH level was highest in the rice paddy (6.18 ± 0.04) and lowest in the mountainous wetland (5.49 ± 0.05), whereas the permafrost exhibited a pH level of 5.96 ± 0.04 (Fig. 3). In addition, the pH exhibited negative correlations with

Table 3 Pearson correlation coefficient between CH_4 flux and abundance of methanogen in different ranges of SOM and pH. SOM of less than 15 %, 15–60 %, and more than 60 % represents mineral soil, organic soil, and peat soil, respectively

	r	*P* value	*n*
Soil organic matter (%)			
>60	0.359	0.040	33
60–15	0.264	0.138	33
<15	0.102	0.254	126
pH			
>6.4	−0.284	0.269	17
6.4–6.0	0.298	0.021	60
6.0–5.6	0.344	0.012	52
<5.6	0.257	0.214	25

both the abundance of methanogen ($r = -0.303$) and the CH_4 flux ($r = -0.199$) (Fig. 5a, b). Samples with a moderate acidic pH level (6.4–5.6) exhibited positive correlations ($r = 0.298$ for pH of 6.4–6.0 and

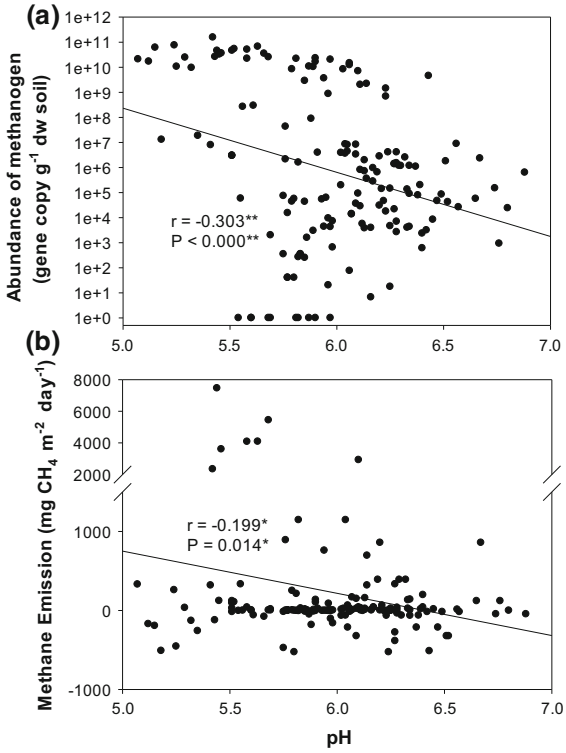

Fig. 5 Correlations between soil pH and abundance of methanogen (**a**), or CH_4 flux (**b**) across all sites

$r = 0.344$ for pH of 6.0–5.6), but samples with higher and lower pH levels did not demonstrate a relationship (Table 3).

Discussion

There was a positive correlation between the annual mean CH_4 flux and the abundance of methanogen when we combined all three types of soil ecosystems (Table 2). This correlation arose because the mountainous wetland showed an extremely high CH_4 flux and a high abundance level of methanogen as compared to the other soil ecosystems (Fig. 3). A large amount of SOM in the mountainous wetland provided the substrate supply for methanogenesis, which may have caused the substantially higher CH_4 flux and the proliferation of methanogen in the mountainous wetland (Shannon and White 1996). The mountainous wetland exhibited higher SOM than the permafrost and the rice paddy since it had been saturated by water consistently and had lower mean temperature, which inhibit decomposition of SOM to CO_2. However, when we considered each ecosystem separately, no significant correlations were found except for the mountainous wetland (Table 3). According to Liu et al. (2011), the abundance of methanogen did not show a relationship with the CH_4 production potential in wetlands in China. Graham et al. (2014) suggested that microbial community indicators, such as abundances or community structures, did not enhance the explanatory power of ecosystem model. Instead, edaphic factors exhibited a more significant relationship with ecosystem functions (Graham et al. 2014). However, Morrissey et al. (2014) have shown that information about microbial community is associated with CH_4 production from tidal freshwater wetland. How much ecosystem process rates can be explained by the microbial community information is still under debate (Carney and Matson 2005), although application of microbial information can enhance the accuracy of ecosystem models (Allison et al. 2010). Based on the results of our research, the microbial abundance was significantly related to the process rate under specific biogeochemical conditions of soil ecosystem, such as a high concentration of SOM and a moderate acidic condition (Tables 2, 3). Except for those biogeochemical conditions, no significant correlation between the CH_4 flux and the abundance of methanogen was found. This result indicates that abundance of methanogen data cannot be applied to the CH_4 flux model in a wide range of biogeochemical conditions in three soil ecosystems: rice paddy, permafrost, and mountainous wetland. However, this result may be biased, as the methanogen community includes a large population of inactive methanogen. This inactive methanogen does not produce CH_4, but it influences the results of a microbial analysis (Thauer 1998). Further studies with RNA-based methods should be conducted to examine the contribution of the active methanogen community, which is actually associated with CH_4 production.

The SOM exhibited a strong positive correlation with the CH_4 flux and abundance of methanogen (Fig. 4a, b). Conrad (1999) reported that a high substrate concentration did increase CH_4 production; thus, the SOM can enhance methanogenic activity (Shannon and White 1996). A correlation between the abundance of methanogen and CH_4 flux was not found under a lower SOM condition, but it increased under a higher SOM condition (Table 3). In a condition with a

low amount of organic matter, substrates for methanogenesis were limited (Shannon and White 1996). Therefore, increases in the abundance of methanogen may not stimulate CH_4 flux proportionally. With a high level of SOM, in contrast, substrates for methanogenesis were ample, and the abundance of methanogen is likely linked directly to changes in CH_4 flux. Interestingly, Morrissey et al. (2014) have shown that abundance of methanogen and CH_4 emission displayed a significant positive correlation when SOM is lower than 15 %. In contrast, permafrost and rice paddy in our study did not show any significant correlation even for samples with lower SOM than 15 %. We speculate that the discrepancy was caused by substantial differences in water availability in the soils. While tidal freshwater wetlands had been saturated constantly (Morrissey et al. 2014), permafrost and rice paddy in our study were not flooded or only temporally flooded. Unsaturated conditions allow oxygen penetration into soils, which inhibits methanogenesis. This may interfere with the relationship between methanogen abundance and methane production. It is noteworthy that similar lack of significant correlation between methanogen abundance and CH_4 production potential was reported with samples with low SOM in natural wetlands in China (Liu et al. 2011).

In addition, this study measured SOM by a loss-on-ignition method; thus, the quality of the organic matter was not considered. Previous studies have shown that the quality and composition of SOM are critical in determining microbial processes in soils (Barnes et al. 2012; Reiche et al. 2010; Shi et al. 2006). Decomposition and fermentation of SOM, which generate substrates for methanogenesis, are regulated by a number of extracellular enzymes (Freeman et al. 1997). Simultaneous measurements of extracellular enzymes and quality of SOM can enhance the mechanistic understanding of GHG emission from wetlands (Morrissey et al. 2014). Microbial decomposition of recalcitrant organic matter is a slow and difficult process (Prescott 2010). Thus, high concentrations of SOM may not always be associated with high rates of methanogenesis if large portion of the SOM is recalcitrant. Another possibility is the presence of phenolics, which inhibit the enzyme activity by 'enzymic latch' (Freeman et al. 2001). Therefore, the quality of the organic matter should be considered in further study for determining the more accurate effect of the substrate concentration for methanogenesis.

The pH was negatively correlated with the abundance of methanogen and the CH_4 flux in a range of 5.25–6.34 (Fig. 5a, b). The mountainous wetland exhibited a low pH, while the CH_4 flux and the abundance of methanogen were high. Because organic acid is emitted as a result of bacterial metabolism, a low pH may associate with high bacterial decomposition activity in a mountainous wetland with a high SOM condition. According to our measurement, there was a positive correlation between CH_4 flux and the abundance of methanogen when the pH ranged from 5.6 to 6.4 (Table 2), while no significant correlation was observed out of that range. Mah and Smith (1981) suggested that most of the methanogens active in near-neutral, not low or high, pH levels. In the present study, the correlation was found only in moderate acidic condition. Wang et al. (1993) observed a significant decrease in methanogenic activity under exceedingly high or low pH conditions. Due to the poor activity of methanogenesis (i.e., a large proportion of inactive methanogen) in relatively high or low pH condition (pH of higher than 6.4 or lower than 5.6), the abundance of methanogen may not represent CH_4 flux accurately in these conditions.

The rice paddy exhibited a higher value of the abundance of methanogen but a statistically identical annual mean CH_4 flux as compared to the permafrost (Fig. 2). Essentially, methanogenesis is a microbial process; thus, it usually follows the Arrhenius law. The annual mean temperatures in the permafrost areas (−5.2 °C in Ny-Ålesund and −2.6 °C in the Nome Complex) were significantly lower than those of the rice paddy (10.2 °C in Haean-myeon) (Table 1). Due to the lower temperature of the permafrost, the CH_4 flux should be smaller than that of the rice paddy if all other conditions are similar. However, the CH_4 flux in the two ecosystems was not significantly different. This observation can be explained in terms of the impact of seasonal changes on the hydrological conditions. The rice paddy has extraordinarily variable cycles of hydrology. This field is submerged during late spring (April) to early fall (September) but is drained the rest of the time. This changes the oxygen availability characteristics significantly, making the area anaerobic during the summer and aerobic during the other seasons. Methanogenesis is strongly inhibited when oxygen is available (Fetzer et al. 1993), for

which the rice paddy in this study may emit CH_4 only in the flooded period. The CH_4 flux from rice paddy in a drained period showed negative values (i.e., CH_4 uptake), resulting in statistically identical annual mean of CH_4 production with permafrost. Seasonal hydrological variation may play an important role in the dynamics of CH_4 flux in a temporary submerged ecosystem, such as a flood plain or a tidal marsh, making it responsible for large proportion of global methane sources (Kirschke et al. 2013).

Conclusion

Overall, this study examined the relationship between the annual mean CH_4 flux and the abundance of methanogen in three soil ecosystems. The relationship has a very narrow range of biogeochemical condition, such as high SOM (more than 60 %), a pH range of 5.6–6.4, and water-saturated, which are considered as a favorable environment for methanogenesis. This result indicates the importance of other biogeochemical conditions when microbial information is considered in CH_4 flux models. For example, microbial information may have only limited importance in CH_4 flux models when a target soil ecosystem is unsaturated and mineral soil. Results of this study would further support fundamental understandings of generalized CH_4 dynamics in different soil ecosystems with various soil characteristics and microbial traits so that more accurate microbial CH_4 flux model can be developed.

The results of this study were originated from three soil ecosystems, so further studies should collect more data from various types of wetland ecosystems (for example, salt marshes, tropical wetlands, and boreal peat areas) with a wide longitudinal distribution to reduce uncertainties in these types of correlation analyses. In addition, SOM quality evaluations, transcriptomic analyses, microbial community structural analyses, and hydrological classifications should be conducted to improve the correlation analyses to discover effective input variables for a more accurate CH_4 flux model.

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